

Hydrocarbon sources in Kachemak Bay Sediments: Improved Discrimination by Specific Compound $\delta^{13}\text{C}$ Measurements

A Final Report Submitted to

**The NOAA/UNH Cooperative Institute for Coastal and Estuarine
Environmental Technology (CICEET)**

Submitted by

**Dr. Susan M. Henrichs
Dr. Donald M. Schell
Ms. Tara Borland
Mr. Timothy Howe
Institute of Marine Science
School of Fisheries and Ocean Sciences
University of Alaska
P.O. Box 757220, Fairbanks, AK**

April 3, 2003



This project was funded by a grant from NOAA/UNH Cooperative Institute for Coastal and Estuarine Environmental Technology, NOAA Grant Number NA97OR0338.



Abstract

Identification of sources of polycyclic aromatic hydrocarbons (PAH) in sediments is an important step in reducing anthropogenic contamination. Identifications based solely on the composition of PAH can be confounded by compositional changes during weathering and biodegradation. Stable isotopic composition of individual PAH offers an additional marker, which can aid in distinguishing sources and which may be less susceptible to alteration. Sediment samples from the Kachemak Bay area were analyzed for both the concentrations and $\delta^{13}\text{C}$ of individual PAH. The major potential sources of PAH to the area were also analyzed to determine which ones were major contributors to the sediments. To determine the stability of the $\delta^{13}\text{C}$ of PAH in crude and diesel oil, microbial degradation experiments using sediment from the Kachemak Bay area, with added North Slope crude, Cook Inlet crude, and diesel oils, were conducted over a one-year period. PAH concentrations decreased and their composition changed, but the $\delta^{13}\text{C}$ of individual PAH remained constant. Hence, $\delta^{13}\text{C}$ of individual PAH has excellent potential as a relatively stable indicator of their sources. Based on isotopic and compositional data, the PAH in Kachemak Bay sediments appear to have mixed sources. Combustion and coal sources are more consistent with the data than are petroleum sources for the sediment PAH.

Keywords: PAH, aromatic, hydrocarbon, $\delta^{13}\text{C}$, Kachemak Bay, Cook Inlet

Introduction

Improved techniques for identifying and quantifying sources of hydrocarbon contaminants to coastal and estuarine sediments, specifically those within the Kachemak Bay (KB) NERR (National Estuarine Research Reserve), are needed. The Cook Inlet Regional Citizen's Advisory Committee (CIRCAC) has had a decade-long monitoring program, investigating whether contaminants in permitted industry discharges were accumulating in Cook Inlet and whether any such accumulations were affecting the indigenous organisms. However, standard approaches have not led to clear identification of the sources of the low levels of hydrocarbons in Cook Inlet sediments (Lees et al., 1999). Petroleum production, marine transportation of crude and refined petroleum products, or discharges from merchant and fishing vessels occur in many areas of Alaska's coastal waters. Therefore, in addition to being a Cook Inlet and KBNERR management issue, identifying sedimentary hydrocarbon sources is an important need for meeting the goals of Alaska's statewide Coastal Management Program.

Although aromatic hydrocarbons make up only about 10% of the complex mixture of compounds in petroleum, they are of special concern because they are among the most toxic, mutagenic, and carcinogenic constituents (Black et al., 1983; White, 1986; Pahlmann and Pelkonen, 1987). Sediments are important reservoirs for PAH (polycyclic aromatic hydrocarbon) contamination in the marine environment (Wakeham and Farrington, 1980; Gschwend and Hites, 1981). Also, there is evidence that sediments are a major source of PAH and other hydrophobic pollutants to marine and lacustrine organisms (McElroy et al., 1989; Thompson et al., 1999). Although sedimentary contamination decreases markedly with time after a spill, due to weathering and biodegradation, aromatic hydrocarbons can persist for at least 20 years (Teal et al., 1992). Given this persistence of PAH in the environment, it is important to develop an accurate and discriminating approach to determining their sources. Identification of the major hydrocarbon sources is often a critical step in reducing inputs to coastal waters and can be important in assigning liability for negligent discharge.

Kachemak Bay includes over 365,000 acres and is the newest addition to the NERR system. It branches from lower Cook Inlet, a large (3300 sq. mi.) glacially formed estuary in southcentral Alaska. Kachemak Bay has high primary productivity, due to upwelling of nutrient-rich water derived from the Gulf of Alaska, and an unusual abundance and diversity of marine life. Although fairly pristine, in part because of flushing provided by Cook Inlet's extreme tides, the KBNERR is currently or potentially affected by human activities. Of greatest significance to this report is offshore oil and gas development. Twelve platforms producing about 35,000 barrels per day are located in the Cook Inlet region, mainly in the upper Inlet about 100 km north of Kachemak Bay. Crude oil is transported to refineries by undersea pipelines and tankers, which also carry refined petroleum products through the Inlet, outbound to the Gulf of Alaska. North Slope crude oil from the Valdez Terminal of the Trans Alaska Pipeline is shipped to the Tesoro refinery at Nikiski, located about 125 km north of the KBNERR on the east side of Cook Inlet. In addition, the KBNERR hosts heavy marine recreational use, resulting in small craft discharges of petroleum products.

Other potential hydrocarbon sources to the KBNERR include airborne combustion products (mainly for PAH), detrital coal, natural seeps within Cook Inlet, and marine and terrestrial organisms. Municipal wastewater from Anchorage, the largest city in the region, contains low levels of pyrogenic and petrogenic hydrocarbons typical of urban runoff, but is not likely to be a significant source to Cook Inlet sediments (Lees et al., 1999). Cook Inlet is surrounded by deposits of mostly immature lignite and subbituminous coals. There are also high grade coals in the Matanuska fields, which are drained by the Matanuska River into upper Cook Inlet. Sediment and bivalve analyses obtained by CIRCAC reveal the presence of aromatic hydrocarbons, particularly perylene, that may be derived from the coal (Lees et al., 1999). The major known oil seep within Cook Inlet is located at Oil Bay, directly west of Kachemak Bay, across Cook Inlet. However, other natural seeps may exist in the Inlet, and also there is potential for transport from eastern Gulf of Alaska seeps such as those at Katalla, via the westward-flowing Alaska Coastal Current.

The standard approach to identifying sources of hydrocarbons is “fingerprinting”. This method involves a detailed comparison of the composition hydrocarbons in sediments and potential source materials (e.g., Boehm et al., 1997). For example, petrogenic PAH generally have lower proportions of the unsubstituted compounds, like fluorene, phenanthrene, or chrysene, compared to certain alkyl-substituted homologues. Pyrogenic PAH, from combustion sources such as fuel, coal, or wood burning, have more high molecular weight (four and five ring) PAH and relatively greater concentrations of the unsubstituted components. Specific petroleum sources can sometimes be distinguished by comparisons of the relative abundances of certain PAH. The many potential natural and anthropogenic sources complicate the assessment of residual oil contamination, as in the case of the *Exxon Valdez* oil spill (Kvendvolden et al., 1995; Page et al., 1996; Short et al., 1999). Weathering can also complicate source identification via the “fingerprinting” approach because, in general, it selectively removes the lighter and more water soluble hydrocarbons, unbranched or noncyclic alkanes, and the less alkyl substituted PAH.

Since 1993, CIRCAC has conducted an Environmental Monitoring Program (EMP) to assess the environmental impacts of the oil industry in Cook Inlet. The monitoring was initially designed to determine if contaminants in permitted industry discharges were accumulating in Cook Inlet and whether any such accumulations were affecting the indigenous organisms. The focus has been on sediments, because of the extremely high sediment loads in the areas of upper Cook Inlet, where the oil industry is concentrated, and because most of the contaminants of concern adsorb rapidly and strongly to sediment particles. The monitoring has included the aforementioned sediment analyses, PAH analyses of mussel and *Macoma balthica* (an infaunal bivalve) tissues, and various assays to assess any toxic effects of hydrocarbons on marine organisms.

The results of the EMP have been summarized by Lees et al. (1999); their review includes virtually all of the environmental hydrocarbon measurements done in Cook Inlet after the 1970s. PAH concentrations in sediments are low, averaging 83 ± 9 ng/g dry weight (Lees et al., 1999). Concentrations show almost no pattern with regard to industrial sources, except for a few high levels in samples from the Kenai River Estuary. Aliphatic hydrocarbon concentrations averaged 910 ± 200 ng/g dry weight. The highest average concentrations of aliphatics were found in Kachemak Bay, 1940 ± 130 ng/g dry weight, and concentrations were also substantially higher than average in the Kenai River Estuary. All Cook Inlet

sediment PAH concentrations were far below those normally regarded as toxic to benthic invertebrates. Total PAH levels in intertidal *Macoma* from two uninhabited bays on the western side of Cook Inlet in 1996 were very low, averaging 350 ng/g dry weight. Subtidal organisms collected in 1993 had even lower values, averaging 56 ng/g dry weight.

Recently CIRCAC collected several coal samples from beach outcrops, including two located on Kachemak Bay. These coal samples contained perylene as the predominant PAH, and also had substantial concentrations of substituted naphthalenes, phenanthrenes, and anthracenes. There was an odd carbon number predominance to the chain length of higher molecular weight n-alkanes, along with the high perylene concentrations, reflecting the immaturity of these deposits (Lees et al., 1999). More mature bituminous and anthracitic coals, such as those found in the Matanuska fields, have a lack of odd-carbon predominance and less perylene, and overall their hydrocarbon “fingerprint” is more similar to petroleum than that of immature coals (Short et al., 1999). PAH in coals are less susceptible to weathering and less bioavailable than petroleum-derived PAH because they are contained within the refractory coal matrix (Short et al., 1999). Lees et al. (1999) employed double ratio plots (C_3 -dibenzothiophene/ C_3 -phenanthrene vs. C_2 -dibenzothiophene/ C_2 -phenanthrene) to conclude that the aromatic hydrocarbon patterns in most Cook Inlet sediment samples resembled those from Cook Inlet wells or Katalla seeps. Most samples did not resemble Alaska North Slope crude, which has high levels of the sulfur-containing dibenzothiophenes. However, some sediment samples also resembled the local low-grade coals or, possibly, a mixture of Cook Inlet and small amounts of Alaska North Slope crude or other petroleum sources.

Lees et al. (1999) also found a positive correlation between levels of C_0 - C_4 naphthalenes and sediment grain size, and high proportions (>50% of total PAH) of naphthalenes in some samples. This was unusual, because aromatic hydrocarbon concentrations are usually greater in fine-grained, organic rich sediments, and because naphthalenes tend to decrease relative to larger PAH during weathering. These patterns were attributed to the presence of particulate coal. Other samples had compositions suggesting fuel or pyrogenic contributions. However, an overall conclusion of their study was that PAH signatures for sediments could not be directly tied to specific sources and that the data suggested undocumented or multiple sources.

The carbon isotopic composition of individual hydrocarbons offers an additional means to distinguish different hydrocarbon sources. Isotopic composition is best used in conjunction with “fingerprinting” since the isotopic composition alone does not necessarily improve discrimination (Mansuy et al., 1997). The controls on carbon isotopic composition of bulk crude oils are fairly well understood. Petroleum generation from source rock results in an average of about one per mil enrichment in $\delta^{13}C$; increased maturity leads to increasing $\delta^{13}C$, and oil to gas cracking enriches the residual petroleum by about 3.5 per mil (Clayton, 1991). Clayton and Bjoroy (1994) show that increasing maturity also leads to increasing $\delta^{13}C$ of virtually all individual compounds measured (including n-alkanes, isoprenoids, branched, cyclic, and aromatic hydrocarbons). In immature sediment samples, the $\delta^{13}C$ of individual biomarkers tends to vary substantially, due to the variety of sources and biogeochemical transformations of sediment organic matter. However, in oils the isotopic composition among components tends to be less variable (Bjoroy et al., 1990; Collister et al., 1992; Clayton and Bjoroy, 1994). This is also apparently true of many of the hydrocarbon

constituents of a brown coal (Schoell et al., 1994), except that hopane derivatives that likely originated from methanotrophic bacteria were about 10 to 30 per mil depleted in $\delta^{13}\text{C}$ relative to the bulk coal. Bitumens from the Uinta Basin show a similar large difference in $\delta^{13}\text{C}$ between the hopanes, probably originating from methanotrophs, and isoprenoids and steranes, which were inferred to come from algae and cyanobacteria. This pattern was reflected in the $\delta^{13}\text{C}$ of the total extractable hydrocarbons (Ruble et al., 1994).

O'Malley et al. (1996) used PAH composition and the $\delta^{13}\text{C}$ of individual PAH to quantify the primary sources of PAH to St. John's Harbor, Newfoundland sediments. The 3-, 4-, and 5-ring PAH derived from combustion sources, fireplaces and automobile exhaust, were on average about 1 ‰ heavier than PAH derived from crankcase oils. (Such oils supply many of the hydrocarbons in municipal storm sewer runoff.) Sedimentary PAH $\delta^{13}\text{C}$ was intermediate between the combustion and crankcase values. Mixing models based on relative concentrations and $\delta^{13}\text{C}$ of 4- and 5-ring PAH indicated that 20-50% of the sedimentary PAH were derived from crankcase oil, while 50-80% were from combustion sources. Inclusion of the isotopic data improved discrimination and quantification of sources, showing crankcase oil contributions to some sediments that were not evident from the "fingerprinting" approach and allowing preliminary separation of automobile and fireplace combustion sources. O'Malley et al. (1994) conducted laboratory experiments and found that photolytic degradation, biodegradation, or evaporation did not affect isotopic composition of several PAH standards.

The specific compound $\delta^{13}\text{C}$ analysis has the major advantage that the $\delta^{13}\text{C}$ of individual hydrocarbons appears to be little-affected by physical weathering or biological decomposition. Mansuy et al. (1997) conducted GC-IRMS analyses of naturally and artificially weathered oils in order to determine whether weathering changed the isotopic composition of individual hydrocarbons, and whether this technique offered improved discrimination sources of weathered oils compared with compositional "fingerprinting." Their results demonstrated that, over two to four months, laboratory biodegradation, evaporation, and water washing did not affect stable isotopic composition of chromatographically resolvable oil components. They also showed that the isotopic composition of individual components of asphaltene pyrolyzates was not much affected by weathering, offering the possibility of relatively unambiguous identification of tarry oil residues. Gasoline range hydrocarbons do show small (0.5 to 1‰), systematic changes in $\delta^{13}\text{C}$ with evaporation (Whiticar and Harris, 2000), but such isotope effects would probably be much smaller for higher molecular weight compounds.

The determination of $\delta^{13}\text{C}$ of individual compounds in standard mixtures can be quite accurate and precise, with reported analytical error of 0.15 to 0.32 ‰ (e.g., Mansuy et al., 1997). However, the measurement is much more difficult in environmental samples, especially those that are highly weathered, so that resolvable components are reduced compared to the unresolvable complex mixture (UCM) (O'Malley et al, 1994;1996; Mansuy et al., 1997). Precision and accuracy are also affected if two components with differing isotopic composition are incompletely resolved. This can be partly compensated for by careful background subtraction and the use of a standard addition approach. In the latter, standards of known composition are added to samples exhibiting the problematic background. Then, the peak selection and background correction are varied to give the correct isotopic composition for the component, and these conditions are then applied to the

analysis of unknown samples (e.g., O'Malley et al., 1996). However, because of these analytical difficulties, it is likely that the most economical approach to routine application of this technique will be to identify a subset of compounds, which have readily measured isotopic composition and which offer discrimination among potential sources.

Objectives

The project objective was to develop and apply a methodology, measurement of $\delta^{13}\text{C}$ of individual hydrocarbons by gas chromatography-isotope ratio mass spectrometry (GC-IRMS), which could provide improved discrimination of hydrocarbon sources in KBNERR sediments. This objective supports the CICEET goal to use NERRs to develop and apply innovative strategies to address anthropogenic contamination and degradation of estuarine ecosystems. In particular, the research was directed at improving techniques for collection of environmental data and determining the sources of hydrocarbon contaminants in Kachemak Bay. Although there have been a few previous applications of this approach, they have been very limited in number and scope (O'Malley et al., 1994; 1996; Mansuy et al, 1997). Further, they used a custom built instrument and a time consuming standard addition method that would not be readily applicable to routine analysis. This technique has never been applied to Alaska's coastal sediments or potential hydrocarbon sources. Similarly, limited studies of weathering effects on isotopic composition have been carried out, but these have not addressed the source materials and weathering conditions relevant to Alaska's coastal sediments.

The specific project objectives were to:

1. Measure the hydrocarbon composition and the $\delta^{13}\text{C}$ of individual hydrocarbons for the major potential hydrocarbon sources to Kachemak Bay. We hypothesized that the $\delta^{13}\text{C}$ of two potential sources of hydrocarbons, low-grade coal and Cook Inlet seep oil, would be distinct, due to the differences in the biogenic source materials from which they are derived. Based on O'Malley et al. (1994), we also hypothesized that pyrogenic PAH would have a distinct isotopic composition.
2. Measure any changes in hydrocarbon composition and the $\delta^{13}\text{C}$ of individual hydrocarbons in the source materials during physical weathering and decomposition by sediment organisms. Based on Mansuy et al. (1997), we hypothesized that the $\delta^{13}\text{C}$ would not change with decomposition.
3. Measure the hydrocarbon composition and the $\delta^{13}\text{C}$ of individual hydrocarbons in sediment samples from Kachemak Bay and adjacent embayments within the KBNERR.
4. Identify the major source(s) of hydrocarbons in KBNERR sediments.

Methods

Sample Collection

Sediment sampling for decomposition experiments was done at the head of Jakolof Bay. Prior to the biodegradation experiments, a large sample of this sediment was sieved through a 1 mm mesh in order to remove macrofauna. These samples were refrigerated at 5°

C until used in experiments. Other samples of sediment were collected from several locations in Jakolof Bay and Kasitsna Bay. Coal samples were obtained from coastal sites along Kachemak Bay near Homer, Alaska. Ash samples from wood stoves in Homer, Alaska, were collected to help determine the cause of the background PAH in the sediment samples. All of the individual samples were placed in clean sample jars and frozen immediately.

Experimental Design

The effect of microbial degradation was investigated using a series of half pint Mason jars. Approximately 70 grams of the Jakolof Bay sieved sediment was added to each jar. Seawater from Kasitsna Bay was placed on top of the sediment. One triplicate set of Mason jars was prepared for each sampling time; however it was later necessary to combine these jars to obtain sufficient PAH for isotopic analysis. The jars were prepared for analysis initially, and at one week, two weeks, three weeks, five weeks, three months and one year. Three different types of oil were used for this experiment. One set of jars contained North Slope crude oil, a second set contained weathered diesel oil, and a third set contained Cook Inlet crude oil. The oil was transferred to the jars using a positive displacement pipette. For both the North Slope and Cook Inlet crude oil, 2.4 μL of the oil was added to each Mason jar, except the ones destined to be incubated for one year. The diesel oil used for these experiments was first weathered for ten days in an open container, shielded from sunlight. This allowed for the evaporation of some of the more volatile and toxic hydrocarbons. For weathered diesel, each jar received 7.2 μL except the one-year jars. For the one-year experiment, 12 μL of crude oil was added to each jar and 36 μL of diesel; five times the amount of oil was used to ensure that sufficient PAH would remain at one year for analysis. During the experiment the jars were kept refrigerated at 5° C. Throughout the experiment, the jars were opened every 48 hours in order to allow recharge of oxygen into the system, as aerobic conditions are necessary for the rapid microbial degradation of hydrocarbons to occur (Leahy and Colwell, 1990). At each sampling time, the jars were removed from the refrigerator and the layer of surface water was removed using a Pasteur pipette. Once the surface water was removed, the sample was placed in a freezer, where it was stored until extracted.

