

**Rapid Survey Protocol for Living Resources
in Louisiana Fresh Marshes**

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Abstract

A significant obstacle to determining the environmental impact of oil spills is the lack of adequate baseline information on the status and trends of living resources in commonly affected areas. Baseline monitoring of living resources could fill these gaps. However, Louisiana has no mechanism in place to insure adequate baseline monitoring. With the development of increasingly sophisticated models that predict oil spill trajectories, it should be possible to mobilize research teams to acquire pre-spill data immediately before a spill reaches a given area. We conducted a review of oil spill impact assessment literature. We then designed a damage assessment that includes many of the lessons learned from previous oil spill impact assessments. Our work defines protocols and procedures for damage assessment of living resources within Louisiana coastal freshwater marshes. Our results are based on interviews with local experts. However, determining the appropriateness of the protocols and procedures will require additional work.

1.0 Introduction

The Oil Pollution Act of 1990 (OPA) addresses oil pollution and establishes liability for the discharge and substantial threat of a discharge of oil to U.S. navigable waters and shorelines. A major goal of the OPA is to restore natural resources that are injured and/or

have lost services as a result of oil spills. The National Oceanic and Atmospheric Administration (NOAA) recently published a final rule to guide trustees in assessing damages to natural resources from a discharge of oil (NOAA 1996). This rule provides a blueprint that enables natural resource trustees to: (1) focus on significant environmental injuries, and (2) plan and implement efficient and effective restoration of the injured natural resources and services. NOAA's rule also encourages public and responsible party involvement in the restoration process.

The environmental impacts of an oil spill are often difficult to determine. The challenge is due, in part, to data gaps in the following areas: (1) the abundance of species in a given area immediately before a spill; (2) how many animals actually died as a result of a spill; and (3) how these deaths, in combination with chronic injury, could affect future population growth (Parsons 1996; Paine et al. 1996 and references therein). Currently, the impact of an oil spill on animal populations is determined by the number of dead oiled animals found after a spill. This method usually underestimates the direct impact of a spill and ignores chronic impacts (Paine et al. 1996).

A significant deficiency in the determination of an oil spill's environmental impact is the lack of adequate baseline information about the status and trends of living resources in the affected area (Paine et al. 1996; Shaw and Bader 1996; Green 1979). Even when "pre-spill" data are available, replication cannot determine whether variations in populations are due to the spill or natural variation in the distribution of animals (Paine et al. 1996). More consistent baseline monitoring of species of special concern could help determine whether oil spills cause fluctuations outside the range of natural variation. However, Louisiana has no mechanism in place to insure adequate baseline monitoring. Additionally, baseline monitoring for all of the Louisiana ecosystems that are potentially threatened by oil spills would be extremely expensive. Even if a long-term ecological monitoring program was in place, the specific needs of emergency spill responders regarding species and populations affected would likely remain unsatisfied (Buddy Goatcher USFWS personal communication).

With the development of increasingly sophisticated models that predict oil spill trajectories (e.g. Walker et al. 1998; Overstreet and Galt 1995), it should be possible to mobilize research teams to acquire pre-spill data immediately before the spill reaches a given area (Paine et al. 1996). During the Exxon Valdez oil spill, between three days and a week were available for rapid assessments (Paine et al. 1996). The information obtained in such a rapid assessment will serve two objectives for the Oil Spill Incident Command: (1) data on living resources present in the potential spill area can be communicated to the planning section and used to minimize impact, and (2) adequate data can be obtained to accurately estimate the impact of the spill as part of the Natural Resource Damage Assessment (NRDA).

The NRDA procedures (NOAA 1996) include pre-incident planning. The NRDA procedures suggest that local natural resource trustees develop scenarios for the types of natural resources and services that may be affected by an incident, and plan appropriate protocols and procedures (Reinharz and Michel 1996). This will alleviate the problems experienced during past oil spill impact assessments when decisions on study design were made quickly (Wiens and Parker 1995; Shaw and Bader 1996) without time to review the lessons learned from previous efforts.

The work described below is focused on freshwater marshes, because they have undergone rapid changes in vegetation composition (Visser et al. 1999), and it is unknown how these changes are affecting living resources. The changes in vegetation communities may negate any historic information on the living resources of Louisiana fresh marshes, since animal communities are strongly affected by the available habitat.

This work defines protocols and procedures for a damage assessment of living resources within Louisiana coastal freshwater marshes based on an extensive literature review. We also interviewed local experts about protocols for different organism groups. However, determining the appropriateness of the protocols and procedures will require additional work, especially since data on Louisiana fresh marsh living resources is very scarce. Most procedures are based on the experts' experiences with sampling the same organism group in other ecosystems.

2.0 Approach

3.0 Lessons Learned

Because oil spills are unplanned environmental accidents, it is usually not possible to study their effects using a nicely balanced, well replicated study design (Eberhardt and Thomas 1991). Nonetheless, it is important that the effects be determined using rigorous statistical procedures (Wiens and Parker 1995). Peterson et al. (2001) provide an excellent discussion of the many factors that affect the outcome of an oil spill assessment. The review below draws heavily on their paper, which contrasts and critiques four studies of the impact of the Exxon Valdez oil spill on intertidal biota.

Table 1. Questions prepared for the interview of experts.

1. What is the best sampling method for your organism group?
 2. What should be the experience level of the sampling personnel?
 3. To what taxonomic level should species be identified in your organism group?
 4. Is there a specific time of day for optimal sampling?
 5. What kinds of abiotic factors are important to note during the sampling?
 6. Are there different habitats that should be sampled?
 7. Is the before-after control-impact (BACI) design the best design to measure impact in your organism group?
 8. How many samples should be taken in each habitat?
 9. Based on your experience, should impact analyses be performed on individual species populations, species diversity, ratios of certain species?
 10. Are there any species that could be used as indicator species?
 11. Do you want to be listed in the manual as an expert that could be contacted to assist with an impact assessment?
-

Table 2. Local experts willing to assist with implementation of this protocol.

Organism Group	Expert	Affiliation
Benthic Invertebrates	Dr. K.R. Carman Dr. J.W. Fleeger	Louisiana State University Louisiana State University
Fish	Dr. D.M. Baltz Dr. F. Jordan	Louisiana State University Loyola University
Amphibians and Reptiles	Dr. J. Bounde	Louisiana Dept. of Wildlife and Fisheries and Louisiana State University
Birds	Dr. P.L. Leberg Dr. E.W. Wischusen	University of Louisiana Lafayette Louisiana State University
Mammals	Dr. G.D. Hartman Dr. E.W. Wischusen	McNeese State University Louisiana State University

3.1 Statistical Approaches to Impact Assessment

Past approaches in impact assessment of oil spills differ dramatically, with major implications for the statistical support required (Peterson et al. 2001). Peterson et al. (2001) state the following: “Testing the null hypothesis of no effect on various species populations is trivial when deaths have been observed.” It is not always possible to determine if a carcass recovered after a spill died as a result of the spill or other causes. Therefore, it is important to determine if post-spill mortality is greater than expected under normal conditions (Paine et al. 1996). Peterson et al. (2001) suggest that the more compelling goal for impact assessment is to estimate the magnitude of the loss and/or the time course to recovery.

Legally, recovery is defined as the return of the resource to conditions that would have prevailed had the spill not occurred (Paine et al. 1996). This may be difficult to determine because natural variation makes it impossible to know what a population or community would have been like in the absence of a spill (Paine et al. 1996). Therefore, the following criteria are often used instead (Paine et al. 1996):

1. a return to pre-spill conditions;
2. establishment of conditions comparable to those within un-oiled/reference areas;
3. stable or increasing populations (though sometimes populations may have been decreasing prior to the spill);
and
4. re-establishment of a healthy biological community.

The rapid survey design outlined in this document is based on Criterion #2.

An important decision to be made in the design is the assessment objective. Objectives fall into two groups (Gilfillan et al. 1999; Peterson et al. 2001):

1. demonstrate that injury occurred somewhere; and
2. determine the impacts for the spill zone as a whole.

Ideally, necessarily limited resources should not be squandered either by devoting extensive effort to sampling even abundant habitats with low sensitivity or by over-sampling rare but sensitive habitats in hopes of detecting small but biologically unimportant differences (Peterson et al. 2001). However, sensitivity is little more than an informed guess, and biological importance is often a value judgment (Peterson et al. 2001). Humphrey et al. (1995) suggest that rather than relying solely on statistical procedures, those conducting impact assessments must acknowledge and incorporate other approaches for drawing inferences from the sampling design (e.g. experimental evidence of toxicity).

The rapid survey design developed here is based on the assumption that the impact determination is representative of the total impact area. The sampling design is a stratified random design stratified on the most common habitats used by different organism groups. Sampling sites will be originally stratified into three impact zones (see discussion below). In the analyses, actual exposure (oiling level and/or toxicity) will be used to determine the impact level.

3.2 Site Selection

The number of sites should be as high as practical (Gilfillan et al. 1999). Increasing the number of sites may mean decreasing the number of samples within a site. More sample sites provide more power to detect impact than fewer sites with more samples within a site (Gilfillan et al. 1999). The rationale for number of sites selected for each organism group is described within each group in the sampling design sections below. However, the sample design is based on expert opinion and requires testing with actual field data from Louisiana freshwater marshes.

One of the assessment problems is that oiling is not assigned at random to sites, so oiled sites may have different characteristics than unoiled sites (Peterson 1993). Consequently the selection of reference areas should ideally mimic the selectivity of the oiling process (Peterson 1993). With the Before-After-Control Impact (BACI) design (Stewart-Oaten et al. 1986; Underwood 1992). The differences between oiled and reference sites can be assessed in the absence of oil by rapidly surveying areas before they are oiled. Ideally, this before-oiling data would come from regular monitoring coastwide. However, in Louisiana monitoring of freshwater organisms is sparse to nonexistent. McDonald et al. (2000) provide guidance on how to analyze species abundance data using the BACI design.

A rapid survey before a spill should also measure background contamination levels. It is likely that Louisiana marsh soils will already be lightly contaminated with petroleum hydrocarbons before a spill, due to their proximity to petrochemical installations or petroleum production facilities. Oil contamination also occurs through run-off, atmospheric fall-out, and spillage from boats (Gill and Robotham 1989). Summers et al. (1993) surveyed sediment contamination in open water areas throughout Louisiana and found several PAH contaminated sites. Rozas et al. (2000) found that all the marsh samples they collected in Galveston Bay contained low levels of petroleum hydrocarbons. DeLaune et al. (1990) have shown that degradation of petroleum hydrocarbons is very slow in anoxic marsh soils, and the investigators postulate that these soils can act as a source of contamination. There is some evidence that some species acclimate to this pollution (Carman et al. 1995), while other species do not (Klerks et al. 1997).

