INVESTIGATION OF THE DEPURATION OF PETROLEUM HYDROCARBONS BY SAND CRABS: AN EVALUATION OF SAND CRABS AS A MONITORING TOOL FOR PETROLEUM HYDROCARBON CONTAMINATION ON SANDY BEACHES

Final Report

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Introduction

Evaluating the exposure to petroleum hydrocarbons and the tissue burdens detected in field samples of animals, requires an understanding of the uptake and release dynamics and the time frame of metabolic processes. This is particularly important for interpreting exposures to PAHs (polycyclic aromatic hydrocarbons), a ubiquitous, water soluble, potentially toxic and highly lipophilic group of contaminants that are widespread in the marine environment (Farrington et al. 1983) and can be a prevalent fraction of the petroleum hydrocarbons in the water following an oil spill.

Uptake of PAHs by gill bearing animals can occur through passive equilibration of a hydrocarbon between the surrounding water and fluids in the animal's tissues and by hydrophobic partitioning between the water and the animal's lipids (Lauren and Rice 1985). The latter can result in high cellular accumulations of hydrocarbons that can exceed equilibrium (Lauren and Rice 1985). Uptake of PAHs can be enhanced by the ventilation of the gills in marine animals such as fish and invertebrates.

Marine animals exposed to PAHs may passively or actively release accumulated hydrocarbons back to the environment as ambient levels of PAH decline (e.g Page et al 2005). Depuration of hydrocarbons can occur via various tissues (gill, digestive tract, endocrine glands). It can occur through a relatively simple gradient-dependent diffusion or may involve active metabolism of PAHs by specialized enzymes.

Differences in the abilities of various marine taxa to metabolize contaminants, such as PAHs, affect the use and interpretation of tissue burden data as a biological indicator of exposure (Watson et al 2004). For example, while marine mussels (*Mytilus* sp) have limited ability to metabolize and biotransform PAHs, thus accumulating them in their tissues, other taxa such as fish and crustaceans may biotransform these compounds to active metabolites fairly rapidly, many of which are toxic, mutagenic or carcinogenic.

An average of 75% of the world's open coast shorelines are sandy beaches (Bascom 1980). For example, in southern California, 74%, 93%, and 66% of the coasts of Santa Barbara, Ventura, Los Angeles Counties, respectively, are sandy beaches (Smith et al. 1976; Dugan et al. 1998). This high prevalence means that sandy beaches are likely to receive the majority of contamination from a spill or other impact associated with coastal and offshore oil and gas activities. This prediction has been confirmed repeatedly in recent years with significant spills of petroleum affecting sandy beaches at Guadalupe, Avila, Santa Ynez River, San Antonio Creek, and Huntington Beach and most recently the 2007 Cosco Busan oil spill that oiled sandy beaches inside and outside of San Francisco Bay.

The sand crab, *Emerita analoga,* occurs intertidally on almost every type and length of ocean beach and is often the dominant macrofaunal species (Dugan et al 2000, 2003). This species has been shown to exhibit sensitive and plastic life history responses to environmental variation and anthropogenic impacts (Fusaro 1978, Siegel and Wenner 1984, Wenner et. al. 1985, 1993, Dugan 1990, Dugan et. al. 1991, 1994, 1996).

Sand crabs filter large volumes of water from the active swash zone of sandy beaches and appear to be able to biologically concentrate lipophilic chemicals, such as PAHs, from water. Sand crabs likely absorb dissolved hydrocarbons fairly rapidly across their gills but may also ingest hydrocarbons while suspension feeding with their plumose 2nd antennae. Sand crabs may passively or actively release accumulated hydrocarbons back to the sea when ambient levels of PAH decline. Thus the concentration of PAH in sand crabs may reflect not only the current level but also recent levels of hydrocarbons.

To date, the sand crab has been used in a few studies to measure pollutants in areas where mussels are not found. Populations of E. *analoga* have been employed as bioindicators (Siegel and Wenner 1984, Wenner 1988) and are bioaccumulators of metals and hydrocarbons (Burnett, 1971, Rossi et. al. 1978, Wenner 1988, Dugan et. al. unpublished, 2004). High concentrations of petroleum hydrocarbons have been reported in E. *analoga* from selected southern California beaches (Rossi et al. 1978, Dugan et al. unpublished data, 2004, DFG unpublished data, Entrix 1996) and recent studies have indicated that sand crabs can accumulate significant concentrations of total hydrocarbons and PAHs in their tissues and eggs on beaches in central and southern California (Dugan et al. 2004). Toxicity of petroleum to sand crabs has been demonstrated indicating a similar response to that of mysids (Barron et al 1999 ab).

