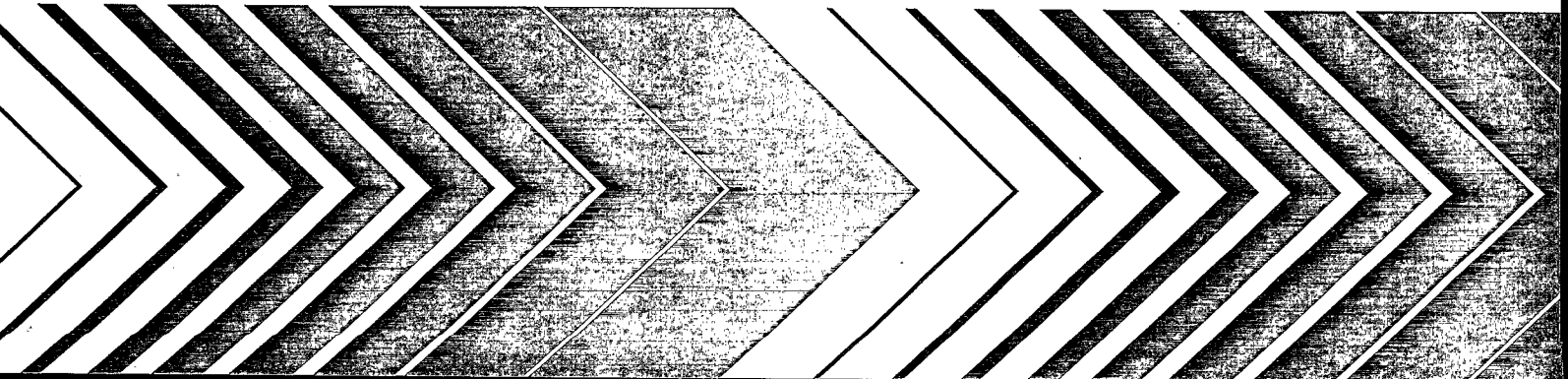
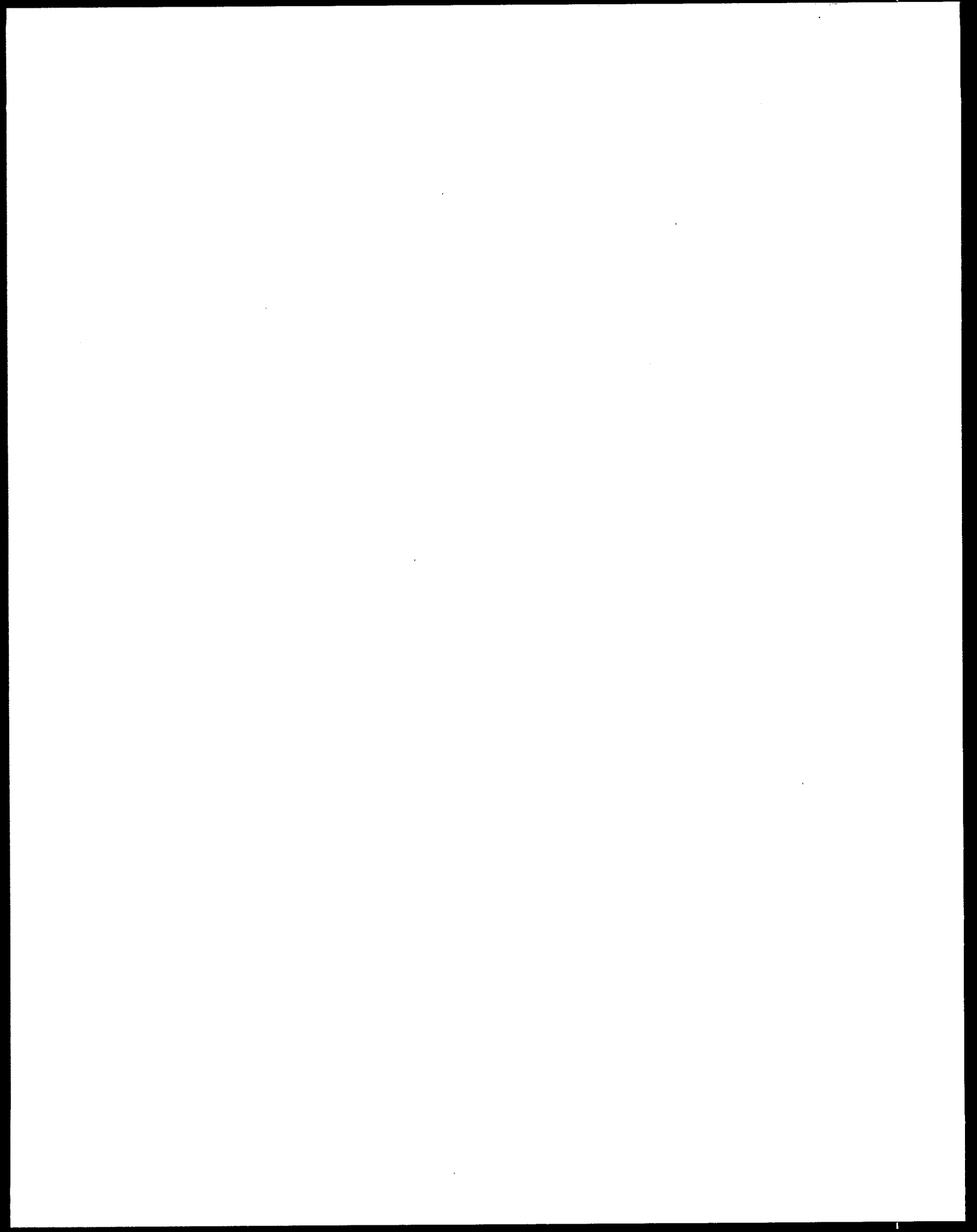




