Monitoring of Recovery of Marshes Impacted by the Chalk Point Oil Spill

Office of Response & Restoration
National Oceanic and Atmospheric Administration
Silver Spring, Maryland

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Submitted to:

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Cover Photography

Top vertical aerial photograph shows the spill area in Swanson Creek in April 2000.

Bottom vertical aerial photograph of the same area in Fall 2004. The round features in the marsh are muskrat huts.

EXECUTIVE SUMMARY

On 7 April 2000, an estimated 140,000 gallons of a mixture of No. 6 and No. 2 fuel oils were released into Swanson Creek, the Patuxent River, and downstream tributaries from a pipeline rupture going into nearby Chalk Point Power Generating Station. The spill affected an estimated 76 acres of brackish marsh, with extensive areas of heavily oiled interior marsh habitat. Federal and State natural resource trustees conducted a Natural Resource Damage Assessment (NRDA) of the spill, which included field studies and chemical analyses of marsh soils for polynuclear aromatic hydrocarbons (PAHs). The restoration plan included creation of 2 hectares of new marsh habitat from uplands.

Because of the predicted long-term persistence of oil-related impacts to wetland functions and services, the National Oceanic and Atmospheric Administration (NOAA) funded a study of the oiled wetlands in 2007, seven years after the initial spill. The study plan consisted of field and laboratory studies to assess the recovery status of the impacted wetlands, as indicated by three metrics: 1) the persistence and weathering status of PAHs in marsh soils; 2) vegetation condition measurements of belowground biomass, stem density, and stem height; and 3) toxicity tests of surficial soils. The objective was to compare these metrics at sites within the heavily oiled interior marsh habitats affected by the spill with similar but unoiled marsh habitats. Studies of the created marsh site were limited to vegetation condition, which will be an important step in documenting the rate of development of the site two years after creation. The study was designed to answer the following questions:

- What is the condition of the vegetation in the heavily oiled marsh seven years post- spill compared to unoiled marshes?
- What is the degree of weathering of oil in the marsh soils?
- What are the sources of the PAHs in the marsh soils
- Is the oil in the marsh soils toxic? If so, at what PAH concentrations?
- What is the above- and belowground biomass at the restored marsh compared to a natural marsh?

Based on statistical analyses of the power of proposed study variables, the study design consisted of:

S. alterniflora:	12 unoiled sites (site name AR for *S. alterniflora* and reference)
	10 oiled sites (site name AH for *S. alterniflora* and heavy oiling)
	6 sites in the created marsh (site name AW for *S. alterniflora* and Washington Creek)
S. cynosuroides:	12 unoiled sites (site name CR for *S. cynosuroides* and reference)
	14 oiled sites (site name CH for *S. cynosuroides* and heavy oiling)

Fieldwork was conducted 29 August-4 September 2007. Sampling sites included those established in 2000 as part of the NRDA studies and additional, randomly located sites based on 2005 digital aerial photography. Statistical tests showed that there were no significant differences in the distance to tidal channels between the oiled and reference sites. Stem density and height were measured in the field. Sediment cores (16-cm diameter) were taken and spilt into 0-10 and

10-20 cm intervals for belowground biomass and PAH analysis. PAHs were analyzed by gas chromatography/mass spectrometry in the selected ion monitoring mode (GC/MS SIM) and included 51 PAHs, 5 individual alkyl isomers, and 3 hopanes/triterpane. Fourteen of the 0-10 cm intervals were selected for ten-day survival whole soil toxicity tests with the amphipod *Ampelisca abdita.*

Visual descriptions in the field varied from no visible oil to black oil filling root cavities. For most of the oiled cores, the total PAHs varied by 1-2 orders of magnitude between the top and bottom intervals, reflecting the high heterogeneity in the oil distribution in the marsh soils. However, there was a tendency for higher PAHs with depth; all of the six samples with over 500 milligrams per kilogram dry weight (mg/kg) total PAHs were from the 10-20 cm interval, equally from both species. The highest value measured was 2,921 mg/kg total PAHs.

Multiple methods were used to infer sources of PAHs in the marsh soils: Pyrogenic Index, fluoranthene/fluoranthene+pyrene ratio, Fl/(Fl+Py), biomakers, double-ratio plots, and total PAH concentrations. Oil weathering was characterized by % PAH depletion ratios for different PAH groups and total PAHs. Nine samples had <1 mg/kg total PAHs, reflecting background levels of pyrogenic PAHs derived from combustion of fossil fuels. Seventeen samples had 1-8 mg/kg total PAHs that contained mixtures of both petrogenic and pyrogenic hydrocarbons. However, 24 samples, half of those collected, very clearly contained petroleum hydrocarbons derived from fossil fuels, with a good match to the source oil. The oil in these 24 samples had lost 22-76% of its initial PAH content in the seven years since the spill. The oil at 10-20 cm was generally less weathered than the top 0-10 cm; all samples with less than 55% total PAH depletion were from the deeper interval of 10-20 cm. There was a clear trend with increased PAH depletion with decreasing total PAH concentrations.

As of 2007, the oil in the marsh soils tended to be less weathered with depth, although this is not a strong trend. Overall, the oil in the marsh soils has undergone little to no additional weathering since Fall 2000, based on comparisons of depletion ratios from samples collected in Fall 2000, Summer 2001, and Summer 2007. There are likely two factors limiting natural weathering processes in the marsh soils: slow physical removal processes and low oxygen availability. The interior marsh habitat is flooded by daily tides through many small channels. During spring tides, there can be 20-30 cm of water in the marsh. The marsh surface has a lot micro-topography with low areas between dense clumps of stems that hold pools of water during low tide. The sediments in these low areas are very soft and water saturated. Obviously, during spring low tides, the marsh soils do drain as low as 30 cm, because the oil penetrated to these depths in some areas. The falling tide drains through dense vegetation. Tidal flushing may have been a mechanism for removal of bulk oil stranded on the surface initially; however, it would not be effective at mobilizing oil from below the marsh surface. There are few bioturbating benthic biota in these marshes. Photo-oxidation does not occur below ground. Therefore, the only other removal mechanism would be microbial degradation.

For the vegetation metrics, stem density and stem height were significantly lower in the oiled versus unoiled sites for *S. alterniflora* but not *S. cynosuroides* habitats. In contrast, belowground biomass was significantly lower in the *S. cynosuroides* habitats but not the *S. alterniflora* habitats. The reasons for these differences (other than that the power was too low,

leading to a false conclusion) may be related to the relative distribution of above- versus belowground biomass and the types of biomass for each species. Because *S. cynosuroides* has more and larger rhizomes and the rhizome biomass has a peak at 10-20 cm, this species may be more likely exposed to the highly concentrated oil that persisted in the cavities along the rhizomes. Some of the black oil observed in the cores occurred along rhizomes, which were partially hollow and dead. Roots and rhizomes in the soil would grow until they encountered zones of oil that would slow growth and could eventually lead to death. *S. alterniflora* has about an equal proportion of roots to rhizomes and the rhizomes are smaller, so any reductions in the biomass of the rhizomes may have had a lesser effect on the overall belowground biomass. Alternatively, the lower belowground biomass of *S. alterniflora* may be in less contact with the oil.

Although there were concerns that copper and nickel could be at concentrations that could contribute to toxicity of the soils, lowest concentration for both metals had the highest soil toxicity, which correlated with the highest total PAH concentrations. Comparisons with sediment toxicity thresholds did not suggest a meaningful contribution to toxicity from copper and nickel in the marsh soils. ESB-TU_{FCV} values for the oiled soils, assuming 5.6% TOC, ranged from 0.01-30.48, with the following distribution and predicted effects based on the toxicity test results:

- 54% (13/24) were <1.0; these soils were not toxic to amphipods in toxicity tests and are not expected exhibit toxic effects.

- 8% (2/24) were between 1.0 and 2.0; the one toxicity test in this range had 66% amphipod survival, thus these soils could have effects on sensitive organisms.

- 38% (9/24) were between 2.0 and 3.0; the two toxicity tests in this range had 3% and 85% survival, respectively, thus these soils are expected to have significant effects on sensitive organisms and effects on many organisms.

- 25% (6/24) were >3.0; the three toxicity tests with values greater than 3.0 had 0-1% amphipod survival; these soils are expected to be toxic to many organisms.

The logistic modeling regression approach accurately predicted the toxicity observed in the *S. cynosuroides* soils for individual PAHs and P_{Max}, though overestimated the toxicity of some of the moderately contaminated *S. alterniflora* soils. Six of the 24 samples (25%) had a P_{Max} value greater than 0.65, which is the value at which 50% of the samples are expected to be toxic. These results are of particular value for long-term monitoring studies of oiled marshes; first, because toxicity is seldom monitored, and second, because the surficial soils (0-10 cm) in some areas were still toxic after seven years. With the slow rates of physical removal and microbial degradation of the oil that penetrated into the marsh soils, it is likely that the toxicity will also persist.

It is of value to compare the 2007 study results with the predicted service losses for the vegetation and soils that were developed during the NRDA. The 2007 study sites were located only in heavily oiled, interior habitats. The recovery curve inputs were converted to the predicted services present for the two marsh types in 2007, shown in Table ES-1. The 2007 study results

are summarized as the ratio of the mean value for oiled sites to that for the unoiled sites, without any consideration for statistical significance. It seems that the predicted service losses for *S. alterniflora* vegetation recovery were underestimated and for *S. cynosuroides* were overestimated. The predicted soil service losses appear to be supported by the 2007 results in that *S. alterniflora* habitats showed no reductions in belowground biomass but 40% of the sites are predicted to still have some toxicity. The *S. cynosuroides* habitats, however, have both a reduced belowground biomass and slightly larger percentage of sites with toxicity.

TABLE ES-1. Comparison of the predicted services present as of 2007 and the 2007 actual results for the marsh and soil services in the interior, heavily oiled habitats.

Resource Category	Predicted Services Present in 2007	2007 Results (Ratio = Value Oiled /Unoiled Sites)
Vegetation		
- *S. alterniflora* interior heavy	100%	Stem density = 63% Stem height = 85%
- *S. cynosuroides* interior heavy	83%	Stem density = 105% Stem height = 94%
Soils		
- *S. alterniflora* interior heavy	85%	ESB-TU_{FCV} values in soil samples 40% >1.0 40% >2.0 30% >3.0 Belowground biomass = 98%
- *S. cynosuroides* interior heavy	57%	ESB-TU_{FCV} values in soil samples 50% >1.0 36% >2.0 21% >3.0 Belowground biomass = 78%

Monitoring of the created marsh two years after construction showed that stem density was at 108% and stem height was at 95% of natural unoiled marshes. Other published studies of created marshes have found that *S. alterniflora* aboveground biomass develops quickly, being comparable with natural marshes within 3-5 years. Thus, the Washington Creek created marsh site is similar to other east coast restoration projects. The belowground biomass development, however, appears to be slower than other marsh creation sites. When comparing the mean belowground biomass, the restored marsh had an average that was only 39% of natural unoiled marshes at 0-10 cm and only 7% of natural unoiled marshes at 10-20 cm. The slower and more variable development of belowground biomass at the Washington Creek marsh creation site is likely, in part, related to the very dense, inorganic, and oxidized sandy clay soils remaining after scrap-down to the appropriate elevation. It may be appropriate to monitor soil physiochemical characteristics in future monitoring studies at the created marsh.

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Monitoring of Recovery of Marshes Impacted by the Chalk Point Oil Spill

Introduction

On 7 April 2000, an estimated 140,000 gallons of a mixture of No. 6 and No. 2 fuel oils were released into Swanson Creek, the Patuxent River, and downstream tributaries from a pipeline rupture going into nearby Chalk Point Power Generating Station, the largest in Maryland. The Federal and State trustees conducted studies of the impact of the oil on marshes as part of the Natural Resource Damage Assessment (NRDA). Based on these studies, an estimated 76 acres of marsh surrounding Swanson Creek were affected. In 2002 an agreement was signed to create 5-6 acres of marsh in Washington Creek to compensate for the damaged marshes. This marsh creation was completed in 2005 using part of what had been a soybean field.

As part of the NRDA for injuries to wetland resources, the trustees used two metrics to represent the lost services and functions of the wetlands as a result of the oil spill: aboveground vegetation and soils. Aboveground vegetation represents a broad range of services related to primary production, habitat structure, recreational and aesthetic value, food chain support, and fish and shellfish production. It was an appropriate metric because it can be readily used for both injury quantification and restoration scaling. Use of a soil-related metric was particularly important for this spill site because of the extensive oil penetration into the soils in areas characterized as heavily oiled and the importance of soil development and biogeochemical cycling to the overall ecological services of wetlands.

Table 1 shows the estimated impacts to ecological service flows for wetland vegetation and soils, for seven of the injury classes based on species, degree of oiling, and habitat (shoreline fringe or interior). The oiling degree (e.g., light, moderate, and heavy) were based on field measurements of the width and height of the oiled band and the percent cover of the oil in the band. The recovery trajectories were based on field studies conducted in 2000-2002 (Michel et al., 2002; NOAA et al., 2002) that showed significant and persistent oil in the marsh soils and variable rates of recovery of the stem height and density for different habitats. The percent (%) services were estimated for three periods: 1) immediately post spill; 2) a first phase of recovery that ended 0.5-1 year after the spill; and 3) a second phase of recovery that ended 5-20 years post spill. For example, all lightly oiled wetland habitats were estimated to have 90% services present (10% loss) immediately after the spill and full recovery to 100% services within 0.5 years, for both vegetation and soils. For heavily oiled *Spartina alterniflora* (smooth cordgrass) in interior habitats, vegetation services were estimated to be 0% immediately after the spill and increase to 50% after one year and return to 100% after five years. However, soil services were estimated to be 25% immediately after the spill, reach 75% by year five, and return to 100% by year ten. *S. cynosuroides* (giant cordgrass) interior habitats were estimated to have the slowest recovery rates (up to 20 years for soil service flows) because of the extensive and deep penetration of oil in the marsh soils.

TABLE 1. Estimated impacts to ecological service flows and recovery rates for seven of the wetland categories oiled as a result of the Chalk Point oil spill that were used in injury quantification.

	VEGETATION			SOILS		
Category	**Services Post Spill (% of Pre-Spill)**	**Recovery Phase 1 %/years**	**Recovery Phase 2 %/years**	**Services Post Spill (% of Pre-Spill)**	**Recovery Phase 1 %/year**	**Recovery Phase 2 %/year**
All Lightly Oiled Shoreline Habitats	90	100/0.5		90	100/0.5	
Typha spp. Heavily Oiled Shoreline	0	100/1		25	60/3	100/10
Typha spp. Heavily Oiled Interior	0	100/1		50	80/5	100/10
S. alterniflora Heavily Oiled Shoreline	0	50/1	100/5	25	80/3	100/5
S. alterniflora Heavily Oiled Interior	0	50/1	100/5	25	75/5	100/10
S. cynosuroides Heavily Oiled Shoreline	0	50/1	100/10	25	60/3	100/10
S. cynosuroides Heavily Oiled Interior	0	50/1	100/10	25	50/5	100/20

Because of the predicted long-term persistence of oil-related impacts to wetland functions and services, the National Oceanic and Atmospheric Administration (NOAA) decided to fund a study of the oiled wetlands in 2007, seven years after the initial spill. The study plan consisted of field and laboratory studies to assess the recovery status of the impacted wetlands, as indicated by three metrics: 1) the persistence and weathering status of polynuclear aromatic hydrocarbons (PAH) in marsh soils; 2) vegetation condition measurements of belowground biomass, stem density, and stem height; and 3) toxicity tests of surficial soils. The objective was to compare these metrics at sites within the heavily oiled interior marsh habitats affected by the spill with similar but unoiled marsh habitats. Studies of the created marsh site were limited to vegetation condition, which will be an important step in documenting the rate of development of the site two years after creation. The study was designed to answer the following questions:

- What is the condition of the vegetation in the heavily oiled marsh seven years post- spill compared to unoiled marshes?
- What is the degree of weathering of oil in the marsh soils?
- What are the sources of the PAHs in the marsh soils
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Summary of Cleanup Methods in Wetlands during the Spill

The Chalk Point oil spill resulted from a leak in a 30.5-centimeter (cm) underground pipeline that supplies oil to the Pepco Chalk Point (now operated by Mirant Chalk Point, LLC) Generating Facility in Aquasco, Maryland (Fig. 1). Approximately 140,000 gallons of fuel oil spilled from the ruptured pipeline in the marsh interior and spread into Swanson Creek, a small tributary of the Patuxent River (Fig. 1). The spilled oil was a mix of No. 6 fuel oil, the product normally transported by the pipeline to generate electricity, and No. 2 fuel oil, a lighter oil that was being used to backflush the pipeline as part of a cleaning procedure.

The marshes of Swanson Creek were heavily oiled, as shown in Figure 2. The marsh adjacent to the leak site contained much of the oil on the first day of the spill. High winds on the second day of the spill pushed the oil deep into the interior of the marshes at the head of Swanson Creek, where thick oil pooled on the marsh surface and in open areas created by muskrats (locally called "eat outs"). Figure 3 shows aerial photographs of the oil in the marshes in the first week after the oil spill.

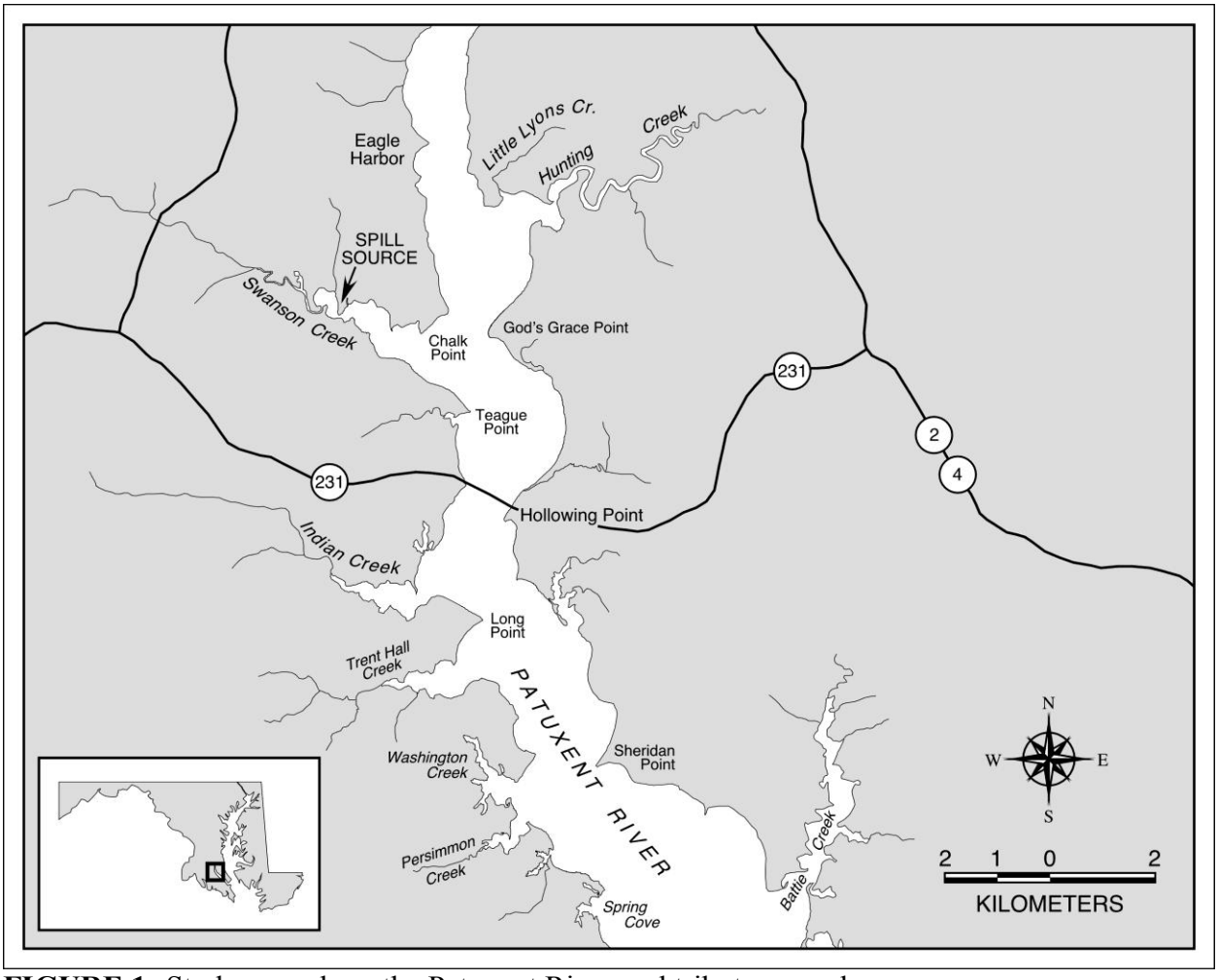


FIGURE 1. Study area along the Patuxent River and tributary creeks.

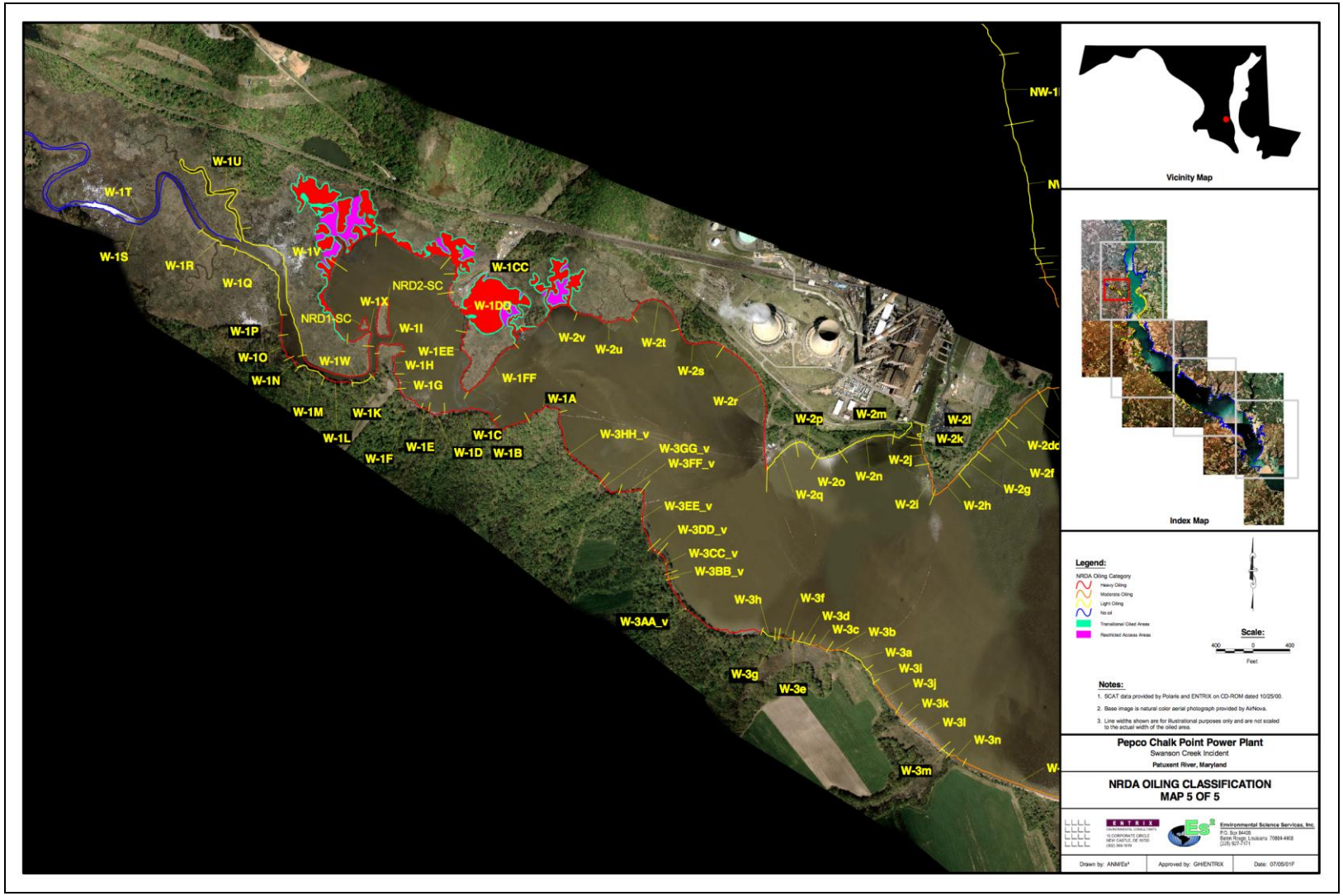


FIGURE 2. Oiling map of Swanson Creek. Lines along the shoreline are used to indicate areas where the oil occurred mostly as a narrow band along the marsh fringe. The red polygons indicate areas of heavy interior oiling. The pink polygons indicate unoiled areas that were surrounded by oiled areas. The areas of interior heavy oiling were the focus of this study.

FIGURE 3. Aerial photographs taken within the first week of the oil spill showing the extensive interior oiling in the marshes at the head of Swanson Creek. Because of the presence of open areas in the marsh (note circular muskrat huts and "eat outs" around the huts), oil pooled on the marsh surface deep inside the marsh as well as coated the vegetation along the marsh adjacent to the tidal creeks.

FIGURE 4. A. Cleanup methods used in the marsh adjacent to the leak site, located to the right of and adjacent to the access road. Note the extensive use of trenching.
B. Flushing on the ground. The trenches were eventually filled and planted.

As a result of the heavy oiling in the marsh area immediately adjacent to and east of the leak site (labeled W1DD on Fig. 2), very intrusive methods were used during the cleanup, including digging of trenches, low- to moderate-pressure and high-volume flushing, extensive manual removal using sorbents, filling of the trenches, and replanting (Gundlach et al., 2003). Figure 4 shows the extent of disruption in this part of the marsh during the cleanup. Figure 4A is an aerial photograph showing the trenches constructed to direct the oil to recovery points. Figure 4B shows the cleanup on the ground in the same area as the aerial photograph. Because of the complexities of the cleanup in this area and the limited funds available, it was not included in this study.

Cleanup methods in other heavily oiled marsh areas at the head of Swanson Creek consisted mostly of manual removal of pooled oil using sorbents, particularly in the two areas immediately to the northeast and northwest of the release site. Figure 5 shows the heavy fringing and interior oiling in the marshes east of the release site, where less intrusive cleanup methods were used. Boardwalk pathways were used to provide worker access for recovery of the pooled oil using sorbents. Nutrients were applied manually and by helicopter several times in the summer of 2000 in the interior areas of zone W-2v east of the break site as part of a biostimulation program. Gundlach et al. (2003) reported that no cleanup was attempted in the interior marshes further to the west (red area in zone W-1V on Fig. 2). Access via small boat was very limited because of the very shallow water levels, even at high tide, and this area was very difficult to access from land.

FIGURE 5. A. Aerial view of the cleanup of the heavy oil in the marshes in Swanson Creek, just to the east of the pipeline break site. Note the extensive use of boardwalks to access the oiled areas. **B.** Workers on boardwalks using sorbents to recover the heavy oil on the marsh surface, to minimize the potential for further damage from trampling.

Study Methods

Study Design

The study design consisted of an oiled versus unoiled comparison of the following variables: vegetation health as indicated by stem density, stem height, and total (live and dead) belowground biomass; oil fate and effects as indicated by polynuclear aromatic hydrocarbon (PAH) concentration and characterization in soils; and toxicity as indicated by sediment bioassay tests. The original study plan was to collect samples at six sampling sites for each combination of fringing and interior, *S. alterniflora* and *S. cynosuroides* marsh types, oiled and unoiled, for a total of 48 sites. A statistical analysis of the power of proposed study variables was conducted (Appendix A). Based on this analysis, the study plan was revised to consist of twelve sampling sites within two habitat combinations each for oiled and unoiled: interior areas for both *S. alterniflora* and *S. cynosuroides* marsh types. The interior parts of the marsh had the longest estimated recovery period (Table 1), and there have been few studies of spill sites with extensive interior oiling. It proved difficult to find homogeneous areas of *S. alterniflora* in the oiled areas in this brackish-water marsh, thus the final distribution of sites consisted of:

S. alterniflora:	12 unoiled sites (site name AR for *S. alterniflora* and reference)
	10 oiled sites (site name AH for *S. alterniflora* and heavy oiling)
	6 sites in the created marsh (site name AW for *S. alterniflora* and Washington Creek)
S. cynosuroides:	12 unoiled sites (site name CR for *S. cynosuroides* and reference)
	14 oiled sites (site name CH for *S. cynosuroides* and heavy oiling)

Stem density and stem height were selected as surrogate measures for aboveground biomass. These variables are easy to measure in the field. Belowground biomass was considered to be one of the key parameters of interest in assessment of vegetation health. Dead belowground biomass contains materials produced over many years, thus would be a good indicator of the impacts of chronic exposures in the seven years since the spill.

PAHs are the components in oil that pose the greatest toxicity risk to biota. The study design focused on the PAH characterization in terms of the degree of weathering of the oil residues, as well as the total PAH concentrations. Sediment samples were collected from all oiled sites and two of the unoiled sites in (AR-5 in Hunting Creek and CR-11 in Trent Hall Creek) for both PAHs and sediment toxicity tests.

To provide a link between the persistence of the oil in the marsh soils (and particularly the PAHs) and toxicity to sediment-dwelling organisms, whole sediment 10-day bioassays were conducted using the infaunal amphipod *Ampelisca abdita*. Although the estuarine amphipod *Leptocheirus plumulosus* is an ecologically important infaunal inhabitant of Chesapeake Bay sediments and commonly used for sediment toxicity testing in the region, it is not as sensitive to PAHs as *A. abdita* (MacDonald, pers. comm., 2007). *A. abdita* is the primary species used in two national sediment monitoring programs (NOAA's National Status & Trends and U.S. Environmental Protection Agency's Environmental Monitoring and Assessment Program, or EMAP). Thus, it was selected for this study.

Site Selection

Color infrared Digital Orthophoto Quarter Quads (USDA, 2005) with ground sample distances of 1 meter (m) were obtained for the study areas. This imagery was classified in ArcGIS 9.2 using an unsupervised parametric spectral classification scheme into 20 classes with default parameters to derive a modified marsh classification. These classes were lumped until a total of five classes remained: three different emergent marsh vegetation classes, one class representing all upland land cover types, and open water. The three emergent marsh vegetation types were dominated by the species *S. alterniflora, S. cynosuroides,* and *Typha* spp. This lumping was accomplished by visual interpretation for the upland and open-water classes, and the use of emergent vegetation community classifications at a series of existing ground control points acquired immediately after the spill (Michel et al., 2002). The five-class, modified marsh type raster was filtered using a 3x3 cell moving window focal majority operation to remove isolated misclassified pixels (ESRI, 2008). The filtered five-class raster data were then converted to a set of vector polygons representing the different marsh types, upland, and open-water areas.

Vector polygons representing oiled marsh areas were derived from aerial photo interpretation of imagery acquired immediately after the spill. Nine random site locations were generated in oiled and unoiled interior areas for both *S. alterniflora* and *S. cynosuroides* marsh types. These nine new sites, together with the three sites in each marsh type reoccupied from previous investigations, constituted the sampling plan.

Global positioning system (GPS) waypoints were generated for each random site location and used to navigate to the point. Some of the pre-selected sites (particularly the unoiled reference sites) proved to be very difficult to access because they were located more than several hundred meters from channels used for access. Furthermore, these deep interior sites were less comparable with the oiled sites, which tended to be closer to marsh channels. In these cases, sites were selected in the general pre-selected areas but not as deep into the marsh. At some sites, the dominant marsh type was different than the type indicated by the imagery classification. If the vegetation was dominated by either *S. alterniflora* or *S. cynosuroides*, the site was used for that marsh type. If the site had a mixed species assemblage, the site was moved to the nearest point with a homogenous plant community dominated by the species of interest.

The locations of the sampling sites are shown in eastern and western Swanson Creek in Figures 6 and 7 (all oiled sites); unoiled sites in Trent Hall Creek (Fig. 8), Hunting Creek (Fig. 9), and Little Lyons Creek (Fig. 10); and the created marsh in Washington Creek (Fig. 11). For Swanson Creek, the April 2000 vertical aerial photograph taken shortly after the spill is used as the base image, indicating the location of the sites relative to the oil visible on the photograph. Parts of Trent Hall Creek were lightly oiled along the marsh edge during the 2000 oil spill; however, there was no interior oiling. Table 2 lists the sites by name, sampling date, type, location, and coordinates. Sites established in 2000 as part of the NRDA studies have sites with numbers 1-3. Where the marsh species changed between 2000 and 2007, the site name was modified to reflect this change (e.g., ACH-1 was originally CH-1, a *S. cynosuroides* site, in 2000, but it was dominated by *S. alterniflora* in 2007, so it was named ACH-1). This same approach was used for the sites established in 2007, that is, site AH-10 became CAH-10 when it was determined in the field that the dominant species was *S. cynosuroides.*

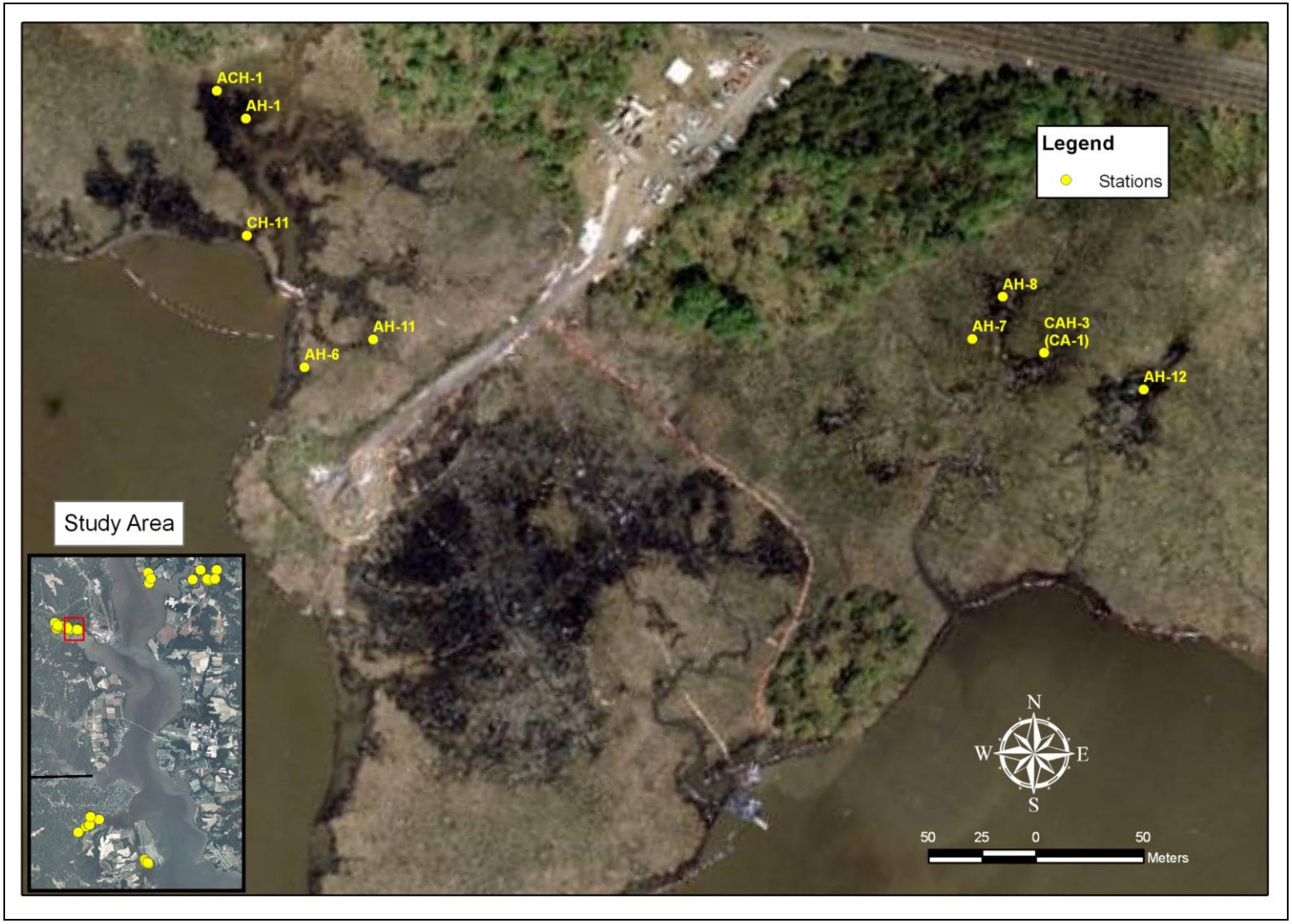


FIGURE 6. Eastern Swanson Creek showing the location of the oiled stations overlain on the April 2000 aerial photograph acquired shortly after the spill. No sites were established in the central area SE of the road because of the extensive response actions including trenching, backfilling, and planting.

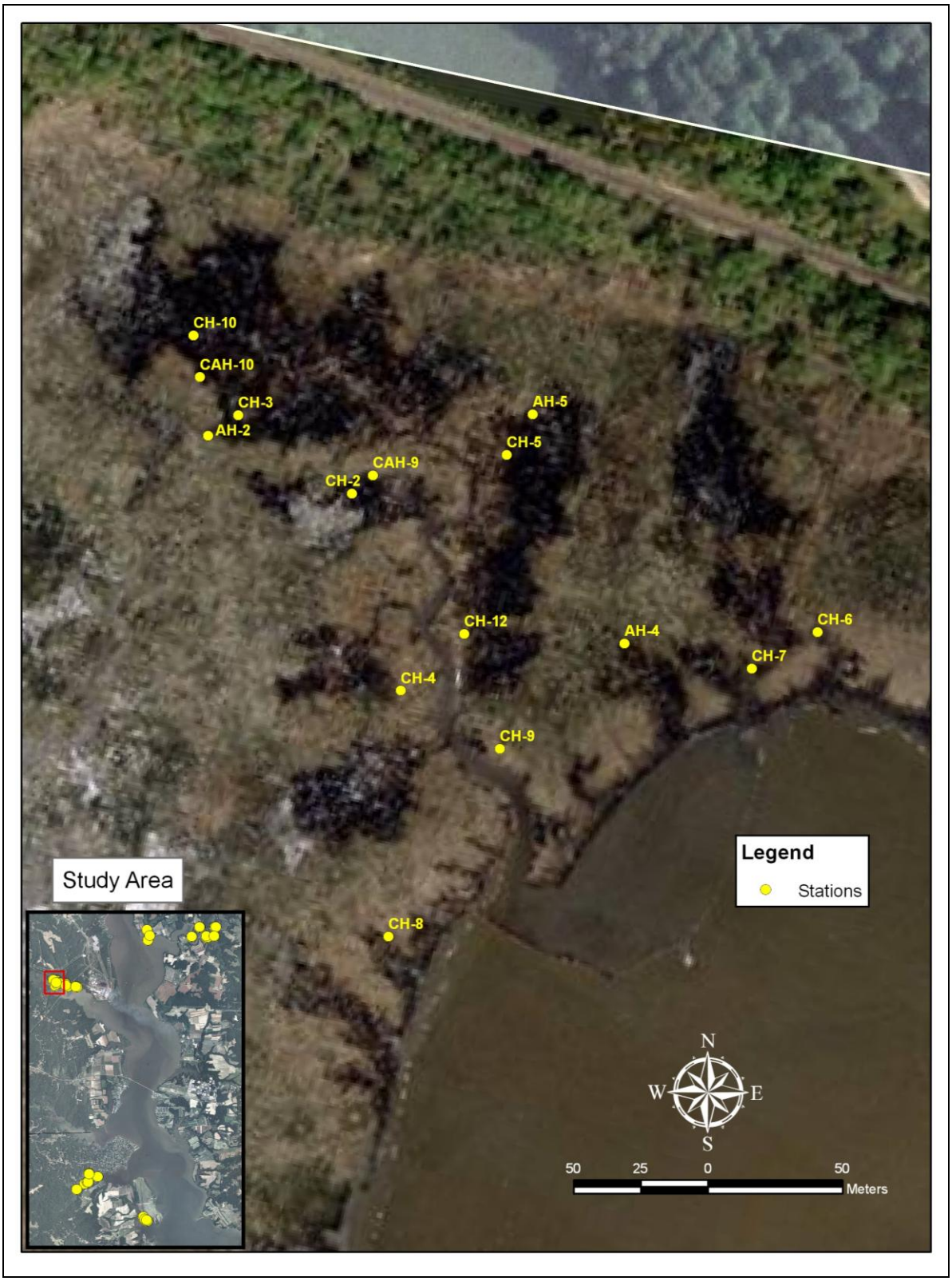


FIGURE 7. Western Swanson Creek showing the location of the oiled stations overlain on the April 2000 aerial photograph acquired shortly after the spill.