Sediment Extraction

All samples were frozen and stored at -40°C until the day before they were extracted. At this time, they were allowed to thaw in a 5°C refrigerator. Each sample was transferred into a pre-weighed thimble. Coal samples were ground using a mortar and pestle prior to being placed into the thimbles, allowing for a more thorough extraction. The coal samples collected from the Homer spit were ground using a Crescent Dental Manufacturing Company Wig L Bug® Amalgamator, as they were too dense for manual grinding. Fritted glass Soxhlet thimbles were used, as they could be cleaned and baked in a muffle furnace, eliminating the need for pre-extraction of cellulose thimbles. Once the sample was completely transferred, the thimble was weighed again, allowing for the wet weight of the sample to be recorded (Table 1). Each sample was then spiked with 400 μL of the internal standard. Each thimble was placed into an extraction apparatus that included a Soxhlet extractor, a condenser and a 500 mL round bottom flask. The round bottom flasks were filled with 150 mL of hexane and 150 mL of methanol. For each new bottle of hexane used,

one blank extraction was run. Of the twenty blanks only three had measurable peaks, with concentrations ranging from 0.02 to 0.7 $\mu\text{g/g}$. These did not interfere with the PAH reported here.

The samples were then extracted for 48 hours at a low heat to minimize solvent loss. During the extraction occasional hexane and methanol rinses helped to wash retained sample from the condenser and to maintain the initial volume of solvents. The reflux rate of the system varied depending upon the sediment type. For the less dense samples the rate was about one cycle every 20 minutes and for denser samples it was about one cycle every 30 minutes. Once the extraction was completed, the extractor was shut down and allowed to cool. Each condenser was rinsed one final time after the samples had completely cooled. The Soxhlets were each removed from the round bottom flasks and any solvent left in the Soxhlet or sediment was rinsed into the round bottom flask three times. The thimble and sediment were dried for 24 hours at 40°C and then weighed in order to obtain a dry weight of the sample extracted (Table 1).

Each extract was then placed into a 1 L separatory funnel. To the extract, 50 mL of organic free water and 100 mL of hexane was added. The funnel was shaken vigorously for 1 minute and then allowed to sit for five minutes. The bottom methanol layer was removed back into the original round bottom flask. The hexane extract was then collected in a clean round bottom flask. The methanol layer was returned to the separatory funnel and extracted twice more with 100 mL of hexane. The hexane extract was stored overnight with 25 g of sodium sulfate.

The hexane extract was then removed from the sodium sulfate and placed into vials for concentration in a Zymark Turbo-vap® II concentration workstation. The Turbo-vap® uses a stream of high-purity nitrogen to bring the sample down to a desired volume. At this point, the triplicate samples were pooled together and brought down to ~20 mL. The sample was then filtered using a vacuum filtration system supplemented with 300 μg of 60 μm silica gel, which had been baked overnight at 450°C. The filtration was repeated 3 times in order to obtain the desired clarity. The sample was then placed back into the Turbo-vap® and brought down to 2 mL, after which it was stored in a sealed vial until injected into the HPLC.

High Performance Liquid Chromatography

Each sample was injected into the HPLC for separation. The purpose of the HPLC was to allow for the separation of alkane and aromatic hydrocarbons. The HPLC system utilized consisted of a Gilson 302 model pump accompanied by a Linear model 200 UV-visible detector set at 254 nm, a Gilson 811B dynamic mixer, a Gilson 201 fraction collector and a Gilson HPLC controller (version 1.20). The preparatory column used contained Phenomenex Phenogel 100-A size exclusion packing, 22.5 X 250 mm. The guard column used contained Phenomenex Phenogel 100-A size exclusion packing, 7.8 X 50 mm. The solvent used was 100% methylene chloride. The solvent flow was 4 mL/min for 30 minutes.

After an initial HPLC separation of a standard demonstrated that all PAH were eluted between 14 and 20 minutes after sample injection, a 2 mL calibration standard of aromatic hydrocarbons, the EPA 610 Polynuclear Aromatic Hydrocarbons Mix, was injected into the HPLC and the column effluent was collected at 1-minute intervals (every 4 mL) from 14 to 20 minutes after injection. Each fraction was then placed in a Turbo-vap and concentrated down to ~1 mL. The fractions were analyzed by gas chromatography by the method of Feder

and Shaw (1996). Based on the results, HPLC effluent collection was begun at 15 minutes and ended at 20 minutes. This allowed for the collection of the majority of aromatics while minimizing interference from alkanes.

For each sample, the aromatic-containing portion of eluent (15-20 minutes) was collected into a vial that was subsequently placed directly into the Turbo-vap. While being concentrated in the Turbo-vap, the sample was rinsed several times with hexane to allow it to replace the methylene chloride. Hexane is less volatile and allowed the samples to be stored for a longer period of time without loss of solvent. The sample was then concentrated a second time to a final volume of ~100 μL . The concentrated sample was stored at 5°C in a 2 mL amber glass vial with a conical insert.

GC-IRMS

In order to determine the correct settings for GC-IRMS of the sediment samples, an entire set of old sediment samples was extracted using essentially the same extraction procedure that would be utilized during the experiment. In order to optimize the GC method, several spiked blanks, crude oil and diesel oil samples were run through the entire procedure. The amount of oil used in each Soxhlet extraction corresponded to the amount of oil added to each sample. For North Slope and Cook Inlet crude oil, 2.4 μL were extracted. For the diesel oil, 7.2 μL was extracted. Analyzing these samples on the GC-IRMS was difficult, as the hydrocarbon peaks were not large enough to be accurately analyzed against the background matrix. Three times the original amount of oil was needed to produce sufficient signal. Therefore, the triplicate jars from the biodegradation experiments were pooled for extraction.

The samples were analyzed by the Alaska Stable Isotope Facility using an HP 6890 Series Gas Chromatograph interfaced to a Finnigan Delta Plus IRMS through the Finnigan GC Combustion III. Just before GC-IRMS, the sample was brought down to a 10 μL volume using a hand-held nitrogen stream. Each day began with blank instrument runs and an internal/external standard run. Approximately 1 μL of sample was injected into the instrument for each run. The instrumental output included the retention time of the peak, the peak height and the $\delta^{13}\text{C}$ of the hydrocarbon composing the peak. The peak height was determined by a baseline to baseline method on the 44 mass. The detection parameters were 0.2 mV/s for the start slope and 0.4 mV/s for the end slope. The background was determined by using the lower of the baseline just before or just after the peak. The “just before” method was used for all of the PAH except for fluoranthene, for which the “just after” method was used. Background correction was applied to the data according to IsodatNT version 1.21 individual BGD background correction with a five second history. The size of the background with respect to each PAH varied from sample to sample. The typical background signal ranged from 50 to 200 mV, and the analytical peak heights of the PAH ranged from 50 mV to 2 V. These results were stored on disk and in hard copy for later analysis. For some samples, it was necessary to manually change the beginning or end point of the peak, to remove the effect of small shoulder peaks. The concentration of each PAH was determined by comparison to the internal standards. The GC-IRMS settings used for running the samples were: Initial temperature – 65°C, Initial time – 2:00 minutes, Rate 1 – 4°C/minute, Column flow – 3.5 mL/minute, Final temperature – 280°C, Final time – 14.25 minutes, Total time – 70 minutes.

The external PAH standard consisted of 5 mg of perylene, 5 mg of chrysene, and 5 mg of phenanthrene in 25 mL of methylene chloride. The internal standard consisted of 23.5 mg of 1-ethylnaphthalene, 18.3 mg of acenaphthene - d10 and 9.5 mg of benzo[b]fluoranthene in 500 mL of hexane. These solutions were run repeatedly on the GC-IRMS, along with a hydrocarbon calibration standard. The calibration standard was Mixture "A" of fifteen n-alkanes (C-16 to C-30) (A. Schimmelmann, Indiana University, <http://php.indiana.edu/~aschimme/hc.html#table>). The mean measured $\delta^{13}\text{C}$ for each of the components was within $\pm 0.5\%$ of the nominal value. The instrument precision (for dry CO_2 gas) was $< \pm 0.3\%$.

Results

The isotopic composition is reported as:

$$\delta^{13}\text{C-PDB} (\text{‰}): \delta^{13}\text{C} = [((^{13}\text{C}/^{12}\text{C})_{\text{sample}} / (^{13}\text{C}/^{12}\text{C})_{\text{standard}}) - 1] * 1000 = \text{‰}.$$

Hydrocarbon standards were analyzed on the GC-IRMS each day before the samples were run. Six hydrocarbons were present in the standard solution. The isotopic compositions obtained for these preliminary standards had standard deviations of 1.4 ‰ or less (Table 2).

The concentrations of fluoranthene and chrysene in the North Slope crude oil microbial degradation experiment decreased within the first seven days (Tables 3-6, Figure 1). A z-test (Table 7) was performed to determine if the initial concentration data point for each hydrocarbon was significantly different than the mean of the remaining data. This test showed that the initial concentration data points of phenanthrene, fluoranthene and chrysene were significantly different than the later data points. Those initial data points were not included in the linear regressions of concentration vs. time. The linear regressions performed on the concentrations of 1-ethylnaphthalene (internal standard), fluorene and perylene included the entire data set, as their initial points were not significantly different from the rest (Tables 3-6, Figure 1). A t-test (Table 7) determined that the slopes of the linear regressions were not significant. The $\delta^{13}\text{C}$ of each PAH vs. time was also analyzed using linear regression. These data sets all had insignificant slopes. For $\delta^{13}\text{C}$ the initial time point was not significantly different from later points and was included in these regressions (Figure 1).

The concentrations of fluorene, phenanthrene and fluoranthene from the Cook Inlet Crude oil experiments decreased within the first fourteen days (Figure 2). A z-test showed that the initial concentration data points of fluorene, phenanthrene and fluoranthene were significantly different than the remaining data points. Those initial data points were not included in the linear regressions. The linear regressions performed on the concentration data of 1-ethylnaphthalene (internal standard), chrysene and perylene included the entire data sets, as their initial data points were not significantly different (Tables 3-6, Figure 2). A t-test determined that the slopes of all of the linear regressions of concentration vs. time were not significant. The isotopic signature for each PAH also remained unchanged, with the exception of $\delta^{13}\text{C}$ of phenanthrene, which had a small slope of $-0.0099 \text{‰ day}^{-1}$ (Figure 2b). This indicates that the phenanthrene was getting isotopically lighter, but to a very small extent.

The concentrations of phenanthrene, chrysene and perylene in the diesel oil experiments decreased within the first seven days (Tables 3-6, Figure 3). A z-test showed

that the initial concentration data points of phenanthrene, chrysene and perylene were significantly different than the later data points. Those initial data points were not included in the linear regressions. The linear regressions performed on the concentration data of 1-ethylnaphthalene (internal standard), fluorene and fluoranthene included all of the data, as their initial data points were not significantly different. A t-test determined that the slopes of the linear regressions were not significant, with the exception of fluorene and phenanthrene, both of which had very small slopes of $0.0001 \mu\text{g g}^{-1} \text{day}^{-1}$. The $\delta^{13}\text{C}$ of each PAH vs. time, from day 1 to day 365, was analyzed using linear regression. All data sets had insignificant slopes (Figure 3).

An f-test (Table 7) was used to determine if the variance of the $\delta^{13}\text{C}$ data from each hydrocarbon in the degradation experiments was significantly different than the variance of the standard runs of that hydrocarbon (Table 12). Fluorene and fluoranthene were not run as standards; therefore, their variances were compared with the standard closest to them in the elution sequence. Fluorene was compared to acenaphthene - d10, and fluoranthene was compared to chrysene. The internal standards all had variances that were statistically similar for all three oil types, with the exception of a smaller variance for 1-ethylnaphthalene from the Cook Inlet Crude oil. Both fluorene and fluoranthene had variances that were significantly greater than those of the standards for all oil types, with the exception of fluoranthene from the diesel oil degradation experiment. Chrysene had a statistically similar variance to the standard in the diesel oil experiment, but not in the North Slope and Cook Inlet crude oil experiments. For all three oil types, phenanthrene and perylene had variances similar to the standard. Phenanthrene and perylene seemed to be the least affected by the matrix of the oil and sediment, allowing their isotopic composition to be measured more precisely. Given the difficulty of measuring $\delta^{13}\text{C}$ for the small concentrations of PAH in these samples, the slightly increased variance for some PAH, compared with the variance for standards, is attributable to analytical error. There is no indication that it resulted from decomposition.

PAH concentration ratios to the largest, most stable hydrocarbon present, perylene, were calculated. Throughout the North Slope Crude oil decomposition experiments, fluorene, phenanthrene and chrysene all maintained a consistent ratio to perylene, with the exception of a significant drop in the phenanthrene and chrysene ratios from day 1 to day 7. The ratios of fluoranthene were more variable, with an initial decrease and then random variation. For the Cook Inlet Crude oil experiment, there was a large decrease in the ratios of all the other PAH to perylene from day 1 to day 14, but from day 35 to day 365 the ratios increased. During the diesel fuel degradation experiment, the chrysene to perylene ratio remained fairly constant throughout the entire experiment. Fluorene, phenanthrene and fluoranthene ratios to perylene were variable.

The $\delta^{13}\text{C}$ of PAH in two crude oils and diesel fuel are shown in Figure 4. The two crude oils were similar within error, except chrysene was slightly heavier for the North Slope (Prudhoe Bay) crude oil. The $\delta^{13}\text{C}$ of the individual PAH within each oil sample were also uniform, except that phenanthrene was isotopically lighter than fluorene and chrysene in North Slope crude oil.

Samples of beach coal from three locations around Kachemak Bay were analyzed (Figure 5). The concentrations of all PAH were greatest in the Mud Bay coal samples and

progressively less in samples from Bishop's Beach and Homer Spit (Tables 9-11). The $\delta^{13}\text{C}$ of individual PAH were similar among the coal samples, within analytical error. The mean phenanthrene, chrysene, and fluorene $\delta^{13}\text{C}$ values were heavier than the mean for perylene (Figure 5).

Sediments from three different locations around Kachemak Bay were analyzed (Tables 8-11). The Jakolof Bay, Jakolof Dock, and Kasitsna Bay sediments had similar $\delta^{13}\text{C}$ values for all of the PAH, except the Kasitsna Bay sediments had lighter $\delta^{13}\text{C}$ values for phenanthrene (Table 9, Figure 6). The concentrations for phenanthrene, chrysene and perylene in the Jakolof Dock sediment were less than those in the Jakolof Bay sediment (Tables 9-11). The concentrations of PAH in Kasitsna Bay sediment were the lowest of all.

Ash and soot samples from two different wood stoves located in Homer, Alaska, were analyzed. The first wood stove burned only wood. The phenanthrene and chrysene $\delta^{13}\text{C}$ values from the first wood stove were lighter than those for the second wood stove, which burned garbage as well as wood (Figure 5). The concentrations of fluorene, phenanthrene and fluoranthene were much less and the concentrations of chrysene and perylene were greater in the wood stove with garbage (Tables 9-11).

Discussion

A commercially available GC-IRMS system was successfully used to measure the isotopic composition of individual PAH in environmental samples. The sensitivity of the measurement was excellent. The $\delta^{13}\text{C}$ of as little as 10 ng of a PAH could be measured precisely. The analytical precision for PAH standards was near 1‰ (= one standard deviation). The analytical precision for internal standards (1-ethynaphthalene, acenaphthalene-d10, and benzo[b]fluoranthene, which were carried through all analytical steps including extraction, HPLC, and evaporative concentration, was similar (Tables 12 and 13). The analytical precision for the five PAH measured in the decomposition experiment samples, fluorene, phenanthrene, fluoranthene, chrysene, and perylene, varied from 1.0 to 2.8‰ among the PAH and different experimental series, and averaged 1.6‰ (Table 12). The slightly poorer precision for these samples, compared with the standards, was probably due to the smaller size of the peaks relative to background. However, also note that these samples were not replicates, but rather samples taken over time during a decomposition experiment, which showed no temporal trend in isotopic composition (see below). While precision for fluoranthene was not much worse than for other PAH in the degradation experiment samples, the analytical variability in oil samples (about 5‰) was too large for the data to be useful, because fluoranthene was a small peak above a complex background.

The most important constraint to use of this method is frequent GC-IRMS breakdown, which ultimately limited the number of samples that could be analyzed during the project to about 100. However, our experience with this earlier model instrument might not apply to those of more recent manufacture. Also, although the instrument's software often performed peak integrations and background corrections accurately, manual corrections were necessary about 10% of the time. Because there was no clear pattern to errors, every peak needed to be checked.

During the biodegradation experiments, there were decreases in fluorene, phenanthrene and fluoranthene concentrations between the first and second sampling times. In all three degradation experiments, perylene, a five-ring PAH, decreased much less than the others. The results are consistent with the findings of Wharfe et al. (1984), that higher molecular weight aromatics hydrocarbons are more environmentally persistent. Herbes and Schwall (1978) also found 4- and 5- ring PAH were least degradable, and that 3- ring hydrocarbons were preferentially degraded by bacteria. Chrysene was also stable in the North Slope and Cook Inlet crude experiments, but was significantly degraded in the diesel fuel experiment. This could be due to the greater solubility or lower viscosity of diesel fuel, which probably allowed it to dissolve or disperse into the interstitial water, making the hydrocarbons more bioavailable, as observed for dispersed and dissolved hydrocarbons in contaminated waters (Leahy and Colwell, 1990). In contrast, both North Slope crude and Cook Inlet crude oils have a higher proportion of very insoluble, high molecular weight hydrocarbons.

Linear regressions were performed on the concentration data from the degradation experiments. The data from day 1 of the experiments were not included as the majority of these data points were significantly greater than the remaining data. Extreme outliers, data points more than two standard deviations away from the mean, were also excluded from the linear regressions. There were a total of six extreme outliers removed from the data sets for the linear regressions, all of which were three-month data points. All of the hydrocarbons analyzed had insignificant slopes, with the exception of fluorene and phenanthrene from the diesel fuel experiment which had significant, but very small slopes of 0.0001. This indicates that after the first week of the experiments, the hydrocarbon bioavailability decreased dramatically; thereafter, the concentrations of the hydrocarbons remained stable for 365 days. Several studies have found that PAH adsorb to sediments, which reduces their bioavailability and makes them more environmentally persistent (Braddock and Richter, 1998; Carmichael et al. 1997; Hatzinger & Alexander. 1995; Weissenfels et al. 1992; Landrum et al. 1992).

The biodegradation experiments for all three oil types demonstrated the stability of the $\delta^{13}\text{C}$ isotopic signatures for the PAH analyzed. The linear regressions performed on the data sets showed insignificant slopes with only one exception. Phenanthrene from the Cook Inlet crude oil experiment did have a statistically significant slope of -0.0099‰ day^{-1} , which amounts to a decrease of about 3 ‰ over the course of the experiment, only about twice the average analytical variability for analysis of phenanthrene standards (Table 12). The phenanthrene became slightly lighter, a trend away from greater similarity to the original sediment PAH. Overall, however, the data are consistent with the hypothesis that as PAH are degraded, the $\delta^{13}\text{C}$ isotopic signature does not change.

The trends in PAH/perylene concentration ratios generally followed patterns expected from decomposition of the added oils. During the initial one to two weeks of the decomposition experiment, initially elevated ratios decreased to values similar to the original Jakolof Bay sediment. Fluorene and fluoranthene had much more variable ratios to perylene than phenanthrene or chrysene, but the variability was probably due to less precise concentration data. Concentrations of these hydrocarbons were low, making them difficult to analyze due to interference from the background matrix. Longer incubation led to little or no further change in PAH/perylene. The concentrations of PAH later in the experiments are

generally less than or equal to the concentrations in the initial sediment before oil addition, consistent with the patterns in PAH/perylene ratios.

Given literature reports of very persistent PAH contamination in sediments (e.g., Teal et al., 1992) and the relatively slow decomposition of radiolabeled aromatic hydrocarbons in Kachemak Bay sediments (Braddock and Richter, 1998), it was surprising that the PAH from added oil disappeared so quickly. There are several possible reasons, including the fact that the sediments used had relatively low organic content and therefore might be expected to adsorb PAH to a lesser extent than organic rich sediments (Terschak, 2002). Another factor is that the oil additions were small, intended to increase the sediment PAH concentration by only a factor of two or three, to better mimic the naturally low levels of sediment contamination in this region. This, however, meant that we could no longer detect oil-derived PAH after about 80% degradation had occurred.