# **Synthetic-Based Drilling Fluids: An Assessment of the Spatial Distribution of Toxicants in Sediments from Gulf of Mexico Drilling Platforms**

**A Report Prepared for the  
Office of Water**





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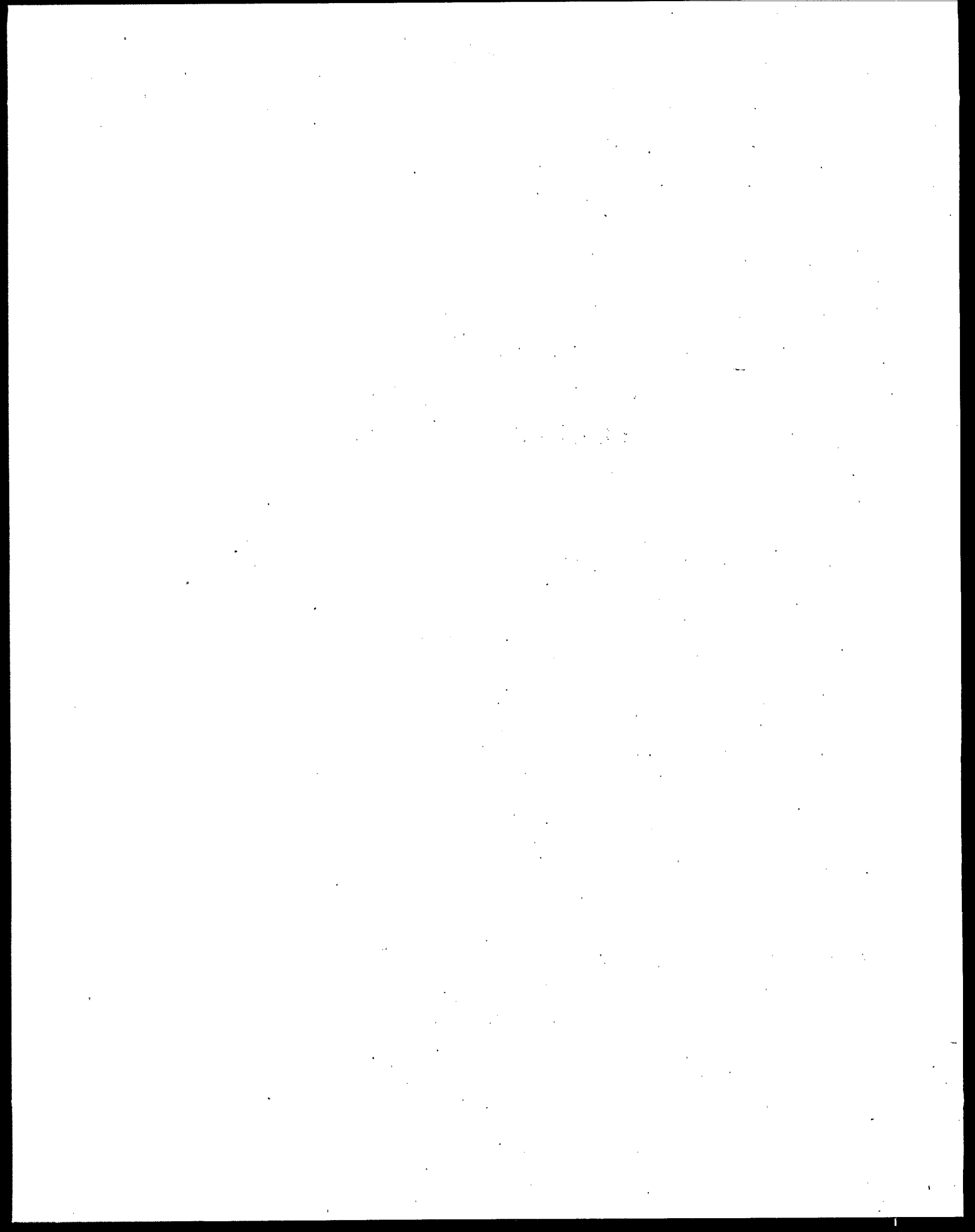
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## Abstract