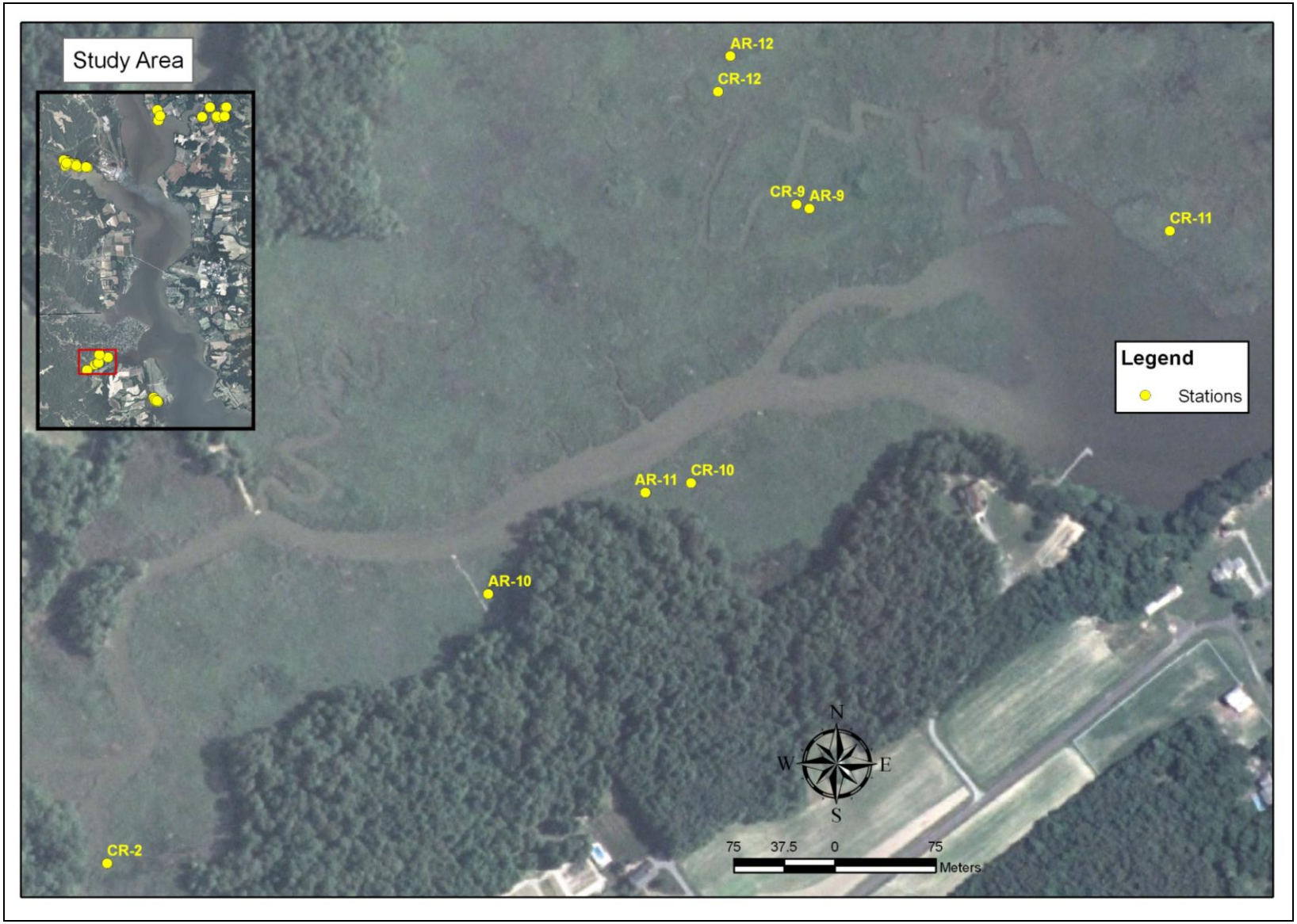


FIGURE 8. Trent Hall Creek showing the location of the unoiled sites overlain on a June 2005 aerial photograph. The lower part of Trent Hall Creek was very lightly oiled along the outer marsh fringe during the 2000 spill.

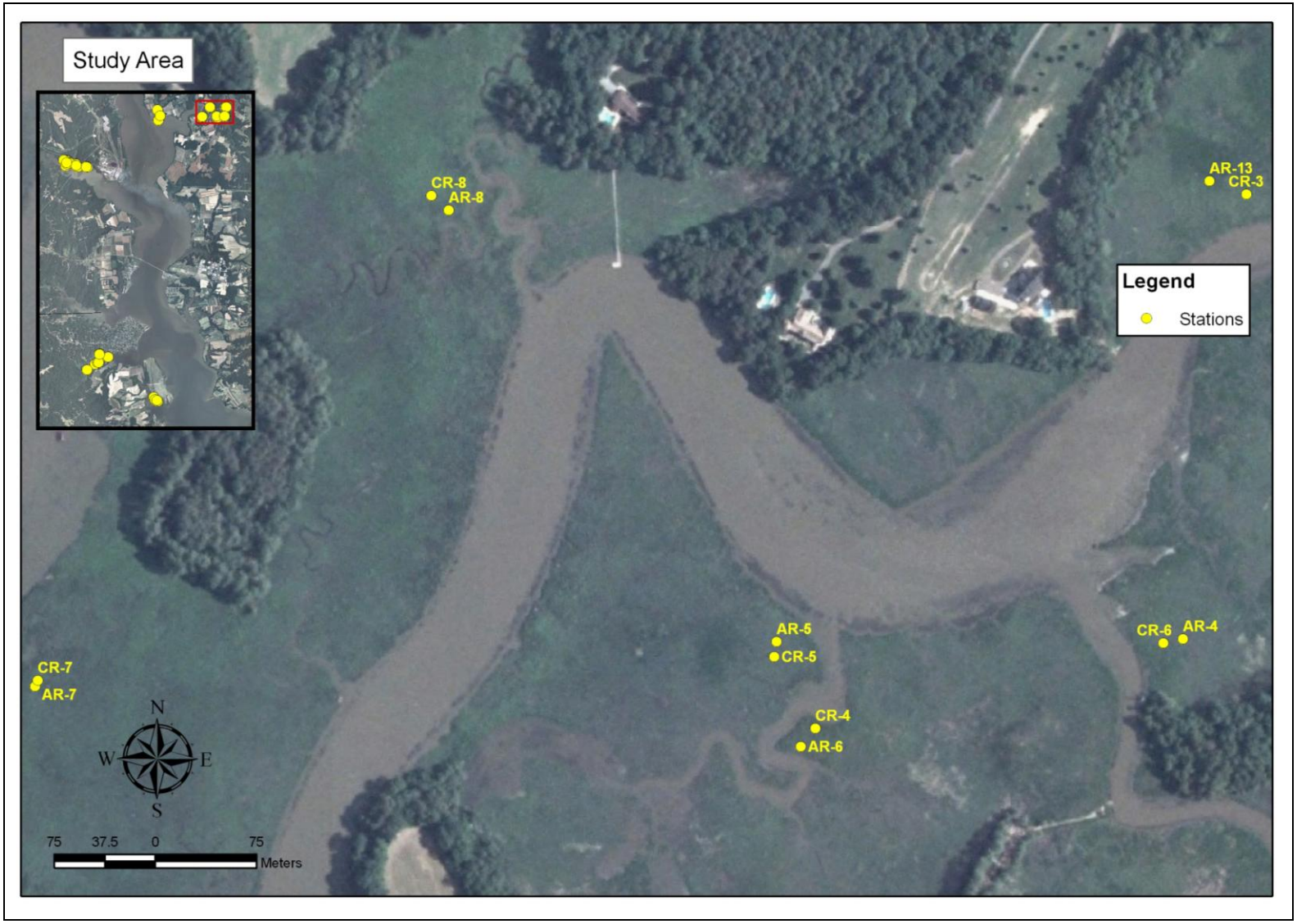


FIGURE 9. Hunting Creek showing the locations of unoiled stations overlain on a June 2005 aerial photograph. All of these sites were established in 2007.

FIGURE 10. Little Lyons Creek showing the location of unoiled stations overlain on a June 2005 aerial photograph. These sites were established in 2000 as unoiled reference sites for the NRDA studies.

FIGURE 11. Washington Creek showing the location of the stations in the created marsh, overlain on an oblique aerial photograph taken in October 2006 that was partially registered to the underlying 2005 vertical aerial photograph (thus the GPS locations are not perfectly registered to the photograph). All sites were located about 2 m inland from the marsh edge at the time of sampling.

TABLE 2. List of sampling sites.

Site ID	Date	Type	Location	Latitude	Longitude
ACH-1	8/29/07	*S. alterniflora* oiled	Swanson Creek	38.54708891	-76.70159815
AH-1	8/29/07	*S. alterniflora* oiled	Swanson Creek	38.54697199	-76.70144401
AH-2	8/30/07	*S. alterniflora* oiled	Swanson Creek	38.54802802	-76.70669401
AH-4	9/1/07	*S. alterniflora* oiled	Swanson Creek	38.5473283	-76.70492426
AH-5	9/1/07	*S. alterniflora* oiled	Swanson Creek	38.54809726	-76.70531217
AH-6	8/29/07	*S. alterniflora* oiled	Swanson Creek	38.54593053	-76.70113463
AH-7	8/29/07	*S. alterniflora* oiled	Swanson Creek	38.54603916	-76.6975693
AH-8	8/29/07	*S. alterniflora* oiled	Swanson Creek	38.54621468	-76.6974046
AH-11	8/30/07	*S. alterniflora* oiled	Swanson Creek	38.54604444	-76.70076784
AH-12	8/29/07	*S. alterniflora* oiled	Swanson Creek	38.54582543	-76.69665392
AR-R1	8/31/07	*S. alterniflora* unoiled	Little Lyons Creek	38.56157997	-76.66643196
AR-R3	8/31/07	*S. alterniflora* unoiled	Little Lyons Creek	38.5650598	-76.6667436
AR-4	8/31/07	*S. alterniflora* unoiled	Hunting Creek	38.56286709	-76.63794564
AR-5	8/31/07	*S. alterniflora* unoiled	Hunting Creek	38.56285812	-76.64140359
AR-6	8/31/07	*S. alterniflora* unoiled	Hunting Creek	38.56215547	-76.64119975
AR-7	9/3/07	*S. alterniflora* unoiled	Hunting Creek	38.56257934	-76.64771374
AR-8	9/3/07	*S. alterniflora* unoiled	Hunting Creek	38.56575365	-76.6441775
AR-9	9/2/07	*S. alterniflora* unoiled	Trent Hall Creek	38.48226411	-76.69114122
AR-10	9/2/07	*S. alterniflora* unoiled	Trent Hall Creek	38.47969808	-76.69388068
AR-11	9/2/07	*S. alterniflora* unoiled	Trent Hall Creek	38.48037173	-76.69254293
AR-12	9/2/07	*S. alterniflora* unoiled	Trent Hall Creek	38.48328444	-76.69180985
AR-13	9/3/07	*S. alterniflora* created	Hunting Creek	38.565929	-76.63770399
AW-1	9/4/07	*S. alterniflora* created	Washington Creek	38.46731918	-76.66703051
AW-2	9/4/07	*S. alterniflora* created	Washington Creek	38.46756376	-76.66790918
AW-3	9/4/07	*S. alterniflora* created	Washington Creek	38.46808822	-76.66864998
AW-4	9/4/07	*S. alterniflora* created	Washington Creek	38.46874117	-76.66881384
AW-5	9/4/07	*S. alterniflora* created	Washington Creek	38.46793617	-76.66818009
AW-6	9/4/07	*S. alterniflora* created	Washington Creek	38.46754918	-76.66735631
CAH-10	8/30/07	*S. cynosuroides oiled*	Swanson Creek	38.54822475	-76.7067293
CAH-3	8/29/07	*S. cynosuroides oiled*	Swanson Creek	38.54597957	-76.69718466
CAH-9	8/30/07	*S. cynosuroides oiled*	Swanson Creek	38.54789341	-76.70599362
CH-2	8/30/07	*S. cynosuroides oiled*	Swanson Creek	38.54783298	-76.70608297
CH-3	8/30/07	*S. cynosuroides oiled*	Swanson Creek	38.54809642	-76.7065661
CH-4	8/30/07	*S. cynosuroides oiled*	Swanson Creek	38.54717265	-76.70587719
CH-5	9/1/07	*S. cynosuroides oiled*	Swanson Creek	38.54796097	-76.70542306
CH-6	9/1/07	*S. cynosuroides oiled*	Swanson Creek	38.54736468	-76.704103
CH-7	9/1/07	*S. cynosuroides oiled*	Swanson Creek	38.54724281	-76.70438396
CH-8	9/1/07	*S. cynosuroides oiled*	Swanson Creek	38.54634988	-76.70593377
CH-9	9/1/07	*S. cynosuroides oiled*	Swanson Creek	38.54697769	-76.70545709
CH-10	8/30/07	*S. cynosuroides oiled*	Swanson Creek	38.54836388	-76.7067557
CH-11	8/29/07	*S. cynosuroides oiled*	Swanson Creek	38.54648131	-76.70144065
CH-12	9/1/07	*S. cynosuroides oiled*	Swanson Creek	38.54736141	-76.70560637
CR-R1	8/31/07	*S. cynosuroides unoiled*	Little Lyons Creek	38.56296214	-76.66555027
CR-2	9/2/07	*S. cynosuroides unoiled*	Trent Hall Creek	38.47790695	-76.69712774
CR-3	9/3/07	*S. cynosuroides unoiled*	Hunting Creek	38.56583864	-76.63738799
CR-4	8/31/07	*S. cynosuroides unoiled*	Hunting Creek	38.56227851	-76.64107318

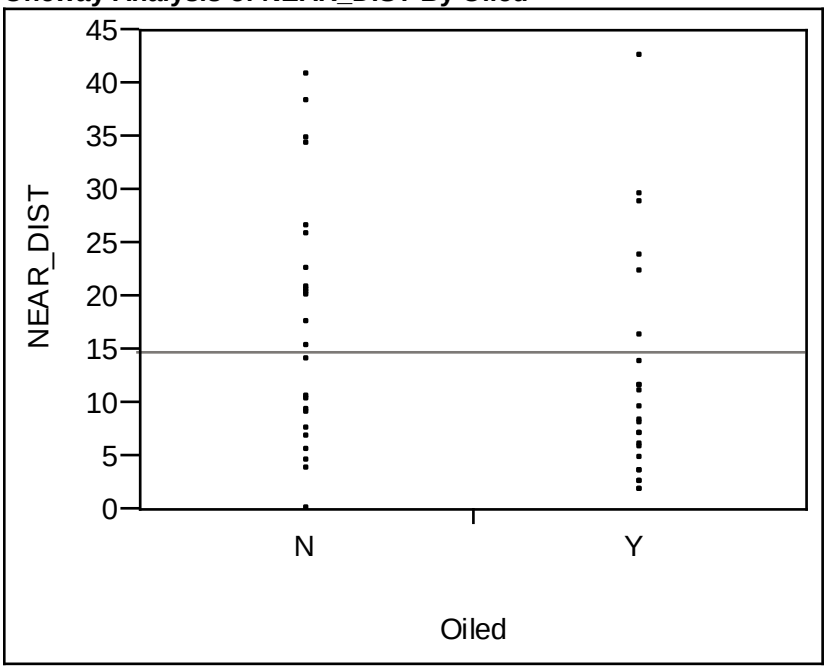
TABLE 2. Cont.

Site ID	Date	Type	Location	Latitude	Longitude
CR-5	8/31/07	*S. cynosuroides* unoiled	Hunting Creek	38.56275712	-76.64142078
CR-6	8/31/07	*S. cynosuroides* unoiled	Hunting Creek	38.5628391	-76.63811009
CR-7	9/3/07	*S. cynosuroides* unoiled	Hunting Creek	38.5626184	-76.6476886
CR-8	9/3/07	*S. cynosuroides* unoiled	Hunting Creek	38.5658513	-76.64432335
CR-9	9/2/07	*S. cynosuroides* unoiled	Trent Hall Creek	38.48229311	-76.69125002
CR-10	9/2/07	*S. cynosuroides* unoiled	Trent Hall Creek	38.48043434	-76.69215728
CR-11	9/2/07	*S. cynosuroides* unoiled	Trent Hall Creek	38.48210771	-76.68808032
CR-12	9/2/07	*S. cynosuroides* unoiled	Trent Hall Creek	38.48304749	-76.69191722

There are many factors that affect marsh vegetation, including salinity, topography and elevation, sediment accumulation rate, marsh subsidence, and distance to tidal channels. The spring tidal range in the study area is 0.55 m. The monthly mean salinity at Long Point, on the north side of Trent Hall Creek, ranged from 7.04 ‰ in May to 11.6 ‰ in October. This part of the Patuxent River has large temporal and spatial variations in salinity; Anderson et al. (1968) reported 24-hour variations in salinity at Benedict (near Swanson Creek) of 2-3.7 ‰.

No studies were identified on which to compare topography, elevation, sedimentation, or subsidence factors. However, we did perform analyses to determine if the reference sites were actually appropriate for comparing to the oiled sites in terms of their distance to tidal channels. All tidal channels with a diameter greater than 3 m were digitized from the most recent aerial photography. A program was written to determine the distance to the nearest tidal channel for each site. The average distance from the water for the sites was 17.9 m for the unoiled sites and 12.1 m for the oiled sites. The distribution of the distances was non-normal for both the oiled and unoiled sites. A log transformation of the distance provided a normal distribution for the oiled sites, but not in the unoiled sites. Therefore, a nonparametric test (Wilcoxon) was used to determine if there was a significant difference in the distance from the tidal channel to the sample sites between the oiled and unoiled sites. Figure 12 shows the results from the Wilcoxon Oneway Analysis, indicating that the sites were not significantly different with a Z value of 0.0650.

Oneway Analysis of NEAR_DIST By Oiled



Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Score Mean	(Mean-Mean0)/Std0
N	24	678	28.2500	1.845
Y	24	498	20.7500	-1.845

2-Sample Test, Normal Approximation

S	Z	Prob>|Z|
498	-1.84546	0.0650

1-way Test, ChiSquare Approximation

ChiSquare	DF	Prob>ChiSq
3.4439	1	0.0635

FIGURE 12. Results of test to determine if the distance to the nearest tidal channel differed for oiled versus unoiled sites, indicating that they were not significantly different.

Field Methods

Vegetation Sampling Methods

At each study site, a 1-m^2 quadrat was placed horizontally over the marsh surface, parallel to the adjacent shoreline. A plastic pole was placed in the upper left corner of the quadrat to anchor it in place during sampling. A pre-printed site identification sheet was placed adjacent to the quadrat and photographs of the quadrat and adjacent habitat were taken.

In-field vegetation measurements consisted of stem height and stem density. Individual plants were segregated according to the location of the main stem as being inside or outside of the quadrat. One researcher used a 1.5-m stick scaled in 2-cm intervals to measure the five tallest individuals of the target species within the quadrat; these data were used to calculate the mean stem height and standard deviation. Stem densities were obtained by counting the number of stems of live plants of each species within the quadrat. Once stem heights and stem densities were recorded, aboveground vegetation was removed from an approximately 20-cm diameter patch in each corner of the quadrat in preparation for substrate sampling.

Substrate Coring Methods

Studies on belowground biomass are scarce because of the difficulty in sampling and high variability due to small core diameters (Good et al., 1982; Gross et al., 1991). Gross et al. (1991) conducted a rigorous study of a short *S. alterniflora* marsh in Lewes, DE to assess the effect of coring tube size on the variability of the belowground biomass estimate. Using a randomized design, they took cores from subplots with coring tubes of different diameters: 3.7 cm, 10.2 cm, 16.5 cm, and 21.5 cm. They found that biomass estimates from the smallest cores had the largest coefficient of variation. Dead belowground biomass estimates approached an asymptote with low variability at 10.2 cm, and live belowground biomass estimates approached an asymptote with low variability at 16.5 cm. Based on these results, a coring tube of 16 cm in diameter was used for the current study at the Chalk Point spill site. Gross et al. (1991) identified another source of variability resulting from heterogeneity in belowground biomass on a small spatial scale (e.g., "clump effect"), where stems occurred in clumps rather than being evenly spaced. To further reduce this source of variability, two cores were taken at each site and combined into one sample for analysis of total belowground biomass. Each core was divided into two intervals, 0-10 cm and 10-20 cm, based on data from Gallagher and Plumley (1979) and Gross et al. (1991) that showed that most of the belowground biomass in southeastern salt marshes occurred in the top 20 cm. Because of limited funding, only total belowground biomass (both live and dead) was measured.

Two manual coring devices were custom designed, based on recommendations made by researchers in the field. Figure 13 shows the coring device and a sample core from one of the unoiled sites. The tube of each coring device, fabricated with 16-gauge stainless steel, measured 40 cm in length and 16 cm in diameter. Weather stripping was affixed to the lid to provide an airtight seal. A circular wooden disk with a diameter slightly less than that of the inner coring tube was attached to a wooden rod to form a plunger, and used to carefully push the core out of the coring tube from the bottom, so not to disturb or contaminate the top of the core.

FIGURE 13. The 16-cm diameter coring tube and an extruded biomass core at station CR-7 ready for sectioning. The top of the core is to the right.

The coring devices were used to extract cores from which chemistry, toxicity, and belowground biomass samples were collected. Toxicity and chemistry sampling required thoroughly cleaned equipment to avoid cross contamination. Therefore, the two coring devices, a knife, and two spoons were cleaned with methylene chloride in the field immediately prior to collection of the cores. Toxicity and chemistry samples were always taken from the first two cores extracted from a site, which were extracted with cleaned coring devices. Cross contamination would not have affected biomass analyses; therefore, biomass samples were obtained from the last two cores from each site with only general cleaning of the coring device.

Shears were used to remove the aboveground vegetation over an approximately 20-cm diameter patch in each corner of the quadrat. A cleaned coring device was driven approximately 30 cm into the soil at one of the four de-vegetated corners. Generally the sharpened edge of the coring tube easily penetrated the marsh soil and roots, but often the coring process required an oscillating rotation of the handles in combination with downward pressure. No corrections were made for compaction as the dense root mat generally provided enough structure to prevent significant compaction. Cores were retaken only when compaction greater than 1-2 cm was noted.

Sample Collection Methods

Samples were placed in three different types of containers. Toxicity samples were placed into 3.8-liter (L), rectangular, plastic bins that were cleaned with Alconox detergent and rinsed with tap water. Chemistry samples were placed into 240-milliliter (mL) glass jars with Teflon-lined caps that were pre-cleaned by the manufacturer. Belowground biomass samples were placed into 1-gallon plastic Zip-Loc bags.

Toxicity samples were a composite of the 0-10 cm intervals from two cores (occasionally three cores were needed to generate the required 2 L of soil volume) at each of the oiled sites and two reference sites. The top 10 cm of the first core was extruded with the plunger, separated with a clean knife, and placed into a pre-cleaned plastic container. The core was then completely extruded and a chemistry sample of the 10-20 cm interval was collected with a clean spoon from the center of the core and placed in a clean, labeled glass jar. A second core was removed from the opposite corner with the second cleaned coring device. This second core was taken to a depth of approximately 20 cm and the top 10 cm were separated and placed in the plastic container. Vegetative material and animals were manually removed from the toxicity sample and the soil homogenized by a researcher wearing latex gloves. A chemistry sample for the 0-10 cm interval was then taken from the toxicity container with a clean spoon and placed in a pre-labeled glass jar. Therefore, the 0-10 cm sample for chemical analysis was a split of the well-homogenized sample for toxicity testing. The 10-20 cm sample for chemical analysis was a single sample from the second core.

Belowground biomass samples were extracted from the remaining corners. For each biomass sample the coring device was driven approximately 30 cm into the soil. The top 20 cm section of an extracted core was precisely measured and separated into 0-10 cm and 10–20 cm intervals. For each site two biomass samples were collected from both depth intervals and combined in labeled Zip-Loc plastic bags. All samples were placed on ice in a large cooler once the sampling of a site was completed.

At the end of each day, belowground biomass samples were processed in the field to remove soil from the root matter. Manual agitation and sieving with a 1.20-mm stainless steel mesh were used to clean the biomass samples in the field. Sieved samples were placed in a plastic bag and stored in a cooler until delivered to the laboratory.

Laboratory Analysis Methods

Belowground Biomass

Each biomass sample was transferred into paper bags for the drying process. Stable weight procedures were conducted by a laboratory technician at the Belle W. Baruch Institute for Marine and Coastal Sciences facility at the University of South Carolina (Columbia, SC). To achieve stable weight, biomass samples were placed in a 60°C oven for a minimum of 3 hours, cooled to room temperature and weighed. This process was repeated until the weight of a biomass sample was within 1 gram (g) of the previous weight. A Mettler PC 4400 Delta Range scale (0.50 g-1000.0 g range and calibrated daily) was used to obtain all of the biomass weights.

Chemical Analysis Methods

Soil samples for chemical analysis were kept cold until they were transferred (in three batches) under chain of custody to the Wye Research and Education Center, University of Maryland (Queenstown, MD**).** At the Wye Center, samples were frozen and held in the dark before being shipped over night on blue ice to B&B Laboratories (College Station, TX) for chemical analyses. All samples were shipped to B&B Laboratories on 4 September 2007. The sealed cooler was delivered to B&B Laboratories on 5 September 2007. There were no discrepancies reported by the laboratory when the samples were logged in. Soil samples were stored in an access-controlled freezer at –20°C until analysis.

PAHs were analyzed by gas chromatography/mass spectrometry in the selected ion monitoring mode (GC/MS SIM) using B&B Laboratories standard operating procedure B&B1006. The analytes included 51 PAHs, 5 individual alkyl isomers, and 3 hopanes/triterpane (listed in Table 3). Appendix B includes all of the PAH results. Quality assurance/quality control (QC) procedures established by B&B Laboratories included analyses of a method blank, laboratory duplicate, matrix spike/matrix spike duplicate, and a sediment standard reference material (SRM) per analytical batch of no more than 20 samples. A standard reference oil (NIST 1582) was also analyzed with the data set. Method blanks were used to determine that sample preparation and analyses were free of contaminants. The duplicate sample was used to determine the precision of the analysis. The matrix spike/matrix spike duplicate was used to measure accuracy and precision of the analysis. The SRM is a material for which a mean and confidence interval are certified for specific analytes. All SRMs are traceable to NIST. SRMs were used to verify analytical accuracy. All QC samples were subject to the identical preparation and analysis steps as samples.

The QC criterion for blanks specified that no more than two target analytes exceed 3X the method detection limits (MDL) listed in Table 3. The QC criteria for spike recoveries were between 40-120%. The QC criterion for the relative percent difference (RPD) for valid spiked duplicates was ±30%. The QC criterion for RPDs for valid duplicates is ±30%. The QC criterion for the sediment SRM was ±20% the laboratory derived mean. The QC criterion for the reference oil SRM was ±15% the laboratory derived mean. Surrogate solutions equivalent to 5-10X the method detection level were prepared for various hydrocarbon analyses. The appropriate surrogate solution was added to every sample including QC samples. The data were corrected based on surrogate recovery up to 100%. The QC criteria for surrogate recoveries were between 40-120%, except d12-perylene which must be greater than 10% and less than 120%. Because many of the samples contained high amounts of oil, extracts required dilution prior to instrumental analysis. Surrogates were re-added to the diluted sample prior to analysis.

The laboratory reported no variances in the procedural blanks and laboratory duplicates. In one matrix spike sample, the recovery of 2-methylnaphthalene exceeded the QC criteria of 40-120% because of the very high oil levels in the sample relative to the spike amounts. In the SRM with one QC batch (ENV1689), naphthalene was detected outside the specified QC recovery limits, for an unknown reason.

TABLE 3. List of PAH analytes and method detection limits (MDL).

PAH (abbreviation)	**MDL** mg/kg dry wt.	**PAH** (abbreviation)	**MDL** mg/kg dry wt.
Naphthalene (C0N)	0.17	C2-Fluoranthenes/Pyrenes (C2F/P)	0.39
C1-Naphthalenes (C1N)	0.33	C3-Fluoranthenes/Pyrenes (C3F/P)	0.39
C2-Naphthalenes (C2N)	0.35	Naphthobenzothiophene (C0NB)	0.20
C3-Naphthalenes (C3N)	0.35	C1-Naphthobenzothiophenes (C1NB)	0.41
C4-Naphthalenes (C4N)	0.35	C2-Naphthobenzothiophenes (C2NB)	0.41
Benzothiophene (BTP)	0.17	C3-Naphthobenzothiophenes (C3NB)	0.41
C1-Benzothiophenes (C1- BTP)	0.35	Benz(a)anthracene (BAA)	0.17
C2-Benzothiophenes (C2- BTP)	0.35	Chrysene (C0C)	0.35
C3-Benzothiophenes (C3- BTP))	0.35	C1-Chrysenes (C1C)	0.35
Biphenyl (BIP)	0.14	C2-Chrysenes (C2C)	0.35
Acenaphthylene (ACY)	0.19	C3-Chrysenes (C3C)	0.35
Acenaphthene (ACE)	0.13	C4-Chrysenes (C4C)	0.35
Dibenzofuran (DBF)	0.20	Benzo(b)fluoranthene (BBF)	0.29
Fluorene (C0F)	0.19	Benzo(k)fluoranthene (BKF)	0.23
C1-Fluorenes (C1F)	0.39	Benzo(e)pyrene (BEP)	0.31
C2-Fluorenes (C2F)	0.39	Benzo(a)pyrene (BAP)	0.22
C3-Fluorenes (C3F)	0.39	Perylene (PER)	1.38
Carbazole (CAR)	0.33	Indeno(1,2,3-c,d)pyrene (IND)	0.28
Anthracene (C0A)	0.19	Dibenzo(a,h)anthracene (DAH)	0.15
Phenanthrene (C0P)	0.14	Benzo(g,h,i)perylene (BGHI)	0.14
C1-Phenanthrenes/Anthracenes (C1P/A)	0.29		
C2-Phenanthrenes/Anthracenes (C2P/A)	0.29	**Individual Alkyl Isomers Hopanes**	
C3-Phenanthrenes/Anthracenes (C3P/A)	0.29	**and Hopanes/Triterpane**	
C4-Phenanthrenes/Anthracenes (C4P/A)	0.29	2-Methylnaphthalene	0.20
Dibenzothiophene (C0D)	0.31	1-Methylnaphthalene	0.13
C1-Dibenzothiophenes (C1D)	0.31	2,6-Dimethylnaphthalene	0.20
C2-Dibenzothiophenes (C2D)	0.31	1,6,7-Trimethylnaphthalene	0.10
C3-Dibenzothiophenes (C3D)	0.31	1-Methylphenanthrene	0.20
Fluoranthene (FL)	0.21	C29-Hopane	1.11
Pyrene (P)	0.19	18a-Oleanane	1.11
C1-Fluoranthenes/Pyrenes (C1F/P)	0.39	C30-Hopane	1.11

Toxicity Study Methods

Ten-day survival whole soil toxicity tests with the amphipod *Ampelisca abdita* were conducted on ten oiled soil samples (plus two reference samples and an in-house control sediment). Samples were selected by Research Planning, Inc. in consultation with the University of Maryland to cover the range of PAH concentrations among the samples. Tests were conducted via Test Method 100.4: *Ampelisca abdita, Eohaustorius estuarius, Leptocheirus plumulosus, or Rhepoxynius abronius* 10-d Survival Test for Sediments given in Section 11 of EPA's "*Methods for Assessing the Toxicity of Sediment-associated Contaminants with Estuarine and Marine Amphipods*" (USEPA, 1994). A summary of the test conditions for *A. abdita* is given in Table 4. The raw data for the toxicity tests from the University of Maryland are included in Appendix C.

Soil samples were press-sieved through a 1-mm mesh stainless steel screen prior to the initiation of the test as discussed in Section 8.3.1 of the USEPA test protocol (USEPA, 1994) to remove any indigenous organisms and debris not removed during collection in the field. The bioassays were initiated on October 5, 2007, which met the sample storage time of ≤8 weeks specified in Section 8.2.1 of the USEPA test protocol (USEPA, 1994) for non-ionic, nonvolatile organic compounds when held in the dark at 4 ºC.

The test endpoint for the toxicity tests was survival. The test acceptability criterion for the toxicity tests was a minimum mean control survival of 90%. Amphipods exposed to five soils from the *S. alterniflora* dominated marshes and the *S. alterniflora* reference soil were run as an experimental unit, while the amphipods exposed to seven soils from the *S. cynosuroides* dominated marshes and *S. cynosuroides* reference soil were run as an experimental unit.

The amphipods used for the initiation of the 10-day tests were 3-5 mm (no mature males or females). Organisms were purchased from Aquatic Research Organisms (Hampton, New Hampshire). Upon receipt of the organisms, amphipods were placed in in-house control sediment with 28 ‰ overlying sea water (Instant Ocean®) and fed *Isochrysis galbana* at a density of ~1 x 10^6 cells/mL twice daily prior to testing. The organisms exhibited swimming behavior upon placement in the holding containers and were opalescent pink in color. The amphipods were acclimated for three days prior to the start of the bioassays.

Static tests (no renewal of overlying water) were conducted in 1-L beakers containing 175 mL sediment and 800 mL overlying water. Twenty organisms were randomly placed in each of five replicate test chambers. The overlying water in the test chambers was well aerated Instant Ocean® at a salinity of 28 ‰. Tests were conducted in a water bath at 20 ± 2°C under a constant photoperiod (24-h L:0-h D) at 500-1,000 lux. The animals were not fed during testing.

Temperature, pH, salinity, and dissolved oxygen were measured in all treatments at the beginning and end of the test. At the end of the 10-d exposures, all replicate amphipod test beaker materials were sieved through a 500-µm sieve to collect surviving organisms. Since *A. abdita* are tube-builders, the sieves were "slapped" forcefully against the surface of the water to ensure that all of the amphipods and tubes were dislodged from the screen. All living animals were counted. Immobile organisms isolated before sieving or from sieved material were considered dead.

Percent survival data were arc sine squared root transformed prior to statistical analyses. Survival of the unoiled reference sediments were 98 and 99%, well within the test acceptability criterion of 90% or greater control survival. No differences ($p < 0.01$) were found in amphipod survival between *S. alterniflora* or *S. cynosuroides* dominated marsh reference sediments and in-house control sediment. The null hypothesis that the *S. alterniflora* group and *S. cynosuroides* group was equal was initially tested using Dunnett's test. Dunnett's test consists of an analysis of variance to determine the error term, which is then used in a multiple comparison test for comparing each of the treatment means with the control mean. The assumptions upon which the uses of Dunnett's test are contingent are that the observations within treatments are independent and normally distributed, with homogeneity of variance. Both groups failed to meet the homogeneity of variance assumption and thus were evaluated by the nonparametric Steel's

Many-One Rank Test. The statistical tests were performed using Toxstat (WEST and Gulley, 1994) at a minimum probability level of 0.05.

TABLE 4. Summary of test conditions and test acceptability criteria for the amphipod *Ampelisca abdita* 10-day survival test.

Test method 100.4	EPA/600/R-94/025 (USEPA, 1994)
Test type:	Whole sediment, static
Test duration:	10 days
Temperature:	20ºC (± 2°C)
Lighting:	Wide-spectrum fluorescent light; 500-1000 lux
Photoperiod:	24 h light; 0 h dark
Test chamber size:	1 L high-form lipless beaker
Test solution volume:	175 mL sediment; 800 mL overlying water (v/v)
Renewal of overlying water:	None
Size of test organism:	3 – 5 mm (no mature males or females)
No. juveniles per test chamber:	20
No. of replicate chambers per concentration:	5
No. organisms per test concentration:	100
Feeding regime:	None
Aeration:	~1 bubble/second via a pipette; ≥ 90% saturation should be maintained
Overlying water:	28 ‰ Instant Ocean®
Overlying water quality:	Temperature, pH, ammonia, salinity, and DO at the beginning and end of the test. Temperature and DO daily.
Pore water quality:	Salinity, ammonia, and pH at the beginning of the test
Test concentrations:	100% sediment
Dilution series:	None
Endpoints:	Survival
Test Acceptability:	Minimum mean control survival of 90%

Results

Marsh Soil Chemistry

Total PAH Concentrations

Oil was not observed on vegetation or on the undisturbed marsh surface. However, oil sheens and black oil droplets were frequently released from the soils during extraction of the cores. Also, oil sheens were released from the shallow bay bottom at the head of Swanson Creek and in some of the creek bottoms when the sediments were disturbed.

Total PAH concentrations in the marsh soils at each site are shown in Table 5, along with the field observations on oiling. These observations include a description of the amount of oil inside the Zip-Loc bags used to store the biomass samples. The total PAH concentrations varied widely, with depth, within the site, and among sites. We often noted in the field that the amount of visible oil would be quite different among the 4-5 cores collected at a site. Because we followed a standard protocol in the sampling order (e.g., toxicity and chemical samples first, then the biomass samples), sometimes the sample for chemical analysis had no visible oil whereas the biomass sample was visibly oiled. Therefore, the degree of oiling at a site is not always indicated by the total PAHs measured in the soil sample.

During the field sampling, it was also noted that the oil distribution varied with depth, and that oil penetration with depth was not uniform; oil was seen along roots, rhizomes, and/or cavities. Figure 14 shows the extruded biomass core at site CH-8. Black oil was visible in root and other cavities throughout the core; note the black area about 10 cm from the top of the core. Free oil droplets accumulated on the water table after this core was extracted. Figures 15 and 16 show maps of the sampling sites with the total PAHs in the 0-10 cm and 10-20 cm core intervals. For most of the oiled cores, total PAHs varied by 1-2 orders of magnitude between the top and bottom intervals, reflecting high heterogeneity in the distribution of oil in marsh soils. However, there was a tendency for higher PAHs with depth; all of the six samples with over 500 milligrams per kilogram dry weight (mg/kg) total PAHs were from the 10-20 cm interval, equally from both species.

Total PAH concentrations were not normally distributed, as indicated by a median value of 7.19 mg/kg and a mean of 236 mg/kg, with a coefficient of variation of 238%. One approach to reduce the effect of the large differences in vertical distribution is to average the two intervals and create a virtual 0-20 cm core, which reduced the coefficient of variation from 238% to 162%. This approach was used in the toxicity assessment results.

TABLE 5. Total PAHs in marsh soils by site.