One of the problems in applying statistical designs to oil spill impacts is the non-random assignment of the impact (Wiens and Parker 1995). If the temporal and spatial variation in environmental setting coincides with the distribution or magnitude of the spill, complete random sampling is not the recommended approach (Wiens and Parker 1995). It is very important to define the environmental setting for the reference areas so that they match the oiled areas (Glasby and Underwood 1998). Without this precaution, differences in environmental settings may wrongly be attributed to oiling (Peterson et al. 2001; Wiens and Parker 1995). For each site, the environmental setting should be described with as many parameters as possible. We list these variables for each species group in the proposed design.

The optimal sampling design is one that combines blocking (stratification based on environmental setting) with some form of randomization (Wiens and Parker 1995; Gilfillan et al. 1999). Randomization is important if sampling results are to be applied to the total spill area (Gilfillan et al. 1999). Determination of the overall impact of a spill must take into account the relative proportions of oiled and unoiled habitats in the general area of the oil spill (Baker et al. 1990 cited in Murphy et al. 1997). Some environmental settings may be more prone to oiling. This can be tested through the correlation between oiling intensity and the environmental setting parameter. If a parameter is highly correlated with oiling intensity, the use of this parameter as a covariant can remove some of the effect of oiling as if oiling were merely part of the background variation among sites (Peterson et al. 2001; Gilfillan et al. 1999).

It is unknown how a future oil spill would distribute over a Louisiana fresh marsh and which habitats may be more prone to oiling due to their geographical settings. Therefore, the recommended design uses a random sampling approach stratified into three impact zones, with the same collection of habitats (described for each organism group below) sampled within each of the zones:

1. High probability of impact. This includes the areas immediately adjacent to the edge of the spill at the start of the survey and areas that are in the predicted path of the spill.

2. Low probability of impact. This includes areas that are outside the predicted path of the spill, but not hydrologically separated from the high impact probability areas.
3. No probability of impact. This includes areas that contain the same habitats as the high impact probability areas, but are hydrologically separated from them (e.g. separated by a substantial ridge).

3.3 Sample Size

Peterson et al. (2001) state: “The aerial extent of a sample is an important design consideration for several reasons. The optimal size of each sample depends on the variable to be estimated. In estimating individual species densities, small sample sizes may be preferable, provided that the sampling effort saved is simply redistributed by utilizing numerous small samples. A larger area of coverage by a sample can achieve better representation by spreading out the sample over a larger range of any natural gradient or across spatial heterogeneity.” If estimation of a community property such as species richness or species diversity from each sample is the goal, then small sample size can lead to greater variation among samples (Pielou 1966). In the recommended design, sample size will be different for the different organism groups and was based on expert opinion.

3.4 Selection of Species to Study

In practice, two categories of species will be selected: those that people care about, and those that are reliable indicators of change (Paine et al. 1996). Long-lived species and residents tend to show less natural variability and may be better indicator species (Day et al. 1997). Rarity of a species may imply a high susceptibility to natural or human caused catastrophe (Paine et al. 1996; Jones and Kaly 1996). The more that different species are tested, the more likely that impacts will be detected by chance alone (Paine et al. 1996). Jones and Kaly (1996) provide an excellent review of the difficulty of selecting appropriate test organisms (indicators) in environmental impact studies.

Humphrey *et al.* (1995) list the following reasons for using a community approach:

1. There is concern for the conservation of diversity.
2. Different species act as “replicates” of each other’s responses.
3. Different species offer a wide range of sensitivity to contamination.
4. Changes at the community level will be better indicators at the ecosystem level compared to the response of a single species.
5. There is no a priori basis for selecting “indicator” species or for considering one species to be more important than another.

The approach for the rapid survey design proposed here is to sample for organism groups and not for particular species. This way, the resulting data can be used to evaluate the impact of a spill on the relative abundance of different species as well as community composition.

3.5 Selection of Test Parameters

Determining the size of species' populations is difficult, because many of the organisms are mobile, and the size of the local population fluctuates due to natural deaths, births, immigration, and emigration. Estimations of population densities are based on samples, but individuals are not uniformly distributed, even in a homogeneous habitat (see the review by Boitani and Fuller 2000). Although all sampling techniques have shortcomings, many of them are useful for determining changes in relative abundance of organisms (Heyer et al. 1994; Wilson et al. 1996).

The interactions between members of a community vary widely, from predation to competition for food and shelter. To truly understand the impact of an oil spill, detailed knowledge about the structure and functioning of the impacted community is needed. The indirect effects of oil on vulnerable species can affect other species in many ways (e.g. through the food web, through reduced predation directly or of competitors, and so on.).

Whether or not effects show up as changes in species abundance, oil may affect demography, especially age structure, birth rates, and individual growth rates. Consequently, a spill could alter population trajectories (Paine et al. 1996; Dunnet 1982; Conan 1982). For some species, these population metrics may be much more spatially and temporally consistent than density and may thus provide a better indication of impact (Paine et al. 1996). Both growth rates and age can be determined in many species with distinct annual growth increments. These species can be used to assess whether oil causes unexpectedly high mortality in certain age classes, whether growth rates change in concert with oiling, and the extent of natural variability in species' growth rates or age structures (Paine et al. 1996; Conan 1982).

For some species, it may be relatively easy to obtain an index of health, such as size distribution or the length to weight ratio of captured animals. This kind of data should be collected where possible. It may be possible to compare growth rates of freshwater mussels with a BACI design. However this would not be part of the rapid survey proposed here. Mussel shells can be collected after the spill event to determine species health.

3.6 Oiling Intensity

During an oil spill, different areas receive different levels of oil. This is true both on a large scale (site to site) but also on a within site scale (Irons et al. 2000). Oiling intensity can be based on the width of the oil layer initially observed. However, oil can be remobilized and redeposited in many sites over time (Peterson et al. 2001). Even when oil was experimentally applied to study its effects, individual substrate samples showed large variations in contamination (DeLaune et al. 1984). Whether to treat contamination as a continuous or categorical factor is an important decision in the design phase (Wiens and Parker 1995). A continuous measure of contamination may fail to reveal injury if one assumes a linear response, but the response instead exhibits a sensitivity threshold. On

the other hand, a measure of contamination based on categories may be sensitive to how the categories are defined and whether a threshold occurs within a category or between categories (Wiens and Parker 1995). Sampling designs that treat contamination as a continuous variable may document contamination effects with greater precision and detect nonlinearity (i.e. thresholds) in responses of the resources (Wiens and Parker 1995).

When comparing oiled areas to unoiled reference areas, the ability to detect oil spill effects on a certain species is affected by the magnitude of the species' movements. A species whose home range bisects the oiled-unoiled border may be less impacted than a species whose home range falls completely within the oiled area. Data should be analyzed at different spatial scales to account for this source of variation (see Irons et al. 2000).

Oil is a complex substance, containing many different chemicals (Gill and Robotham 1989), with different toxicological effects. This is worth considering because, except among petroleum chemists, there is a strong tendency to gloss over the great variety of contaminants and their properties (Clark 1982a). The lighter, more volatile fractions of oils are acutely toxic to most types of living organisms (Shales et al. 1989). For example, refined oils are more toxic than crude oils (Rossi et al. 1976; Carr and Reish 1977; Klerks and Nyman 1999). The water soluble fraction of oils may be more harmful than oiled sediments (Stickle et al. 1990), or it may be the opposite for some species (Klerks and Nyman 1999). Solubility of hydrocarbons is significantly higher in fresh water than in saline water (Sutton and Calder 1974). In low energy environments, the long residence time of oil may lead to chemical transformations resulting from biodegradation, which can produce toxic reaction products (Vandermeulen 1982).

Some test of the toxicity of the water in the sampling area should be performed. Klerks and Nyman (1999) tested the toxicity of one crude oil and one diesel oil that had a high likelihood of being spilled in Louisiana's fresh marshes. They found that toxicity was generally higher for benthic organisms than for water column organisms. Although the toxicity of these oils was highly correlated with several chemical analyses, the predictive ability of these relationships was very low (Klerks and Nyman 1999). This is probably due to the complex mixtures of different oil constituents. In addition, the standardized toxicity test may have had a higher correlation with actual biological impact than the detailed chemical analyses of the oil constituents present at each site. Overton et al. (1997) found a strong positive correlation between light aromatic hydrocarbons and toxicity measured using the Microtox® Assay, but a low correlation with total petroleum hydrocarbon and total target aromatic carbon. Overton's findings indicate that the Microtox® Assay could be a good method for estimating how toxic a spill was to aquatic organisms. However, this supposition needs to be confirmed. The importance of using soil extraction techniques that minimize oil loss (e.g. glass laboratory where possible, polyvinyl difluoride filters) and use of extraction chemicals that are not themselves toxic (e.g. $\text{Ca}(\text{NO}_3)_2$) improve the reliability of bioassays (van Gestel et al. 2001). The Microtox® Assay is based on the inhibition of the bioluminescence of bacteria and is considered a cost effective method for assessing biological responses to mixtures of toxic

chemicals (Eisman et al. 1991; Salanitro et al. 1997; Overton et al. 1997). This test can be performed on both water column and soil samples (Salanitro et al. 1997).

The other possibility is to use different benthic and water column species as assay organisms. For example, *Chironomus tentans* (benthic invertebrate), *Daphnia pulex* (water column invertebrate), and *Oryzias latipes* (small fish) have been successfully used as assay species (Klerks and Nyman 1999). Another possibility is the Ceriodaphnia Assay, which is based on the survival and/or fecundity of this invertebrate zooplankton species (Stewart et al. 1990). At least one benthic and one water column test organism are recommended, because different parts of the ecosystem have different susceptibilities to contamination depending on the type of oil spilled. Although none of the organisms used in previous bioassays are native to Louisiana, they are easily maintained in laboratory settings. In addition, personnel with experience in performing these assays are available locally. Additional information on toxicity test methods for aquatic organisms can be found in APHA (1989).

Toxicity of water and sediments should be determined at the same location that biological samples are collected. To account for different levels of contamination over time, contamination samples should, at a minimum, be taken each time that biological samples are taken. The information on contamination level can then be used either as a covariate or as a blocking factor (no oil, medium oil, and high oil as blocks) in the impact analysis. Before deciding the range of the different blocks, one should plot the measure of oiling level (e.g. total PAH) versus the species or community variable of interest. This procedure will help determine if any threshold levels exist.

3.7 Chain of Custody

Maintaining a chain of custody form for all samples is usually not the standard operating mode for most biologists. However, maintaining the chain of custody is good scientific practice and will increase the legal standing of the data gathered. An example of a chain of custody form is provided in Figure 1.