Our study was designed mainly to evaluate the GC-IRMS method for better characterization of sediment contaminants, rather than to comprehensively assess the sources of PAH to sediments of Kachemak Bay. Hence we analyzed only a limited number of sediment samples and source materials. The potential source materials analyzed included Cook Inlet crude oil, North Slope crude oil, and diesel fuel; wood stove ash; and local coals. Unfortunately, most of these materials had rather similar $\delta^{13}\text{C}$ for the PAH analyzed (Figure 7). In particular, the hypothesis that the $\delta^{13}\text{C}$ of the PAH in Kachemak Bay region coals would be less than that of PAH in Cook Inlet or North Slope crude oil or diesel was not supported by the data. If a difference exists, it is not more than 3‰, and variability within the oil or coal groups is of similar magnitude. The $\delta^{13}\text{C}$ of chrysene and fluorene in Cook Inlet and North Slope crude oils were significantly different, with the Cook Inlet PAH being isotopically lighter in both cases. The Jakolof Bay sediment PAH isotopic composition could be distinguished from that of most source materials (Figure 7), based on the $\delta^{13}\text{C}$ of phenanthrene. Tentatively, the isotopic composition could be explained as a mixture of one of the coals (Bishop's Beach) and ash from burning garbage. However, at this time we have completed only two analyses of ash from garbage, and its composition might be quite variable depending on what was burned and the combustion conditions.

Conclusions

The $\delta^{13}\text{C}$ of four PAH, fluorene, phenanthrene, chrysene and perylene, was measured with good precision, averaging $\pm 1.6\text{‰}$ (1 standard deviation), for quantities as small as 10 ng. The $\delta^{13}\text{C}$ of fluoranthene was also measured with comparable precision, except in oils. Polycyclic aromatic hydrocarbons, in added North Slope and Cook Inlet crude oils and diesel fuel, were significantly degraded in sediments from Jakolof Bay, Alaska, over a one to two week period. Little or no further decomposition occurred over a one-year period. The lighter hydrocarbons decomposed more rapidly than heavier ones. Despite the decomposition, the $\delta^{13}\text{C}$ of the PAH was unchanged in nearly all experiments. These results support the hypothesis that the $\delta^{13}\text{C}$ isotopic fingerprint could be a valuable tool for determining the source of weathered crude oils. However, most of the PAH sources investigated for this study, North Slope and Cook Inlet crude oils, diesel fuel, and local coals, had similar PAH $\delta^{13}\text{C}$. Only the two crude oils had statistically significant differences in isotopic

composition. The phenanthrene $\delta^{13}\text{C}$ for sediment samples from Jakolof Bay was heavier than that of most of the source materials analyzed, except for ash from a wood stove that burned garbage and Bishop's Beach coal.

Technology Transfer and Management Application

Copies of this report have been provided to the Kachemak Bay NERR manager and the Cook Inlet Citizen's Regional Advisory Council, simultaneously with submission to CICEET. The report and some additional project information is also publicly available, via our web site, <http://www.ims.uaf.edu/PAH/>.

Scientific and Academic Achievement

Borland, Tara. 2003. The Effects of Microbial Degradation on the $\delta^{13}\text{C}$ of Individual Polycyclic Aromatic Hydrocarbons in Crude and Diesel Oils. M.S. Thesis, University of Alaska Fairbanks (will graduate in Summer, 2003).

Literature Cited

- Bjoroy, M., K. Hall, and J. Jumeau. 1990. Stable carbon isotope ratio analysis on single components in crude oils by direct GC-isotope analysis. *Trends Anal. Chem.* 9: 331-337.
- Black, J. A., W. J. Birge, A. G. Westerman, and P. C. Francis. 1983. Comparative aquatic toxicology of aromatic hydrocarbons. *Fundam. Appl. Toxicol.* 3: 353-358.
- Boehm, P. D., G. S. Douglas, W. A. Burns, P. J. Mankiewicz, D. S. Page, and A. E. Bence. 1997. Application of petroleum hydrocarbon chemical fingerprinting and allocation techniques after the *Exxon Valdez* oil spill. *Mar. Pollution Bull.* 8: 599-613.
- Braddock, J. F. and Z. D. Richter. 1998. Microbial degradation of aromatic hydrocarbons in marine sediments. Final Report to the U.S. Department of the Interior, Minerals Management Service, Alaska OCS Region, Task Order 11986, OCS Study MMS 97-0041. University of Alaska Coastal Marine Institute, Fairbanks, AK, 82 pp.
- Carmichael, L. M., R. F. Christman, and F. K. Pfaender. 1997. Desorption and mineralization kinetics of phenanthrene and chrysene in contaminated soils. *Environ. Sci. Technol.* 31: 126-132.
- Clayton, C. J. 1991. Effect of maturity on carbon isotope ratios of oils and condensates. *Organic Geochem.* 17: 887-899.
- Clayton, C. J., and M. Bjoroy. 1994. Effect of maturity on $^{13}\text{C}/^{12}\text{C}$ ratios of individual compounds in North Sea oils. *Organic Geochem.* 21: 737-750.
- Collister, J. W., R. E. Summons, E. Lichtfouse, and J. M. Hayes. 1992. An isotopic study of the Green River oil shale. *Organic Geochem.* 19: 265-276.
- Feder, H. M., and D. G. Shaw. 1996. Environmental Studies in Port Valdez, Alaska, Final Report to the Alyeska Pipeline Service Company. University of Alaska, Fairbanks, AK, 304 pp.
- Gschwend, P. M. and R. Hites. 1981. Fluxes of polycyclic aromatic hydrocarbons to marine and lacustrine sediments of the northeast United States. *Geochim. Cosmochim. Acta* 45: 2359-2367.
- Hatzinger, P. B., and M. Alexander. 1995. Effect of aging of chemicals in soil on their biodegradability and extractability. *Environ. Sci. Technol.* 29: 537-545.
- Herbes, S. E. and L. R. Schwall. 1978. Microbial transformation of polycyclic aromatic hydrocarbons in pristine and petroleum-contaminated sediments. *Appl. Environ. Microbiol.* 35: 306-316.
- Kvendvolden, K. A., F. D. Hostettler, P. R. Carlson, J. B. Rapp, C. N. Threlkeld, and A. Warden. 1995. Ubiquitous tarballs with a California source signature on the shorelines of Prince William Sound. *Environ. Sci. Technol.* 29: 2684-2694.

- Landrum, P. F., B. J. Eadie, and W. R. Faust. 1992. Variation in the bioavailability of polycyclic aromatic hydrocarbons to the Amphipod *Diporeia* (Spp.) with sediment aging. *Environ. Toxicol. Chem.* 11: 1197-1208.
- Leahy, J.G. and R. R. Colwell. 1990. Microbial degradation of hydrocarbons in the environment. *Microbiol. Rev.* 54: 305-315.
- Lees, D. C., J. R. Payne, and W. B. Driskell. 1999. Technical evaluation of the environmental monitoring program for Cook Inlet Regional Citizen's Advisory Council. Unpublished report, Littoral Ecological and Environmental Services, 168 pp.
- Mansuy, L., R. P. Philip, and J. Allen. 1997. Source identification of oil spills based on the isotopic composition of individual components in weathered oil samples. *Environ. Sci. Technol.* 31: 3417-3425.
- McElroy, A. E., J. W. Farrington and J. M. Teal. 1989. Bioavailability of polycyclic aromatic hydrocarbons in the aquatic environment. *In* *Metabolism of Polycyclic Aromatic Hydrocarbons in the Aquatic Environment*, Varanasi, U., ed. CRC Press, Boca Raton, FL, pp. 1-39.
- O'Malley, V. P., T. A. Abrajano, and J. Hellou. 1994. Determination of the $^{13}\text{C}/^{12}\text{C}$ ratios of individual PAH from environmental samples: can PAH sources be apportioned? *Organic Geochem.* 21: 809-822.
- O'Malley, V. P., T. A. Abrajano, and J. Hellou. 1996. Stable carbon isotopic apportionment of individual polycyclic aromatic hydrocarbons in St. John's Harbour, Newfoundland. *Environ. Sci. Technol.* 30: 634-639.
- Page, D. S., P. D. Boehm, G. S. Douglas, A. E. Bence, W. A. Burns, and P. J. Mankiewicz. 1996. The natural petroleum hydrocarbon background in subtidal sediments of Prince William Sound, Alaska, USA. *Environ. Toxicol. Chem.* 15: 1261-1286.
- Pahlman, R. and O. Pelkonen. 1987. Mutagenicity studies of different polycyclic aromatic hydrocarbons: the significance of enzymatic factors and molecular structures. *Carcinogenesis* 8: 773-778.
- Ruble, T. E., A. J. Bakel, and R. P. Philp. 1994. Compound specific isotopic variability in Uinta Basin native bitumens: paleoenvironmental implications. *Organic Geochem.* 21: 661-671.
- Schoell, M., B. R. T. Simoneit, and T.-G. Wang. 1994. Organic geochemistry and coal petrology of tertiary brown coal in the Zhoujing mine, Baise basin, South China. 4. Biomarker sources inferred from stable carbon isotope compositions of individual compounds. *Organic Geochem.* 21: 713-719.
- Short, J. W., K. A. Kvendvolden, P. R. Carlson, F. D. Hostettler, R. J. Rosenbauer, and B. A. Wright. 1999. Natural hydrocarbon background in benthic sediments of Prince William Sound Alaska: oil vs. coal. *Environ. Sci. Technol.* 33: 34-42.
- Terschak, J. A. 2002. Phenanthrene adsorption and desorption by melanoidins and marine sediment humic acids. Ph.D. thesis, University of Alaska Fairbanks.

- Teal, J. M., J. W. Farrington, K. A. Burns, J. J. Stegeman, B. W. Tripp, B. Woodin, and C. Phinney. 1992. The West Falmouth oil spill after 20 years: Fate of fuel oil compounds and effects on animals. *Mar. Pollution Bull.* 24: 607-614.
- Thompson, B. Anderson, J. Hunt, K. Taberski, and B. Phillips. 1999. Relationships between sediment contamination and toxicity in San Francisco Bay. *Mar. Environ. Res.* 48: 285-309.
- Wakeham, S., and J. W. Farrington. 1980. Hydrocarbons in contemporary aquatic sediments. *In Contaminants and Sediments, Volume 1*, Baker, R. A., ed. Ann Arbor Science Publishers, pp. 3-31.
- Weissenfels, W. D., H. J. Klewer, and J. Langhoff. 1992. Adsorption of polycyclic aromatic hydrocarbons (PAHs) by soil particles: Influence on biodegradability and biotoxicity. *Appl. Microbiol. Biotechnol.* 36: 689-696.
- Wharfe, J.R., S. R. Wilson, and R. A. Dines. 1984. Observations on the fish populations of an east coast estuary. *Mar. Poll. Bull.* 15: 133-136.
- White, K. L. 1986. An overview of immunotoxicology and carcinogenic polycyclic aromatic hydrocarbons. *Environ. Carcinogen Rev.* C4: 163-202.
- Whiticar, M. J., and S. A. Harris. 2000. Compound specific isotope correlation (CSIC): Biogeochemical fingerprints of organic compounds. *GeoCanada 2000*, <http://cseg.ca/conferences/2000/910.PDF> (extended abstract).

Table 1. Wet and dry weights of samples extracted.

Sample #	Sample I.D.	Wet Weight (g)	Dry Weight (g)	Water (%)
J01.001	North Slope Initial	271.95	125.08	54.01
J01.011	North Slope 1 week	240.32	86.19	64.14
J01.021	North Slope 2 weeks	284.58	118.92	58.21
J01.031	North Slope 3 weeks	243.59	84.01	65.51
J01.051	North Slope 5 weeks	235.49	68.16	71.06
J01.141	North Slope 3 months	234.09	78.62	66.41
J01.521	North Slope 1 year	209.49	64.32	69.30
J02.001	Cook Initial	285.10	108.16	62.06
J02.011	Cook 1 week	257.77	91.27	66.90
J02.021	Cook 2 weeks	274.72	97.58	64.48
J02.031	Cook 3 weeks	254.97	85.07	66.64
J02.051	Cook 5 weeks	293.11	105.50	64.01
J02.141	Cook 3 months	281.52	103.60	63.20
J02.521	Cook 1 year	260.42	84.22	67.66
J03.001	Diesel Initial	243.85	92.31	62.14
J03.011	Diesel 1 week	226.64	77.89	65.63
J03.021	Diesel 2 weeks	277.72	108.04	61.10
J03.031	Diesel 3 weeks	213.07	77.34	63.70
J03.051	Diesel 5 weeks	217.76	63.94	70.64
J03.141	Diesel 3 months	222.26	71.47	67.84
J03.521	Diesel 1 year	339.15	122.95	63.75
C01.1	Mud Bay Coal	2.68	2.51	6.34
C01.2		4.00	3.74	6.50
C01.3		6.08	5.89	3.13
C01.4		8.00	7.34	8.25
C02.1	Jakolof Bay Sediment	70.10	58.96	15.89
C02.2		66.36	56.62	19.68
C02.3		70.23	59.68	15.02
C02.4		n/a	18.66	n/a
C03.1	Jakolof Bay Sediment Sieved	45.86	18.24	60.23
C03.2		50.21	17.28	65.58
C04.1	Jakolof Dock Sediment	79.39	63.59	19.90
C04.2		76.12	64.06	15.84
C04.3		61.65	54.28	11.95
C05.1	Kasitsna Bay Sediment	52.72	49.18	6.71
C05.2		61.53	58.98	4.14
C06.1	Homer Spit Coal	3.20	2.55	20.31
C06.2		5.71	4.92	13.84
C06.3		4.59	3.65	20.48
C07.1	Bishop's Beach Coal	5.71	4.79	16.11
C07.2		5.83	4.79	17.84
C07.3		6.99	5.68	18.74
C08.1	Woodstove ash (w/garbage)	1.52	1.49	1.97
C08.2		2.20	2.17	1.36
C08.3		2.52	2.49	1.19
C09.1	Woodstove ash	1.41	1.31	7.09
C09.2		1.71	1.63	4.68
C09.3		1.99	1.91	4.02

Table 2. $\delta^{13}\text{C}$ of the initial daily PAH standards.

Date	1-Ethynaphthalene $\delta^{13}\text{C-PDB} (\text{‰})^*$	Acenaphthene-d10 $\delta^{13}\text{C-PDB} (\text{‰})^*$	Phenanthrene $\delta^{13}\text{C-PDB} (\text{‰})^*$	Chrysene $\delta^{13}\text{C-PDB} (\text{‰})^*$	Benzo [b] fluoranthene $\delta^{13}\text{C-PDB} (\text{‰})^*$	Perylene $\delta^{13}\text{C-PDB} (\text{‰})^*$
<i>3-May-01</i>	-24.8	-24.9	-24.0	-24.4	-27.3	-25.7
<i>4-May-01</i>	-25.7	-25.8	-23.9	-24.5	-26.5	-25.8
<i>15-Jun-01</i>	-24.6	-23.8	-23.7	-24.6	-27.1	-25.5
<i>9-Jul-01</i>	-24.7	-22.9	-22.7	-22.2	-24.8	-24.0
13-Jul-01	-27.5	-23.2	-23.8	-22.8	-25.4	-25.8
20-Jul-01	-25.8	-23.5	-23.5	-23.5	-25.5	-24.7
23-Jul-01	-25.1	-24.3	-23.9	-24.1	-24.9	-25.4
24-Jul-01	-27.3	-23.7	-23.8	-23.2	-25.2	-25.1
27-Jul-01	-27.7	-23.8	-23.9	-24.3	-26.2	-25.7
10-Aug-01	-27.1	-23.4	-23.4	-23.0	-23.2	-26.0
2-Apr-02	-25.7	-23.5	-22.2	-22.9	-27.8	-24.6
3-Apr-02	-32.0	-24.7	-22.4	-23.9	-25.1	-25.6
2-May-02	-25.3	-21.9	-21.8	-22.0	-23.5	-22.8
3-May-02	-25.7	-21.5	-22.2	-22.7	-23.7	-23.9
8-May-02	-25.7	-22.6	-21.7	-21.1	-28.7	-22.7
9-May-02	-25.5	-21.4	-22.2	-22.2	-24.7	-23.1
10-May-02	-26.6	-22.4	-21.9	-22.3	-24.1	-23.1
16-May-02	-25.8	-23.0	-22.0	-22.4	-24.5	-23.4
30-May-02	-26.2	-23.3	-22.4	-22.3	-26.6	-21.6
31-May-02	-26.7	-22.4	-21.8	-22.0	-22.9	-22.9
4-Jun-02	-25.4	-22.1	-21.6	-21.9	-23.7	-23.0
5-Jun-02	-26.1	-22.0	-22.1	-22.5	-24.9	-23.7
17-Jun-02	-26.9	-22.6	-22.1	-22.0	-25.7	-23.7
18-Jun-02	-26.0	-22.5	-22.6	-23.2	-25.4	-24.3
20-Jun-02	-25.8	-23.5	-22.0	-22.3	-25.3	-23.2
12-Jul-02	-28.1	-22.2	-21.8	-21.7	-23.4	-22.5
24-Jul-02	-26.9	-22.1	-22.0	-22.5	-24.4	-23.4
25-Jul-02	-25.6	-21.7	-20.5	-20.2	-24.7	-23.2
26-Jul-02	-25.2	-21.3	-22.1	-22.2	-24.5	-23.2
29-Jul-02	-26.6	-21.9	-21.5	-22.7	-26.8	-24.5
Mean	-26.5	-22.7	-22.4	-22.5	-25.0	-23.9
Standard deviation	1.4	0.9	0.9	0.9	1.4	1.2

*The italicized data were obtained from a different internal standard solution and are not included in the mean and standard deviation calculations.

Table 3. Concentration and $\delta^{13}\text{C}$ of the internal standards from the microbial degradation experiment samples.

Sample#	Sample I.D.	1-Ethyl-naphthalene $\delta^{13}\text{C-PDB} (\text{‰})^*$	1-Ethyl-naphthalene Conc. ($\mu\text{g/g}$)	Acenaphthene-d10 $\delta^{13}\text{C-PDB} (\text{‰})^*$	Benzo [b] fluoranthene $\delta^{13}\text{C-PDB} (\text{‰})^*$
J01.001	North Slope Initial	-27.1	0.221	-23.6	-25.7
J01.011	North Slope 1 Week	-25.2	0.300	-22.3	-23.7
J01.021	North Slope 2 Weeks	-27.4	0.142	-22.5	-24.0
J01.031a	North Slope 3 Weeks	-27.1	0.151	-22.2	-23.8
J01.031b		-25.2	0.181	n/a	-21.8
J01.051	North Slope 5 Weeks	-26.5	0.404	-22.0	-23.3
J01.141	North Slope 3 Months	-26.3	0.752	-22.7	-25.9
J01.521	North Slope 1 Year	-25.6	0.441	-22.1	-23.4
J02.001	Cook Initial	-26.5	0.445	-23.0	-25.9
J02.021	Cook 2 Weeks	-25.8	0.326	-22.2	-24.2
J02.031a	Cook 3 Weeks	-26.7	0.409	-22.7	-23.6
J02.031b		-25.7	0.416	-22.0	-23.9
J02.031c		-26.0	0.422	-22.5	-23.9
J02.031d		-25.7	0.410	-22.2	-24.2
J02.051a	Cook 5 Weeks	-28.4	0.196	-23.8	-26.1
J02.051b		-25.7	0.187	-23.3	-25.1
J02.051c		-25.7	0.184	-22.8	-25.2
J02.141	Cook 3 Months	-26.6	0.321	-22.9	-26.1
J02.521	Cook 1 Year	-26.3	0.366	-24.8	-24.1
J03.001	Diesel Initial	-26.8	0.360	-22.8	-25.7
J03.011	Diesel 1 Week	-23.2	0.286	-22.9	-23.6
J03.021	Diesel 2 Weeks	-25.3	0.284	-22.1	-23.8
J03.031	Diesel 3 Weeks	-26.2	0.373	-22.8	-24.6
J03.051a	Diesel 5 Weeks	-25.7	0.313	-24.5	-24.9
J03.051b		-26.0	0.311	-24.0	-24.6
J03.051c		-26.2	0.326	-24.0	-24.8
J03.051d		-26.3	0.342	-24.6	-25.0
J03.141a	Diesel 3 Months	-26.8	0.401	-23.5	-26.3
J03.141b		-26.8	0.381	-24.1	-25.6
J03.521a	Diesel 1 Year	-25.5	0.187	-20.5	-23.4
J03.521b		-26.6	0.200	-20.6	-23.6
J03.521c		-24.7	0.171	-21.0	-23.3

*The notation $\delta^{13}\text{C-PDB}(\text{‰})$ is defined within the text. Outliers are printed in a smaller font.