Station	Total PAHs (mg/kg)	Field Observations
ACH-1 (0-10 cm)	45.8	Silver and rainbow sheen on water table; light stain on both biomass bags
ACH-1 (10-20 cm)	1.90	
AH-1 (0-10 cm)	178.4	Black oil droplets on water table; biomass bag 0-10 cm moderate stain, 10-20 cm heavy stain
AH-1 (10-20 cm)	1,011	
AH-2 (0-10 cm)	43.9	Open area with very stunted *S. alterniflora*; large patch of *Scirpus* in upper right; both biomass bags no visible oil
AH-2 (10-20 cm)	1.8	
AH-4 (0-10 cm)	2.07	No visible oil; both biomass bags no visible oil
AH-4 (10-20 cm)	0.62	
AH-5 (0-10 cm)	156.4	Light rainbow sheen on water table; biomass bags no visible oil
AH-5 (10-20 cm)	5.33	
AH-6 (0-10 cm)	8.51	Black oil 7-20 cm in core; black oil droplets on water table; biomass bag 0-10 cm clean, 10-20 cm heavy stain
AH-6 (10-20 cm)	2,921	
AH-7 (0-10 cm)	1.62	Silver sheen on water table; biomass bags no visible oil
AH-7 (10-20 cm)	1.29	
AH-8 (0-10 cm)	151.1	Both biomass bags light stain
AH-8 (10-20 cm)	188.8	
AH-11 (0-10 cm)	5.87	No visible oil; both biomass bags no visible oil
AH-11 (10-20 cm)	2.1	
AH-12 (0-10 cm)	41.0	Black oil 10-20 cm in cavities; biomass bag 0-10 cm light stain, 10-20 cm heavy stain
AH-12 (10-20 cm)	2,084	
AR-5 (0-10 cm)	0.82	No visible oil; biomass bags no visible oil
CA-1 (0-10 cm)	5.52	No visible oil; biomass bags no visible oil
CA-1 (10-20 cm)	3.82	
CAH-10 (0-10 cm)	264.2	Both biomass bags light stain
CAH-10 (10-20 cm)	0.68	
CAH-9 (0-10 cm)	9.9	Both biomass bags light stain
CAH-9 (10-20 cm)	266.5	
CH-2 (0-10 cm)	4.72	Biomass bag 0-10 cm light stain, 10-20 cm heavy stain
CH-2 (10-20 cm)	255.6	
CH-3 (0-10 cm)	5.07	Moved site 3 m to north because *Scirpus* dominated former quadrat sites; both biomass bags no visible oil
CH-3 (10-20 cm)	1.98	
CH-4 (0-10 cm)	0.68	No visible oil; both biomass bags no visible oil
CH-4 (10-20 cm)	1.07	
CH-5 (0-10 cm)	10.85	Black oil in cavities 2-30 cm; biomass bag 0-10 cm moderate stain; 10-20 cm heavy stain
CH-5 (10-20 cm)	796.7	
CH-6 (0-10 cm)	2.18	Black oil in cavities 0-30 cm in biomass core; no visible oil in chemistry cores; biomass bag 0-10 cm v. light stain; 10-20 cm moderate stain
CH-6 (10-20 cm)	23.52	
CH-7 (0-10 cm)	3.36	No visible oil; biomass bags no visible oil
CH-7 (10-20 cm)	0.82	
CH-8 (0-10 cm)	91.86	Black oil 0-20 cm in first core; black oil in both biomass cores; biomass bag 0-10 cm moderate stain; 10-20 cm very heavy stain
CH-8 (10-20 cm)	729.4	
CH-9 (10-20 cm)	0.58	No visible oil in first 2 cores; biomass core rainbow sheen 10-20 cm; biomass bags no visible oil
CH-9 (10-20 cm)	0.91	
CH-10 (0-10 cm)	453.4	Biomass bag 0-10 cm moderate stain; 10-20 cm heavy stain
CH-10 (10-20 cm)	11.09	
CH-11 (0-10 cm)	139.8	Black oil droplets on water table; biomass bag 0-10 cm moderate stain; 10-20 cm heavy stain
CH-11 (10-20 cm)	1,384	
CH-12 (0-10 cm)	3.43	No visible oil but oily smell in first 2 cores; biomass core 0-10 cm rainbow sheen; both biomass bags no visible oil
CH-12 (10-20 cm)	0.64	
CR-11 (0-10 cm)	0.78	No visible oil; both biomass bags no visible oil

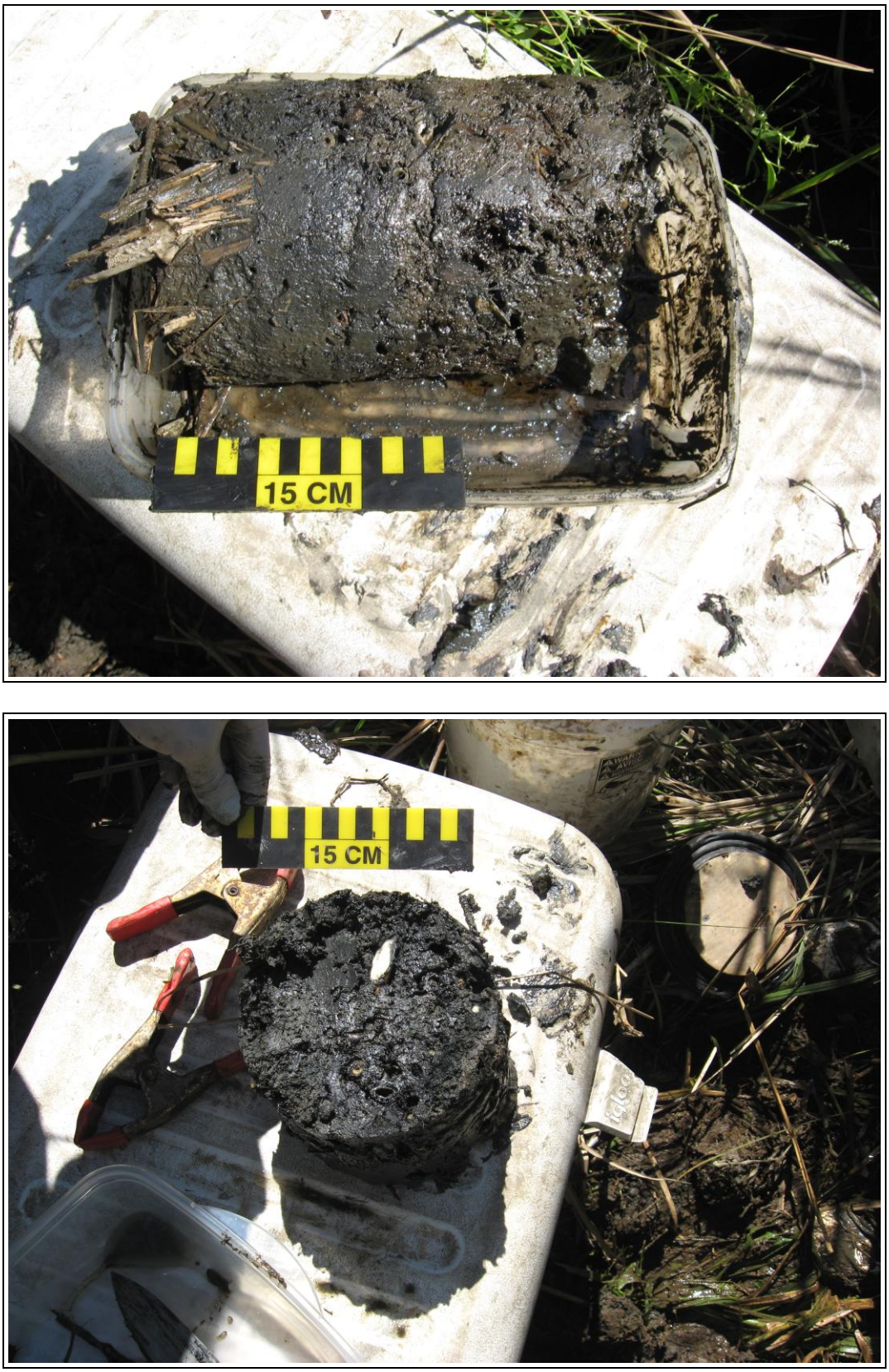


FIGURE 14. Marsh soil core at site CH-8. Black oil was visible in root and other cavities. Free oil droplets accumulated on the water table.

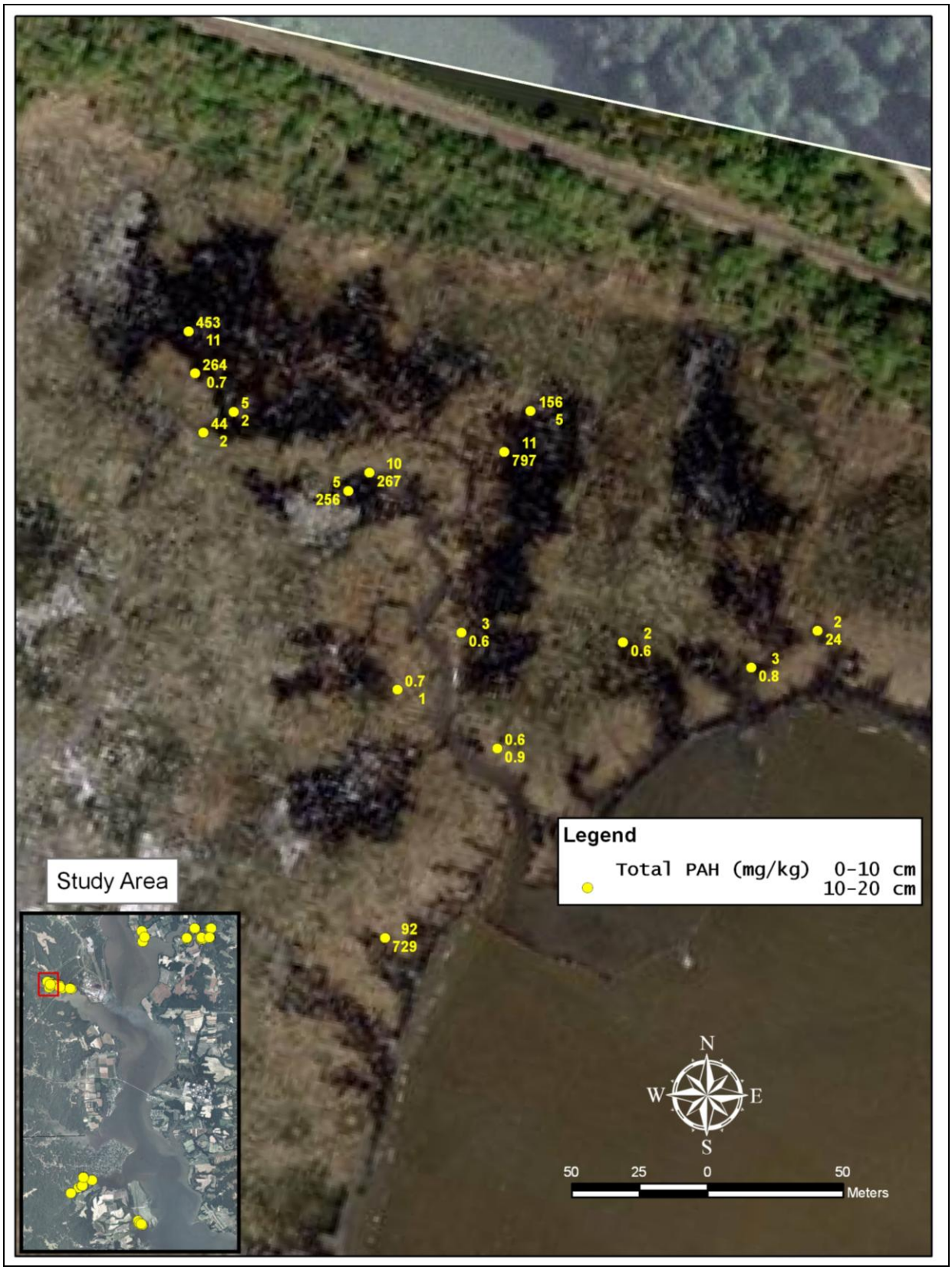


FIGURE 15. Total PAHs (mg/kg) in the top (0-10 cm) and bottom (10-20 cm) core intervals for western Swanson Creek oiled sites.

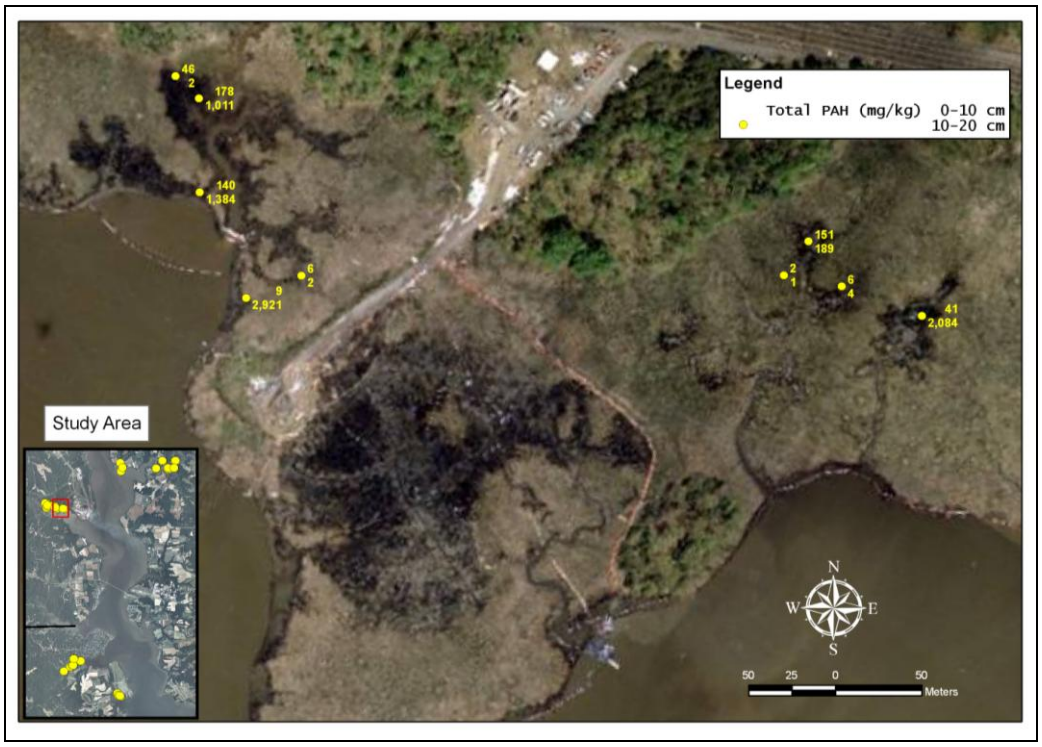


FIGURE 16. Total PAHs (mg/kg) in the top (0-10 cm) and bottom (10-20 cm) core intervals for eastern Swanson Creek oiled sites.

Oil Source Analysis

Two ratio approaches were used to infer sources of PAHs in the marsh soils: Pyrogenic Index[1] (Wang et al., 1999); and fluoranthene/fluoranthene+pyrene ratio, Fl/(Fl+Py). Figure 17 plots the Pyrogenic Index versus total PAHs for samples and source oils; Figure 18 plots Pyrogenic Index versus Fl/(Fl+Py), showing the source oils and different PAH concentration ranges. The Fl/(Fl+Py) ratio for samples with <1 mg/kg total PAHs ranged from 0.52-0.57; a ratio >0.50 indicates dominance by combustion of biomass and coal (Yunker at al., 2002), consistent with the presence of a coal-fired power plant at Chalk Point. For these samples, the Pyrogenic Index was 0.40-0.68, indicating that PAHs were dominantly from combustion sources. This index was <0.50 for some samples because they also contained 10-30% naphthalenes, with the alkylated homologues being most abundant, likely reflecting the heavy use of motorized watercraft in the area. Marr et al. (1999) determined the PAH concentrations in ten commercial gasolines in California, finding that naphthalene ranged from 69-2,600 mg/L and contributed 97% of the total concentration of the 16 PAH priority pollutants measured. That study did not measure the alkylated homologues. Nine samples had total PAHs <1 mg/kg: two from unoiled sites, six from the deeper interval in the core, and one from the surface interval. Figure 19 (upper left) shows the PAH distribution in a representative background sample. Note the abundance of naphthalenes and the dominance of 4- and 5-ringed PAHs.

[1] Pyrogenic Index = acenaphthelene+acenaphthene+anthracene+fluoranthene+pyrene+benz(a)antrhacene+benzofluoranthenes+benzopyrenes+perlylene+dibenzoantrhacene+benzoperylene/C_0-C_4naphthalenes+C_0-C_3fluorenes+C_0-C_4phenanthrenes+C_0-C_4dibenzothiophenes+C_0-C_4chrysenes

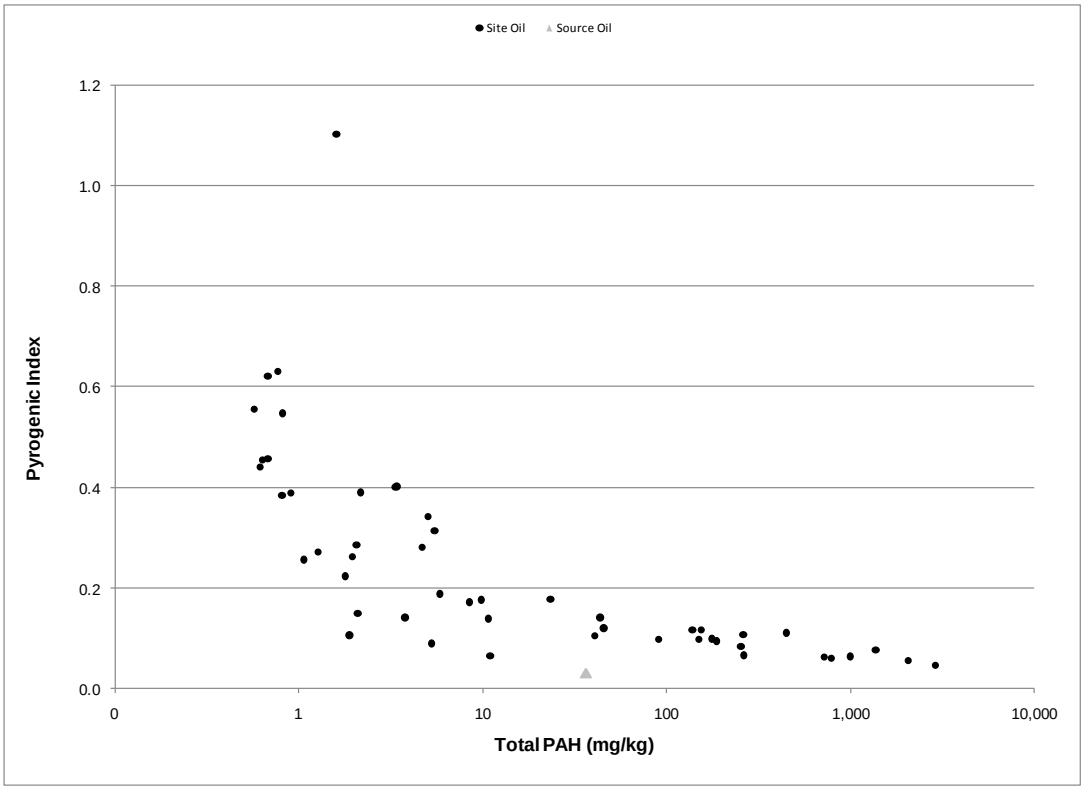


FIGURE 17. Pyrogenic Index for oiled marsh soils and the source oil versus total PAH, for three groups: <1 mg/kg as low background with mostly pyrogenic PAHs; 1-8 mg/kg as background with mixed PAH sources; and >8 mg/kg as oiled with mostly petrogenic PAHs.

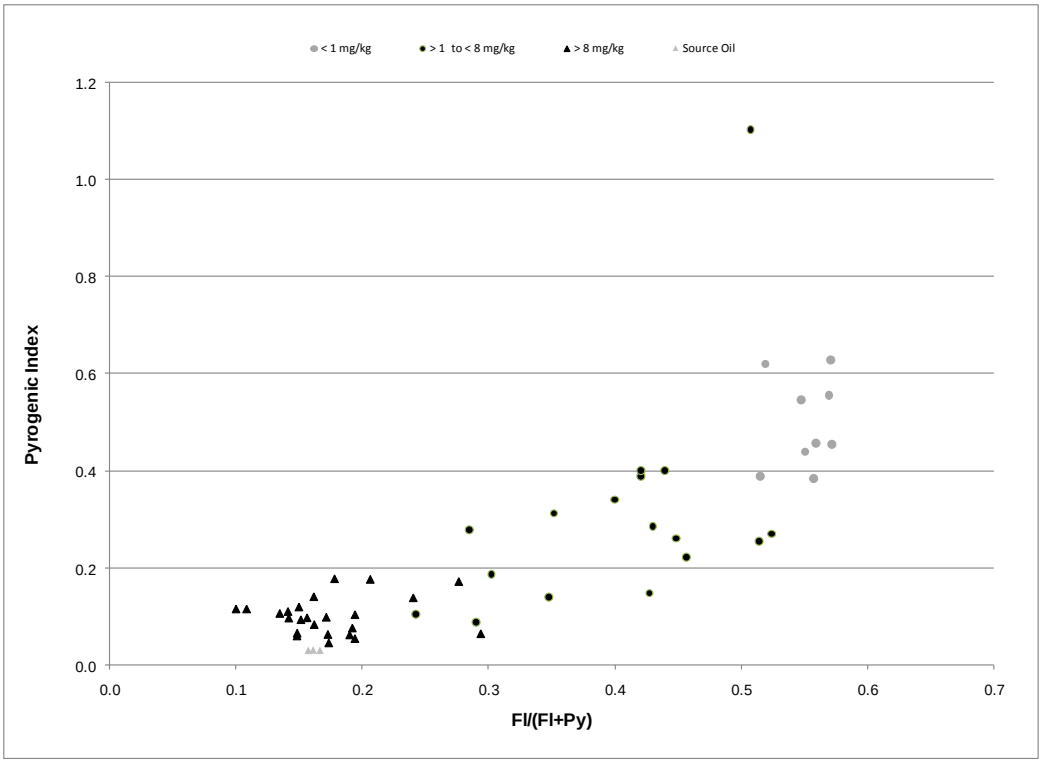


FIGURE 18. Petrogenic Index vs. fluoranthene/fluoranthene+pyrene ratio in oiled marsh soils.

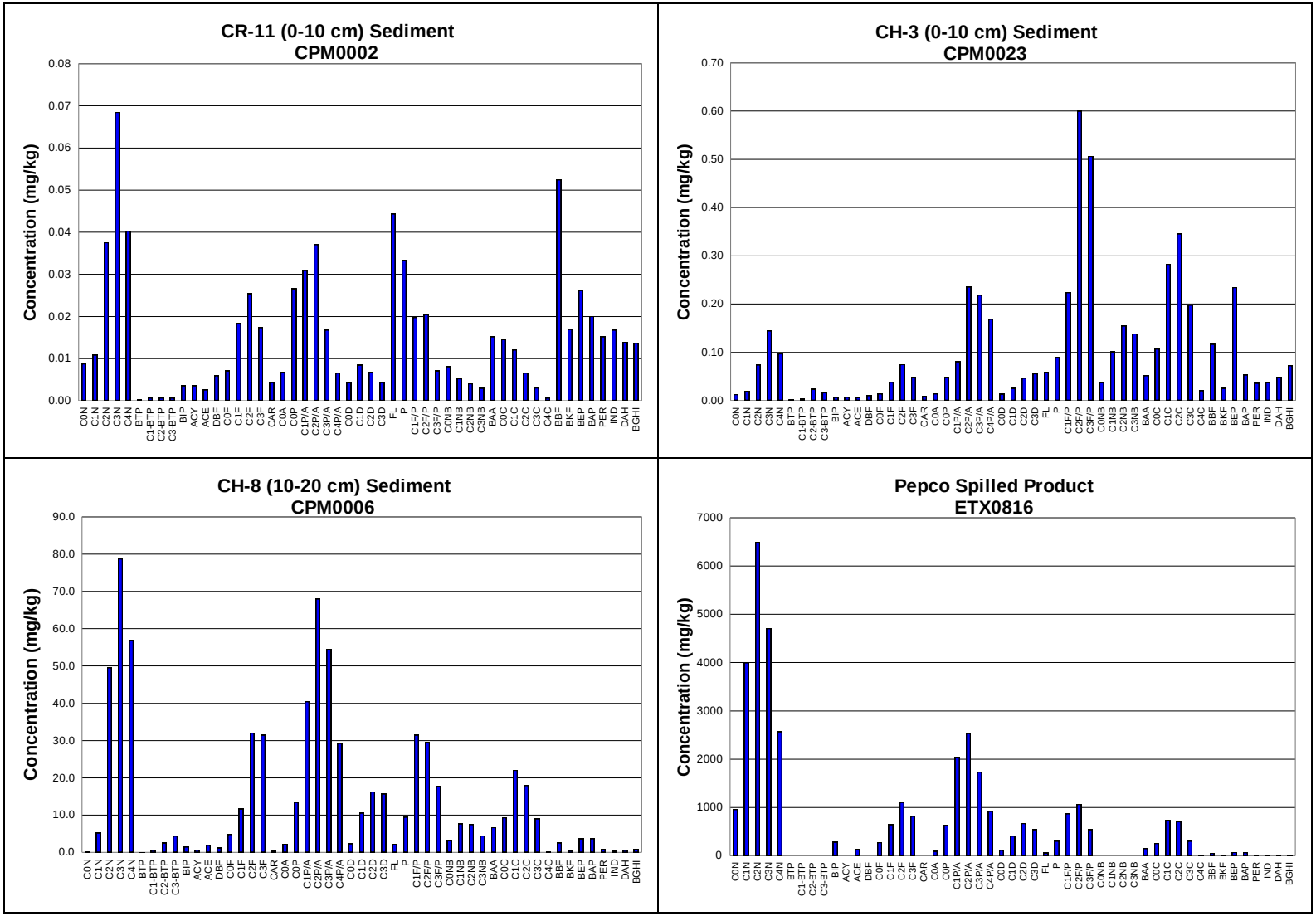


FIGURE 19. Representative PAH distributions in samples containing total PAHs <1 mg/kg (upper left), 1-8 mg/kg (upper right), >8 mg/kg (lower left), and the source oil (lower right). All of the samples with >8 mg/kg had PAH distributions that matched the source oil. Refer to Table 3 for PAH abbreviations.

There were seventeen samples with total PAHs from 1 to 8 mg/kg; these samples contained mixtures of both petrogenic and pyrogenic hydrocarbons. The Fl/(Fl+Py) ratio in these samples ranged from 0.30-0.45 (Fig. 18); Yunker et al. (2002) determined that a ratio of 0.40-0.50 indicated PAHs derived from liquid fuel combustion and <0.40 indicated PAHs derived from fossil fuels. The Pyrogenic Index for these samples ranged from 0.11 to 0.43 (Fig. 17), indicating that these PAHs originated from a variety of sources. Figure 19 (upper right) shows a representative sample in this range.

Samples with more than 8 mg/kg total PAHs had a Fl/(Fl+Py) ratio of 0.1-0.29; the source oil had a ratio of 0.16 (Fig. 18). The Pyrogenic Index for these samples ranged from 0.05 to 0.18 (Fig. 17); the source oil had a Pyrogenic Index of 0.03. These 24 samples, half of those collected, clearly contained petroleum hydrocarbons derived from fossil fuels, with a good match to the source oil (as discussed further below). The maximum total PAHs in the soils were 2,921 mg/kg at AH-6 at 10-20 cm. Figure 19 (lower left) shows a representative sample. The PAH distribution in the source oil, collected directly from a hole in the pipeline shortly after the spill, is shown in the lower right of Figure 19.

Another established fingerprinting approach to determine the source of oil in environmental samples is to use petroleum biomarkers, which are very resistant to degradation. Furthermore, the rates of degradation are affected equally so their ratio in weathered samples depends only on their ratio in the source oil. Much of the early work with biomarkers was conducted by the oil exploration community; however, this research has now been applied to fingerprinting of oil spills, most notably the T/V *Exxon Valdez* (Short et al., 1999; Boehm et al., 2001).

The analytes measured in the 2007 samples included two biomarkers, C-29 hopane and C-30 hopane. Wang et al. (1994) found that the ratio of these two compounds was a reliable source indicator as found in the 22-year old *Arrow* oil spill. Heavy refined products, such as the oil spilled into Swanson Creek in 2000, can have relatively high concentrations of biomarkers. Only C-30 hopane was reported in the source oils sampled and analyzed in 2000. Without quantification of at least two biomarkers in both, the oil and soil samples, it is not possible to match the spilled oil to the oil in the samples collected in 2007. However, the plot of the ratio of C-29 hopane to C-30 hopane versus total PAHs in the oiled marsh soils in 2007 does provide further insight to the source of the oil contamination (Fig. 20). Considering that the spilled oil was potentially a poorly blended mixture of two refined products (a No. 2 fuel oil was being used to clean the pipeline containing a No. 6 fuel oil), the ratio for most of the samples containing greater than 8 mg/kg had a relatively narrow range of 0.57-0.82. Samples containing less than 8 mg/kg had a much larger range in the ratio, indicating different oil sources or a mixture of sources.

The PAH distributions in all 2007 samples were visually evaluated, and the results further supported the conclusion that those samples with >8 mg/kg were dominated by petrogenic hydrocarbons that closely matched the spilled oil. All of the samples exhibited some degree of degradation. Two approaches were used to evaluate and quantify weathering: 1) double ratio plots of PAHs; and 2) depletion ratios for PAH compounds.

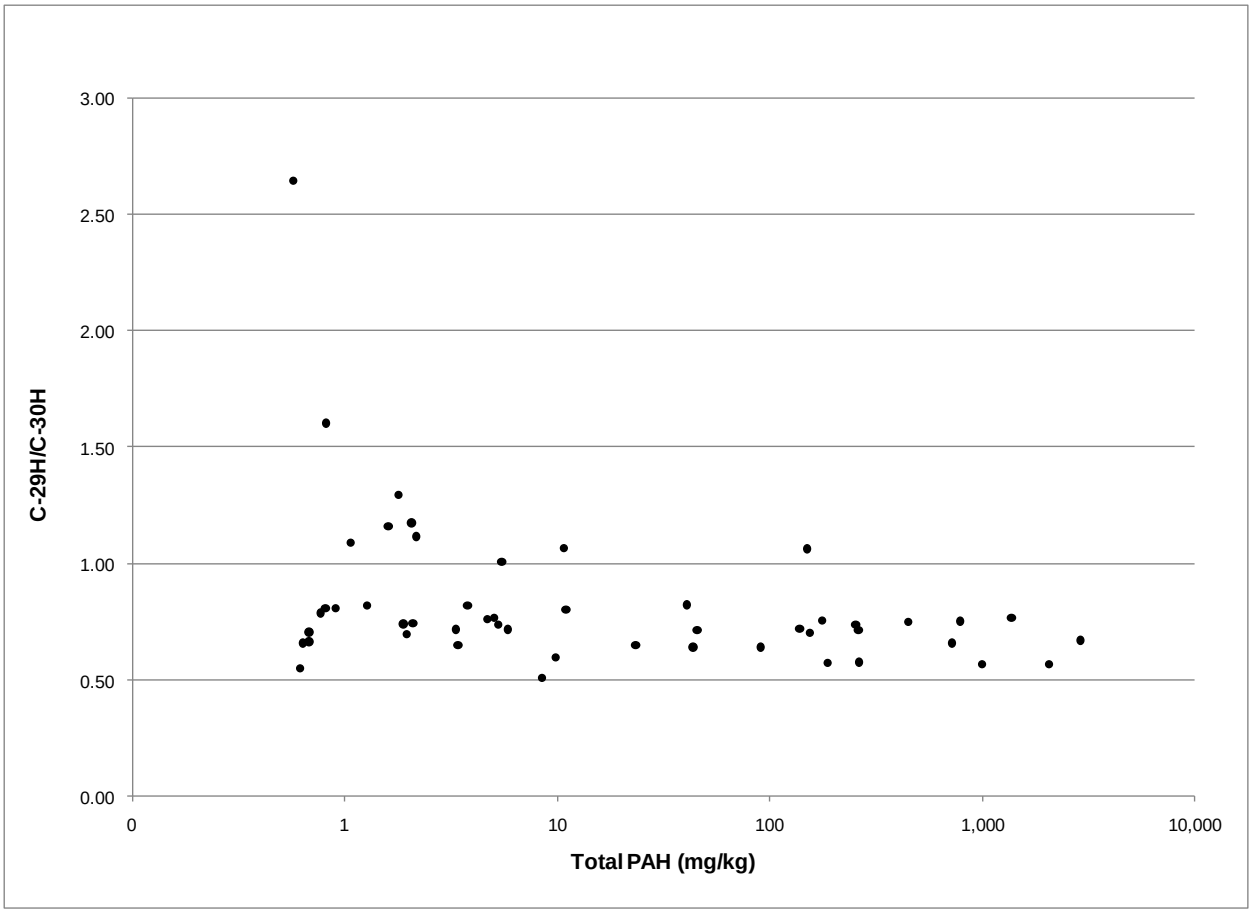


FIGURE 20. Plot of the biomarkers C-29 hopane/C-30 hopane versus total PAHs for all samples collected in 2007. For the samples greater than 8 mg/kg total PAH, the ratio generally ranged between 0.57-0.82 indicating a common oil source.

Figure 21 shows a double-ratio plot of C_2-phenanthrenes/C_2-chrysenes vs. C_3-phenanthrenes/C_3-chrysenes for the 24 samples that contained >8 mg/kg. The samples plot in a linear trend from the upper right to the lower left of the graph, indicating an increasing degree of microbial weathering. There is a difference in the rate of weathering of the oil with depth; samples from 0-10 cm generally plot towards the lower left of the graph indicating a higher degree of weathering, whereas the 10-20 cm samples plot more towards the upper right indicating less weathering. Surprisingly, there does not appear to be any difference in the degree of weathering of the oil in the soils in the *S. alterniflora* versus *S. cynosuroides* habitats. During the NRDA injury assessment and scaling, the oil impacts and persistence was predicted to be higher for the *S. cynosuroides* habitats (see Table 1) based on the available data on oil loading and temporal changes from 2000 to 2001 (see Table 6 and compare the PAH concentrations between habitats). However, it appears that oil persistence and weathering after seven years are similar for interior habitats of both species.

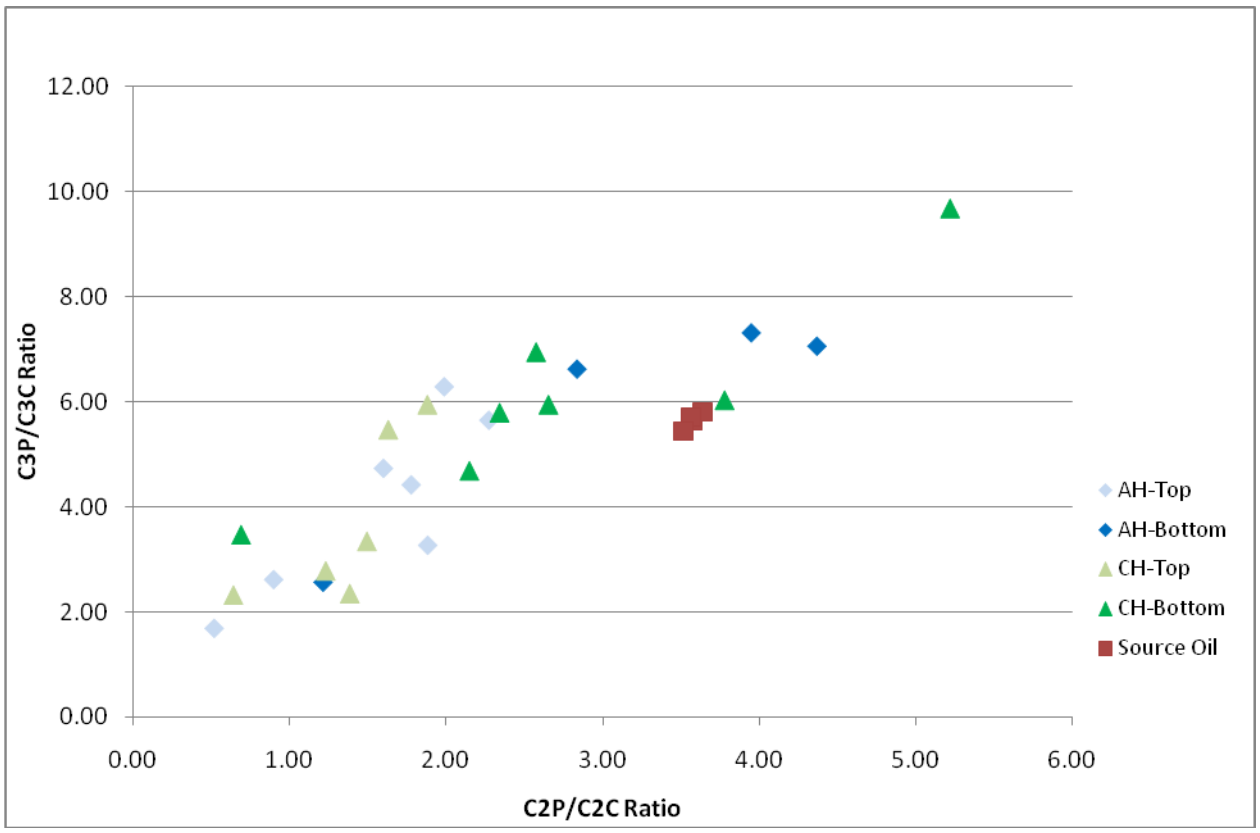


FIGURE 21. Double-ratio plot of C_2-phenanthrenes/C_2-chrysenes vs. C_3-phenanthrenes/C_3-chrysenes in the 24 samples that contained >8 mg/kg in 2007 and three source oil samples. There were no clear differences in weathering rate by marsh type; however, weathering was slower with depth.

Oil Weathering Trends

Analysis of PAH weathering trends was conducted for the 24 samples that contained more than 8 mg/kg because the PAHs in these samples were dominated by petroleum hydrocarbons from the spilled oil, rather than complex mixtures from multiple sources. PAH depletion ratios were calculated for these samples following the methods by Douglas et al. (1996) using C_2-chrysene as the conserved internal marker within the oil to act as a standard. We first attempted to use C-30 hopane as the internal marker, which was the only stable biomarker measured in the source oil in 2000. We determined that there was a problem with the reported value for C-30 hopane in the source oil because calculations resulted in many negative depletion values. Zhu et al. (2001) recommends chrysene as an internal marker if data for stable biomarkers are not available.

For the source oil, we used the average PAH concentration of three samples analyzed by the same laboratory in 2000. The individual PAH depletion, corrected for oil loss, was determined by:

$$\% \text{ PAH depletion} = [1-((PAH_1/PAH_0)(C_2\text{-chrysene}_0/C_2\text{-chrysene}_1))] \times 100$$

where PAH_1 is the concentration of individual PAHs in the marsh soil sample, and PAH_0 is the concentration of individual PAHs in the source oil. C_2-chrysene$_0$ is the concentration of this PAH in the source oil and C_2-chrysene$_1$ is the concentration of this PAH in the marsh soil sample. This equation was applied to the 24 samples collected in 2007 that contained greater than 8 mg/kg. The results, shown in Figure 22, indicated that the oil in these samples had lost 22-76% of its initial PAH content in the seven years since the spill, with the exception of one sample (with 11.09 mg/kg) that had a negative depletion ratio, indicating that it contained other sources of PAH. There was a clear trend with increased PAH depletion with decreasing total PAH concentration.

Another factor affecting oil weathering rates is depth of penetration into the marsh soils. Figure 22 shows that the oil at 10-20 cm was generally less weathered than the top 0-10 cm. All samples with less than 55% total PAH depletion were from the deeper interval of 10-20 cm.

The rate of degradation of different groups of PAH compounds followed the pattern noted by others, namely the degradation rate decreases with ring size. Figure 23 (top) shows that naphthalenes (2-ringed PAH) are the most degraded with 19 of the 24 samples at 90 to nearly 100% depletion. The four samples on the right with 60-80% naphthalene depletion contain >40 mg/kg.

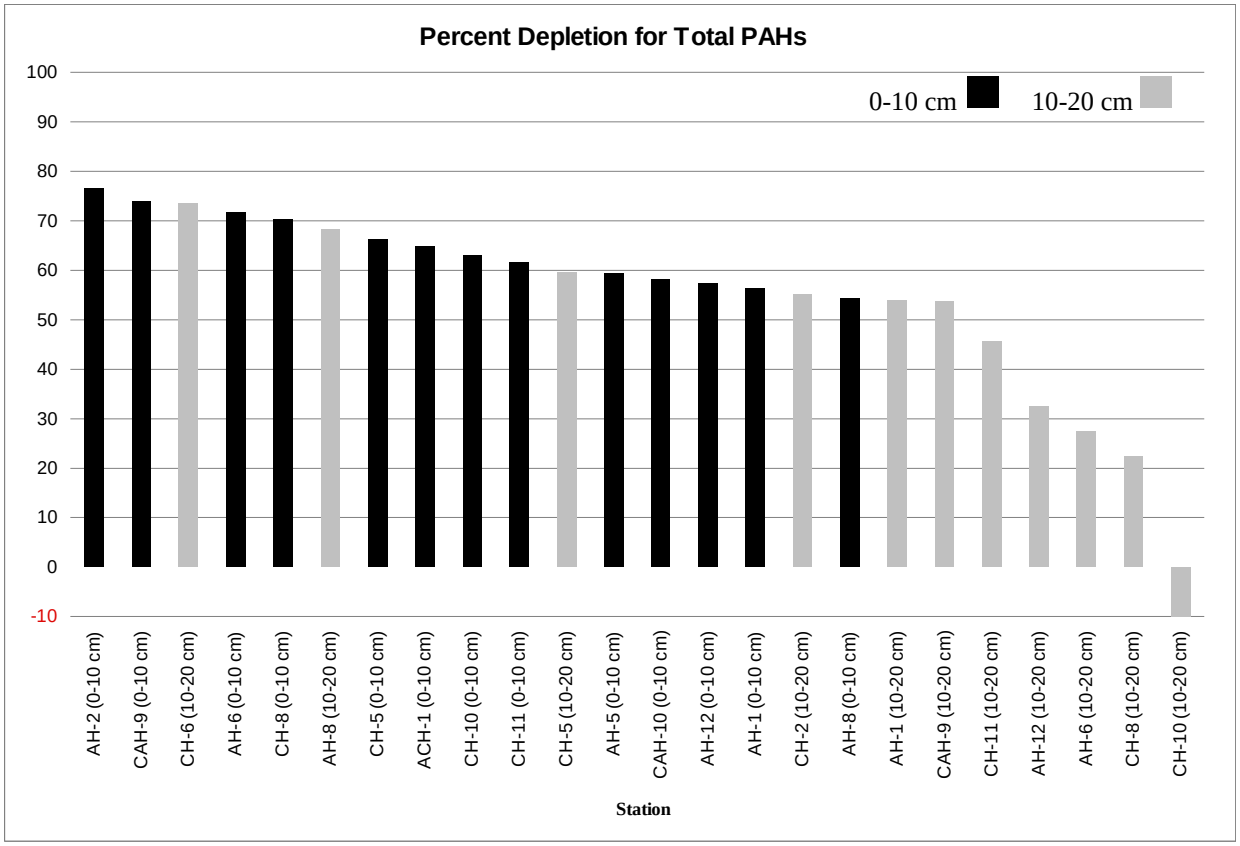


FIGURE 22. Total PAH depletion ratios in samples collected in 2007 that contained >8 mg/kg.