Use of the amphipods, *Leptocheirus plumulosus* and *Ampelisca abdita*, in these bioassays presented no major difficulties in the execution of these test protocols. Sensitivity to the toxicants was exhibited by *L. plumulosus* and survival of control animals was good suggesting the suitability of this organism for use. Continued application of these species to evaluations of field-collected sediments contaminated with Synthetic-based drilling fluids (SBF) is encouraged and should enhance our understanding of the toxicity of these products. Data from this initial screening with *Leptocheirus plumulosus* indicate sensitivity of this species to sediments collected within a 150 meter radius of platform 1 (GI95) and demonstrate the spatial distribution of contaminants along a gradient. Sediments within the vicinity of the other two platforms, (platform 2, SMI57C and platform 3, ST148) proved to be less toxic than those from platform 1 but serve to illustrate the sensitivity of the organism, *L. plumulosus* to a range of SBF. Data from tests with *Ampelisca abdita* indicated a lower sensitivity to the field-collected samples than was observed with *L. plumulosus*. Survival values in the range of the control suggested an apparent lack of toxicity from any of the sites to this organism above those of background. Procedural delays were thought to have reduced the overall responsiveness of *Ampelisca abdita* in these tests. Measures of sample variability indicated variability between replicate samples from the same grab and between sequential grabs. Variability denoted in composite samples suggests additional research should be conducted to improve the protocol to achieve sample homogenization. Coarse-sieving of field-collected sediments should also be explored to ascertain if such procedural modifications might also reduce sample variability.

## 1. INTRODUCTION

Increasing pressure from industry has prompted the U.S. Environmental Protection Agency (EPA) to consider the expansion of current regulatory guidelines for oil drilling to include language to facilitate more wide-spread use of synthetic-based drilling fluids (SBF) in the United States. Synthetic-based fluids are currently in use in US coastal waters although no specific limitations for SBF have been set forth in current guidelines (EPA, 1996). Historically, use of SBF has been greatest in the North Sea (Friedheim and Conn, 1996); however, use of these agents in the US has grown appreciably (as many as 300 wells in the Gulf of Mexico have been drilled with SBF) since the initiation of drilling of the first well using SBF in the Gulf of Mexico in 1992 (Candler et al., 1997).

Synthetic-based fluids have been described as being more effective than water-base muds (WBM) and oil-based muds (OBM) and considered more environmentally benign than their predecessors (Veil et al., 1996; Burke and Veil, 1995; Candler et al., 1993; Friedheim et al., 1991). Despite indications of some

environmental benefits use of the agents in US waters remains tenuous until questions can be addressed about the environmental safety of these agents and the appropriateness of toxicity tests currently described in the coastal guideline to adequately assess the potential impact of SBF on benthic species.

Synthetic-based fluids are muds prepared for drilling purposes which are manufactured from materials containing no detectable levels of priority pollutants. All major mud suppliers are said to offer SBF, and formulations of these materials are routinely prepared using vegetable esters, polyalpha olefins (PAO), internal/isomerized olefins (IO), linear alpha olefins (LAO) and ethers (Candler et al., 1997). Traditional base-fluids such as diesel and mineral oil, are not designated SBF since they are not synthesized. Conversely, they are refined from crude oil and are known to contribute to the toxicity of drilling fluids (they release aromatics into the water fraction of the fluids). Concern regarding the use of SBF is related largely to questions about the biodegradability and toxicity of SBF-coated cuttings, since cutting piles accumulate on the seafloor and synthetics may account for as much as 12% of the material adhering to the surface of the cuttings (Friedheim and Conn, 1966).

Because toxicity protocols, currently incorporated in the coastal guidelines, were designed to assess water-column effects (EPA, 1996), limited toxicological data are available on the potential impact of Synthetic-based fluids on North American benthic species (Candler et al., 1997; Candler, 1997; Hood, 1997 a & b). One seafloor study has been completed which directly addresses the impacts of SBF discharges on benthic fauna of the Gulf of Mexico (Candler et al., 1995) and suggests diminished biological effects of an SBF when compared with an OBM.

Evaluation of several benthic endpoints (species richness, diversity and number of individuals) and total petroleum hydrocarbon (TPH) concentrations indicate a smaller area of transition (than for OBM) surrounding the PAO discharge platform with community level effects approaching background for the benthic fauna.

Laboratory investigations with benthic species have focused on use of the amphipods, *Corophium volutator*, *Rhepoxinius abronius*, *Ampelisca abdita* and *Leptocheirus plumulosus*, and deal almost exclusively with the toxicity of the base fluids (Candler et al., 1997; unpublished data by Candler, 1997; Hood, 1997a). Contaminants of interest have included enhanced mineral oils (EMO), internal/isomerized olefins (IO), and polyalpha olefins (PAO). Some data exist on the toxicity of a used, whole synthetic-base mud (Hood, 1997b) and indicate some product toxicity. The reported LC<sub>50</sub> for the used SBF ranged between 692 mg/kg and 3, 600 mg/kg in 10-day

(definitive) sediment toxicity tests with *R. abronius*, *A. abdita* and *L. plumulosus*. Ranking of species sensitivity to this product indicated *Leptocheirus* > *Rhepoxinius* > *Ampelisca*.