Table 4. Concentration and $\delta^{13}\text{C}$ of fluorene and phenanthrene from the microbial degradation experiment samples.

Sample#	Sample I.D.	Fluorene $\delta^{13}\text{C-PDB}(\text{‰})^*$	Fluorene Conc. ($\mu\text{g/g}$)	Phenanthrene $\delta^{13}\text{C-PDB}(\text{‰})^*$	Phenanthrene Conc. ($\mu\text{g/g}$)
J01.001	North Slope Initial	-21.2	0.004	n/a	0.150
J01.011	North Slope 1 Week	-22.5	0.003	-23.1	0.029
J01.021	North Slope 2 Weeks	-22.6	0.002	-22.7	0.016
J01.031a	North Slope 3 Weeks	-22.5	0.001	-22.2	0.007
J01.031b		-20.3	0.007	-22.7	0.041
J01.051	North Slope 5 Weeks	-20.3	0.006	-22.8	0.032
J01.141	North Slope 3 Months	-25.1	0.006	-23.4	0.056
J01.521	North Slope 1 Year	-21.4	0.005	-21.9	0.023
J02.001	Cook Initial	-28.4	0.048	-23.0	0.386
J02.021	Cook 2 Weeks	-24.4	0.013	-25.4	0.042
J02.031a	Cook 3 Weeks	-21.3	0.008	-23.6	0.043
J02.031b		-22.8	0.008	-24.0	0.046
J02.031c		-23.6	0.019	-24.4	0.051
J02.031d		-26.8	0.009	-25.0	0.052
J02.051a	Cook 5 Weeks	-24.7	0.000	-23.5	0.003
J02.051b		-28.2	0.001	-27.0	0.005
J02.051c		-22.8	0.008	-23.8	0.008
J02.141	Cook 3 Months	-25.7	0.005	-26.3	0.079
J02.521	Cook 1 Year	-23.0	0.010	-27.7	0.066
J03.001	Diesel Initial	-26.0	0.013	-20.1	0.092
J03.011	Diesel 1 Week	-20.0	0.025	-22.4	0.023
J03.021	Diesel 2 Weeks	-20.5	0.016	-25.6	0.018
J03.031	Diesel 3 Weeks	-23.0	0.025	-22.4	0.029
J03.051a	Diesel 5 Weeks	-25.6	0.000	-22.9	0.016
J03.051b		-13.7	0.007	-22.8	0.009
J03.051c		-30.2	0.002	-23.2	0.011
J03.051d		-20.1	0.000	-23.6	0.013
J03.141a	Diesel 3 Months	n/a	n/a	-24.9	0.051
J03.141b		-24.6	0.006	-23.8	0.032
J03.521a	Diesel 1 Year	-21.0	0.056	-23.4	0.047
J03.521b		-20.4	0.034	-24.0	0.024
J03.521c		-21.3	0.063	-23.0	0.056

*The notation $\delta^{13}\text{C-PDB}(\text{‰})$ is defined within the text. Outliers are printed in a smaller font.

Table 5. Concentration and $\delta^{13}\text{C}$ of fluoranthene and chrysene from the microbial degradation experiment samples.

Sample#	Sample I.D.	Fluoranthene $\delta^{13}\text{C-PDB} (\text{‰})^*$	Fluoranthene Conc. ($\mu\text{g/g}$)	Chrysene $\delta^{13}\text{C-PDB} (\text{‰})^*$	Chrysene Conc. ($\mu\text{g/g}$)
J01.001	North Slope Initial	-23.6	0.198	-26.5	0.053
J01.011	North Slope 1 Week	-24.1	0.027	-21.9	0.012
J01.021	North Slope 2 Weeks	-27.9	0.017	-24.6	0.005
J01.031a	North Slope 3 Weeks	n/a	0.003	-21.4	0.003
J01.031b		-26.3	0.071	-21.3	0.012
J01.051	North Slope 5 Weeks	-21.1	0.025	-22.1	0.022
J01.141	North Slope 3 Months	-26.2	0.067	-24.1	0.023
J01.521	North Slope 1 Year	-20.5	0.014	-23.2	0.012
J02.001	Cook Initial	-24.3	0.097	-27.4	0.025
J02.021	Cook 2 Weeks	-26.9	0.014	-22.2	0.019
J02.031a	Cook 3 Weeks	-21.1	0.014	-23.7	0.018
J02.031b		-22.6	0.013	-22.4	0.023
J02.031c		-22.3	0.018	-21.7	0.024
J02.031d		-23.4	0.016	-23.2	0.020
J02.051a	Cook 5 Weeks	n/a	0.001	-25.7	0.033
J02.051b		n/a	0.001	-25.9	0.003
J02.051c		n/a	0.001	-22.6	0.005
J02.141	Cook 3 Months	n/a	0.000	-27.1	0.043
J02.521	Cook 1 Year	-22.1	0.040	-25.7	0.023
J03.001	Diesel Initial	-25.0	0.006	-22.7	0.013
J03.011	Diesel 1 Week	-22.4	0.018	-21.2	0.007
J03.021	Diesel 2 Weeks	n/a	0.012	-21.7	0.004
J03.031	Diesel 3 Weeks	n/a	0.026	-23.7	0.004
J03.051a	Diesel 5 Weeks	-20.2	0.010	-24.5	0.007
J03.051b		-25.2	0.010	-25.7	0.033
J03.051c		-21.6	0.011	-23.1	0.003
J03.051d		-26.0	0.012	-20.6	0.003
J03.141a	Diesel 3 Months	n/a	0.000	-24.0	0.027
J03.141b		n/a	0.037	-24.0	0.025
J03.521a	Diesel 1 Year	-25.3	0.016	-21.9	0.009
J03.521b		-24.7	0.003	-21.7	0.006
J03.521c		-25.2	0.007	-21.2	0.012

*The notation $\delta^{13}\text{C-PDB}(\text{‰})$ is defined within the text. Outliers are printed in a smaller font.

Table 6. Concentration and $\delta^{13}\text{C}$ of perylene from the microbial degradation experiment samples.

Sample#	Sample I.D.	Perylene $\delta^{13}\text{C-PDB} (\text{‰})^*$	Perylene Conc. ($\mu\text{g/g}$)
J01.001	North Slope Initial	-28.7	0.083
J01.011	North Slope 1 Week	-26.9	0.075
J01.021	North Slope 2 Weeks	-27.2	0.041
J01.031a	North Slope 3 Weeks	-28.4	0.018
J01.031b		-24.8	0.055
J01.051	North Slope 5 Weeks	-26.8	0.112
J01.141	North Slope 3 Months	-28.8	0.154
J01.521	North Slope 1 Year	-28.4	0.087
J02.001	Cook Initial	-28.4	0.098
J02.021	Cook 2 Weeks	-28.9	0.097
J02.031a	Cook 3 Weeks	-25.9	0.101
J02.031b		-26.5	0.091
J02.031c		-25.9	0.096
J02.031d		-27.2	0.094
J02.051a	Cook 5 Weeks	-31.3	0.007
J02.051b		-30.4	0.005
J02.051c		-29.8	0.005
J02.141	Cook 3 Months	-30.8	0.210
J02.521	Cook 1 Year	-28.7	0.059
J03.001	Diesel Initial	-30.7	0.137
J03.011	Diesel 1 Week	-28.2	0.071
J03.021	Diesel 2 Weeks	-28.4	0.028
J03.031	Diesel 3 Weeks	-28.5	0.059
J03.051a	Diesel 5 Weeks	-29.1	0.057
J03.051b		-26.8	0.050
J03.051c		-27.6	0.056
J03.051d		-28.7	0.061
J03.141a	Diesel 3 Months	-31.4	0.160
J03.141b		-29.7	0.180
J03.521a	Diesel 1 Year	-27.9	0.068
J03.521b		-28.3	0.047
J03.521c		-27.6	0.000

*The notation $\delta^{13}\text{C-PDB}(\text{‰})$ is defined within the text. Outliers are printed in a smaller font.

Table 7. Statistical tests.

Test	Formula	Description
t-test	$t = b / [(\text{Sy}^*\text{x}) / \text{SXX}]$	$b = \text{slope}$ $\text{Sy}^*\text{x} = [\sum(y-\hat{y})^2 / (n-2)]^{-0.5}$ $\text{SXX} = \sum X^2 - [(\sum X)^2] / n$
z-test	$z = (y - \mu_0) / \sigma$	$z = 1.64$ for 95% confidence $\mu_0 = \text{null hypothesis}$ $\sigma = \text{standard deviation}$
f-test	$F = s_1^2 / s_2^2$	$s_1^2 = \text{variation of population 1}$ $s_2^2 = \text{variation of population 2}$

Table 8. Concentration and $\delta^{13}\text{C}$ of 1-ethylnaphthalene, acenaphthene-d10, and benzo [b] fluoranthene in sediment, coal, crude oil, and diesel fuel samples. These PAH were internal standards added to the samples before extraction.

Sample#	Sample I.D.	1-Ethylnaphthalene $\delta^{13}\text{C-PDB}$ (‰)	1-Ethylnaphthalene Conc. ($\mu\text{g/g}$)*	Acenaphthene-d10 $\delta^{13}\text{C-PDB}$ (‰)	Benzo [b] fluoranthene $\delta^{13}\text{C-PDB}$ (‰)
C02.1	Jakolof Bay Sediment	-24.5	0.059	-23.3	24.9
C02.2		-27.6	0.39	-23.8	-26.2
C02.3		-26.4	0.16	-22.9	-25.0
C02.4		-23.8	0.28	-25.0	-25.8
C03.1	Jakolof Bay Sediment Sieved	-27.3	1.20	-23.1	-25.6
C03.2		-24.5	1.06	-23.8	-25.7
C04.1	Jakolof Dock Sediment	-27.6	0.43	-22.5	-24.7
C04.2		-26.2	0.19	-22.9	-24.1
C04.3		-24.3	0.42	-22.8	-24.4
C05.1	Kasitsna Bay Sediment	-26.5	0.22	-21.2	-25.7
C05.2		-26.2	0.14	-22.2	-24.5
C01.1	Mud Bay Coal	-27.3	4.5	-24.5	-26.0
C01.2		-25.6	2.3	-22.3	-23.2
C01.3a		-27.3	2.2	-24.3	-26.0
C01.3b		-28.0	2.2	-23.5	-24.9
C01.3c		-26.2	2.2	-22.9	-25.3
C01.4		-26.9	1.1	-22.4	-21.6
C06.1	Homer Spit Coal	-29.6	5.9	-22.8	-24.1
C06.2		-27.8	2.2	-26.9	-27.8
C06.3		-28.9	6.8	-26.0	-29.9
C07.1	Bishop's Beach Coal	-25.8	2.6	-23.2	-23.2
		-24.8	4.0	-21.6	-26.2
		-27.8	3.1	-23.8	-23.5
C08.1	Woodstove ash (w/garbage)	-26.3	8.4	-22.4	-23.8
C08.3		-27.2	6.6	-23.8	-22.6
C09.1	Woodstove ash	n/a	n/a	-22.4	-23.8
		-25.0	4.5	-23.1	-23.3
		-30.0	1.8	-24.6	-23.3
PX3	North Slope Crude Oil	-27.8	1500	-24.5	-26.5
PX3.2		-27.4	1600	-23.7	-25.4
PX3.3		-27.0	2200	-24.0	-24.6
PX3.4		-27.2	2400	-24.8	-24.3

Table 8. (continued)

CX3	Cook Inlet Crude Oil	-27.9	1000	-24.7	-26.8
CX3.2		-26.5	1300	-22.4	-23.5
CX3.3		-28.8	2800	-25.8	-22.7
CX3.4		-27.4	2100	-25.1	-24.8
DX3	Diesel Oil	-27.7	790	-23.0	-25.9
DX3.2		-28.0	420	-25.1	-23.3
DX3.3		-26.4	320	-22.7	-24.2
DX3.4		-28.7	1260	-21.6	-25.3

*Sediment and coal concentrations are in units of $\mu\text{g/g}$ dry weight. Oil and diesel concentrations are in units of $\mu\text{g/g}$ of crude oil or diesel fuel.

Table 9. Concentration and $\delta^{13}\text{C}$ of fluorene and phenanthrene in the sediment, coal, crude oil, and diesel fuel samples.

Sample#	Sample I.D.	Fluorene $\delta^{13}\text{C-PDB}$ (‰)	Fluorene Conc. ($\mu\text{g/g}$)*	Phenanthrene $\delta^{13}\text{C-PDB}$ (‰)	Phenanthrene Conc. ($\mu\text{g/g}$)*
C02.1	Jakolof Bay Sediment	-20.3	0.002	n/a	n/a
C02.2		-26.9	0.002	-20.3	0.030
C02.3		-21.2	0.002	-20.6	0.002
C02.4		-24.5	0.004	-21.6	0.34
C03.1	Jakolof Bay Sediment Sieved	-24.0	0.009	-22.3	0.18
C03.2		-22.0	0.006	-21.2	0.43
C04.1	Jakolof Dock Sediment	-24.5	0.002	-20.4	0.075
C04.2		-27.8	0.001	-22.4	0.018
C04.3		-23.3	0.000	-21.0	0.13
C05.1	Kasitsna Bay Sediment	-24.3	0.0003	-23.3	0.011
C05.2		-26.1	0.0002	-23.0	0.007
C01.1	Mud Bay Coal	-28.6	0.025	-24.6	0.096
C01.2		-24.1	0.34	-25.2	0.16
C01.3a		-25.5	0.95	-24.8	0.48
C01.3b		-24.2	1.04	-23.7	0.50
C01.3c		-23.6	1.02	-22.6	0.50
C01.4		-28.1	0.063	-26.2	0.52
C06.1	Homer Spit Coal	-21.8	1.28	-22.8	0.14
C06.2		-25.9	0.033	-28.4	0.12
C06.3		-26.1	0.12	-22.8	0.077
C07.1	Bishop's Beach Coal	-24.7	0.10	-22.9	0.067
C07.2		-24.4	0.033	-25.5	0.008
C07.3		-20.9	0.042	-21.6	0.028
C08.1	Woodstove ash (w/garbage)	-23.2	0.005	-22.3	0.027
C08.3		-28.5	0.032	-22.7	0.016
C09.1	Woodstove ash	-22.4	0.015	-26.0	0.15
C09.2		-24.5	0.025	-24.2	0.17
C09.3		-26.4	0.063	-24.7	0.59
PX3	North Slope Crude Oil	-22.4	14	-27.1	56
PX3.2		-21.0	59	-27.3	140
PX3.3		-22.4	28	-27.5	83
PX3.4		-19.6	28	-27.1	89

Table 9. (continued)

CX3	Cook Inlet Crude Oil	-29.8	26	-29.6	190
CX3.2		-23.5	110	-25.8	300
CX3.3		-28.3	33	-23.1	120
CX3.4		-27.2	110	-26.5	240
DX3	Diesel Oil	-22.4	290	-26.1	200
DX3.2		-22.1	200	-23.5	90
DX3.3		-22.4	110	-24.2	36
DX3.4		-26.0	140	-27.1	33

*Sediment and coal concentrations are in units of $\mu\text{g/g}$ dry weight. Oil and diesel concentrations are in units of $\mu\text{g/g}$ of crude oil or diesel fuel.

Table 10. Concentration and $\delta^{13}\text{C}$ of fluoranthene and chrysene in sediment, coal, crude oil, and diesel fuel samples.

Sample#	Sample I.D.	Fluoranthene $\delta^{13}\text{C}$ -PDB (‰)	Fluoranthene Conc. ($\mu\text{g/g}$)*	Chrysene $\delta^{13}\text{C}$ -PDB (‰)	Chrysene Conc. ($\mu\text{g/g}$)*
C02.1	Jakolof Bay Sediment	-28.6	0.002	-23.2	0.22
C02.2		-23.8	0.004	-25.7	0.034
C02.3		n/a	n/a	-26.9	0.002
C02.4		n/a	n/a	-22.8	0.25
C03.1	Jakolof Bay Sediment Sieved	-22.7	0.031	-24.1	0.024
C03.2		n/a	n/a	-22.6	0.052
C04.1	Jakolof Dock Sediment	-29.7	0.17	-23.7	0.23
C04.2		-23.6	0.037	-23.2	0.051
C04.3		-27.8	0.12	-24.4	0.074
C05.1	Kasitsna Bay Sediment	n/a	0.006	-25.4	0.007
C05.2		-21.6	0.005	-25.7	0.007
C01.1	Mud Bay Coal	-25.0	0.20	-25.6	1.9
C01.2		-23.7	0.19	-21.0	1.3
C01.3a		-28.5	0.21	-22.1	1.0
C01.3b		-27.6	0.22	-24.8	2.9
C01.3c		-27.8	0.071	-25.0	2.9
C01.4		-26.7	1.7	n/a	n/a
C06.1	Homer Spit Coal	-23.3	0.68	-26.2	0.15
C06.2		-28.3	0.44	-28.3	0.089
C06.3		-22.9	0.099	-22.9	0.033
C07.1	Bishop's Beach Coal	-24.0	0.053	-25.9	0.078
C07.2		-25.3	0.19	-23.7	0.058
C07.3		-22.1	0.077	-28.1	0.12
C08.1	Woodstove ash (w/garbage)	-21.4	0.000	-20.1	0.021
C08.3		-27.5	0.000	n/a	n/a
C09.1	Woodstove ash	-21.0	0.021	-22.7	0.003
C09.2		-22.5	0.025	-28.6	0.000
C09.3		-28.9	0.25	-28.9	0.000
PX3	North Slope Crude Oil	-24.6	11	-23.6	46
PX3.2		-27.9	18	-25.0	57
PX3.3		-33.7	5.6	-20.9	11
PX3.4		-28.3	5.6	-20.6	22

Table 10. (continued)

CX3	Cook Inlet Crude Oil	-29.8	23	-26.3	54
CX3.2		-22.0	18	-25.5	34
CX3.3		-33.6	11	-24.5	17
CX3.4		-29.3	39	-25.8	17
DX3	Diesel Oil	n/a	n/a	n/a	n/a
DX3.2		-21.2	7.8	-22.7	0.90
DX3.3		-21.4	0.73	n/a	n/a
DX3.4		-32.4	1.8	n/a	n/a

*Sediment and coal concentrations are in units of $\mu\text{g/g}$ dry weight. Oil and diesel concentrations are in units of $\mu\text{g/g}$ of crude oil or diesel fuel.

Table 11. Concentration and $\delta^{13}\text{C}$ of perylene in sediment, coal, crude oil, and diesel fuel samples.

