Toxicological monitoring and protocol development for abandoned pipeline removal in Louisiana

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TOXICOLOGICAL MONITORING AND PROTOCOL DEVELOPMENT FOR ABANDONED PIPELINE REMOVAL IN LOUISIANA

A Thesis

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College In partial fulfillment of the Requirements for the degree of Master of Science

In

The Department of Environmental Studies

By

Forest Wootten
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# TABLE OF CONTENTS

ACKNOWLEDGMENTS .............................................................................................................. ii

LIST OF TABLES ..................................................................................................................... v

LIST OF FIGURES ................................................................................................................... vi

ABSTRACT .............................................................................................................................. viii

1. INTRODUCTION ................................................................................................................. 1

2. LITERATURE REVIEW ....................................................................................................... 3
   2.1 Calcasieu River Basin ................................................................................................. 3
   2.2 Site History ................................................................................................................ 3
   2.3 Current Oil/Gas Infrastructure Programs ..................................................................... 5
   2.4 Louisiana Coastal Zone Management ....................................................................... 6
   2.5 Louisiana Water Quality Monitoring ....................................................................... 7
   2.6 Biomonitoring ............................................................................................................. 8
   2.7 Ploidy ........................................................................................................................ 15
   2.8 Semipermeable Membrane Devices ......................................................................... 16

3. MATERIALS AND METHODS ............................................................................................ 18
   3.1 Site Description and Selection .................................................................................. 18
   3.2 Objectives and Hypothesis ....................................................................................... 18
   3.3 Permitting .................................................................................................................. 19
   3.4 Soil Sampling ............................................................................................................. 19
   3.5 Soil Analysis .............................................................................................................. 19
   3.6 Soil Gas Chromatography Extraction ..................................................................... 20
   3.7 Soil Gas Chromatography Analysis ......................................................................... 20
   3.8 Soil Calculations ....................................................................................................... 22
   3.9 Monitoring System Deployment ............................................................................. 24
   3.10 Sediment Disturbance Event .................................................................................. 27
   3.11 Hydrology Analysis ................................................................................................. 28
   3.12 Oyster Sampling ..................................................................................................... 28
   3.13 Oyster Condition and Color Index ......................................................................... 29
   3.14 Oyster Hydrocarbon Analysis .............................................................................. 30
   3.15 Oyster Metals Analysis ......................................................................................... 30
   3.16 Aerial Imagery ........................................................................................................ 32
   3.17 Statistical Analysis ................................................................................................. 32

4. RESULTS AND DISCUSSION ......................................................................................... 33
   4.1 Hydrology of the Site ............................................................................................... 33
   4.2 Total Hydrocarbons ............................................................................................... 37
   4.3 Metals Analysis ....................................................................................................... 41
LIST OF TABLES

Table 3.1 Compounds Quantified on Gas Chromatography .................................................. 22
Table 3.2 GPS Locations of Test ......................................................................................... 26
Table 3.3 Wavelength (nm) for each metal analyzed with ICP/MS ..................................... 31
Table 4.1. Total discharge of water (Q) for each transect ...................................................... 36
Table 4.2. Concentrations (µg/mg) of alkanes and PAHs in sediments ................................. 38
Table 4.3. Affect of ploidy on concentration (µg/mg) of hydrocarbons following disturbance event ......................................................................................................................... 40
Table 4.4 Lead Concentrations (µg/mg) by test cage ............................................................ 46
Table 4.5. Metal concentrations (µg/mg) of oyster dry tissue weight over time .................. 49
Table 4.6. Average concentration (mg/kg) of diploid versus triploid oysters ..................... 50
Table 4.7 Physical water quality parameters on Lake Calcasieu ........................................ 52
LIST OF FIGURES

Figure 2.1 Several abandoned pipelines cut at shoreline..........................................................4
Figure 3.1 Assembled Oyster Stack..........................................................................................25
Figure 3.2 Locations of the oyster sampling locations..............................................................26
Figure 3.3 Sediment Perturbation created by trawling of weights from boat............................27
Figure 4.1. Water flow across the Big Lake oil field on 2008 July 12....................................33
Figure 4.2. Depth profile of Transect A....................................................................................34
Figure 4.3 Velocity of water at each sampled location across Transect A...............................35
Figure 4.4. Depth profile of Transect B.....................................................................................35
Figure 4.5 Velocity of water at each sampled location across Transect B...............................36
Figure 4.6 Mean concentration of alkanes extracted from oyster tissue.................................39
Figure 4.7 Mean concentration of PAHs extracted from oyster tissue over time....................40
Figure 4.8 Percent change in metals concentration of oyster tissue as compared to Day 0......41
Figure 4.9 Percent change in metals concentration of oyster tissue as compared to Day 0......42
Figure 4.10 Arsenic concentrations over time..........................................................................43
Figure 4.11 Mean chromium concentration over time.............................................................44
Figure 4.12 Mean copper concentration at each sampling location........................................44
Figure 4.13 Mean iron concentration at each sampling location..............................................45
Figure 4.14 Mean nickel concentration at each sampling location..........................................46
Figure 4.15 Mean Zinc concentration at each sampling location............................................47
Figure 4.16 Mean barium concentration at each sampling location.........................................48
Figure 4.17 Mean aluminum concentration at each sampling location....................................49
Figure 4.18 Estimation of Oyster Health from each sampling location....................................51
Figure 4.19 Condition Index based on oyster Ploidy…………………………………………………………52

Figure 4.20 Oysters observed for color index……………………………………………………………………53
ABSTRACT

Oil/Gas extraction have left Louisiana with a legacy of abandoned infrastructure across the State. Presently, the State has developed guidelines for the removal of abandoned vessels and abandoned on shore facilities. No such guidelines exist, however, for the network of abandoned pipelines present throughout the coastal zone of Louisiana.

A pipeline removal was simulated in Lake Calcasieu, LA. The site was chosen because of the presence of many abandoned pipelines and previous work done on sight to remove the on shore infrastructure. In addition, the Calcasieu is an industrial water body, with potentially hazardous pollutants sequestered in the sediments of the lake bottom. Several industrial facilities discharge effluents into the water body, and a superfund site exists as a result. The sediment plume created during the perturbation event was monitored with triploid and diploid oysters to assess the toxicological consequences of the sediment plume.

Oyster tissue was analyzed for alkanes, Polynuclear Aromatic Hydrocarbons, hexachlorobutadiene, trace metals and organo metals. No difference was seen among concentrations of analytes between diploid and triploid oysters. In addition, the condition index of diploid oysters matched those of the triploid oysters, suggest spawning did not occur during the field study.

Test Cage 3 oysters were most affected by the perturbation event and displayed significant (p < .05) increases in total hydrocarbon concentration and in 13 of 16 metals tested. These increases corresponding with significant (p < .05) drop in the condition of Test cage 3 oysters 3 days following perturbation, from 6.6 to 4.8. No other Test Cage oysters displayed a clear response in body concentrations to the perturbation event. As such, the northern and southern range of the sediment plume can be demonstrated though analysis of the oyster tissue.
Such data would be of critical importance in determining any deleterious affects to the aquatic ecosystem attributable to the sediment plume as a result of pipeline removal.
INTRODUCTION

Oil and gas industries have been active in Louisiana for about 100 years as the first oil well was drilled in 1901 near Jennings, Louisiana (LDNR). Beginning in the twentieth century, the industry became an economic leader in the state and currently remains an integral member of the state’s business, social and environmental landscape. In 2006/2007, Louisiana generated an estimated $522.5 million from oil/gas royalties alone, an all time high (LDNR).

As a result, however, of such extensive exploration and drilling activities over the last century, the State has inherited a legacy of extraction infrastructure including drilling wells, refineries, tank batteries and pipelines. Many of these facilities remain abandoned well after active operations have ceased. In many cases, ownership and liability of these facilities is ambiguous, complicating attempts to remove or remediate the structures.

Pipelines in particular present a unique challenge for the state, as thousands of miles of pipelines remain abandoned and buried, often crossing private, state and federal properties. Many of these pipelines have become partially exposed, creating potential hazards along navigation routes. In addition, these pipelines may rest in sediments heavily polluted from years of industrial activity.

Thus far, no state protocol exists for the removal of abandoned pipelines in Louisiana. The Louisiana Department of Environmental Quality has established RECAP guidelines for wells and tank batteries, but no state approved protocol has been developed and enacted for pipeline removal. This has created concern among industry leaders who are cautious of removing pipelines that may release pollution currently sequestered in the sediments.
The focus of this study was to identify the potential toxicological hazards of pipeline removal in coastal Louisiana, and develop a suggested protocol for the toxicological assessment and monitoring of pipeline removal projects. To this end, a site was selected in the Lake Calcasieu River basin where several abandoned pipelines lay exposed as they descend into the river channel. The sediments of the area were monitored for chemical and physical parameters as potential pollutant concerns. In addition, a biomonitoring system was set up using triploid and diploid oysters and semi permeable membrane devices (SPMDs). A pipeline removal was simulated to create a sediment disturbance. Following the sediment disturbance, aerial imagery was taken to visually identify the sediment movement in the water column. The deployed oysters were collected before and after the disturbance event to assess any detrimental impacts to the water quality and aquatic system as a result of the sediment disturbance. The oyster tissue was analyzed for specific pollutant parameters, namely PAH’s, alkanes and metals. Additionally, a condition index was calculated for the harvested oysters to determine what if any stress was caused the oysters as a result of the disturbance.

A further comparison was done to assess the level of accumulation of each chemical parameter between the diploid oysters, triploid oysters and SPMD’s to evaluate any differences between each monitoring system. The results of the work have been used to support a suggested protocol for pre-removal assessment and post-removal toxicological monitoring of abandoned pipelines in coastal Louisiana.
LITERATURE REVIEW

2.1 Calcasieu River Basin

The Calcasieu river basin is and has been an important economic and industrial force in Louisiana. Encompassing nearly 4,105 square miles, the Calcasieu River supports a variety of industries, including shipping, oil/gas development and industrial plants (LDEQ, 1999; LDWF, 2005). These activities, along with heavy agricultural use within the watershed, have had a significant impact on the environmental quality of the Calcasieu river system (Waldon, 1996).

Currently, the 37 mile long Calcasieu River has an informational advisory for fish consumption due to low levels of chemical contamination (LDEQ, 2004). Hexachlorobenzene, Hexachloro-1,3-butadiene and PCBs are all listed as causes for the advisory. Suspected sources include industrial effluents lawfully discharged under permit (LDEQ, 2004). Hexachlorobenzene and Hexachloro-1,3-butadiene are listed under the Clean Water Act (CWA) as chemicals of bioaccumulation concern (LDEQ, 2004). In addition, due to the long history of oil/gas exploration, PAHs and alkanes are a concern in local pockets throughout the river and lake system.

2.2 Site History

The Calcasieu River site was chosen for pipeline removal and monitoring due to the presence of several abandoned flowlines extending out into a state water bottom. The well heads and pipelines were originally constructed under State Lease 2406, in the Big Lake oil field. Pipelines originally converged at an onshore tank battery which was removed in 2001. It is believed the pipelines were installed in the 1950’s by Standard Oil, although clear documentation of ownership does not exist. The selected site met the needs of the state and private sector
companies involved at this site, and the pipelines are a potential hazard and obstruction to navigation.

![Figure 2.1 Several abandoned pipelines cut at shoreline](image)

The pipelines sit directly atop the sediment bed and sink below the sediments moving into the shipping channel. A significant sediment plume would be generated during the removal of the pipelines. The pipelines have been drained and capped and are not believed to pose a significant risk for direct oil leakage or spill. However, the sediment plume from the removal event is cause for concern for any party attempting to remove the pipelines. Sediment particles, particularly silting and clay particles have a high affinity for a wide spectrum of pollutants including those associated with oil/gas activities (NOAA, 2007). Thus sediments present a particular risk to the aquatic system as they act as a repository for toxic material (Kanz et al,
1993). The risk of releasing sequestered pollutants into the aquatic environment necessitates the need for monitoring and testing guidelines before any major pipeline removals and sediment disturbances take place.

2.3 Current Oil/Gas Infrastructure Programs

The Louisiana Oil Spill Coordinators Office (LOSCO) is the primary state agency tasked with preventing spills and other damages to Louisiana’s natural systems due to oil/gas operations. The first program concerning abandoned infrastructure removal, the Abandoned Barge Program, was initiated in 1993 by LOSCO to mitigate the risk of oil spills from abandoned barges in Louisiana (OSPAR, Act). The program is allocated up to 1,000,000 dollars per year for activities by the Louisiana Oil Spill Prevention and Response Act (OSPAR) and LOSCO has partnered with the United States Coast Guard and the Environmental Protection Agency in the Joint Operating Procedure (JOP) to maximize resources. When a barge is identified as an oil spill risk and flagged for removal, JOP first attempts to identify ownership. If no ownership exists, either the federal agencies or LOSCO takes responsibility for removal of the barge, depending on the threat of midnight dumping. LOSCO reports that while JOP has removed several barges, individual owners have taken the initiative to remove barges themselves since the formation of JOP (LOSCO, 2009).

A second program coordinated by LOSCO is the Abandoned Facilities Program. The facilities program seeks to minimize the risk to human health and the natural environment from potential oil spills at abandoned facilities in the state. Such facilities include wells, tank batteries, onshore facilities, rigs and pits. Funds for the program are allocated through OSPAR and partnerships have been formed with JOP, along with the Louisiana Division of Natural Resources and the United States Army Corps of Engineers (LOSCO, 2009).
Neither of the two programs deals specifically with abandoned pipeline removal. However, these programs can serve as a model for funding and partnership with state and federal agencies in regards to pipeline removal. In addition, a component of OSPAR has been to develop biological monitoring guidelines for aquatic systems.

## 2.4 Louisiana Coastal Zone Management

In 1972, the Federal Government passed the Coastal Zone Management Act allowing individual states to establish their own Coastal Management Programs. Louisiana followed suit and passed the State and Local Coastal Resources Management Act, creating the Louisiana Coastal Resources Program (LCRP). The program was originated precisely to help the state encourage economic development within the defined coastal zone while actively protecting and preserving the natural features of coastal lands and waters. The Coastal Management Division (CMD) is the agency responsible for implementing the LCRP (CMD, 2009).

The principal management tool of the CMD is the requirement of Coastal Use Permits (CUP) for any project within the coastal zone deemed to have a direct or significant impact to the coastal waters of Louisiana. Applicants must submit a CUP application to the CMD for review and the application acts as the single application for all state and local permits required for the project. The permit allows for inter-agency and public comment and is intended to streamline the permitting process as the CMD forwards the application to all relevant agencies. For example, if a federal 401 permit is required under the Clean Water Act (CWA) the United States Army Corps of Engineers (CWA) issues the 401 permit. A 401 permit can not be issued by the USACOE unless the Louisiana Department of Environmental Quality issues a water quality certification assuring that the state’s water quality standards will not be violated by the proposed
project. The CUP serves as the application for review by USACOE and LDEQ for issuance of their required permits as deemed necessary (CMD, 2009).

The CMD also establishes Coastal Use General Permits which allow a variety of activities to occur. Application for a general permit requires the initial submission of a CUP by the applicant, and if appropriate the CMD can authorize the project under the general permit or require an individual CUP from the applicant (CMD, 2009).

In 2005 the CMD issued a General Permit allowing for the installation, replacement, maintenance or removal of up to 10,000 linear feet of pipeline; the general permit expired in 2007 (CMD, 2009). All significant pipeline removals in the coastal zone today require a full CUP from each individual applicant.

2.5 Louisiana Water Quality Monitoring

Since the 1950’s the Louisiana Department of Environmental Quality has maintained statewide data on surface water conditions. The federal Clean Water Act of 1972 provided further guidance for the state in prioritizing its Water Quality Monitoring Network. Section 303(d) of the CWA calls for the state to develop Total Maximum Daily Loads (TMDL’s) for state waters. Section 305(b) of the CWA requires the state to complete an assessment of all subsections of the state water bodies. The 305(b) report is used to develop the TMDL’s as required by section 303(d).

Monitoring efforts include testing samples of ambient water, industrial/municipal effluents, fish, shellfish and sediment samples. Samples are tested for a variety of parameters, ranging from Dissolved Oxygen, Total Suspended Solids, Biological Oxygen Demand to specific pollutant analysis. LDEQ has developed an EPA approved Quality Assurance Project Plan to
ensure the quality of data collected. Any data used in 305(b) reporting or 303(d) listing must follow LDEQ’s Quality Assurance Project Plan (LDEQ, 2004).

Data assists the LDEQ in determining if water bodies support designated uses. The designated use of a water body determines the data parameters most appropriate for testing. The uses include Primary Contact Recreation, Secondary Contact recreation, Fish and Wildlife Propagation, Drinking Water Source, Outstanding Natural Resource, Agriculture, Oyster Production and Limited Aquatic and Wildlife (LDEQ, 2004). Attainment is determined by what percentage of the water body meets the specified water quality criteria.

LDEQ also conducts biotoxicity tests in response to unique events such as fish kills, spills or special research studies. Whole Effluent Testing (WET) guidelines have been established for several candidate species. For fresh water samples, LDEQ conducts a chronic vertebrate test and a chronic invertebrate test (LDEQ, 2004). For saline samples, a chronic and an acute vertebrate test are generally conducted according to the EPA’s WET guidelines (LDEQ, 2004). WET testing, along with physical and chemical monitoring, represent the most complete analysis currently available for environmental samples. A number of different test organisms and toxicity endpoints have been developed for both chronic and acute tests depending on the salinity of the sample (Greenstein et al, 2008). Estuarine sediments common to the coastal zone pose a unique challenge for laboratory toxicity test, as the large salinity range makes replication challenging. However several specific tests have been developed for estuarine sediments (Davoren et al, 2005 and Dekker, 2006)

2.6 Biomonitoring

Historically, mussels and oysters have been used in the United States for monitoring of aquatic systems. As the organisms are filter feeders, analyzing the flesh of bivalves has proven a
useful system for gauging existing pollution in the aquatic system. An individual oyster filter 4 to 40 liters of water per hour, removing suspended solids, pathogens, sediments and other debris from the water (Volety et al, 2008).

Active filtering leads to accumulation of chemical pollutants from the water column and associated sediments, making oysters an ideal biomonitoring system (Byrne, 2001). Oysters will accumulate pollutants dissolved in the water column, and pollutants adhered to disturbed and suspended sediments. The surface area and charge of sediments, particularly clay particles, causes pollutants to be sequestered in the sediment layer (Burton et al, 2001). Sequestration of pollutants creates concern for any project, such as a pipeline removal, where sediment plumes in the water column results. In addition, the sessile nature of oysters allows for site specific pollution monitoring.

The EPA mussel watch program was initiated in 1976 and is currently operated by the National Oceanic and Atmospheric Administration (NOAA). NOAA’s National Status and Trends Program (NS&T) Mussel Watch Project Contaminant Monitoring uses Crassostrea virginica in Louisiana other Gulf Coast states to assess aquatic pollution and toxic body burden levels for aquatic organisms. The Mussel Watch program has over 300 sites nationwide and 20 test sites located in Louisiana waters. Other species of mussel and oyster are used in other parts of the country, as appropriate.