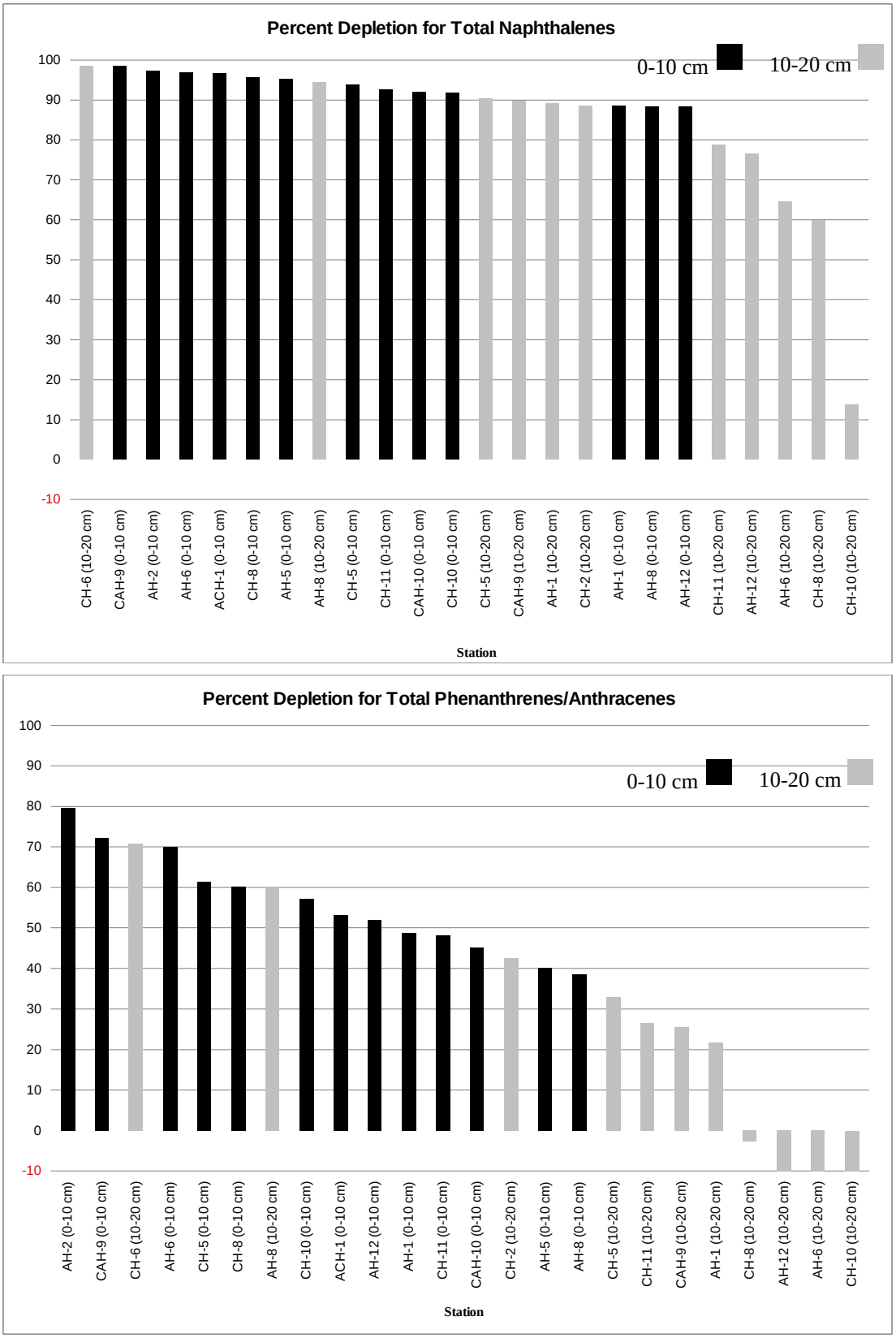


FIGURE 23. PAH depletion ratios for naphthalenes (top) and phenanthrenes/anthracenes (bottom).

The sample with the lowest depletion ratio for naphthalenes was CH-10 (10-20 cm), containing a relatively low 11.09 mg/kg total PAH. The biomass core at this site was heavily oiled; obviously, the chemistry sample included only a small amount of an oiled area and further indicates the heterogeneity of the oil distribution in the marsh soils.

The 0-10 cm samples were homogenized in the field for 5-10 minutes prior to the collection of a subsample for chemical analysis, whereas the 10-20 cm samples were quickly collected from the core and placed directly into the sampling container. Thus, some of the more volatile components (e.g., naphthalenes) could have been lost via evaporation during sampling. However, many of the samples from the 10-20 cm interval had similar depletion ratios (Fig. 22). Furthermore, loss of naphthalenes during sample handling is more critical immediately following a spill, rather than after seven years of volatilization and leaching into pore water.

The phenanthrenes/anthracenes (3-ringed PAH) were only moderately degraded (bottom chart in Fig. 23), although there was a broad range in the depletion ratio among samples. Degradation of these compounds was also slower with depth; eight of the eleven deeper cores had depletion ratios for phenanthrenes/anthracenes of less than 0.35. Though not shown, the dibenzothiophenes had a similar pattern.

The depletion ratio for fluoranthenes/pyrenes and for chrysenes (4-ringed PAH) were low, generally less than 10% after seven years post spill. These depletion curves indicate that oil weathering is driven by microbial degradation rather than by physical removal processes.

Figure 19 (lower right) shows the average PAH distribution in the three source oil samples. It is important to note that naphthalenes comprise 50% of the total PAHs in the source oil; heavy refined products vary widely in PAH distribution depending on the types of oils used to create the product. Heavy refined oils used for power production are blended to meet viscosity and heat value requirements, so they often are mixed with lighter cuts of oil. Also, naphthalenes would be the dominant PAHs in the No. 2 fuel oil used to backflush the pipeline at the time of the release. Naphthalenes are the most volatile and readily degraded of all the PAHs and, at 50% of the total PAHs, their degradation drives the total PAH depletion ratio.

Sites numbered as –1, –2, and –3 were established in 2000 as part of the NRDA injury assessment studies (Michel et al., 2002), and there are some PAH results for samples collected in 2000 and 2001 (Table 6). With such heterogeneous oil distribution and variation in sampling intervals, it is difficult to make temporal comparisons. However, for the few sites and intervals that were collected from the same location, there is a general trend of decreasing total PAHs over time. Table 6 (bottom row) also shows the mean total PAH depletion ratios for all samples collected in 2000, 2001, and 2007 containing >8 mg/kg. These data indicate little change in the degree of oil degradation since 2000.

TABLE 6. Comparison of total PAHs in marsh soils over time. With high spatial variability within a site and different sampling intervals, only general trends can be assessed.

Site	Interval (cm)	PAH (mg/kg) 2000	PAH (mg/kg) 2001	PAH (mg/kg) 2007
S. cynosuroides				
CH-1	0-5	472	2,160	45.8
	5-10	722	1,216	
	10-15	189		1.9
	18-20	6	372	
CH-2	0-5	2,600	4,326	
	0-10			4.7
	13-15		2,186	
	10-20			255
S. alterniflora				
AH-1	0-5	2	3	
	0-10			178
	10-20			1,010
AH-2	0-5	202	54	43.9
	8-10	194	8	
	10-20			1.8
	20-25		14	
AH-3	0-5	886	1	5.5
	5-10			
	10-20			3.8
Average total PAH depletion for samples with total PAH >8 mg/kg		55.84±20.5% (n=9)	59.50±11.6% (n=14)	57.76±14.3% (n=24)

Vegetation Condition

The marshes in the study area are brackish marshes, as indicated by the mixture of *S. alterniflora* and *S. cynosuroides* throughout, as well as the occurrence of *Polygonum* spp. (smartweed) at 19 of the 48 sites, and *Scirpus* spp. at 10 sites. *Amaranthus* spp. (water hemp) was also common, occurring at 21 sites. *Typha* spp. were common along the upper parts of the creeks and adjacent to uplands, but did not occur in any of the sites studied. Three of the six heavily oiled interior sites established in 2000 had a marked change in dominant species, one switched from *S. alterniflora* to *S. cynosuroides*, and two switched from *S. cynosuroides* to *S. alterniflora.*

During the field surveys, all large areas were typically covered with vegetation. However, the *S. alterniflora* sites had some locally bare areas. No bare zones in the previously oiled areas were visible in the 2004 vertical aerial photograph shown on the cover of this report. Vegetation in the vicinity of muskrat huts showed evidence of grazing; these areas were avoided during site selection.

Vegetation condition for *S. alterniflora* and *S. cynosuroides* habitats was indicated by three variables: stem density, stem height, and total belowground biomass. For each vegetation variable, a two-sample pooled variance t-test was carried out comparing plot group means between oiled and unoiled sites. The data were assumed to be normally distributed, independent, and of equal variance. Where evidence for unequal variances existed, t-tests using unpooled variance were carried out. Summary statistics for all variables are shown in Table 7; also included are the data on stem density and stem height measured in 2000 and 2001 as part of the NRDA studies (Michel et al., 2002).

Stem density of *S. alterniflora* in the unoiled sites averaged 223 stems/m^2 and was similar to that measured in 2001. In oiled sites, stem density in 2007 averaged 141.2 stems/m^2 and was reduced by 37% compared to that of unoiled sites. Figure 24 shows scatter and box plots for stem density for sites in *S. alterniflora* habitats. Average stem density was significantly different (t=2.999, df=20, p=0.007) between oiled and unoiled sites in 2007. This decrease in stem density in *S. alterniflora* habitats has persisted since 2001.

Stem density of *S. cynosuroides* in the unoiled sites averaged 94 stems/m^2 and was similar to that measured in 2001 but higher than in 2000. In the oiled sites, stem density in 2007 was slightly higher (5% increase over the unoiled sites), averaging 99.1 stems/m^2. Figure 24 shows scatter and box plots for stem density for *S. cynosuroides* habitats. Average *S. cynosuroides* stem height was not statistically different between oiled and unoiled sites in 2007. Stem density in the oiled *S. cynosuroides* marshes increased since 2000, whereas it initially decreased then remained steady in the unoiled marshes, suggesting recovery of this metric.

TABLE 7. Summary statistics for stem density, stem height, and belowground biomass for heavily oiled and unoiled areas in *S. alterniflora* and *S. cynosuroides* dominated habitats.

Date	No. of Sites	Mean Stem Density (#/m^2)		Mean Stem Height (m)		Mean Belowground Biomass 0-10 cm (g/m^2)		Mean Belowground Biomass 10-20 cm (g/m^2)	
		Oiled	**Unoiled**	**Oiled**	**Unoiled**	**Oiled**	**Unoiled**	**Oiled**	**Unoiled**
	S. alterniflora								
7/2000	3	261	150	1.30	1.17	--	--	--	--
7/2001	3	95	233	1.02	1.02	--	--	--	--
8-9/2007	10-12	141*	223	1.06*	1.25	3,163	3,343	3,291	3,195
8-9/2007	10-12	CV= 46%	CV= 28%	CV= 23%	CV= 11%	CV= 22%	CV= 33%	CV= 27%	CV =32%
	S. cynosuroides								
7/2000	3	45	141	1.56	2.09	--	--	--	--
7/2001	3	77	94	1.35	1.62	--	--	--	--
8-9/2007	12-14	99	94	1.96	2.08	3,784*	4,823	3,670*	4,618
8-9/2007	12-14	CV= 39%	CV= 36%	CV= 19%	CV= 15%	CV= 37%	CV= 26%	CV= 27%	CV= 23%

- * = significant difference between oiled and unoiled sites in 2007; CV = coefficient of variation

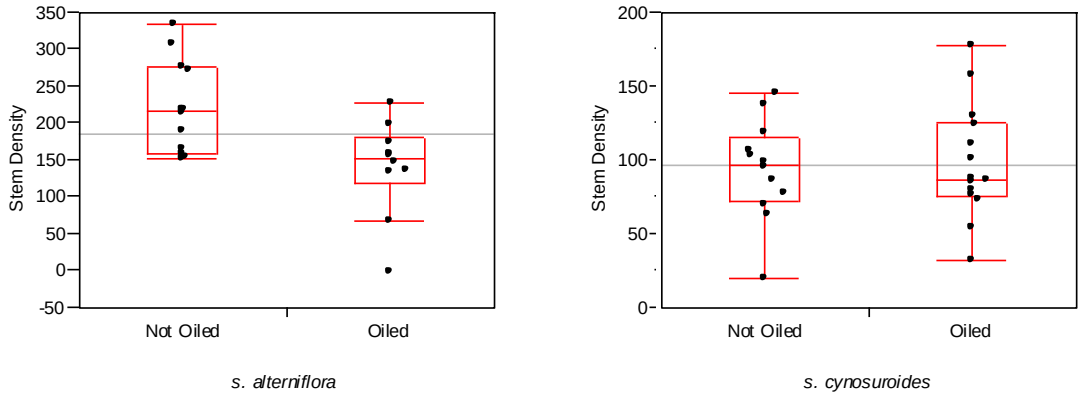


FIGURE 24. Scatter and box plots of stem density (#/m^2) in both oiled and unoiled sites for *S. alterniflora* and *S. cynosuroides* dominated habitats. Grey lines indicate grand means.

Stem height of *S. alterniflora* in the unoiled sites averaged 125 cm in 2007 and was slightly higher than in 2000. In the oiled areas, stem height in 2007 averaged 106.3 cm, a reduction of 15% compared to the unoiled sites. This decrease compared to the unoiled sites has persisted since 2001. Figure 25 shows scatter and box plots for stem height for sites in *S. alterniflora* habitats. There is moderate evidence (t=2.286, df=20, p=0.033) for significant differences in average between oiled and unoiled sites. Due to evidence for unequal variances (*Brown-Forsythe* f=4.559, df=1, p=0.045), testing using the unpooled variances was performed. Testing unpooled variances only slightly weakens (t =2.181, df=13.909, p=0.047) the original conclusion.

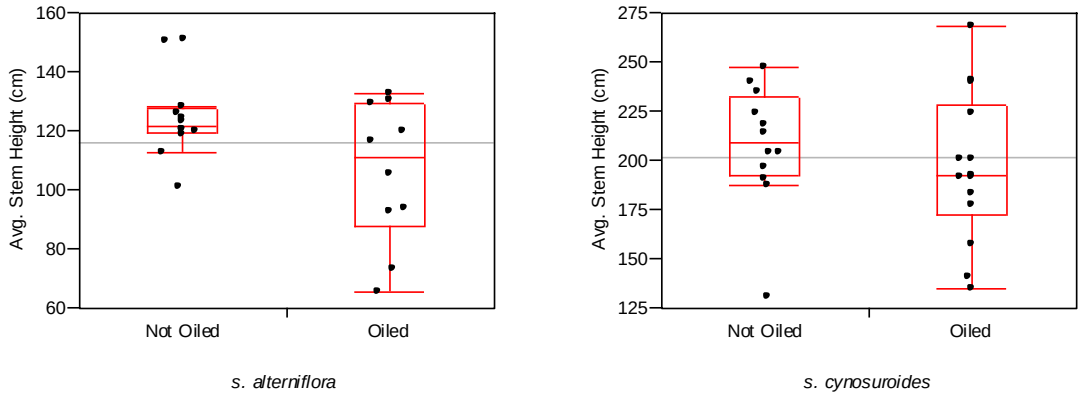


FIGURE 25. Scatter and box plots of average stem height (cm) in both oiled and unoiled sites for *S. alterniflora* and *S. cynosuroides* dominated habitats. Grey lines indicate grand means.

Stem height of *S. cynosuroides* in the unoiled sites averaged 208 cm in 2007, which is similar to that measured in 2000, but higher than in 2001. In the oiled sites, stem height was slightly lower (6% decrease compared to the unoiled sites), averaging 196.5 cm. Figure 25 shows scatter and box plots for stem height for sites in *S. cynosuroides* habitats. Average stem height was not statistically different between oiled and unoiled sites in 2007. Stem height has increased since 2000, suggesting recovery of aboveground vegetation in the heavily oiled *S. cynosuroides* habitats.

Belowground biomass in *S. alterniflora* habitats was similar for both oiled and unoiled areas at both 0-10 cm or 10-20 cm depth in 2007. For sites in *S. cynosuroides* habitats, there is weak to moderate evidence for significant differences in belowground biomass between oiled and unoiled areas at the 0-10 cm (t=1.984, df=20, p=0.059) depth range and moderate evidence at the 10-20 cm (t=2.334, df=20, p=0.028) depth range. Figure 26 shows scatter and box plots for belowground biomass at both depth ranges for both species for oiled and unoiled sites.

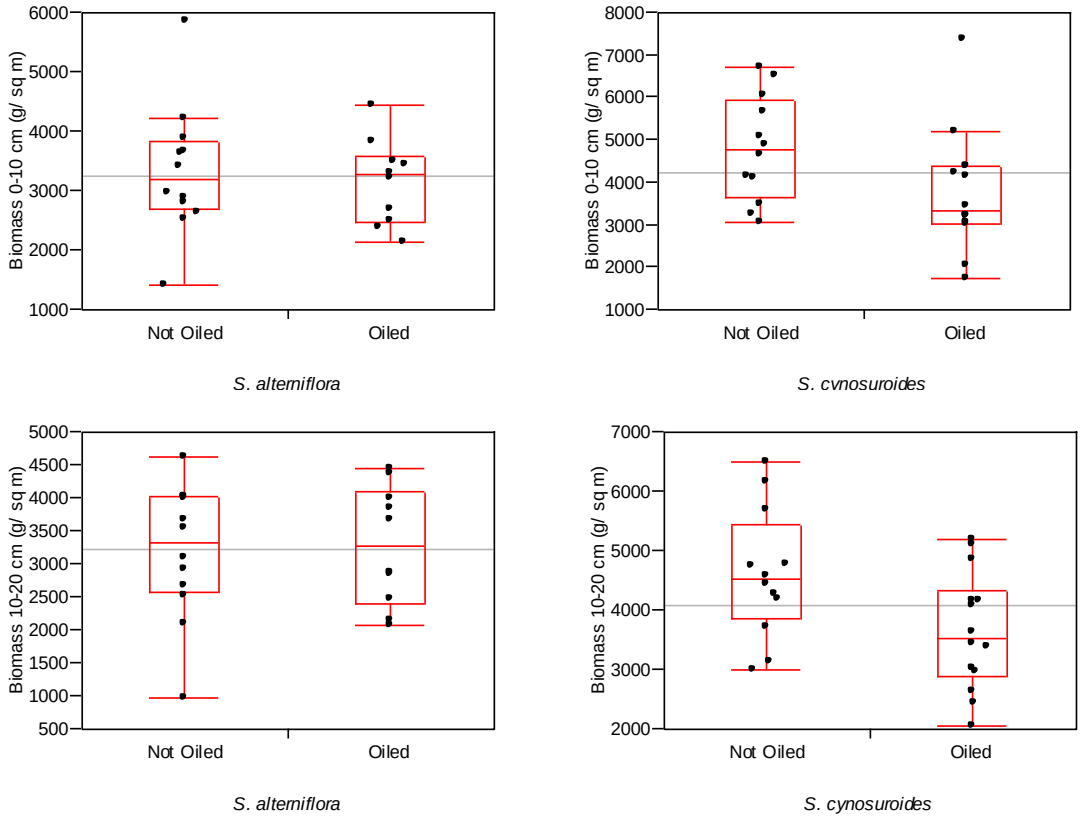


FIGURE 26. Scatter and box plots of below -10 cm below the surface (top) and 10-20 cm below the surface (bottom) in both oiled and unoiled sites for *S. alterniflora* and *S. cynosuroides* dominated habitats. Grey lines indicate grand means.

Marsh Creation Monitoring

The marsh creation project in Washington Creek was completed in October 2005. The project consisted of removal of 0.75-1.0 m of upland sediments in mid-marsh areas and up to 1.5 m in the channel bottom. About 2 hectares of the surface were planted at 60-cm spacing on center. The plants had a root mass of approximately 4x4x5 cm and were 30 cm in height. Each plant was 3-6 months old and had at least 3 stems. Species planted were: *S. alterniflora* from mid-tide (+0.7 feet above mean low water of 0.0 feet) to spring high water (+1.8 feet), and *S. patens* overlapping about 5 m with the *S. alterniflora* (+1.65 feet) up to the lip of the berm (+4.0 feet). A goose exclusion fence and string were in place the entire first year to avoid decimation of young plants by non-migratory Canadian geese before their root systems developed. Each plant was fertilized with one ounce of Osmocote (3-4 month 19-6-21, or similar) when planted.

Figure 11 shows the distribution of the six sampling sites; they were all in *S. alterniflora* planted habitat and located about 2 m from the outer marsh edge in September 2007. Samples were not taken for chemical analysis. Figures 27 and 28 show photographs of each site of the quadrat and one of the two extruded biomass cores taken at each site. The vegetation appeared healthy. Note that the soils were inorganic, consisting of muddy, oxidized, fine-grained sand. Table 8 shows the data collected and the mean values for unoiled *S. alterniflora* from Table 7.

TABLE 8. Vegetation metrics for the six *S. alterniflora* created marsh sites in Washington Creek and the twelve unoiled *S. alterniflora* sites.

Site	Stem Height (m)	Stem Density (#/m^2)	Belowground Biomass (g/m^2) 0-10 cm	Belowground Biomass (g/m^2) 10-20 cm
AW-1	1.31	271	7,895*	346
AW-2	1.18	223	912	340
AW-3	1.09	237	2,285	396
AW-4	1.13	231	503	72
AW-5	1.34	196	1,392	120
AW-6	1.13	296	1,373	128
Mean CV	1.20±0.10 9%	242±36 15%	1,293±665 51%	234±142 61%
Mean of unoiled *S. alterniflora* (n=12)	1.25±0.14	223.3±62.9	3,343±1,090	3,195±1,011

* Value extraordinarily high, but we were not able to identify cause of anomaly. Mean excludes this value.

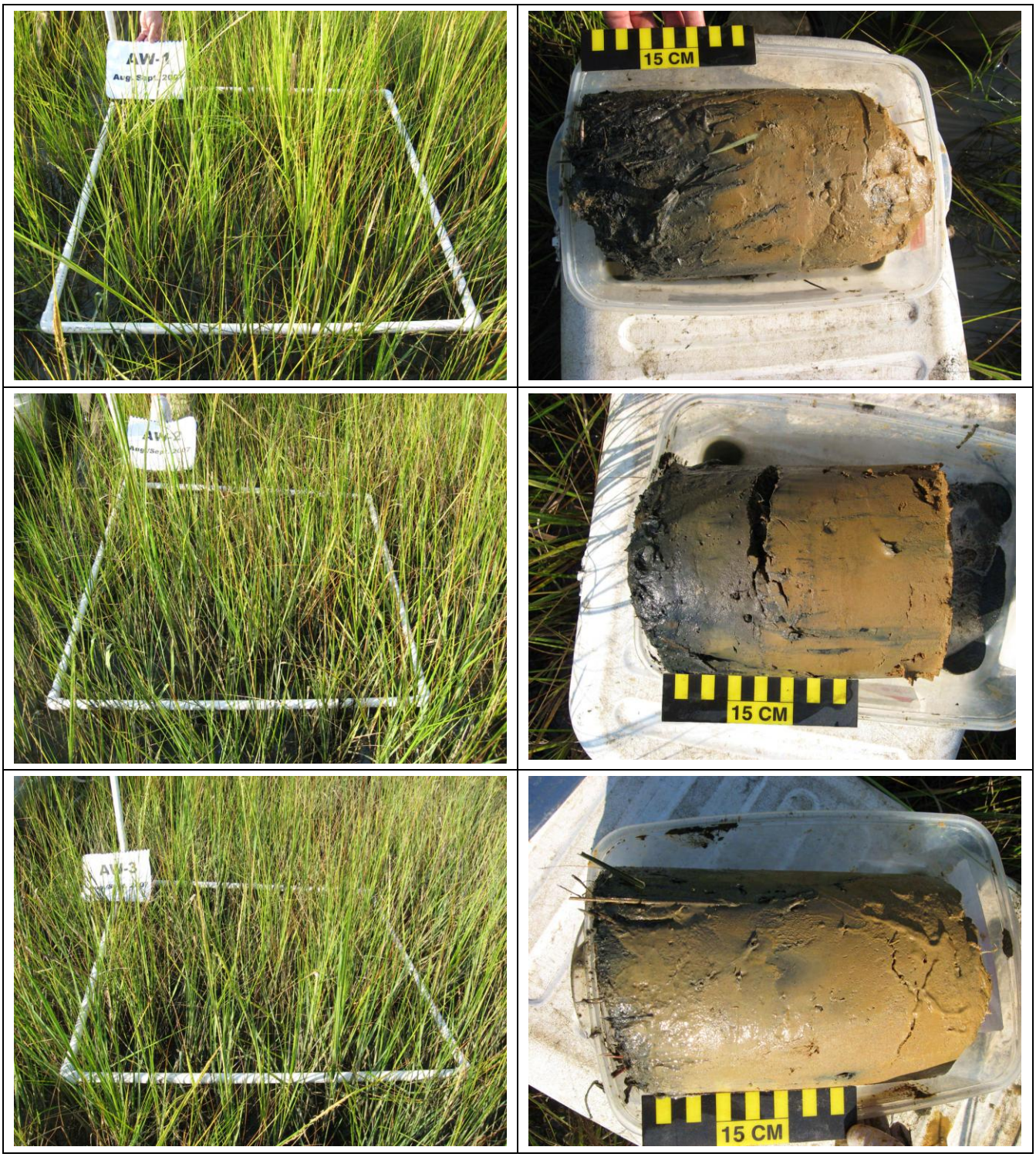


FIGURE 27. Photographs of one of the two cores extracted from sites AW-1, AW-2, and AW-3 at the created marsh in Washington Creek, taken on 4 September 2007.

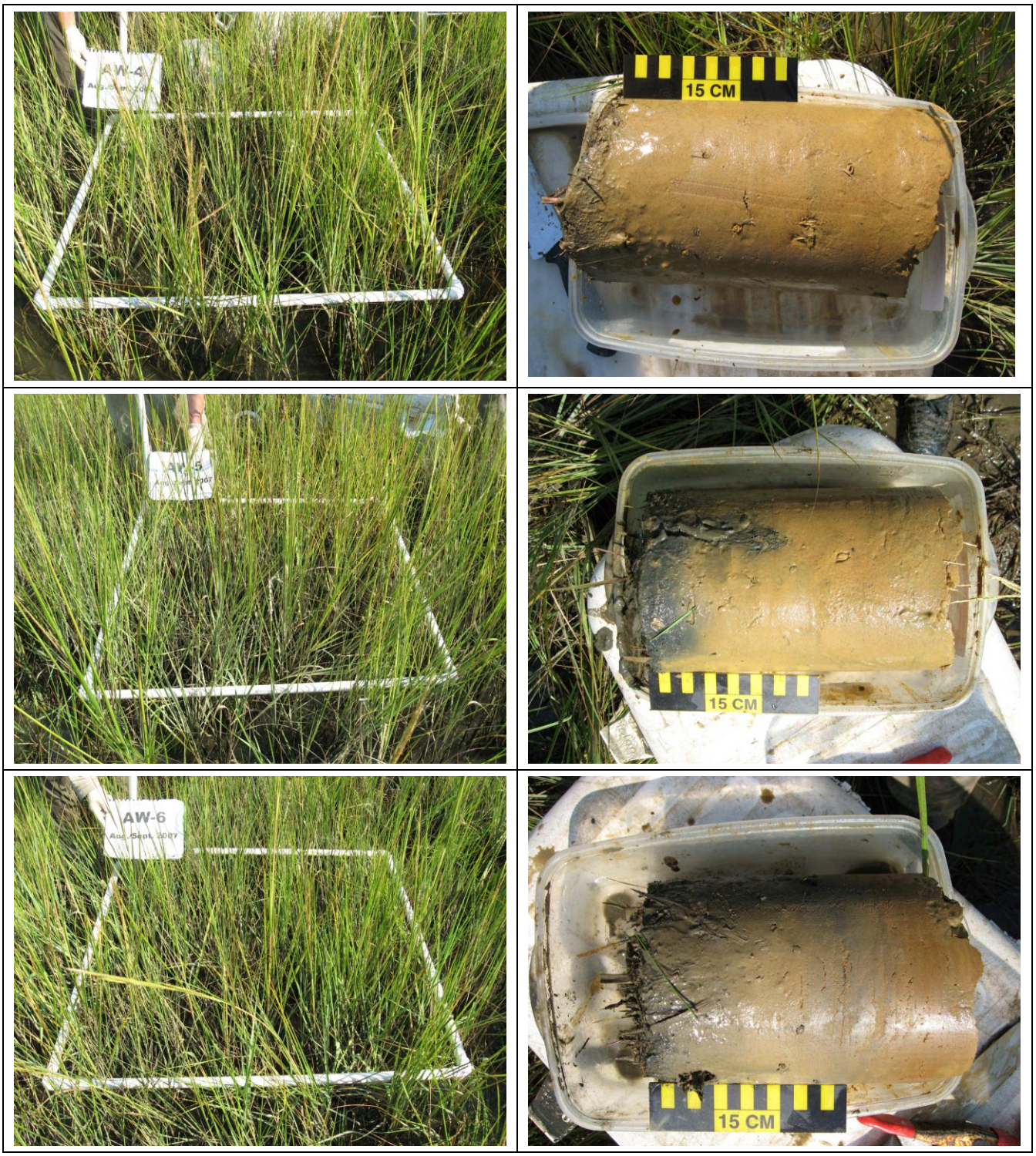


FIGURE 28. Photographs of one of the two cores extracted from sites AW-4, AW-5, and AW-6 at the created marsh in Washington Creek, taken on 4 September 2007.

Site AW-1 was located closest to the mouth of the channel thus it had the highest degree of flushing and the most well-developed root mass (compare the cores in Figs. 27 and 28). Belowground biomass weight for the top 10 cm was six times the average of the other sites, 30% higher than the highest unoiled *S. alterniflora* site, and 2.3 times the average of the unoiled *S. alterniflora* sites from natural marshes. We could not determine the cause of this anomalous measurement. The lab did not report any observations on the presence of sediments or anything else unusual about this sample. Visual comparisons in Figures 27 and 28 suggest that belowground biomass in the top 10 cm should have been similar to the other cores. Therefore, we decided to remove this outlier site from the calculations of the mean and standard deviations for the created marsh. .

Stem density and stem height of created *S. alterniflora* marsh two years post planting were, on average, 108% and 95%, respectively, of the metrics seen in twelve sites of natural unoiled *S. alterniflora* marshes in the area. Other published studies of created marshes have found that *S. alterniflora* aboveground biomass develops quickly, being comparable with natural marshes within 3-5 years (Seneca et al., 1985; Broome et al., 1986; Craft et al., 2002). Thus, the Washington Creek site is similar to other east coast restoration projects.

The belowground biomass development, however, appears to be slower than in other marsh creation sites. When comparing the mean belowground biomass, the restored marsh was on average 39% of the natural unoiled marshes at 0-10 cm and 7% of natural unoiled marshes at 10-20 cm. Broome et al. (1986) reported belowground biomass at 100% of natural marshes at 3 years. Craft et al. (2002) found *S. alterniflora* belowground biomass in a created marsh to reach equivalence to the natural marsh within 2 years. There is also higher variability in the mean belowground biomass results for both depth intervals (CV=51-61%) at the restored marsh compared to the natural unoiled *S. alterniflora* marshes (CV=32-33%).

The slower and more variable development of belowground biomass at the Washington Creek marsh creation site is likely, in part, related to the soils of the created marsh. As can be seen in the core photographs, the native soils remaining after scrap-down to the appropriate elevation were dense, inorganic, and oxidized. The site furthest from the mouth of the created channel (AW-4) had the lowest belowground biomass and almost no accumulation of organic matter even in the surface. Craft et al. (2002) found that, even after 15 years, created marshes planted in the "C" horizon of soils, with high bulk density, acidity, and metals retained these characteristics. They found that soil characteristics developed faster at lower tidal elevations where the marsh surface was more frequently inundated. It may be appropriate to monitor soil physiochemical characteristics in future monitoring studies at the created marsh.

Soil Toxicity Studies

The soil toxicity results (Table 9) are compared in Tables 10 and 11 with three sediment quality guidelines used to screen contaminants in sediments for potential toxicity.

1. The Effects Range-Low (ERL) and Effects Range-Medium (ERM) are frequently used sediment quality guidelines to estimate the probabilities of observing sediment toxicity (Long et al., 1998).

2. Field et al. (2002) and USEPA (2005) developed a series of logistic regression models for 22 individual PAHs that quantify relationships between the concentrations of sediment-associated contaminants and toxicity to the marine amphipods, *A. abdita* and *Rhepoxynius abronius*. In contrast to the sediment quality guidelines, USEPA logistic regression models provide specific sediment concentrations (Tp values) that would result in specific probabilities of observing toxicity (20, 50, and 80%). Tables 10 and 11 compare the Tp values for individual PAHs, copper, and nickel, and the maximum predicted PAH toxicity probability (P_{Max}) in the soil samples in this study.
3. Equilibrium Partitioning Sediment Benchmark Toxic Unit Final Chronic Value (ESB-TU_{FCV}) for PAH mixtures for the protection of benthic organisms (USEPA, 2003) based on 34 PAHs in sediments. The 34 PAHs summed in the calculation of this value include most of the PAHs measured in the samples (listed in Table 3) with the exception of benzothiophene, dibenzothiophene, and naphthobenzothiophene (and their alkylated homologues), and C1- and C2-fluoranthenes/pyrenes, which are minor contributors to the total PAHs in the samples. Two values are reported in Tables 10 and 11 for the range of reported Total Organic Carbon (TOC) concentrations from previous studies at the sites because TOC was not measured in the individual samples collected in 2007.

The amphipods in the *S. alterniflora* and *S. cynosuroides* marsh reference soils met the test acceptability criterion of 90% survival (Table 8). Mean survival was 98 and 99% at the end of the 10-day test for *S. alterniflora* and *S. cynosuroides*, respectively. In-house control survival was 96%. The raw data for both tests, which include initial pore water measurements, water quality data for each treatment, survival data, and statistical analyses, are shown in Appendix C.

TABLE 9. Soil chemical composition and survival data for the amphipods exposed to the samples from *S. alterniflora* and *S. cynosuroides* sampling sites.

Sample	Total PAH (mg/kg)	Copper (mg/kg)	Nickel (mg/kg)	No. of Reps	Mean Survival (%)	Sign. (p = 0.05)
S. alterniflora						
AR-5 (Ref.)	0.82	21	30	5	98	
AH-11	5.87	15	28	5	100	
AH-6	8.51	18	38	5	97	
AH-2	43.94	17	46	5	96	
AH-5	156.4	20	39	5	85	*
AH-1	178.4	14	43	5	0	*
S. cynosuroides						
CR-11 (Ref.)	0.78	20	28	5	99	
CH-6	2.18	19	33	5	94	
CH-3	5.07	18	40	5	97	
CH-5	10.85	20	28	5	91	
CH-8	91.86	21	39	5	66	*
CH-11	139.7	18	26	5	3	*
CAH-10	264.2	18	41	5	1	*
CH-10	453.4	19	43	5	0	*

TABLE 10. Comparison of sediment quality guidelines with the analytes found in the oiled *S. alterniflora* soils. The samples that exceed the ERM or ESB-TU_{FCV} = 1 are bolded.

Analyte	ERL	ERM	T20	T50	T80	AR-5 (Ref.)	AH-11	AH-6	AH-2	AH-5	AH-1
% Amphipod Survival						98	100	97	96	85	0
PAHs (mg/kg dry weight sediment)											
Acenaphthene	0.016	0.500	0.019	0.120	0.710	0.003	0.003	0.010	0.057	0.028	0.259
Acenaphthylene	0.044	0.640	0.014	0.140	1.420	0.004	0.003	0.008	0.030	0.038	0.116
Anthracene	0.085	1.100	0.034	0.290	2.490	0.007	0.011	0.018	0.077	0.197	0.374
Benzo(a)anthracene	0.261	1.600	0.061	0.470	3.530	0.010	0.054	0.105	0.382	1.320	**1.940**
Benzo(a)pyrene	0.430	1.600	0.069	0.520	3.910	0.017	0.052	0.084	0.372	1.480	1.500
Chrysene	0.384	2.800	0.082	0.650	5.190	0.015	0.119	0.141	0.806	**2.840**	**3.030**
Dibenz(a,h)anthracene	0.063	0.260	0.019	0.110	0.690	0.012	0.032	0.040	0.130	0.258	**0.266**
Fluoranthene	0.600	5.100	0.120	1.030	8.950	0.040	0.036	0.057	0.096	0.424	0.663
Fluorene	0.019	0.540	0.019	0.110	0.660	0.010	0.010	0.023	0.162	0.153	**0.676**
2-Methylnaphthalene	0.070	0.670	0.021	0.130	0.770	0.006	0.011	0.012	0.038	0.042	0.076
Naphthalene	0.160	2.100	0.030	0.220	1.570	0.008	0.008	0.011	0.019	0.017	0.025
Phenanthrene	0.240	1.500	0.068	0.460	3.060	0.029	0.034	0.030	0.040	0.051	0.031
Pyrene	0.665	2.600	0.120	0.930	6.980	0.033	0.084	0.150	0.498	**3.48**	**3.20**
Total PAHs						0.82	5.87	8.51	43.9	156.4	178.4
P_{Max}						*0.16*	*0.28*	*0.31*	*0.57*	*0.73*	*0.80*
ESB-TU_{FCV} (5.6% TOC)						0.02	0.09	0.13	0.61	**2.56**	**3.17**
ESB-TU_{FCV} (26.8% TOC)						0.00	0.02	0.03	0.13	0.53	0.66
Metals (mg/kg dry weight sediment)											
Copper	34	270	32	94	280	21	15	18	17	20	14
Nickel	20.9	51.6	15	47	150	30	28	38	46	39	43

TABLE 11. Comparison of sediment quality guidelines with the analytes found in the oiled *S. cynosuroides* soils. The samples that exceed the ERM or ESB-TU_{FCV} = 1 are bolded.

Analyte	ERL	ERM	T20	T50	T80	CR-11 (Ref.)	CH-6	CH-3	CH-5	CH-8	CH-11	CAH-10	CH-10
% Amphipod Survival						99	94	97	91	66	3	1	0
PAHs (mg/kg dry weight sediment)													
Acenaphthene	0.016	0.500	0.019	0.120	0.710	0.003	0.002	0.007	0.008	0.046	0.092	0.214	0.454
Acenaphthylene	0.044	0.640	0.014	0.140	1.420	0.004	0.003	0.007	0.008	0.035	0.066	0.136	0.241
Anthracene	0.085	1.100	0.034	0.290	2.490	0.007	0.006	0.014	0.015	0.084	0.149	0.517	0.799
Benzo(a)anthracene	0.261	1.600	0.061	0.470	3.530	0.015	0.024	0.052	0.122	0.502	1.120	**2.300**	**4.740**
Benzo(a)pyrene	0.430	1.600	0.069	0.520	3.910	0.020	0.030	0.053	0.085	0.707	1.450	**2.180**	**4.740**
Chrysene	0.384	2.800	0.082	0.650	5.190	0.015	0.033	0.107	0.164	1.550	2.340	**4.550**	**8.830**
Dibenz(a,h)anthracene	0.063	0.260	0.019	0.110	0.690	0.014	0.024	0.049	0.032	0.207	0.248	**0.416**	**1.010**
Fluoranthene	0.600	5.100	0.120	1.030	8.950	0.044	0.031	0.059	0.058	0.187	0.322	0.586	1.070
Fluorene	0.019	0.540	0.019	0.110	0.660	0.007	0.008	0.015	0.016	0.122	0.190	0.638	**1.070**
2-Methyl naphthalene	0.070	0.670	0.021	0.130	0.770	0.033	0.042	0.089	0.182	**1.130**	**2.890**	**3.760**	**6.500**
Naphthalene	0.160	2.100	0.030	0.220	1.570	0.009	0.008	0.013	0.014	0.017	0.033	0.017	0.028
Phenanthrene	0.240	1.500	0.068	0.460	3.060	0.0267	0.023	0.048	0.037	0.144	0.250	0.121	0.223
Pyrene	0.665	2.600	0.120	0.930	6.980	0.033	0.042	0.089	0.182	1.130	**2.890**	**3.760**	**6.500**
Total PAHs						0.78	2.18	5.07	10.85	91.86	139.7	264.2	453.4
P_{Max}						*0.17*	*0.23*	*0.35*	*0.29*	*0.64*	*0.70*	*0.80*	*0.85*
ESB-TU_{FCV} (5.6% TOC)						0.02	0.03	0.07	0.17	**1.45**	**2.35**	**4.31**	**7.70**
ESB-TU_{FCV} (26.8% TOC)						0.00	0.01	0.02	0.04	0.30	0.49	0.90	**1.61**
Metals (mg/kg dry weight sediment)													
Copper	34	270	32	94	280	20	19	18	20	21	18	18	19
Nickel	20.9	51.6	15	47	150	28	33	40	28	39	26	41	43

Survival of amphipods exposed to five oiled *S. alterniflora* soils is summarized in Table 9. Exposure to samples from AH-11, AH-6, and AH-2 (total PAH concentration 5.87, 8.51, and 43.94 mg/kg, respectively) did not have an effect on survival which ranged from 96 to 100%. Samples from AH-5 and AH-1, which contained total PAH concentrations of 156.4 and 178.4 mg/kg, were toxic to the amphipod.