Information gaps exist and suggest the need for continued research on SBF to enhance our understanding of the toxicity and potential hazards associated with the discharge of drilling fluids and cuttings, contaminated with synthetic material, into sub-tropical waters such as the Gulf of Mexico. This study represents a singular attempt to augment the current data to provide information on the toxicity of three product types, IO, LAO, and a combined ester-olefin mixture, currently in use on drilling platforms in the Gulf of Mexico.

This study was designed to provide a qualitative assessment of a series of field sites in the Gulf of Mexico for the Office of Water, with an indication of the potential hazards associated with the field application/use of synthetic-based drilling fluids (SBF). Additionally, this report supplies information on the relative sensitivity of two infaunal amphipods, *Leptocheirus plumulosus* and *Ampelisca abdita*, to these agents and discusses the feasibility of adapting a standardized protocol, such as the 10-day acute sediment toxicity test (EPA, 1994), to the evaluation of a non-homogeneous geochemical matrix of SBF mixed with sediment.

The use of *L. plumulosus* and *A. abdita* in this study, and their consideration for use in Agency guidance for Synthetic-based fluids, is intended to complement current regulatory trends toward use of amphipods for the assessment of sediment-associated contaminants and is intended to complement works previously performed on this unique group of products. Both *Leptocheirus plumulosus* and *Ampelisca abdita* have been used routinely for the evaluation of the toxicity of marine and estuarine sediments. Their sensitivity to a range of toxicants has been documented in the scientific literature, and the method reviewed extensively. Guidance documents (ASTM, 1993; EPA, 1994) have been prepared for these acute bioassays and serve to substantiate the credibility of these protocols for use in ecological risk assessment.

Toxicity testing was conducted according to EPA Guidelines as specified in *Methods for assessing the toxicity of sediment-associated contaminants with estuarine and marine amphipods* (EPA, 1994). Testing was conducted at Gulf Ecology Division, NHEERL, U.S. Environmental Protection Agency, Gulf Breeze, FL, and utilized undiluted, sediment samples collected in the vicinity of three drilling platforms (GI95, SMI 57C and ST 148) during a reconnaissance survey conducted aboard the research vessel S.S. Anderson (EPA) August 18-22, 1997.

## 2. TEST SUBSTANCE

Field samples containing synthetic drilling fluids were received from George Gibson, U.S. Environmental Protection Agency, on August 23, 1997. Samples were contained in sample jars of a variable nature, i.e., size (1- 1.5 liter) and construction (glass or high-density polyethylene). Upon receipt in the laboratory, the samples were stored in the dark in an environmental chamber at approximately 4°C. Prior to their use in the bioassay, each sample underwent a visual inspection to assess spoilage. An absence of a foul odor and black spots on the surface of the sediments were noted for each sample, indicating suitability for testing.

## 3. TEST ORGANISMS

Field samples were evaluated using one marine amphipod, *Ampelisca abdita*, and one estuarine amphipod, *Leptocheirus plumulosus*. *Leptocheirus plumulosus* were purchased from Chesapeake Cultures (P.O. Box 507, Hayes, VA 23072, 804 693-4046) and were received on August 29, 1997. *Ampelisca abdita* were purchased from East Coast Amphipod (16 Ayrault St., Suite 1, Newport, RI 02840, 401 849-4631). The amphipods were collected and shipped on September 3, 1997, and received on September 5, 1997, after a 36 H delay in shipment.

**Feeding:** No food was supplied to the amphipods during holding and testing.

**Age/Length:** *Leptocheirus plumulosus* used in this study were mixed-age adults ranging in size from 2 to 4 mm. *Ampelisca abdita* were juveniles, ranging in size from 0.71 mm to 1.18 mm.

**Receipt/Handling:** Water quality parameters of the overlying water contained in each organism shipping container were measured and recorded upon arrival at the laboratory. *Leptocheirus* were shipped in water only, thus transfer of these organisms to containers of freshly, aerated seawater was accomplished by pouring the contents of a container through a 125 µm sieve. Amphipods retained on the screen of the sieve were flushed from the screen with a gentle stream of saline (20 ppt) water into 2L Carolina culture dishes containing 20 ppt oxygenated seawater. Bowls were aerated and the organisms were held at 20±1°C until the randomization process was initiated.

As was the case with *Leptocheirus*, water quality parameters of the overlying water of each shipping container of *Ampelisca* were measured and recorded upon arrival at the laboratory. Collection of *Ampelisca* was slightly different, however, as the amphipods had been shipped in sediment obtained from their collection site. Using a rubber spatula, sediment containing the

amphipods was removed from the shipping container and placed on the surface of a 500  $\mu\text{m}$  sieve. The sieve was placed inside a large polyethylene tub and ambient sea water gently sprayed over the surface to facilitate removal of the sediments.