Traditionally, NS&T Mussel Watch Program subjects sediment and tissue samples to standard analytical testing including, organochlorines (pesticides), metals, Polynuclear Aromatic Hydrocarbons (PAH’s) and Polychlorinated Biphenols (PCBS’s). Additional toxicity testing is conducted using sediment samples, with microtox and P450 assays commonly used by the Mussel Watch Program.
PAH’s have been identified as one of the most carcinogenic constituents of oil and oil products (Marcus, 1985). PAH’s are man-made compounds generated during the combustion of fossil fuels and PAH’s released into the atmosphere during combustion can enter aquatic system. As such, PAH’s have become a standard monitoring analyte when assessing water quality. Oyster have been used to monitor PAH levels in coastal waters for decades, and the impacts and fate of PAH’s in the aquatic environment is well documented (Bravo et al, 1979). Studies have implicated PAH’s in genotoxicity for aquatic organisms as well as having adverse effects on heart and respiration rates of mussels (Cachot et al, 2006; Marcus, 1985). Additional inputs into the aquatic environment are derived from petroleum formation and petroleum spills (Hwang et al, 2008).

PCB’s are commonly used as a benchmark toxicant and have been banned since 1979. The compounds are entirely anthropogenic in source and are regulated by the federal government under Toxic Substances Control Act. PCB’s have been shown as highly carcinogenic, as well as having a wide range of impacts on aquatic organisms. Several studies have been conducted measuring PCB’s in shellfish around the world, indicating the ubiquitous nature of PCB pollution in the aquatic environment (Encomio, 2000).

Other commonly tested parameters of aquatic pollution include metals. Metals are more complicated as there are both natural and anthropogenic sources. Environmental sediment samples typically contain many different metals of varying concentrations. While no individual metal may be toxic at the present concentration, the synergistic affects of multiple trace metal exposure can increase toxicity dramatically to an organism (Verriopoulus, 1988). Additionally, many metals such as nickel are essential for biological development but toxic at high concentrations. Within the Calcasieu River, Copper, Cadmium and Lead have all appeared as
metals of concern. Both Copper and Cadmium are believed to be a product of industrial effluent, while sources of Lead are unknown.

For any study of metals toxicity, it is important to identify whether the metal exists in an organic or inorganic form. Heavy metals are a toxicity concern, with the inorganic forms of mercury, silver, copper and zinc proving the most toxic to oyster larvae (Calabrese et al, 1973). Several studies of metal toxicity have been conducted on oyster larvae and adult oysters alike. Oysters prove a valuable tool for monitoring metal contamination over time, and the bioaccumulation and depuration of metals out of oyster tissue (Okazaki, 1976). As with hydrocarbons such as PAHs, metals are also sequestered to the surface of sediments, posing a particular risk to filter feeders such as oysters (Ettajani, 2006). Metal sequestration in sediments can be affected by physical parameters such as salinity and pH of the water body (Cox, 2005). Individual metals can also be used as representatives for other trace metals. Aluminum, for example, has been shown to be a good indicator of other trace and heavy metals, and is thus a commonly monitored metal (Kanz, 1993).

Along with the industrial activity along the Calcasieu, use of metals by for oil/gas extraction would suggest elevated levels of metals within Calcasieu River sediments. Viscosity control agents such as bentonite typically have high levels of trace metals such as Lead, Cadmium, Aluminum and Chromium (Kanz, 1993). Zinc, lead, iron and barium have all been used as weighting agents in drilling muds, with barite (BaSO₄) the most commonly used. The low solubility (0.25g/L) and high specific gravity of barium make barium a particular concern for sessile and benthic organisms, such as oysters (Kanz, 1993). Indeed, the oyster and benthic algae have been shown as the most sensitive organisms to drilling mud toxicity (Kanz, 1993).
When using a biomonitoring system such as oyster, several endpoints are used to
determine the stress of the organism as a result of pollutant exposure including, the condition
index, lysosomal integrity and genetic toxicity (Ringwood et al, 1998; Ringwood et al, 2002).
With oysters, most larval toxicity studies are generally carried out in the laboratory, while adult
tests can utilize field harvested samples (Butler et al, 1992). In conjunction with the body
burden of analytes determined with Gas Chromatography/Mass Spectrophotometry for PAH’s
and PCBS or Induced Plasma Coupling for Metals, sublethal endpoints allow for a broader
understanding of the pollutant or pollution event on the aquatic system.

Condition Indexes have long been used for evaluating the health of _Crassostrea virginica_
populations (Mercado-Sivla, 2005). A condition index typically measures the ratio of tissue
weight to shell cavity volume. The A. E. Hopkins Condition Index is among the most commonly
used, calculating condition as (dry tissue weight * 100) / Shell Cavity Volume (Lawerence et al,
1982). Condition indexes are attractive as they represent a quick and cheap method for
evaluating the general health of an oyster population. In oysters, metabolic energy leftover after
reproduction and maintenance is converted to biomass (Volety et al, 2008), and a condition
index provides an indicator of how well an oyster has used available shell space for growth
(Lawerence et al, 1982). An oyster stressed from exposure to aquatic or sedimentary pollution
will convert less energy to biomass and will have a reduced condition index value. Condition
Indexes have also been used to quantify the impact of parasites on overall oyster health
(Mercado-Silva et al, 2005). Thus a condition index is a useful tool for quickly evaluating the
affects of water quality and water pollution on the growth and health of an oyster.

It is crucial to standardize methods for Calculating Condition Indexes to make any
meaningful conclusions from the data. Several studies have examined the Hopkins formula, and
determined no real accuracy is gained from using dry tissue weight as opposed to wet tissue weight. However, wet weights can be affected by variable draining time following collection (Lawrence, 1982). Similarly, internal cavity can be measured by a variety of methods. Several methods can be used, however studies have demonstrated a 1:1 ration between cavity volume and the weight of the cavity contents (Lawrence, 1982). Thus, cavity volume can and has been calculated as the difference between the measured weight in air of the whole oyster and the measured weight in air of the shells. This is the most simple, and cost effective method for determining shell cavity volume.

While the condition index is a useful and cost-effective tool for assessing general oyster population health, its interpretation is limited. Particularly with field samples exposed to several possible stressors, changes in condition index can not be definitively attributed to one individual stressor.

Lysosomal integrity is a second readily used endpoint for oyster toxicity. PCB’s, PAH’s and metals have all been implicated in disruption of cellular membranes (Hwang et al, 2008). Lysosomal destabilization assays have been developed for assessment of membrane health in aquatic organisms and can provide valuable consideration for evaluating the relative health of biomonitoring organisms, particularly in response to pollutant stress. Hwang et al demonstrated a correlation between PAH concentration and organismal health as a concentration of 2,100 ng/g total PAH’s caused a 50 percent destabilization of lysosomes. Following a 20 day elimination of PAH’s, membrane stabilization showed quick recovery (Hwang et al, 2008). The results indicate lysosomal assays may provide a more sensitive and early warning parameter to aquatic pollution although it is not widely used by federal or state agencies currently.
Genetic toxicity is a third major endpoint for toxicity studies. Evaluation of gamete number and quality are easily conducted on fertile oysters (His et al, 1997; Volety et al, 2008). Additional studies evaluate larval recruitment and juvenile oyster growth and a gonadal index evaluates the gamete number and health. Recent studies have implicated sediment PAH’s, along with metals and PCB’s with increased genetic toxicity (Chacot et al 2006; Volety et al, 2008). Juvenile growth is measured in the field by deploying juvenile oysters in cages and measuring shell width and length over time (Volety et al, 2008). Depending on time, needs and budget constraints, careful consideration should be given to which specific bioassay is to be conducted prior to any experiment (Johnson et al, 2004).

The NS&T Mussel Watch Program has yielded over twenty years of data, demonstrating trends in aquatic pollution and pollution hot spots in the coastal United States. Mussel Watch data indicates that levels of organic pollutants, namely low and high molecular weight PAH’s, PCB’s, DDT, Chlorodane, Dieldrin and Butylin have all decreased over time (NOAA, 2007). Trace and heavy metal contamination remains constant across the United States. In Louisiana specifically, only one of the Mussel Watch sites, located in Lake Pontchartrain, is considered to have elevated organic pollutants, with 40 parts-per-billion chlordanes and 96 parts-per-billion PCB’s (NOAA, 2007). The Mussel Watch Program samples at two locations in the Calcasieu water body. One sampling sit is located in the north of Lake Calcasieu and the other in the southern edge of the Lake between St. John’s Island and Rabbit Island.

Recently, an environmental impact assessment has been initiated by the EPA, NOAA, the Food and Drug Administration (FDA) and the U.S. Geologic Survey (USGS) to determine the effect of Hurricanes Katrina and Rita on coastal waters from Texas to Alabama. In addition to the standard analytes and tests conducted by the Mussel Watch Program, the specialized
assessment includes testing sediment samples for polybrominated flame retardants (PBDE’s), Fipronil (an insecticide), and Clostridium perfringens. C. perfringens is a pathogenic microorganism whose spores persist in sediments and soil (NOAA, 2007).

2.7 Ploidy

Typically, C. virginica exist as diploid oysters in the wild. However, advances in aquaculture has enabled the production of triploid organisms. Cells of triploid organisms contain a 3\textsuperscript{rd} set of chromosomes (Gagnarie \textit{et al}, 2006). Triploid oysters can be induced either by a chemical or physical treatment, or by crossing tetraploid males with diploid females (Gagnarie \textit{et al}, 2006). Triploids have gained increased attention as a commercial product, as they have several physiological distinctions from diploids. First, unlike diploid oysters, triploid oysters do not spawn. Normal diploid oysters are induced to spawn by increased temperatures during the warmer months. Prior to spawning, oysters store lipid and glycogen tissue in preparation for the spawning period.

As a result, triploid oysters spend less metabolic energy on gamete production, and invest more energy for somatic sell growth throughout the year (Gagnarie \textit{et al}, 2006). Specifically, triploid oysters have higher total body glycogen and gonad glycogen levels than diploid oysters during gametogenesis (Hand, 1999; Lingfeng \textit{et al}, 2007). As a result, triploid oysters grow at a faster rate and retain their flesh weight throughout the year. In some oysters, Triploids have been found to be on average 30-40 % heavier, reaching market weight 6 months faster than diploid oysters (Hand, 2004)

Some evidence exists that triploid oysters are also more resistant to stress than diploid organisms, and thus may present a healthy crop. However, mortality differences between diploid and triploid organisms are unclear (Gagnarie \textit{et al}, 2006). One concern with triploid oysters is
the potential for increased body burden of pollutants in triploid oysters. As oysters spawn, the lipid and glycogen content decreases. Higher lipid content has been shown to facilitate higher PAH concentration, and so triploid oysters may accumulate and retain more toxicants than diploid oysters (Marcus, 1985). Glycogen utilization by oysters during spawning has also been shown to be retarded by environmental stress and exposure to toxicants, such as hexachlorobenzene, a toxicant of concern in Lake Calcasieu (Baturo et al, 1995).

In addition, the condition index between populations of diploid and triploid oysters may vary due to these physiological differences, and not as a result of environmental stress. During gametogenesis, diploid oysters lose significant amounts of carbohydrate material, decreasing their overall meat weight (Lingfeng 2007). As meat weight is a primary component of a condition index calculation, it may prove an unreliable indicator of oyster health for spawning oysters. Thus a seasonal consideration must be given when calculating condition indices, or determining body burden concentrations of target pollutants, particularly with diploid oysters.

2.8 Semipermeable Membrane Devices (SPMDs)

One obvious advantage to SPMDs for pollution monitoring is the absence of mortality. Any biotic monitoring system is obviously susceptible to organism death which restricts the use of biomonitoring systems. With oysters, salinity gradients may prevent deployment in certain areas. In addition, diseases such as Perkinsus marinas, better known as dermo, could devastate a deployed oyster cage for monitoring of chemical or physical pollution (Voltey et al, 2008).

Several studies have attempted to correlate the accumulation of lipid soluble pollutants in SPMD’s to that of oysters and other mussels. Whereas oysters are filter feeder, SPMD’s must rely strictly on passive diffusion of compounds and studies have repeatedly shown higher levels of pollutants in tissues of bivalves than SPMD’s in field and laboratory studies (Huckins et al,
However, the general conclusion is that SPMD’s accumulate the same analytes as mussels and have value as a monitoring system if the results can be correlated with approved methods, like those for tissue sampling in NOAA’s mussel watch (Huckins et al., 2004).
3

Materials and Methods

3.1 Site Description and Selection

Pipelines located on the Big Lake Oil Field on the east bank of the Calcasieu River were identified for this pilot study. The site was chosen due the presence of abandoned pipelines exposed on state water bottoms, and land access to the pipes for heavy machinery. An initial site visit was conducted on February 10, 2008 to evaluate the site and experimental setup. A survey of available bivalve populations revealed the need to deploy bivalves on site for biomonitoring.

3.2 Objectives and Hypothesis

1. Develop a functional protocol for toxicological monitoring and assessment of pipeline removal projects in Louisiana

2. Deploy biomonitoring organisms on site for collection and subsequent analysis of PAH’s, Alkanes, and Metals on Day 0, 7, 10, 14, 21 and 35 (Day 7 representing disturbance event)

3. Compare the ability of diploid and triploid C. virginica oysters to act as biomonitoring organisms by evaluating relative levels of PAH’s, Alkanes and Metals in the tissues of the two test organisms

4. Evaluate the potential use of Semi-Permeable Membrane Devices as a possible alternative to the use of C. virginica

5. Collect discharge and flow data to determine the predicted movement of a sediment pulse event.

6. Collect aerial imagery of the disturbance event to compare the visual movement of sediment with levels of pollutant compounds found in the monitoring organisms.
Hypothesis:  

1. Following the disturbance event, levels of PAH’s, Alkanes and Metals within the tissues of the test organisms will increase relative to the proximity of the organism to the physical disturbance event.

2. Triploid oysters will show higher accumulation of alkanes, PAH’s and metals than will diploid oysters.

3.3 Permitting

A Coastal Use Permit (CUP) was obtained from the Coastal Management Division of the Louisiana Department of Natural Resources for permission to deploy oyster cages and remove up to 50 linear ft of the abandoned pipelines. The application was 1st submitted in December 2007, approved in April of 2008, and work on the permit was initiated on June 10 2008, when the oyster cages were first deployed.

Because only a small segment of each pipeline was to be removed, the permit required the remaining section to be capped and buried at least 3 ft below the water bottom.

3.4 Soil Sampling

Soil samples were collected from the site on March 24, 2008. Submerged sediment samples were taken at points within the pipeline network, and at points both north and south of the pipelines within the water body. Sediment was collected from 1-6 inches deep using a manual coring drill. Samples were immediately placed into zip lock bags, labeled and transported on ice in a closed cooler back to Baton Rouge. Once in the laboratory, sediment was filtered using a 2 mm sieve to remove rocks, and then stored in a 4 degree refrigerator in unused amber sampling jars.

3.5 Soil Analysis

Soil samples were analyzed using Gas Chromatography/Mass Spec for PAH’s, Alkanes,
and hexachlorobutadiene. Physical characteristics of the soil were determined to assess the predicted suspension time and potential movement within water of the sediment once disturbed by the process of pipeline removal.

3.6 Soil Gas Chromatography Extraction

Sediment samples were first extracted following EPA Method 3550b. Briefly, 50 g wet weight of sediment were prepared in triplicate for analysis. Each sample was mixed in a 500 ml sterile beaker with granular anhydrous sodium sulfate to reduce water content of the samples. Dicloromethane (DCM) was added so as to completely cover each sediment sample, and 1 ml of the surrogate standard (100 μg/mL Phenanthrene-d10 and 100 μg/mL 5-alpha Androstane) was added to the each sample. Quality control standards require recovery of 70% - 120 % of the surrogate standard. Samples were than sonicated for 5 minute intervals three different times using a Fisher Sonic Dismembrator Model 300. After sonication the liquid from each sample was separated by filtering under a fume hood through a 0.45μm filter packed with granular anhydrous sodium sulfate. Liquid extract was collected in a 250 ml round bottom flask. The samples were again covered with DCM and sonicated and decanted a second and third time.

The round bottom flasks with decanted liquid were reduced in volume to 5 ml using a roto-evaporator and 45°C water bath. Condensed volume was transferred into 25 ml centrifuge tube, and the round bottom flasks were rinsed with DCM to remove any remaining hydrocarbon analytes. 1 ml was than taken from each sample and placed into an amber autosampling GC vial, along with 10μL of internal standard (1000 μg/mL each of Napthalene-d8, Acenaphthen-d10, Chrysene-d12, and Perylene-d12).

3.7 Soil Gas Chromatography Analysis

GC analysis was conducted according to Modified EPA SW846-8270. This modified
method allows for the analysis of total hydrocarbons in the samples, represented by PAH’s and nC10-cC35 alkanes. The method was also altered to include analysis of hexachlorobutadiene. In all 77 compounds were quantified with GC/MS analysis. Of these, 4 internal standards (Napthalene-d8, Acenaphthen-d10, Chrysene-d12, and Perylene-d12) were used to determine the concentration of each experimental analyte. The two surrogate standards (Phenanthrene-d10, 5-alpha Androstane) were used to determine percent recovery of hydrocarbons during extraction.

Prior to running the extracted samples, a 5 point calibration curve (0.5ppm, 1ppm, 5ppm, 10ppm, 25 ppm) was conducted for each individual analyte, including hexachlorobutadiene, as required by the Modified Method. Calibration curves were also conducted for the internal and surrogate standards.

The gas chromatogram oven (Hewlett Packard Model 5890) and the mass spectrometer (5972 Series Hewlett Packard Mass Selective Detector) were tuned prior to use. The sample sequence was programmed and vials loaded into an auto sampler. The GC/MS was operated in selective ion mode (SIM) with the injector set at 250°C and the detector set at 300°C. The GC column (30m x 0.25 mm ID 0.25μm film thickness silicone-coated fused-silica capillary column) was initially set at 55°C and held for 3 minutes. The temperature was then gradually increased to 280°C at a rate of 5.0°C per minute. Once at 280°C, the temperature was increased to 300°C at a rate of 1.5°C per minute. This process resulted in a total run time of 65.33 min per sample.