Amphipod survival in seven oiled *S. cynosuroides* soils is summarized in Table 9. Exposure to samples from CH-6, CH-3, and CH-5 (total PAH concentration 2.18, 5.07, and 10.85 mg/kg, respectively), did not have an effect on survival, which ranged from 91 to 97%. Samples from CH-8, CH-11, CAH-10, and CH-10, which contained total PAH concentrations of 91.86, 139.7, 264.2, and 453.4 mg/kg, were toxic to the amphipod.

Copper and nickel were measured in the marsh soil samples used in the toxicity tests because there were concerns that these metals could be at concentrations that could contribute soil toxicity. The Chalk Point power plant had been implicated in early studies as a source of localized copper pollution from copper and nickel condenser tubing. Wright and Zamuda (1991) found high levels of both dissolved and particulate copper, with a high ratio of particulate: dissolved copper at the power plant's point of discharge.

The concentrations of copper in the marsh soils ranged from 14-21 mg/kg; nickel ranged from 26-46 mg/kg (Table 9). There is clearly no relationship between copper or nickel concentrations and the measured soil toxicity. The lowest concentration for both metals had the highest soil toxicity, which correlated with the highest total PAH concentration. Copper concentrations did not exceed the ERL of 34 mg/kg. Nickel concentrations did not exceed the ERM of 51.6 mg/kg in any of the marsh samples, but all the samples had nickel above the ERL of 20.9 mg/kg (Tables 10 and 11). Copper and nickel did not exceed USEPA's T80 or T50 toxicity probability concentrations in any marsh sample. Copper did not exceed the T20 in any marsh sample. These multiple lines of evidence do not suggest a meaningful contribution of copper and nickel to toxicity of marsh soils.

The ERL or ERM for total PAHs were not included because they are based on the 16 priority pollutant PAHs and do not include the alkylated homologues which dominate the PAHs in oils. Exceedences of ERM values by individual PAH analytes were accurate predictors of toxicity for individual PAHs in the *S. alterniflora* and *S. cynosuroides* marsh soils. The most toxic samples, causing ≥97% mortality (AH-1, CH-11, CAH-10, and CH-10), had multiple individual analytes present at concentrations exceeding their ERM. The slightly less toxic samples, AH-5 (85% survival) had two individual PAHs present that exceeded its ERM value, and CH-8 (66% survival) had one individual PAH exceeding an ERM. All other samples, from both the *S. alterniflora* and *S. cynosuroides* dominated marsh soils, were not toxic to *Ampelisca*. None of the non-toxic soils had individual compounds that exceeded ERM values.

The ESB-TU_{FCV} predictions of toxicity with TOC of 5.6% in the marsh soils closely matched the measured amphipod toxicity. Values less than 1.0 are not expected to have toxic effects, and all the samples with ESB-TU_{FCV} values less than 1.0 had >90% survival. The four samples with <3% survival had ESB-TU_{FCV} values of 2.35-7.70. Two samples had intermediate survival: CH-8 had 66% survival and ESB-TU_{FCV} of 1.45, and AH-5 had 85% survival and ESB-

TU_{FCV} of 2.56. The ESB-TU_{FCV} values calculated using the upper value of 26.8% TOC at did not correlate well with the actual measured toxicity in the soils. Only one sample was >1.0, even though toxicity was observed in six of the samples. These TOC levels include the roots and rhizomes; therefore, the lower TOC value of 5.6% is likely to be representative of the fine-grained organic carbon associated with the soils that affects the dissolution of PAHs in pore water.

Because of the high degree of vertical variability in the PAH concentrations in the upper and lower core intervals, a virtual 0-20 cm core was created by averaging the values for the 0-10 cm and 10-20 cm intervals. Then, the ESB-TU_{FCV} value was calculated using this average PAH concentration and the high/low values for TOC. Figure 29 shows the calculated ESB-TU_{FCV} values for the 26 "virtual 0-20 cm cores" for which PAHs were measured using the low and high TOC values; however, the values for the 5.6% TOC are considered to be the better predictor of toxicity in the Swanson Creek marsh soils.

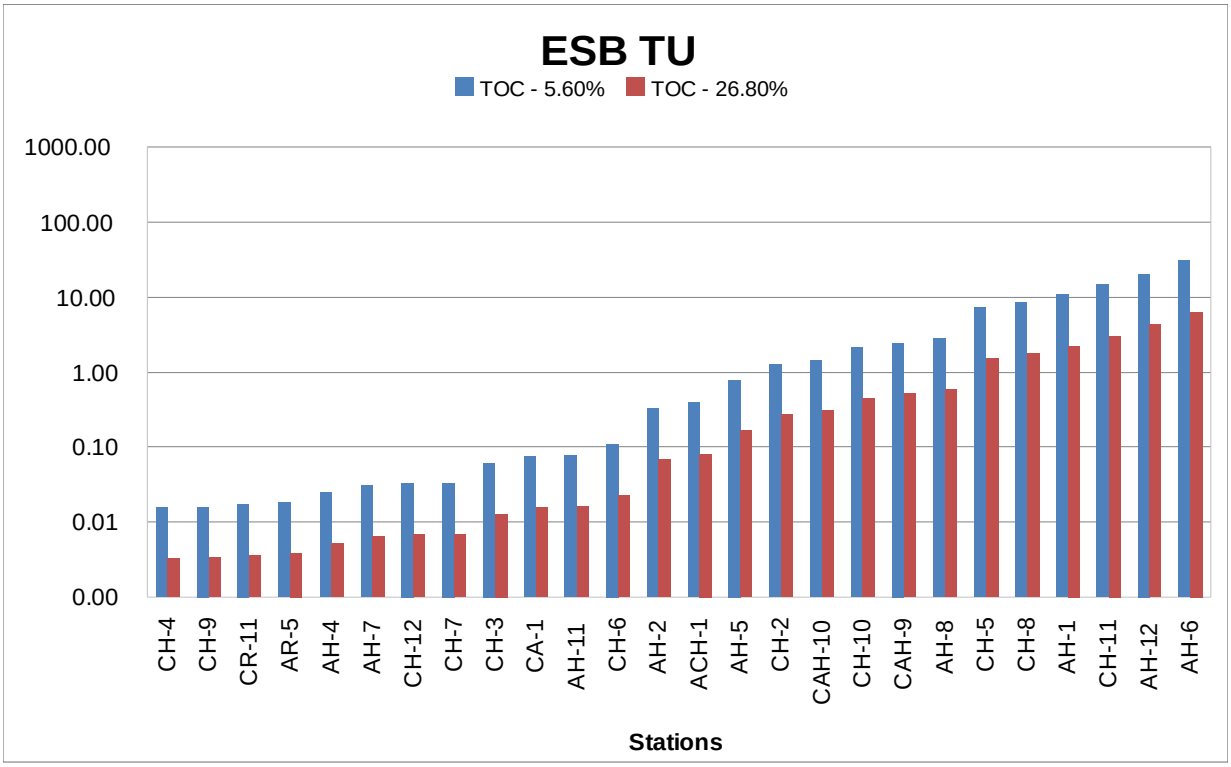


FIGURE 29. ESB-TU_{FCV} values (the sum of 34 PAHs averaged for the 0-20 cm interval) for the 26 marsh soil samples using both the low and high range of TOC reported in the marsh soils.

ESB-TU_{FCV} values for the oiled soils, assuming 5.6% TOC, ranged from 0.01-30.48, with the following distribution and predicted effects based on the toxicity test results:

- 54% (13/24) were <1.0; these soils were not toxic in amphipod toxicity tests and are not expected exhibit toxic effects.

- 8% (2/24) were between 1.0 and 2.0; one toxicity test in this range had 66% amphipod survival, thus these soils could have effects on sensitive organisms.

- 38% (9/24) were between 2.0 and 3.0; two toxicity tests in this range had 3% and 85% amphipod survival, thus soils are expected to have significant effects on sensitive organisms and effects on many organisms.

- 25% (6/24) were >3.0; three toxicity tests with values greater than 3.0 had 0-1% amphipod survival; these soils are expected to be toxic to many organisms.

The logistic modeling regression approach accurately predicted the toxicity observed in the *S. cynosuroides* soils for individual PAH and P_{Max}, though overestimated the toxicity of some of the moderately contaminated *S. alterniflora* soils. The underestimation may be attributable to the uncertainty in the actual TOC concentration in the *Spartina*-dominated marsh soils. USEPA Tp values were derived from a large database in which the average TOC content was 1.97% (USEPA, 2003). TOC concentrations were not measured in the current study; however, TOC measurements of samples of oiled marsh soils in 2000 ranged from 8.7 to 11.6% (B&B Laboratories, 2001, unpublished data) and in 2002 ranged from 5.6 to 26.8% (Mendelssohn and Slocum, 2004). The actual TOC concentration in a specific sample significantly affects the calculated toxicity, for both approaches.

Figure 30 shows plots of the percent amphipod survival test results versus the calculated ESB-TU_{FCV} values (top) and the maximum predicted probability (P_{Max}) of toxicity using the logistic modeling regression (bottom) for the "virtual 20-cm" cores, using 5.6% TOC. The plots clearly demonstrate the utility of both approaches, particularly with such a limited dataset.

Figure 31 shows the calculated P_{Max} values for the marsh soils for which PAHs were measured using the 5.6% TOC values, with different bars for the 0-10 cm, 10-20 cm and the "virtual 0-20 cm" intervals. Six of the 24 samples (25%) had a P_{Max} value greater than 0.65. According to Field (pers. comm., 2008), P_{Max} values = 0.65 had approximately 50% incidence of toxicity to marine amphipods in the national database used by Field et al. (2002) to derive the logistic regression model for sediment chemical mixtures. These same six samples also had ESB-TU_{FCV} values >3.0. Thus, the soils at 25% of the sampling sites contained PAHs at concentrations that pose significant toxic risks to benthic organisms.

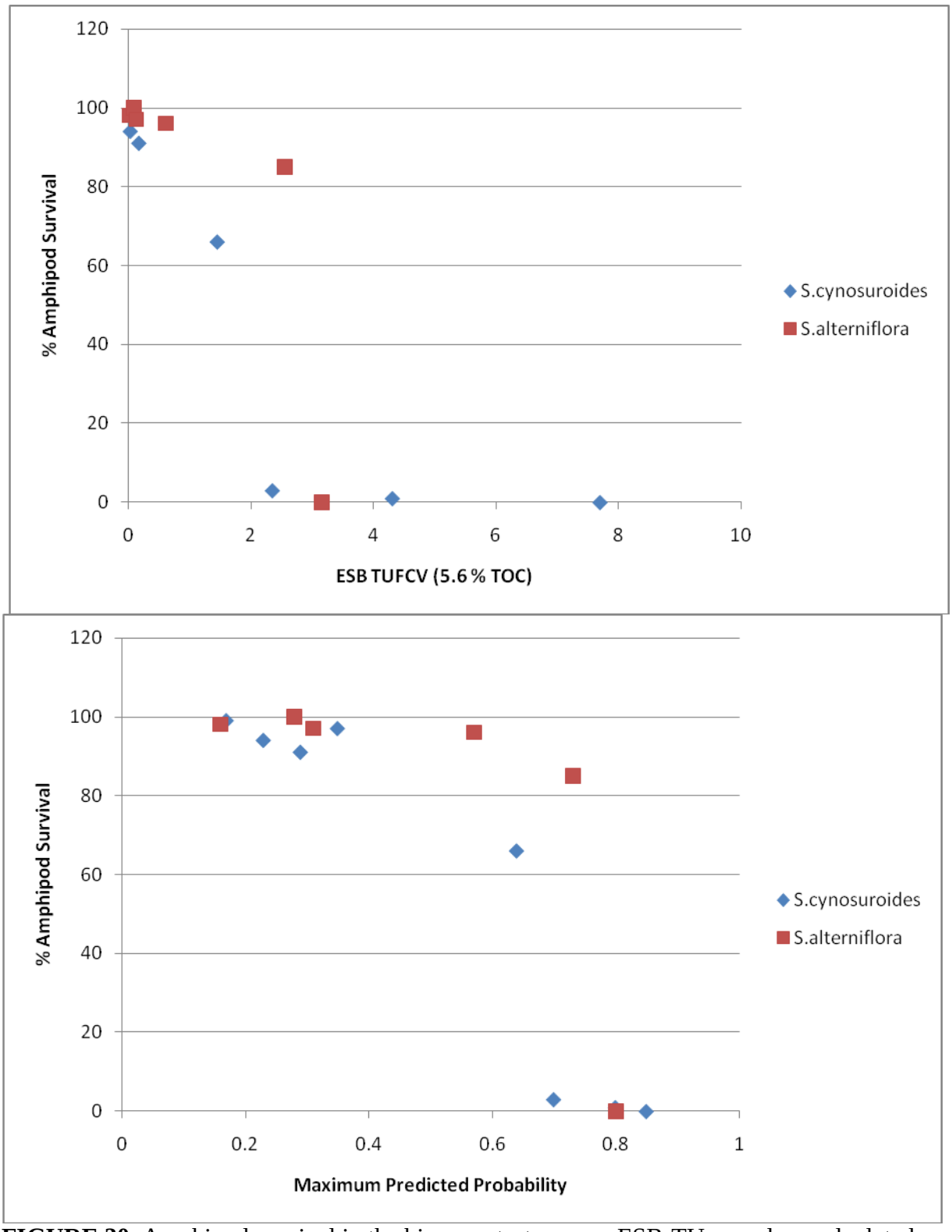


FIGURE 30. Amphipod survival in the bioassay tests versus ESB-TU_{FCV} values calculated as the sum of 34 PAHs (top) and the maximum predicted probability (P_{Max}) toxicity using the logistic modeling regression as the sum of 22 PAHs (bottom), for the "virtual 20-cm" cores by species, using 5.6% TOC.

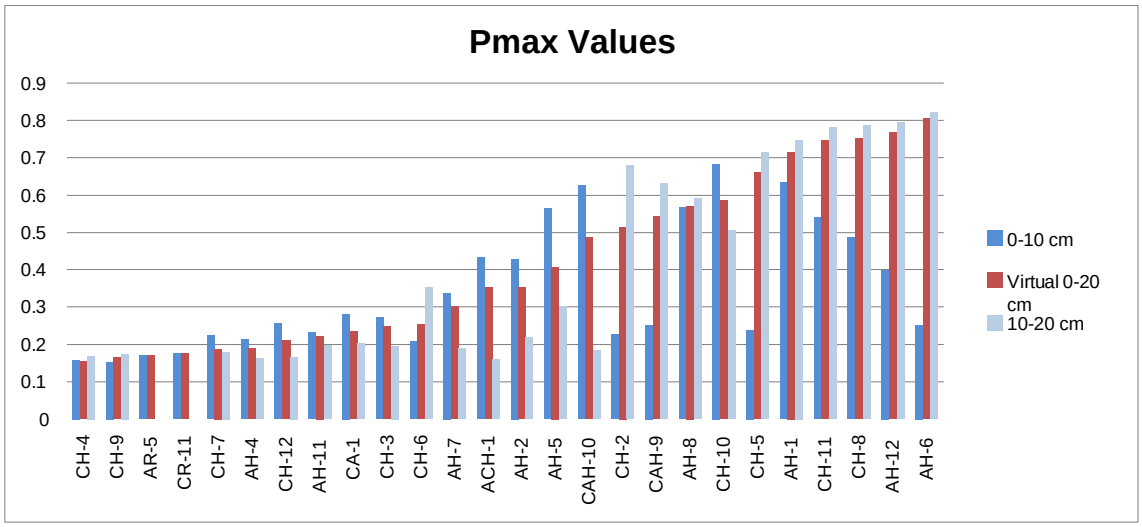


FIGURE 31. Maximum predicted probability (P_{Max}) toxicity values for the 26 marsh soil samples (assuming 5.6% TOC) for the individual 0-10 cm and 10-20 cm intervals and the "virtual 0=20 cm" interval, listed in order of increasing P_{Max} for the 0-20 cm virtual interval.

Discussion

This study was conducted seven years after the Chalk Point oil spill, focusing on the most heavily oiled interior brackish marsh habitats of *S. alterniflora* and *S. cynosuroides* in Swanson Creek . The spilled oil was a mixture of No. 6 fuel oil and No. 2 fuel oil. The oil had pooled on the marsh surface, particularly in open, unvegetated areas created by muskrat grazing. We avoided the area immediately adjacent to the pipeline break where aggressive cleanup was conducted. Sites were located in oiled marshes on either side of the release site where some cleanup efforts were conducted, using manual removal with sorbents by crews working from boardwalks. Sites were located at the very head of Swanson Creek where it was reported that little or no cleanup was attempted. Therefore, the results of this study are representative of these conditions.

The study site is characterized as a brackish-water marsh, with mixed stands of *S. alterniflora, S. cynosuroides, Typha* spp., and *Scirpus* spp., with minor occurrence of *Polygonum* spp. and *Amaranthus* spp. *S. cynosuroides* usually occurred as a continuous band of vegetation along creek banks and large zones throughout the marsh platform. *S. alterniflora* generally occurred as smaller patches interspersed in the marsh and it was harder to find mono-specific stands.

One important consideration is the composition of the spilled oils, because it was a mix of two separate oil types. Table 12 is a comparison of the oil spilled into Swanson Creek with the source oil from other spills of heavy refined products, showing the wide variability in total PAHs; however, most of the oils have naphthalenes as the most common PAHs. The Swanson Creek source oil had total PAHs that are somewhat higher than other heavy refined products, probably reflecting the additional contribution of the No. 2 fuel oil in the pipeline at the time of the release. However, it is not considered to be unusual in the total PAHs or the amount of naphthalenes present.

TABLE 12. Comparison of PAHs in heavy refined oil products (based on unpub. data from Environment Canada, Louisiana State University, and Alpha Woods Hole Group).

Oil Name	Total PAH (mg/kg)	% Naphthalenes in PAH
No. 6 fuel oil	8,070	54
IFO-180	8,646	43
Average of 4 Canadian bunker fuels	16,504	54
Low API Gravity oil (LAPIO)	21,322	58
Cosco Busan IFO-380	35,381	33
Swanson Creek oil	**36,600**	**50**
New Carissa bunker fuel	64,300	51

Based on analysis of the PAH results, the marsh soil samples can be divided into three groups:

1. Those with <1 mg/kg total PAHs that are dominated by pyrogenic hydrocarbons plus naphthalenes and represent "background."
2. Those with 1-8 mg/kg total PAHs that are a mixture of multiple sources of pyrogenic and petrogenic hydrocarbons, including the source oil from the Swanson Creek spill.
3. Those with >8 mg/kg that are dominated by petrogenic hydrocarbons that match the source oil from the Swanson Creek spill.

Half of the samples (24 out of 48) collected in 2007 fall into the third group, with about equal representation from both 0-10 cm and 10-20 cm intervals. The highest PAH concentrations were in soils from 10-20 cm, with all the samples containing >700 mg/kg being from the lower interval. At nine oiled sites, total PAHs increased with depth, usually by 1-2 orders of magnitude, indicating lower overall weathering rates with depth. At six oiled sites, total PAHs decreased with depth, with the concentration in the lower interval below 8 mg/kg, indicating that at these sites little oil penetrated beyond 10 cm into the soils. Five of these sites were in areas where there was no cleanup. No differences in PAH concentrations, distribution with depth, or degree of weathering were detected in samples from marshes that were manually cleaned or nutrients applied versus those that were reportedly not cleaned (at the very head of Swanson Creek), probably because of the high degree of spatial variability.

In the oiled areas, 15 out of 24 sites had total PAHs greater than 8 mg/kg in marsh soils (either depth interval). All but one of the samples with >8 mg/kg left a stain inside the biomass plastic bags (keeping in mind that separate cores were used to collect the chemistry and biomass samples). However, these observations indicate that sites in the third group have been contaminated by whole liquid oil. Only one of the samples with less than 8 mg/kg left a stain inside the plastic bag, supporting the conclusion that these sites had minor amounts of the spilled oil remaining.

Four sites in the oiled area had low total PAHs (less than about 2 mg/kg in both depth intervals) and no visible oil or only rainbow sheens observed in the field: AH-4, AH-7, CH-4, and CH-9. All of these sites were located close to the edge of visibly oiled areas in Figures 15 and 16 and likely were less oiled initially. Five sites in the oiled area had total PAHs in both core intervals that did not exceed 6 mg/kg and no visible oil or only rainbow sheen observed in the field: AH-11, CA-1, CH-3, CH-7, and CH-12. Two of these sites (CA-1 and CH-3) were established in 2000 in obviously oiled areas, and chemical analysis of the sediments showed high levels of oil and PAH contamination in both 2000 and 2001. It is likely that the oil at these sites has undergone significant natural removal, that there is significant spatial variation in the distribution of the oil in the marsh soils, or a combination of both.

As of 2007, the oil in the marsh soils tended to be less weathered with depth (Figs. 21, 22, and 23), although this is not a strong trend. Overall, the oil in the marsh soils has undergone little to no additional weathering since Fall 2000. Figure 32 shows the average and standard deviations for depletion ratios for total PAHs and individual PAH groups for the samples collected in 2000, 2001, and 2007 that contain greater than 8 mg/kg. Although there are many

differences in the sampling locations and depths for these three periods, the results support the conclusion that there has been very little change in the PAH distributions since the Fall of 2000.

In a study of the effectiveness of nutrient addition in stimulating microbial degradation of the oil in two marsh areas, Entrix (2002) sampled the top 5 cm at six stations two times during 2000 (July and September) and 21 stations five times during 2001 (May, June, August, September, and October). Total PAH concentrations in 2000 were on the order of 1,800-2,600 mg/kg, whereas they averaged 482 mg/kg in October 2001 (range 1.34-3,068 mg/kg). Entrix (2002) noted that most of the weathering occurred by September 2000, in the first six months after the spill. This weathering likely occurred while the oil was pooled on the marsh surface. By Spring 2001, there were few changes in the overall degree of PAH weathering (compared to September 2000). By October 2001, there was again little change in the overall PAH weathering or depletion ratios, but there was more variability within areas. There were no differences in PAH weathering in the surficial soils between those sites treated with nutrients compared to those without nutrient addition, indicating that nutrients were not a limiting factor in microbial degradation in these marshes.

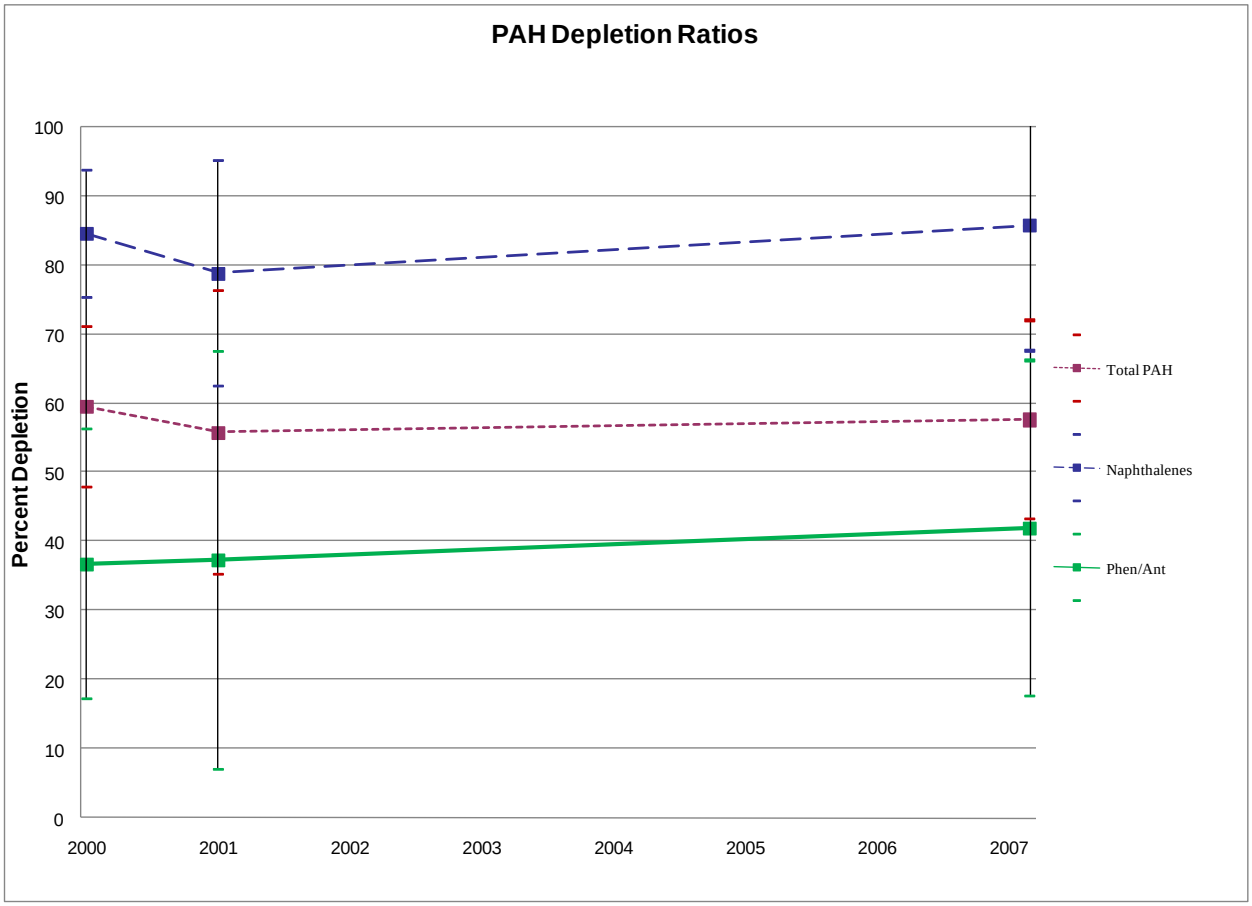


FIGURE 32. Mean and standard deviation PAH depletion ratios for total PAH, naphthalenes, and phenanthrenes/anthracenes in the Swanson Creek oiled marshes in Fall 2000 (n=9), Summer 2001 (n=14), and Summer 2007 (n=24) that contain more than 8 mg/kg.

Figure 33 shows a plot of total PAH % depletion versus total PAH concentrations in two sets of marsh soil samples: 21 samples from 0-5 cm collected in October 2001 as part of the

nutrient biostimulation monitoring and 24 samples collected in August/September 2007 that exceeded 8 mg/kg in this study from both 0-10 cm and 10-20 cm intervals. It should be noted that 15 of the 21 samples collected in 2001 were from the same general areas sampled in 2007. Even though they are from different depths for the two sampling periods, the similarity in the degree of PAH weathering is remarkable. Both sample sets exhibit a clear relationship between total PAH concentrations and the degree of PAH degradation. There appears to be more degradation of moderately oiled soils in 2001 when the sampling interval was 0-5 cm compared to 2007 when the sampling interval was 0-10 cm, suggesting that degradation processes were faster at the surface. Entrix (2002) reported that percent depletions for most of the n-alkanes from n-C_{12} to n-C_{34} were greater than 95% by October 2001, which indicates that microbial degradation was occurring in these surface soils.

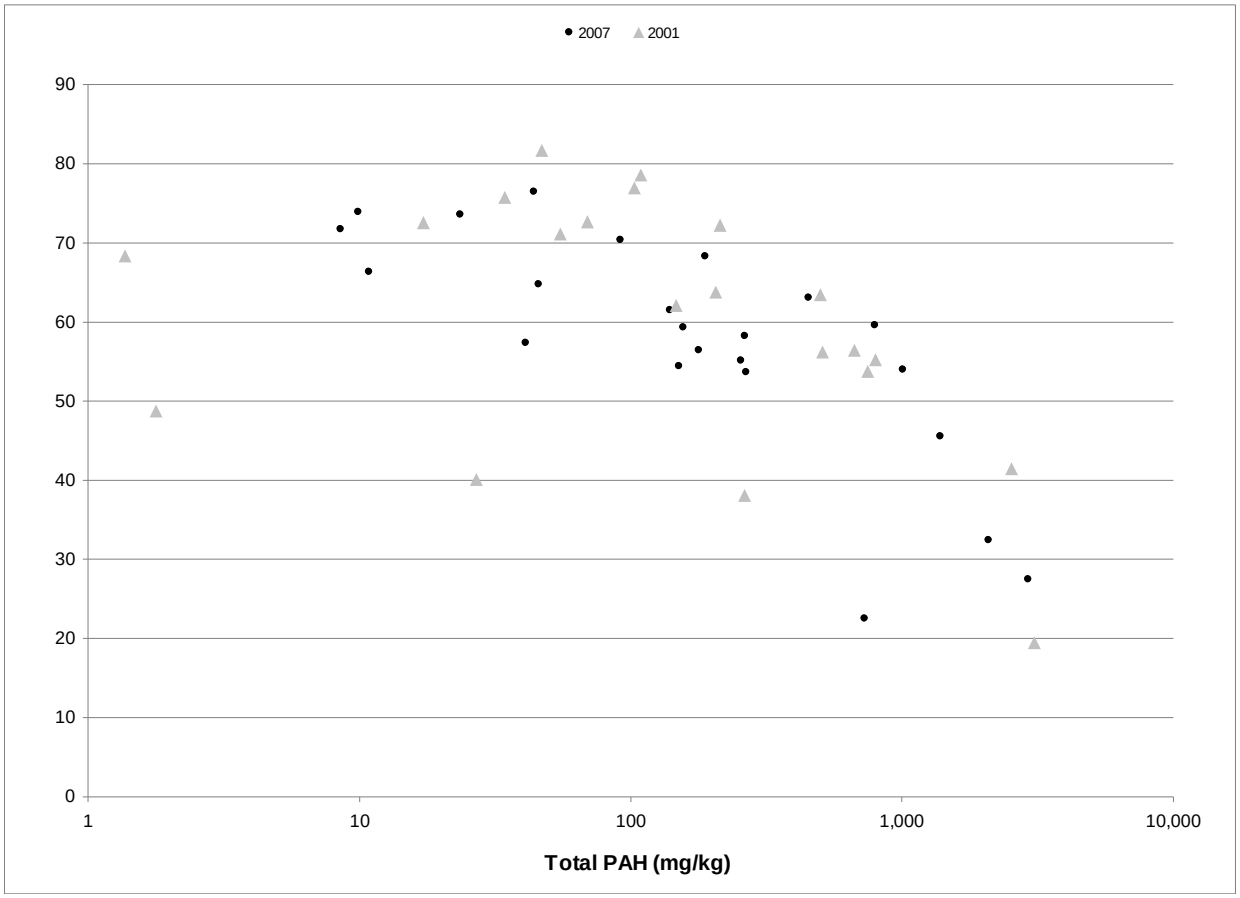


FIGURE 33. Comparison of total PAH depletion ratios for samples collected in 2001 vs. 2007.

Based on the PAH data reported by Entrix (2002) and discussed in this report, it appears that there has been very little weathering of the PAHs in the marsh soils since Fall 2000. What are the limiting factors to natural weathering processes in the marsh soils? There are likely two: slow physical removal processes and low oxygen availability. The interior marsh habitat is flooded by daily tides through many small channels. During spring tides, there can be 20-30 cm of water in the marsh. The marsh surface has a lot micro-topography with low areas between

dense clumps of stems that hold pools of water during low tide. Sediments in these low areas are soft and water saturated. Obviously, during spring low tides, the marsh soils do drain as low as 30 cm, because the oil penetrated to these depths in some areas. The falling tide drains through dense vegetation. Tidal flushing may have been an initial mechanism for removal of bulk oil stranded on the surface; however, it would not be effective at mobilizing oil from below the marsh surface. There are few bioturbating benthic organisms in these marshes. Photo-oxidation does not occur below ground. The only other removal mechanism would be microbial degradation.

Mendelssohn and Slocum (2004) measured the soil oxidation-reduction intensity (Eh) in Swanson Creek soils as part of their study of soil cellulose decomposition. At seven of eight sites, the Eh at 2 cm averaged -41 milliVolts (mV) (range of 31$\pm$62 to -130$\pm$3 mV); at 15 cm it averaged -84 mV (range of 0$\pm$24 to -137$\pm$30 mV). Thus, the soils are highly anaerobic. Zhu et al. (2001), in the U.S Environmental Protection Agency guidance manual for bioremediation of oil in freshwater wetlands, state that "…in freshwater wetland environments, petroleum degradation is likely to be limited by oxygen availability." In some of the case studies cited, no enhanced oil degradation occurred in anaerobic soils even when nutrients were added. Mendelssohn and Lin (2002) showed that nutrient addition to *S. alterniflora* marsh cores only increased PAH degradation in the top 5 cm in treatments where oxidants were applied in addition to bioremediation agents under drained conditions. There were no differences when the cores were flooded. Reddy et al. (2002) found that the oil from the West Falmouth spill persisted in marsh soils at depths of 6-28 cm for 30 years, and only n-alkanes and the more water-soluble and volatile compounds had been lost.

With few mechanisms in the marsh interior for physical removal of the oil, and the slow rate of degradation expected under anaerobic conditions, it is likely that the oil will persist in these areas of the marsh for decades. Overall degradation rates in the surface soils may be faster where respiration by roots and tidal flushing can be a source of oxygen.

The impact of the oil on vegetation condition seven years post spill varied by species. For *S. alterniflora*, stem density and stem height were significantly lower in oiled sites compared to unoiled sites, but no differences were seen for belowground biomass. For *S. cynosuroides*, the results were opposite, with belowground biomass significantly lower in oiled sites but not stem density and stem height. The reasons for these differences (other than that power was too low, leading to a false conclusion) may be related to the relative distribution of above- versus belowground biomass and the types of biomass for each species. The study by Schubauer and Hopkinson (1984) of both species in a brackish marsh in Georgia provides interesting insights to possible factors. Table 13 compares the key characteristics of each species.

Because *S. cynosuroides* has more and larger rhizomes, and the rhizome biomass has a peak at 10-20 cm, this species may be more exposed to oil that is at higher concentrations and more persistent in the cavities along the rhizomes. Some of the black oil that we observed in the cores occurred along rhizomes, some of which were hollow and dead. Figure 18 is a good example; note the heavily oiled hollow rhizomes in the lower photograph which is the top of the

TABLE 13. Characteristics of each species that might be a factor in oil exposure and response.

Characteristic	*S. alterniflora*	*S. cynosuroides*
Ratio of below- to aboveground biomass	3.5	2.3
Ratio of live-to-dead aboveground biomass, annual	0.52:1	0.24:1
Peak aboveground biomass	July (733 g/m^2)	October (1,234 g/m^2)
Ratio of live-to-dead belowground biomass	0.20:1	0.26:1
% live belowground biomass made up of rhizomes in growing season	53%	90-94%
Vertical distribution of rhizomes	Relatively even throughout 0-40 cm	Strong peak at 10-20 cm

10-20 cm core. Roots and rhizomes in the soil would grow until they encountered zones of oil that would slow growth and could eventually lead to death. *S. alterniflora* has about an equal proportion of roots to rhizomes and the rhizomes are smaller, so any reductions in the biomass of the rhizomes may have had a lesser effect on the overall belowground biomass. Alternatively, the lower belowground biomass of *S. alterniflora* may be in less direct contact with the oil. In the only published study we found on the effects of oil on *S. cynosuroides*, Ferrell et al. (1984) conducted greenhouse experiments with both *S. alterniflora* and *S. cynosuroides*. They suggested that the impacts of oil added to the substrate to both species were caused by decreased root and rhizome growth, but they did not note any differences between *S. alterniflora* and *S. cynosuroides* in the short-term test results. With live root turnover of about 1-2 years (Schubauer and Hopkinson, 1984), the reduced belowground biomass observed in *S. cynosuroides* habitats would reflect current stress from exposure to the residual oil.

Several studies have determined the oil concentrations that could affect salt marsh species, mostly in controlled experiments. Most of these studies report total oil loading either as the amount of oil mixed into the soils prior to transplanting, or applied to the soil surface and thus reported as volume/area. Few studies reported the PAH concentrations. However, it is clear it can take relatively high levels of oil in marsh soils to affect plants. Alexander and Webb (1987) found that it took greater than 10,500 mg/kg of a light crude oil to affect stem density of *S. alterniflora*. Krebs and Tanner (1981) found that 2,000 mg/kg of a No. 6 fuel oil spill in the Potomac River had no effect or a slight positive effect on *S. alterniflora* aboveground metrics, but 10,000 mg/kg resulted in mortality.

Lin et al. (2002) homogeneously mixed fresh No. 2 fuel oil into marsh soils and transplanted *S. alterniflora* stems that were free of soil and rhizomes into different oil dosages. They found significant reductions in belowground biomass (about 50% decrease) after three months of exposure at oil dosages greater than 29,000 mg/kg dry soil, which equated to PAH concentrations greater than 1,600 mg/kg. However, they saw impacts to aboveground biomass, stem density, and shoot height at above 57,000 mg/kg oil. They also observed significant

stimulation of belowground biomass at the 7,000 mg/kg oil dose rate, which equated to about 400 mg/kg PAHs. Other studies have also reported stimulated growth (Hershner and Moore, 1977; Lin and Mendelssohn, 1996). However, it is difficult to compare exposure to a No. 2 fuel oil that was well-mixed in the soil in their greenhouse experiment with the highly variable oil distribution in the Swanson Creek marsh soils. The measured PAH concentrations in the collected sample from a site is only a general indication of the degree of oiling in the marsh soils; it is not representative of the dose to the roots and rhizomes because the oil was highly concentrated in some cavities. Thus, only a fraction of the roots and rhizomes are actually exposed, and the exposure would be high enough to cause mortality and prevent re-growth.

Mendelssohn and Slocum (2004) did not find any effect of oil on decomposition rates of organic matter in the marsh soils in Swanson Creek, as measured using the cellulose (cotton) strip technique in a study conducted in August 2002, two years after the spill. Their study consisted of seven oiled sites of varying degrees of oiling and one reference site. Total PAH concentrations were comparable to those found in 2007, although they reported a significant decrease in oil with depth at their sites. Other naturally occurring environmental factors appeared to control decomposition rates, such as salinity, pH, and depth. Our study also suggests that decomposition rates have not been affected, in that the belowground biomass is lower in the oiled areas. To be more definitive, measurements of both live and dead belowground biomass would be needed.

Oiled *S. alterniflora* sites had reduced stem density and stem height compared to unoiled sites whereas *S. cynosuroides* did not. Reduced stem density and height are commonly reported in the literature for oiled *S. alterniflora* (e.g., Krebs and Tanner, 1981; Lin and Mendelssohn, 1996). The denser, shorter *S. alterniflora* vegetation may be more sensitive to oil exposure, compared to the tall and rigid culms of *S. cynosuroides*.

The fourteen amphipod toxicity tests correlated well with the predicted toxicity using ESB-TU_{FCV} values, with samples showing some toxicity at values between 1.0-2.0 and 0% survival at values >3.0. Extrapolating predicted toxicity from ESB-TU_{FCV} values for the average PAHs in the 0-20 cm interval to the 24 heavily oiled sites, 11 sites or 46% had a value >1.0 and thus are likely to cause toxic effects. However, 25% of the sites had a value >3.0 and thus contained PAHs at concentrations that could cause chronic toxicity even seven years after the spill. The toxicity tests also correlated well with the maximum predicted toxicity, P_{Max}, using the logistic regression model approach. These results are of particular value for long-term monitoring studies of oiled marshes; first, because toxicity is seldom monitored, and second, because the surficial soils (0-10 cm) in some areas still exhibit toxicity after seven years. With the slow rates of physical removal and microbial degradation of the oil that penetrated into the marsh soils, it is likely that the toxicity will also persist.

Comparison with Predicted Service Losses

It is of value to compare the 2007 study results with the predicted service losses for the vegetation and soils that were developed during the NRDA. The 2007 study sites were located only in heavily oiled, interior habitats. The recovery curve inputs for in Table 1 were converted

to the predicted services present for the two marsh types in 2007, shown in Table 14. The 2007 study results are summarized as the ratio of the mean value for oiled sites to that for the unoiled sites, without any consideration for statistical significance. It seems that the predicted service losses for *S. alterniflora* vegetation recovery were underestimated and for *S. cynosuroides* were overestimated. The predicted soil service losses appear to be supported by the 2007 results in that *S. alterniflora* habitats showed no reductions in belowground biomass but 40% of the sites are predicted to still have some toxicity. The *S. cynosuroides* habitats, however, have both a reduced belowground biomass and slightly larger percentage of sites with toxicity.