Most of the sediment passed through, leaving the amphipods behind. A fine spray of water was again passed over the sieve to ensure organisms had been flushed from their tubes. Amphipods retained on the screen of the sieve were flushed from the screen with a gentle stream of 28 ppt water into 2L Carolina culture dishes containing 20 ppt oxygenated seawater (28 ppt). The tubes were examined to see if any amphipods were present. Amphipods found adhering to the water surface were removed from the containers by placing a fine-meshed screen just below the surface of the water and gently lifting them out. These organisms were added to the culture dishes containing animals previously collected. The bowls were aerated and the organisms were held in environmental chambers maintained at  $28 \pm 1^\circ\text{C}$  until the randomization process was initiated.

#### 4. REFERENCE & FIELD-COLLECTED SEDIMENTS

The reference sediment for these tests was sediment collected by the supplier. In the case of *Leptocheirus*, no sediment was received from the vendor; therefore a sediment obtained from a non-polluted region of the Pensacola Bay Estuary was used as a reference sediment. The sediments were coarse-sieved through a 2,000  $\mu\text{m}$  stainless steel sieve and fine-sieved through a 500  $\mu\text{m}$  stainless steel sieve to remove any large organisms that were confused with or preyed upon the amphipods.

Field-collected sediments were not wet-sieved, in an attempt to maintain the integrity of their geochemical properties. Stainless steel forceps were used, however, to remove large objects (shell and other debris) and predators from the field samples prior to use in the bioassay. Samples collected from multiple benthic grabs were homogenized by stirring by hand. Samples from the same benthic grab were similarly homogenized, if the samples were received in more than one storage container.

#### 5. OVERLYING WATER

In the case of *Leptocheirus*, the overlying water added to the exposure chambers after the addition of the sediment-test was 20 ppt natural, filtered sea water. The overlying water added to *A. abdita* exposure chambers was 28 ppt natural, filtered sea water. Synthetic sea water was prepared by adding a brine solution (prepared from a commercial preparation of dried, balanced sea salts [Forty Fathoms Sea Salts, Baltimore, MD]) to natural sea water (20 ppt filtered seawater) to obtain a seawater mixture of 28 ppt salinity. The resultant solution was aerated and allowed to age for several days prior to use. Water for tests with *Ampelisca* was maintained in a water bath at  $20^\circ\text{C}$ , and seawater for *Leptocheirus* maintained at  $25^\circ\text{C}$ .

#### 6. EXPOSURE CHAMBERS

Exposure chambers were one-liter glass beakers. Glassware was acid-washed prior to use, rinsed five times with deionized water and air-dried prior to affixing sample labels to each exposure vessel. Beakers contained approximately 2 cm of field-collected sediment. Sediments were weighed to ensure equivalent amounts of material were delivered to each beaker (average weight of sediment disbursed = 253.47 g). Sea water (800 ml, 20 ppt and 28 ppt salinity, respectively, for *L. plumulosus* and *A. abdita*) was added to each exposure chamber to bring the total volume of sediment and overlying water to 1 liter.

#### 7. TEST CONCENTRATION

Because a limited amount of sediment was received, the test was conducted as a screening bioassay. As such, a geometric series of concentrations of the sediments was not tested. Rather, replicate samples (3) of the undiluted field-collected sediment were evaluated against a control in the 10 day acute test.

Exposure chambers containing control and field-collected samples were placed onto a water table maintained at  $20^\circ\text{C}$ . Each exposure chamber was covered with a watch glass containing a small hole used for insertion of an aeration apparatus. Gentle aeration of each exposure chamber was established using flexible air-line tubing fitted with a 1 ml serological glass pipette. The flexible air-line tubing was connected to a gang valve, and the tapered end of the pipette inserted through the hole of the watch glass, suspending it to a depth approximately 2 cm below the surface of the water in the exposure chamber. Temperature, dissolved oxygen, pH and salinity were measured in the overlying water in each treatment and control.

#### 8. PREPARATION OF TEST ORGANISM

Organisms maintained overnight in two-liter Carolina dishes were randomly distributed using a fire-polished, wide-bore dropping pipette to 10 ml glass beaker cups containing approximately 10 ml of sea water. Five organisms were randomly delivered into each cup; a total of twelve cups were prepared for each treatment and control.

#### 9. TEST INITIATION

The test was initiated when twenty organisms, introduced into each exposure chamber by gently pouring the 10-ml cups over a fine-meshed screen, were transferred from the screen into the overlying water of the exposure chamber. This procedure was repeated until the required number of organisms (20) was introduced into the exposure chambers.