Table 3.1 lists all the individual alkanes and PAHs analyzed from the soil samples collected on site. Compound homologues were not analyzed. Light weight ringed hydrocarbons such as benzene and toluene were excluded from analysis, as the primary focus was lipid soluble compounds that would have high affinity for sediments and be readily absorbed and stored with oyster tissue.
Table 3.1 Compounds Quantified on Gas Chromatography

<table>
<thead>
<tr>
<th>Internal Standard</th>
<th>Surrogate Standard</th>
<th>n-Alkanes</th>
<th>PAH's</th>
</tr>
</thead>
<tbody>
<tr>
<td>Napthalene-d8</td>
<td>Phenanthrene-d10</td>
<td>nC-10 Decane</td>
<td>Naphthalene</td>
</tr>
<tr>
<td>Acenaphthen-d10</td>
<td>Androstane</td>
<td>nC-11 undecane</td>
<td>Fluorene</td>
</tr>
<tr>
<td>Chrycene-d12</td>
<td></td>
<td>nC-12 Dodecane</td>
<td>Dibenzothiophene</td>
</tr>
<tr>
<td>Perylene-d12</td>
<td></td>
<td>nC-13 Tridecane</td>
<td>Phenanthrene</td>
</tr>
<tr>
<td></td>
<td></td>
<td>nC-14 Tetradecane</td>
<td>Anthracene</td>
</tr>
<tr>
<td></td>
<td></td>
<td>nC-15 Pentadecane</td>
<td>Fluoranthene</td>
</tr>
<tr>
<td></td>
<td></td>
<td>nC-16 Hexadecane</td>
<td>NBT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>nC-17 Heptadecane</td>
<td>Benzo (a) Antracene</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pristane</td>
<td>Chrysene</td>
</tr>
<tr>
<td></td>
<td></td>
<td>nC-18 Octadecane</td>
<td>Benzo (b) Fluoranthene</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Phytane</td>
<td>Benzo (k) Fouoranthe</td>
</tr>
<tr>
<td></td>
<td></td>
<td>nC-19 Nonadecane</td>
<td>Benzo (e) Pyrene</td>
</tr>
<tr>
<td></td>
<td></td>
<td>nC-20 Eicosane</td>
<td>Benzo (a) Pyrene</td>
</tr>
<tr>
<td></td>
<td></td>
<td>nC-21 Heneicosane</td>
<td>Perylene</td>
</tr>
<tr>
<td></td>
<td></td>
<td>nC-22 Docosane</td>
<td>Indeno (1,2,3-cd) Pyrene</td>
</tr>
<tr>
<td></td>
<td></td>
<td>nC-23 Tricosane</td>
<td>Dibeno (a,h) anthracene</td>
</tr>
<tr>
<td></td>
<td></td>
<td>nC-24 Tetracosane</td>
<td>Benzo (g,h,i) perylene</td>
</tr>
<tr>
<td></td>
<td></td>
<td>nC-25 Pentacosane</td>
<td>Pyrene</td>
</tr>
<tr>
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<td></td>
<td>nC-26 Hexacosane</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>nC-27 Heptacosane</td>
<td></td>
</tr>
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<td></td>
<td></td>
<td>nC-28 Octacosane</td>
<td></td>
</tr>
<tr>
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<td>nC-29 Nonacosane</td>
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<td></td>
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<td>nC-32 Dotriacontane</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>nC-33 Tritiacontane</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>nC-34 Tetratriacontance</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>nC-35 Pentatriacontane</td>
<td></td>
</tr>
</tbody>
</table>

3.8 Soil Calculations

Following integration of absorbance peaks using ChemStation Data Analysis software, concentrations were calculated with the following formula.

Relative Response Factor (RRF)

\[
RRF = \frac{(A_x \cdot C_{i_s})}{(A_{is} \cdot C_x)}
\]

Where:

\(A_x\) = area of analyte in calibration standard
C_{is} = \text{concentration of the internal standard (ng)}

A_{is} = \text{area of the internal standard}

C_{x} = \text{concentration of calibration standard (ng)}

**Concentrations of Analytes**

\[
[C] (\text{ng/mg or ng/mL}) = \frac{(A_{x} \times I_{x} \times V_{\text{fin}} \times 1000 \times DF)}{(A_{is} \times \text{RRF} \times V_{i} \times M \text{ or } V_{ini})}
\]

Where:

[C] = \text{concentration}

A_{x} = \text{area/target response of analyte}

I_{x} = \text{amount of internal standard injected (ng)}

V_{\text{fin}} = \text{final volume of the total extract (ml)}

1000 = \text{conversion factor (1000 ng in a μl)}

DF = \text{dilution factor}

A_{is} = \text{area/target response of internal standard}

\text{RRF} = \text{average relative response of internal standard}

V_{i} = \text{volume of sample injected (μL)}

M = \text{mass of sample (mg)}

V_{ini} = \text{initial volume of sample (ml)}

**Percent Surrogate Standard Recovery for Quality Control**

\[
[C]_{ss} (\text{ng/mg or ng/mL}) = \frac{(V_{ss} \times Css)}{M \text{ or } V_{ini} \times 1000}
\]

Where:

[C]_{ss} = \text{concentration of surrogate standard}

V_{ss} = \text{volume of surrogate standard added to sample (ml)}

Css = \text{concentration of surrogate standard (μg/ml)}
M = mass of sample (mg)

\[ V_{\text{ini}} = \text{initial volume of sample (ml)} \]

1000 = conversion factor

### 3.9 Monitoring System Deployment

Oyster Sampling stations were prepared using welded PVC covered steel mesh 20 x 24 x 6 in Shellfish Trays from Atlantic Aquaculture. Each individual tray had a hinged top that was secured shut using plastic zip ties. Full sampling stations were constructed by stacking five trays, one on top of the next.

The bottom tray of each stack was filled with bricks to anchor the stack in place in the water. The second tray of each stack was filled with approximately ~200 diploid oysters and secured to the bottom tray using a bungee cord. The third tray in each stack contained three SPMDs and was secured to the second tray with a bungee cord. The SPMDs were attached at each end to the side of the cage using a zip tie, and allowed to float in the interior of the cage. A steel deployment cage designed for SPMD deployment can be purchased to protect the membranes from physical abrasion and puncturing while deployed in the field. The fourth cage contained ~200 triploid oysters. The fifth cage was empty, and a 24 in diameter orange buoy was attached to the fifth cage to identify each station in the water. Each individual cage was closed with a plastic zip tie, and each cage was fastened to the one below it with a bungee cord forming the sampling station, as seen in Figure 3.1.

Four sampling stations were assembled in total, each containing diploid oysters, triploid oysters and SPMD’s. Three stations were deployed within the pipeline network area to monitor the sediment plume, and a fourth reference cage was deployed to provide data on ambient pollution levels within the river system. The height difference between the second cage
containing diploid oysters and the third cage containing triploid oysters was less than 8 in. This difference of height was not deemed significant enough to dramatically alter the kind and quantity of pollutants the oysters would be exposed to during the experiments duration.

![Figure 3.1 Assembled Oyster Stack](image)

On June 10, 2008 oysters and semipermeable membranes were deployed for monitoring on location in Lake Calcasieu. Oysters were obtained from Dr. John Supan and the Louisiana State University Oyster Hatchery in Grand Isle, LA, and the SPMD’s were ordered from Environmental Sampling Technologies. The oysters were transported from Grand Isle to Lake Calcasieu in coolers packed covered with burlap cloth and ice the morning of the 6th. Stacks
were assembled on location and deployed in the water that same day using a small flat bottom bateau. Assembled stacks were lifted from the bateau and dropped into the water at the permitted locations. Oysters were obtained from the Louisiana State Oyster Hatchery on Grand isle, LA.

**Table 3.2 GPS Locations of Test Cages**

<table>
<thead>
<tr>
<th>Control</th>
<th>Sampling 1</th>
<th>Station 2</th>
<th>Station 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>N30°02.374'</td>
<td>N30°02.254'</td>
<td>N30°02.186'</td>
<td>N30°02.128'</td>
</tr>
<tr>
<td>W93°17.080'</td>
<td>W93°16.938'</td>
<td>W93°17.892'</td>
<td>W93°17.845'</td>
</tr>
</tbody>
</table>

**Figure 3.2** Locations of the oyster sampling locations
3.10 Sediment Disturbance Event

Our permit and initial aim allowed for the physical removal of pipeline from the water. Removed pipeline was to be surveyed for Natural Occurring Radioactive Material (NORM) by contractors from PSC services. Depending on the presence of NORM, removed pipeline would either be cut into segments and disposed of in a landfill of site, or brought to a treatment facility for remediation of NORM prior to disposal.

As required by the permit, all affected landowners were notified of the work plan. However, a dispute over landownership with a private landowner prevented us from mobilizing heavy machinery from the adjacent land to remove the pipe. As such, we continued with our monitoring program by simulating the sediment disturbance event expected from pipeline removal. The sediment disturbance event was created by dragging four 25 pound weights along the length of a pipeline from a boat. The weights were dragged repeatedly along the linear tract of the pipeline for about 45 minutes.

Figure 3.3 Sediment Perturbation created by trawling of weights from boat
3.11 Hydrology Analysis

Flow data was collected on site after the sediment disturbance event. Two separate transects were conducted using a SunTek Flowtracker Handheld Acoustic Doppler Velocimeter (ADV). The two transects moved South to North and East to West away from the shore to a central meeting point out over open water. Each transect was 100 ft long, and flow data of the water was collected every 10 ft along the transect. At water depths of less than 1 meter, only one data point was collected at 60% of the total water height above the bottom. For water depths greater than 1 meter, two data points were collected, one at 20% and one at 80% total water height above the bottom. Data points collected reflected total water flow in a direction perpendicular to the transect.

Data collected with ADV was then analyzed using SunTek Flow Tracker v2.11 software. Flow data was used to determine the direction and total water discharge across the length of each transect. The total discharge from each transect was used to determine the direction and discharge of water in Lake Calcasieu during the sediment disturbance event. This data was used as a predictor of sediment movement and thus a predictor of PAH, alkanes and metal exposure for each sampling station.

3.12 Oyster Sampling

Oysters were sampled from each stack on Day 0, 1, 3, 7, 14, 35. Oyster cages were accessed by wading out to each cage. Cages were dismantled from top to bottom, one cage at a time by removing the bungee cords to separate each cage, and then cutting the zip ties to open the cage. A minimum of 15 diploid and 15 triploid oysters were collected from each station. Oysters were initially placed into labeled zip lock bags. Once back on shore, the collected
oysters were immediately placed in a cooler on ice and transported back to the laboratory in Baton Rouge. Oysters were then stored in a 4\(^0\) Celsius refrigerator until analysis.

### 3.13 Oyster Condition and Color Index

Each oyster collected was first washed and cleaned of as much dirt, mud and vegetative growth as possible. Each oyster was then labeled and measured for shell length, width and wet weight. Oysters were then shucked, and oyster meat wet weight and empty shell weight recorded. Each oyster meat was examined for coloration and texture, and assigned a qualitative Color Index value. All the shells and three oyster meats were then left to dry in an oven for 48 hours, when a consistent weight could be recorded. Dry shell and dry meat weight were then collected for each shell and the three dried oyster meats. Water content of the shells and oysters was calculated by dividing the dry weight by the wet weight and multiplying by 100. The average meat water content calculated from the three dried oysters was used to estimate the dry meat weight of the other oysters for purposes of the Condition Index calculations.

For each sampling location, 12 triploid and 12 diploid oysters were used to calculate the condition index for each testing time.

Condition Indexes were calculated based on the ratio of shell weight to meat weight of an oyster. Two different formulas were used to calculate two Condition Indexes:

- **Condition Index 1** = \[
  \left\{ \frac{\text{Dry Tissue Weight (g)}}{\text{Shell Cavity Volume}} \right\} \times 100
  \]
- **Condition Index 2** = \[
  \left\{ \frac{\text{Dry Tissue Weight (g)}}{\text{Dry Shell Cavity Volume (g)}} \right\} \times 100
  \]

\*Shell Cavity Volume = Whole Oyster Weight (g) – Shell Weight (g)

Shell cavity volume can also be calculated as a measure of water displaced by the shells (Mercado-Silva, 2005). Research has demonstrated a linear relationship between calculating shell cavity through weight subtraction vs volume displacement (Shumacker, 1998). Condition
Indexes from each sampling cage were compared to determine how proximity to the sediment disturbance event impacted the growth of the oysters.

3.14 Oyster Hydrocarbon Analysis

Oyster tissue was analyzed for the same alkanes and PAHs used for the soil analysis. In addition, hexachlorobutadiene concentrations in oyster tissue were also tested.

Three wet oyster meats were weighed for each GC/MS sample, and blended into a slurry. 100 ml of 20% KOH solution was added to blended sample, and 1 ml of surrogate standard added to the mixture. The solution was transferred into 250 ml round bottom flasks, and refluxed for 2 hours. The solution was allowed to cool, and transferred into a seperatory funnel. 100 ml Pentane was added to the separatory funnel, mixed and the pentane eluted off. This process was repeated three times to remove hydrocarbons from the refluxed mixture. Pentane Effluent was than filtered through sodium sulfate and rinsed with DSMO. The resulting volume was then roto-vaporated down to 5 ml, and prepared for injection into the GC. Injection was conducted as stated above, using the same sequence, method and internal standards. An adjustment to the calculations was made to account for the slightly varying wet oyster weight in each sample. Whereas M (mass) = 50000 mg for each sediment sample, mass varied based on the size of the three oysters used for each sample.

3.15 Oyster Metals Analysis

Oysters were analyzed for metal concentration using Inductively Coupled Plasma Mass Spec (ICP/MS). A minimum of 1 g dry oyster tissue is needed for metals analysis. Metals analysis is generally conducted using wet tissue. Water was assumed to comprise 80% of oyster wet weight, so 5-7 g of wet oyster tissue was used for ICP/MS analysis. Individual oysters were blended into a slurry, and 5-7 grams of the slurry was weighed and digested for ICP/MS analysis.
Slurred oyster tissue was placed into test tube vials, and 5 ml concentrated Nitric Acid was added to digest the 5-7 g wet oyster tissue slurry. Overnight digestion on a hot plate resulted in full digestion of the oyster tissue into the acid solution, with minimal solids remaining. Once oyster tissue had completed dissolved in the acid, concentrations of each acid solution were diluted to 50 ml for injection into the ICP.

Metals concentrations of each sample were quantified based on wavelength absorption of 2 ppm and 10 ppm standards. Each metal was observed at the wavelengths shown in table 3. Where concentrations differed by more than 5% between wavelengths, the values at each wavelength were averaged together to produce a final concentration. Each sample was analyzed in triplicate, with three different slurries tested for each sample.

**Table 3.3** Wavelength (nm) for each metal analyzed with ICP/MS

<table>
<thead>
<tr>
<th>Element</th>
<th>Wavelength 1</th>
<th>Wavelength 2</th>
<th>Wavelength 3</th>
<th>Wavelength 4</th>
<th>Wavelength 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aluminum</td>
<td>257.509</td>
<td>308.215</td>
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<tr>
<td>Arsenic</td>
<td>188.98</td>
<td>193.696</td>
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<tr>
<td>Barium</td>
<td>455.403</td>
<td>585.367</td>
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<td></td>
</tr>
<tr>
<td>Calcium</td>
<td>317.933</td>
<td>396.847</td>
<td>430.253</td>
<td>612.222</td>
<td>616.217</td>
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<tr>
<td>Cadmium</td>
<td>214.439</td>
<td>226.502</td>
<td>228.802</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cobalt</td>
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<tr>
<td>Chromium</td>
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<tr>
<td>Copper</td>
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<td>324.754</td>
<td>327.395</td>
<td></td>
<td></td>
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<tr>
<td>Iron</td>
<td>238.204</td>
<td>238.863</td>
<td>259.837</td>
<td>263.105</td>
<td>370.792</td>
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<td></td>
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<td>Sodium</td>
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<td>330.298</td>
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<td>615.423</td>
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<td>Nickel</td>
<td>216.555</td>
<td>231.604</td>
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</tr>
<tr>
<td>Phosphorous</td>
<td>213.618</td>
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<tr>
<td>Lead</td>
<td>220.353</td>
<td>405.781</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zinc</td>
<td>206.2</td>
<td>213.857</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
3.16 Aerial Imagery

Overhead photography of the work site was captured using a remote controlled plane with a digital camera tied to the plane. The plane and camera could be remote operated to collect photos of the site.

3.17 Statistical Analysis

An independent t-test was used to compare the statistical significance of triploid and diploid oysters. Mean values from each test cage were pooled to generate a mean body burden concentration for total hydrocarbons, metals, and average condition index. An independent t-test was conducted to compare differences between body burden and condition index values between diploid and triploid oysters.

A paired t-test was conducted to analysis changes in body burden concentrations and condition index over time within each test cage. Diploid and triploid oysters were not found to be statistically different in concentration of pollutants or condition index, and mean values for diploid and triploid oysters were pooled to generate a mean value for body burden concentrations within each test cage. Changes in concentration overtime were analyzed with a paired t-test to assess evaluate changes from baseline values to day 3 values and so on. A confidence interval of p < 0.05 was used for all statistical analysis.
RESULTS AND DISCUSSION

4.1 Hydrology of the Site

The test site in the Big Lake oil field is located on the east bank of the Calcasieu River approximately twelve miles south of Lake Charles, Louisiana. Two hydrological transects were established to capture the prevalent water discharge on the day of the sediment disturbance. Figure 4.1 shows the orientation of the two transects in relation to the sediment perturbation and Test Cages. Two transects were conducted to allow for a 360 degree estimation of discharge direction. The Big Lake Oil Field location results in two dominant discharge drivers, river flow north to south toward the Gulf of Mexico and tidal influx which can bring water south to north.

Figure 4.1 Water flow across the Big Lake oil field on 2008 July 12
When water depth along the transect was three feet or less, flow was recorded once at a depth equal to 40% of the total water depth. When water depth along the transect was three feet or greater, flow was recorded twice 20% and 80% the total water depth at that point along the transect. The two flow measurements were then averaged together to estimate the predominant flow of water across the transect at that sampling location. Flow was recorded as the velocity of water moving across the transect. Velocity measurements at each point along each transect were separately added together to determine the speed and direction of flow across each transect.

Figure 4.2 and Figure 4.3 show the depth profile and velocity measurements for transect A. The total velocity across transect A was -5.3 ft/s. Figure 4.4 and Figure 4.5 show the depth profile and velocity measurements for transect B.

The total velocity across transect B was -16.2 ft/s. The negative values represent movement of water north across transect A, and west across transect B. Using the area encompassed by each transect, discharge (Q north and Q west) was calculated for each transect.
Figure 4.3  Velocity of water at each sampled location across Transect A. Positive values indicate water moving east across the transect. Negative values indicate a western movement. The values were summed together netting a -5.3 ft/s velocity across transect A.

The discharge across transect A (Q north = 160.3 ft$^3$/s) and the discharge across transect B (Q west = 520.6 ft$^3$/s) values were treated as vectors and added to yield a total discharge, Q total, of 544 ft$^3$/s in a northwest direction.

Figure 4.4. Depth profile of Transect B.
Figure 4.5. Velocity of water at each sampled location across Transect B. Positive values indicate water moving east across the transect. Negative values indicate a western movement. The values were summed together, resulting in a -16.2 ft/s movement across transect B.

Table 4.1. Total discharge of water (Q) for each transect. Each sampling location was assumed to represent 10 linear feet along the transect. Area was calculated using the depth and width of each sample location. Discharge (Q) was calculated by multiplying the area by the flow at that location. The discharge values at each location were then added to give a total net discharge across each transect.