3.8 Number of Sampling Dates

Oil that is not collected during cleanup can disappear from the environment through evaporation and degradation by light and microbial consumers into other carbon based compounds (Wolfe et al. 1994). The lightest, most toxic fractions probably evaporate within the first ten days of the spill (Wolfe et al. 1994). Mills (1997, cited in Rozas et al. 2000) studied the natural degradation of a suite of petroleum analytes from the 1994 Baytown spill in an oligohaline marsh in Texas, and found that more than 95% of the total resolved hydrocarbons biodegraded in approximately 150 days. In contrast, a marsh may take over ten years to recover from a thick spill or a spill that penetrates deep into the sediment (Sell et al. 1995; Wolfe et al. 1994). The high organic content of the marsh sediments, the low energy nature of the marsh environment, and the low oxygen content of marsh all retard the removal of oil through physical processes and microbial degradation (Teal et al. 1992; DeLaune et al. 1990). In general, recovery time should be

positively correlated with initial toxicity and concentrations of oil on the marsh surface (Rozas et al. 2000). Most communities of organisms in temperate waters will recover from a large oil spill within two years, with some effects detectable up to 20 years after the spill (Clark 1982b; Teal et al. 1992).

Chain of Custody Record

Part A: Sample Collection Information

Sample site: _____

Latitude: _____

Longitude: _____

Collection date: _____

Collection time: _____

Sample type*: _____

Sample numbers: _____

Collected by: _____

Affiliation: _____

Signature: _____

Storage location: _____

Storage time: _____

Storage temperature: _____ C or F

Part B: Sample Transfer Information

Transfer date: _____

Transfer time: _____

Relinquished by: _____

Affiliation: _____

Signature: _____

Accepted by: _____

Affiliation: _____

Signature: _____

Storage location: _____

Storage time: _____

Storage temperature: _____ C or F

Part C: Sample Processing Information

Processing date: _____

Processing time: _____

Processed by: _____

Affiliation: _____

Signature: _____

Figure 1. Example of a chain of custody form.

The number of dates on which sampling is conducted, and the time period encompassed by the sampling design affect investigators' ability to detect and quantify oiling effects and to infer recovery trajectories (Stewart-Oaten et al. 1986; Wiens and Parker 1995). Repeated sampling allows treatment x time interactions to be tested in statistical analyses and provides estimates of how the differences between oiled and

reference sites change through time. A detrimental effect of an environmental perturbation is much more serious biologically if it lasts a longer period of time, so repeated sampling to document recovery with confidence is a critical component of a good assessment design (Peterson et al. 2001). The natural seasonal variation in the density of certain species should be carefully noted so normal declines or increases are not attributed to oil spills or oil spill recoveries (Paine et al. 1996).

In addition, repeated sampling allows detection of any delayed effects. These could include indirect effects operating within a community such as trophic cascades (Schoener 1993). In addition, foods contaminated by low to moderate concentrations of oil are readily eaten by some fish (Christiansen and George 1995). This trend could have delayed effects on the reproduction of these fish as well as on species higher up into the food chain. Alternatively, oil induced alterations in the density of one species can affect the density of another species that is not directly impacted by oil (Paine et al. 1996). Therefore, oil effects may cause—or be obscured by—longer term indirect effects that propagate through species assemblages (Paine et al. 1996). Both oiled and reference areas need to be studied through time, so that natural impacts can be separated from oil impacts. For example, bird populations in Prince William Sound, Alaska seemed to be recovering three years after the Exxon Valdez spill. However, nine years after the spill, some species were still negatively impacted, most likely through reworked oil and oil impacts on food sources (Irons et al. 2000).

The rapid survey design provided here does not make any recommendation as to how often the initial survey should be repeated. However, the above discussion should be considered when making these decisions.

4.0 Survey Design

The recommended design for each species group is outlined below. This design is based mainly on the recommendations of the various experts that were interviewed for this project (see Appendix B). Since very little research has been done on the animals that live in the Louisiana fresh marsh, this recommended design should be field tested to determine the utility of different sampling techniques that were developed for other habitats. In addition, the number of samples needed to determine an ecologically significant impact needs to be fine tuned using standard statistical methods considering both Type I and Type II errors (Osenberg et al. 1994; Mapstone 1995).

Each sampling design uses the stratified random sampling by impact zone (see Section 1.3.2). Random sampling should be done using the following steps:

1. Identify the three impact zones on the quads surrounding the spill.
2. Lay a grid of 0.25 km^2 cells (0.5 km on each side) over the three impact zones. Assign consecutive numbers to the cells that contain at least 50% marsh within each zone.

3. Use a random number table to select 10 cells within each zone. Assign each cell a new site number in the order that they were chosen. If three sites are needed for one of the organism groups, the first three sites are used.

4.1 Invertebrates

4.1.1 Introduction

Benthic invertebrates play an important role in aquatic ecosystems. They promote decomposition and nutrient cycling and are the most important food source for many of the vertebrate species (Boesch et al. 1976). Benthic invertebrates living in sediments are largely sedentary and have relatively short life spans. Shorter life cycles suggest more rapid responses at the community level, but greater temporal variability (Stewart and Loar 1994). Within the benthos, each species is capable of different types of responses to different contaminants as well as different responses to sediments in the benthos. This diverse array of responses make benthic invertebrates a superb template for environmental impact assessment (Peterson et al. 1996).

The review of rapid assessment approaches using benthic invertebrates by Resh et al. (1995) is an excellent introduction to the factors that should be considered when designing an invertebrate survey. Several rapid assessment methods for freshwater streams have been developed (see Resh et al. 1995), and could be adapted for oil spill impact assessments in freshwater wetlands. These assessment methods rely heavily on the fact that some invertebrate species are more sensitive to contamination than other groups. The degree of impact is then based on the ratio of these groups. The sensitivity of crustaceans to contaminants is well established (Coul and Chandler 1992; Peterson et al. 1996; Moore and Stevenson 1997). Amphipods in particular could be indicators of oiling impacts (Gomez-Gesteira and Dauvin 2000). The trophic structure of the benthic community is also affected by contamination (Brown et al. 2000) and could be used to determine the impact of oil spills. These kinds of metrics need to be further developed with controlled experiments (see Grouns et al. 1997) using Louisiana fresh marsh invertebrate communities. Several invertebrates that may occur in freshwater wetlands, including mollusks, crawfish, and insects, are considered to be in peril (Louisiana Natural Heritage Program 1996). If these species occur in an area impacted by a spill, special consideration should be given in the impact assessment. Odum et al. (1984) wrote that documentation of the benthos in tidal freshwater is scarce. This seems to be the case in the southeastern United States, and particularly in Louisiana. The only published sources found for this study were for a Louisiana forested wetland (Pratt 1998; Vittor 1981), and for intermediate marshes (Gaston and Young 1992). Chabreck (1973) provides a list of invertebrates that occur in freshwater marsh ponds.

Gomez-Gesteira and Dauvin (2000) summarize the previous findings on the impact of oil spills on marine benthic species and communities as follows:

1. Species sensitive to hydrocarbons (crustaceans and especially amphipods) disappear rapidly and show very high initial mortality.
2. The initial impact is correlated with the importance of sensitive species in natural conditions.
3. In some cases, insensitive species or opportunistic species, especially polychaetes, which usually proliferate after an increase in organic matter, show important increases in abundance one to three years after the stress.
4. The duration of colonization of affected species after an oil spill generally surpasses 10 years.

Total meiofauna abundance seemed unaffected by South Louisiana Crude oil in a Louisiana salt marsh within a four month period after oiling (Fleeger and Chandler 1983; Smith et al. 1984). To survive these oiled conditions, the meiofauna must have a high tolerance for the hydrocarbon stress and low oxygen conditions associated with an oil layer covering the substrate (Fleeger and Chandler 1983). However, invertebrate diversity decreased in a Texas freshwater stream community as the result of an oil spill. Complete recovery of the community had not occurred 26 months after the spill (Harrel 1985). This illustrates that total meiofauna abundance is not a good indicator of oiling impacts and that analyses should be performed on both individual species abundance and community composition indices.

4.1.2 Sampling Design

Use the first five cells within each impact zone. Within each cell, choose the site that is most likely to receive oiling during a spill (e.g. where water exchange between the marsh and open water takes place). These selected sites are the sampling areas. At each site, two samples should be taken in each of three habitats: (1) marsh substrate, (2) marsh vegetation, and (3) open water bottom. Six samples per site and 15 sites result in a total of 90 samples.

4.1.3 Sampling Method

4.1.3.1 Biological Sampling

Previous studies have used 2.54 cm (1 inch) diameter coring tubes (Smith et al. 1984), or 6 cm (2.4 inch) diameter coring tubes (DeLaune et al. 1984). In the salt marsh, 90% of meiofauna occurs in the top 4 cm of the substrate (Smith et al. 1984). The depth of sampling necessary in the fresh marsh is unknown. The right sampling size should be determined in a field trial. In the absence of such a trial, the larger sample size (6.7 cm diameter core) up to 10 cm deep is recommended. Samples should be fixed in the field in a buffered 10% formalin-rose bengal solution and kept in jars.

For open water bottom samples, a Barrett-type coring device can be used to obtain a 10 cm deep 6.7 cm diameter core. These samples should be processed in the same way as the marsh substrate samples.

In the salt marsh, 0.25-m² plots have been used to count the large snails present (DeLaune et al. 1984). The benthic crew should harvest a 0.15-m² plot of vegetation. This vegetation should be stored in a plastic bag and returned to a laboratory, where it can be examined for epifauna.

4.1.3.2 Environmental Sampling

Marine copepod densities are related to tidal variation (Palmer and Brandt 1981), and species composition differs with different substrate characteristics. Freshwater benthic invertebrate communities are also affected by changes in water levels and sediment composition (Lindgarth and Chapman 2001). The following abiotic parameters should be measured at each site:

1. Grain size of the substrate (% sand vs. silt/clay). At each site (marsh and open water), one 50 cc core should be collected from each of the two substrates. The cores should be brought back to the laboratory for processing.
2. Percentage organic matter. At each site (marsh and open water), one 50 cc core should be collected from the two substrates and stored in a watertight plastic bag or centrifuge tube. The cores should be brought back to the laboratory for processing.
3. Redox potential of the soil should be measured at 5 cm depth. Redox measurements should be made using a portable millivolt meter and a saturated calomel reference electrode (see Faulkner et al. 1989). It is important to let the electrode calibrate for at least 15 minutes. This can be accomplished by installing the electrodes before the other sampling is started and by taking the readings after all other sampling is completed.
4. Water level (above or below the marsh) to the nearest cm.
5. Interstitial salinity. A 50cc core should be collected from the two substrates and stored in a watertight plastic bag or centrifuge tube. The cores should be brought back to the laboratory for processing.
6. Toxicity. At each site (marsh and open water), two extra cores (same size as organism cores) should be collected from the two substrates. In addition, 500 ml of surface water (including floating oil when present) should be collected for bioassays from each site. The cores should be stored on ice and brought back to the laboratory for processing.
7. Water quality parameters such as dissolved oxygen in the water column, pH, salinity, and turbidity should be measured in the field. Two surface water samples should be collected in a precleaned 50 ml polyethylene bottle. One bottle should be fixed in the field with an anti-oxidant buffer solution for sulfide determination (Swenson and Turner 1994). Both bottles should be stored on ice and brought back to the laboratory for processing.