#### 10. TEST MONITORING & TERMINATION

The bioassay was performed under condition of continuous light in accordance with the recommended test conditions (Table 1). Aeration of the exposure chambers was continuous, each

chamber was observed daily and the airflow adjusted, as appropriate, to ensure maintenance of dissolved oxygen at  $\geq 90\%$ . Temperature, dissolved oxygen (D.O.), pH and salinity were measured in the overlying water of each treatment and control at the start and conclusion of the test. Water quality parameters (D.O., pH, salinity and temperature) were monitored daily for a single representative of each treatment and control group.

The test was terminated 10 days after introduction of the amphipods to the exposure chambers. Beginning with the control treatment, the contents of each replicate were poured onto the screen of a 500- $\mu\text{m}$  nylon, sieve held over a plastic tub. The organisms were typically retained on the screen while most of the sediment passed through the screen. A fine spray of water was passed over the sieve to remove any sediments adhering to the surface of the screen.

Animals were collected from the sieve any passing through the screen were recovered from the surface of the water retained in the tub (refer to the prior description of this technique) and all were placed in a finger bowl. The finger bowls were placed on an illuminated light table and the number of surviving organisms determined. In cases where mortality was questionable, determination of survival was made by examining animals under a dissecting scope.

### 11. DEVIATIONS FROM PROTOCOL

Sediment toxicity tests with *L. plumulosus* and *A. abdita* included a few departures from the standard testing protocols. One deviation from the guidelines was the use of only three replicate samples of each sediment evaluated, rather than the prescribed minimum of four (EPA, 1994). This was necessitated by the limited volume of material received and the need to conduct testing with two test organisms. Because a reduced number of replicates was used, test acceptability was modified to include a minimum control survival of 80%.

Another deviation from recommended test guidelines was the use of sediments beyond the suggested 14 day storage limit (ASTM, 1996). Sediments used in tests with *Ampelisca* were beyond this limit at the initiation of the 10-day acute sediment toxicity test. This was due to the initial receipt of a batch of organisms of inferior quality and a delay in receipt (36 H) of the replacement shipment of amphipods. Visual and olfactory inspection of the sediments used in tests with *A. abdita*, however, indicated no apparent loss of quality for these samples. Because animals were shipped at temperatures and salinities matching those used in the toxicity tests, animals were used without a period of acclimation as is generally recommended.

### 12. STATISTICAL ANALYSIS

The determination of 10-day  $\text{LC}_{50}$  values was not performed, as a geometric series of sediment concentrations had not been

evaluated for each of the field-collected sediments. The mean percent (%) survival was calculated for each replicate group of samples and served as the basis for comparison. To compare sites, split samples from composites (although not statistically true replicates) were used to compare sample variability (attributed to the homogenization procedure) and to test the sensitivity of the two test organisms, *L. plumulosus* and *A. abdita*. Replicate samples from the same benthic grab were used to compare within-grab variability.

### 13. RESULTS

*Leptocheirus*: The bioassay was terminated after 10 days of exposure, and the survival for each treatment group determined. Survival data for *Leptocheirus plumulosus* are tabulated in Table 2. Data from tests with *Ampelisca abdita* are shown in Table 3. Data from tests with *Leptocheirus* indicate a high degree of toxicity (0 - 65% survival) for sites within a 150 m (1G1, 1G3, 1G7 and 1G10) radius of drilling Platform 1 (GI 95). Although survival at the platform reference site (1 R3 A+B) was slightly reduced compared with that noted in the control sediment (C-17) (83.3% compared to 95%), it was significantly different from the four test sites (1G1, 1G3, 1G7 and 1G10) referred to above.

Sediment samples collected at sites adjoining Platforms 2 (SMI 57C) and 3 (ST 148) were far less toxic than those collected in the vicinity of Platform 1. The lowest recorded survival (81.7%) for any of the sediments for stations surrounding Platform 2 was observed with sediments from station 2G2. Although sediment samples from stations within the survey area of Platform 3 demonstrated lower toxicity than sediments from Platform 1, they tended to be slightly more toxic than samples obtained from sites near Platform 2. Sediments from the reference site for Platform 2 (2R1) were of equivalent toxicity (95%) to the control sediment (C17). A survival value of 86.7% was recorded for the sediments from the Platform 3 reference site (3R2).

*Ampelisca*: Tests with *Ampelisca* indicated lower survival (86.67%) with the control treatments (i.e., *Ampelisca* control sediment) than was observed with *Leptocheirus* (95%). Contrary to indications of *L. plumulosus* adaptability to Pensacola Bay sediments (C17), this sediment proved unsuitable for habitation by *A. abdita* (0% survival). Survival of amphipods treated with sediments from Platforms 1, 2 and 3 indicated no adverse toxicity beyond that demonstrated for the control treatment. The lowest survival value was 83.3% and was recorded for organisms treated with sediments from 1G10B and 3G1A+B. In one case, survival (91.7%) for one of the field-collected sediments (2G6) exceeded that recorded for the *Ampelisca* control sediment (86.7%). Toxicity for the two reference sites (1R3A+B and 3R2) evaluated in this series of acute toxicity tests indicated comparability to that determined for



the control sediment (85.0% and 88.3 % survival, respectively, compared with 86.7% for the control).