<table>
<thead>
<tr>
<th>TRANSECT A</th>
<th>Width (ft)</th>
<th>Depth (ft)</th>
<th>Flow (ft/s)</th>
<th>Q (ft³/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>3.35</td>
<td>-2.5939</td>
<td>-86.9</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.9</td>
<td>0.0325</td>
<td>0.2925</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1.4</td>
<td>-0.5006</td>
<td>-7.008</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1.7</td>
<td>0.5606</td>
<td>9.5302</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1.65</td>
<td>0.0189</td>
<td>0.3119</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1.9</td>
<td>0.1371</td>
<td>2.6049</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>2.4</td>
<td>0.3346</td>
<td>8.0304</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>2.7</td>
<td>-1.3022</td>
<td>-35.16</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>2.9</td>
<td>0.5946</td>
<td>17.243</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>2.8</td>
<td>-2.2919</td>
<td>-64.17</td>
<td></td>
</tr>
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<td>10</td>
<td>3.1</td>
<td>0.5645</td>
<td>17.5</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>3.2</td>
<td>-0.5512</td>
<td>-17.64</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>3.4</td>
<td>-0.2928</td>
<td>-4.978</td>
<td></td>
</tr>
</tbody>
</table>

Q = -160.3

<table>
<thead>
<tr>
<th>TRANSECT B</th>
<th>Width (ft)</th>
<th>Depth (ft)</th>
<th>Flow (ft/s)</th>
<th>Q (ft³/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>3</td>
<td>-0.2731</td>
<td>-4.0965</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>3</td>
<td>-0.5463</td>
<td>-16.389</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>3.1</td>
<td>0.6611</td>
<td>20.4941</td>
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<tr>
<td>10</td>
<td>3.2</td>
<td>0.546</td>
<td>17.472</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>3.1</td>
<td>-2.5173</td>
<td>-78.0363</td>
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</tr>
<tr>
<td>10</td>
<td>3.2</td>
<td>-2.2783</td>
<td>-72.9056</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>3.3</td>
<td>-3.2966</td>
<td>-108.788</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>3.3</td>
<td>-1.8405</td>
<td>-60.7365</td>
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<tr>
<td>10</td>
<td>3.3</td>
<td>-1.4237</td>
<td>-46.9821</td>
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</tr>
<tr>
<td>10</td>
<td>3.3</td>
<td>-0.3952</td>
<td>-13.0416</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>3.3</td>
<td>-3.8542</td>
<td>-127.189</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>3.3</td>
<td>-0.5846</td>
<td>-19.2918</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>3.3</td>
<td>-0.2707</td>
<td>-8.9331</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>3.3</td>
<td>-0.1353</td>
<td>-2.23245</td>
<td></td>
</tr>
</tbody>
</table>

Q = -520.655
Total discharge (Q) indicated water moved in an overall northwesterly direction the morning of the disturbance event. On-site water movement varied due to wind patterns, precipitation and tidal activity in the Calcasieu watershed. Because of the spatial variability it is difficult to deploy monitoring stations in advance to anticipate for expected water flow patterns on the day of actual sediment perturbation. As a result, the four sampling stations were positioned to capture sediment plume movement in any potential direction. The hydrological survey was conducted the day of the perturbation event to document suspended sediment movement specifically related to the disturbance. Based on the hydrological data and the location of the sampling stations, Station 3 was exposed to the greatest sediment load due.

4.2 Total Hydrocarbons

Sediments and oyster tissue were analyzed for alkanes and polynuclear aromatic hydrocarbons (PAHs). Alkanes and PAHs were chosen as primary analytes because of their high concentrations in oil and high relative toxicity. Sediment analysis was conducted prior to oyster deployment to assess preexisting quantities of both alkanes and PAHs. Table 4.2 shows the mean hydrocarbon values of the area sediments. Pipeline removal at the current location created a large sediment plume and sediment analysis was used to determine the potential of the plume to release sequestered alkanes and PAHs.

Alkanes and PAHs were analyzed from oyster tissue at times before and after the disturbance event to assess the body burden of both types of compounds. Changes in hydrocarbon concentration in oyster tissues illustrate the accumulation of alkanes and PAHs by the organisms following the disturbance event. Additionally, tissue from diploid and triploid oysters was compared to determine the effect of ploidy and metabolic activity on accumulation of hydrocarbons. Oysters were deployed three weeks prior to collection to allow the organisms
Table 4.2. Concentrations (μg/mg) of alkanes and PAHs in sediments.

<table>
<thead>
<tr>
<th>Compound</th>
<th>μg/mg</th>
<th>Compound</th>
<th>μg/mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>nC-10 Decane</td>
<td>0.01</td>
<td>Phenanthrene-d10 SS #1</td>
<td>33.06</td>
</tr>
<tr>
<td>nC-11 Undecane</td>
<td>0.01</td>
<td>5-alpha Androstane SS #2</td>
<td>118.31</td>
</tr>
<tr>
<td>nC-12 Dodecane</td>
<td>0</td>
<td>nC-25 Pentacosane</td>
<td>0.09</td>
</tr>
<tr>
<td>nC-13 Tridecane</td>
<td>0</td>
<td>nC-26 Hexacosane</td>
<td>0.04</td>
</tr>
<tr>
<td>nC-14 Tetradecane</td>
<td>0.01</td>
<td>nC-27 Heptacosane</td>
<td>0.22</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>0</td>
<td>nC-28 Octacosane</td>
<td>0.03</td>
</tr>
<tr>
<td>nC-15 Pentadecane</td>
<td>0.06</td>
<td>nC-29 Nonacosane</td>
<td>0.08</td>
</tr>
<tr>
<td>nC-16 Hexadecane</td>
<td>1.06</td>
<td>Fluoranthene</td>
<td>0.04</td>
</tr>
<tr>
<td>nC-17 Heptadecane</td>
<td>0.14</td>
<td>Pyrene</td>
<td>0.26</td>
</tr>
<tr>
<td>Pristane</td>
<td>3.46</td>
<td>Naphtobenzothiophene</td>
<td>0.01</td>
</tr>
<tr>
<td>nC-18 Octadecane</td>
<td>0.64</td>
<td>Benzo (a) Anthracene</td>
<td>0.13</td>
</tr>
<tr>
<td>Phytane</td>
<td>1.66</td>
<td>Chrysene</td>
<td>0.12</td>
</tr>
<tr>
<td>nC-19 Nonadecane</td>
<td>0.17</td>
<td>nC-30 Triacontane</td>
<td>0.02</td>
</tr>
<tr>
<td>nC-20 Eicosane</td>
<td>0.05</td>
<td>nC-31 Hentriaccontane</td>
<td>0.34</td>
</tr>
<tr>
<td>nC-21 Heneicosane</td>
<td>0.05</td>
<td>nC-32 Dotriacontane</td>
<td>0.1</td>
</tr>
<tr>
<td>nC-22 Docosane</td>
<td>0.03</td>
<td>nC-33 Tritriacontane</td>
<td>0.1</td>
</tr>
<tr>
<td>nC-23 Tricosane</td>
<td>0.04</td>
<td>nC-34 Tetratriaccontane</td>
<td>0.14</td>
</tr>
<tr>
<td>nC-24 Tetracosane</td>
<td>0.04</td>
<td>nC-35 Pentatriaccontane</td>
<td>0.16</td>
</tr>
<tr>
<td>Fluorene</td>
<td>0.02</td>
<td>Benzo (b) Fluoranthene</td>
<td>0.1</td>
</tr>
<tr>
<td>Dibenzothiophene</td>
<td>0.01</td>
<td>Benzo (k) Fluoranthene</td>
<td>0.04</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>0.14</td>
<td>Benzo (e) Pyrene</td>
<td>0.05</td>
</tr>
<tr>
<td>Anthracene</td>
<td>0.04</td>
<td>Benzo (a) Pyrene</td>
<td>0.08</td>
</tr>
<tr>
<td>Dibenzo (a,h) anthracene</td>
<td>0.01</td>
<td>Perylene</td>
<td>0.02</td>
</tr>
<tr>
<td>Benzo (g,h,i) perylene</td>
<td>0.05</td>
<td>Indeno (1,2,3 - cd) Pyrene</td>
<td>0.05</td>
</tr>
</tbody>
</table>

to acclimatize to the Lake Calcasieu conditions, and provide baseline concentration values.

Three weeks of uptake and depuration of various compounds reflected background levels of alkanes and PAHs in the aquatic system. Baseline samples were collected the morning prior to the sediment disturbance event (Day = 0).

The average concentration of alkanes in all oyster tissue, independent of ploidy, from all four sampling cages was determined (Figure 4.6).
Concentrations of alkanes extracted from oyster tissue increased by sixty-three percent three days after the disturbance event relative to baseline levels. Over the next eleven days, concentrations of alkanes declined to baseline concentrations, indicating that the oysters were impacted by perturbation of the sediments with respect to alkanes.

In a manner similar to the alkanes, the concentrations of PAHs increased following perturbation, shown in Figure 4.7. On Day 3, PAH concentration increased twenty-three percent in extracted oyster tissue. PAH concentrations in the oyster tissue peaked on Day 7 and subsequently returned to baseline levels. The oysters were impacted by the sediment disturbance as seen in the increase in PAH body burden. The spike in Day 7 PAH concentrations represents a prolonged metabolism of PAHs as compared to alkanes. Ploidy did not appear to have a significant impact on body burden of hydrocarbons concentrations present in the sampled oysters (Table 4.3). Both diploid and triploid oysters were deployed in an attempt to quantify differences between the two organisms for use in biomonitoring systems.
Figure 4.7. Mean concentration of PAHs extracted from oyster tissue over time.

Diploid oysters allocate metabolic energy to spawn in the warm summer months, decreasing their glycogen and lipid content. The sterile triploid oysters do not spawn and retain a higher flesh weight than diploid oysters and thus might carry a greater hydrocarbon body burden.

Table 4.3. Affect of ploidy on concentration (μg/mg) of hydrocarbons following disturbance event.

<table>
<thead>
<tr>
<th></th>
<th>Baseline Diploid (μg/mg)</th>
<th>Day 3 Diploid (μg/mg)</th>
<th>Day 7 Diploid (μg/mg)</th>
<th>Day 14 Diploid (μg/mg)</th>
<th>Average (μg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkanes</td>
<td>18.792</td>
<td>39.18</td>
<td>28.0128</td>
<td>24.425</td>
<td>27.60245</td>
</tr>
<tr>
<td>PAHs</td>
<td>0.227</td>
<td>0.299</td>
<td>0.4158</td>
<td>0.2453</td>
<td>0.296775</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Baseline Triploid (μg/mg)</th>
<th>Day 3 Triploid (μg/mg)</th>
<th>Day 7 Triploid (μg/mg)</th>
<th>Day 14 Triploid (μg/mg)</th>
<th>Average (μg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkanes</td>
<td>22.448</td>
<td>28.3947</td>
<td>33.7928</td>
<td>28.611</td>
<td>28.311625</td>
</tr>
<tr>
<td>PAHs</td>
<td>0.2871</td>
<td>0.334</td>
<td>0.7325</td>
<td>0.425</td>
<td>0.44465</td>
</tr>
</tbody>
</table>

The diploid and triploid organisms concentrated alkanes at almost identical levels, with triploid organisms retaining PAHs at a higher concentration on average. Additionally, triploid organisms demonstrated a delayed assimilation of both alkanes and PAHs over time as well as a delayed depuration of hydrocarbons. Triploid oysters appear to retain both alkanes and PAHs at
higher concentrations longer than diploid oysters. But both organisms effectively demonstrated
the impact of sediment perturbation by an increase in hydrocarbon body burden on Day 3. This
indicates that either organism can provide an effective biomonitoring system for surveying the
release of hydrocarbons during removal of buried structures such as pipelines.

4.3 Metals Analysis

Metals analysis was conducted at 5 different times over the course of 36 days. A total of
16 trace and organo metals were analyzed, all of which are shown in Figure 4.8 and Figure 4.9.
Oyster were collected from each sampling location, and metals analyzed to determine the impact
of ploidy and cage location on metal body burden before and after sediment perturbation. In
Figure 4.8 and 4.9, all samples were pooled together to illustrate the general increase in metal
concentration between day 0 and day 3. Day 0 concentrations represent baseline levels, while
day 3 reflects the impact of the sediment plume. For all 16 metals, an increase in concentration
was seen between day 0 and day 3. The affects of ploidy and location are separated in later
graphs.

![Change in Metal Concentration](image)

**Figure 4.8** Percent change in metals concentration of oyster tissue as compared to Day 0.
Figure 4.9 Percent change in metals concentration of oyster tissue as compared to Day 0.

Because concentrations for each of the sixteen metals tested demonstrated an increase on Day 3 following the sediment disturbance event, the deployed oysters were an effective tool for identifying the amount of sequestered metals released into the aquatic environment following sediment disturbance.

Figure 4.10 graphs the affect of the disturbance event on Arsenic body burden to deployed oysters. Arsenic concentrations increased from Day 0 to Day 3 in all 4 sample cages. Triploid and Diploid oyster concentrations from each sampling cage were pooled together for statistical analysis. No significant difference was seen in arsenic concentrations between diploid and triploid organisms. Test Cage 3 concentrations increased significantly from Day 0 to Day, from $0.2 \pm 0.019 \mu g/mg$ to $0.4 \pm 0.012 \mu g/mg$ ($p < 0.05$). Arsenic concentrations trend back towards the baseline concentrations. Body burden concentrations are within the normal range of arsenic content for an estuarine oyster. Taken alone, these arsenic concentrations do not constitute a health concern for the organism or consumption of the organisms. However as a monitoring tool, arsenic appears useful for determining the spatial extent of the sediment plume. The hydrological data previously discussed suggests the plume predominately affected Test Cage
3. The arsenic analysis helps to ground truth this conclusion, as only Test Cage 3 oysters showed a statistically significant increase in arsenic concentrations following sediment perturbation. This increase can be attributed to increased exposure to sediment bound arsenic for Test Cage 3 oysters. Additionally, the data suggests that the down stream sampling locations, Test Cage 2 and Test Cage 3, perhaps were not impacted by the sediment perturbation, and can be assumed to be outside the spatial reach of the plume.

![Arsenic Concentrations by Sampling Location](image)

**Figure 4.10** Mean Arsenic concentrations over time

A second spike in body burden is seen in Test Cage 2 oysters on day 14. By day 14, the sediment plume has long dissipated, so any increases in concentration past day 3 do not result from the sediment plume.

Chromium concentrations are shown in Figure 4.11. Concentrations increased from day 0 to day 3 in all three test cages. Only the increase in Test Cage 3 was statistically significant, spiking from $0.06 \pm 0.036 \mu g/mg$ to $0.28 \pm 0.15 \mu g/mg$ ($p < 0.05$). Again diploid and triploid samples were pooled to increase sample size for statistical analysis. As with Arsenic concentrations, Test Cage 3 displayed the lowest initial concentration of Chromium of the four sample cages deployed.
Figure 4.11. Mean chromium concentration over time

Copper concentrations are displayed in Figure 4.12. Test cage 3 oysters again display the only statistically significant increase in concentrations following exposure to the sediment plume, 2.5 ± 0.9 μg/mg to 4.7 ± 1.3 μg/mg (p < 0.05).

Figure 4.12 Mean copper concentration at each sampling location.
In addition, the pattern is continued whereby Test Cage 3 oysters demonstrate the lowest baseline concentration for copper. It should also be noted that as with chromium and arsenic, copper concentrations in Test Cage 2 oysters actually peak later on day 14.

Iron concentrations shown in Figure 4.13 continue the trend observed in the metals data.

**Figure 4.13** Mean iron concentration at each sampling location.

Test Cage 3 displayed the largest single increase in concentration from day 0 to day 3, 8.6 ± 3.2 μg/mg to 31.7 ± 14.1 μg/mg (p < 0.05). Iron adhered to sediments is not a specific toxicity concern as with other trace metals such as arsenic. However due to its prevalence in sediments, iron provides an important marker for determining the extent of impact the sediment disturbance has on the aquatic environment. In addition, a precipitous drop in iron as seen in the reference oyster samples may in fact be an indication of oyster stress. Studies have shown iron to be a naturally fluctuating metal within oyster tissue. However as concentrations in all three test cages increased while the reference cage decreased, increases in iron resulted from the filtration of iron-containing sediments by the oysters. Another important component is again Iron concentrations are lowest in Test Cage 3 oysters.
Nickel body burden in oyster tissue shown in Figure 4.14 seem to confirm the two major trends; Test Cage 3 oysters demonstrate the largest response to the sediment event and Test Cage 3 oysters have the lowest initial concentration. However, none of the changes from the baseline concentration to Day 3 concentrations were statistically significant (p < .05). It should also be noted that again, Test Cage 2 oyster body burdens were highest at a later date, in this case Day 36. Again, however, the differences in Nickel concentrations in Test Cage 2 were not statistically significant.

![Nickel Concentration by Sampling Location](image)

**Figure 4.14** Mean nickel concentration at each sampling location.

Lead concentrations are shown in Table 4.4. Lead concentrations were found in very low concentrations, and no statistical argument can be made based on the data. However it should be noted that Test Cage 3 oysters had the lowest initial lead concentrations of all the sampling locations.

<table>
<thead>
<tr>
<th></th>
<th>Reference</th>
<th>Test Cage 1</th>
<th>Test Cage 2</th>
<th>Test Cage 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>0.03±0.021</td>
<td>0.028±0.019</td>
<td>0.025±0.012</td>
<td>0.017±0.013</td>
</tr>
<tr>
<td>Day 3</td>
<td>0.028±0.018</td>
<td>0.013±0.001</td>
<td>0.042±0.019</td>
<td>0.044±0.026</td>
</tr>
<tr>
<td>Day 7</td>
<td>0.045±0.023</td>
<td>0.049±0.022</td>
<td>0.039±0.011</td>
<td>0.042±0.017</td>
</tr>
<tr>
<td>Day 14</td>
<td>0.017±0.013</td>
<td>0.016±0.009</td>
<td>0.022±0.01</td>
<td>0.027±0.02</td>
</tr>
<tr>
<td>Day 36</td>
<td>0.024±0.013</td>
<td>0.017±0.015</td>
<td>0.026±0.018</td>
<td>0.024±0.018</td>
</tr>
</tbody>
</table>

**Table 4.4** Lead Concentrations (μg/mg) by test cage
Neither Nickel nor Lead concentrations are high enough to be of any toxic concern before or after the sediment event. The low baseline concentrations in Test Cage 3 for both Nickel and Lead continue to suggest Test Cage 3 oysters had the lowest background exposure to trace metal pollutants.

Zinc concentration shown in Figure 4.15 again displays Test Cage 3 oysters with the lowest baseline concentration, followed by the largest concentration increase between day 0 and day 3. Zinc concentrations more than doubled in Test Cage 3, increasing from 33.4±13.2 to 72.9±15.8 μg/mg between day 0 and day 3. Additionally, Test Cage 3 initial concentrations were considerably lower than the other sampling locations. These results are consistent with the pattern seen in other trace metals analyzed. The lack of any statistically significant change in the other cages provides indicates the sediment plume created had a very localized impact, and did not extend to reach the other test cages or the reference cage.