Sample#	Sample I.D.	Perylene $\delta^{13}\text{C-PDB}$ (‰)	Perylene Conc. ($\mu\text{g/g}$)*
C02.1	Jakolof Bay Sediment	-24.7	1.2
C02.2		-27.4	0.001
C02.3		-27.0	0.021
C02.4		-26.3	0.34
C03.1	Jakolof Bay Sediment Sieved	-22.4	0.013
C03.2		-27.7	0.23
C04.1	Jakolof Dock Sediment	-28.2	0.039
C04.2		-23.3	0.019
C04.3		-25.4	0.035
C05.1	Kasitsna Bay Sediment	-28.4	0.003
C05.2		-24.4	0.005
C01.1	Mud Bay Coal	-28.9	2.8
C01.2		-26.4	2.7
C01.3a		-28.1	0.99
C01.3b		-29.1	0.95
C01.3c		-27.1	0.98
C01.4		-26.9	2.4
C06.1	Homer Spit Coal	-26.6	0.93
C06.2		-31.0	0.62
C06.3		-34.0	0.47
C07.1	Bishop's Beach Coal	-26.2	1.4
C07.2		-27.7	0.36
C07.3		-27.7	0.33
C08.1	Woodstove ash (w/garbage)	-25.1	0.02
C08.3		n/a	n/a
C09.1	Woodstove ash	-20.8	0.009
C09.2		-25.6	0.098
C09.3		-25.9	0.000
PX3	North Slope Crude Oil	-28.9	77
PX3.2		-26.9	8.8
PX3.3		n/a	n/a
PX3.4		-25.4	22
CX3	Cook Inlet Crude Oil	-25.5	40
CX3.2		-27.0	28
CX3.3		-25.4	22
CX3.4		-28.6	5.6
DX3	Diesel Oil	n/a	n/a
DX3.2		-22.7	4.4
DX3.3		n/a	n/a
DX3.4		n/a	n/a

*Sediment and coal concentrations are in units of $\mu\text{g/g}$ dry weight. Oil and diesel concentrations are in units of $\mu\text{g/g}$ of crude oil or diesel fuel.

Table 12. The standard deviations of $\delta^{13}\text{C}$ data from the microbial degradation experiments and the hydrocarbon standards.

PAH	North Slope Crude	Cook Inlet Crude	Diesel Fuel	Standard
1-ethynaphthalene	0.8	0.3	1.2	1.4
acenaphthene - d10	0.5	0.9	1.2	0.9
benzo [b] fluoranthene	1.2	1.0	1.0	1.4
fluorene	1.6	1.9	2.2	0.9
phenanthrene	0.5	1.6	1.7	0.9
fluoranthene	2.8	2.2	1.3	0.9
chrysene	1.8	2.2	1.1	0.9
perylene	1.0	1.6	1.2	1.2

Table 13. The standard deviations of $\delta^{13}\text{C}$ data from the analysis of crude oils, diesel fuel, and the hydrocarbon standards.

PAH	North Slope Crude	Cook Inlet Crude	Diesel Fuel	Standard
1-ethynaphthalene	0.3	1.0	1.0	1.4
acenaphthene - d10	0.5	1.5	1.5	0.9
benzo [b] fluoranthene	1.0	1.8	1.2	1.4
fluorene	1.3	2.7	1.9	0.9
phenanthrene	0.2	2.7	1.6	0.9
fluoranthene	3.8	4.9	5.6	0.9
chrysene	2.1	0.8	n/a	0.9
perylene	1.8	1.5	n/a	1.2

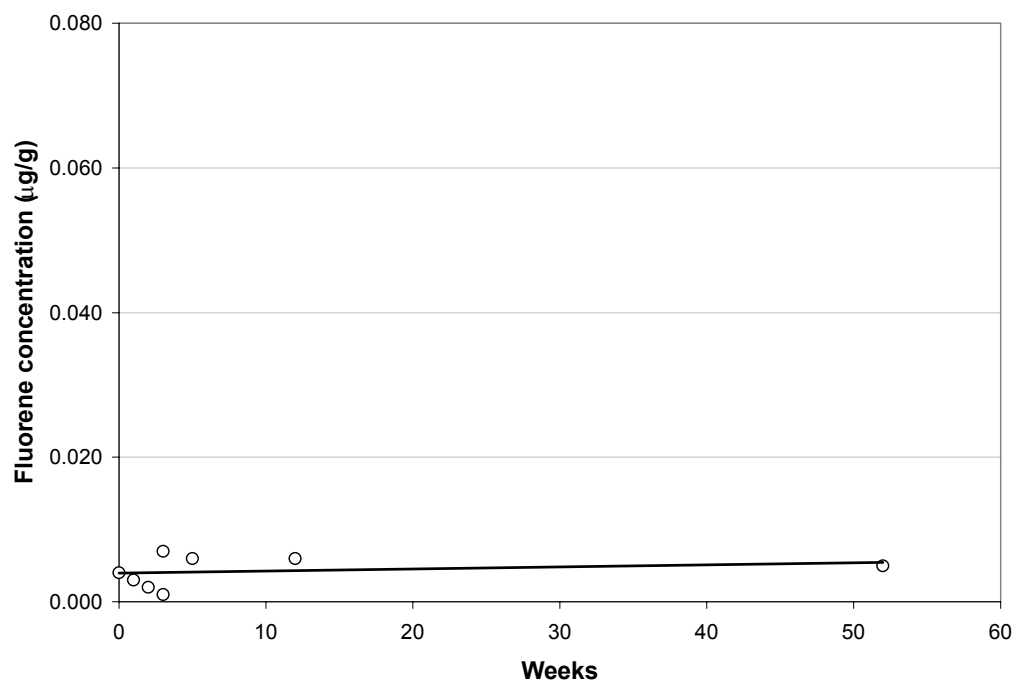
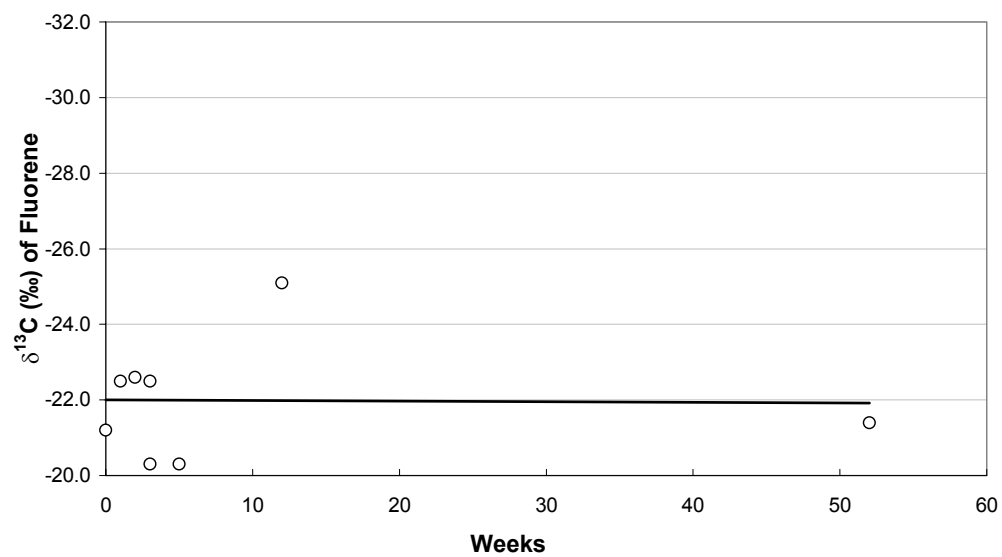


Figure 1a. Linear regressions of the $\delta^{13}\text{C}$ values and concentrations of fluorene from the North Slope crude oil microbial degradation experiment.

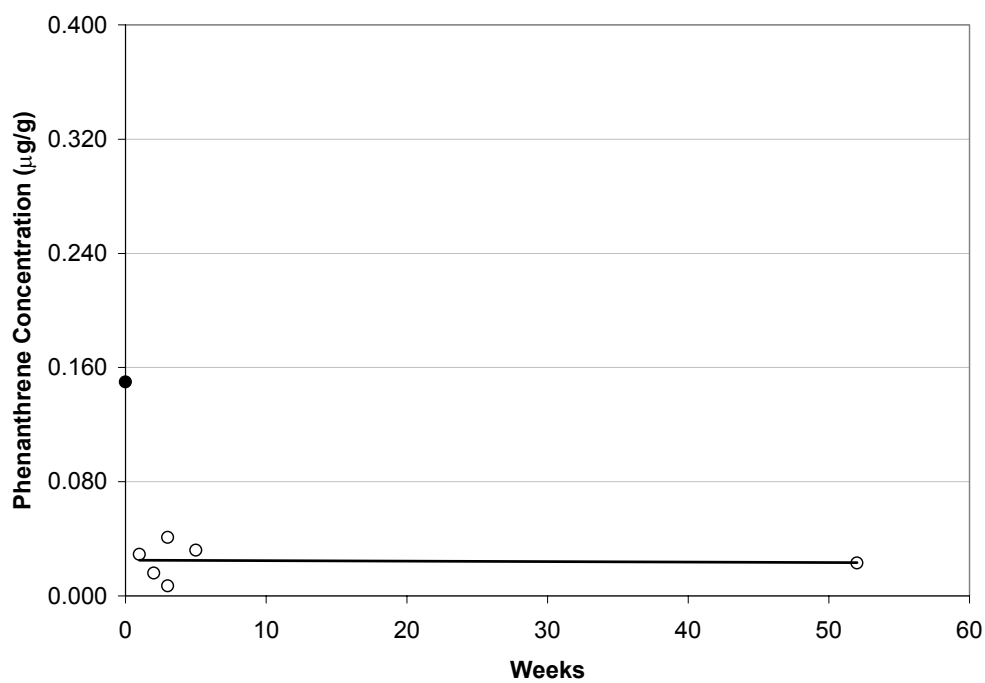
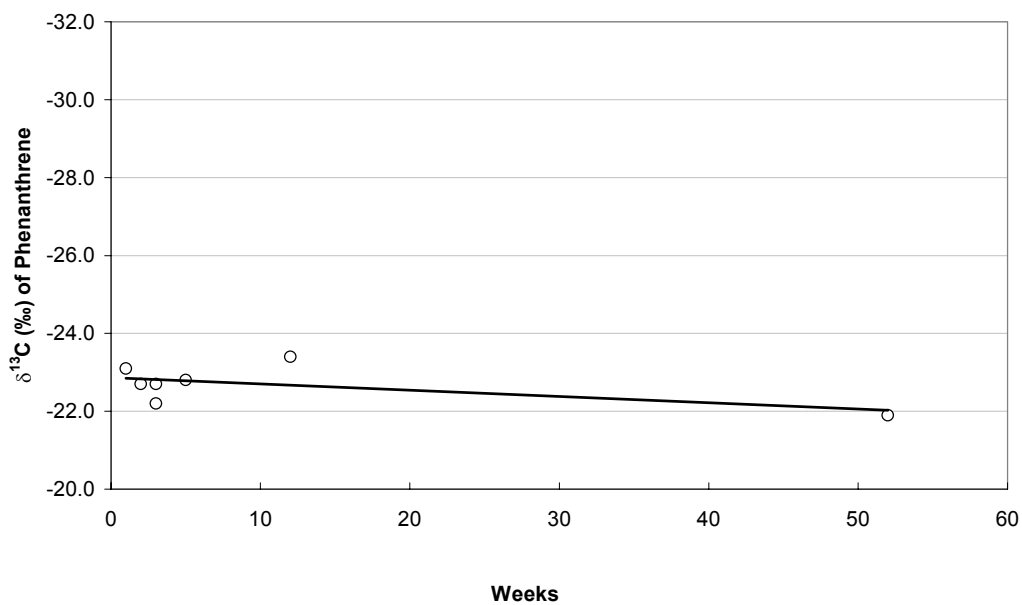


Figure 1b. Linear regressions of the $\delta^{13}\text{C}$ values and concentrations of phenanthrene from the North Slope crude oil microbial degradation experiment. Solid circles are initial concentrations omitted from the regression (see text).

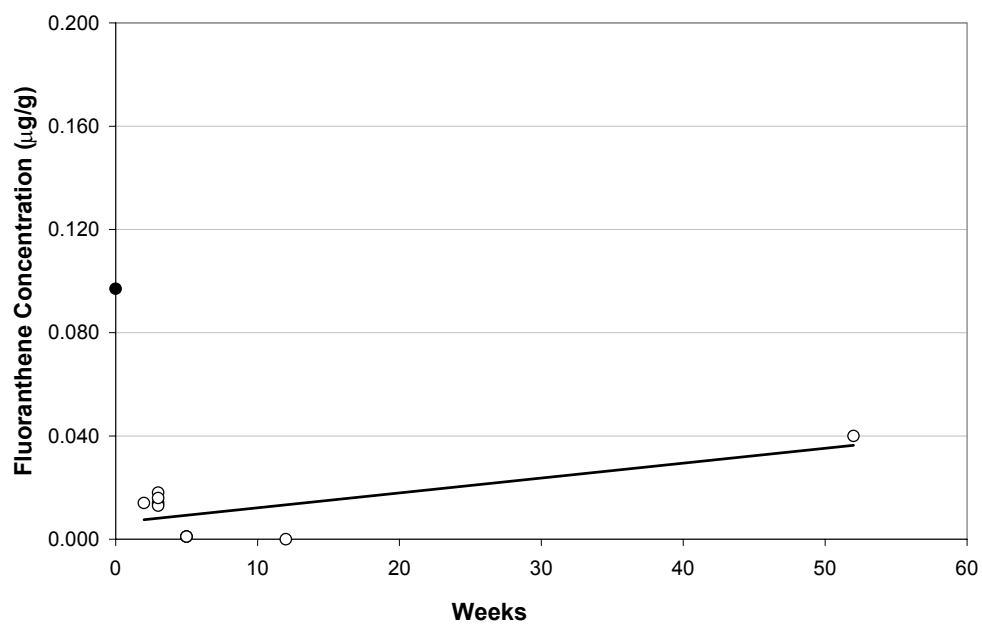
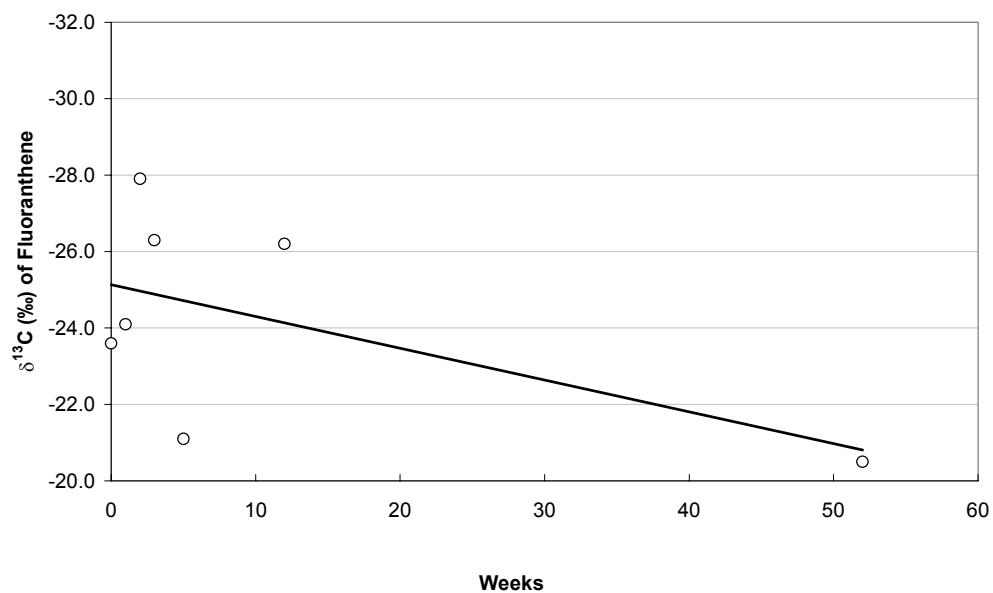


Figure 1c. Linear regressions of the $\delta^{13}\text{C}$ values and concentrations of fluoranthene from the North Slope crude oil microbial degradation experiment. Solid circles are initial concentrations omitted from the regression (see text).

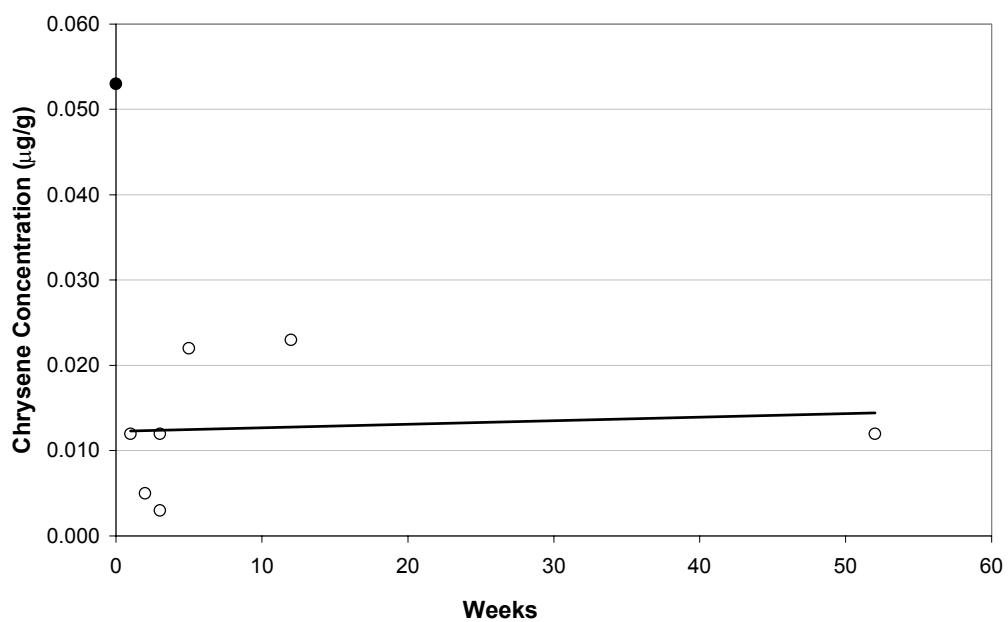
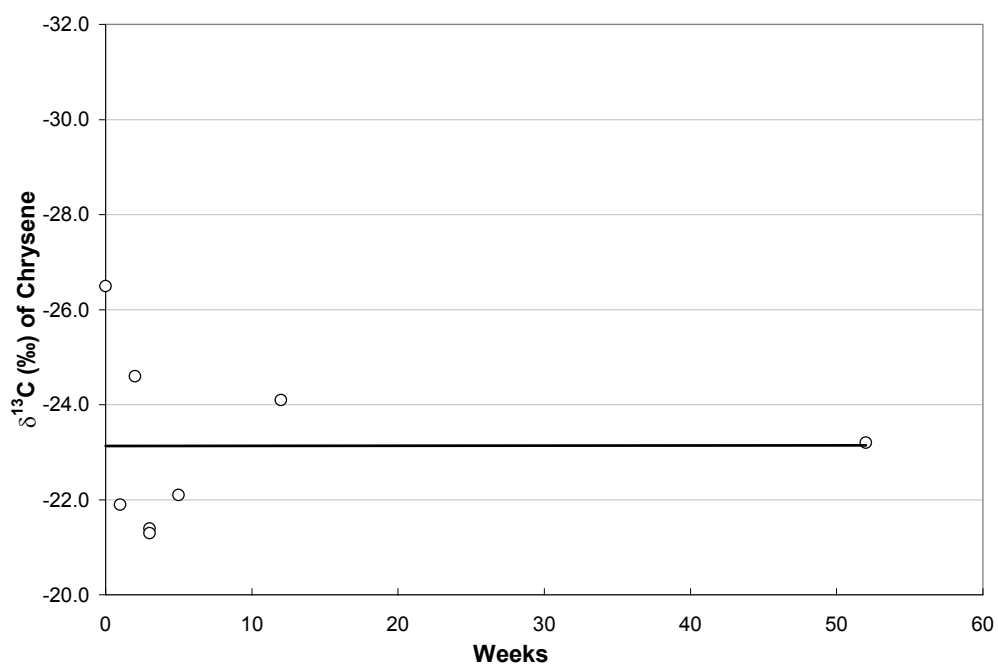


Figure 1d. Linear regressions of the $\delta^{13}\text{C}$ values and concentrations of chrysene from the North Slope crude oil microbial degradation experiment. Solid circles are initial concentrations omitted from the regression (see text).