*Reference Toxicant:* Toxicity evaluations involving the reference toxicant, copper sulfate were performed at concentrations in excess of the 96-hour LC<sub>50</sub> of copper sulfate, for both *L. plumulosus* and *A. abdita* to ensure demonstration of a lethal effect. In both cases, all animals had expired within 24 H of their initial exposure.

#### 14. CONCLUSIONS

Use of the amphipods, *Leptocheirus plumulosus* and *Ampelisca abdita*, in these bioassays presented no major difficulties in the execution of these test protocols. Sensitivity to the toxicants was exhibited by both organisms, and survival of control animals was good, indicative of the suitability of *L. plumulosus* and *A. abdita* for use. Continued application of these species to evaluations of field-collected sediments contaminated with synthetic-based fluids (SBF) is encouraged and is expected to enhance our understanding of the toxicity of these products.

Data from this initial reconnaissance survey indicate toxicity associated with sediments recovered from sites surrounding at least one of the drilling platforms (Platform 1; GI 95). Although toxic responses were limited, only one (*Leptocheirus plumulosus*) of the two species tested (*L. plumulosus* and *Ampelisca abdita*) at these sites warrant closer examination, because of the extreme degree of toxicity (0% survival) denoted for at least one site (1G1) in close proximity (50 m) to the point of discharge for Platform 1. Toxicity was also clearly evident at other sites within a 150 m radius of this platform (1G3, 1G7 and 1G10), although survival was not nearly as limited for animals exposed to these sediments as for those treated with sediments from 1G1.

These data, coupled with data from the reference site for this platform (1R3), clearly indicate the spatial distribution of toxicants beyond the point of discharge and illustrate the dilution of a pollutant along a geographic gradient. Similarly, recent work by Candler et al. (1995) demonstrated the distribution of contaminants within a 200 m radius of a synthetic-base well (characterized as using PAO) in the Gulf of Mexico.

Quantitative analysis and pollutant characterization have not yet been completed for these samples, thus the nature of the contaminant(s) associated with these samples or the identity of the agents eliciting this toxic response is not yet clear. Petroleum is suspected of having contributed to the toxic response noted with *Leptocheirus*, as a smell of petroleum products was clearly evident when these samples were distributed to the test chambers. Furthermore introduction of water to the beakers containing these sediments resulted in the formation of a surface sheen. Because oil can be quite toxic to

aquatic life (Neff and Anderson, 1981), the presence of petroleum and petroleum by-products may serve to mask or enhance the toxicity associated with any synthetic materials discharged from the drilling platform.

Comparison of data from 10 day, static acute sediment tests with the two target organisms, *L. plumulosus* and *A. abdita*, indicate comparative sensitivity of *Leptocheirus* > *Ampelisca*. *Ampelisca abdita* was seen to be less susceptible to the effects of exposure to the synthetic muds than was *Leptocheirus*, despite the lower numbers of amphipods noted in sediments from both the control treatments and the reference sites at the conclusion of the test. The lower survival counts denoted in *A. abdita* control sediments were attributed to stress experienced by the animals prior to initiation of the test (i.e., the 36 H shipping delay).

Comparison of survival data from treatments involving exposure to sediments from multiple (benthic) grabs at a collection site indicates a small degree of variability among sequential grabs. Hand mixing of composite sample tended not to reduce the variability associated with replicate samples, although it appeared not to distort the toxic effect noted in the individual grab samples. Additional research should be conducted to see if other methods of mixing might further reduce the variability among sample replicates. Considerable amounts of shell and other marine debris were associated with the samples making recovery and counting of the amphipods rather time consuming. The presence of this material was also thought to partially contribute to the variability observed among sample replicates. Additional studies should be initiated to assess the effect of coarse-sieving of these sediments on the sample toxicity.

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Table 1. Test conditions for conducting a 10-d sediment toxicity test with *Ampelisca abdita*, *Eohaustorius estuarius*, *Leptocheirus plumulosus*, or *Rhepoxynius abronius*.