Objectives

The primary objective of our study was to determine the ability of sand crabs to depurate petroleum hydrocarbons and to estimate the relative half life (T½) of PAHs in sand crabs. This information is needed to enhance the understanding and interpretation of tissue burdens of petroleum hydrocarbons in this widespread intertidal species.

This project also continued collaborative research that has been underway for the last several years by DFG, the Marine Science Institute at the University of California, Santa Barbara (MSI), and the Central Coast Regional Water Quality Control Board.

Methods and Sampling Design

Field collections

Forty replicates of adult female sand crabs (each replicate consisted of 5-10 adult sand crabs, n = 311) were collected using clean techniques (Dugan et al 2004) at Sands Beach, located directly adjacent to and west of Coal Oil Point in Santa Barbara County (Figure 1, 2), a beach known to be contaminated with petroleum hydrocarbons from natural seepage on September 28, 2005. The majority of crabs were larger individuals (18-25 mm in carapace length, CL) and approximately one third of the individuals were smaller female crabs (10-15 mm CL) due to the scattered distribution and low density of larger crabs in the intertidal and swash zone. This sample of crabs took ~16 person-hours to collect and was transported to the marine laboratory for processing.

To serve as a control for the study, fifteen replicates (n = 144) of adult female sand crabs were collected using clean techniques from an uncontaminated beach, Surf Beach, located north of Point Conception on Vandenberg Air Force Base in Santa Barbara County (Figure 1), on September 29, 2005. These crabs were uniformly large individuals (21-30 mm CL) as is typical of beaches north of Point Conception and were easily collected from dense aggregations of animals located in the swash zone (Figure 2). This sample of crabs took ~3 person-hours to collect and was transported to the marine laboratory for processing.

Three replicates each of sand and water and duplicates and one triplicate were collected from the contaminated (Sands Beach) and the clean beach (Surf Beach) on October 24, 2005. These were shipped to the DFG Water Pollution Control Laboratory (WPCL) in Rancho Cordova for analysis on the 25th of October 2005.

Figure 1. Locations of contaminated (Sands Beach) and uncontaminated control (Surf Beach) beaches in Santa Barbara County where samples of sand crabs, *Emerita analoga*, were collected for tissue analysis and the laboratory experiment.


Figure 2. Field collections of sand crabs for experimental laboratory culture at the contaminated beach (Sands-left) and the control beach (Surf- right).

Laboratory setup and experimental maintenance

The majority of the sand crabs transported to the marine laboratory at UC Santa Barbara were sorted and then maintained in flow-through seawater aquaria with clean sand and running seawater for six months. The laboratory set up for the experiment consisted of six 10 gallon glass aquaria with flow-through filtered seawater supplied through detergent-cleaned tygon tubing and pvc seawater spray bars (Figure 3ab). Filtered seawater ran through the system for at least 2 days before any crabs were introduced to the aquaria. Approximately 3 inches of cleaned # 20 sandblasting sand was placed in the bottom of each aquaria and then was flushed with seawater for at least 24 hours before introducing the live sand crabs. The laboratory lights were placed on a 12 hour on/off cycle and an air stone connected to an aquarium-grade air pump by tygon tubing was provided in each aquarium.

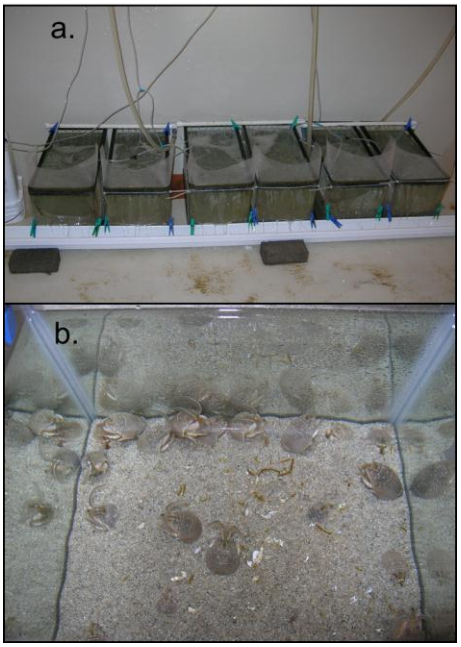

Figure 3a. Laboratory culture aquaria and apparatus used in the experiment.
Figure 3b. Sand crabs feeding on brine shrimp nauplii with plumose 2nd antennae in laboratory culture.