4.1.4 Sampling Processing

4.1.4.1 Biological Samples

A graded stack of sieves should be used to separate the organisms from the substrate matrix. Start with a 2 mm mesh sieve to remove coarse organic matter, followed by a 1 mm mesh sieve, and a 0.5 mm sieve. The correct sieve size depends on the abundance and size of organisms in the samples and should be determined in a field trial of the methods (James et al. 1995). The following sources could be consulted for species identification Heard (1982), Fauchald (1977).

Vegetation samples should be carefully examined for epifauna. Details on sample processing need to be further developed in collaboration with a benthic organism expert.

4.1.4.2 Substrate Characteristic Samples

Very detailed descriptions of many of the sample processing and sample collection techniques described below can be found in Swenson and Turner (1994).

4.1.4.2.1 Grain Size

Soil samples are processed following Folk (1974). First the sample is dried to constant weight in a 70°C oven and then weighed to the nearest mg. Next, the sample is homogenized using a mortar and pestle and/or a laboratory grinder. A 15 mg subsample (weighed to the nearest 0.0001 g) is obtained. Salts are removed by agitating the subsample in 150 ml of distilled water. Salts will be dissolved in the water and removed by centrifuging the suspension and decanting the water. Organic matter is removed through oxidation with hydrogen peroxide. The subsample is dried to constant weight and weighed to the nearest 0.0001 g. The subsample is then washed using a #230 mesh sieve (to collect sands) and a #120 mesh sieve (to collect silts). Both fractions should be dried to constant weight in a 70°C oven and weighed to the nearest mg. The clay fraction is estimated by subtracting the dry weight of the silt and fine sand fractions from the total dry weight of the subsample.

4.1.4.2.2. Percentage Organic Matter

Percentage organic matter can be determined using the loss on ignition method. The sample should be dried to constant weight and then homogenized using a mortar and pestle and/or a laboratory grinder. A subsample (~0.75 g) is taken and placed into a clean, preweighed, and labeled crucible. The sample is weighed, then the crucible containing the sample is burned at 500°C for 60 minutes. Percentage organic matter is calculated as $1 - (\text{weight of the subsample after burning} / \text{weight of the subsample before burning})$.

4.1.4.2.3 Interstitial Salinity

Interstitial water is separated from the soil matrix using a centrifuge. Water is carefully decanted into a vial. The 100 ml subsample is titrated using a Haake-Buchler Digital Cloridometer.

4.1.4.2.4 Sulfide

Sulfide can be measured using a sulfide electrode following the procedures outlined in Swenson and Turner (1994).

4.1.4.3 Toxicity

Microtox® Assays should be performed by a laboratory that is equipped for this survey. The LSU Department of Environmental Studies has experience with this assay (Overton et al. 1997). Bioassays with water column and benthic species should be performed using procedures similar to those employed by Klerks and Nyman (1999).

4.1.4.4 Suspended Sediments

Suspended load is determined by filtering 50 ml of water through a glass fiber filter that was pre-combusted at 1022°F (550 °C) and pre-weighed (Whatn Type GF/F or equivalent). The filters are dried at 140 °F (60 °C) and re-weighed to determine total suspended load in mg/l. The filters are then combusted at 1022 °F (550 °C), cooled, and re-weighed to estimate percent organic by loss on ignition (APHA, 1992).

4.1.5 Personnel Qualifications

Field personnel should be trained in procedures for collecting and preserving benthic samples and associated abiotic sampling. Field personnel should also be trained in chain of custody procedures. Both of these training courses could be accomplished in a half day field trip.

Laboratory personnel examining biological samples should be trained in benthic organism identification and should be supervised by a benthic invertebrate specialist. Laboratory personnel making physical parameter determinations can be trained within a day. Toxicity bioassays should be performed by experienced laboratory personnel and should be sent to qualified laboratories.

4.1.6 Number of Field Personnel Required

One crew of three people should be able to sample four sites per day (this includes all the habitats and physical parameters at one site). If 15 total sites are sampled, this

means that four crews of three people are required for benthic invertebrate sampling. The number of sites a crew can sample in one day needs to be tested.

4.2 Fish and Macro Invertebrates

4.2.1 Introduction

Fish are known to the public, contain prey and predatory species, can amplify concentrations of materials that bioaccumulate, occur abundantly in freshwater systems, are sampled relatively easily with simple equipment, and are commercially and recreationally important (Stewart and Loar 1994). These qualities make fish an ideal indicator of oil spill effects using a rapid prespill sampling survey.

Fish kills are commonly associated with oil spills in fresh water (Shales et al. 1989). Oil on the water surface can prevent gaseous diffusion, causing oxygen stress in some freshwater fish. This occurs when the fish come to the water surface for air; they then become directly exposed to the oil (Mitchell and Bennet 1972). There are significant interspecies as well as life stage differences in the toxicity of different oil fractions, but in general, lighter oil compounds are more toxic (Shales et al. 1989). Eggs and larval fish are much more susceptible to oil spill effects than adult fish (McIntyre 1982). Hydrocarbons are rapidly taken up by adult fish, but they also rapidly disappear when the fish are placed in clean water, suggesting that fish have the metabolic capacity to remove hydrocarbons (Shales et al. 1989). McDonald et al. (1996) conclude that sediment concentrations of PAH ranging from 3,000 to 10,000 :g/g appear to be required before significant hepatic EROD activity is induced in fish. The exposure levels of fish can be assessed by measuring bile PAH metabolites (Escartin and Porte 1999).

4.2.2 Sampling Design

The recommended sampling design consists of nine sites, with three habitats sampled at each site. Three sites are located within each of the three impact zones (high, low, none). Site selection will follow the selection of the benthic invertebrate sites, using the first three randomly selected cells. At each site, three habitats are sampled: marsh, marsh edge, and open water.

Three crews are deployed to sample three sites at the same time. Each set of three will include two impact sites and one control site. The crew captains will communicate by radio or phone so that gear deployment takes place at the same time (which should equate to the same water level conditions) at each of the three sites. Each crew will sample two impact sites and one control site, so that the crew is not a factor in the impact determination. A sample allocation of sites and crews is provided in Table 3. Since little is known about the distribution of small fish and macroinvertebrates in Louisiana's fresh marshes, we recommend additional study of this technique as well as the number of samples needed for a statistically sound design.

Table 3. Allocation of sampling sites to three nekton crews.

Crew Number	High Impact Site Number	Low Impact Site Number	No Impact Site Number
1	1	2	3
2	2	3	1
3	3	1	2

4.2.3 Field Sampling

4.2.3.1 Biological Sampling

The drop sampling technique provides a quantitative, instantaneous evaluation within a determined area. The technique described below is based on Arrivillaga and Baltz (1999). A 1-m² square metal throw trap that samples in water up to 1 m deep is recommended (Chick et al. 1992; Jordan et al. 1997). The throw trap should be suspended from a boom extending 2 m (7 feet) beyond the bow of the boat and approximately 0.4 m (1.3 ft) above the water. A pull-pin release mechanism should be used to drop the throw trap on randomly selected sites within a habitat. After the deployment of three throw traps, sample collection begins. In dense vegetation, above-ground plant material needs to be harvested from the sampler and stored in a plastic bag, so that epifauna can be removed in the laboratory. After removing the vegetation from the sample box, the box needs to be swept thoroughly with a fitting 5 mm mesh landing net. This is followed by a thorough dip netting with a smaller net covering the entire basal area until three repeated passes yield no more organisms.

Small specimens should be preserved directly in 95% ethanol, while larger organisms should be preserved with 10% buffered formalin solution, and later transferred to 95% ethanol.

4.2.3.2 Environmental Sampling

The following abiotic parameters should be measured for each sample box:

1. depth of water (cm)
2. water temperature
3. water salinity
4. dissolved oxygen (water)
5. turbidity
6. description of vegetation present in the sample box. Include cover to the nearest 5%, dominant species, and other species observed.
7. description of substrate beneath the samples
8. description of sampling characteristics. Include distance of the sample box to the marsh edge (m), visibility of the bottom, evacuation success.
9. toxicity information gathered for invertebrates (See Sections 4.1.3.2 and 4.1.4.3.)

4.2.4 Report to Incident Command

The following information should be reported back to the incident command immediately after all sites are sampled: approximate density of fish at each site, presence of rare and/or endangered species, any other observations that can assist with planning of spill control and cleanup. A standard reporting form for each site is provided in Figure 2.

4.2.5 Laboratory Processing

All specimens should be identified to the species level (Hoese and Moore 1998). Each specimen should be weighed and its length recorded.

4.2.6 Personnel Qualifications

Field personnel should be trained in the correct operation of the drop sampling apparatus as well as organism retrieval and preservation. Crew captains need to be able to coordinate simultaneous sampling at different locations. Laboratory personnel need to be trained in the identification of fish and macro-invertebrates.

4.3 Reptiles and Amphibians

4.3.1 Introduction

Amphibian skins are permeable, so they may readily absorb the water soluble components of oil (Shales et al. 1989). In contrast, reptiles have impermeable skins and may be impacted by the physical effects of oiling (Shales et al. 1989). Oil spill related kills of frogs, snakes, and turtles have been documented in freshwater areas (Shales et al. 1989; Masnik et al. 1976).

Incident Command Information

A. Site Description

Sample site: _____

Latitude: _____

Longitude: _____

Collection date: _____

B. Animal Information

Organism group sampled: _____

Density of animals: HIGH MEDIUM LOW (circle one).

Diversity of animals: HIGH MEDIUM LOW (circle one).

Presence of rare or endangered species: YES NO (circle one).

Name rare or endangered species observed: _____

Observations on other organisms: _____

C. Other Information
 Substrate character: ORGANIC MUDDY SANDY (circle one).

Other comments: _____

Figure 2. Example form for reporting information back to the incident command.

4.3.2 Sampling Design

The North American Amphibian Monitoring Program (NAAMP) uses 10 stops per route to describe an area, with stops at least 0.5 miles apart (USGS 2002). The NAAMP sampling design will be followed in this protocol, with 10 randomly selected stations within each of the three impact zones (see Section 4.0).