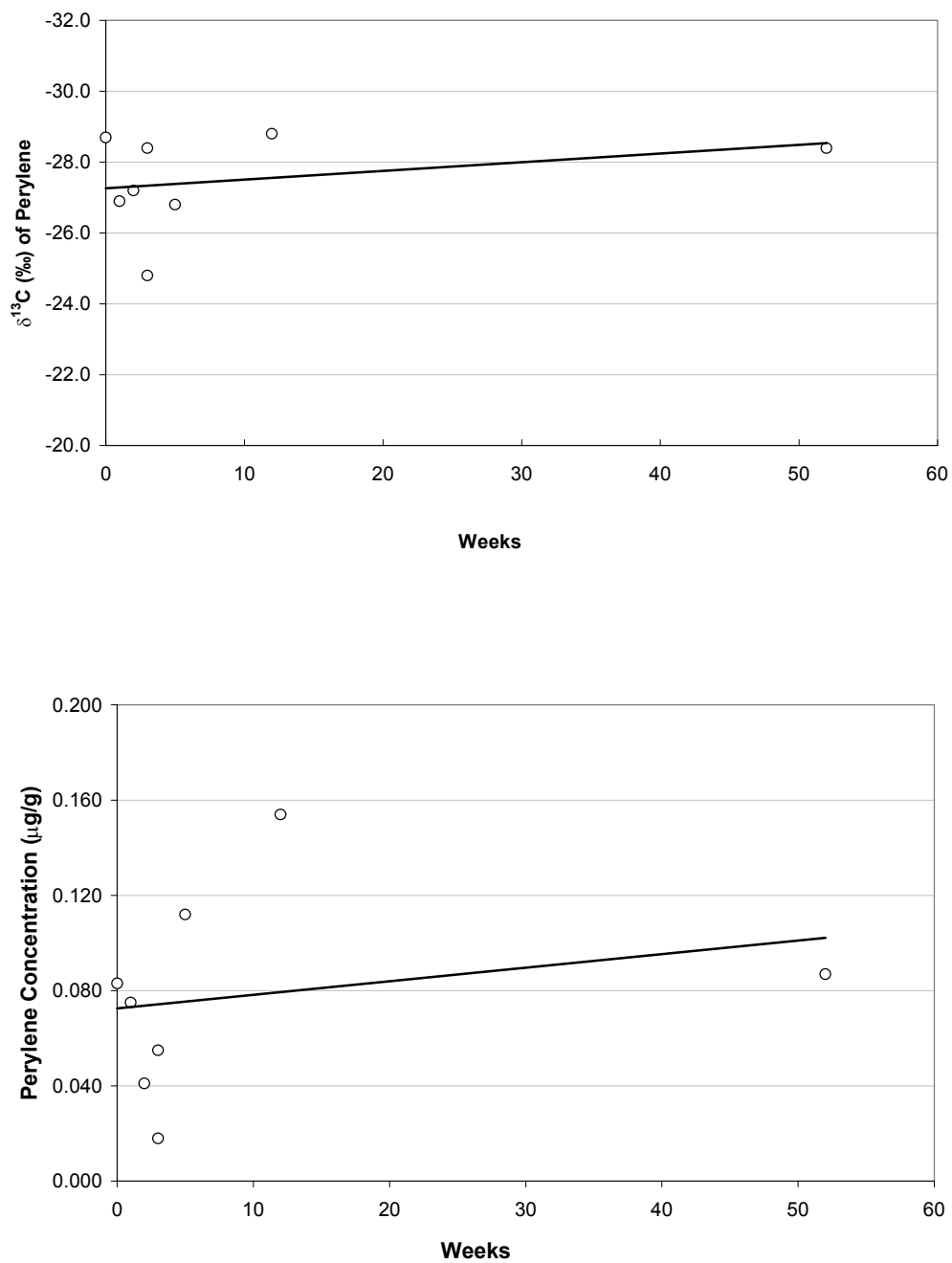


Figure 1e. Linear regressions of the $\delta^{13}\text{C}$ values and concentrations of perylene from the North Slope crude oil microbial degradation experiment.

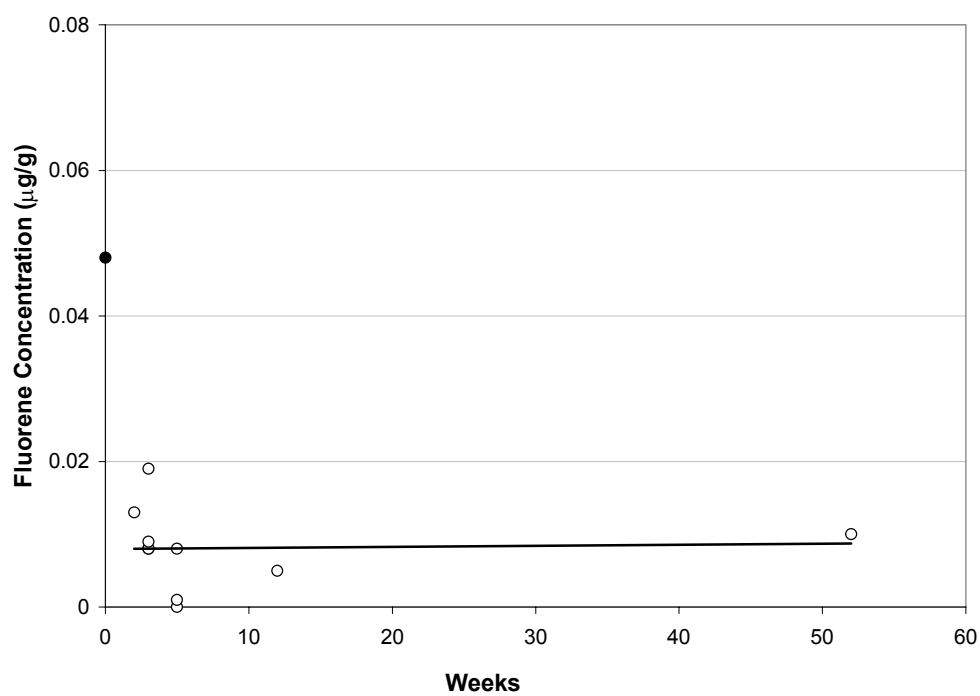
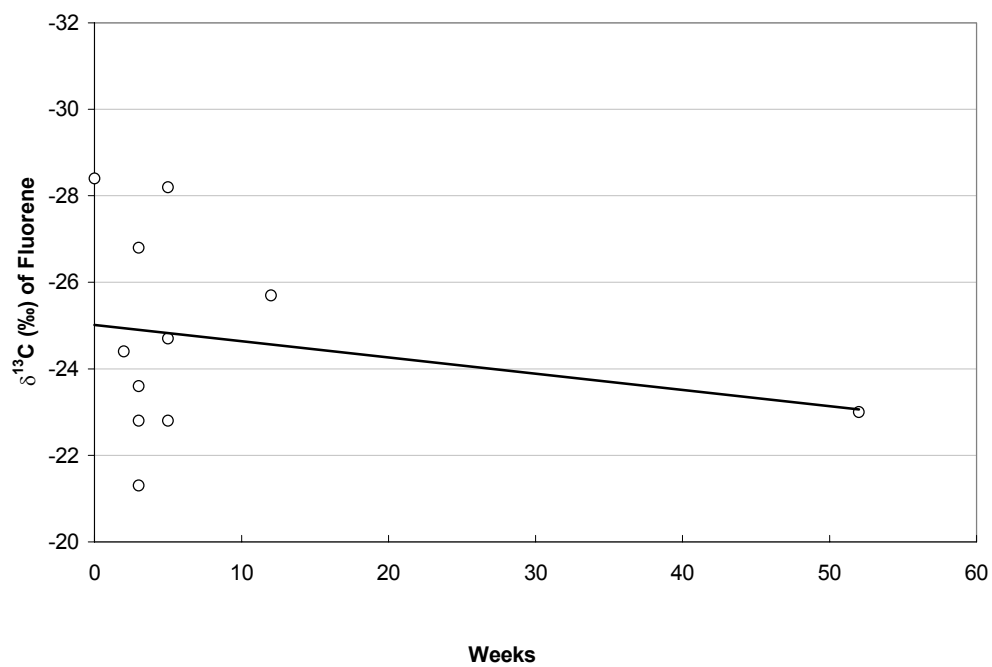


Figure 2a. Linear regressions of the $\delta^{13}\text{C}$ values and concentrations of fluorene from the Cook Inlet crude oil microbial degradation experiment. Solid circles are initial concentrations omitted from the regression (see text).

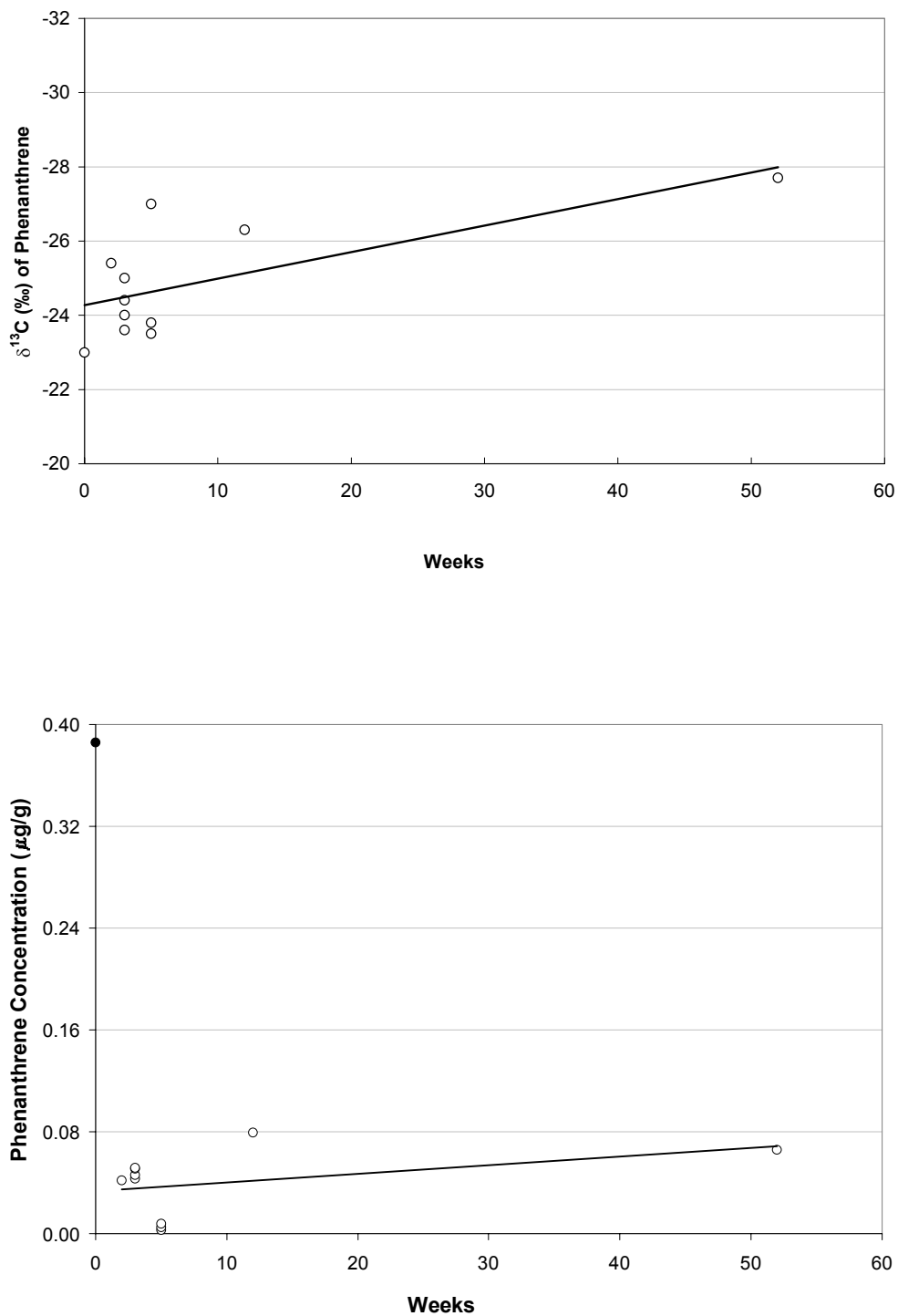


Figure 2b. Linear regressions of the $\delta^{13}\text{C}$ values and concentrations of phenanthrene from the Cook Inlet crude oil microbial degradation experiment. Solid circles are initial concentrations omitted from the regression (see text).

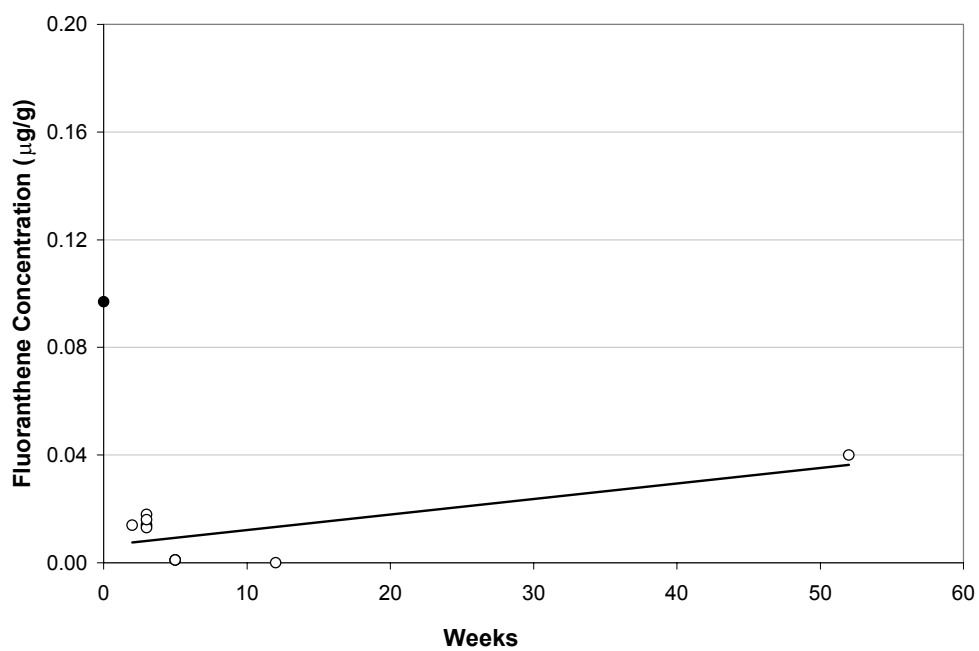
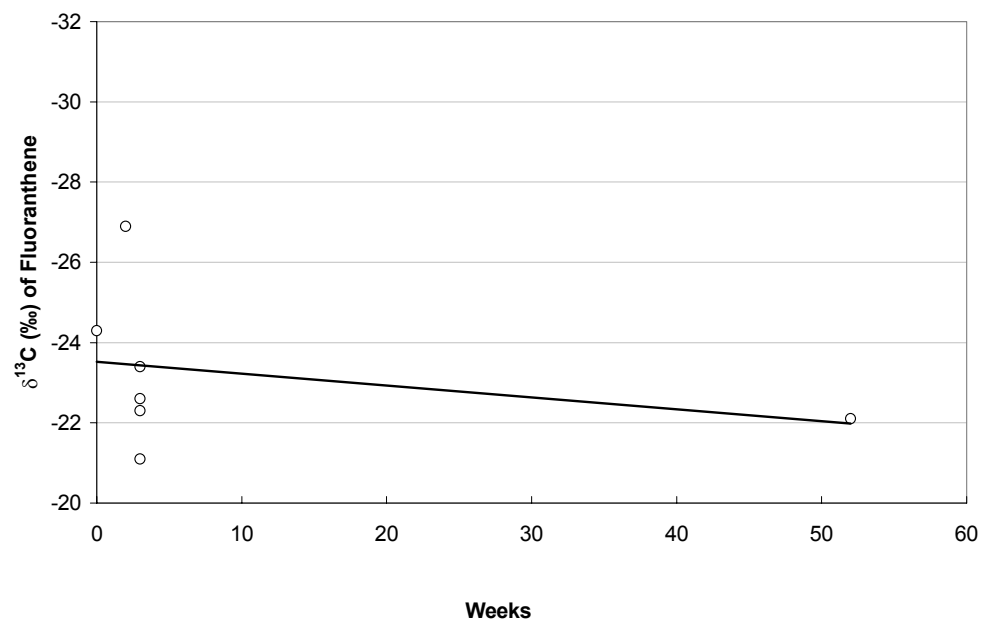


Figure 2c. Linear regressions of the $\delta^{13}\text{C}$ values and concentrations of fluoranthene from the Cook Inlet crude oil microbial degradation experiment. Solid circles are initial concentrations omitted from the regression (see text).

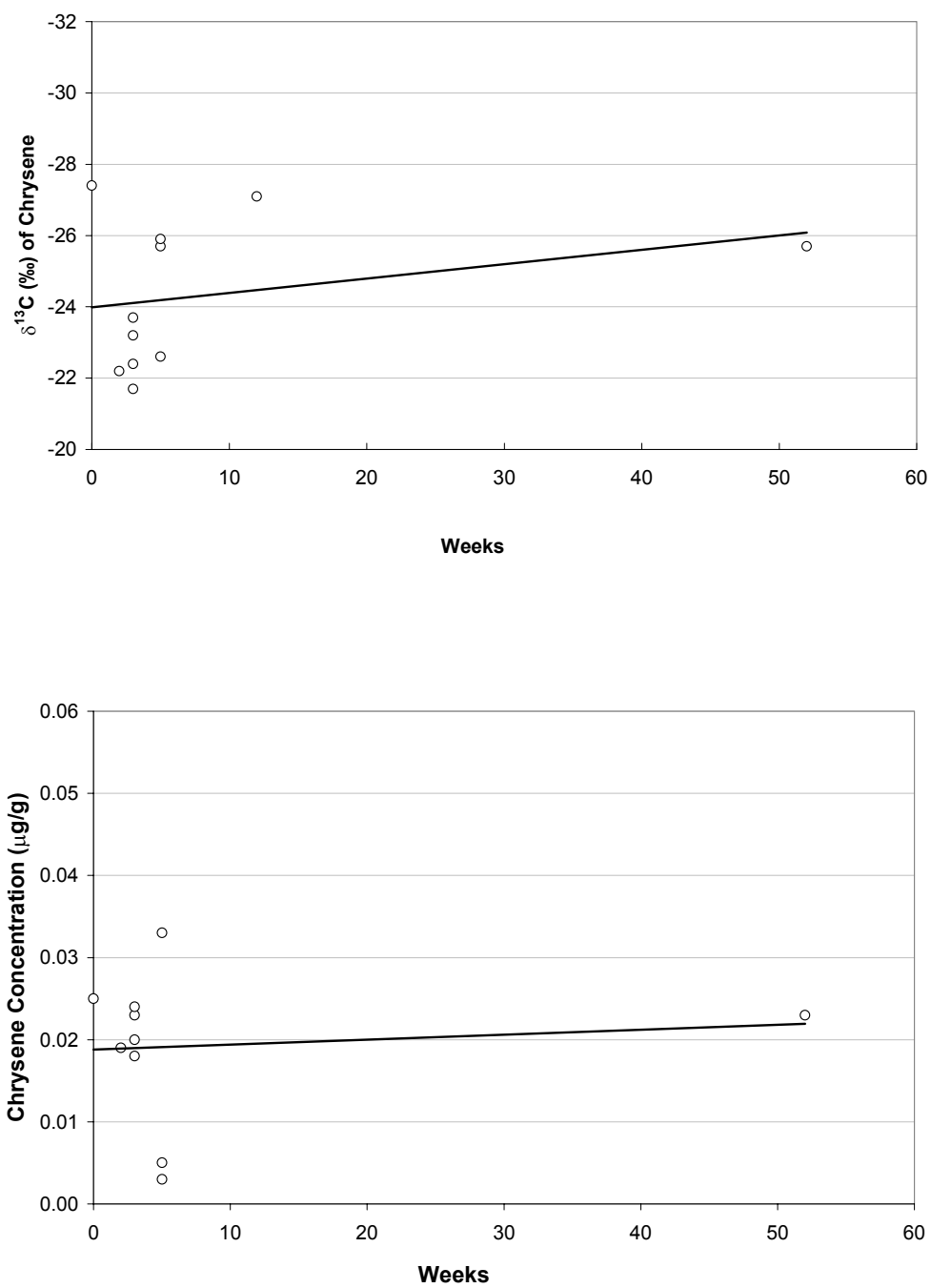


Figure 2d. Linear regressions of the $\delta^{13}\text{C}$ values and concentrations of chrysene from the Cook Inlet crude oil microbial degradation experiment.