![Zinc Concentrations by Sampling Location](image)

**Figure 4.15** Mean Zinc concentration at each sampling location.
Furthermore, Test Cage 2 oysters again spike in not immediately following the sediment plume on day 3, but later on day 14 and day 36. This growing phenomenon appears to not be a statistical anomaly, but perhaps corresponds to a secondary exposure event localized around Test Cage 2 on Day 14.

Figure 4.16 shows barium concentrations following the same pattern as described in other metals. Only Test Cage 3 showed a significant change in concentrations from Day 0 – Day 3 (0.16 ± 0.1 to 0.48 ± 0.19 μg/mg; p<.05). In addition, the baseline concentrations are again the lowest within Test Cage 3. Also, Test Cage 2 oysters demonstrate a significant increase well after the documented sediment disturbance, peaking on day 14.

Figure 4.17 illustrates the impact of the sediment plume on aluminum concentrations in the sediments. Much like iron concentrations, aluminum concentrations decrease over time in the reference oysters. Importantly, aluminum concentrations again significantly increase in Test Cage 3 oysters from Day 0 to Day 3 (7.1 ± 6.7 to 36.6 ± 14.1 μg/mg; p<.05). Also, Test Cage 3 oysters again have the lowest trace metal baseline concentration.
Unlike many of the trace metals tested, no delayed spike was seen in either Test Cage 2 or Test Cage 3 oysters.

**Table 4.5.** Metal concentrations (μg/mg) of oyster dry tissue weight over time.

<table>
<thead>
<tr>
<th></th>
<th>Ca</th>
<th>Cd</th>
<th>K</th>
<th>Mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>1609±107</td>
<td>0.492±0.13</td>
<td>1260±312</td>
<td>223±22.8</td>
</tr>
<tr>
<td>Day 3</td>
<td>2249±167</td>
<td>0.606±0.15</td>
<td>1959±284</td>
<td>256±37.1</td>
</tr>
<tr>
<td>Day 7</td>
<td>1407±95</td>
<td>0.561±0.11</td>
<td>1394±173</td>
<td>284±19.9</td>
</tr>
<tr>
<td>Day 14</td>
<td>935±103</td>
<td>0.527±0.19</td>
<td>1856±190</td>
<td>283±29.3</td>
</tr>
<tr>
<td>Day 36</td>
<td>2006±212</td>
<td>0.43±0.14</td>
<td>1227±111</td>
<td>368±29.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Mn</th>
<th>Na</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>2.9±1.2</td>
<td>1068±87.5</td>
<td>1190±201</td>
</tr>
<tr>
<td>Day 3</td>
<td>3.4±0.98</td>
<td>1099±184.2</td>
<td>1536±192</td>
</tr>
<tr>
<td>Day 7</td>
<td>5.2±1.87</td>
<td>1229±164.9</td>
<td>1583±185</td>
</tr>
<tr>
<td>Day 14</td>
<td>3.8±1.54</td>
<td>875±68.4</td>
<td>1668±193</td>
</tr>
<tr>
<td>Day 36</td>
<td>4.4±1.76</td>
<td>1553±97.3</td>
<td>1039±98</td>
</tr>
</tbody>
</table>

Additional organo and trace metals quantified are listed in Table 4.5. Table 4.5 shows the mean values for each metal after data from each sampling cage was pooled. Calcium,
Potassium and Phosphorous concentrations in the oyster tissues all increased significantly immediately following the sediment disturbance, between day 0 and day 3.

As Table 4.4 shows, there was no significant difference for any metal when comparing mean concentrations between triploid and diploid oysters. As with hydrocarbons, ploidy appeared to have little impact on the bioaccumulation of metals in oyster tissue. Both triploid and diploid organisms displayed almost identical concentrations for trace and organo metals.

**Table 4.6.** Average concentration (mg/kg) of diploid versus triploid oysters.

<table>
<thead>
<tr>
<th>Element</th>
<th>Diploid</th>
<th>Triploid</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al</td>
<td>20.820</td>
<td>22.174</td>
<td>-1.354</td>
</tr>
<tr>
<td>As</td>
<td>0.306</td>
<td>0.324</td>
<td>-0.018</td>
</tr>
<tr>
<td>Ca</td>
<td>418.000</td>
<td>402.600</td>
<td>15.400</td>
</tr>
<tr>
<td>Cd</td>
<td>0.132</td>
<td>0.128</td>
<td>0.004</td>
</tr>
<tr>
<td>Cr</td>
<td>0.150</td>
<td>0.126</td>
<td>0.024</td>
</tr>
<tr>
<td>Cu</td>
<td>5.582</td>
<td>5.080</td>
<td>0.502</td>
</tr>
<tr>
<td>Fe</td>
<td>19.324</td>
<td>20.942</td>
<td>-1.618</td>
</tr>
<tr>
<td>K</td>
<td>371.800</td>
<td>397.800</td>
<td>-26.000</td>
</tr>
</tbody>
</table>

**4.4 Condition Index**

The condition index was calculated for each oyster collected. Condition indexes used a ratio of dry tissue weight to wet tissue weight. Figure 4.18 shows the condition indexes for the oyster over time. Each sampling cage was detrimentally affected over time following the sediment disturbance. Considering the low trace metal and hydrocarbon baseline concentrations, it is not surprising test cage 3 demonstrates the highest initial condition index. Test cage 3 oysters had the lowest initial concentrations of both total hydrocarbons and metals, and thus were the healthiest oysters initially. Test cage 3 oysters also received the bulk of the sediment exposure due to the perturbation event.
Figure 4.18 Estimation of Oyster Health from each sampling location

The condition index bears this out, as test cage 3 oysters demonstrated the largest percent drop in condition index from day 0 to day 3 samples. This corresponds with the dramatic increase in metal and hydrocarbon concentrations seen in test cage 3.

Similarly, Test Cage 2 also demonstrated relatively low baseline concentrations of trace metals and hydrocarbons, and Test Cage 2 oysters have a very healthy baseline condition index. The Reference Cage and Test Cage 1, meanwhile, consistently had higher baseline concentrations for trace metals and hydrocarbons. As Figure 4.18 shows, the condition index for oysters at these two sampling locations are initially lower than either Test Cage 2 or 3, presumably attributable to the higher baseline levels of trace and organo metals.

The worst condition value for any samples come from Test Cage 2 on Day 14. As has been shown, Test Cage 2 body burden concentrations unexpectedly spiked at Day 14 for barium, zinc, copper and arsenic. The condition index value for Test Cage 2 oysters at day 14 confirm these values were not due to mishandling or analytical error, but reflect true body burden concentrations at this time and location. In addition, these increased levels had a detrimental
impact on the oysters, as evidenced by the drop in condition from day 7 to day 14, corresponding with the increase in metal body burdens over that time.

As with metals, the impact of ploidy on condition index was examined. Figure 4.19 shows the differences in ploidy between diploid and triploid organisms. Figure 4.19 shows the general decline in condition over time, but the important component in the difference in values between the two organisms. No statistical difference was seen in the condition of triploid vs. diploid oysters.

![Condition Index](image)

**Figure 4.19** Condition Index based on oyster Ploidy

Figure 4.18 is a significant graph, as diploid oysters spawn during the summer warmer summer months.

**Table 4.7** Physical water quality parameters on Lake Calcasieu

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Day 3</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 36</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dissolved Oxygen (mg/L)</td>
<td>5</td>
<td>5.5</td>
<td>4</td>
<td>4</td>
<td>4.5</td>
</tr>
<tr>
<td>Salinity (ppt)</td>
<td>7.8</td>
<td>9.1</td>
<td>6.4</td>
<td>11</td>
<td>7.6</td>
</tr>
<tr>
<td>Temperature (C)</td>
<td>24.3</td>
<td>24.8</td>
<td>24.4</td>
<td>25.4</td>
<td>26.6</td>
</tr>
</tbody>
</table>

Table 4.7 shows the DO, salinity and temperature of the water on site over the course of the monitoring program. There were no dramatic shifts in any parameter to cause concern to the
oysters or their health. The water temperature was warm enough for the diploid organisms to spawn.

In addition, a visual inspection was used to determine the color index of each oyster collected. The color index rated oyster appearance on 1-5 scale, with 1 being the healthiest and 5 being very discolored and marked. Figure 4.20 displays a photo of 3 oyster meats. The first two oyster meats had no discoloration or marks, and were rated a 1 on the color index. The third oyster had a darker brown color all over with a few very dark spots, and was rated a 3 on the color index. The color index values from cage to cage and over time did not differ significantly, nor were any changes seen between diploid and triploid organisms.

![Figure 4.20 Oysters observed for color index](image)

Hexachlorobutadiene in oyster tissue was also quantified as a pollutant of interest. Our GC/MS data did not show any detectable levels of hexachlorobutadiene in the sampled oyster tissue.
5

Conclusions

5.1 Summary of Findings

Sediment perturbation had a demonstrated effect on oysters in test cage 3. Analysis of test cage 3 oysters showed a statistically significant (p < .05) increase in 13 of 16 tested metals three days following sediment perturbation. Hydrological and visual data indicate the sediment plume moved directly over test cage 3, and away from the down stream test cages. Not surprisingly, the direct impact of the sediment plume is most prominent when comparing changes in metal concentration in Day 3. Test cage 3 oysters also demonstrated the largest percent decline in condition index of any of the cages from day 0 to day 3. This drop in test cage 3 oyster condition corresponds directly with the increase body burden of metals and hydrocarbons due to the sediment perturbation event. As condition indices are considered an accurate estimation of general oyster health, it can be concluded that the sediment plume had a deleterious impact on the oysters in test cage 3. This negative impact can not be attributable to any particular metal or PAH found at dangerously high concentrations. Instead, the decrease likely reflects a synergistic affect of the combined exposure of the metals and hydrocarbons, along the possible physical affects of the sediment particles themselves on the oysters.

Triploid and diploid oysters did not show any statistical significance for any of the analytes measured, thus proving the null hypothesis. However, the condition index also did not vary between triploid and diploid oysters. Nor did the condition index ever widely fluctuate among diploid oysters, suggesting spawning did not occur during monitoring and sampling. Thus, it can not be concluded that diploid and triploid oysters will always provide similar monitoring results. Had the experiment been conducted later in the year, in August and
September, a spawning event may have occurred providing a difference in condition index and body burden concentrations as a result of physiological realities, and not as a result of exposure to suspended sediments.

A clear trend exists whereby Test Cage 2 and 3 have lower baseline concentrations of Hydrocarbons and Trace metals than Test Cage 1 or the reference cage. Test Cage 2 and 3 were placed directly within the pipeline network, while the other two cages were placed outside the pipelines. One would expect to find a more compact water bottom along pipelines and near a tank battery. Industry practice involves dredging and filling of areas for the installation and maintenance of pipelines. In addition, pipelines are required to be buried below the water bottom. With over 50 years of pipeline work on location, the water bottom has been compacted with fill material, consisting of larger pebbles and rocks than normally found in the deltaic river systems of Louisiana.

The reference cage and Test Cage 1 were deployed outside the pipeline network, on a less altered water bottom far more representative of the natural river systems. As a result, these cages were more exposed to background sediment events than either Test Cage 2 or 3. Consideration may be given in the future to provide a more consistent ground surface for each deployed cage. This conclusion is supported by the water bottom assessment found in Appendix B.

Neither Test Cage 1 nor the reference cage demonstrated any consistent response to the perturbation event, suggesting that our sediment disturbance did not extend beyond the immediate vicinity. Test cage 1 and 2 were downstream of the sediment event, and did not demonstrate a clear pattern of increasing body burden, or have the pronounced decline in condition as test cage 3. The reference cage, well north of the sediment disturbance, showed zero correlation with the sediment events. The deployment strategy thus establishes the northern
and southern extent of the sediment plume impact, beyond which the aquatic environment was
unaffected by the sediment plume. Additional cages progressing out into the shipping channel
would be needed to complete the spatial extent of the sediment plume. This confirms the utility
of using oysters as a monitoring system, as data clearly demonstrates locations where the
sediment disturbance was felt and areas where it could not be detected above background
pollution and natural fluctuations within oyster tissue.

Our deployment of SPMDs was not successful as they ruptured during deployment. The
SPMDs have been used in other sites and are potentially very useful as an alternative to oysters,
particularly in areas where oysters can not survive do to excessive pollution or salinity
constraints. Great care however must be taken to protect the SPMDs, and some form of
protection is needed if using SPMDs.

5.2 Future Considerations

A laboratory toxicity test using sediment samples should be conducted to confirm the
decrease in Test Cage 3 oyster condition index after sediment perturbation in the field was a
result of exposure to the sediments of the disturbance event. In addition, a laboratory study will
allow for a more accurate determination of the time needed for oysters to recover following a
sediment event and exposure to high levels of PAH’s, alkanes and metals. Sediment samples
could be spiked, and then oysters placed in a clean environment and allowed to depurate out
compounds. In the field, secondary exposure events can not be controlled. Indeed, spikes in
Zinc and Barium levels on day 14 and spikes of PAH’s on day 7 suggest secondary exposure
events that may have affected the condition index of the oysters at these later dates.

Biomonitoring systems could be further tested by cooperated with new pipeline
installation or removal of other physical structures in the coastal zone.
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Coastal Management Division (CMD). “Permits/Mitigation” Louisiana Department of Natural Resources, April 11, 2009


Louisiana Department of Natural Resources (LDNR) “State oil and gas revenue hits record high for FY 06-07” Press Release. July 13, 2007


APPENDIX A

SUGGESTED PROTOCOL FOR FLOWLINE/PIPELINE REMOVAL IN COASTAL LOUISIANA
APPENDIX A: TABLE OF CONTENTS

Introduction..................................................................................................................63

II. Pre-Site Assessment..................................................................................................63
   1. Site Survey...........................................................................................................63
      1.1 Site Identification.........................................................................................63
      1.2 Establishing Pipeline Owner/Liable Party.................................................64
      1.3 State Designated Use and Monitoring.......................................................66
   2. Assess Potential Toxicological Threats to Removal...........................................69
   3 Exploratory Sediment Analysis..........................................................................69
      3.1 Sampling Strategies.....................................................................................70
      3.2 Quality Control.............................................................................................70
      3.3 Sampling equipment....................................................................................71
         3.3.1 Core Sample..........................................................................................71
         3.3.2 Grab Sample..........................................................................................71
      3.4 Sample Storage.............................................................................................72
      3.5 Physical Analysis.........................................................................................72
      3.6 Chemical Analysis.......................................................................................72
      3.7 Biological and Toxicological Analysis......................................................75
   4 Hydrological Assessment of Area........................................................................75
      4.1 Evaluate Factors Influencing Water Discharge.........................................76
      4.2 Determine Direction of Water Discharge.................................................76
   5. Determine Course of Action................................................................................76
   6. Permit Application...............................................................................................78
   7. Aerial/Visual Surveillance...................................................................................79

II Post Removal Assessment......................................................................................79
   8. Sample Stations..................................................................................................79
      8.1 Deploy Sample Stations.............................................................................79
      8.2 Sample Station Design...............................................................................82
   9 Sample Monitoring Analysis...............................................................................82
      9.1 Collection of Oyster Tissue.........................................................................82
      9.2 GC/MS Analysis.........................................................................................83
      9.3 Metal Analysis.............................................................................................84
      9.4 Condition Index..........................................................................................85
      9.5 Water Sample Collection...........................................................................86
      9.6 Pipeline Disposal........................................................................................86
Introduction

Abandoned Pipeline Removal in Coastal Louisiana

Undocumented and obsolete pipelines are prevalent across coastal Louisiana and removal of the pipelines can prove difficult. Often the pipelines were installed by companies no longer solvent and private ownership is difficult to confirm. Segments of the pipeline can cross several different property lines, further complicating issues of ownership and liability. Over time, sections of the oil and gasline infrastructure can become exposed and present a potential hazard and or obstruction to navigable water bodies. As it stands, no standard protocol exists to guide the removal and remediation of the abandoned pipelines.

When removing abandoned pipelines, care is given to prevent contamination of the sediment and water column by oil and other hydrocarbons. Precautions are taken to plug and flush pipelines to ensure no residual spill occurs during removal. However additional consideration should be given to potentially hazardous impacts of the sediments surrounding and covering the pipeline as the sediments act as sinks for chemical contaminants. The large sediment disturbance caused by removing a pipeline may release sequestered pollutants into the aquatic system causing environmental and human health hazards. As a result, private companies are reluctant to voluntarily remove their abandoned pipelines for fear of releasing a pollutant into the aquatic environment, compromising other resources such as fisheries or oyster leases.

The current protocol is designed to help guide ownership resolution and environmental risk analysis prior to pipeline removal. In addition, a monitoring strategy is proposed to help objectively quantify the impact of pipeline removal to the ecology of an aquatic system.

I. Pre-Site Assessment

1. Site Survey
Abandoned pipelines present a unique challenge for removal and remediation due to the duration and ambiguity in ownership. Many abandoned pipelines cover several linear kilometers, crossing public and private lands and different geographical distinctions. Additionally, records for pipelines installed prior to 1976 are incomplete. When firm documentation cannot be found identifying the owner of the pipeline, the location of the pipeline becomes a central issue to its removal. Finally, property boundaries are delineated differently depending on whether a property is considered a lake or river or tidal shoreline.

1.1 Site Identification

*Land Ownership Survey* – Sections of the same pipeline may be found on private property, State land and water bottoms or federal land and water bottoms. Property ownership is particularly difficult to clarify at the land/water interface.

All water bodies affected by pipeline removal need to be verified as navigable or non-navigable water bodies. For non-navigable water bodies, adjacent landowners own the title to the center of the lake or river. For navigable water bodies, private ownership typically ends at the high water mark. Below the high water mark, the water bed is owned by the State as a public highway for commerce. By law, waters are deemed navigable whereby a tidal influence is felt on the water body. Water bodies can also be deemed navigable-in-fact; the water body is non-tidal but has been used for navigation in the past. The distinctions are critical to obtaining permits for pipeline removal. An experienced land surveyor should be hired to determine the boundaries of public and private ownership within the project area. All private owners and land managers adjacent to the project locale must informed of the planned actions prior to pipeline removal. Pipelines slated for removal over public water bottoms may require mobilization access across private property; notification of adjacent private property owners should be
anticipated as part of the permitting process. The Coastal Use Permit requires such notification for work proposed in the Coastal Zone of Louisiana. Additionally, any obstruction to a navigable water body of the United States is forbidden by law except when permitted by the United States Army Corps of Engineers. Exposed pipelines on navigable water bodies may be deemed obstructions to navigation.

In addition to ownership and boundary surveys, important biological, geological or cultural features must be documented near the potential work zone. Permitting agencies consider the date in order to determine whether a specific project, such as pipeline removal, could potentially compromise the designated uses of an area.

1.2 Establishing Pipeline Owner/Liable Party

Identification of pipeline, where possible, is essential for determining ownership and liability for the abandoned pipeline. As many abandoned pipelines were installed and abandoned prior to the formation of the Coastal Zone Management Division, accurate information on pipelines installed before 1976 can be difficult to obtain.