4.3.3 Sampling Methods

4.3.3.1 Amphibian and Small Reptile Sampling

Most marsh species can be trapped using funnel traps (locally known as minnow traps) arranged along a trap line. Each trap should be positioned so that part of the trap is out of the water, and trapped animals can breathe (Dr. Jeff Bounde personal communication). Each trap should also be shaded either within the existing plant canopy or with a piece of wood (Heyer et al. 1994). Traps should be deployed during the afternoon and checked the next morning. Because traps can contain dangerous animals, personnel should be trained in the correct procedures for handling the traps (Heyer et al.

1994). Each organism trapped should be identified as to species, weighed to the nearest gram, identified as male or female (where possible), and assigned to an age class (juvenile, sub-adult, adult).

Open water species can be trapped using hoop nets. Part of the net should be suspended above the water, so that trapped animals have the opportunity to breathe. These nets can be deployed at the same time as the minnow traps.

4.3.3.2 Frog Sampling

In the vast majority of frog species, males in reproductive condition use distinctive species specific calls to advertise their position to potential mates and rivals (Wells 1977). Therefore, call surveys are a very reliable method for determining the different frog species that are present and their relative abundance. Call surveys are the recommended method to use in Louisiana marshes (Dr. Jeff Bounde personal communication). The sampling plan follows the methods of the NAAMP (USGS 2002), with sampling starting at least one hour after sunset. At each station, the observer listens for five minutes, and then records the amphibian calling index for each species heard. The numerical classification recommended by the NAAMP is as follows:

1. Individuals can be counted; there is space between calls.
2. Calls of individuals can be distinguished, but there is some overlapping of calls.
3. Full chorus, calls are constant, continuous and overlapping.

4.3.3.3 Alligator Sampling

Night light surveys are an effective way to determine the relative abundance of alligators (Woodward and Marion 1978). The number of alligators counted along the same transect are strongly affected by water temperature and nocturnal light (Woodward and Marion 1978). It is therefore recommended that all surveys be conducted with similar water temperatures and during the same phase of the moon. Water temperature should be determined at the beginning and end of each transect to the nearest °C. The moon phase should also be recorded, and the water level noted relative to the marsh surface.

A Q-beam spotlight is moved in an arc of approximately 160 degrees in front of a boat traveling at constant speed to detect the red reflection of alligator eyes above the water (Woodward and Marion 1978). When an alligator is spotted, an effort is made to approach it and record the length to the nearest foot.

4.3.3.4 Environmental Sampling

Weather data are especially important in the interpretation of herpetology studies (Heyer et al. 1994; Woodward and Marion 1978). Temperature, precipitation, and night light conditions influence the activity and thereby the observations of many amphibians and reptiles. It is important to measure the following parameters:

1. Precipitation during the survey (install a rain gauge at each of the trapping line stations) and in the 24 hours before the survey (use data from the nearest meteorological station). For alligator night counts and frog call surveys, note if precipitation occurred during the survey.
2. Water temperature at the beginning of each survey.
3. Estimate percentage cloud cover at the beginning and end of the survey to the nearest 10%.
4. Wind speed and direction at the beginning and end of the survey.
5. Moon phase.
6. Water column toxicity. For the first five sites, the information gathered for invertebrates can be used (see Sections 4.1.3.2 and 4.1.4.3). At Sites 6 through 10, 500 ml of water should be collected and processed following the procedures outlined in the invertebrate section.
7. Describe vegetation of the major habitats sampled.

4.3.4 Report to Incident Command

The following information should be reported back to the incident command, immediately after all sites are sampled: approximate density of amphibians and reptiles at each site, presence of rare and/or endangered species, any other observations that can assist with planning of spill control and cleanup. A standard reporting form for each site is provided in Figure 2.

4.3.5 Personnel Qualifications

Personnel participating in the frog survey should be trained in the identification of frog calls. A tape with all frog calls should be made available, so that personnel can check their identification skills.

4.4 Reptiles and Amphibians

4.4.1 Introduction

Large numbers of waterfowl and herons have been killed in previous freshwater oil spills (Shales et al. 1989). This is similar to the number of birds killed in offshore oil spills (Dunnet 1982; Day et al. 1997; Irons et al. 2000). Other documented sublethal effects are the transfer of oil from breast feathers to eggs, which resulted in decreased hatching success (Parnell et al. 1985), and reduced reproductive output as a result of ingested oil (Shales et al. 1989). Behavioral studies have shown that waterfowl tend to avoid oiled waters after initial contact (Custer and Albers 1980). Chabreck (1973) noted that birds avoided ponds contaminated with oil for at least six months after a spill. Oil spill impacts depend on the feeding habits of the birds studied. Birds that dive in the water for their food are more affected than surface feeders (Irons et al. 2000; Day et al. 1997). The impact of an oil spill and the ability to detect this impact depend on the home

range of the species in relationship to the length of a survey transect (Irons et al. 2000) and differ between resident and migratory species (Day et al. 1997).

A sensitivity index has been used to rate the vulnerability of each bird species to be used for planning (King and Sanger 1979). A similar vulnerability index could be developed for Louisiana marsh birds.

4.4.2 Sampling Design

Murphy et al. (1997) suggest that enough stations are needed to detect statistically significant changes (100% increase, or 50% decrease) in bird densities. Previous studies using point counts in uplands recommend that 25 to 30 point counts be performed per area of interest (Hutto et al. 1986 and references therein). To maximize boat use and crews, I suggest that open water and marsh stations be paired so that a crew of two observers can sample the station pair within a 15 minute period. Stations can be spaced so that no more than five minutes of travel time are needed between stations. With three hours of survey time available (see sampling method), this would result in a maximum of nine sampling stations per habitat per crew. Therefore, three crews would be needed to perform this survey. Sample stations should be allocated to crews in such a way that each crew samples a combination of impact level zones (Table 4). This assures that measured impacts are not a function of crew bias.

Table 4. Allocation of sampling sites to three bird crews.

Crew Number	High Impact Site Number	Low Impact Site Number	No Impact Site Number
1	1,2,3	4,5,6	7,8,9
2	4,5,6	7,8,9	1,2,3
3	7,8,9	1,2,3	4,5,6

4.4.3 Sampling Design

4.4.3.1 Biological Sampling

Unlimited distance point counts are time efficient and easily used in rugged terrain, especially in patchy habitats where transects are inappropriate (Gutzwiller 1991). Hutto et al. (1986) give an excellent account of how to maximize the efficiency of this kind of method. A similar study with different count duration, different sampling radii, a different distance between stations, and enough point counts to describe the bird community should be performed in the Louisiana fresh marsh.

In the absence of this study, the following sampling method is recommended based on knowledge of the freshwater habitat and the likely detection limits for most bird species. Sampling stations are equally distributed among open water stations surveyed from a boat, and marsh stations surveyed from a raised platform (e.g. deer stand) at the

marsh edge. At each point, an observer records the number of birds of each species within a 50 mile radius as well as the abundance of individuals of each species detected beyond the 50 mile radius. Recording starts as the observer approaches the imaginary 50 mile circle (so that birds that are flushed by the approach are counted), and continues for 10 minutes (Gibbons et al. 1996). Birds that were originally detected outside the 50 mile radius that entered the circle during the 10 minute observation are counted as belonging within the circle. Diurnal variation in the detectability of birds is widely acknowledged, and most bird counts are confined to the early morning hours (Conner and Dickson 1980). Therefore, all sampling should be performed within three hours after sunrise.

4.4.3.2 Environmental Sampling

Because point counts only require a relatively short time, they can be markedly influenced by weather conditions (Gibbons et al. 1996). Therefore, it is important that these weather conditions be noted. Post-spill sampling should be performed under the same weather conditions as those encountered during the rapid survey. It is important to measure the following parameters:

1. Precipitation during the survey. No counts should be performed in heavy rain (i.e. low visibility). It should be noted on the field sheet if light rain occurred during the survey.
2. Air temperature at the beginning of the survey.
3. Estimate percentage cloud cover at the beginning survey to the nearest 10%.
4. Wind speed and direction at the beginning of the survey.
5. Water column toxicity (use data from the amphibian survey).
6. Description of the dominant vegetation in the survey area.

4.4.4 Report To Incident Command

The following information should be reported to the incident command immediately after all sites are sampled: approximate density of birds at each site, presence of rare and/or endangered species, any other observations that can assist with planning of spill control and cleanup. A standard reporting form for each site is provided in Figure 2.

4.4.5 Personnel Qualifications

Each crew member should be able to reliably identify all common wading birds, waterfowl, raptors, and marsh birds expected to occur in the area. The common species and their identifying characteristics could be taught to crews in a day. Each crew member should carry a guide to help with the identification of rarer species (e.g. Peterson Guide).

4.5 Mammals

4.5.1 Introduction

Mammals are likely to be affected by oil spills through soiling of the pelt, which reduces control of body heat (McEwan et al. 1974). Mammals are also affected when they ingest oil either from drinking water or contaminated food sources (Shales et al. 1989). Heavy mortality of muskrat has been reported from previous freshwater oil spills (Shales et al. 1989).

4.5.2 Sampling Design

A 20.3x20.3x80.0 cm unbaited live trap has been shown to effectively capture nutria, muskrat, raccoon, mink, river otter, and opossum in a Louisiana brackish marsh. Nutria and muskrat were the most abundant animals captured, with less than two animals per 100 trap nights (Robicheaux and Linscombe 1978). Abernethy et al. (1985) used peanut baited snap traps in a floating fresh marsh in the early spring with a capture rate of 0.6 to 5.1 animals per 100 trap nights. Fulvous harvest mouse and rice rat were the only two species captured in the marsh. On spoil banks, house mice and white-footed mice were captured in addition to both marsh species. Capture rates on the spoil banks were higher (4.4 to 10.3 animals per 100 trap nights) than in the marsh. Small mammal species diversity and abundance may be low in Louisiana freshwater marshes compared to intermediate and brackish marshes (Martin et al. 1991).

Based on this information and the recommendations of the interviewed experts, the design below is recommended. However, this design should be thoroughly tested and adjusted in a field trial. The recommended design consists of 10 randomly selected study sites in each of the three impact zones (see Section 4.0). At each site, a trap line will be established with five trapping stations spaced at 20 m intervals. At each trapping station, two traps of each of the three types (see Section 4.6.3.1) will be deployed. This creates a total of 10 traps of each size at each site and 100 traps of each size within each impact zone. When possible, traps should be deployed for at least two nights, because of the low trap rates expected based on previous research.