| Parameter                                   | Conditions  |
|---|---|
| 1. Test type:                               | Whole sediment toxicity test, static  |
| 2. Temperature:                             | 15°C: <i>E. estuarius</i> and <i>R. abronius</i><br>20°C: <i>A. abdita</i><br>25°C: <i>L. plumulosus</i>  |
| 3. Salinity:                                | 20ppt: <i>E. estuarius</i> and <i>L. plumulosus</i><br>28 ppt: <i>A. abdita</i> and <i>R. abronius</i>  |
| 4. Light quality:                           | Wide-spectrum fluorescent lights  |
| 5. Illuminance:                             | 500 - 1000 lux  |
| 6. Photoperiod:                             | 24L:0D  |
| 7. Test chamber:                            | 1-L glass beaker or jar with ~10 cm I.D.  |
| 8. Sediment volume:                         | 175 mL (2 cm)   |
| 9. Overlying water volume:                  | 800 mL  |
| 10. Renewal of overlying water:             | None  |
| 11. Size and life stage of amphipods:       | <i>A. abdita</i> : 3-5 mm (no mature males or females)<br><i>E. estuarius</i> : 3-5 mm<br><i>L. plumulosus</i> : 2- 4 mm (no mature males or females)<br><i>R. abronius</i> : 3-5 mm    |
| 12. Number of organisms/ chamber:           | 20 per test chamber   |
| 13. Number of replicate chambers/treatment: | Depends on objectives of test. At a minimum, four replicates must be used.  |
| 14. Feeding:                                | None  |
| 15. Aeration:                               | Water in each test chamber should be aerated overnight before start of test, and throughout the test; aeration at rate that maintains 90% saturation of dissolved oxygen concentration. |
| 16. Overlying water:                        | Clean sea water, natural or reconstituted water.  |
| 17. Overlying water quality:                | Temperature daily. pH, ammonia, salinity, and DO of overlying water at least at test start and end. Salinity, ammonia, and pH of pore water.  |
| 18. Test duration:                          | 10 d  |
| 19. Endpoints:                              | Survival (reburial optional for <i>E. estuarius</i> , <i>L. plumulosus</i> , and <i>R. abronius</i> )   |
| 20. Test acceptability:                     | Minimum mean control survival of 90% and satisfaction of performance-based criteria specifications outlined in the guidance document.   |

Table 2. Survival data for *Leptocheirus plumulosus* exposed to field-collected sediments containing synthetic based drilling muds.

| Descriptor        | Site ID        | Distance from Platform | Survival (%) | Standard Error of the Mean |
|-------------------|----------------|------------------------|--------------|----------------------------|
| <i>Platform 1</i> | 1G1            | 50                     | 0            | 0                          |
|                   | 1G1 rep4       | 50                     | 0            | 0                          |
|                   | 1G3 A          | 50                     | 51.67        | 3.33                       |
|                   | 1G3 B          | 50                     | 56.67        | 8.82                       |
|                   | 1G3 A+B        | 50                     | 55.0         | 8.66                       |
|                   | 1G7 A          | 150                    | 61.67        | 8.82                       |
|                   | 1G7 B          | 150                    | 63.33        | 9.28                       |
|                   | 1G7 A+B        | 150                    | 65.0         | 8.66                       |
|                   | 1G10 A         | 100                    | 51.67        | 3.33                       |
|                   | 1G10 B         | 100                    | 56.67        | 12.02                      |
|                   | 1G10 A+B       | 100                    | 58.3         | 8.33                       |
|                   | 1R3 A+B        | 2000                   | 83.3         | 3.33                       |
| <i>Platform 2</i> | 2G2            | 50                     | 81.67        | 1.67                       |
|                   | 2G6            | 150                    | 90.0         | 5.77                       |
|                   | 2G9            | 100                    | 91.67        | 4.41                       |
|                   | 2R1            | 2000                   | 95.0         | 0                          |
| <i>Platform 3</i> | 3G1 A+B        | 50                     | 86.67        | 3.33                       |
|                   | 3G5 A+B        | 150                    | 90.0         | 5.77                       |
|                   | 3R2            | 1000                   | 86.67        | 3.33                       |
| <i>Controls</i>   | C-17           | NA                     | 95           | 2.89                       |
|                   | Copper sulfate | NA                     | 0            | 0                          |

Table 3. Survival data for *Ampelisca abdita* exposed to field-collected sediments containing synthetic based drilling muds.

| Descriptor        | Site ID                           | Distance form Platform (m) | Survival (%) | Standard Error of the Mean |
|-------------------|-----------------------------------|----------------------------|--------------|----------------------------|
| <i>Platform 1</i> | 1G3 A                             | 50                         | 86.67        | 8.82                       |
|                   | 1G3 B                             | 50                         | 85.0         | 7.64                       |
|                   | 1G10 B                            | 100                        | 83.33        | 6.67                       |
|                   | 1R3 A+B                           | 2000                       | 85.0         | 2.89                       |
| <i>Platform 2</i> | 2G2                               | 50                         | 85.0         | 0                          |
|                   | 2G6                               | 150                        | 91.67        | 4.41                       |
|                   | 2G9                               | 100                        | 83.33        | 3.33                       |
| <i>Platform 3</i> | 3G1 A+B                           | 50                         | 83.33        | 6.67                       |
|                   | 3G5 A+B                           | 150                        | 85.0         | 2.89                       |
|                   | 3R2                               | 1000                       | 88.33        | 4.41                       |
| <i>Controls</i>   | <i>Ampelisca</i> Control Sediment | NA                         | 86.67        | 3.33                       |
|                   | C-17                              | NA                         | 0            | 0                          |
|                   | Cu                                | NA                         | 0            | 0                          |