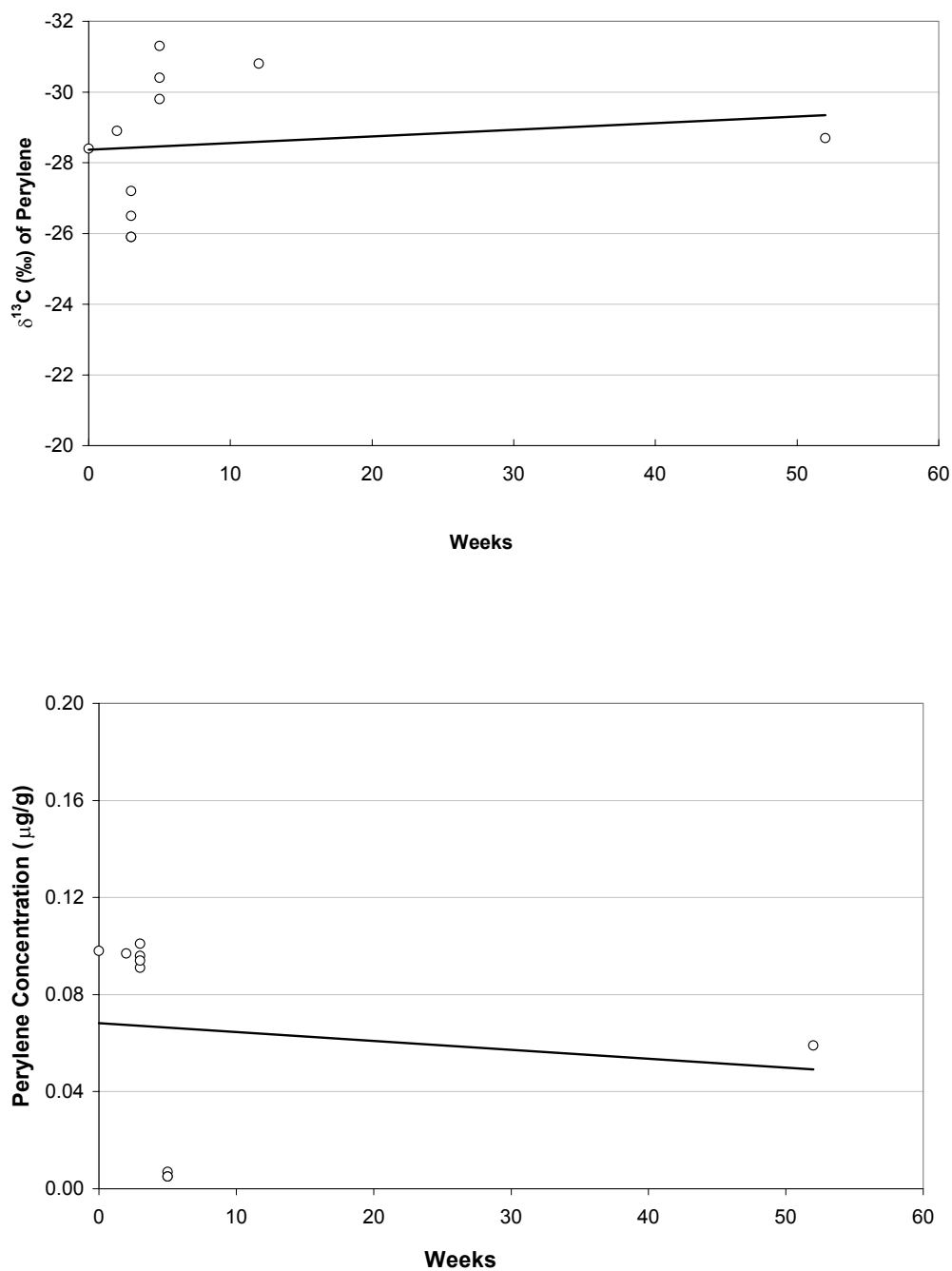


Figure 2e. Linear regressions of the $\delta^{13}\text{C}$ values and concentrations of fluoranthene from the Cook Inlet crude oil microbial degradation experiment.

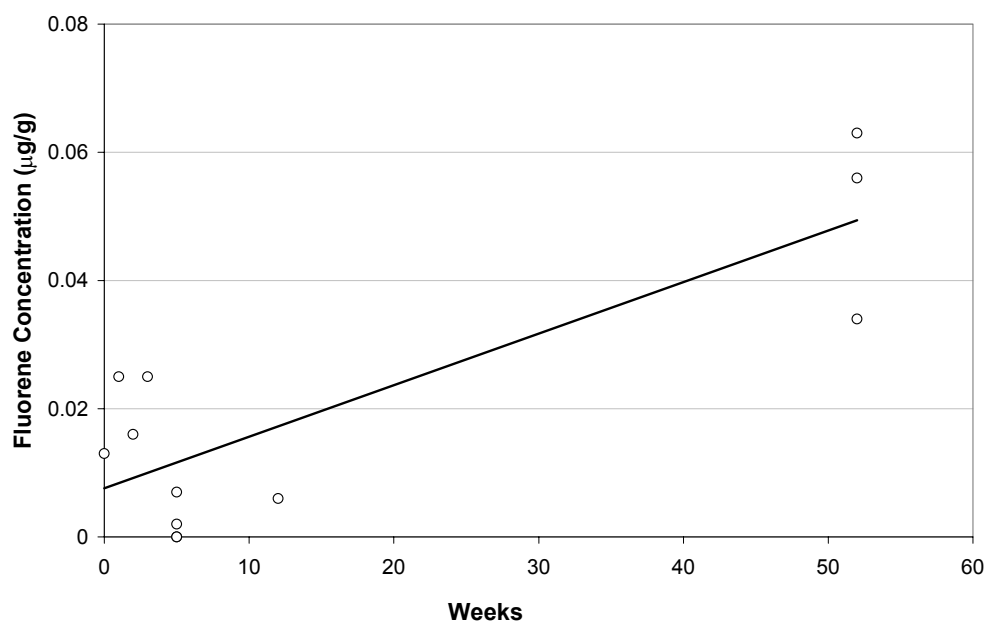
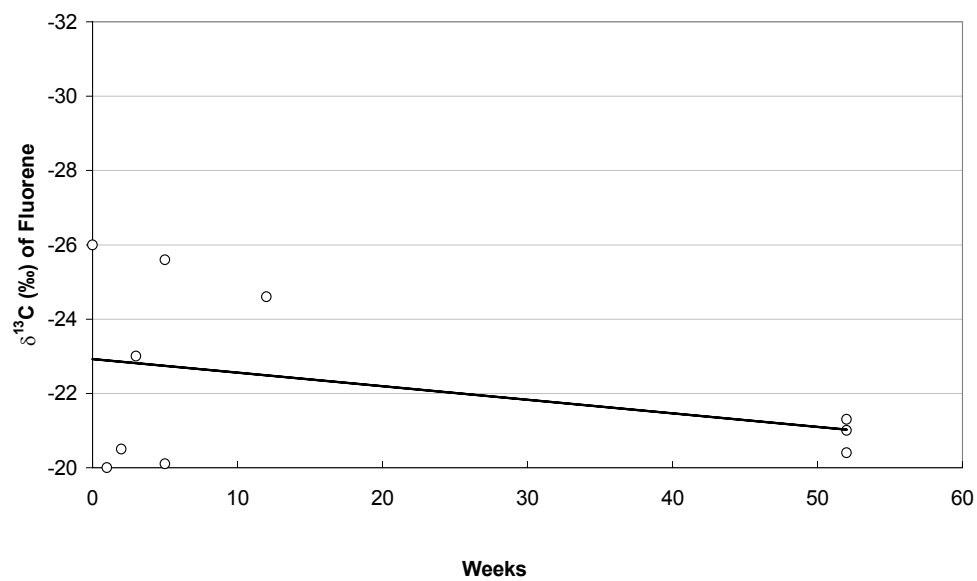


Figure 3a. Linear regressions of the $\delta^{13}\text{C}$ values and concentrations of fluorene from the diesel fuel microbial degradation experiment.

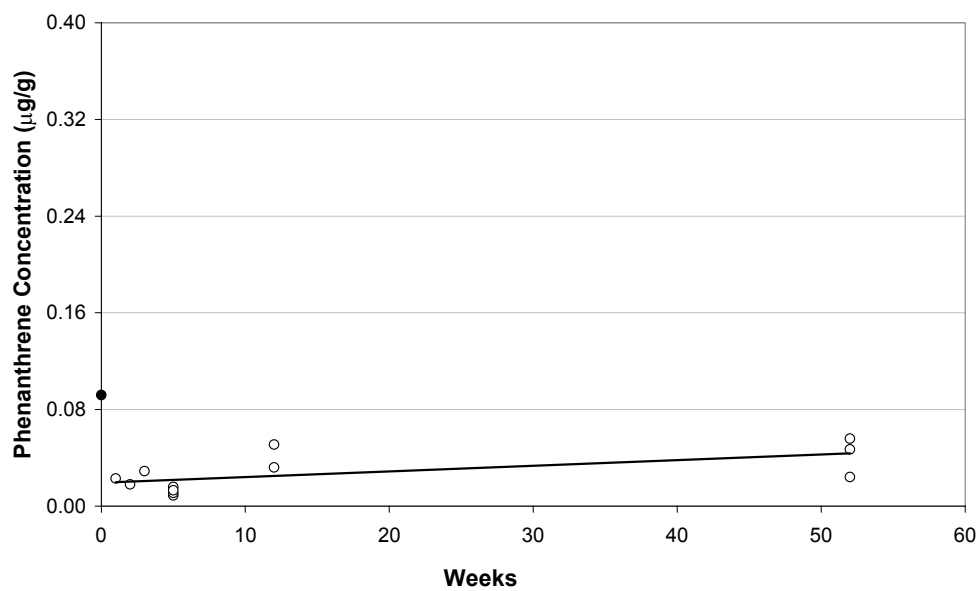
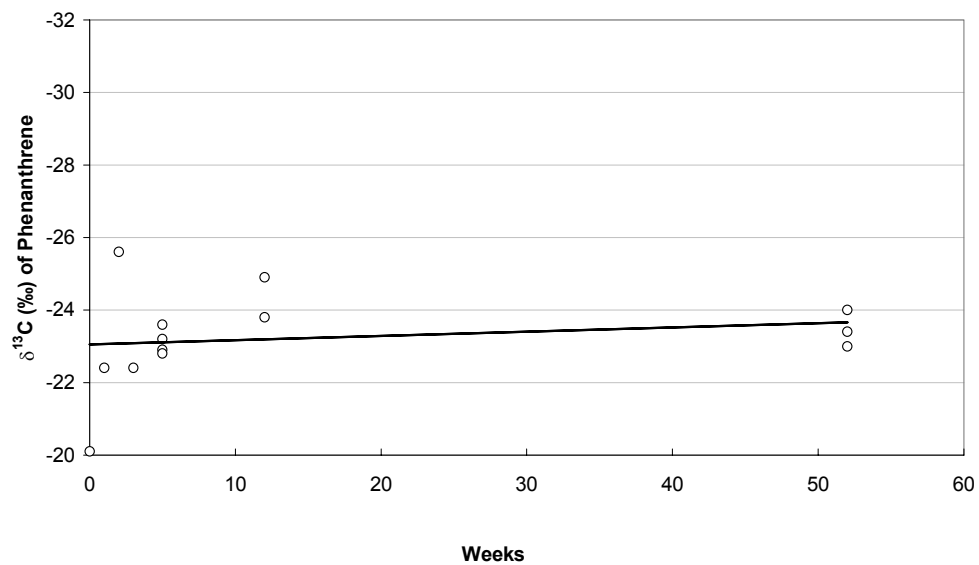


Figure 3b. Linear regressions of the $\delta^{13}\text{C}$ values and concentrations of phenanthrene from the diesel fuel microbial degradation experiment. Solid circles are initial concentrations omitted from the regression (see text).

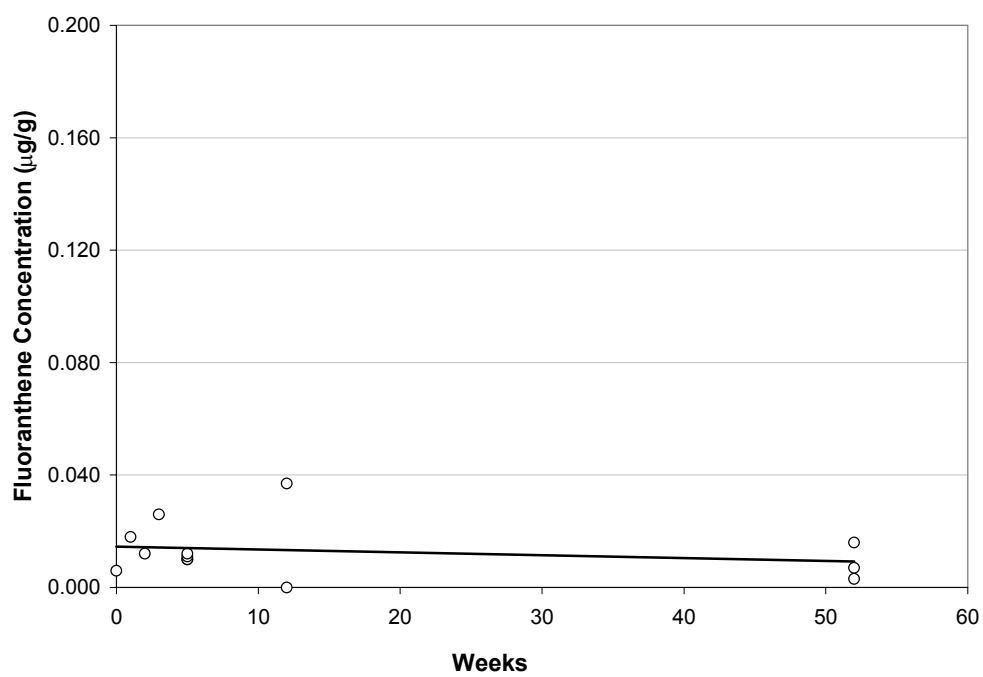
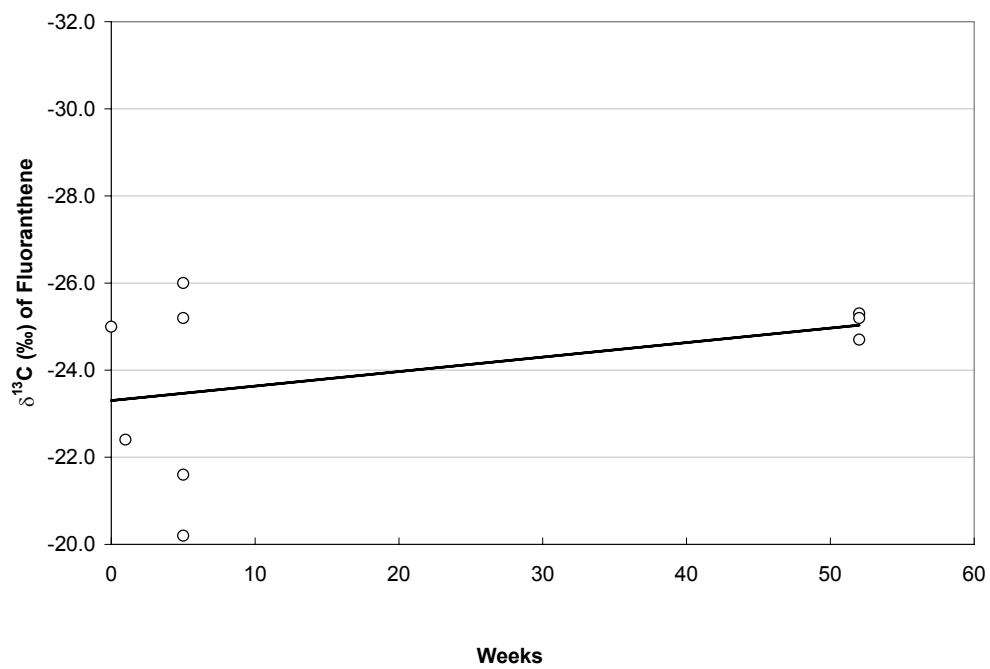


Figure 3c. Linear regressions of the $\delta^{13}\text{C}$ values and concentrations of fluoranthene from the diesel fuel microbial degradation experiment.

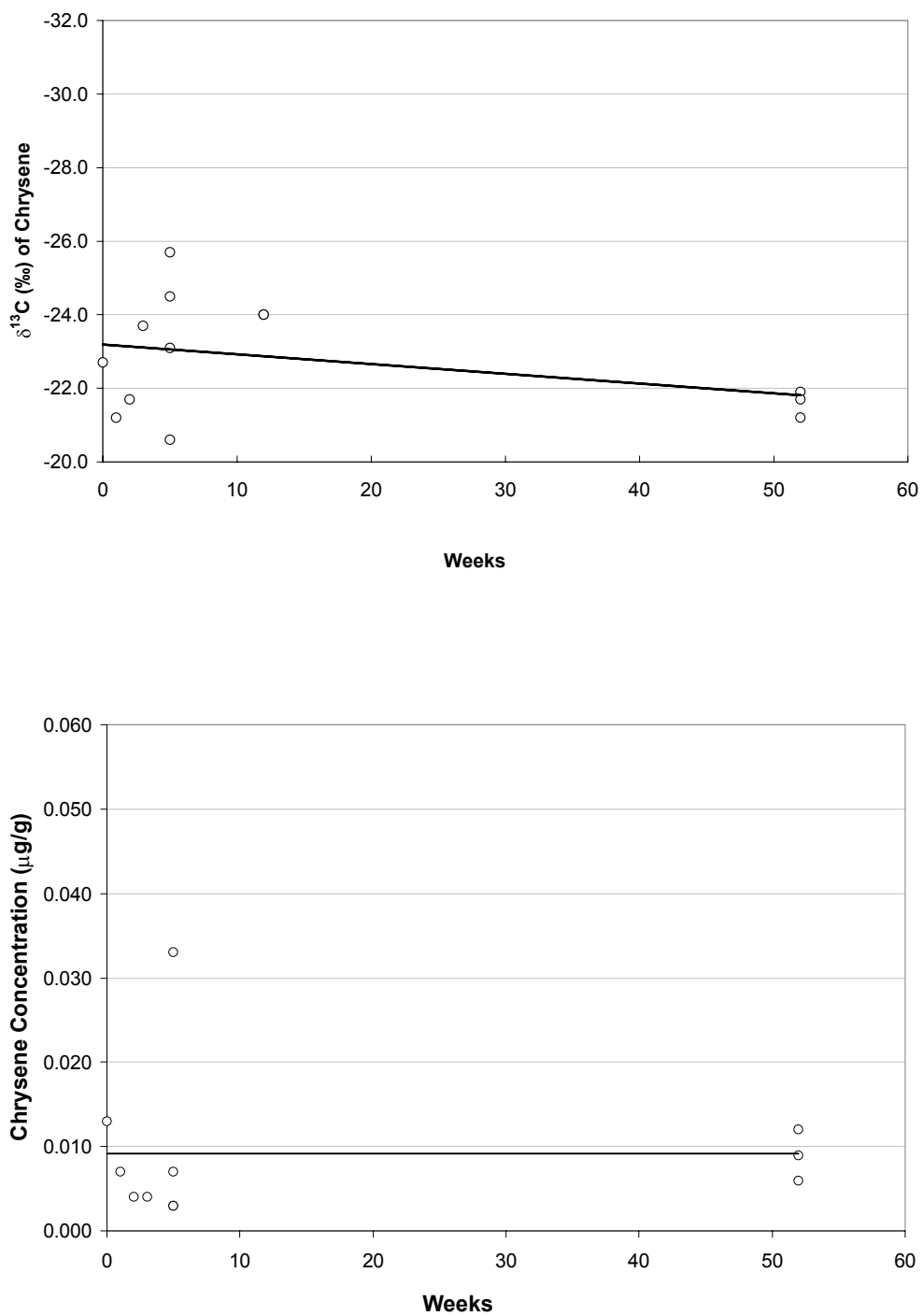


Figure 3d. Linear regressions of the $\delta^{13}\text{C}$ values and concentrations of chrysene from the diesel fuel microbial degradation experiment.