4.5.3 Sampling Method

Trapping is very time consuming, especially for species that occur in low densities (Sutherland 1996), but this may be the only way to sample mammals in a Louisiana marsh. Whitetailed deer, Louisiana brown bear, and feral hogs are the only mammals that will not be covered with a sampling design based on trapping. Notes on signs of presence of these mammals (e.g. scat) should be made by the personnel deploying the traps.

4.5.3.1 Biological Sampling

Numerous kinds of traps can be used to capture mammals. The appropriate trap depends on the size of the mammal (Wilson et al. 1996). Trappable marsh mammals range in size from a 20 lb nutria to a 5g least shrew (Lowery 1974). Therefore, each trap line will have three types of traps:

1. large live trap targeting large furbearers—approximately 8" x 8" x 31.5" (e.g. Tomahawk trap) baited with carrots;
2. small live trap targeting small rodents—approximately 3.5" x 3" x 9" (e.g. Sherman trap) baited with bird seed; and
3. small snap trap targeting shrews—Museum Special mouse trap baited with chunky peanut butter.

The baits used in the traps should be tested (Wilson et al. 1996). In the past, researchers have used rolled oats, peanut butter, sardines, birdseed, apple slices, and combinations thereof as bait for small rodents. Ant proofing the snap traps (Beltyukova and Spassky 1989; Chabreck et al. 1986) should be tested as well. Ants can quickly devour a dead rodent and leave very little evidence for identification (Dr. Greg Hartman McNeese State University, personal communication). In the marsh, live traps should be placed on plywood platforms over a buoyant material (e.g. Styrofoam) so that they will not flood with the tides (Wolfe 1985). Traps will be deployed during the day and checked at dusk and the next morning. Length and weight of each organism caught should be measured and recorded.

4.5.3.2 Environmental Sampling

Mammals may be more active on dark, warm, and dry nights (Sutherland 1996). In aquatic environments, water level may influence the activity of mammals (Wilson et al. 1996). Vegetation composition affects the distribution of different species (Kincaid et al. 1983; Spencer and Cameron 1988). Therefore, environmental sampling for mammals is exactly the same as environmental sampling for amphibians and reptiles (see Section 4.4.3.4).

4.5.4 Report to Incident Command

The following information should be reported back to incident command, immediately after all sites are sampled: approximate density of mammals at each site, presence of rare and/or endangered species, any other observations that can assist with planning of spill control and cleanup. A standard reporting form for each site is provided in Figure 2.

4.5.5 Report to Incident Command

Each crew member should be able to reliably identify all mammals expected to occur in the area. The common species and their identifying characteristics could be taught to crews in half a day. Each crew member should carry a guide to help with the identification of rarer species (e.g. Lowery 1974).

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6.0 Appendix A

6.0 Appendix A: List of vertebrate species known to occur in Louisiana Fresh Marshes

Organism Group	Scientific Name	Common Name
Amphibians	<i>Ambystoma tigrinum</i>	Tiger salamander
	<i>Hemidactylum scutatum</i>	Four toed salamander
Reptiles	<i>Alligator mississippiensis</i>	American alligator
	<i>Farancia erythrograma</i>	Rainbow snake
Fish	<i>Achirus lineatus</i>	Lined sole
	<i>Acipenser oxyrinchus</i>	Atlantic sturgeon
	<i>Alosa chrysochloris</i>	Skipjack herring
	<i>Ameiurus melas</i>	Black bullhead
	<i>Ameiurus natalis</i>	Yellow bullhead
	<i>Amia calva</i>	Bowfin
	<i>Anchoa mitchilli</i>	Bay anchovy
	<i>Anguilla rostrata</i>	American eel
	<i>Aphredoderus sayanus</i>	Pirate perch
	<i>Aplodinotus grunnius</i>	Freshwater drum
	<i>Archosargus probatocephalus</i>	Sheepshead
	<i>Brevoortia patronus</i>	Gulf menhaden
	<i>Carassius auratus</i>	Goldfish
		River carpsucker

<i>Carpiodes carpio</i>	Quillback
<i>Carpiodes cyprinus</i>	Flier
<i>Centrarchus macropterus</i>	Bay whiff
<i>Citharichthys spilopterus</i>	Grass carp
<i>Ctenopharyngodon idella</i>	Common carp
<i>Cyprinus carpio</i>	Roughtail stingray
<i>Dasyatis centroura</i>	Gizzard shad
<i>Dorosoma cepedianum</i>	Threadfin shad
<i>Dorosoma petenense</i>	Ladyfish
<i>Elops saurus</i>	Fringed flounder
<i>Etropus crossotus</i>	Violet goby
<i>Gobioides broussoneti</i>	Blue catfish
<i>Ictalurus furcatus</i>	Channel catfish
<i>Ictalurus punctatus</i>	Smallmouth buffalo
<i>Ictiobus bubalus</i>	Bigmouth buffalo
<i>Ictiobus cyprinellus</i>	Black buffalo
<i>Ictiobus niger</i>	Spot
<i>Leiostomas xanthurus</i>	Spotted gar
<i>Lepisosteus oculatus</i>	Shortnose gar
<i>Lepisosteus platostomus</i>	Alligator gar
<i>Lepisosteus spatula</i>	Warmouth
<i>Lepomis gulosus</i>	Orangespotted sunfish
<i>Lepomis humilis</i>	Bluegill
<i>Lepomis macrochirus</i>	Longear sunfish
<i>Lepomis megalotis</i>	Redear sunfish
<i>Lepomis microlophus</i>	spotted sunfish
<i>Lepomis punctatus miniatus</i>	Hybrid sunfish
<i>Lepomis spp.</i>	Bantam sunfish
<i>Lepomis symmetricus</i>	Inland silverside
<i>Menidia beryllina</i>	Atlantic croaker
<i>Micropogonias undulatus</i>	Spotted bass
<i>Micropterus punctulatus</i>	Largemouth bass
<i>Micropterus salmoides</i>	Spotted sucker
<i>Minytrema melanops</i>	White bass
<i>Morone chrysops</i>	Yellow bass
<i>Morone mississippiensis Morone saxatilis</i>	Striped bass
<i>Morone sp.</i>	Hybrid striped bass
<i>Mugil cephalus</i>	Striped mullet
<i>Myrophis punctatus</i>	Speckled worm eel
<i>Notemigonus crysoleucas</i>	Golden shiner
<i>Noturus spp.</i>	Madtoms
<i>Paralichthys lethostigma</i>	Southern flounder
<i>Percina caprodes</i>	Logperch
<i>Polyodon spathula</i>	Paddlefish
<i>Pomoxis annularis</i>	White crappie
<i>Pomoxis nigromaculatus</i>	Black crappie

	<i>Pylodictis olivaris</i> <i>Strongylura marina</i> <i>Syngnathus scovelli</i> <i>Trinectes maculatus</i>	Flathead catfish Atlantic needlefish Gulf pipefish Hogchoaker Minnows Catfish Darters
Birds	<i>Aix sponsa</i> <i>Ajaia ajaja</i> <i>Anas acuta</i> <i>Anas americana</i> <i>Anas clypeata</i> <i>Anas crecca</i> <i>Anas discors</i> <i>Anas fulvigula</i> <i>Anas platyrhynchos</i> <i>Anas strepera</i> <i>Anhinga anhinga</i> <i>Ardea alba</i> <i>Ardea herodias</i> <i>Aythya affinis</i> <i>Aythya collaris</i> <i>Aythya valisineria</i> <i>Bubulcus ibis</i> <i>Charadrius alexandrinus</i> <i>Charadrius melodus</i> <i>Charadrius wilsonia</i> <i>Egretta caerulea</i> <i>Egretta rufescens</i> <i>Egretta thula</i> <i>Egretta tricolor</i> <i>Elanoides forficatus</i> <i>Eudocimus albus</i> <i>Fulica americana</i> <i>Gallinula chloropus</i> <i>Larus atricilla</i> <i>Lophodytes cucullatus</i> <i>Nyctanassa violacea</i> <i>Nycticorax nycticorax</i> <i>Pelecanus occidentalis</i> <i>Phalacrocorax olivaceus</i> <i>Plegadis falcinellus</i> <i>Podilymbus podiceps</i> <i>Porphyryula martinica</i> <i>Rynchops niger</i> <i>Sterna antillarum</i>	Wood Duck Roseate spoonbill Northern pintail American wigeon Northern shoveler Green-winged teal Blue-winged teal Mottled duck Mallard Gadwall Anhinga Great egret Green blue heron Lesser scaup Ring-necked duck Canvasback Cattle egret Snowy plover Piping plover Wilson's plover Little blue heron Reddish egret Snowy egret Tricolored heron Swallow-tailed kite White ibis American coot Common Gallinule Laughing gull Hooded merganser Yellow-crowned night-heron Black-crowned night-heron Brown pelican Olivaceous cormorant Glossy ibis

	<i>Sterna caspia</i> <i>Sterna forsteri</i> <i>Sterna maxima</i> <i>Sterna nilotica</i> <i>Sterna sandvicensis</i>	Pie-billed Grebe Purple Gallinule Black skimmer Least tern Caspian tern Forster's tern Royal tern Gull-billed tern Sandwich tern Passerines Raptors
Mammals	<i>Lutra canadensis</i> <i>Mustela frenata</i> <i>Mustela vison</i> <i>Myocastor coypus</i> <i>Ondatra zibethicus</i> <i>Procyon lotor</i> <i>Trichechus manatus</i> <i>Ursus americanus luteolus</i>	Northern river otter Long-tailed weasel Mink Nutria Muskrat Common raccoon West Indian manatee Louisiana black bear Rabbits Small rodents

7.0 Appendix B

7.1 Dr. Donald M. Baltz

Professor, Department of Oceanography and Coastal Sciences/Coastal Fisheries Institute, Louisiana State University. Interviewed on March 14, 2002. Organism group discussed was macro invertebrates and small fish.

1. What is the best sampling method for your organism group?

Drop box sampling (1 m²). Box should be completely emptied of organisms. First with a sweep net of the box dimensions, followed by thorough dip netting with a smaller net covering the entire basal area until repeated passes (three) produce no more animals.

2. What should be the experience level of the sampling personnel?

Crew leaders need to be trained to do concurrent sampling of three sites (see question 4) and on drop sampling technique (e.g. being stealthy in approaching the sampling site)

3. To what level should animals be identified in your organism group?

To the species level.

4. Is there a specific time of day for optimal sampling?

No. But sampling should be done with similar water level conditions at all sites. This can be best accomplished by having three crews sample two impact and one control site at the same time. The crews should be in radio communication to coordinate drop times and habitat types: open water of given depth, marsh edge with or without vegetation, flood marsh of certain plant type, or mixed.