TABLE 14. Comparison of the predicted services present as of 2007 and the 2007 actual results for the marsh and soil services in the interior, heavily oiled habitats.

Resource Category	Predicted Services Present in 2007	2007 Results (Ratio = Value Oiled /Unoiled Sites)
Vegetation		
- *S. alterniflora* interior heavy	100%	Stem density = 63% Stem height = 85%
- *S. cynosuroides* interior heavy	83%	Stem density = 105% Stem height = 94%
Soils		
- *S. alterniflora* interior heavy	85%	ESB-TU_{FCV} values in soil samples 40% >1.0 40% >2.0 30% >3.0 Belowground biomass = 98%
- *S. cynosuroides* interior heavy	57%	ESB-TU_{FCV} values in soil samples 50% >1.0 36% >2.0 21% >3.0 Belowground biomass = 78%

Recommendations for Future Studies

It is recommended to continue monitoring the oiled marshes in Swanson Creek to track the physical removal and degradation of the oil in the marsh soils. Such data provide the basis for estimation of the impacts and recovery rates for future spills in brackish marshes, a habitat type for which there are very few case studies. The only published reports on the impacts of oil on *S. cynosuroides* were the greenhouse experiments of Ferrell et al. (1984) and the Swanson Creek studies. There are very few good monitoring studies for interior oiling. The 2007 monitoring results indicate that the residual oil has undergone only limited degradation since 2000. It is recommended that the sites be monitored at ten years post-spill and include PAH loading and weathering in marsh soils, vegetative condition indicators, and soil toxicity tests. This triad

provides the linkages between oil exposure and effects to both the habitat and biota who utilize it, answering the "so what?" question about oil persistence deep in marsh soils.

Vegetation health metrics in future studies should include measurement of both live and dead belowground biomass. We have hypothesized that the rhizomes of *S. cynosuroides* continue to be affected by the residual oil in the marsh soils. It would be important to determine the basis for the differences in impacts between the two *Spartina* species.

Soil samples should be analyzed for total organic carbon, grain size, and water content. An evaluation should be made on whether or not to include the roots and rhizomes in the total organic carbon analysis. The issue is whether carbon in the belowground biomass has effects on octanol:water partition coefficients and thus on the calculation of sediment quality benchmarks.

Future field studies should include excavation of trenches in the marsh soils to get better visual observations on the vertical distribution of the oil. This information would then be used to develop a conceptual model explaining the considerable vertical heterogeneity. Such a model would then be used in future sampling designs that would address these issues (e.g., more composite samples for chemical and toxicity analyses).

Because the Chalk Point site has the potential to provide long-term monitoring results for multiple uses, it is recommended that NOAA convene a group of technical staff and managers to discuss possible topics such as: 1) determining recovery curves for sediment toxicity and vegetation metrics; 2) optimizing sample/statistical design for spill response; 3) studying oil degradation; 4) consideration of including synoptic sampling of the benthic community to verify predicted toxicity from the amphipod toxicity tests and PAH concentrations; 5) expanding the study area to include additional species (e.g., *Typha*) and other degrees of oiling; 6) including photo-activation in the toxicity studies; 7) monitoring for habitat restoration success (saltmarsh and oyster reef); and 8) outreach opportunities.

Future efforts should incorporate additional tests species and procedures to more accurately assess sediment quality. For example, there is a 17-day *A. abdita* survival and growth test that provides growth as a chronic endpoint. Also, it is recommended that future testing encompass both lethal and sublethal (development and reproduction) endpoints. One ecologically important lower trophic-level community of high potential value in toxicology is the rapidly reproducing meiobenthos. Meiobenthos play an important role in the cycling of carbon and nutrients, and they provide an important food source for larger invertebrates and juvenile fish species in estuarine and marine ecosystems (Coull, 1990; Gee, 1989). Critical components of the meiobenthic community are copepods, a taxa that comprises 10 to 40% of this community and maintains the structure of marine and estuarine food webs (Coull, 1990; Gee, 1989). Over the last decades, reliable and cost effective tests have been successfully adapted to several copepods species including the copepod *Amphiasucs tenuiremis*. A test that is highly desirable to assess sediment quality (described in Chandler and Green, 1996) and that has been consistently implemented (Kovatch et al., 1999; Bejarano et al., 2004) is the *A. tenuiremis* 14-day chronic life cycle test. Through this test multiple endpoints are quantified simultaneously (i.e., stage specific survival and reproductive outputs) under logistically simple and reliable laboratory conditions.

Summary

The brackish marshes in Swanson Creek heavily oiled by the April 2000 spill of 140,000 gallons of a mixture of No. 6 and No. 2 fuel oils have not recovered after seven years. Background concentrations of PAHs were <1 mg/kg dry weight, and samples with 1-8 mg/kg PAHs were considered to contain mixed pyrogenic and petrogenic sources. All of the samples with more than 8 mg/kg PAHs were determined to be contaminated with the spilled oil using various fingerprinting approaches.

The oil remaining in soils has high vertical and horizontal heterogeneity, and liquid black oil was concentrated along root and rhizome cavities. Total PAH concentrations in the soils in the heavily oiled marsh ranged from as low as 0.58 mg/kg to as high as 2,920 mg/kg. At the 24 sites sampled, PAH concentrations in the 0-10 cm and 10-20 cm intervals increased with depth at nine sites, decreased with depth at six sites, and were at or close to background in both depths at nine sites. Because of this high variability, the PAH concentrations in the two depth intervals were averaged to create a "virtual" 0-20 cm for analysis of predicted toxicity.

Total PAH degradation in the samples containing more than 8 mg/kg PAHs ranged from 22-77%, with 19 out of 23 samples in the range of 54-77%. The oil was less weathered with depth; all of the samples with less than 55% total PAH depletion were from the deeper interval of 10-20 cm. The depletion ratios for total naphthalenes (2-ringed PAHs) ranged from 60-98%, whereas total phenanthrenes/anthracenes (3-ringed PAHs) ranged from 20-80%. The 4-ringed PAHs (fluoranthenes, pyrenes, and chrysenes) showed only 10% depletion. These patterns reflect the decreasing rate of microbial degradation with increasing molecular weight and number of rings. The samples with the highest PAH concentrations had the lowest depletion ratios. PAH depletion ratios for total PAH, total naphthalenes, and total phenanthrenes/ anthracenes for samples collected in 2000 and 2001 were either the same or only slightly lower than samples collected in the same general areas in 2007. Therefore, the oil in the marsh soils has undergone minimal further degradation since the Fall of 2000, a period of seven years.

Impacts to vegetation varied by species. *S. alterniflora* in the oiled areas had reduced stem height (37% lower) and density (15% lower), but no detectable differences in belowground biomass, compared to unoiled areas. *S. cynosuroides* in the oiled areas was the opposite, with no detectable differences in stem height or density, but reduced belowground biomass (21 and 22% in the 0-10 and 10-20 cm intervals). These different responses likely reflect the different characteristics of each species.

Toxicity test results (amphipods, 10-day mortality endpoint) correlated well with the predicted toxicity using the ESB-TU_{FCV} values (sum of 34 PAHs) using the estimated value of 5.6% TOC. Using this sediment quality benchmark, 46% of the samples had ESB-TU_{FCV} values >1.0, indicating some degree of toxicity; 38% were >2.0, indicating a moderate risk of toxicity. However, 25% of the samples had ESB-TU_{FCV} values >3.0 and thus exhibit significant toxicity seven years after the spill.

The Chalk Point spill site provides a good opportunity to monitor oil persistence and effects in a brackish marsh habitat. Recommendations have been made to improve the value of further studies.

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APPENDIX A

Statistical Power Analysis

Monitoring of Recovery of Marshes Impacted by the Chalk Point Oil Spill
Statistical Power Analysis

Zach Nixon, Research Planning, Inc.

Introduction

Field and laboratory studies will be conducted to assess the recovery status of the wetlands impacted by the April 2000 spill of an estimated 126,000 gallons of a mixture of No. 6 and No. 2 fuel oils into Swanson Creek off the Patuxent River from a ruptured pipeline feeding the nearby Chalk Point Power Generating Station. Recovery status will be evaluated via three classes of metric: below-ground biomass, stem density, and stem height, persistence and weathering status of polycyclic aromatic hydrocarbons (PAH) in marsh soils, and amphipod toxicity tests. Similar but reduced in scope studies will be conducted at a restoration marsh site, which will be an important step in documenting the services provided by the restoration project.

The statistical power of these studies to evaluate hypotheses related to differences in oiled and unoiled marshes is of concern when examining recovery status. This investigation examines the statistical power of proposed tests of variables related to marsh structure and function. Specifically:

1.) below-ground biomass
2.) stem density
3.) stem height

Existing Data

During the wetland injury assessment studies conducted in 2000 and 2001, stations (n=3) were established in four wetland types (*Spartina alterniflora, S. cynosuroides, Typha spp.*, and *Scirpus spp.*), two habitats (interior and shoreline), and two degrees of oiling (heavy and moderate). The study site locations were selected to be representative of each combination of factors which were used to define eight habitat categories. At each of the study sites, the following data were collected: percent vegetative cover, stem density stem height by species, water depth, distance to the shoreline edge, oil observations on vegetation and soils, biota present, and sediment cores for chemical analysis of total petroleum hydrocarbons (TPH) and polycyclic aromatic hydrocarbons (PAH).

Proposed Study Design

The initially proposed sampling design reexamined all the existing sites for heavily oiled *S. alterniflora* and *S. cynosuroides*, both shoreline and interior, and included three additional sites within each of these categories for a total n = 6 per category. Six sites were to be included in restored areas as well. Table A-1 enumerates proposed sites by category for this sampling design.

TABLE A-1. Proposed sampling sites by category.

Category	Existing Sites	New Sites	Total Sites
S. alterniflora, heavy, interior	3	3	6
S. alterniflora, heavy, shoreline	3	3	6
S. cynosuroides, heavy, interior	3	3	6
S. cynosuroides, heavy, shoreline	3	3	6
S. alterniflora, reference, interior	3	3	6
S. alterniflora, reference, shoreline	3	3	6
S. cynosuroides, reference, interior	3	3	6
S. cynosuroides, reference, shoreline	3	3	6
Restored marsh, interior	0	6	6

At each site, the following variables will be measured: below-ground biomass via coring, and stem density and stem height as surrogates for above-ground biomass. At each site, sediment cores for chemical analysis of PAH and toxicity testing will also be collected, but these variables are not part of this analysis.

Statistical Power Analysis

The statistical power of a test is the probability that a significance test at a fixed significance level will reject the null hypothesis given a particular alternative value of the parameter of interest. In estimating prospective statistical power, the following pieces of information are required: the significance level (α), the sample size, an estimate of variation, and the size of effect we are interested in – the alternative value referred to above. This investigation examines the statistical power of two-sample, one-sided t-tests carried out between oiled and control groups in each of the above categories. Sample size is derived from the study plan, although the effects of variable sample sizes are also investigated. Power is evaluated in each case at three different significance levels: 0.05, 0.1 and 0.2. It should be noted that evaluation of power is based on the parametric t-test, which can only be used when the data are normally distributed. Ecological data are often not normally distributed, indicating that nonparametric tools may be required to investigate recovery status rather than the t-test.

Variability in plant height and stem density can be estimated directly from those data collected previously. Table A-2 contains means and pooled standard deviations for all sites, by species, from 2000 and 2001 that will be revisited. Variability for below-ground biomass, which was not measured in 2000 or 2001, must be estimated from literature. Table A-3 contains means and calculated or reported standard deviations for *S. alterniflora* and *S. cynosuroides* below-ground biomass from several literature sources.

The effect sizes of interest are more difficult to estimate. Data from 2000 and 2001 indicate TPH concentrations at oiled sites ranging up 40,000 ppm. Lin *et al.* (2002) report experimental results that indicate decreases of below-ground biomass of approximately 40% of control at similar crude oil concentrations. As such, a minimum effect size of 40% of the mean is used here. It is important to note that effects sizes calculated thusly are somewhat arbitrary. Other effects sizes, might be selected, such as a different percentage of the mean, or a fixed number.

TABLE A-2. Sample means ± 1 standard deviation (pooled) from 2000 and 2001 by species for above-ground biomass proxy variables.

Species	Height (m)	Density (ind. / m^2)
S. alterniflora	1.13 ± 0.21	254 ± 160
S. cynosuroides	1.61 ± 0.43	140 ± 101

TABLE A-3. Literature reported means ± 1 standard deviation (calculated from SE in parentheses) for below-ground biomass variables. * indicates values estimated from graphs. Means for total below-ground biomass for *S. alterniflora* are sums of live and dead reported values.

Species	Source	N	Live (g/m^2)	Dead (g/m^2)	Total (g/m^2)	Loc.
S. alterniflora (tall)	Gross *et al.*, 1991	29	1044 ± 414 (77)	2968 ± 2563 (476)	4012	DE
S. alterniflora (short)	Gross *et al.*, 1991	68	913 ± 527 (64)	5925 ± 2111 (256)	6838	DE
	Gross *et al.*, 1991	25	893 ± 265 (53)	4320 ± 2225 (445)	5213	VA
S. cynosuroides	Hackney *et al.*, 1986*	24			7500 ± 1469 (300)	MS

Lin *et al.* also report that there were no significant differences in stem density, stem height, or actual above ground biomass across those same oil concentrations. Previous investigations in 2000 and 2001 demonstrated no significant differences in plant height or stem density between oiled and unoiled areas, but similar effects sizes for those variables are calculated here for completeness. Table A-4 contains the final variables used in this power analysis. Note that because no direct figures were available or could be calculated, the estimated standard deviation for *S. alterniflora* was calculated from the estimated mean and the average coefficient of variation (0.5) of all below ground biomass variables for that species.

TABLE A-4. Values used in power calculations.

Species	Variable	Mean	Effect Size (40% of mean)	SD
S. alterniflora	Height (m)	1.13	0.45	0.21
	Density (ind/m^2)	254	102	160
	Live biomass (g/m^2)	950	380	400
	Dead biomass (g/m^2)	4400	1760	2300
	Total belowground biomass (g/m^2)	5350	2140	2675
S. cynosuroides	Height (m)	1.61	0.64	0.43
	Density (ind/m^2)	140	56	101
	Total belowground biomass (g/m^2)	7500	3000	1469

Figure A-1 contains results of the power analysis as power-effect size curves for fixed sample size (n=6) at three significance levels for above-ground biomass proxy variables. Figure A-2 displays similar power-effect size curves for below-ground biomass variables. Vertical lines in Figure A-2 indicate the anticipated effect size. These figures should be used to interpret the effect on statistical power of attempting to detect different sized effects using the proposed fixed per group sample size.

Results indicate that power is below optimal for investigating differences in dead below-ground biomass of *S. alterniflora* and marginal for investigating differences in live below-ground biomass of that same species. Power is adequate for investigating differences in below-ground biomass of *S. cynosuroides.*

Figure A-3 and Figure A-4 display power-sample size curves for fixed effect size at three significance levels for above-ground and below-ground biomass variables respectively. These figures should be used to interpret the effect on statistical power of changing the per group sample size while detecting a fixed effect size. Table A-5 lists the minimum sample sizes necessary to achieve 80% power given the postulated effects sizes.

TABLE A-5. Minimum sample size to achieve 80% power given postulated effects sizes at three different significance levels.

Species	Variable	N (α=0.2)	N (α=0.1)	N (α=0.05)
S. alterniflora	Height (m)	2	3	4
	Density (ind/m^2)	15	23	32
	Live biomass (g/m^2)	7	11	15
	Dead biomass (g/m^2)	10	16	22
	Total biomass (g/m^2)	10	15	21
S. cynosuroides	Height (m)	3	5	7
	Density (ind/m^2)	19	30	41
	Total biomass (g/m^2)	2	3	4

Note that the initially proposed per group sample size (n=6) does not give sufficient power for several variables at any of the significance levels given the proposed effects sizes. Investigations of data from 2000 and 2001 reveal that there are almost no significant differences between the above ground biomass proxy variables between shoreline and interior sites. Figure 5 and Figure 6 display box plots for above ground proxy variables from 2000 and 2001 by location. As such, it is recommended that the shoreline and interior groups be merged to increase the per group sample size to 12. This will provide sufficient power at the α=0.2 significance level for all variables except stem density. Combining live and dead belowground biomass measurements for *S. alterniflora* or only evaluating live below-ground biomass may enable sufficient power at the more rigorous α=0.1 significance level for most variables. Table A-6 contains revised proposed per group sample sizes.

TABLE A-6. Revised sampling sites by category.

Category	Existing Sites	New Sites	Total Sites
S. alterniflora, heavy	3	9	12
S. cynosuroides, heavy	3	9	12
S. alterniflora, reference	3	9	12
S. cynosuroides, reference	3	9	12
Restored marsh, interior	0	6	6

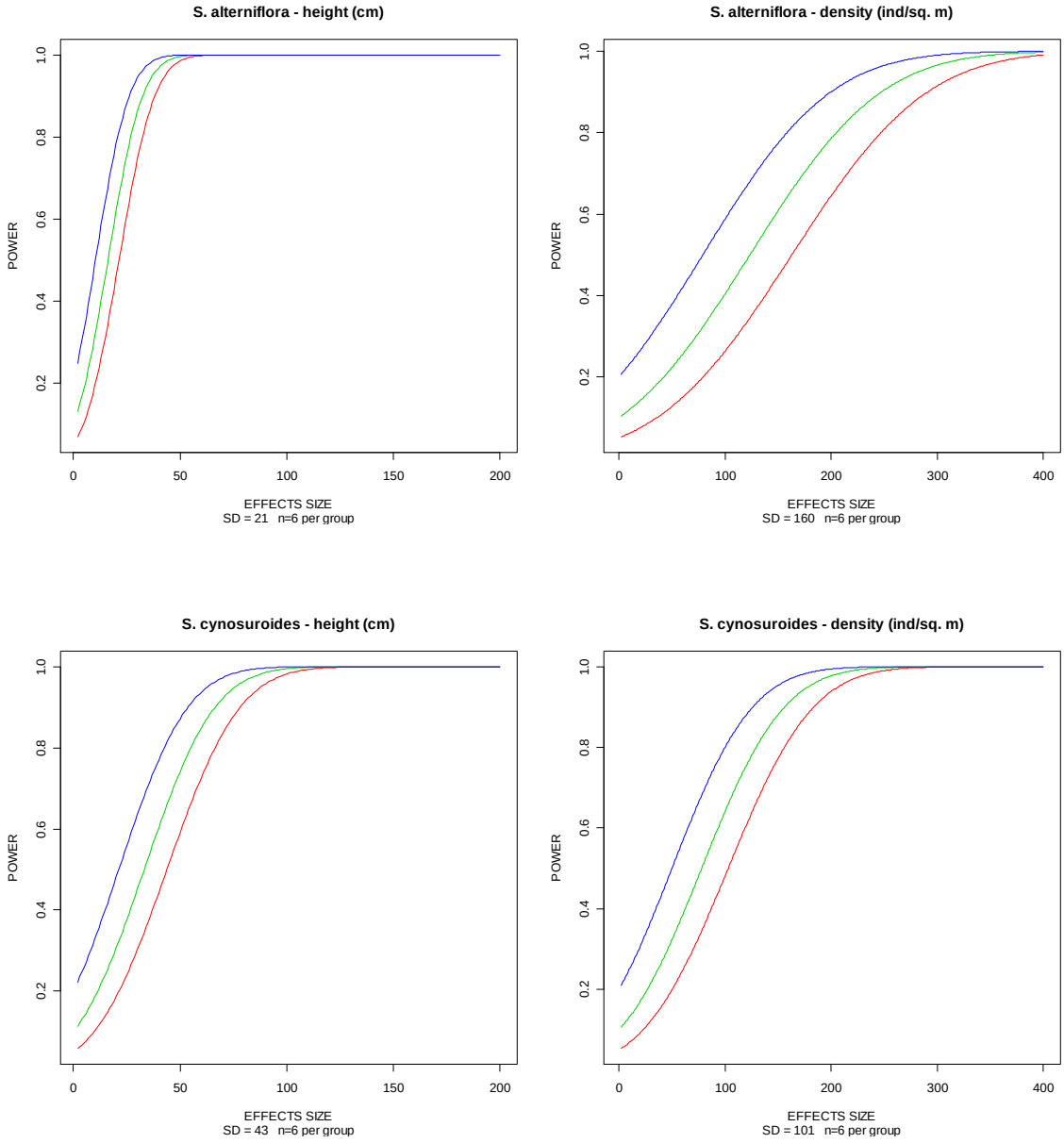

FIGURE A-1. Power-effect size curves for fixed per group sample size (n=6) at three significance (red: $\alpha = 0.05$, green: $\alpha = 0.1$, blue: $\alpha = 0.2$) levels for above-ground biomass proxy variables.

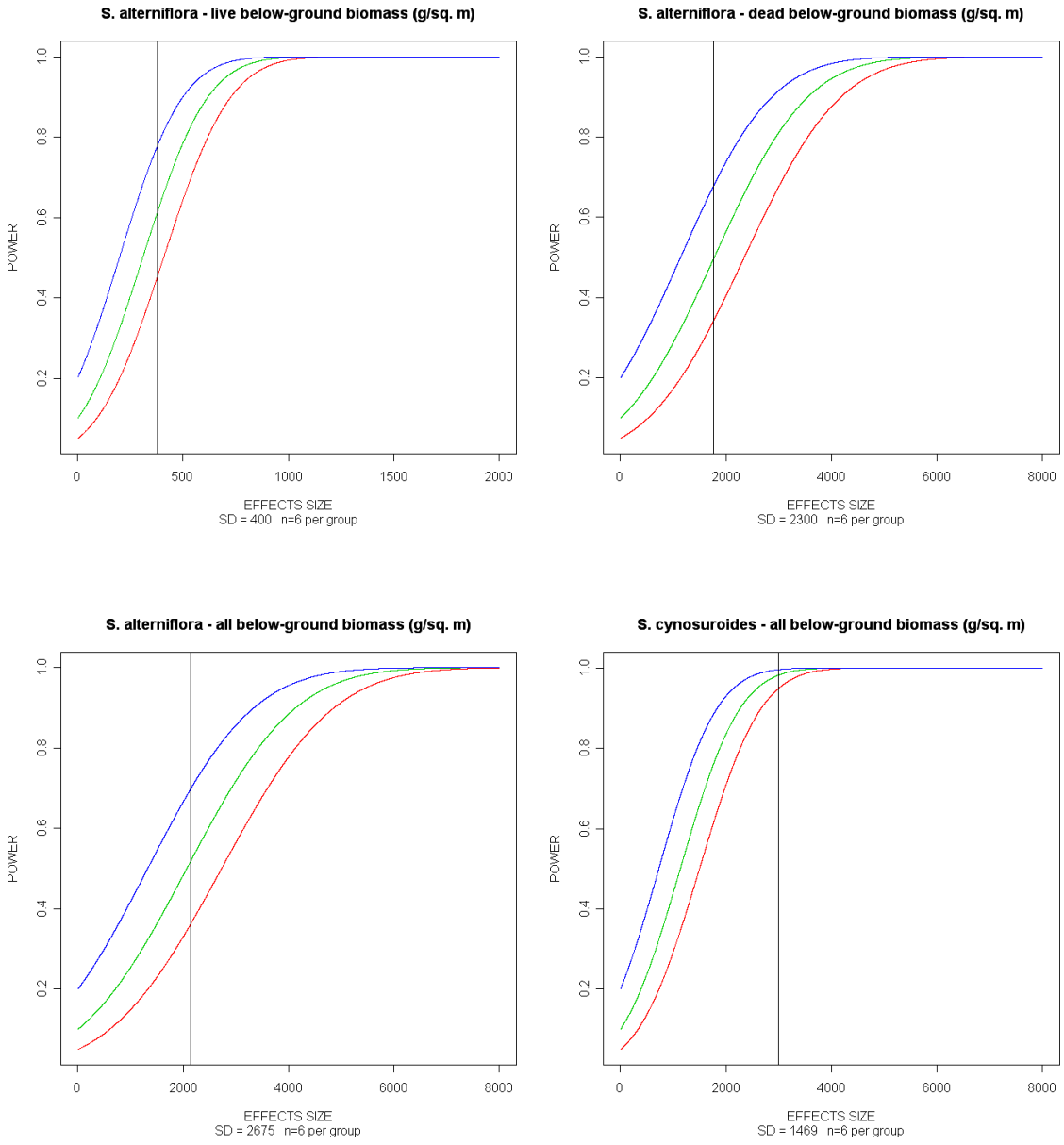

FIGURE A-2. Power-effect size curves for fixed per group sample size (n=6) at three significance (red: α =0.05, green: α =0.1, blue: α =0.2) levels for below-ground biomass variables. Vertical line indicates anticipated effect size.

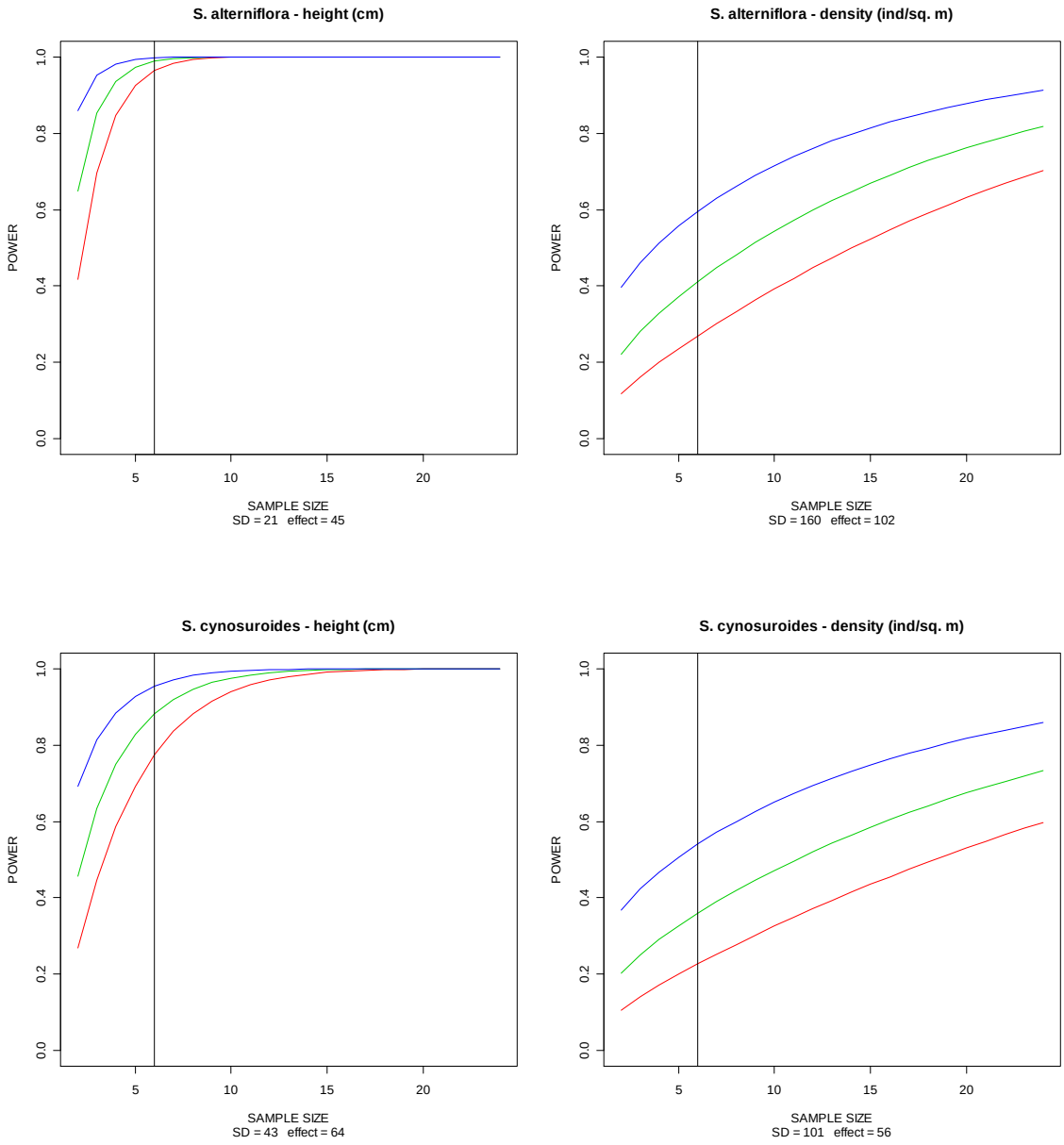

FIGURE A-3. Power-sample size curves for fixed effect size at three significance (red: α =0.05, green: α =0.1, blue: α =0.2) levels for above-ground biomass proxy variables. Vertical line indicates initial per group sample size.

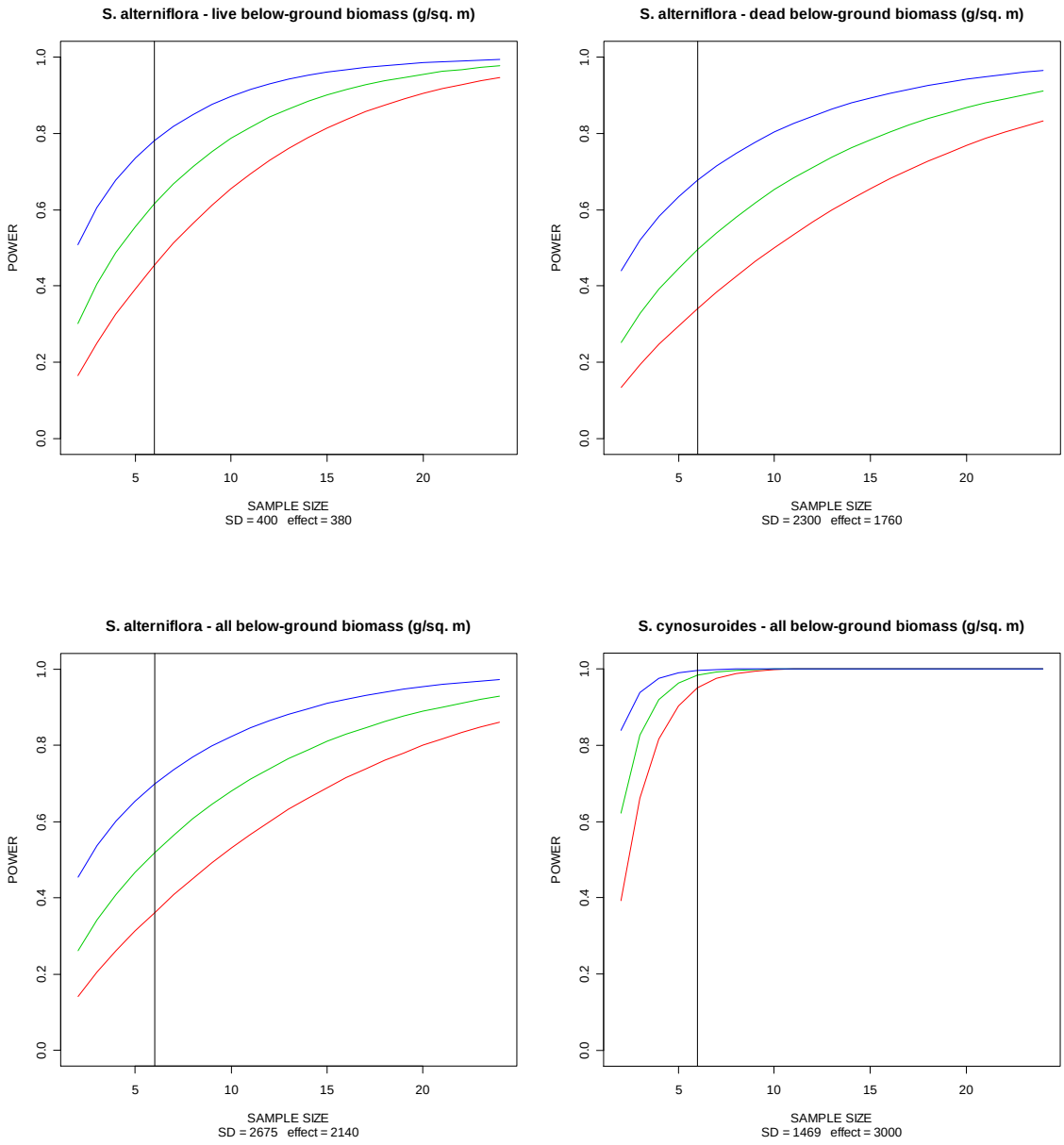

FIGURE A-4. Power-sample size curves for fixed effect size at three significance (red: α =0.05, green: α =0.1, blue: α =0.2) levels for below-ground biomass variables. Vertical line indicates initial per group sample size.

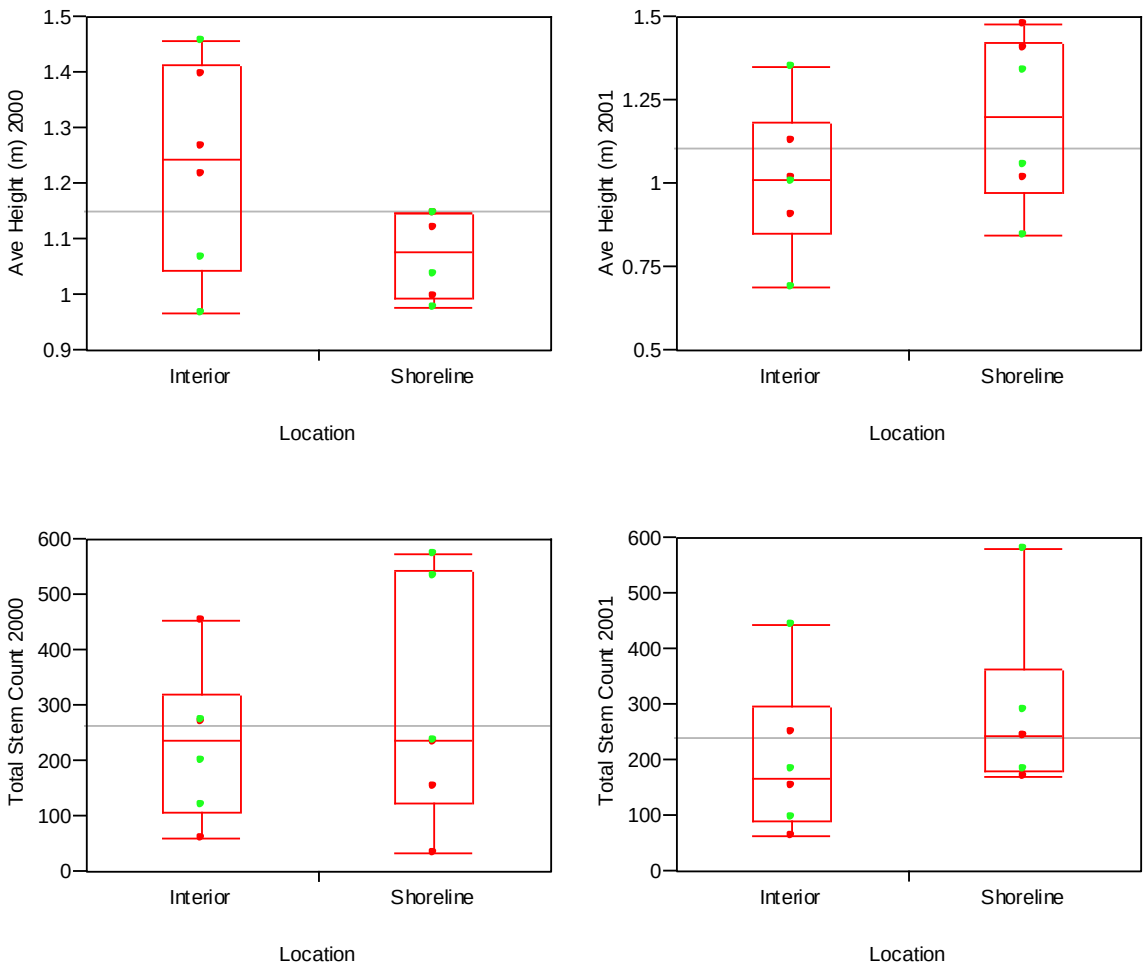

FIGURE A-5. Box plots for above ground proxy variables from 2000 and 2001 by location for *S. alterniflora* sites. Green markers are reference sites and red markers are oiled sites. Only the average height in 2000 variable was significantly different at α=0.1.

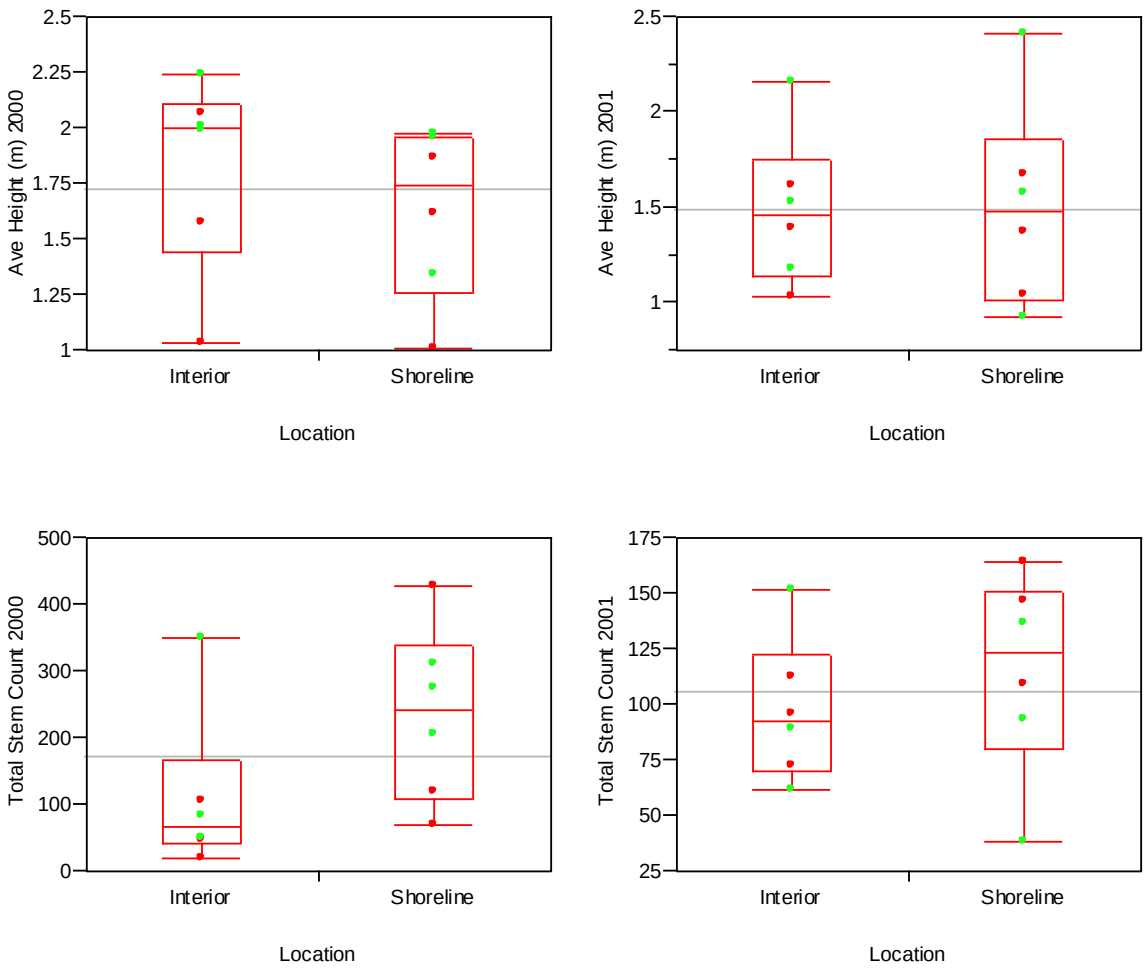

FIGURE A-6. Box plots for above ground proxy variables from 2000 and 2001 by location for *S. cynosuroides* sites. Green markers are reference sites and red markers are oiled sites. No variables were significantly different at α=0.1.