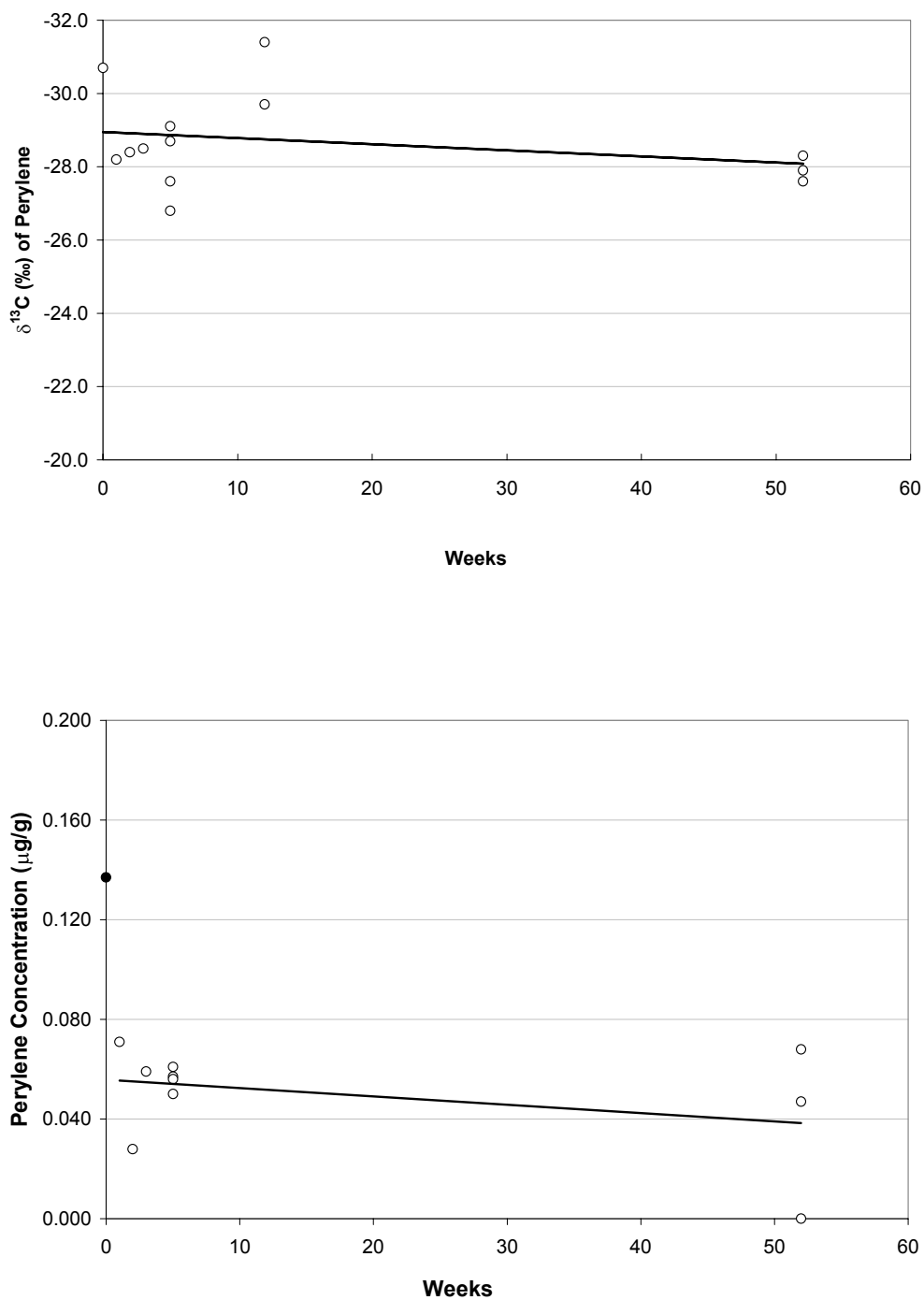


Figure 3e. Linear regressions of the $\delta^{13}\text{C}$ values and concentrations of perylene from the diesel fuel microbial degradation experiment. Solid circles are initial concentrations omitted from the regression (see text).

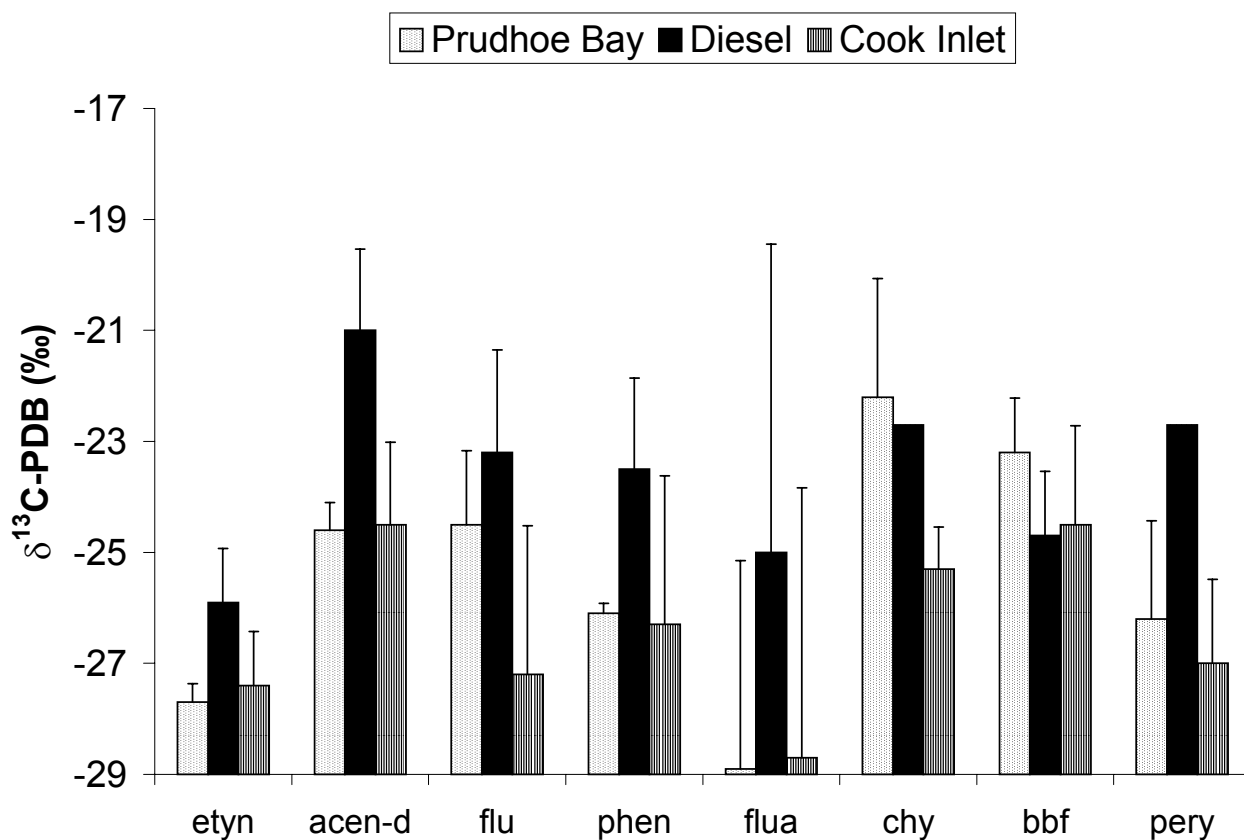


Figure 4. $\delta^{13}\text{C}$ of PAH in crude oil and diesel fuel samples. Error bars represent one standard deviation of the data. etyn = 1-ethylnaphthalene, acen-d = acenaphthene-d10, flu = fluorene, phen = phenanthrene, flua = fluoranthene, chy = chrysene, bbf= benzo [b] fluoranthene, and pery = perylene. Etyn, aden-d, and bbf are internal standards added before solvent extraction.

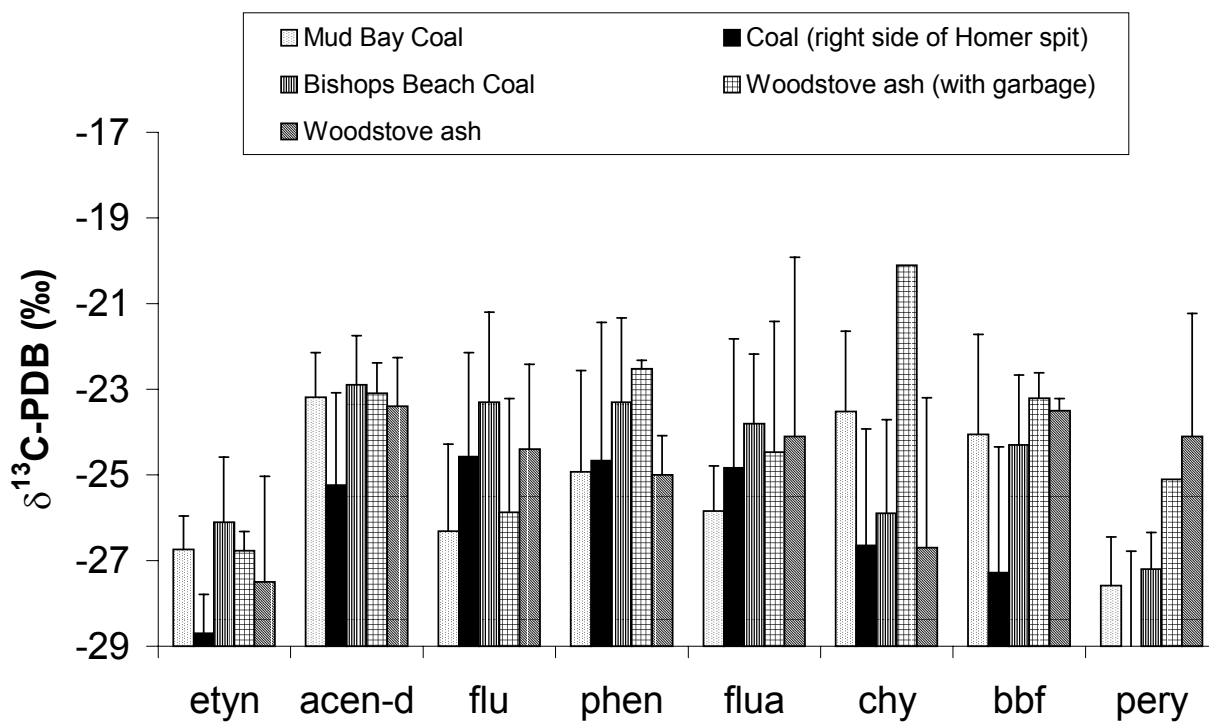


Figure 5. $\delta^{13}\text{C}$ of PAH in coal and ash. Error bars represent one standard deviation of the data, or $\frac{1}{2}$ of the difference between two replicates for the ash samples. etyn = 1-ethylnaphthalene, acen-d = acenaphthene-d10, flu = fluorene, phen = phenanthrene, flua = fluoranthene, chy = chrysene, bbf = benzo [b] fluoranthene, and pery = perylene. Etyn, acen-d, and bbf are internal standards added before solvent extraction.

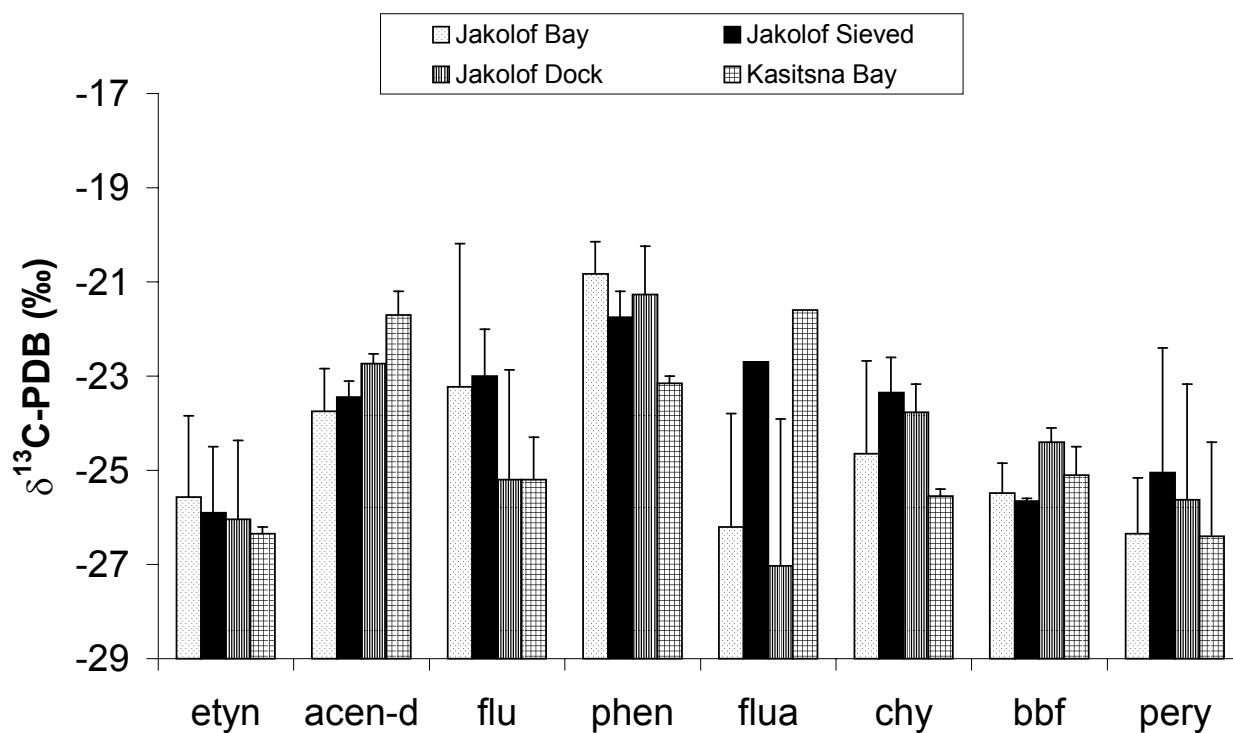


Figure 6. $\delta^{13}\text{C}$ of PAH in sediments. Error bars represent one standard deviation of the data, or $\frac{1}{2}$ of the difference between two replicates for the Kasitsna Bay and sieved Jakolof Bay sediments. etyn = 1-ethylnaphthalene, acen-d = acenaphthene-d10, flu = fluorene, phen = phenanthrene, flua = fluoranthene, chy = chrysene, bbf = benzo [b] fluoranthene, and pery = perylene. Etn, aden-d, and bbf are internal standards added before solvent extraction.

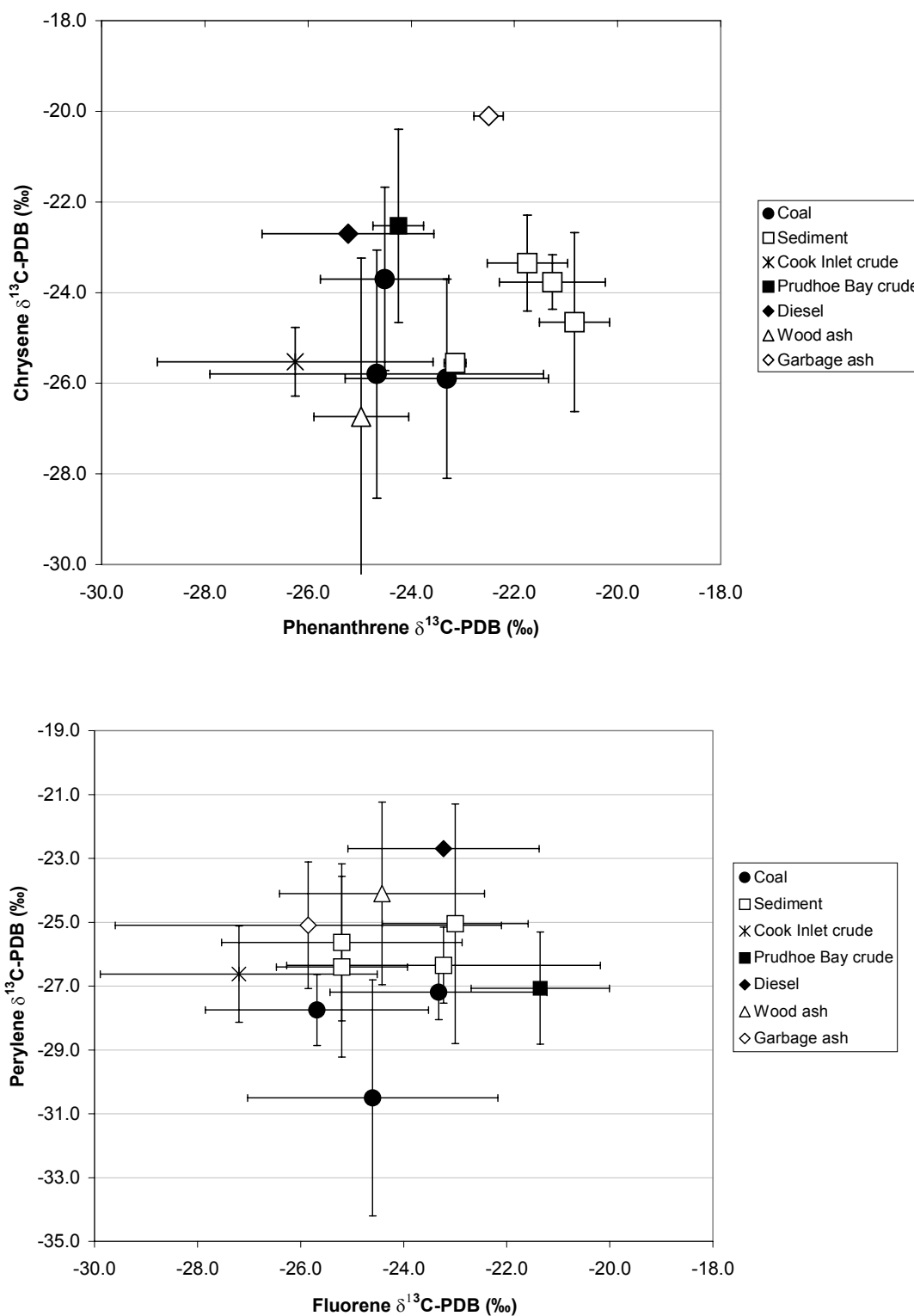


Figure 7. $\delta^{13}\text{C}$ of PAH extracted from sediment samples compared with $\delta^{13}\text{C}$ of PAH from coal, crude oil, diesel fuel, and ash. Error bars represent 1 standard deviation of data from replicate analyses.