A precedent has been set by both the Abandoned Barge Program and Abandoned Facilities Program for determining ownership and responsibility for removal of abandoned infrastructure. In both programs a private owner is identified and responsible for removal when possible. If a private owner can not be identified, responsibility for remediation of the abandoned infrastructure falls to either a state or federal agency. In Louisiana, funds for these programs have been allocated under OSPAR however no funds have been specifically assigned for the removal of abandoned pipelines. Additionally, private landowners can be held liable for waste and pollution on their property, even when such material represents a legacy from another
landowner. A private landowner could potentially be held accountable for pipeline removal. However, the majority of pipelines exist on state owned water bottoms.

Louisiana DNR maintains current and historical records of all state oil and gas field leases. With these records and other archival searches, a determination can be made identifying when a pipeline was installed and by whom. In some instances, the original company does not exist but their assets are often purchased by other private sector companies. If a chain of ownership can be established from the original company to a present day operator, private ownership of the abandoned pipeline is identifiable.

Additionally, the pipeline in question may connect with a well head or tank battery that has since been plugged or abandoned. The Abandoned Facility Program includes well heads and tank batteries within its scope of activities. If ownership is determined for the tank or wellhead associated with an abandoned pipeline, than ownership may also apply to the pipeline in question.

1.3 State Designated Use and Monitoring

The Louisiana Department of Environmental Quality conducts a state wide Surface Water Monitoring Program. The data collected is used to determine water quality in the area with respect to state and national standards. The data establishes if a specific water body is in support of its “designated use” as each water body in the state is given such a use.

When considering the removal of a pipeline, the designated uses of the affected water body must be identified. The designated uses (Table 1) are evaluated by LDEQ based on analytical parameters to determine if the specific water body is sufficiently supporting the designated uses. The most recent and comprehensive data on Louisiana water bodies can be
found in the 2004 Water Quality Inventory Report submitted to the EPA, available through the EPA or LDEQ offices.

**Table 1. Designated Uses for Louisiana Water Bodies**

<table>
<thead>
<tr>
<th>Rivers/Streams</th>
<th>Lakes</th>
<th>Estuaries</th>
<th>Wetlands</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary Contact Recreation</td>
<td>Primary Contact Recreation</td>
<td>Primary Contact Recreation</td>
<td>Primary Contact Recreation</td>
</tr>
<tr>
<td>Secondary Contact Recreation</td>
<td>Secondary Contact Recreation</td>
<td>Secondary Contact Recreation</td>
<td>Secondary Contact Recreation</td>
</tr>
<tr>
<td>Recreation</td>
<td>Recreation</td>
<td>Recreation</td>
<td>Recreation</td>
</tr>
<tr>
<td>Fish/Wildlife Propagation</td>
<td>Fish/Wildlife Propagation</td>
<td>Fish/Wildlife Propagation</td>
<td>Fish/Wildlife Propagation</td>
</tr>
<tr>
<td>Drinking Water Source</td>
<td>Drinking Water Source</td>
<td>Shellfish Propagation</td>
<td>Drinking Water Source</td>
</tr>
<tr>
<td>Outstanding Natural Resource</td>
<td>Agriculture</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shellfish Propagation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agriculture</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Limited Aquatic Life/Wildlife</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In addition to listing designated uses, the 2004 IR lists all the possible parameters for testing and all advisories for water bodies in the state. The advisories provide useful information for tailoring a site-specific toxicological sampling and monitoring program for each pipeline removal project. As part of the integrated reports, LDEQ has developed water body specific
Total Maximum Daily Loads (TMDLs) for specific parameters such as dissolved oxygen and heavy metals. All TMDLs should be identified and the degree of attainment for the TMDLs noted for all water bodies under consideration.

A second critical data set maintained to determine environmental quality and human health concerns of the state’s coastal resources is the National Status and Trends (NS&T) Mussel Watch program. Mussel Watch methodology has been used to collect baseline data for over twenty years and is commonly used to determine the environmental consequences of specific events. For example, Mussel watch data and methods were used to by NOAA to assess the impact of Hurricane Katrina to the aquatic system. Mussel Watch data includes nationwide body burden levels in oyster tissue for PAH’s, trace and heavy metals, pesticides, butylins and condition indexes. Prior to any pipeline removal, Mussel Watch data should be examined to determine proximate locations of neighboring Mussel Watch stations and the parameters and data collected at the stations. Along with the IR report, Mussel Watch data should be used to determine baseline environmental quality of the site and any toxicological trends present in the area. The data sets assist in identifying any unique analytical parameters of concern to a specific site. In addition data will be useful for evaluating any environmental or health impacts as a result of a pipeline removal.

In addition to an ownership and boundary survey, important biological, geological or cultural features should also be documented near the potential work zone. Such features are considered when determining the various uses of a water body or wetland area. The State identifies uses such as water source, surface recreation, wildlife refuge and monitors whether an area is in support of its designated uses. The primary state and federal monitoring programs include the Water Quality Monitoring Program and the Mussel Watch Program. Such data and
consideration will be used by permitting agencies to determine whether a specific project, such as pipeline removal, may compromise the designated uses of an area.

2. **Assess Potential Toxicological Threats to Removal**

   Sediments present a unique concern for projects within the coastal zone. The physical properties of sediment particles result in the sequestering of pollutant and toxic material within the benthic layers. Pollutants can include common, conventional pollutants associated with oil and gas activities. However, pipelines may run through sediments where accumulation of other pollutants from non-extraction industries has occurred. These legacy pollutants are of particular concern to any party accepting liability for pipeline removal. Industrial facilities have permits to discharge highly toxic pollutants that can be concentrated in the sediment layer. A sediment plume created by pipeline removal can act as a bolus dose for highly toxic pollutants.

   In either case, the release of sequestered pollutants during pipeline removal presents the primary environmental concern when considering the extraction of abandoned pipelines. Prior to any removal, an exploratory analysis of the sediments should be conducted to quantify the toxicological risks of sediment disturbance. Any party accepting responsibility and performing a pipeline removal should take care to document potential legacy pollutants, and seek a hold harmless agreement from the state before any removal. The objective of a site study and sediment analysis is to identify specific pollutants of concern. In many cases, pollutants of concern will represent legacy pollutants from the permitted discharge of other entities not related to the installation and operation of pipelines.

3. **Exploratory Sediment Analysis**

   For a complete technical guide, the National Oceanic and Atmospheric Administration Technical Memorandum for Organic Contaminant Analytical Methods of the National Status and
Trends Program should be referenced. The complete document can be found at http://www.ccma.nos.noaa.gov/publications/organicsmethods.pdf

3.1. Sampling Strategies

A sampling strategy is employed to create a spatial distribution of sediment pollutants along the entire work zone. Samples should be collected within 15 meters of a linear transect encompassing the full length of the pipeline to be removed. The samples should be collected alternatively on either side of the full length of the pipeline. Considering the high clay/silt content of Louisiana sediments, sediments should be sampled 20 meters apart from one another with triplicate samples collected at each location. Each sample location can be documented using a handheld GPS unit. Any sampling plan must collect samples representing the full lateral area of the work zone.

Pollution levels in sediments have been demonstrated to fluctuate over time, particularly in regards to organic pollutants such as PAH’s. Because of temporal variations in pollutant levels found in sediments, samples should be collected as near to removal date as possible. Preliminary samples can be collected well in advance of any pipeline removal to gauge relative pollutant values, however, sediment sampling should be conducted within 3 months of the pipeline removal. Several different procedures and protocols for sampling collection, storage and analysis have been developed. These methods are briefly outlined below. Full methodology for collection and storage of sediments should follow the JAMP-guidelines developed as part of OSPAR, and all analytical work should follow the protocols established by the NS&T Mussel Watch Program.

3.2. Quality Control
It is important that all samples be collected in a standardized method. A percentage of sampling locations should be collected twice (in duplicate) to further minimize human and methodology errors during sampling collection, storage and analysis. A standard sampling checklist, to be completed by the samplers, should be developed and adopted for each project.

Collected oyster tissue and sediments must be delivered to laboratories within 6 months, and analysis conducted within 30 days of arrival in a laboratory, according to standard testing methods. Collected samples can not be retested at later dates and be considered valid. It is the prerogative of the individual party conducting the pipeline removal, but sending samples to multiple independent laboratories provides additional quality assurances on the validity of your data.

3.3. Sampling Equipment

Two principle techniques can be used to sample, depending on the specific project needs: A core Sample and a Grab Sample.

3.3.1 Core Sample:

A core sample allows for sub-sampling of the core to analysis specific layers within the core. These layers represent sedimentation over time, and can provide a useful view of the pollution loading within the system over several decades. In its simplest form, a core sampler includes a hollow cylinder that is manually pushed into the sediment bottom. The top of the cylinder is then sealed, and the core is pulled up. This technique is very applicable to areas within coastal Louisiana, where very shallow water conditions exist. A long list of coring equipment has been developed, ranging in cost and utility. Depending on the specific needs of a project, namely water depth, different coring equipment will be appropriate.

3.3.2. Grab Sample:
Grab samplers collect undisturbed sediment samples and are commonly used for collecting spatial data on pollutant levels. Grab samples collect from the bottom surface and penetrate down to varying depths, usually 8-12 cm. Again the landscape of each project may necessitate different equipment, however grab samplers would be very useful in collected sediments along a pipeline transect.

3.4. Sample Storage

Once collected, samples should be stored and transported in either glass or polyethylene containers. Plastic containers should not be used, unless the container is polyethylene. Plastics other than polyethylene or polytetrafluorethene can result in absorption of PAH’s, understating the concentrations present in the sample (JAMP Guideline). Many pollutants such as PAH’s can photo-degrade. Care should be given to minimize exposure of samples to light. Amber Jars are ideal for storage of samples. Samples should be transported to the laboratory on ice to slow biological metabolism. Core samples are typically transported within their cores, kept vertical to avoid disturbing the core. The objective is to analysis sediments that represent the conditions on site as accurately as possible, minimizing the influence of sampling.

3.5. Physical Analysis

Dry weight and particulate grain size should be calculated. Dry weight will be used to quantify specific chemical contaminants during chemical analysis of the sediments. Grain size provides a rough estimate of chemical contaminate pollution, as smaller size sediment particles tend to have a higher potential for sequestering pollutants. Additionally, sediment size will roughly indicate the suspension time of the sediments following the disturbance from pipeline removal.

3.6. Chemical Analysis
Standard chemical analysis for sediments should include at a minimum Total Hydrocarbons (THCs) and metals. THCs should include PAHs and c10-c35 alkanes, while metals should include trace and organo metals. While concentrations for any individual hydrocarbon or metal may be of toxicological concern, the synergistic effects of the pollutants may prove toxic to aquatic organisms. In addition, data has shown tissue analysis of these compounds can definitively demonstrate the extent and range of the sediment plume in the aquatic system.

Table 2. Standard GC/MS compounds for Total Hydrocarbons associated with oil

<table>
<thead>
<tr>
<th>Internal Standard</th>
<th>Surrogate Standard</th>
<th>n-Alkanes</th>
<th>PAH’s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene-d8</td>
<td>Phenanthrene-d10</td>
<td>nC-10 Decane</td>
<td>Naphthalene</td>
</tr>
<tr>
<td>Acenaphthen-d10</td>
<td>Androstane</td>
<td>nC-11 undecane</td>
<td>Fluorene</td>
</tr>
<tr>
<td>Chrysene-d12</td>
<td></td>
<td>nC-12 Dodecane</td>
<td>Dibenzothiophene</td>
</tr>
<tr>
<td>Perylene-d12</td>
<td></td>
<td>nC-13 Tridecane</td>
<td>Phenanthrene</td>
</tr>
<tr>
<td></td>
<td></td>
<td>nC-14 Tetradecane</td>
<td>Anthracene</td>
</tr>
<tr>
<td></td>
<td></td>
<td>nC-15 Pentadecane</td>
<td>Fluoranthen</td>
</tr>
<tr>
<td></td>
<td></td>
<td>nC-16 Hexadecane</td>
<td>NBT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>nC-17 Heptadecane</td>
<td>Benzo (a) Anthracene</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pristane</td>
<td>Chrysene</td>
</tr>
<tr>
<td></td>
<td></td>
<td>nC-18 Octadecane</td>
<td>Benzo (b) Fluoranthen</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Phytane</td>
<td>Benzo (k) Fouoranthen</td>
</tr>
<tr>
<td></td>
<td></td>
<td>nC-19 Nonadecane</td>
<td>Benzo (e) Pyrene</td>
</tr>
<tr>
<td></td>
<td></td>
<td>nC-20 Eicosane</td>
<td>Benzo (a) Pyrene</td>
</tr>
<tr>
<td></td>
<td></td>
<td>nC-21 Heneicosane</td>
<td>Perylene</td>
</tr>
<tr>
<td></td>
<td></td>
<td>nC-22 Docosane</td>
<td>Indeno (1,2,3-cd) Pyrene</td>
</tr>
<tr>
<td></td>
<td></td>
<td>nC-23 Tricosane</td>
<td>Dibeno (a,h) anthracene</td>
</tr>
<tr>
<td></td>
<td></td>
<td>nC-24 Tetracosane</td>
<td>Benzo (g,h,i) perylene</td>
</tr>
<tr>
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<td></td>
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<td>Pyrene</td>
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<td></td>
<td>nC-33 Tritriacontane</td>
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<td></td>
<td></td>
<td>nC-34 Tetratriacontance</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>nC-35 Pentatriacontane</td>
<td></td>
</tr>
</tbody>
</table>
Figure 1 Flowchart for the extraction and quantification of PAHs and other organic contaminants. Figure taken from the NOAA method for organic contaminant analysis (Kimbrough, 2006)

Quality Control is necessary with chemical analysis of samples. All GC work should include sample blanks, triplicate samples, surrogate compounds to ensure extraction efficiency, and internal standards of known concentrations.
Depending on the specific site history and site concerns, analysis can be tailored to include PCBs, flame retardants (PBDEs), chlorinated hydrocarbons and other chemicals of concern. In our study, Hexachlorobutadiene was analyzed due to the historical and ongoing permitted dumping of hexachlorobutadiene into the Calcasieu water system, and the upstream presence of bayou D’Inde, a registered superfund site with high contamination of hexachlorobutadiene.

It is unreasonable to test for every possible pollutant, thus pre-site evaluations are crucial to identify the most likely causes of sediment contamination at a particular site.

3.7. Biological and Toxicological Analysis

Toxicological tests should be conducted on collected sediments. A wide variety of EPA approved target organisms are available for testing. For LDEQ water quality surveys, chronic vertebrate and chronic invertebrate testing is generally conducted for fresh water environments. Chronic vertebrate and acute vertebrate tests are usually conducted for marine samples.

Before conducting a pipeline removal, one chronic and one acute test should be conducted to determine the toxicity of the sediments to be disturbed. Sediment tests can be conducted using whole sediments or elutriates. Elutriate sediment tests are more appropriate for pipeline removal studies, as it is the sediment disturbance and affect on the water column that is of primary concern. As sediment perturbation is the primary environmental concern, a toxicological test using collected sediments will prove an extremely valuable data set in the event of later litigation following a pipeline removal. As oysters are the most valuable commercial product and most sensitive organism to local pollution events, it is strongly encouraged a toxicity test utilize oysters as a test organism. Additionally, sediments can be analyzed for bacterial counts and pathological indicators.
4 Hydrological Assessment of Area

A hydrological analysis of the area should be conducted prior to any deployment of monitoring stations. The monitoring stations are designed to serve two purposes: 1. Provide a real world data set on the deleterious affects of pipeline removal to aquatic organisms 2. Establish the extent of area impacted by the pipeline removal. In order to properly deploy oyster cages, the prevailing hydrology of the area must be known.

4.1 Evaluate Factors Influencing Water Discharge

Depending on location, water movement can be dominated by tidal action, winds, and river movement. In Louisiana, discharge in water bodies is generally very small, as coastal water bodies are generally very slow moving. However consideration should be given to the prevailing tides and other factors when considering placement of sampling cages.

4.2 Determine Direction of Water Discharge

Two perpendicular transects should be conducted at the site of pipeline removal and sediment perturbation. A flowmeter can be used to determine water velocity across each transect. Measurements should be taken every 10 ft. Measurements can be taken once (at 40% depth) in areas of depths less than 3 ft. Measurements should be taken twice (at 20% and 80% depth) in areas of depths greater than 3 ft. Total discharge across the transects is calculated using the area encompassed by the transect and the velocity measurements.

Hydrology measurements should be conducted twice, once to determine placement and again immediately before pipeline removal.

5. Determine Course of Action

Two primary options exist for abandoned pipelines in the Coastal Zone: 1 Bury in Place or 2. Remove pipelines and dispose of site. Following a survey of valuable resources in the area,
a sediment analysis and hydrological analysis, a decision can be made about the appropriate
course of action for an abandoned pipeline, particular an exposed pipeline. Decisions should be
based on the demonstrated toxicity of the sediments surrounding the pipelines. The hydrology
data and physical analysis of the particles can be used to estimate the expected reach and
direction of any sediment perturbation caused by pipeline removal.

This protocol and study is designed to provide guidance on monitoring abandoned
pipeline removal. However in instances where toxicity tests on sampled sediments and chemical
analysis reveal highly polluted and toxic sediments, the preferred environmental action may well
be burying the pipeline on site. This can remove the obstruction with a minimum release of
sequester pollutants.

6. Permit Application

If pipeline removal is to occur within the Coastal Zone of Louisiana, application for a
Coastal Use Permit will almost certainly be required. Any project deemed to have a direct and
significant impact to the coastal waters of Louisiana requires a CUP. Projects may be exempted,
depending on the location of the project. If a project is occurring on land 5 ft above sea level or
within a fastland a CUP is not required. Additionally, General Permits are occasionally
authorized by the Secretary of the Division of Natural Resources that cover specifically defined
projects. For example, a General Permit was authorized in the past to allow for the repair and
removal of 10,000 linear feet of pipeline through vegetated wetlands in specific parishes. If such
a General Permit exists and has not yet expired, an individual could apply for authorization
through the General Permit. A first step would be to contact the CMD, and inquire about the
need for an individual CUP.
If and when the CMD determines a CUP is required, the application should be submitted as early as possible in the process, as permitting can take considerable time. While permitting applications are being analyzed, background information, such as sediment analysis, can be conducted.

In Louisiana, the permitting process for work within the Coastal Zone has been streamlined through the administration of the Coastal Management Division. All information pertaining to CUP applications and requirements are available through the CMD. The CUP is meant to function as the single application for all necessary permits. Once a CUP has been submitted, the CMD will forward the application on to all other relevant permitting agencies for review and comment. The CUP will be reviewed by the Louisiana Department of Wildlife and Fisheries, Louisiana Department of Health and Hospitals, LDEQ, the State Land Office, Louisiana Department of Natural Resources and Coastal Restoration Division, Louisiana Department of Culture, Recreation, and Tourism, and Louisiana Department of Transportation and Development. These agencies have a write to comment and request changes to the work plan. Specific agencies may have specific requirements before agreeing to a CUP, such as the LDEQ water bottoms assessment. This assessment would include the presence of any significant biological resources, such as existing or historical oyster reefs present in and near the affected area. In addition, federal review will occur through the USACOE.