5. What kind of abiotic factors are important to note during the sampling?

Will provide copy of field sheet that has all the different abiotic factors that need to be measured and/or noted. This includes data on the adequacy of the drop sample deployment, up to nine environmental/WQ variables, and quality of drop, whether or not bottom was visible.

6. Are there different (micro-) habitats that should be sampled?

Three habitats should be included if possible: 1. flooded marsh, 2. marsh edge \pm 1 m from plant-water interface, 3. open water $>$ 1 m from marsh edge.

7. Is the before-after control-impact (BACI) design the best design to measure impact in your organism group?

Yes, especially with sufficient replication.

8. How many samples should be taken in each (micro-) habitat?

As many as possible. It should be possible to have each crew sample three different locations and three sites per location in a long day, fewer in winter. With three crews, this comes to six impacted sites and three control sites, with three habitats sampled within each site. During a testing phase of the survey technique cumulative diversity estimates should be made to assist with sample size determinations using Delury depletion estimates should also be used to determine adequate netting effort. This will ensure a complete sample ~95%.

9. Based on your experience, should impact analyses be performed on individual species populations, species diversity, ratio of certain species?

Community structure based on rank order abundance should be tested with Kendall's W in the BACI design format (i.e., across BACI community characterizations). Another good indicator may be derived from the community diversity and other measures of fishes, crustaceans, and gastropods (see Arrivillaga and Baltz 1999). This indicator showed large differences between seagrass beds and adjacent sandflats.

10. Are there any species that could be used as indicator species?

I do not know of any, but you might consult the literature for species that are sensitive to oiling. Analysis of rarer species in the samples may also be useful to determine if any of them are sensitive to oiling.

11. Do you want to be listed in the manual as an expert that could be contacted to assist with an impact assessment?

Yes, but I am especially interested in assisting with the training of the response teams.

7.2 Dr. Jeff Bounde

Biologist, Fur and Refuge Division, Louisiana Department of Wildlife and Fisheries, and Research Associate, Museum of Natural History, Louisiana State University. Interviewed on April 8, 2002. Organism group discussed was amphibians and reptiles.

1. What is the best sampling method for your organism group?

A variety of methods is necessary for this diverse group of organisms. Sampling methods should include: 1. trapping (using minnow traps and hoop nets), 2. visual

survey transects, 3. call surveys, and 4. alligator night counts. Note: minnow traps and hoop nets should be installed such that trapped organisms can reach the water surface and breathe.

2. What should be the experience level of the sampling personnel?

Personnel with a bachelor's degree in biology or wildlife can be trained in the different sampling techniques as well as species identification. Tapes with the calls of the species of frogs occurring in these habitats, as well as a field manual with color pictures of each species should be provided to the sampling personnel.

3. To what level should animals be identified in your organism group?

To the species level; most of the time there is only one species within each genus.

4. Is there a specific time of day for optimal sampling?

Yes, trapping has to be done overnight, visual transects have to be done during the day, call surveys are best within a couple of hours after dark, alligator night surveys have to be done after dark.

5. What kind of abiotic factors are important to note during the sampling?

Date and time of survey, recent (last 24 hours) and current (during survey) weather conditions. This includes: precipitation (inches), air temperature (high, low, current), water temperature (current), cloudiness (average percent cover), wind speed and direction.

6. Are there different (micro-) habitats that should be sampled?

Marsh and open water. Spoil banks could be a third habitat, but oiling on spoil banks is probably minimal. Species composition on the spoil banks is quite different from the species composition of the marsh.

7. Is the before-after control-impact (BACI) design the best design to measure impact in your organism group?

Yes, in the absence of good baseline data.

8. How many samples should be taken in each (micro-) habitat?

- 20 minnow traps in each of the two areas (oiled vs. control).
- 10 hoop nets in each area.
- 10 visual transects (length?) in each area.
- Survey at least five miles of waterways for alligators.

9. Based on your experience, should impact analyses be performed on individual species populations, species diversity, ratio of certain species?

After the spill, it should be relatively easy to identify dead amphibian and reptile species floating on the water surface. This should give a reasonable estimate of the number of organisms killed.

10. Are there any species that could be used as indicator species?

There are no endangered species of reptiles and amphibians in Louisiana freshwater marshes. However, Amphiumas are very sensitive to pollution and may be most affected by a spill. These species are difficult to sample since they live in the soil beneath waterways, but any type of contamination kills them, and the dead bodies float to the top. They are sometimes trapped in the minnow traps, but usually one per 10 traps.

11. Do you want to be listed in the manual as an expert that could be contacted to assist with an impact assessment?

Yes.

7.3 Dr. Kevin R. Carmen

Professor, Biological Sciences Department, Louisiana State University. Interviewed on April 17, 2002. Organism group discussed was benthic invertebrates.

1. What is the best sampling method for your organism group?

I have no experience with this marsh type, but probably some type of coring device should be used. It is very important that the surface of the sediment is undisturbed while taking the sample.

2. What should be the experience level of the sampling personnel?

Personnel need to be trained on how to collect a core, how to section the core vertically, and how to preserve the sample.

3. To what level should animals be identified in your organism group?

To the species level. It is easy to be misled about impacts when organisms are only identified to the higher taxa levels. Identification to higher taxa may be sufficient for large impacts. Detection of smaller, but still biologically significant, impacts requires identification to the species level.

4. Is there a specific time of day for optimal sampling?

Sampling before and after the spill at both control and impact stations should be performed with similar water levels at mid-day. Several benthic organisms migrate into the water column at night.

5. What kind of abiotic factors are important to note during the sampling?

- Grain size of the substrate (% sand vs. silt/clay)
- Redox potential or other measurement of oxygen availability in the substrate
- Sulfide and Ammonium concentrations (these are often affected by oiling)
- Standard water quality parameters (conductivity, temperature etc.)

6. Are there different (micro-) habitats that should be sampled?

Again, I would like to stress my unfamiliarity with this marsh type. Based on my experience in saline marshes oil is most likely to affect the marsh edge habitats, therefore the first priority would be to sample this habitat. Deep water habitats may be affected when tar balls sink to the bottom, therefore deep water habitats would be second priority. Ideally, a transect of samples from the marsh edge to the deep water would be collected. To reduce costs, samples from impacted areas would only be processed if contamination was confirmed with PAH analyses.

7. Is the before-after control-impact (BACI) design the best design to measure impact in your organism group?

Yes, especially with sufficient replication.

8. How many samples should be taken in each (micro-) habitat?

Five control and five impact sites, with three reps and two habitats could be a good starting point. What you really need is a power analysis based on data from this habitat. In the absence of this, I would take all the samples and start with processing one sample per site. Then determine the amount of variation in impact and a control sites and decide if processing of the other samples is necessary.

9. Based on your experience, should impact analyses be performed on individual species populations, species diversity, ratio of certain species?

My experience is that ratios between taxa can be very misleading. They can be used when it has been shown that the species composition within each taxa has not changed. I would recommend looking at changes in species composition through diversity indices and multivariate analyses.

10. Are there any species that could be used as indicator species?

This is difficult to answer without knowing the assemblage of species that occur in freshwater marshes. Although it has been shown that in general crustaceans are more sensitive to oil, this should be tested in the laboratory with the species that normally occur in the freshwater marshes of Louisiana. In the salt marsh, we have found that increased benthic microalgal production is a good indicator of contamination. This is a combined effect of increased ammonium in the soil and

reduced grazing by benthic invertebrates affected by the contamination. It needs to be tested if the same process occurs in the freshwater marsh.

11. Do you want to be listed in the manual as an expert that could be contacted to assist with an impact assessment?

Yes, although I am not an expert in freshwater environments.

7.4 Dr. John W. Fleeger

Professor, Biological Sciences Department, Louisiana State University. Interviewed on March 14, 2002. Organism group discussed was benthic invertebrates.

1. What is the best sampling method for your organism group?

Core sampling combined with sampling of plant stems.

2. What should be the experience level of the sampling personnel?

Sampling and preparation of samples requires minimal training. Identifying animals requires more training.

3. To what level should animals be identified in your organism group?

This depends on the composition of the samples. Large impacts can usually be detected through identification to the family level. Some families are more sensitive to oil spills than others. Samples should be preserved and stored, so that more in depth identification can be done if necessary.

Another thing to consider is the sieve size used to extract the organisms. This is again dependent on the composition of the samples. A graded stack of sieves should be used. I would start with a 2mm mesh sieve to remove coarse organic matter, followed by a 1 mm mesh sieve, and a 0.5 mm sieve. But the correct sieve size depends on the abundance and size of organisms in the samples and should be determined. Currently, our knowledge of benthic invertebrates in Louisiana fresh marshes is very limited. There may be some more information on this in the theses of Fred Sklar and Rick Pratt.

4. **Is there a specific time of day for optimal sampling?**

No.

5. **What kind of abiotic factors are important to note during the sampling?**

- Grain size of the substrate (% sand vs. silt/clay)
- Percentage organic matter of the substrate
- Redox potential or other measurement of oxygen availability in the substrate.

6. **Are there different (micro-) habitats that should be sampled?**

At a minimum both the substrate and the plant stems should be sampled as micro-habitats in the marsh. The number of other habitats (e.g. open water bottoms) is difficult to answer, because it usually involves a trade off between number of samples and number of habitats sampled. I would try to sample the habitat that is most sensitive to oil spills. Which of these habitats is most sensitive needs to be determined first.

7. **Is the before-after control-impact (BACI) design the best design to measure impact in your organism group?**

Yes, especially with sufficient replication.

8. **How many samples should be taken in each (micro-) habitat?**

Depends on the design. In general it is better to sample more sites than having more replication within a site. Five control and five impact sites, with two reps and two micro-habitats could be a good starting point.

9. **Based on your experience, should impact analyses be performed on individual species populations, species diversity, ratio of certain species?**

This is difficult to answer without having a better idea about the species composition of the samples. Some research suggests that the ratio between

sensitive taxa and not-sensitive taxa is a good indicator of impact. Other research has shown that sensitivity is very dependent on the contamination source. Determination of sensitive taxa to different oils is required before these kind of ratios can be determined.

10. Are there any species that could be used as indicator species?

In general, crustaceans are more sensitive to oil than deposit feeders. Of the crustaceans, amphipods may be the most sensitive group and may be used as an indicator species.

11. Do you want to be listed in the manual as an expert that could be contacted to assist with an impact assessment?

Yes.

7.5 Dr. Gregory D. Hartman

Associate Professor, Department of Biological and Environmental Sciences, McNeese State University. Interviewed on April 19, 2002. Organism group discussed was mammals.

1. What is the best sampling method for your organism group?

Trapping with a variety of traps, including snap traps that target the smallest mammals (e.g. shrews), small live traps for small mammals (e.g. mice), and larger traps for medium-sized herbivores and small carnivores (e.g. nutria, raccoon, otter). Snap traps should be baited with chunky peanut butter and live traps with bird seed. It may also be possible to establish some track stations, especially along the water's edge.