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APPENDIX B

Analytical Results for Polynulcear Aromatic Hydrocarbons (PAH)

Research Planning-Chalk Point Marsh Study
Polycyclic Aromatic Hydrocarbon Data
Client Submitted Samples

Sample Name	CPM0036.D	CPM0040.D	CPM0042.D	CPM0035.D	CPM0022.D	CPM0048.D	CPM0005.D	CPM0003.D
Client Name	ACH-1 (0-10 cm)	ACH-1 (10-20 cm)	AH-1 (0-10 cm)	AH-1 (10-20 cm)	AH-2 (0-10 cm)	AH-2 (10-20 cm)	AH-4 (0-10 cm)	AH-4 (10-20 cm)
Matrix	Sediment	Sediment	Sediment	Sediment	Sediment	Sediment	Sediment	Sediment
Collection Date	08/29/07	08/29/07	08/29/07	08/29/07	NA	NA	09/01/07	09/01/07
Received Date	09/05/07	09/05/07	09/05/07	09/05/07	09/05/07	09/05/07	09/05/07	09/05/07
Extraction Date	09/10/07	09/11/07	09/10/07	09/11/07	09/10/07	09/11/07	09/10/07	09/10/07
Extraction Batch	ENV 1689	ENV 1690	ENV 1689	ENV 1690	ENV 1688	ENV 1690	ENV 1688	ENV 1689
Date Acquired	09/14/07	09/16/07	09/14/07	09/16/07	09/15/07	09/16/07	09/15/07	09/14/07
Method	PAH-2002	PAH-2002	PAH-2002	PAH-2002	PAH-2002	PAH-2002	PAH-2002	PAH-2002
Sample Dry Weight (g)	5.0	5.0	5.0	5.0	5.0	5.0	12.2	12.1
% Moisture	76	53	75	55	82	78	86	83
% Dry	24	47	25	45	18	22	14	17
Dilution	2x	NA	5x	10x	5x	2x	2x	2x
Target Compounds	Su Corrected Conc. (ng/dry g)	Su Corrected Conc. (ng/dry g)	Su Corrected Conc. (ng/dry g)	Su Corrected Conc. (ng/dry g)	Su Corrected Conc. (ng/dry g)	Su Corrected Conc. (ng/dry g)	Su Corrected Conc. (ng/dry g)	Su Corrected Conc. (ng/dry g)
Naphthalene	4.8	3.5	24.9	270	18.7	9.6	12.0	6.8
C1-Naphthalenes	14.8	6.4	77.2	1060	39.7	16.0	18.1	8.8
C2-Naphthalenes	56.6	38.6	1320	6890	304	85.5	64.5	34.5
C3-Naphthalenes	657	86.5	8770	43900	1190	212	114	61.9
C4-Naphthalenes	1510	69.2	13600	68100	1030	147	70.2	38.5
Benzothiophene	0.6	0.3	8.8	30.1	7.9	3.9	1.0	0.7
C1-Benzothiophenes	6.7	2.6	119	318	23.7	11.7	2.7	2.8
C2-Benzothiophenes	9.7	3.3	202	826	53.7	18.6	13.4	3.1
C3-Benzothiophenes	30.0	5.2	765	3570	118.0	15.6	10.5	4.4
Biphenyl	4.7	2.1	28.5	462	16.9	8.7	6.0	3.3
Acenaphthylene	8.0	1.4	116	334	29.9	5.2	3.9	2.8
Acenaphthene	8.2	2.1	259	805	57.0	14.6	5.1	3.7
Dibenzofuran	8.3	3.2	157	582	61.6	10.0	10.9	5.1
Fluorene	28.4	5.9	676	2360	162	28.0	12.0	8.0
C1-Fluorenes	198	15.6	2280	8610	359	36.5	26.5	10.7
C2-Fluorenes	1430	39.9	8040	37200	711	78.4	46.3	19.2
C3-Fluorenes	2370	60.8	10800	39500	925	55.7	43.4	14.9
Carbazole	10.3	0.9	93.4	435	16.1	3.6	4.0	2.1
Anthracene	38.0	2.5	374	1520	76.8	14.4	7.6	4.4
Phenanthrene	12.4	8.5	31.0	3750	39.5	44.2	37.6	20.4
C1-Phenanthrene/Anthracenes	683	27.6	2780	39400	355	92.2	52.6	19.5
C2-Phenanthrene/Anthracenes	3980	110	13900	119000	1850	138	160	23.8
C3-Phenanthrene/Anthracenes	5250	246	17800	130600	3610	84.1	120	16.5
C4-Phenanthrene/Anthracenes	3140	167	10300	75100	2250	41.9	90.4	13.2
Dibenzothiophene	19.9	2.5	332	1550	123.0	17.9	7.7	3.5
C1-Dibenzothiophene	208	6.6	1430	11100	202	28.4	15.0	5.4
C2-Dibenzothiophene	1040	25.6	3970	29700	525	30.9	27.6	5.5
C3-Dibenzothiophene	1480	63.1	4770	35300	916	22.7	32.7	6.8
Fluoranthene	165	7.6	663	3240	96.1	46.7	40.6	30.2
Pyrene	935	23.8	3200	15500	498	55.5	53.8	24.6
C1-Fluoranthenes/Pyrenes	3340	93.1	10800	42400	2370	51.1	93.2	20.7
C2-Fluoranthenes/Pyrenes	4150	156	13700	59000	5300	55.3	184	26.4
C3-Fluoranthenes/Pyrenes	2150	87.8	8650	40700	4290	26.5	94.3	13.1
Naphthobenzothiophene	366	9.4	933	4280	223	8.3	28.3	5.6
C1-Naphthobenzothiophenes	909	31.0	2530	11800	1050	10.9	47.7	9.3
C2-Naphthobenzothiophenes	1150	43.0	2850	12800	1530	8.9	48.2	4.2
C3-Naphthobenzothiophenes	669	28.1	1720	6910	1120	6.4	26.9	3.1
Benz(a)anthracene	495	10.5	1940	7380	382	10.7	21.1	9.7
Chrysene	1010	38.3	3030	15700	866	28.6	37.9	12.8
C1-Chrysenes	2710	115	7570	45600	2960	35.1	74.9	10.3
C2-Chrysenes	2490	110	7830	42000	3570	21.4	64.4	7.8
C3-Chrysenes	1110	48.2	4030	19700	2140	9.6	26.4	3.1
C4-Chrysenes	37.7	2.9	93.2	497	95.3	<2.1	<0.9	<0.9
Benzo(b)fluoranthene	402	19.2	995	4900	379	39.0	55.5	27.8
Benzo(k)fluoranthene	71.9	3.6	189	693	59.6	11.3	16.9	5.0
Benzo(e)pyrene	621	32.8	1780	6100	959	22.1	49.6	11.3
Benzo(a)pyrene	415	11.5	1500	5180	372	18.3	20.1	12.0
Perylene	111	8.8	424	1330	161	18.3	16.8	6.6
Indeno(1,2,3-c,d)pyrene	63.3	3.1	184	532	98.6	9.9	15.9	9.5
Dibenzo(a,h)anthracene	94.5	6.4	266	840	130	18.3	25.1	10.1
Benzo(g,h,i)perylene	155	6.6	477	1320	216	10.9	16.0	8.5
Total PAHs	45828	1904	178378	1010674	43937	1798	2073	622
Individual Alkyl Isomers and Hopanes								
2-Methylnaphthalene	15.9	3.2	75.9	780	38.4	10.6	13.9	6.0
1-Methylnaphthalene	7.8	6.6	48.3	835	20.4	13.8	13.4	8.5
2,6-Dimethylnaphthalene	25.4	15.6	58.7	1340	48.5	29.0	27.3	14.7
1,6,7-Trimethylnaphthalene	99.4	9.1	1040	4840	229	25.6	13.9	8.8
1-Methylphenanthrene	185	5.1	771	8730	118	22.1	13.0	6.4
C29-Hopane	445	45.7	1350	3230	1090	42.0	121	9.4
18a-Oleanane	161	20.7	466	1390	438	<6.6	<2.7	<2.8
C30-Hopane	622	61.7	1790	5670	1700	32.4	103	17.1
17a, 21b (H)-Hopane								
Surrogate (Su)	Su Recovery (%)	Su Recovery (%)	Su Recovery (%)	Su Recovery (%)	Su Recovery (%)	Su Recovery (%)	Su Recovery (%)	Su Recovery (%)
Naphthalene-d8	91	91	99	103	89	97	99	90
Acenaphthene-d10	88	98	99	90	90	95	98	82
Phenanthrene-d10	89	93	89	91	98	74	99	87
Chrysene-d12	59	56	75	69	96	60	79	52
Perylene-d12	80	71	87	84	93	78	96	80

Research Planning-Chalk Point Marsh Study
Polycyclic Aromatic Hydrocarbon Data
Client Submitted Samples

Sample Name	CPM0009.D	CPM0004.D	CPM0030.D	CPM0041.D	CPM0032.D	CPM0034.D	CPM0015.D	CPM0033.D
Client Name	AH-5 (0-10 cm)	AH-5 (10-20 cm)	AH-6 (0-10 cm)	AH-6 (10-20 cm)	AH-7 (0-10 cm)	AH-7 (10-20 cm)	AH-8 (0-10 cm)	AH-8 (10-20 cm)
Matrix	Sediment	Sediment	Sediment	Sediment	Sediment	Sediment	Sediment	Sediment
Collection Date	09/01/07	09/01/07	NA	08/29/07	08/29/07	08/29/07	08/29/07	08/29/07
Received Date	09/05/07	09/05/07	09/05/07	09/05/07	09/05/07	09/05/07	09/05/07	09/05/07
Extraction Date	09/10/07	09/10/07	09/10/07	09/11/07	09/10/07	09/11/07	09/10/07	09/11/07
Extraction Batch	ENV 1688	ENV 1689	ENV 1688	ENV 1690	ENV 1688	ENV 1690	ENV 1688	ENV 1690
Date Acquired	09/15/07	09/14/07	09/15/07	09/16/07	09/15/07	09/16/07	09/15/07	09/16/07
Method	PAH-2002	PAH-2002	PAH-2002	PAH-2002	PAH-2002	PAH-2002	PAH-2002	PAH-2002
Sample Dry Weight (g)	5.0	13.0	5.0	5.0	12.0	5.0	5.0	5.1
% Moisture	75	76	78	72	82	83	79	78
% Dry	25	24	22	28	18	17	21	22
Dilution	5x	2x	2x	40x	2x	NA	10x	10x
Target Compounds	Su Corrected Conc. (ng/dry g)	Su Corrected Conc. (ng/dry g)	Su Corrected Conc. (ng/dry g)	Su Corrected Conc. (ng/dry g)	Su Corrected Conc. (ng/dry g)	Su Corrected Conc. (ng/dry g)	Su Corrected Conc. (ng/dry g)	Su Corrected Conc. (ng/dry g)
Naphthalene	17.0	10.7	11.2	114	12.7	8.0	28.5	21.3
C1-Naphthalenes	42.4	32.5	13.5	17700	18.1	13.9	82.4	62.7
C2-Naphthalenes	262	105	68.0	163600	41.6	69.8	1350	894
C3-Naphthalenes	2950	566	196	314200	57.4	160	8740	6500
C4-Naphthalenes	6030	614	201	223100	29.2	112	9150	9030
Benzothiophene	2.6	2.4	4.0	264	0.7	0.6	6.9	5.5
C1-Benzothiophenes	24.1	7.6	3.0	4300	1.9	1.6	85.6	39.1
C2-Benzothiophenes	52.9	15.5	19	10900	4.1	12.3	177	141
C3-Benzothiophenes	195	40.0	24	19000	8.5	13.2	527	575
Biphenyl	15.0	9.9	4.5	4170	5.5	5.1	20.1	22.6
Acenaphthylene	38.1	7.9	7.5	2730	4.6	4.5	127	113
Acenaphthene	28.2	16.3	9.9	7780	2.5	5.7	170	112
Dibenzofuran	34.8	16.0	13.3	6010	8.7	10.1	156	81.0
Fluorene	153	64.2	22.6	18600	7.1	18.1	406	117
C1-Fluorenes	1130	169	67.8	39600	22.0	27.7	2060	1040
C2-Fluorenes	4400	367	161	111600	40.3	51.9	5670	4720
C3-Fluorenes	6690	268	190	101900	24.5	36.9	5610	5190
Carbazole	<4.9	2.8	5.0	1890	8.0	2.4	<9.6	80.7
Anthracene	197	27.7	17.7	7610	5.9	8.4	456	367
Phenanthrene	51.3	20.2	29.9	54600	48.8	37.8	128	82.7
C1-Phenanthrene/Anthracenes	1520	173	97.7	177100	54.1	61.1	2390	1590
C2-Phenanthrene/Anthracenes	14600	480	517	303900	61.3	93.6	14400	13800
C3-Phenanthrene/Anthracenes	21800	372	779	284800	48.2	51.0	17500	21000
C4-Phenanthrene/Anthracenes	11400	174	505	145400	25.4	22.7	9000	14800
Dibenzothiophene	212	58.4	22.0	10200	7.5	7.8	733	753
C1-Dibenzothiophene	760	95.7	46.3	41700	12.9	14.5	1690	1240
C2-Dibenzothiophene	3680	134	133	73600	15.9	16.0	4050	3420
C3-Dibenzothiophene	5990	108	207	82200	14.1	12.2	4580	5700
Fluoranthene	424	46.6	57.3	6700	58.8	38.5	458	359
Pyrene	3480	114	150	31900	57.0	34.9	2470	2010
C1-Fluoranthenes/Pyrenes	10200	220	517	88700	48.1	27.6	8490	8120
C2-Fluoranthenes/Pyrenes	12400	261	892	116200	45.3	29.4	10500	17400
C3-Fluoranthenes/Pyrenes	7230	95.9	558	74800	24.8	16.6	6660	15300
Naphthobenzothiophene	1150	19.8	65.0	10500	13.3	7.9	947	543
C1-Naphthobenzothiophenes	3490	46.2	217	23600	17.2	7.9	2570	2320
C2-Naphthobenzothiophenes	4020	44.3	307	25100	19.4	8.3	2660	3390
C3-Naphthobenzothiophenes	1850	25.0	163	14000	11.1	3.6	1470	2970
Benz(a)anthracene	1320	34.2	105	16300	19.4	11.8	1300	1310
Chrysene	2840	45.9	141	33100	30.7	24.4	2430	3550
C1-Chrysenes	8120	118	516	95800	32.8	23.8	6640	12200
C2-Chrysenes	7350	111	576	77000	26.1	17.4	6340	11400
C3-Chrysenes	3470	50.9	298	38900	11.4	6.1	3100	8210
C4-Chrysenes	77.6	1.3	13.5	879	<0.9	<1	83.5	332
Benzo(b)fluoranthene	1520	32.0	121	8740	152	37.5	950	1150
Benzo(k)fluoranthene	157	10.0	29.4	1390	54.3	13.3	149	141
Benzo(e)pyrene	2300	24.0	171	11400	105	21.5	1730	2540
Benzo(a)pyrene	1480	24.1	84.0	9880	102	18.4	1070	1440
Perylene	364	12.9	53.5	2590	26.8	23.5	371	625
Indeno(1,2,3-c,d)pyrene	181	9.7	24.0	969	41.8	12.3	239	359
Dibenzo(a,h)anthracene	258	10.3	40.3	1510	85.2	13.6	285	406
Benzo(g,h,i)perylene	408	11.0	38.0	2310	41.5	10.9	840	1200
Total PAHs	156365	5326	8513	2920836	1616	1288	151046	188773
Individual Alkyl Isomers and Hopane								
2-Methylnaphthalene	42.2	37.2	12.0	527	15.8	9.5	72.8	56.0
1-Methylnaphthalene	20.4	14.3	8.1	26700	11.3	11.8	50.1	39.7
2,6-Dimethylnaphthalene	43.8	25.5	17.2	70100	16.5	28.1	155	66.1
1,6,7-Trimethylnaphthalene	292	90.7	27.3	31500	6.6	14.8	1190	443
1-Methylphenanthrene	320	65.3	31.1	38000	13.6	11.0	718	456
C29-Hopane	1770	27.4	170	8060	247	31.0	3800	2900
18a-Oleanane	606	<2.6	77.2	2840	37	<3.3	1150	1630
C30-Hopane	2520	37.2	333	12000	213	37.8	3570	5050
17a, 21b (H)-Hopane								
Surrogate (Su)	Su Recovery (%)	Su Recovery (%)	Su Recovery (%)	Su Recovery (%)	Su Recovery (%)	Su Recovery (%)	Su Recovery (%)	Su Recovery (%)
Naphthalene-d8	89	83	94	99	98	66	93	99
Acenaphthene-d10	89	81	95	98	99	79	90	97
Phenanthrene-d10	97	81	99	99	64	82	95	98
Chrysene-d12	57	62	74	71	84	64	68	81
Perylene-d12	90	72	95	85	98	68	89	91

Research Planning-Chalk Point Marsh Study
Polycyclic Aromatic Hydrocarbon Data
Client Submitted Samples

Sample Name	CPM0028.D	CPM0045.D	CPM0039.D	CPM0037.D	CPM0011.D	CPM0014.D	CPM0038.D	CPM0050.D
Client Name	AH-11 (0-10 cm)	AH-11 (10-20 cm)	AH-12 (0-10 cm)	AH-12 (10-20 cm)	AR-5 (0-10 cm)	CA-1 (0-10 cm)	CA-1 (10-20 cm)	CAH-9 (0-10 cm)
Matrix	Sediment	Sediment	Sediment	Sediment	Sediment	Sediment	Sediment	Sediment
Collection Date	NA	NA	08/29/07	08/29/07	08/31/07	08/29/07	08/29/07	08/30/07
Received Date	09/05/07	09/05/07	09/05/07	09/05/07	09/05/07	09/05/07	09/05/07	09/05/07
Extraction Date	09/10/07	09/11/07	09/10/07	09/11/07	09/10/07	09/10/07	09/11/07	09/10/07
Extraction Batch	ENV 1688	ENV 1690	ENV 1689	ENV 1690	ENV 1688	ENV 1688	ENV 1690	ENV 1689
Date Acquired	09/15/07	09/16/07	09/14/07	09/16/07	09/15/07	09/15/07	09/16/07	09/14/07
Method	PAH-2002	PAH-2002	PAH-2002	PAH-2002	PAH-2002	PAH-2002	PAH-2002	PAH-2002
Sample Dry Weight (g)	13.0	5.0	5.1	5.0	15.1	5.0	5.1	5.0
% Moisture	83	72	76	74	77	80	73	78
% Dry	17	28	24	26	23	20	27	22
Dilution	2x	2x	5x	20x	2x	2x	NA	2x
Target Compounds	Su Corrected Conc. (ng/dry g)	Su Corrected Conc. (ng/dry g)	Su Corrected Conc. (ng/dry g)	Su Corrected Conc. (ng/dry g)	Su Corrected Conc. (ng/dry g)	Su Corrected Conc. (ng/dry g)	Su Corrected Conc. (ng/dry g)	Su Corrected Conc. (ng/dry g)
Naphthalene	7.7	9.1	6.8	151	7.9	14.3	10.6	5.3
C1-Naphthalenes	12.6	21.6	22.4	1660	9.2	22.8	23.0	8.1
C2-Naphthalenes	40.0	105	297	31500	41.4	45.2	98.6	26.3
C3-Naphthalenes	98.2	257	2360	174900	84.8	76.0	247	91.8
C4-Naphthalenes	129	205	2940	157200	48.9	56.9	220	167
Benzothiophene	0.8	1.3	2.6	49.8	0.6	0.7	1.0	0.8
C1-Benzothiophenes	2.2	2.6	18.3	571	<0.7	1.9	5.9	0.5
C2-Benzothiophenes	11	10.1	39.2	4290	<0.7	11.1	7.7	8.8
C3-Benzothiophenes	9	15.5	142	13400	<0.7	13.0	12.4	6.5
Biphenyl	4.6	7.4	7.4	262	3.7	6.2	7.4	2.5
Acenaphthylene	3.3	4.9	33.4	1690	3.8	6.5	4.8	5.1
Acenaphthene	3.4	9.8	37.2	4450	2.8	2.9	6.5	3.8
Dibenzofuran	7.1	10.1	31.6	3700	7.3	9.3	10.7	4.5
Fluorene	10.3	20.8	104	6510	10.0	7.9	22.3	9.6
C1-Fluorenes	37.1	43.0	422	28100	19.5	40.9	45.9	24.5
C2-Fluorenes	105	103	1690	86200	30.4	71.0	132	147
C3-Fluorenes	110	76.9	1770	76000	20.8	90.6	138	273
Carbazole	4.7	4.4	13.4	462	3.1	8.3	2.8	3.5
Anthracene	11.4	10.4	115	6370	7.3	14.2	11.6	12.2
Phenanthrene	34.1	49.0	20.9	7040	28.9	37.7	35.8	16.9
C1-Phenanthrene/Anthracenes	93.3	89.5	449	109800	31.7	75.9	92.2	55.0
C2-Phenanthrene/Anthracenes	381	180	3460	257600	46.1	243	242	460
C3-Phenanthrene/Anthracenes	588	146	3790	233200	16.5	432	405	1010
C4-Phenanthrene/Anthracenes	426	75.3	2090	128200	9.0	292	262	700
Dibenzothiophene	13.2	14.0	163	11600	5.3	8.4	11.9	11.8
C1-Dibenzothiophene	31.7	28.2	382	35300	8.3	20.7	27.6	31.6
C2-Dibenzothiophene	98.3	46.4	988	63700	8.2	61.0	63.5	122
C3-Dibenzothiophene	162	43.8	1060	61600	4.8	117	116	292
Fluoranthene	36.3	39.5	151	6100	40.1	49.2	41.9	45.0
Pyrene	83.9	52.9	626	25300	33.1	90.7	78.6	173
C1-Fluoranthenes/Pyrenes	328	62.3	2300	76200	20.0	343	179	734
C2-Fluoranthenes/Pyrenes	588	56.1	3100	97100	25.3	589	265	1030
C3-Fluoranthenes/Pyrenes	392	30.0	2410	68900	7.2	468	142	920
Naphthobenzothiophene	40.7	9.0	215	7590	8.4	41.9	17.7	63.8
C1-Naphthobenzothiophenes	127	13.0	565	19400	8.8	119	44.8	198
C2-Naphthobenzothiophenes	186	11.1	725	20300	4.5	184	56.5	286
C3-Naphthobenzothiophenes	118	5.8	552	10500	2.1	134	33.8	232
Benz(a)anthracene	53.9	11.6	476	14600	10.3	62.9	24.2	144
Chrysene	119	29.1	771	29000	14.9	78.2	58.2	163
C1-Chrysenes	357	36.7	2070	76700	10.2	297	164	632
C2-Chrysenes	351	24.3	1840	59000	5.9	369	155	724
C3-Chrysenes	180	10.4	1160	33000	<0.7	221	68.4	436
C4-Chrysenes	8.2	<2.1	47.6	793	<0.7	17	3.3	14.1
Benzo(b)fluoranthene	107	34.0	219	7310	40.2	115	58.2	113
Benzo(k)fluoranthene	18.0	9.6	30.8	1140	14.9	27.3	16.6	21.4
Benzo(e)pyrene	173	15.6	358	10100	19.7	215	52.2	195
Benzo(a)pyrene	52.2	14.3	269	8620	16.6	66.5	32.2	92.2
Perylene	29.9	12.4	131	2340	45.4	33.9	18.8	49.0
Indeno(1,2,3-c,d)pyrene	20.3	7.9	81	792	12.0	45.8	13.4	28.9
Dibenzo(a,h)anthracene	32.1	19.1	81	1300	12.5	53.6	12.3	37.5
Benzo(g,h,i)perylene	35.9	8.2	376	2230	10.5	112	16.0	64.8
Total PAHs	5872	2103	41010	2083821	823	5520	3816	9897
Individual Alkyl Isomers and Hopane								
2-Methylnaphthalene	11.4	14.3	21.9	396	6.3	20.1	19.6	7.6
1-Methylnaphthalene	7.4	18.7	14.3	2150	7.6	13.9	15.4	5.5
2,6-Dimethylnaphthalene	17.6	40.7	25.8	4720	19.6	20.3	47.3	10.1
1,6,7-Trimethylnaphthalene	11.9	25.2	385	21800	10.2	9.5	23.3	8.8
1-Methylphenanthrene	27.1	22.7	211	23100	7.1	19.1	15.4	21.9
C29-Hopane	256	21.6	544	5380	58.7	981	57.5	222
18a-Oleanane	88.6	<6.6	272	2240	<2.2	241	7.7	82.0
C30-Hopane	357	29.1	660	9470	36.6	973	70.2	372
17a, 21b (H)-Hopane								
Surrogate (Su)	Su Recovery (%)	Su Recovery (%)	Su Recovery (%)	Su Recovery (%)	Su Recovery (%)	Su Recovery (%)	Su Recovery (%)	Su Recovery (%)
Naphthalene-d8	98	98	88	97	98	98	72	88
Acenaphthene-d10	94	97	84	99	92	98	89	81
Phenanthrene-d10	97	69	97	98	99	97	95	87
Chrysene-d12	99	61	76	52	67	74	64	68
Perylene-d12	96	77	78	91	97	97	73	77

Research Planning-Chalk Point Marsh Study
Polycyclic Aromatic Hydrocarbon Data
Client Submitted Samples

Sample Name	CPM0044.D	CPM0027.D	CPM0026.D	CPM0025.D	CPM0020.D	CPM0023.D	CPM0047.D	CPM0046.D
Client Name	CAH-9 (10-20 cm)	CAH-10 (0-10 cm)	CAH-10 (10-20 cm)	CH-2 (0-10 cm)	CH-2 (10-20 cm)	CH-3 (0-10 cm)	CH-3 (10-20 cm)	CH-4 (0-10 cm)
Matrix	Sediment	Sediment	Sediment	Sediment	Sediment	Sediment	Sediment	Sediment
Collection Date	08/30/07	08/30/07	08/30/07	08/30/07	08/30/07	08/30/07	08/30/07	08/30/07
Received Date	09/05/07	09/05/07	09/05/07	09/05/07	09/05/07	09/05/07	09/05/07	09/05/07
Extraction Date	09/11/07	09/10/07	09/10/07	09/10/07	09/10/07	09/10/07	09/11/07	09/10/07
Extraction Batch	ENV 1690	ENV 1688	ENV 1689	ENV 1688	ENV 1689	ENV 1688	ENV 1690	ENV 1689
Date Acquired	09/16/07	09/15/07	09/14/07	09/15/07	09/14/07	09/15/07	09/16/07	09/14/07
Method	PAH-2002	PAH-2002	PAH-2002	PAH-2002	PAH-2002	PAH-2002	PAH-2002	PAH-2002
Sample Dry Weight (g)	5.0	5.1	10.1	13.5	5.0	10.0	5.0	15.0
% Moisture	73	79	77	72	66	82	75	71
% Dry	27	21	23	28	34	18	25	29
Dilution	5x	10x	2x	2x	5x	2x	2x	2x
Target Compounds	Su Corrected Conc. (ng/dry g)	Su Corrected Conc. (ng/dry g)	Su Corrected Conc. (ng/dry g)	Su Corrected Conc. (ng/dry g)	Su Corrected Conc. (ng/dry g)	Su Corrected Conc. (ng/dry g)	Su Corrected Conc. (ng/dry g)	Su Corrected Conc. (ng/dry g)
Naphthalene	19.5	17.1	5.2	8.3	19.9	13.0	8.9	3.8
C1-Naphthalenes	67.6	57.9	8.4	11.9	60.7	19.8	15.1	5.2
C2-Naphthalenes	1290	1520	32.8	26.8	1870	74.3	71.1	15.9
C3-Naphthalenes	11300	11800	62.3	56.3	11400	145	167	30.6
C4-Naphthalenes	17000	11900	33.5	56.9	19600	97.3	116	20.0
Benzothiophene	10.0	7.9	1.0	1.3	10.0	1.2	0.9	0.3
C1-Benzothiophenes	82.8	89.5	6.6	1.4	137	3.3	7.2	0.3
C2-Benzothiophenes	186	267	9	8.4	313	23.8	5.8	1.7
C3-Benzothiophenes	834	638	9	8.6	1070	16.7	10.3	3.5
Biphenyl	22.7	31.6	5.0	3.6	38.2	6.9	6.2	1.6
Acenaphthylene	98.6	136	3.0	5.2	162	7.2	5.3	2.7
Acenaphthene	178	214	10.1	3.7	373	6.6	8.8	1.6
Dibenzofuran	150	151	3.5	6.2	194	10.5	7.9	2.9
Fluorene	654	638	16.3	8.3	1030	14.6	20.4	5.5
C1-Fluorenes	2470	3020	20.9	25.7	3210	38.7	26.0	6.8
C2-Fluorenes	10100	7680	30.8	56.8	11400	75.2	57.6	13.9
C3-Fluorenes	13100	7850	26.8	42.1	13900	49.4	45.4	12.5
Carbazole	69.2	107	1.5	3.1	70.8	8.9	4.0	1.9
Anthracene	448	517	7.1	9.1	538	14.2	10.6	4.1
Phenanthrene	63.3	121	13.6	27.4	281	48.1	44.1	15.7
C1-Phenanthrene/Anthracenes	4120	5340	21.8	43.1	5310	81.0	70.9	14.2
C2-Phenanthrene/Anthracenes	28300	22700	33.1	154	23400	236	102	23.0
C3-Phenanthrene/Anthracenes	37800	30000	13.4	319	26000	219	100	17.2
C4-Phenanthrene/Anthracenes	21300	15800	8.0	266	15000	169	79.6	15.7
Dibenzothiophene	708	601	7.6	10.4	630	13.4	11.7	2.7
C1-Dibenzothiophene	2520	2090	6.8	15.8	2470	25.7	19.2	3.9
C2-Dibenzothiophene	8250	5980	6.3	37.1	6140	47.0	25.0	5.3
C3-Dibenzothiophene	10400	7190	3.8	83.0	7170	56.1	25.3	6.3
Fluoranthene	644	586	33.8	31.6	770	59.3	45.0	31.9
Pyrene	3690	3760	26.6	79.4	3980	89.0	55.3	29.5
C1-Fluoranthenes/Pyrenes	11700	16900	19.8	265	15000	224	68.5	29.3
C2-Fluoranthenes/Pyrenes	16300	25100	27.1	568	17200	600	112	40.4
C3-Fluoranthenes/Pyrenes	10200	17600	9.7	447	11000	507	88.3	29.2
Naphthobenzothiophene	1030	1580	6.7	43.5	1340	37.8	10.5	6.6
C1-Naphthobenzothiophenes	3400	4600	3.4	128	3910	101	19.1	9.1
C2-Naphthobenzothiophenes	3710	6000	3.6	189	4680	155	28.5	15.1
C3-Naphthobenzothiophenes	2150	3280	2.5	112	2640	139	25.6	14.2
Benz(a)anthracene	1760	2300	8.7	46.6	3410	51.9	14.1	11.7
Chrysene	4630	4550	13.1	125	4390	107	39.1	21.1
C1-Chrysenes	12400	13300	14.5	322	12700	283	64.3	25.4
C2-Chrysenes	11000	12100	5.9	364	10900	346	79.4	29.9
C3-Chrysenes	5440	5040	2.3	207	5540	198	47.3	20.8
C4-Chrysenes	170	184	<1	9.9	163	21.0	4.6	1.8
Benzo(b)fluoranthene	1520	1850	21.1	103	1290	117	52.4	32.6
Benzo(k)fluoranthene	240	229	8.8	17.9	231	25.6	15.3	10.4
Benzo(e)pyrene	2150	4520	12.1	184	1660	235	49.0	22.2
Benzo(a)pyrene	1520	2180	12.7	50.4	1590	53.3	23.4	16.2
Perylene	407	640	13.4	38.6	443	36.1	12.7	14.2
Indeno(1,2,3-c,d)pyrene	181	295	9.0	22.7	194	37.5	14.8	12.4
Dibenzo(a,h)anthracene	282	416	14.1	29.9	302	49.3	16.7	9.0
Benzo(g,h,i)perylene	462	709	8.8	41.1	517	72.7	19.1	13.5
Total PAHs	266528	264183	685	4725	255648	5067	1977	685
Individual Alkyl Isomers and Hopane								
2-Methylnaphthalene	66.5	40.6	7.4	11.4	32.7	14.9	10.2	4.4
1-Methylnaphthalene	36.6	46.9	6.2	6.2	69.1	14.9	12.9	4.1
2,6-Dimethylnaphthalene	68.0	116	11.2	10.6	185	33.7	30.1	6.6
1,6,7-Trimethylnaphthalene	1590	1510	7.2	5.8	1160	17.3	19.9	4.1
1-Methylphenanthrene	839	1250	4.6	12.0	1750	19.0	15.9	4.1
C29-Hopane	1380	4070	15.4	270	1390	588	104	43.1
18a-Oleanane	509	1350	<3.3	101	468	182	39.7	14.9
C30-Hopane	2390	5680	21.8	354	1880	766	149	64.9
17a, 21b (H)-Hopane								
Surrogate (Su)	Su Recovery (%)	Su Recovery (%)	Su Recovery (%)	Su Recovery (%)	Su Recovery (%)	Su Recovery (%)	Su Recovery (%)	Su Recovery (%)
Naphthalene-d8	94	85	84	93	97	96	97	80
Acenaphthene-d10	98	87	81	93	99	96	94	75
Phenanthrene-d10	95	96	72	93	83	64	73	75
Chrysene-d12	54	74	72	99	67	98	63	63
Perylene-d12	87	88	79	91	78	95	86	73

Research Planning-Chalk Point Marsh Study
Polycyclic Aromatic Hydrocarbon Data
Client Submitted Samples

Sample Name	CPM0043.D	CPM0016.D	CPM0017.D	CPM0013.D	CPM0012.D	CPM0001.D	CPM0008.D	CPM0019.D
Client Name	CH-4 (10-20 cm)	CH-5 (0-10 cm)	CH-5 (10-20 cm)	CH-6 (0-10 cm)	CH-6 (10-20 cm)	CH-7 (0-10 cm)	CH-7 (10-20 cm)	CH-8 (0-10 cm)
Matrix	Sediment	Sediment	Sediment	Sediment	Sediment	Sediment	Sediment	Sediment
Collection Date	08/30/07	09/01/07	09/01/07	09/01/07	09/01/07	09/01/07	09/01/07	09/01/07
Received Date	09/05/07	09/05/07	09/05/07	09/05/07	09/05/07	09/05/07	09/05/07	09/05/07
Extraction Date	09/11/07	09/10/07	09/10/07	09/10/07	09/10/07	09/10/07	09/10/07	09/10/07
Extraction Batch	ENV 1690	ENV 1688	ENV 1689	ENV 1688	ENV 1689	ENV 1688	ENV 1689	ENV 1688
Date Acquired	09/16/07	09/14/07	09/15/07	09/15/07	09/14/07	09/14/07	09/14/07	09/16/07
Method	PAH-2002	PAH-2002	PAH-2002	PAH-2002	PAH-2002	PAH-2002	PAH-2002	PAH-2002
Sample Dry Weight (g)	5.0	5.0	5.1	15.0	5.0	12.8	15.0	5.0
% Moisture	63	73	67	77	74	74	69	68
% Dry	37	27	33	23	26	26	31	32
Dilution	NA	2x	10x	2x	2x	2x	2x	5x
Target Compounds	Su Corrected Conc. (ng/dry g)	Su Corrected Conc. (ng/dry g)	Su Corrected Conc. (ng/dry g)	Su Corrected Conc. (ng/dry g)	Su Corrected Conc. (ng/dry g)	Su Corrected Conc. (ng/dry g)	Su Corrected Conc. (ng/dry g)	Su Corrected Conc. (ng/dry g)
Naphthalene	8.5	13.8	48.3	7.6	5.7	10.9	6.8	17.0
C1-Naphthalenes	13.3	18.9	125	10.7	10.0	16.5	10.5	39.0
C2-Naphthalenes	69.5	102	4460	34.6	42.2	53.3	56.9	322
C3-Naphthalenes	157	431	42800	64.0	169	102	123	2490
C4-Naphthalenes	107	432	49000	44.6	400	65.3	75.9	4020
Benzothiophene	0.7	1.8	20.9	0.5	0.8	0.7	0.5	8.2
C1-Benzothiophenes	3.3	5.0	213	7.2	8.8	5.4	3.6	44.3
C2-Benzothiophenes	4.4	11.0	595	8.5	6.7	12.1	5.1	76.1
C3-Benzothiophenes	8.8	21.2	2900	9.2	13.8	7.9	8.0	153
Biphenyl	4.6	6.2	47.2	3.2	3.1	5.1	3.4	18.8
Acenaphthylene	5.0	8.0	265	3.4	7.4	5.3	4.2	34.8
Acenaphthene	4.8	7.6	527	2.5	4.9	3.6	4.2	46.2
Dibenzofuran	8.1	10.9	336	5.6	4.9	8.5	6.5	33.9
Fluorene	12.5	16.5	1380	7.5	19.7	10.3	15.4	122
C1-Fluorenes	22.0	85.8	5600	18.0	62.9	24.1	19.4	550
C2-Fluorenes	43.7	242	24800	32.9	361	44.7	34.8	2180
C3-Fluorenes	25.7	323	26200	24.5	618	35.9	20.0	2940
Carbazole	2.6	7.0	378	2.7	6.6	5.2	1.7	36.2
Anthracene	6.7	15.1	1000	5.5	22.5	8.7	6.2	86.4
Phenanthrene	35.4	37.1	1260	23.0	20.7	46.6	23.1	144
C1-Phenanthrene/Anthracenes	51.3	245	31800	32.4	126	54.9	25.7	2090
C2-Phenanthrene/Anthracenes	57.2	851	88200	82.4	1170	104	26.4	7270
C3-Phenanthrene/Anthracenes	37.5	956	104900	119	2400	137	11.6	10000
C4-Phenanthrene/Anthracenes	16.6	578	57300	83.9	1860	90.9	4.8	6960
Dibenzothiophene	6.9	8.1	890	5.2	28.5	8.3	5.6	80.8
C1-Dibenzothiophene	12.3	67.0	8260	10.2	64.0	14.8	8.2	620
C2-Dibenzothiophene	13.6	213	22000	21.3	304	27.0	7.2	1800
C3-Dibenzothiophene	10.4	262	27100	34.0	668	38.1	4.4	2760
Fluoranthene	34.3	57.6	1970	30.6	64.6	48.7	36.8	187
Pyrene	32.4	182	11300	42.1	298	62.1	29.2	1130
C1-Fluoranthenes/Pyrenes	23.3	589	34700	102	1790	156	19.6	4480
C2-Fluoranthenes/Pyrenes	29.7	937	52200	217	2830	343	29.9	8450
C3-Fluoranthenes/Pyrenes	10.2	773	31000	175	1460	275	6.0	7230
Naphthobenzothiophene	6.1	81.7	3130	16.4	97.3	22.3	4.9	299
C1-Naphthobenzothiophenes	5.4	227	10400	50.4	514	86.6	3.7	1060
C2-Naphthobenzothiophenes	3.7	331	11700	69.7	781	117	3.5	1680
C3-Naphthobenzothiophenes	1.6	212	6360	52.3	551	109	2.1	1480
Benz(a)anthracene	9.5	122	5520	23.6	278	38.2	10.4	502
Chrysene	18.5	164	13500	32.9	529	87.3	12.5	1550
C1-Chrysenes	16.8	591	39700	123	1780	193	8.7	5130
C2-Chrysenes	11.2	617	37700	138	1700	238	5.0	5930
C3-Chrysenes	4.3	408	18100	81.4	690	136	2.4	3600
C4-Chrysenes	1.2	18.1	664	3.7	28.5	10.9	<0.7	142
Benzo(b)fluoranthene	34.0	102	3740	71.3	310	101	29.7	640
Benzo(k)fluoranthene	10.2	28.7	597	19.3	55.9	23.8	10.4	100
Benzo(e)pyrene	16.6	179	4930	97.7	552	166	15.6	1410
Benzo(a)pyrene	14.3	84.9	3520	29.1	338	41.3	14.7	707
Perylene	11.8	46.6	982	28.2	127	44.9	19.5	355
Indeno(1,2,3-c,d)pyrene	9.9	33.0	491	19.3	74.1	33.4	10.7	166
Dibenzo(a,h)anthracene	6.1	32.5	800	23.6	92.8	29.0	7.0	207
Benzo(g,h,i)perylene	8.4	59.7	1290	28.5	168	52.7	9.5	477
Total PAHs	1069	10853	796699	2179	23518	3362	815	91855
Individual Alkyl Isomers and Hopane								
2-Methylnaphthalene	7.8	16.8	112	8.7	9.0	13.2	7.1	33.9
1-Methylnaphthalene	12.6	11.3	78.4	7.3	7.2	11.5	10.4	25.6
2,6-Dimethylnaphthalene	29.2	30.6	506	17.0	16.1	24.5	25.8	39.0
1,6,7-Trimethylnaphthalene	16.8	48.5	4620	7.5	15.5	12.0	15.9	208
1-Methylphenanthrene	10.3	61.7	6780	7.9	26.2	13.2	7.2	542
C29-Hopane	41	438	3970	279	597	290	27.8	1890
18a-Oleanane	<3.3	104	1280	57.0	219	108	<2.2	742
C30-Hopane	37.1	411	5280	250	919	404	34.3	2950
17a, 21b (H)-Hopane								
Surrogate (Su)	Su Recovery (%)	Su Recovery (%)	Su Recovery (%)	Su Recovery (%)	Su Recovery (%)	Su Recovery (%)	Su Recovery (%)	Su Recovery (%)
Naphthalene-d8	79	97	99	93	78	99	86	94
Acenaphthene-d10	96	98	93	96	69	99	81	96
Phenanthrene-d10	95	93	99	99	87	86	71	98
Chrysene-d12	57	71	69	65	68	87	61	54
Perylene-d12	64	88	78	99	76	97	72	76