The application process involves a questionnaire about the project, plats and drawings demonstrating the work and equipment to be used, and a landowner waiver form. All information can be submitted on-line, and CMD officials will provide assistance with the application.
Following approval, a public notice will be issued allowing for a public commenting period, and if requested public meeting to discuss the project. A period of 6 months should be expected from initial application to full and final approval to commence work. Work must be initiated within 2 years of CUP issuance, and completed within 5 years of CUP issuance. The CMD staffs field investigators to ensure compliance. Any project operating without a CUP is subject to fines and jail time.

7. Aerial/Visual Surveillance

Prior to any pipeline removal, aerial imagery is highly encouraged to acutely document the present conditions of all vegetation and wetland features. In addition, visual imagery of any manmade structures, such as access roads or piers should be recorded prior to mobilization of heavy equipment on site.

Following pipeline removal and mobilization of site, a second set of images should be collected to again record the condition of area vegetation and manmade structures. Such a data set will be useful in the event of any liability claims reporting a loss of wetlands or physical damage to any manmade structures.

As such imagery is primarily to protect against damage claims and not intended to be a scientific data set, images can be collected in the most cost effective manner.

II Post Removal Assessment

8 Sample Stations

Oysters should be used as the primary biomonitoring organism. The sessile nature of oysters and their filter feeding makes oysters an ideal organism for documenting sediment disturbance and aquatic pollution. In addition, federal standards for sampling and analysis of
oysters are well developed, and an extensive database exists on ambient oyster body burden concentrations.

8.1 Deploy Sample Stations

Oyster Sample stations should be deployed no less than two weeks prior to pipeline removal. Stations locations will be determined based on the presence of particle resources of concern, such as an oyster lease, and expected movement of the sediment plume. A primary goal of the sampling stations is to quantitatively determine what area was impacted by the sediment plume. As such, the sampling stations should be deployed to provide data in each direction. A simple deployment strategy would place sample stations in a diamond shape around the work site, with additional sample at distances expected to exceed the reach of the sediment plume. Again time, money and resources are a consideration. However the value in deploying sampling stations comes producing a data set defining where the sediment plume impacted the aquatic environment, and where the sediment plume did not impact the aquatic environment.

At a minimum, four cages should be deployed, with at least one cage far enough removed from the sediment plume to provide a reference data set on ambient pollution in the water column throughout the monitoring experiment.
Figure 2 Suggested Deployment Strategy for Pipeline Removal Monitoring

An ideal deployment strategy would consist of cages radiating out from the pipeline removal area as shown. The three test cages in the immediate vicinity of the pipeline removal event will provide the best information on the effect of sediment perturbation during pipeline removal on oyster health and condition. The three extent cages are deployed primarily to
determine the reach of the sediment plume. If the sediment plume is transported far enough to impact the extent cages, a statistical analysis should show significant increases a majority of compounds and elements tested.

8.2 Sample Station Design

Sample Stations need to be constructed to withstand deployment in the field for 8 weeks, and provide easy access for sampling. *Crassostrea viginica* is the oyster to use in Louisiana. If monitoring over the summer months, the use of triploid *C. viginica* oysters is highly encouraged. The impact of spawning on body burden concentrations and condition indices could produce misleading information. Triploid oysters do not spawn, and thus will provide consistent results year round. No difference has been found in body concentrations of pollutants of diploid and triploid oysters when spawning does not occur, so diploid oysters are an acceptable organism during the cooler months. Oysters can be obtained from hatcheries within the state of Louisiana.

Construction of sample stations should use a simple design where the oysters are elevated above the water bottom surface. Two PVC-steel coated cages can be stacked on top of each other. The bottom cage can be filled with weights to anchor the structure in place, while the top cage can be filled with oysters. Cages can be permanently fastened together, or simply attached using zip ties or bungee cords. Additional empty cage can be fastened to provide a surface for the sampler to stand on while collected samples. It is important to provide some kind of surface for a sampler to stand, as you do not want to expose oysters to sediments churned up during the sampling process.

9.1 Collection of Oyster Tissue

Oysters should be collected immediately prior to pipeline removal (Day 0), and again on day 3, day 14 and day 36 following pipeline removal. Samples can be collected in zip lock bags,
and transported on ice to a laboratory. Samples should be stored in a 4 C freezer, and should be analyzed within 14 days of harvesting.

No fewer than 30 oysters should be collected from each cage on each day. Every oyster should be weighed and measured. At least 9 oysters should be dedicated for GS/MS analysis, 3 oysters for metal analysis, and 15 oysters for condition analysis.

9.2 GC/MS Analysis

Figure 3 Flow chart for PAH and organic contaminant analysis from oyster tissue. Figure taken from NOAA approved method for organic contaminant analysis (Kimbrough, 2006)
All GC/MS work should be conducted in triplicate samples. One sample should constitute 3 oysters tissues blended together for analysis. Assuming all pollutants to be analyzed are lipid soluble, a single extraction technique can be used. Briefly, 3 oysters should be blended into a slurry for each sample, with a minimum of triplicate samples for each location. A surrogate standard added to provide quality assurance of the extraction techniques. The oyster slurry should be weighed, and 100 mL of 20% KOH and methonal added to the slurry. The oyster slurry should then be refluxed for ~2 hrs. Hexane should be added to the resulting solution, and filtered out over sodium sulfate and washed with DCM. The solubility in hexane of identified pollutants of concern, such as hexachlorobutadiene or PCBs to be tested should be confirmed. A modification of the standard method for oyster extraction may be modified as necessary, or separate samples run for Total Hydrocarbons and other priority pollutants.

9.3 Metal Analysis

For in-depth consultation on metals testing from oyster tissues, the National Oceanic and Atmospheric Administration Technical Memorandum on Major and Trace Element Analytical Methods of the National Status and Trends Program should be consulted.

Briefly, Inductively Coupled Plasma Atomic Emission Spectroscopy should be used for metals analysis. Trace and organo metals should be tested. At least 5 g of wet weight are needed. Triplicate samples are required, so each individual oyster should be blended separately, and 5 g of tissue removed from an oyster removed for analysis. Tissue is digested in acid and then analyzed for all programmed samples.
Table 4. Wavelength absorption for metals using ICP/AES analysis

<table>
<thead>
<tr>
<th>Element</th>
<th>Wavelength 1</th>
<th>Wavelength 2</th>
<th>Wavelength 3</th>
<th>Wavelength 4</th>
<th>Wavelength 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aluminum</td>
<td>257.509</td>
<td>308.215</td>
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<td>Arsenic</td>
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<td>Barium</td>
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<tr>
<td>Calcium</td>
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<td>430.253</td>
<td>612.222</td>
<td>616.217</td>
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<td>Cadmium</td>
<td>214.439</td>
<td>226.502</td>
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<tr>
<td>Cobalt</td>
<td>228.615</td>
<td>238.892</td>
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<td>Chromium</td>
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<td>266.602</td>
<td>267.716</td>
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<td>224.7</td>
<td>324.754</td>
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<td>Iron</td>
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<td>259.837</td>
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<td>Potassium</td>
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<td>Magnesium</td>
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<td>263.817</td>
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<td>330.298</td>
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<td>Phosphorous</td>
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<tr>
<td>Zinc</td>
<td>206.2</td>
<td>213.857</td>
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</tr>
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</table>

Particularly in areas where drilling muds may have been deposited, metals such as barium and zinc are good candidates for metals analysis. Organo metals should also be included, as concentrations, particularly of iron, can be high in the sediment layer. Again while not a specific toxicological concern, organo metals can still spike in oysters exposed to a sediment perturbation, achieving the goal of defining the borders of sediment plume transport within the water column.

9.4 Condition Index

A condition index should be conducted on all oysters left after analysis, and at a minimum 12 oysters per site used. A condition index is calculated by determining the ration of dry shell weight to dry oyster weight. All oysters should be shucked, and wet weights of shells
and meats determined. Oyster shell and meats should then be dried in an oven until a constant weight is obtained.

Several formula variations exist for calculating a condition index.

Condition Index 1 = [\{\text{Dry Tissue Weight (g)} / \text{Shell Cavity Volume}\} * 100]
Condition Index 2 = [\{\text{Dry Tissue Weight (g)} / \text{Dry Shell Cavity Volume (g)}\} * 100]

*Shell Cavity Volume = \text{Whole Oyster Weight (g)} – \text{Shell Weight (g)}

The condition index, along with extracted tissue data, provide the most useful data set to assess the toxicological impacts of a pipeline removal and associated sediment plume. Condition index can be affected artificially by spawning, which results in a decrease in gonad glycogen stores. This process can underestimate the dry oyster meat weight, providing an artificial decrease in condition index not associated with an actual decline in oyster health or condition. As such, sterile triploid oysters are recommended, particularly for monitoring events during summer months.

9.5 Water Sample Collection

Water samples should be collected following pipeline removal around the sampling cages. Suspended sediment analysis can be conducted on water samples, to confirm the movement of the sediment plume around sampling cages. For total suspended solids analysis, ASTM method 2540 should be followed.

9.6 Pipeline Disposal

Finally, once pipelines have been removed, a survey for Naturally Occuring Radioactive Material (NORM) should be conducted. The presence of NORM will require pipelines be decontaminated prior to disposal in conventional landfills.
APPENDIX B

PERMIT AND SURVEY DOCUMENTS

Figure 1. Coastal Use Permit Application

Joint Permit Application
For Work Within the Louisiana Coastal Zone

Louisiana Department of Natural Resources
Coastal Management Division

U.S. Army Corps of Engineers (CDE)
New Orleans District

Application Number: 4697  Permit Number: P20083176  Date Received: 02/13/2008

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Step 1 of 15 - Applicant Information

Applicant: Ralph Fontier
Applicant Type: GOVERNMENT AGENCY

Mailing Addr: LSU 126C Energy Coast & Environment Building
Baton Rouge, LA 70803

Contact Info: Ralph Fontier
Phone: (225) 673-4207  Fax: (225) 673-4286  Email: rfontier@lsu.edu

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Step 2 of 15 - Agent Information

Agent Name:
Mailing Addr: 

Contact Info: 
Phone: -  Fax: -  Email: 

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Step 3 of 15 - Permit Type

☒ Coastal Use Permit (CUP) ☐ Solicitation of Views (SOV) ☐ Request for Determination (RFD)

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Step 4 of 15 - Pre-Application Activity

a. Have you participated in a Pre-Application or Geological Review Meeting for the proposed project?

☒ No  ☐ Yes  Date meeting was held:

Attendees:
(Individual or Company Rep) (CDE Representative) (CDE Representative)

b. Have you obtained an official wetland determination from the CDE for the project site?

☒ No  ☐ Yes  If Yes, Please upload a copy with your application:

c. Is this application a mitigation plan for another CUP?

☒ No  ☐ Yes  Permit Number:

---

87
Step 5 of 15 - Project Information

a. Describe the project.

The current project is a risk-based assessment and subsequent protocol development for the removal of abandoned oil production pipelines/tees from Lake Calcasieu of Cameron Parish, Louisiana. The protocol will be based on the existing LA DEQ RECAP guidelines. The pipeline extraction will be conducted by Dushill employing a zipper method to remove the abandoned flowline. Working from land and using existing access stone roads and lots, the contractors will cut the pipeline approximately 20 feet from the bank. In order to cut the pipeline, a 4' x 4' x 4' volume will be excavated as necessary to expose the abandoned line. The sediment will remain on site; there will be no disposal of sediments or dredged materials elsewhere. From shore, the cut pipeline will then be secured by a nylon choker and pulled from the lake using a backhoe. The remaining pipeline will be capped at the point of cut. A crew will be on site with oil booms and other implements to catch any overflow of the pipeline contents if necessary. All removed pipelines and materials will be disposed of in a legal and lawful manner by the contractor. No dredging other than the previously mention excavation site is anticipated for the current project and access by water will not be required. LSU will conduct onsite toxicity monitoring of pipeline removal following the ASTM protocol. Caged bivalves (Crassostrea virginica) will be placed in four locations at the removal site. The bivalves will be put in metal frame cages, 3' x 3' x 3', and placed on stands made from rebar and located on the lake bottom. The cages will be placed in the lake 1 month prior to pipeline extraction and removed at the conclusion of the study. No dredging is anticipated for the placement of the caged bivalves. After several months onsite, the organisms will undergo extensive analysis in order to determine the presence or non-presence of heavy metals, polycyclic aromatic hydrocarbons (PAHs), and other compounds.

b. Is this application a change to an existing permit?

☐ No  ☐ Yes  Permit Number:

Permit Number:

b. Have you previously applied for a permit or emergency authorization for all or any part of the proposed project?

☐ No  ☐ Yes

<table>
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<th>Permit Number</th>
<th>Decision Status</th>
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<td>P20011204</td>
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Step 6 of 15 - Project Location

a. Physical Location

Street: 
City: 
Parish: Cameron 
Zip: 

b. Latitude and Longitude

Created On: 02/13/2008  Page: 2
**Joint Permit Application**
For Work Within the Louisiana Coastal Zone

<table>
<thead>
<tr>
<th>Louisiana Department of Natural Resources</th>
<th>U.S. Army Corps of Engineers (COE)</th>
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<tr>
<td>Coastal Management Division</td>
<td>New Orleans District</td>
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**a. Section, Township, and Range**

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<th>Range #</th>
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**b. Lot, Track, Parcel, or Subdivision Name**

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**e. Site Direction**

START - I-10 toward Lake Charles. Exit #34 to merge onto I-212 W toward Loop. Exit #4 to Pheen Lake Rd. Turn L at Nelson Rd. Turn R at W Gauthier Rd. Turn L at Big Lake Rd./LA-334. Continue for 7 miles. Look for yellow gate and end of a stone road.

**END**

---

**Step 7 of 15 - Adjacent Landowners**

Adjacent Landowner 1: John & Betty Huber  
Mailing Address: LA 334  
Lake Charles, LA 70601

---

**Step 6 of 15 - Project Specifics**

\(\text{a. Project Name and/or Title: Abandoned Flowine Removal Monitoring and Evaluation}\\)

\(\text{b. Project Type: Non-Residential}\\)

\(\text{c. What will be done for the proposed project?}\\)
d. Why is the proposed project needed?

To provide a scientific basis for the evaluation and design of abandoned flowline removal. Site serves as useful model of area.

---

Step 9 of 15 - Project Status

a. Proposed start date: 02/25/2008
Proposed completion date: 10/25/2008

b. Is any of the project work in progress?

[X] No  [ ] Yes

c. Is any of the project work completed?

[X] No  [ ] Yes

---

Step 10 of 15 - Structures, Materials, and Methods for the Proposed Project

a. Excavations

[X] Non-Vegetated Waterbottoms: 3 yd³  0 Acres  [ ] Vegetated Waterbottoms: 0 yd³  0 Acres  [ ] Wetlands: 0 yd³  0 Acres  [ ] Non-Wet Areas: 0 yd³  0 Acres

b. Fill Areas
c. Fill Materials

- Concrete: \( yd^3 \)
- Crushed Stone or Gravel: \( yd^3 \)
- Native Material: \( yd^3 \)
- Other: \( yd^3 \)

- Rock: \( yd^3 \)
- Sand: \( yd^3 \)
- Topsoil/Dirt: \( yd^3 \)

- Other: \( yd^3 \)

d. What equipment will be used for the proposed project?

- Airboat
- Backhoe
- Barge Mounted Bucket Dredge
- Barge Mounted Drilling Rig

- Bulldozer/Grader
- Dragline/Excavator
- Handjet
- Land Based Drilling Rig

- Marsh Buggy
- Other Wheeled Vehicles
- Self Propelled Pipe Laying Barge
- Tugboat

- Other:

---

Step 11 of 15 - Project Alternatives

a. What alternative locations, methods, and access routes were considered to avoid impact to wetlands and/or waterbottoms?

Compared an alternative location near Cocodrie, LA as well as access to current site by water. Site in Cocodrie did not provide a sufficient environment for evaluation, and access to Cameron Parish site via water is not possible at low tide events.

b. What efforts were made to minimize impact to wetlands and/or waterbottoms?

All traffic will be conducted on existing stone roads and lots. During removal activities, contractor emergency response personnel will be on site and will provide containment equipment if needed.

---

Step 12 of 15 - Permit Type and Owners
Joint Permit Application
For Work Within the Louisiana Coastal Zone

a. Are you applying for a Coastal Use Permit?
   - [ ] No
   - [x] Yes

b. Are you the sole landowner/oyster lease holder?
   - [x] Yes
   - [ ] No

   - The applicant is an owner of the property on which the proposed described activity is to occur.
   - The applicant has made reasonable effort to determine the identity and current address of the owner(s) of the land on which the proposed described activity is to occur, which included, a search of the public records of the parish in which the proposed activity is to occur.
   - The applicant hereby attests that a copy of the application has been distributed to the following landowners/oyster lease holders:

   Landowner/Oyster Lease Holder 1: Hebert-Geer Company, LLC.
   Mailing Address: 349 Miguel Street
   Lake Charles, LA 70607

   Landowner/Oyster Lease Holder 2: Dorothy Louise Hebert-Toibert
   Mailing Address: 152 Oliver Street
   Lake Charles, LA 70607

c. Does the proposed activity present potential impacts to vegetated wetlands?
   - [ ] No
   - [ ] Yes
   - [x] Not Sure

---

Step 15 of 15 - Maps and Drawing Instructions

Note: CMD Compiled Plats consist of a complete and current set of plats that have been pieced together by CMD using only the most current portions of the plat files provided by the applicant/agent. All out-of-date plats have been excluded.

---

Step 14 of 15 - Payment

The fee for this permit is: $ 100.00
Step 15 of 15 - Payment Processed

Applicant Information

Applicant Name:  Ralph Porlier  
Address:  LSU 1285 Energy Coast & Environment Building  
          Baton Rouge, LA 70803

To the best of my knowledge the proposed activity described in this permit application complies with, and will be conducted in a manner that is consistent with, the Louisiana Coastal Management Program.
Figure 2 Plan Map for Coastal Use Permit

GPS Coordinates of Sampling Locations

1. N30°02.374' W93°17.080'
2. N30°02.254' W93°16.938'
3. N30°02.186' W93°17.892'
4. N30°02.128' W93°17.845'

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Figure 3 Cross Section Map for Coastal Use Permit

CROSS-SECTIONS:

OYSTERS TRAP

PIPELINE

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A short draft vessel (0.5 ft) will be used to access cut/cap zone and oyster cages as necessary.

*Sediment cover is intermittent over the pipelines at our shown cut/cap zones. We will only cut where the pipeline is visible above the sediment cover.
Figure 4 Approval of Coastal Use Permit

DEPARTMENT OF NATURAL RESOURCES
COASTAL MANAGEMENT DIVISION

P.O. BOX 44487
BATON ROUGE, LOUISIANA 70804-4487
(225) 442-2000
1-800-267-4019

COASTAL USE PERMIT CONSISTENCY DETERMINATION

C.U.P. No.: P289071868
C.O.E. No.: MVN-2008-0090SWKK

NAME: PORTIER, RALPH
LSU 1285 ENERGY COAST & ENVIRONMENT BUILDING
BATON ROUGE, LA 70863
Attn: Ralph Portier

LOCATION: Cameron Parish, LA
Located in Lake Calcasieu near the northeastern shore; in the Big/Grand Lake Field;
Sections 8 and 9, T12S-R9W; the northern end of the removal and test zone is Lat 30° 02' 22.44"N, Long
093° 17' 04.65"W; the southern end of the removal and test zone is Lat 30° 02' 07.68"N, Long 93° 17'
50.70"W.