2. What should be the experience level of the sampling personnel?

Sampling personnel should be trained in how to set the different traps, the handling of trapped animals, identification of live animals and partial remains of animals. Prior experience of sampling personnel with the handling of live animals could be very important.

3. To what level should animals be identified in your organism group?

To the species level.

4. Is there a specific time of day for optimal sampling?

Yes, overnight sampling for most species.

5. What kind of abiotic factors are important to note during the sampling?

Precipitation during the last few days, phase of the moon, and temperature.

6. Are there different (micro-) habitats that should be sampled?

Whatever habitats are out there, such as distinct vegetation zones. It is difficult to identify these, without having a specific place in mind.

7. Is the before-after control-impact (BACI) design the best design to measure impact in your organism group?

Yes.

8. How many samples should be taken in each (micro-) habitat?

A Calhoun line probably is adequate. Two traps per station along a transect line. Sampling will likely have to vary to some degree as a function of habitat.

9. Based on your experience, should impact analyses be performed on individual species populations, species diversity, ratio of certain species?

All of the above, using relative trapping success as an indicator/estimator of population size for each species.

10. Are there any species that could be used as indicator species?

Do not know if certain mammal species may be more sensitive to oil than other mammal species.

11. Do you want to be listed in the manual as an expert that could be contacted to assist with an impact assessment?

Yes.

7.6 Dr. Frank Jordan

Professor, Department of Biological Sciences, Loyola University. Interviewed on May 23, 2002. Organism group discussed was macro-invertebrates and small fish.

1. What is the best sampling method for your organism group?

Quantative sampling using throw traps should provide a good estimate of the relative abundance of fishes and macro-invertebrates. Qualitative sampling using dip nets should provide a good estimate of the species diversity in the area.

2. What should be the experience level of the sampling personnel?

Personnel needs minimal training to do the sampling. Specimens should be preserved in the field, so that they can be identified in the laboratory. Identification will require more training. Voucher samples should be prepared and stored.

3. To what level should animals be identified in your organism group?

To the species level, where possible (e.g. larval species and some insects maybe impossible to key out to species level).

4. Is there a specific time of day for optimal sampling?

No. Research has shown that very few differences occur between day and night. These differences are much smaller than those expected from an oil spill. Daytime would be best, because it is easier to sample during the day.

5. What kind of abiotic factors are important to note during the sampling?

Dissolved oxygen, salinity/conductivity, oiling level, density and type of vegetation present are the most important factors. pH and ammonium level could also be important.

6. Are there different (micro-) habitats that should be sampled?

Open water and the different vegetative habitats that occur within the spill zone.

7. Is the before-after control-impact (BACI) design the best design to measure impact in your organism group?

Yes.

8. How many samples should be taken in each (micro-) habitat?

As many as possible. At least three quantitative samples per site. The number of sites depends on the effect size that is expected. This really should be tested further.

9. Based on your experience, should impact analyses be performed on individual species populations, species diversity, ratio of certain species?

All of those. If a biotic index is developed for these habitats it could be a good test parameter. Health indices (e.g. the length/weight ratio) may also be of use (see Schmitt, R. J. and C. W. Osenberg. 1996. Detecting ecological impacts: Concepts and applications in coastal habitats. Academic Press: New York, NY, USA.)

10. Are there any species that could be used as indicator species?

Not that I know of. Most fresh marsh species are fairly tolerant of perturbations in the environment.

11. Do you want to be listed in the manual as an expert that could be contacted to assist with an impact assessment?

Yes.

7.7 Dr. Paul L. Leberg

Associate Professor, Department of Biology,
University of Louisiana-Lafayette

Interviewed on March 28, 2002.

Organism groups discussed was birds. Dr. Leberg is also an expert on bats, but mentioned that bat densities are low in fresh water marshes, due to the lack of roost sites. Bats should be considered when determining oil spill impacts in forested areas.

1. What is the best sampling method for your organism group?

For birds, there could be two approaches. The first method is to do point counts from the ground. This method is especially applicable to passerines. The second methods involves aerial transects, preferably from a helicopter. Conspicuous birds (waterfowl and waders) can then be counted. During the breeding season the flight should also includes counts at rookery sites within the area, augmented by ground surveys.

2. What should be the experience level of the sampling personnel?

They should be able to identify the birds both visually and through song (for the point counts).

3. To what level should animals be identified in your organism group?

To the species level.

4. Is there a specific time of day for optimal sampling?

Yes, birds should be surveyed within three hours after sunrise. Aerial surveys would be best in the early morning and late afternoon. Rookeries can be surveyed any time of the day.

5. **What kind of abiotic factors are important to note during the sampling?**

Wind speed, cloud cover, precipitation, temperature, and time of day.

6. **Are there different (micro-) habitats that should be sampled?**

Point counts will sample both marsh edge and interior at the same time. Aerial surveys would sample all habitats at the same time.

7. **Is the before-after control-impact (BACI) design the best design to measure impact in your organism group?**

Yes, but having more before data than a quick survey would be much better.

8. **How many samples should be taken in each (micro-) habitat?**

Based on my experience in different environmental settings than the fresh marsh, 20 point counts per distinct habitat type are sufficient. Distinct habitat in this case would mean distinct vegetation communities. This sample size should be tested before implementation of the design.

9. **Based on your experience, should impact analyses be performed on individual species populations, species diversity, ratio of certain species?**

Individual species.

10. **Are there any species that could be used as indicator species?**

Seabirds, waders, and waterfowl. Passerines may be affected more by cleanup activities than the actual spill.

11. Do you want to be listed in the manual as an expert that could be contacted to assist with an impact assessment?

Yes.

7.8 Dr. Nancy N. Rabalais

Faculty, Louisiana Universities Marine Consortium. Interviewed on March 25, 2002.
Organism group discussed was benthic invertebrates.

1. What is the best sampling method for your organism group?

Eckman grab for open water bottoms. Coring in vegetated areas.

2. What should be the experience level of the sampling personnel?

Sampling and preparation of samples requires minimal training. One half day training session should be sufficient to train a field crew. Crew should also be trained in chain of custody procedures. Identifying animals requires a lot more training.

3. To what level should animals be identified in your organism group?

This depends on the composition of the samples and the kind of analyses that are necessary to detect impacts. Identification to the species level could require a lot of time.

4. Is there a specific time of day for optimal sampling?

No, but water level at the time of sampling could be very important.

5. What kind of abiotic factors are important to note during the sampling?

Water level, salinity, temperature, grain size of the substrate, total organic carbon in the substrate, and contaminant levels. The micro-toxicity test may also be useful (contact Maud Walsh for more information).

6. Are there different (micro-) habitats that should be sampled?

Yes, plant stems, soils, and open water bottoms.

7. Is the before-after control-impact (BACI) design the best design to measure impact in your organism group?

Yes.

8. How many samples should be taken in each (micro-) habitat?

This depends on the kind of analyses that will be performed to detect impacts. If species diversity is used, species area curves can be used to determine the number of samples that are necessary. The number of samples also depends on the size of the sample. The rule of thumb for the marine environment is five samples (Eckman grabs). This rule is probably based on the costs associated with processing a sample.

9. Based on your experience, should impact analyses be performed on individual species populations, species diversity, ratio of certain species?

This is difficult to answer. Assessments that have been developed for streams and the marine environment may not be applicable to fresh water marshes. This requires research that as far as I know has not taken place.

10. Are there any species that could be used as indicator species?

It may be that freshwater mussels could be used. Their growth rate, tissue concentration of contaminants, and tissue production rates may be used to determine impacts. This needs additional research, before it could be used.

11. Do you want to be listed in the manual as an expert that could be contacted to assist with an impact assessment?

No. You may want to contact some of the consulting companies that do this work routinely (e.g. Barry Vittor).

7.9 Dr. E. William Wischusen

Associate Professor, Department of Biological Sciences, Louisiana State University.
Interviewed on March 26, 2002. Organism groups discussed were birds and mammals.

1. What is the best sampling method for your organism group?

This depends on what kind of characteristics you want to measure. Dr. Visser clarified that the primary interest would be a measure of relative abundance of animals.

For birds, there could be two approaches. The first method is to do visual counts from the ground. At each station the number and species within a certain radius from the observer would be counted. This could involve some way to raise the observer (e.g. deer stand) and binoculars or spotting scopes. The second involves a flight over the area and taking pictures. Conspicuous birds (waterfowl and waders) can then be counted.

For mammals there could be two approaches as well. The first is life trapping using both smaller and larger traps. The second method involves spotlight surveys at night. This method is only applicable to larger animals (e.g. deer)

2. What should be the experience level of the sampling personnel?

The more experienced the better, but at a minimum they should be able to identify the species. It is possible to provide a field manual to those species that are expected in the fresh marsh habitat and train personnel to identify these with half a day of training. Complete identification guides should be available to the sampling personnel as well.

3. To what level should animals be identified in your organism group?

To the species level, when possible (approximately 99% of the time). With the visual identification of birds, sometimes an individual can only be assigned to a group (e.g. unidentified heron).

4. Is there a specific time of day for optimal sampling?

Yes, birds should be surveyed in the early morning and late afternoon. The timing of the surveys should be done relative to sunrise and sunset, not a specific hour.

Yes, most mammals are nocturnal, life traps should be checked in the morning and in the early night (a few hours after sunset???)

5. What kind of abiotic factors are important to note during the sampling?

Weather characteristics including wind speed, cloud cover, precipitation, fog, temperature.

6. Are there different (micro-) habitats that should be sampled?

-Three habitats for birds: 1. marsh, 2. marsh edge, and 3. open water.

-Three habitats for mammals: 1. marsh interior, 2. marsh edge, and 3. high ground

7. Is the before-after control-impact (BACI) design the best design to measure impact in your organism group?

Yes, but having more before data than a quick survey would be much better. One day we may know enough that we can predict the impact of an oil spill through population models.

8. How many samples should be taken in each (micro-) habitat?

As many as possible. For mammals the rule of thumb is that you need 100 trap nights. This means ~10 traps per habitat at 10 stations. The more nights you can sample the better.

9. Based on your experience, should impact analyses be performed on individual species populations, species diversity, ratio of certain species?

Relative abundance of species. It is very likely that some species will be differentially impacted by an oil spill, primarily from indirect effects through the

food chain. Filter feeders (e.g. ibis and spoonbill species) may be directly affected, while insectivorous species may show a delayed impact.

10. Are there any species that could be used as indicator species?

See reply to question 9.

11. Do you want to be listed in the manual as an expert that could be contacted to assist with an impact assessment?

Yes.