Research Planning-Chalk Point Marsh Study
Polycyclic Aromatic Hydrocarbon Data
Client Submitted Samples

Sample Name	CPM0006.D	CPM0018.D	CPM0021.D	CPM0049.D	CPM0024.D	CPM0031.D	CPM0029.D	CPM0010.D
Client Name	CH-8 (10-20 cm)	CH-9 (0-10 cm)	CH-9 (10-20 cm)	CH-10 (0-10 cm)	CH-10 (10-20 cm)	CH-11 (0-10 cm)	CH-11 (10-20 cm)	CH-12 (0-10 cm)
Matrix	Sediment	Sediment	Sediment	Sediment	Sediment	Sediment	Sediment	Sediment
Collection Date	09/01/07	09/01/07	09/01/07	08/30/07	08/30/07	08/29/07	08/29/07	09/01/07
Received Date	09/05/07	09/05/07	09/05/07	09/05/07	09/05/07	09/05/07	09/05/07	09/05/07
Extraction Date	09/10/07	09/10/07	09/10/07	09/10/07	09/10/07	09/10/07	09/10/07	09/10/07
Extraction Batch	ENV 1689	ENV 1689	ENV 1689	ENV 1689	ENV 1689	ENV 1688	ENV 1689	ENV 1688
Date Acquired	09/14/07	09/14/07	09/14/07	09/14/07	09/14/07	09/15/07	09/14/07	09/15/07
Method	PAH-2002	PAH-2002	PAH-2002	PAH-2002	PAH-2002	PAH-2002	PAH-2002	PAH-2002
Sample Dry Weight (g)	5.0	13.1	15.0	5.0	15.0	5.0	5.0	15.0
% Moisture	70	66	69	77	74	74	68	75
% Dry	30	34	31	23	26	26	32	25
Dilution	10x	2x	2x	20x	2x	5x	20x	2x
Target Compounds	Su Corrected Conc. (ng/dry g)	Su Corrected Conc. (ng/dry g)	Su Corrected Conc. (ng/dry g)	Su Corrected Conc. (ng/dry g)	Su Corrected Conc. (ng/dry g)	Su Corrected Conc. (ng/dry g)	Su Corrected Conc. (ng/dry g)	Su Corrected Conc. (ng/dry g)
Naphthalene	122.0	4.9	4.8	27.8	24.7	33.4	82.9	10.0
C1-Naphthalenes	5230	6.4	8.0	96.1	61.8	115	1550	16.1
C2-Naphthalenes	49600	34.3	40.7	2430	454	1360	42400	59.3
C3-Naphthalenes	78800	67.9	87.3	21500	1940	5340	120500	110
C4-Naphthalenes	56900	40.6	56.5	27000	1270	6840	106900	68.0
Benzothiophene	85.5	0.3	0.3	6.8	5.8	11.7	121	0.6
C1-Benzothiophenes	623	3.7	0.9	151	22.5	80.2	967	5.2
C2-Benzothiophenes	2540	3.0	2.9	372	71.4	128	2710	9.6
C3-Benzothiophenes	4430	4.4	5.7	1290	168	286	7120	9.5
Biphenyl	1510	2.6	3.0	35.7	16.6	27.9	135	5.4
Acenaphthylene	689	3.3	3.3	241	27.3	66.2	841	5.0
Acenaphthene	1990	2.8	3.3	454	87.1	92.4	2290	3.5
Dibenzofuran	1210	4.1	5.0	253	72.6	63.8	944	7.9
Fluorene	4880	8.4	14.0	1070	267	190	4380	10.2
C1-Fluorenes	11700	11.4	16.5	4160	440	875	16500	24.8
C2-Fluorenes	32100	22.3	34.4	17400	773	3750	57100	37.3
C3-Fluorenes	31500	12.7	23.6	19400	472	5240	57000	29.2
Carbazole	300	1.1	1.6	162	4.9	43.1	744	5.2
Anthracene	2140	5.0	4.9	799	50.8	149	2880	8.9
Phenanthrene	13500	14.9	18.4	223	54.8	250	7960	42.9
C1-Phenanthrene/Anthracenes	40500	18.6	24.4	9690	461	2950	61700	51.4
C2-Phenanthrene/Anthracenes	68000	19.1	35.3	35000	861	11300	128900	131
C3-Phenanthrene/Anthracenes	54600	8.4	29.0	42100	565	17900	125500	176
C4-Phenanthrene/Anthracenes	29300	4.8	19.6	25200	266	8000	74200	128
Dibenzothiophene	2370	4.2	5.1	623	96.3	66.4	1930	8.9
C1-Dibenzothiophene	10600	5.4	7.8	3420	189	693	15400	15.2
C2-Dibenzothiophene	16300	5.9	7.9	9430	191	2870	33500	31.8
C3-Dibenzothiophene	15700	3.4	8.1	12000	145	4900	34500	46.6
Fluoranthene	2230	33.3	32.0	1070	57.0	322	4950	45.0
Pyrene	9500	25.1	30.1	6500	137	2890	20800	61.9
C1-Fluoranthenes/Pyrenes	31500	16.6	32.1	30600	332	9260	81400	159
C2-Fluoranthenes/Pyrenes	29500	15.8	42.1	41600	373	11700	85100	311
C3-Fluoranthenes/Pyrenes	17700	6.3	32.0	24300	161	6720	39100	302
Naphthobenzothiophene	3210	4.8	5.8	2120	27.3	918	5860	21.9
C1-Naphthobenzothiophenes	7720	4.0	8.3	6320	69.1	2780	17200	77.8
C2-Naphthobenzothiophenes	7510	2.4	12.9	7970	71.1	3330	19200	116
C3-Naphthobenzothiophenes	4530	1.5	11.7	6210	40.4	1440	10400	99.0
Benz(a)anthracene	6770	9.5	11.3	4740	60.6	1120	13400	35.1
Chrysene	9380	10.8	17.5	8830	91.4	2340	19900	74.4
C1-Chrysenes	22100	7.3	24.6	22300	206	6860	52300	179
C2-Chrysenes	18000	3.7	27.8	23500	165	6950	48600	218
C3-Chrysenes	9050	1.9	17.8	12600	58.4	3270	21100	142
C4-Chrysenes	212	<0.3	<0.7	376	2.0	63.1	643	9.6
Benzo(b)fluoranthene	2560	22.7	30.8	2800	45.4	1330	6940	119
Benzo(k)fluoranthene	554	7.7	10.2	552	8.6	176	1190	26.8
Benzo(e)pyrene	3690	12.6	21.0	6580	36.0	2100	9680	180
Benzo(a)pyrene	3740	12.5	15.4	4740	31.0	1450	9150	36.0
Perylene	836	28.4	22.4	1600	20.9	345	2390	37.2
Indeno(1,2,3-c,d)pyrene	406	10.7	13.1	720	10.8	154	1100	30.5
Dibenzo(a,h)anthracene	586	6.4	9.3	1010	13.1	248	1680	41.4
Benzo(g,h,i)perylene	943	9.9	13.8	1860	13.8	362	2820	54.1
Total PAHs	729447	578	914	453432	11090	139749	1383658	3434
Individual Alkyl Isomers and Hopane								
2-Methylnaphthalene	464	4.0	5.6	83.6	68.3	72.0	78.1	12.9
1-Methylnaphthalene	8650	6.6	7.7	72.8	30.2	103	2640	11.3
2,6-Dimethylnaphthalene	26800	11.9	18.6	126	36.1	434	20600	25.6
1,6,7-Trimethylnaphthalene	7640	4.3	12.8	3350	282	706	14700	13.7
1-Methylphenanthrene	10300	4.4	6.6	2480	146	588	11900	12.5
C29-Hopane	2430	71.1	43.6	5820	39.9	1910	6960	323
18a-Oleanane	956	<1.1	<2.2	2000	6.6	609	2260	120
C30-Hopane	3690	26.9	53.8	7760	49.7	2650	9090	498
17a, 21b (H)-Hopane								
Surrogate (Su)	Su Recovery (%)	Su Recovery (%)	Su Recovery (%)	Su Recovery (%)	Su Recovery (%)	Su Recovery (%)	Su Recovery (%)	Su Recovery (%)
Naphthalene-d8	99	93	90	98	94	78	99	99
Acenaphthene-d10	94	86	88	88	92	78	90	99
Phenanthrene-d10	85	80	84	95	97	96	76	88
Chrysene-d12	76	72	76	80	71	57	48	95
Perylene-d12	83	82	85	82	82	92	79	98

Research Planning-Chalk Point Marsh Study
Polycyclic Aromatic Hydrocarbon Data
Client Submitted Samples

Sample Name	CPM0007.D	CPM0002.D	ETX0816A.D	ETX0816B.D	ETX0816C.D	
Client Name	CH-12 (10-20 cm)	CR-11 (0-10 cm)	Source Oil 4" Hole at excavation site	Source Oil 4" Hole at excavation site	Source Oil 4" Hole at excavation site	Mean of three source oils
Matrix	Sediment	Sediment	Petroleum	Petroleum	Petroleum	
Collection Date	09/01/07	09/03/07	4/19/00	4/19/00	4/19/00	
Received Date	09/05/07	09/05/07	4/20/00	4/20/00	4/20/00	
Extraction Date	09/10/07	09/10/07	NA	NA	NA	
Extraction Batch	ENV 1689	ENV 1688	NA	NA	NA	
Date Acquired	09/14/07	09/14/07	10/30/00	10/30/00	10/30/00	
Method	PAH-2002	PAH-2002	PAH	PAH	PAH	
Sample Dry Weight (g)	15.0	15.0	3.44	1.72	1.72	
% Moisture	69	77				
% Dry	31	23				
Dilution	2x	2x	2x	2x	2x	

Target Compounds	Su Corrected Conc. (ng/dry g)	Su Corrected Conc. (ng/dry g)	Su Corrected Conc. (ng/mg)	Su Corrected Conc. (ng/mg)	Su Corrected Conc. (ng/mg)	Average Conc. (ng/mg)	Std. Dev	%RSD
Naphthalene	5.0	8.8	919	919	960	933	24.1	2.6
C1-Naphthalenes	9.0	10.9	3890	3930	4000	3940	55.7	1.4
C2-Naphthalenes	41.5	37.6	6370	6270	6500	6380	115	1.8
C3-Naphthalenes	80.5	68.5	4720	4640	4710	4690	43.6	0.9
C4-Naphthalenes	47.8	40.3	2480	2550	2570	2533	47.3	1.9
Benzothiophene	0.4	0.3						
C1-Benzothiophenes	3.2	<0.7						
C2-Benzothiophenes	3.0	<0.7						
C3-Benzothiophenes	4.2	<0.7						
Biphenyl	2.7	3.6	273	274	282	276	4.8	1.8
Acenaphthylene	3.2	3.6	1.0					
Acenaphthene	3.3	2.6	137	140	142	140	2.6	1.9
Dibenzofuran	4.6	6.0						
Fluorene	11.8	7.1	274	270	277	274	3.8	1.4
C1-Fluorenes	12.7	18.3	654	652	645	650	4.3	0.7
C2-Fluorenes	25.3	25.4	1110	1100	1110	1107	5.8	0.5
C3-Fluorenes	14.9	17.4	914	901	821	879	50.4	5.7
Carbazole	1.3	4.4						
Anthracene	4.8	6.8	94.7	92.3	93.5	93.5	1.2	1.3
Phenanthrene	16.3	26.6	612	630	641	628	14.6	2.3
C1-Phenanthrene/Anthracenes	19.7	31.0	2030	2020	2040	2030	10.0	0.5
C2-Phenanthrene/Anthracenes	23.5	37.1	2510	2460	2540	2503	40.4	1.6
C3-Phenanthrene/Anthracenes	9.3	16.8	1730	1760	1740	1743	15.1	0.9
C4-Phenanthrene/Anthracenes	6.9	6.6	875	851	925	883	37.8	4.3
Dibenzothiophene	4.0	4.3	123	123	121	123	1.1	0.9
C1-Dibenzothiophene	5.5	8.5	421	409	413	414	6.1	1.5
C2-Dibenzothiophene	5.5	6.8	676	648	673	666	15.4	2.3
C3-Dibenzothiophene	3.0	4.4	523	516	543	527	14.2	2.7
Fluoranthene	31.6	44.4	60.2	55.6	61.1	59.0	3.0	5.0
Pyrene	23.6	33.3	314	298	306	306	7.9	2.6
C1-Fluoranthenes/Pyrenes	15.9	19.8	885	874	875	878	5.8	0.7
C2-Fluoranthenes/Pyrenes	39.6	20.5	1000	1020	1070	1030	36.1	3.5
C3-Fluoranthenes/Pyrenes	6.6	7.1	542	530	543	538	7.5	1.4
Naphthobenzothiophene	4.3	8.1						
C1-Naphthobenzothiophenes	4.1	5.3						
C2-Naphthobenzothiophenes	2.7	4.1						
C3-Naphthobenzothiophenes	1.7	3.0						
Benz(a)anthracene	9.2	15.2	152	145	151	149	4.0	2.7
Chrysene	10.4	14.6	266	261	261	263	2.9	1.1
C1-Chrysenes	6.9	12.1	742	708	731	727	17.2	2.4
C2-Chrysenes	4.1	6.6	714	676	712	701	21.2	3.0
C3-Chrysenes	1.7	3.1	318	303	305	308	8.0	2.6
C4-Chrysenes	<0.7	<0.7	7.0	6.7	6.8	6.8	0.1	2.1
Benzo(b)fluoranthene	23.6	52.5	47.8	50.4	53.8	51	3.0	5.9
Benzo(k)fluoranthene	9.6	17.0	8.5	7.7	8.0	8.1	0.4	5.4
Benzo(e)pyrene	12.8	26.2	78.2	75.9	74.9	76	1.7	2.2
Benzo(a)pyrene	15.0	20.1	72.4	66.4	67.4	69	3.2	4.7
Perylene	14.9	15.2	20.9	19.1	19.6	20	0.9	4.6
Indeno(1,2,3-c,d)pyrene	9.8	16.8	11.6	11.9	11.0	11	0.5	4.0
Dibenzo(a,h)anthracene	7.4	13.9	14.3	14.5	13.4	14	0.6	4.3
Benzo(g,h,i)perylene	9.3	13.7	24.1	21.9	22.0	23	1.2	5.5
Total PAHs	638	776	36600	36300	37000	36633	351	1.0
Individual Alkyl Isomers and Hopane								
2-Methylnaphthalene	5.8	8.2	3620	3660	3750	3677	66.6	1.8
1-Methylnaphthalene	9.2	8.2	2030	2050	2070	2050	20.0	1.0
2,6-Dimethylnaphthalene	18.7	15.8	2700	2700	2790	2730	52.0	1.9
1,6,7-Trimethylnaphthalene	5.3	8.4	466	459	466	464	4.0	0.9
1-Methylphenanthrene	5.0	6.4	423	411	419	418	5.9	1.4
C29-Hopane	19.9	35.0						
18a-Oleanane	<2.2	<2.2						
C30-Hopane	30.2	44.4						
17a, 21b (H)-Hopane			574	503	589	555.3	45.9	8.3

Surrogate (Su)	Su Recovery (%)	Su Recovery (%)	Su Recovery (%)	Su Recovery (%)	Su Recovery (%)
Naphthalene-d8	87	95	100	100	102
Acenaphthene-d10	81	97	104	108	110
Phenanthrene-d10	74	97	102	105	103
Chrysene-d12	67	76	84	84	83
Perylene-d12	80	97	99	94	89

APPENDIX C

Toxicity Test Results

ATTACHMENT 4

WATER QUALITY DATA, SURVIVAL DATA, AND STATISTICAL ANALYSES FOR THE *SPARTINA ALTERNIFLORA* AND *S. CYNOSUROIDES* TESTS

(25 Pages)

Chalk Point - Amphipod *Ampelisca abdita* 10-Day Survival Test
Pore Water Quality Data

Day 0 **Date:** 10/5/07 (FRI)

Sediment	Salinity (ppt)	Ammonia (mg/L)	Dissolved Oxygen (mg/L)
AR-5 (Ref.)	24	2.91	6.3
AH-11	24	2.80	5.8
AH-6	23	3.61	5.7
AH-2	24	3.23	5.8
AH-5	23	3.76	5.6
AH-1	23	3.89	5.5
CR-11 (Ref.)	24	3.96	5.7
CH-6	24	3.84	5.8
CH-3	23	3.98	5.7
CH-5	24	4.07	5.6
CH-8	24	3.75	5.7
CH-11	24	3.94	5.8
CAH-10	23	4.13	5.6
CH-10	24	4.16	5.6
WREC (Con.)	22	1.01	6.2

Chalk Point - Amphipod *Ampelisca abdita* 10-Day Survival Test
Overlying Water Quality Data

Day 0 **Date:** 10/5/07 (FRI)

Sediment	Temperature (°C)	pH	Ammonia (mg/L)	Salinity (ppt)	Dissolved Oxygen (mg/L)
AR-5 (Ref.)	20.0	7.81	0.43	28	7.1
AH-11	20.0	7.98	0.07	28	7.1
AH-6	20.0	7.94	1.36	28	7.2
AH-2	20.0	7.78	1.00	28	7.1
AH-5	20.0	7.77	1.20	28	7.1
AH-1	20.0	7.81	0.97	28	7.1
CR-11 (Ref.)	20.0	7.87	1.30	28	7.2
CH-6	20.0	7.80	1.17	28	7.2
CH-3	20.0	7.81	1.13	28	7.2
CH-5	20.0	7.93	2.30	28	7.0
CH-8	20.0	7.85	1.19	28	7.1
CH-11	20.0	7.67	1.69	28	7.0
CAH-10	20.0	8.04	1.01	28	7.4
CH-10	20.0	7.81	0.90	28	7.3
WREC (Con.)	20.0	8.10	0.53	28	7.1

Chalk Point - Amphipod *Ampelisca abdita* 10-Day Survival Test
Overlying Water Quality Data

Day 1 **Date:** 10/6/07 (SAT)

Beaker	Sediment	Rep.	Temperature (°C)	Dissolved Oxygen (mg/L)	Observations
52	AR-5 (Ref.)	01	20.0	7.1	clear, some tubes starting
43	AH-11	01	20.0	7.2	clear, some tubes starting
67	AH-6	01	20.0	7.2	clear, some tubes starting
22	AH-2	01	20.0	7.1	slightly cloudy, surface "fluffy looking"
26	AH-5	01	20.0	6.9	clear, small holes/tubes
8	AH-1	01	20.1	6.7	8-9 dead - around from surface, no tubes
39	CR-11 (Ref.)	01	20.0	6.9	clear, surface is very irregular
41	CH-6	01	20.0	7.2	clear, some tubes
54	CH-3	01	20.0	7.3	clear, surface irregular
47	CH-5	01	20.0	7.0	clear, surface irregular
24	CH-8	01	20.0	7.2	clear, surface irregular
14	CH-11	01	20.1	7.4	clear, some tubes
9	CAH-10	01	20.1	7.4	clear, small holes/tubes
38	CH-10	01	20.0	7.1	clear, small holes/tubes
60	WREC (Con.)	01	20.0	7.1	clear, some tubes starting

- water bath temp - 20°C
- lights on
- air flowing to all beakers

Chalk Point - Amphipod *Ampelisca abdita* 10-Day Survival Test
Overlying Water Quality Data

Day 2 **Date:** 10/7/07 (SUN)

Beaker	Sediment	Rep.	Temperature (°C)	Dissolved Oxygen (mg/L)	Observations
55	AR-5 (Ref.)	02	20.2	7.2	clear, small tubes starting
17	AH-11	02	20.2	7.2	tubes starting
16	AH-6	02	20.2	7.3	some tubes
42	AH-2	02	20.2	7.1	small tubes
75	AH-5	02	20.2	7.0	some tubes
19	AH-1	02	20.2	7.3	lots of dead bodies - visible on sediment
72	CR-11 (Ref.)	02	20.2	7.1	some tubes
12	CH-6	02	20.2	7.1	some tubes
15	CH-3	02	20.2	7.2	some tubes
3	CH-5	02	20.2	7.3	some tubes
63	CH-8	02	20.2	7.4	some tubes
44	CH-11	02	20.2	7.4	a few tubes
62	CAH-10	02	20.2	7.2	surface is mucky, no tubes visible
51	CH-10	02	20.2	7.2	surface "oily" looking
28	WREC (Con.)	02	20.2	7.2	lots of tubes

- water bath temp ~20.2
- Lights on
- Air flowing to all beakers

Chalk Point - Amphipod *Ampelisca abdita* 10-Day Survival Test
Overlying Water Quality Data

Day 3 **Date:** 10/8/07 (mon)

Beaker	Sediment	Rep.	Temperature (°C)	Dissolved Oxygen (mg/L)	Observations
23	AR-5 (Ref.)	03	20.3	7.0	some tubes
49	AH-11	03	20.3	6.6	small tubes
71	AH-6	03	20.3	6.7	small tubes
69	AH-2	03	20.3	6.9	small tubes
33	AH-5	03	20.3	7.3	a few tubes
57	AH-1	03	20.3	7.3	surface is "fluffy" - no tubes visible
10	CR-11 (Ref.)	03	20.3	7.1	surface very irregular, a few tubes
68	CH-6	03	20.3	7.0	a few tubes
58	CH-3	03	20.3	6.9	many tubes
50	CH-5	03	20.3	7.1	some tubes
1	CH-8	03	20.3	7.2	a few tubes
25	CH-11	03	20.3	6.9	a few tubes
29	CAH-10	03	20.3	7.0	many bodies on sediment
5	CH-10	03	20.3	7.2	surface irregular, no tubes visible
46	WREC (Con.)	03	20.3	7.2	many tubes on edges

- water bath temp - 20.3°C
- lights on
- Air flowing to all beakers

Chalk Point - Amphipod *Ampelisca abdita* 10-Day Survival Test
Overlying Water Quality Data

Day 4 **Date:** 10/9/07 (TUE)

Beaker	Sediment	Rep.	Temperature (°C)	Dissolved Oxygen (mg/L)	Observations
70 (10)	AR-5 (Ref.)	04	20.1	6.9	some tubes
30	AH-11	04	20.1	6.9	many tubes
2	AH-6	04	20.1	7.1	many tubes
6	AH-2	04	20.1	7.2	many tubes
53	AH-5	04	20.1	7.3	some tubes
74	AH-1	04	20.1	7.3	many dead bodies - visible
45	CR-11 (Ref.)	04	20.1	7.1	surface very irregular, some tubes
73	CH-6	04	20.1	7.2	many tubes
18	CH-3	04	20.1	7.3	many tubes
21	CH-5	04	20.1	7.1	many tubes
35	CH-8	04	20.1	6.9	surface irregular, some tubes
32	CH-11	04	20.1	7.2	surface irregular, some tubes
65	CAH-10	04	20.1	7.4	surface kind of "fluffy"
56	CH-10	04	20.1	7.4	surface pretty smooth
66	WREC (Con.)	04	20.1	7.1	

- water bath temp - 20.1
- lights on
- Air flow to all beakers

Chalk Point - Amphipod *Ampelisca abdita* 10-Day Survival Test
Overlying Water Quality Data

Day 5 **Date:** 10/10/07 (wed)

Beaker	Sediment	Rep.	Temperature (°C)	Dissolved Oxygen (mg/L)	Observations
7	AR-5 (Ref.)	05	20.1	7.1	small tubes
64	AH-11	05	20.1	6.9	many small tubes
37	AH-6	05	20.1	7.1	many small tubes
31	AH-2	05	20.1	7.3	many small tubes
48	AH-5	05	20.1	7.3	some tubes
34	AH-1	05	20.1	7.3	sediment "fluffy", no tubes visible
4	CR-11 (Ref.)	05	20.1	7.3	surface irregular, some tubes
27	CH-6	05	20.1	7.1	some tubes
61	CH-3	05	20.1	7.0	many tubes
59	CH-5	05	20.1	6.9	surface irregular, some tubes
40	CH-8	05	20.1	7.0	a few tubes, a few burrows visible
13	CH-11	05	20.1	6.8	surface irregular, a few tubes
20	CAH-10	05	20.1	7.4	surface irregular
36	CH-10	05	20.1	7.4	surface irregular
11	WREC (Con.)	05	20.1	7.3	many tubes

- water bath temp - 20.1
- Air flowing to all beakers
- lights on

Chalk Point - Amphipod *Ampelisca abdita* 10-Day Survival Test
Overlying Water Quality Data

Day 6 **Date:** 10/11/07 (Thur)

Beaker	Sediment	Rep.	Temperature (°C)	Dissolved Oxygen (mg/L)	Observations
52	AR-5 (Ref.)	01	20.0	7.1	some tubes
43	AH-11	01	20.0	6.9	many tubes
67	AH-6	01	20.0	7.1	many tubes
22	AH-2	01	20.0	7.0	many small tubes
26	AH-5	01	20.0	6.9	a few tubes
8	AH-1	01	20.0	6.8	no tubes visible
39	CR-11 (Ref.)	01	20.0	6.9	surface very irregular, some tubes
41	CH-6	01	20.0	7.1	some tubes
54	CH-3	01	20.0	7.1	some tubes
47	CH-5	01	20.0	6.9	very irregular, some tubes
24	CH-8	01	20.0	6.8	some tubes
14	CH-11	01	20.0	7.1	no tubes visible
9	CAH-10	01	20.0	7.4	a few small tubes
38	CH-10	01	20.0	7.4	no tubes visible
60	WREC (Con.)	01	20.0	7.2	many tubes

water bath temp - 20.0°C
Air flowing to all beakers
lights on

Chalk Point - Amphipod *Ampelisca abdita* 10-Day Survival Test
Overlying Water Quality Data

Day 7 **Date:** 10/12/07 (FR)

Beaker	Sediment	Rep.	Temperature (°C)	Dissolved Oxygen (mg/L)	Observations
55	AR-5 (Ref.)	02	20.0	7.2	some tubes
17	AH-11	02	20.0	7.2	many tubes
16	AH-6	02	20.0	7.3	lots tubes
42	AH-2	02	20.0	7.2	many tubes
75	AH-5	02	20.0	7.0	surface irregular, some tubes
19	AH-1	02	20.0	7.2	surface irregular, no tubes visible
72	CR-11 (Ref.)	02	20.0	7.3	many tubes along edges
12	CH-6	02	20.0	7.2	many tubes
15	CH-3	02	20.0	7.2	many tubes
3	CH-5	02	20.0	7.4	many tubes
63	CH-8	02	20.0	7.4	surface irregular, some tubes
44	CH-11	02	20.0	7.3	surface irregular, few tubes
62	CAH-10	02	20.0	7.4	surface irregular, no tubes visible
51	CH-10	02	20.0	7.5	surface irregular, few tubes
28	WREC (Con.)	02	20.0	7.2	many tubes

- water bath temp - 20.0
- Air flowing to all beakers
- lights on

Chalk Point - Amphipod *Ampelisca abdita* 10-Day Survival Test
Overlying Water Quality Data

Day 8 **Date:** 10/13/07 (SAT)

	Sediment	Rep.	Temperature (°C)	Dissolved Oxygen (mg/L)	Observations
Beaker 23	AR-5 (Ref.)	03	20.0	7.3	few tubes
49	AH-11	03	20.0	7.2	many tubes
71	AH-6	03	20.0	7.2	many tubes
69	AH-2	03	20.0	7.1	many tubes
33	AH-5	03	20.0	7.0	some tubes
57	AH-1	03	20.0	7.2	No tubes visible
10	CR-11 (Ref.)	03	20.0	7.1	many tubes
68	CH-6	03	20.0	7.1	many tubes
58	CH-3	03	20.0	7.3	many tubes
50	CH-5	03	20.0	7.2	many tubes
1	CH-8	03	20.0	7.3	some tubes
25	CH-11	03	20.0	7.3	some tubes
29	CAH-10	03	20.0	7.3	few tubes
5	CH-10	03	20.0	7.4	few tubes
46	WREC (Con.)	03	20.0	7.2	many tubes

- water bath temp - 20.0
- Air flowing to all beakers
- lights on

Chalk Point - Amphipod *Ampelisca abdita* 10-Day Survival Test
Overlying Water Quality Data

Day 9 **Date:** 10/14/07 (SUN)

Sediment	Temperature (°C)	pH	Ammonia (mg/L)	Salinity (ppt)	Dissolved Oxygen (mg/L)
AR-5 (Ref.)	20.0	7.73	0.69	28	6.9
AH-11	20.0	7.82	0.23	28	6.9
AH-6	20.0	7.80	1.65	28	7.1
AH-2	20.0	7.62	1.15	28	7.1
AH-5	20.0	7.39	1.37	28	7.2
AH-1	20.0	7.68	1.19	28	7.1
CR-11 (Ref.)	20.0	7.71	1.39	28	7.2
CH-6	20.0	7.68	1.31	28	7.0
CH-3	20.0	7.72	1.28	28	7.0
CH-5	20.0	7.68	2.46	28	7.3
CH-8	20.0	7.81	1.35	28	7.4
CH-11	20.0	7.81	1.85	28	7.1
CAH-10	20.0	7.79	1.17	28	7.4
CH-10	20.0	7.61	1.04	28	7.4
WREC (Con.)	20.0	8.14	0.79	28	7.2

water bath temp - 20.0
Air flowing to all beakers

Chalk Point - Amphipod *Ampelisca abdita* 10-Day Survival Test
Overlying Water Quality Data

Day 10 **Date:** 10/15/07 (MON)

Beaker	Sediment	Rep.	Temperature (°C)	Dissolved Oxygen (mg/L)	Observations
70	AR-5 (Ref.)	04	20.0	7.1	many tubes
30	AH-11	04	20.0	7.1	many tubes
2	AH-6	04	20.0	7.2	many tubes
6	AH-2	04	20.0	7.0	many tubes
53	AH-5	04	20.0	7.0	many tubes
74	AH-1	04	20.0	6.9	no tubes visible
45	CR-11 (Ref.)	04	20.0	7.1	many tubes
73	CH-6	04	20.0	7.0	many tubes
18	CH-3	04	20.0	7.2	many tubes
21	CH-5	04	20.0	7.1	many tubes
35	CH-8	04	20.0	7.0	some tubes
32	CH-11	04	20.0	7.2	some tubes
65	CAH-10	04	20.0	7.2	a few tubes
56	CH-10	04	20.0	7.3	a few tubes
66	WREC (Con.)	04	20.0	7.2	many tubes

Final Day of Test

Chalk Point- Amphipod Ampelisca abdita 10-Day Survival Test
Day 10- Survival Data

Day 10 Date: 10/15/07 (MON)

Sediment	Rep.	No. Alive	Observations
AR-5 (Ref.)	01	20	
AR-5 (Ref.)	02	19	
AR-5 (Ref.)	03	20	
AR-5 (Ref.)	04	19	
AR-5 (Ref.)	05	20	
AH-11	01	20	
AH-11	02	20	
AH-11	03	20	
AH-11	04	20	
AH-11	05	20	
AH-6	01	19	
AH-6	02	20	
AH-6	03	19	
AH-6	04	19	
AH-6	05	20	
AH-2	01	20	
AH-2	02	19	
AH-2	03	18	
AH-2	04	19	
AH-2	05	20	
AH-5	01	19	
AH-5	02	17	
AH-5	03	18	
AH-5	04	15	
AH-5	05	16	

Chalk Point- Amphipod Ampelisca abdita 10-Day Survival Test
Day 10- Survival Data

Day 10 Date: 10/15/07 (Mon

Sediment	Rep.	No. Alive	Observations
AH-1	01	0	
AH-1	02	0	
AH-1	03	0	
AH-1	04	0	
AH-1	05	0	
CR-11 (Ref.)	01	20	
CR-11 (Ref.)	02	19	
CR-11 (Ref.)	03	20	
CR-11 (Ref.)	04	20	
CR-11 (Ref.)	05	20	
CH-6	01	20	
CH-6	02	19	
CH-6	03	19	
CH-6	04	18	
CH-6	05	18	
CH-3	01	20	
CH-3	02	18	
CH-3	03	19	
CH-3	04	20	
CH-3	05	20	
CH-5	01	18	
CH-5	02	17	
CH-5	03	19	
CH-5	04	20	
CH-5	05	17	

Chalk Point- Amphipod Ampelisca abdita 10-Day Survival Test
Day 10- Survival Data

Day 10 Date: 10/15/07 (mon)

Sediment	Rep.	No. Alive	Observations
CH-8	01	15	
CH-8	02	13	
CH-8	03	14	
CH-8	04	13	
CH-8	05	11	
CH-11	01	2	
CH-11	02	0	
CH-11	03	1	
CH-11	04	0	
CH-11	05	0	
CAH-10	01	0	
CAH-10	02	1	
CAH-10	03	0	
CAH-10	04	0	
CAH-10	05	0	
CH-10	01	0	
CH-10	02	0	
CH-10	03	0	
CH-10	04	0	
CH-10	05	0	
WREC-CON	01	20	
WREC-CON	02	20	
WREC-CON	03	19	
WREC-CON	04	18	
WREC-CON	05	19	

Title: chalk point sediment- AR Ref vs. WREC CON
File: ar5 Transform: NO TRANSFORMATION

t-Test of Solvent and Blank Controls Ho: GRP1 Mean = GRP2 Mean

GRP1 (Solvent cntl) Mean =	0.9600	Calculated t value =	-0.8944
GRP2 (Blank cntl) Mean =	0.9800	Degrees of freedom =	8
Difference in means =	-0.0200		

2-sided t value (0.05, 8) = 2.3060 No significant difference at alpha=0.05
2-sided t value (0.01, 8) = 3.3554 No significant difference at alpha=0.01

WARNING: This procedure assumes normality and equal variances!

GRP1 - A. thaliana control
GRP2 - S. alterniflora control

Title: chalk point 10-d ampelisca test- AH samples
File: arsur Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's Test for Normality

D = 0.1175
W = 0.9637

Critical W = 0.9000 (alpha = 0.01 , N = 30)
W = 0.9270 (alpha = 0.05 , N = 30)

Data PASS normality test (alpha = 0.01). Continue analysis.

Title: chalk point 10-d ampelisca test- AH samples
File: arsur Transform: ARC SINE(SQUARE ROOT(Y))

Hartley's Test for Homogeneity of Variance
Bartlett's Test for Homogeneity of Variance

These two tests can not be performed because at least one group has zero variance.

Data FAIL to meet homogeneity of variance assumption.
Additional transformations are useless.

Title: chalk point 10-d ampelisca test- AH samples
File: arsur Transform: ARC SINE(SQUARE ROOT(Y))

Summary Statistics on Transformed Data TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	AR-5 (ref)	5	1.3453	1.4588	1.4134
2	AH-11	5	1.4588	1.4588	1.4588
3	AH-6	5	1.3453	1.4588	1.3907
4	AH-2	5	1.2490	1.4588	1.3714
5	AH-5	5	1.0472	1.3453	1.1844
6	AH-1	5	0.1120	0.1120	0.1120

Title: chalk point 10-d ampelisca test- AH samples
File: arsur Transform: ARC SINE(SQUARE ROOT(Y))

Summary Statistics on Transformed Data TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	AR-5 (ref)	0.0039	0.0622	0.0278	4.3977
2	AH-11	0.0000	0.0000	0.0000	0.0000
3	AH-6	0.0039	0.0622	0.0278	4.4694
4	AH-2	0.0079	0.0889	0.0397	6.4809
5	AH-5	0.0137	0.1172	0.0524	9.8992
6	AH-1	0.0000	0.0000	0.0000	0.0000

Title: chalk point 10-d ampelisca test- AH samples
File: arsur Transform: ARC SINE(SQUARE ROOT(Y))

Steel's Many-One Rank Test - Ho: Control<Treatment

GROUP	IDENTIFICATION	%	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	DF	SIG 0.05
1	AR-5 (ref)	98	1.4134				
2	AH-11	100	1.4588	32.50	16.00	5.00	
3	AH-6	97	1.3907	25.00	16.00	5.00	
4	AH-2	96	1.3714	24.00	16.00	5.00	
5	AH-5	85	1.1844	16.00	16.00	5.00	*
6	AH-1	0	0.1120	15.00	16.00	5.00	*

Critical values are 1 tailed (k = 5)

Title: Chalk point- cr11 vs. wrec con
File: cr11 Transform: NO TRANSFORMATION

t-Test of Solvent and Blank Controls Ho: GRP1 Mean = GRP2 Mean

GRP1 (Solvent cntl) Mean =	0.9600	Calculated t value =	-1.4142
GRP2 (Blank cntl) Mean =	0.9900	Degrees of freedom =	8
Difference in means =	-0.0300		

2-sided t value (0.05, 8) = 2.3060 No significant difference at alpha=0.05
2-sided t value (0.01, 8) = 3.3554 No significant difference at alpha=0.01

WARNING: This procedure assumes normality and equal variances!

GRP1 - H. Anna cntl
GRP2 - S. cyprinorides cntl

Title: chalk point 10-d ampelisca test- CH samples
File: chsur Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's Test for Normality

D = 0.2073
W = 0.9804

Critical W = 0.9190 (alpha = 0.01 , N = 40)
W = 0.9400 (alpha = 0.05 , N = 40)

Data PASS normality test (alpha = 0.01). Continue analysis.

Title: chalk point 10-d ampelisca test- CH samples
File: chsur Transform: ARC SINE(SQUARE ROOT(Y))

Hartley's Test for Homogeneity of Variance
Bartlett's Test for Homogeneity of Variance

These two tests can not be performed because at least one group has zero variance.

Data FAIL to meet homogeneity of variance assumption.
Additional transformations are useless.

Title: chalk point 10-d ampelisca test- CH samples
File: chsur Transform: ARC SINE(SQUARE ROOT(Y))

Summary Statistics on Transformed Data TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	cr-11 (ref)	5	1.3453	1.4588	1.4361
2	CH-6	5	1.2490	1.4588	1.3295
3	CH-3	5	1.2490	1.4588	1.3941
4	ch-5	5	1.1731	1.4588	1.2799
5	ch-8	5	0.8355	1.0472	0.9499
6	ch-11	5	0.1120	0.3218	0.1767
7	cah-10	5	0.1120	0.2255	0.1347
8	ch-10	5	0.1120	0.1120	0.1120

Title: chalk point 10-d ampelisca test- CH samples
File: chsur Transform: ARC SINE(SQUARE ROOT(Y))

Summary Statistics on Transformed Data TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	cr-11 (ref)	0.0026	0.0507	0.0227	3.5339
2	CH-6	0.0075	0.0868	0.0388	6.5306
3	CH-3	0.0090	0.0948	0.0424	6.8018
4	ch-5	0.0150	0.1225	0.0548	9.5720
5	ch-8	0.0061	0.0784	0.0350	8.2487
6	ch-11	0.0090	0.0948	0.0424	53.6711
7	cah-10	0.0026	0.0507	0.0227	37.6656
8	ch-10	0.0000	0.0000	0.0000	0.0000

Title: chalk point 10-d ampelisca test- CH samples
File: chsur Transform: ARC SINE(SQUARE ROOT(Y))

Steel's Many-One Rank Test - Ho: Control<Treatment

GROUP	IDENTIFICATION	%	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	DF	SIG 0.05
1	cr-11 (ref)	99	1.4361				
2	CH-6	94	1.3295	19.00	16.00	5.00	
3	CH-3	97	1.3941	24.50	16.00	5.00	
4	ch-5	91	1.2799	18.50	16.00	5.00	
5	ch-8	46	0.9499	15.00	16.00	5.00	*
6	ch-11	3	0.1767	15.00	16.00	5.00	*
7	cah-10	1	0.1347	15.00	16.00	5.00	*
8	ch-10	0	0.1120	15.00	16.00	5.00	*

Critical values are 1 tailed (k = 7)

Aquatic Research Organisms
Data Sheet

Collection Date: 9/30/07
Time: 8:30 A.M
Field Temperature:
Water: 18 °C Air: 20 °C
Physical Data: In sediment jar.
Animal Collected: *A. abdita*
Number Collected: 16000+ Sediment Provided: Yes (No)

Jar Number	No. of Amphipods	Amphipod Size		Sediment
1		<1.7mm >1.18mm		
	2800	<1.18mm >.71mm	X	some
2		<1.7mm >1.18mm		
	≈800	<1.18mm >.71mm	X	some
3		<1.7mm >1.18mm		
		<1.18mm >.71mm		
4		<1.7mm >1.18mm		
		<1.18mm >.71mm		
5		<1.7mm >1.18mm		
		<1.18mm >.71mm		
6		<1.7mm >1.18mm		
		<1.18mm >.71mm		
7		<1.7mm >1.18mm		
		<1.18mm >.71mm		
8		<1.7mm >1.18mm		
		<1.18mm >.71mm		
9		<1.7mm >1.18mm		
		<1.18mm >.71mm		
10		<1.7mm >1.18mm		
		<1.18mm >.71mm		

Location: Blackwater river Hampton, NH
Animals shipped in Blackwater river sediment
Collected By: Norman Bradt

Comment: Spirulina added to holding jar before shipping.
Holding jar aerated with pure O_2 before packing.
Control sediment not sieved

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