DESCRIPTION: Proposed research project to monitor hydrocarbon and heavy metal toxicity resulting from
the removal of oil pipelines and to develop a subsequent protocol for future pipeline removal in
coastal Louisiana. Segments of abandoned pipelines will be removed and the remaining pipelines
will be capped approx. 20 feet from the shoreline. Cages (each one cubic yard in size) containing
bivalves will be placed in four locations at the removal site; the bivalves will later be analyzed for
toxicity uptake. No dredging will occur for access, flowline removal, or anchoring of the bivalve
cages.

In accordance with the rules and regulations of the Louisiana Coastal Resources Program and Louisiana R.S. 49, Sections
214.21 to 214.41, the State and Local Coastal Resources Management Act of 1976, as amended, the permittee agrees to:

1. Carry out, perform, and/or operate the use in accordance with the permit conditions, plans and specifications approved by
the Department of Natural Resources.
2. Comply with any permit conditions imposed by the Department of Natural Resources.
3. Adjust, alter, or remove any structure or other physical evidence of the permitted use if, in the opinion of the Department
of Natural Resources, it proves to be beyond the scope of the use as approved or is abandoned.
4. Provide, if required by the Department of Natural Resources, an acceptable surety bond in an appropriate amount to
ensure adjustment, alteration, or removal should the Department of Natural Resources determine it necessary.
5. Hold and save the State of Louisiana, the local government, the department, and their officers and employees harmless
from any damage to persons or property which might result from the use, including the work, activity, or structure permitted.
6. Certify that the use has been completed in an acceptable and satisfactory manner and in accordance with the plans
and specifications approved by the Department of Natural Resources. The Department of Natural Resources may, when
appropriate, require such certification to be given by a registered professional engineer.
7. All terms of the permit shall be subject to all applicable federal and state laws and regulations.
8. This permit, or a copy thereof, shall be available for inspection at the site of work at all times during operations.
9. The applicant will notify the Coastal Management Division of the date on which initiation of the permitted activity
described under the "Coastal Use Description" began. The applicant shall notify the Coastal Management Division by
mailing the enclosed green initiation card on the date of initiation of the coastal use.
10. Unless specified elsewhere in this permit, this permit authorizes the initiation of the coastal use described under
"Coastal Use Description" for two years from the date of the signature of the Secretary or his designee. If the coastal use
is not initiated within this two year period, then this permit will expire and the applicant will be required to submit a
new application. Initiation of the coastal use, for purposes of this permit, means the actual physical beginning of the use
of activity for which the permit is required. Initiation does not include preoperatory activities, such as movement of
equipment onto the coastal use site, expenditure of funds, contracting out of work, or performing activities which
by themselves do not require a permit. In addition, the permittee must, in good faith and with due diligence, reasonably
progress toward completion of the project once the coastal use has been initiated.
11. The following special conditions must also be met in order for the use to meet the guidelines of the Coastal
Resources Program:

a. The ends of the lines to be left in place must be capped and buried a minimum of 3 feet below the water bottom.
b. This permit does not convey any property rights, mineral rights, or exclusive privileges; nor does it authorize injury to property.

c. The oyster baskets must be marked/lighted in accordance with U. S. Coast Guard regulations.

d. The oyster baskets will be removed from the water bottom immediately upon concusion of the data gathering portion of the experiment.

e. Standard LDWF Provisions for Coastal Use Permits (CUPs) in currently Productive Public Oyster Seed Grounds (LDWF retains the right to amend permit provisions as needed):

Applicant shall be liable for, and shall compensate the state for, any damages to the oyster seed grounds caused by Applicant or Applicant's contractors during any work done under this permit. Prior to commencement of the permitted activity, Applicant will also provide LDWF with the name of an individual in authority who can be contacted regarding any work done under the permit.

Compensation for impacts to the public oyster seed grounds shall be in the form of the planting cullch material (i.e. crushed concrete, limestone, oyster shell, etc) at the rate of 1 cubic yard per acre of impacted area for barren, non-supportive areas of the seed grounds, 50 cubic yards per acre of impacted area for supportive areas, and 157 cubic yards per acre of impacted area for reef areas plus the value of any living oyster resources destroyed. Applicant shall bear the expense of acquisition and deposition of cullch. The cullch shall be deposited by Applicant, Applicant's contractor, or sub-contractor, under the direct supervision of LDWF, and shall be deposited at a time, place, and in a manner prescribed by the Department. In lieu of planting cullch material, Applicant may make payment directly to the Public Oyster Seed Ground Development Account.

Applicant shall not discharge any drilling and/or workover effluent except for coagulated filtered water into the waters in the areas of the proposed activity. Discharge rate of water shall not exceed the rate of filtering.

Applicant shall not discharge any produced waters into the waters in the areas of proposed activity.

Applicant, Applicant's contractors and sub-contractors shall not discharge any human waste from any vessel that does not meet or exceed the requirements of the Department of Health and Hospitals.

If access route traverses a currently productive public oyster area, Applicant shall secure approval of the access route from LDWF and shall ingress and egress to the project location only along the approved route.

Applicant shall establish and maintain, until the permitted activity is complete, along the access route appropriate access route markings for vessels traveling to and from the project location. These markings may be subject to applicable local, state, and federal navigation requirements. These markings shall be sufficient to be used during day and night operations as well as in any climatic and sea condition which may occur during permitted activities.

Applicant shall provide legal representation and indemnification to LDWF for any and all lawsuits and legal claims that may be filed or made against LDWF as a result of the activities by Applicant.

This permit specifically does not authorize prop washing, wheel washing, dredging, or jetting beyond what is shown in the application and drawings. Any changes or variances in the location, access route, volume of material moved and/or magnitude of the area of impact shall require formal application to, and prior written authorization from, the Department of Natural Resources (DNR). The decision by DNR whether to authorize these changes will require consultation by DNR with LDWF in strict adherence to all applicable provisions of the February 3, 2005 Memorandum of Agreement between those two agencies.

Applicant shall have at the project location float booms for containing any spills.
At the discretion of the Secretary or Deputy Assistant Secretary of the Louisiana Department of Wildlife and Fisheries, any activities may be suspended until more favorable conditions prevail.

Applicant shall provide a letter of completion and as-built drawings of the completed project to the LDWF no later than 60 days following completion of the permitted activity.

At the discretion of LDWF, a post-project bottom contour and side-scan survey may be required. The results of these surveys will be made available to the Department, upon request.

Applicant shall remove or spread any dredged material which is greater than 0.5 feet above original bottom contours.

At the discretion of LDWF, the Applicant may be required to return all or part of water bottoms to pre-project conditions.

All vessels utilized under this permit shall be of such size and loaded in such a manner as to not impact the water bottoms over which they pass.

f. Permittee is subject to all applicable state laws related to damages which are demonstrated to have been caused by this action.

g. Permittee shall allow representatives of the Coastal Management Division or authorized agents to make periodic, unannounced inspections to assure the activity being performed is in accordance with the conditions of this permit.

h. Permittee shall comply with all applicable state laws regarding the need to contact the Louisiana One Call (LOC) system (1-800-272-3020) to locate any buried cables and pipelines.

i. This permit authorizes the initiation of the Coastal Use described under "Coastal Use Description" for two (2) years from the date of the signature of the Secretary or his designee. Initiation of the Coastal Use, for purposes of this permit, means the actual physical beginning of the use or activity for which the permit is required. Initiation does not include preparatory activities, such as movement of equipment onto the Coastal Use site, expenditure of funds, contracting out of work, or performing activities which by themselves do not require a permit. In addition, Permittee must, in good faith and with due diligence, reasonably progress toward completion of the project once the Coastal Use has been initiated. If the Coastal Use is not initiated within this two (2) year period, an extension may be granted pursuant to the requirements contained in the Rules and Procedures for Coastal Use Permits (Title 43.I.723.D.). Please note that a request for permit extension MUST be made no sooner than one hundred eighty (180) days and no later than sixty (60) days prior to the expiration of the permit.

The expiration date of this permit is five (5) years from the date of the signature of the Secretary or his designee.

Upon expiration of this permit, a new Coastal Use Permit will be required for completion of any unfinished or uncompleted work items and for any maintenance activities involving dredging or fill that may become necessary. Other types of maintenance activities may also require a new Coastal Use Permit.

*************** End of Conditions ***************
By accepting this permit the applicant agrees to its terms and conditions.

I affix my signature and issue this permit this 22nd day of May, 2008

THE DEPARTMENT OF NATURAL RESOURCES

Jim Rives, Administrator
Coastal Management Division

This agreement becomes binding when signed by Administrator of the Coastal Management Division, Department of Natural Resources.

Attachments
Figure 5 Landowner Notification Letter as Required by Coastal Use Permit

Department of Natural Resources
Coastal Management Division
P. O. Box 44487
Baton Rouge, LA 70804-4487

Applicant’s Name:_____________________________________________________

Brief project description:
Calcacieu Lake, Big Lake Site Abandoned Flow Line Removal and Assessment

I, ____________________________________________, representing ____________________________.

☐ Hereby waive my right to request that any necessary compensatory mitigation for the above referenced project be performed on my property.

☐ Hereby notify the Coastal Management Division of my request to have any necessary compensatory mitigation for the above referenced project implemented on my property if a suitable plan can be developed. I understand that I have an obligation to develop, or to assist the applicant in developing, the mitigation project that I would like to have implemented.

_________________________  _______________________
Signature                     Date
Operations Division
Western Evaluation Section

BASEFILE: MVN 2008-00905 WKK

Mr. Ralph Portier
LSU 1285
Energy Coast & Environment Building
Baton Rouge, Louisiana 70803

Dear Mr. Portier:

The proposed work to dredge for removal of abandoned pipelines in Calcasieu Lake, located near the community of Lake Charles, in Cameron Parish, Louisiana, is authorized under Category I of the Programmatic General Permit provided that all conditions of the permit are met.

This authorization has a blanket water quality certification from the Louisiana Department of Environmental Quality (DEQ), Office of Environmental Services. As such, no additional authorization from DEQ is required.

However, prior to commencing work on your project, you must obtain approvals from state and local agencies as required by law and by terms of this permit. These approvals include, but are not limited to, a permit or waiver from the Coastal Management Division of the Louisiana Department of Natural Resources.

If the work is initiated within two (2) years of the date of this letter, the authorization remains valid for a total of five (5) years from the date of this letter. If the work is not initiated within two (2) years, this authorization becomes null and void.

We ask that you utilize the following link to complete and submit a Customer Service Survey: http://per2.awp.usace.army.mil/survey.html. The New Orleans District Regulatory Branch is committed to improving our service to you and would like your honest opinions of how we are doing. If you do not have internet access you may request a hard copy of the Customer Service Survey by calling (504) 862-2257. Your input is important to us, thank you for your time.
April 11, 2008

Jim Rives, Administrator
Louisiana Department of Natural Resources
Coastal Management Division
P.O. Box 44487
Baton Rouge, LA 70804-4487

RE: Application Number: P20071866
Applicant: Ralph Portier
Notice Date: April 10, 2008
Location: Calcasieu Lake

Dear Mr. Rives:

The professional staff of the Department of Wildlife and Fisheries reviewed the public notice as referenced above. The following recommendations have been provided by the appropriate biologist(s):

**Marine Fisheries:**

1. Applicant shall be liable for, and shall compensate the state for, any damages to the oyster seed grounds caused by Applicant or Applicant’s contractors during any work done under this permit. Prior to commencement of the permitted activity, Applicant will also provide LDWF with the name of an individual in authority who can be contacted regarding any work done under the permit.

2. Compensation for impacts to the public oyster seed grounds shall be in the form of the planting of cultch material (i.e. crushed concrete, limestone, oyster shell, etc) at the rate of 1 cubic yard per acre of impacted area for barren, non-supportive areas of the seed grounds, 50 cubic yards per acre of impacted area for supportive areas, and 187 cubic yards per acre of impacted area for reef areas plus the value of any living oyster resources destroyed. Applicant shall bear the expense of acquisition and deposition of cultch. The cultch shall be deposited by the Applicant, Applicant’s contractor, or subcontractor, under the direct supervision of LDWF, and shall be deposited at a time, place, and in a manner prescribed by the Department. In lieu of planting cultch material, the Applicant may make payment directly to the Public Oyster Seed Ground Development Account.
3. Applicant shall not discharge any drilling and/or workover effluent except for flocculated filtered water into the waters in the areas of the proposed activity. Discharge rate of water shall not exceed the rate of filtering.

4. Applicant shall not discharge any produced waters into the waters in the areas of proposed activity.

5. Applicant, Applicant's contractors and sub-contractors shall not discharge any human waste from any vessel that does not meet or exceed the requirements of the Department of Health and Hospitals.

6. If access route traverses a currently productive public oyster area, the Applicant shall secure approval of the access route from LDWF and shall ingress and egress to the project location only along the approved route.

7. Applicant shall establish and maintain, until the project is complete, along the access route appropriate access route markings for vessels traveling to and from the project location. These markings may be subject to applicable local, state, and federal navigation requirements. These markings shall be sufficient to be used during day and night operations as well as in any climatic and sea condition which may occur during permitted activities.

8. Applicant shall provide legal representation and indemnification to LDWF for any and all lawsuits and legal claims that may be filed or made against LDWF as a result of the activities by Applicant.

9. This permit specifically does not authorize prop washing, wheel washing, dredging, or jetting beyond what is shown in the application and drawings. Any changes or variances in the location, access route, volume of material moved and/or magnitude of the area of impact shall require formal application to, and prior written authorization from, the Department of Natural Resources (DNR). The decision by DNR whether to authorize those changes will require consultation by DNR with LDWF in strict adherence to all applicable provisions of the February 3, 2005 Memorandum of Agreement between those two agencies.

10. Applicant shall have at the project location float booms for containing any spills.

11. At the discretion of the Secretary or Deputy Assistant Secretary of the Louisiana Department of Wildlife and Fisheries, any activities may be suspended until more favorable conditions prevail.

12. Applicant shall provide a letter of completion and as-built drawings of the completed project to the Department no later than 60 days following completion of the permitted activity.
13. At the discretion of LDWF, a post-project bottom contour and side-scan survey may be required. The results of these surveys will be made available to the Department, upon request.

14. Applicant shall remove or spread any dredged material which is greater than 0.5 feet above original bottom contours.

15. At the discretion of LDWF, the Applicant may be required to return all or part of water bottoms to pre-project conditions.

16. All vessels utilized under this permit shall be of such size and loaded in such a manner as to not impact the water bottoms over which they pass.

The Department of Wildlife and Fisheries appreciates the opportunity to review and provide recommendations to you regarding the proposed activity. Please contact me should you need further assistance.

Sincerely,

Venise Ortego, Permits Coordinator

Office: (225) 763-3595
Email: vortegeo@wflouisiana.gov

c: Christy Lavergne, Biologist Supervisor
    Dr. Ralph Portier, LSU Department of Environmental Sciences
Figure 8 On Site Well Information for State Lease 2406

Well Information

Review Well Information

<table>
<thead>
<tr>
<th>SERIAL</th>
<th>WELL NAME</th>
<th>WELL NUMBER OR ID</th>
<th>FIELD PAIR</th>
<th>PROD TYPE</th>
<th>SRC</th>
<th>THT</th>
<th>AEP</th>
<th>EFFECTIVE DATE</th>
<th>API NUM</th>
</tr>
</thead>
<tbody>
<tr>
<td>59418</td>
<td>M H OSL 2657</td>
<td>001</td>
<td>1188</td>
<td>12</td>
<td>26</td>
<td>029</td>
<td>128</td>
<td>09/1/1976</td>
<td>17022030303030</td>
</tr>
</tbody>
</table>

Prmt Date: Spud Date: Stat Date: Stcd
12/03/1966 12/30/1966 09/20/1971 01

Well Surface Coordinates

Surface Longitude: Surface Latitude: Lambert X: Lambert Y: Ground Elevation: Zone: Datum:
93-16-62 93-16-62 113348 504315 0 0 NAD83

Well Surface Coordinates Generated By Dnr

UTM X: 1132547 UTM Y: 619535 Longitude: 89 Latitude: 89

472757 3598366 10 3331817 3852293 63 2632477 1450354 65 3939027 3596526

Bottom Hole Coord

Effective Date: End Date: Plungeback: Total Depth: True Vertical Depth: Measured Depth: Lat: Dec: Lnt Dec: Long Dec: Lnt Min: Long Min: Coordinate System:
12/01/1976 13400 13415 21 1383092 504270 0 0

Well History

Scout Info

Report Date: Well Status: Measured Depth: True Vertical Depth: Detail:
03/31/1966 COMPLETION DATE: UPPER PERF: LOWER PERF: SANDS: RESERVOIR:
03/31/1966 12797 1287 MARG H

Well Tests

09/30/1971 1138 0 0 0 0 0 0 0 0 0

Well Allowables

Effective Date: End Date: Luw Type: Luw Code: Allowable: Estimated Potential: Current Allowable Type:
LEASUNIT: WELL PRODUCTION


Casing

Completion Date: Casing: Wellbore Size: Casing Weight: Upper Depth: Lower Depth: Cement Sacks: Test Pressure: Hours Under Pressure: Test Date: Casing Pulled: Creation Process:
09/30/1971 1138 0 0 0 0 0 0 0 0 0

09/30/1971 1138 0 0 0 0 0 0 0 0 0

Plug and Abandon

P and A Date: Casing Type: Casing Out Type: Casing Out Depth: Nug Weight: Left: Comments:
09/30/1971

Plugs

Plug Type: Upper Plug Depth: Lower Plug Depth: Backs of Cement: Slurry Weight:
1850 2150 1850 12630 0 0 0 0

Tubing and Packers

Completion Date: Tubing Size: Tubing Lower Depth: Tubing Upper Depth: Packers Depth:
03/31/1966 233000 12632 0 0

03/31/1966 12632 0 0 0

03/31/1966 12632 0 0 0

105
Figure 9 Oil Lease Survey
Figure 11 Bottom Water Survey Conducted for 2001 Battery Removal
VITA

Forest Wootten was born in January 1984, in New Orleans to Ernst and Pamela Wootten. Forest graduated from Metairie Park Country Day High School in May of 2002. Forest enrolled in the University of Richmond, and earned a Bachelor of Science in Biology in 2006. Following graduation, he worked as a hack site attendant for the peregrine fund, monitoring the release of Aplomado Falcons back into west Texas and southern New Mexico. After the Peregrine Fund, he worked for with the Colorado State Parks and Natural Areas from 2006-2007 before enrolling in the environmental science program at Louisiana State University to pursue his master’s degree. With the completion of this thesis and other requirements, Forest will receive his Master of Science degree in May 2009.