Replicates of crabs from Sands Beach were divided by size and into four groups and distributed into glass aquaria. Maximum densities of large crabs were 48 individuals, and of small crabs were 100 individuals for this site. All small crabs were placed in one aquarium. The large crabs from Sands Beach were equally distributed among 4 glass aquaria initially then condensed equally to three aquaria on day 11, October 10, 2005. The crabs from the control beach were initially placed in a single aquarium immediately following collection with a maximum density of 96 individuals. These crabs were split into two aquaria on Day 10, October 10, 2005.

All dead crabs and molts were removed several times per week and the sand in each aquarium was gently stirred by hand to oxygenate the deeper sand layers twice a week. Molted exuvia and dead crabs were counted and the carapace length of dead crabs was measured. All dead crabs and exuvia were discarded. Latex gloves were worn during any contact with the crabs, water or aquaria.

Brine shrimp cysts were prepared and hatched in filtered seawater in cleaned glass containers under lights. The resulting freshly hatched nauplii were used to feed crabs in each aquarium 3 times each week. During feeding, the lab lights and the flow-through seawater was turned off for 1 hour to allow the crabs to feed on the brine shrimp nauplii. This culture and feeding program was continued for the duration of the experiment.

Tissue samples
Three time zero (T-0) replicates of sand crabs from the contaminated and the control beaches were collected, placed in cleaned foil envelopes and frozen for future analysis of petroleum levels on September 29th (Sands) and September 30th (Surf). Sand crabs in culture in laboratory aquaria were sampled periodically as detailed in our work plan (see Table 1). Equal numbers of crabs from each aquarium were used to make up the contents of each replicate to avoid the introduction of any aquaria-specific effects to our results.

Table 1. Sampling design for sand crab tissues.

Sample	Day	n	Date	Site	Sample Composition
T-0	0	3	Sept. 29, 2005	Contaminated	4-5 large 2-3 small crabs
T-0	0	3	Sept. 30, 2005	Control	4-5 large crabs
T-1	2	5	Oct. 1, 2005	Contaminated	4 large, 2 small crabs
T-2	4	5	Oct. 3, 2005	Contaminated	4 large, 2 small crabs
T-3	8	5	Oct. 7, 2005	Contaminated	4 large, 2 small crabs
T-4	16	5	Oct. 15, 2005	Contaminated	3 large, 2 small crabs
T-5	22	5	Oct. 21, 2005	Contaminated	3 large, 2 small crabs
T-6	44	5	Nov. 12, 2005	Contaminated	3 large, 2 small crabs
T-7	90	5	Dec. 28, 2005	Contaminated	3 large, 2 small crabs
T-7	89	5	Dec. 28, 2005	Control	4 large crabs
T-8	176	5	Mar. 24, 2006	Contaminated	3 large, 2 small crabs

Inclement weather, very high swell, and erosive beach conditions during weeks 12-16 prevented the planned additional field collections of crabs from the contaminated beach (Sands). The samples of crabs from Sands collected from the experimental aquaria on day 176 completed the time course for the laboratory experiment. Five replicates of sand crabs were sampled on day 90 from the control (Surf Beach) culture for tissue analysis.

Laboratory Analyses
Frozen crab samples were kept at UC Santa Barbara until the completion of the experiment then all samples were shipped to the WPCL for analysis of polycyclic aromatic hydrocarbons (PAHs) and biomarkers.

Water, sand and crabs were analyzed at the WPCL. The samples were analyzed by PAHs and biomarkers. Sand crabs were washed with deionized water and homogenized using a Brinkman B-400 homogenizer prior to analysis. Water samples were extracted using liquid-liquid extraction. Homogenized crab tissue and sand were extracted using a pressurized fluid extraction (Dionex ASE-200) and extracts were cleaned up using gel permeation chromatography and silica gel alumina column chromatography. Sample extracts were analyzed using gas chromatography mass spectrometry (Agilent 6890/5973) using selected ion monitoring (SIM) for quantitation of PAHs and GC-MS full scan mode to facilitate petroleum fingerprinting. Sand crabs were also analyzed for moisture and lipid content.

PAH Analysis Quality Control: Tissue homogenates were extracted in sets of 18. A method blank, matrix spike and matrix spike duplicate (MS/MSD), standard reference material (SRM) and sample duplicate were extracted with each set. GC-MS instrument calibration was verified every 10 hours. All QC results met State Water Resources Control Board Surface Water Ambient Monitoring Program (SWAMP) quality assurance acceptance criteria.

Statistical analyses
Temporal changes in tissue burdens of petroleum hydrocarbons were examined using results of PAH analyses. These were compared among sampling times and replicates from the contaminated and control beaches using ANOVA and regression analyses. Concentrations of PAHs in sand crabs were statistically compared among samples of

sand crabs from the contaminated site at T-0 and T-8 (final) using repeated measures ANOVA and from the control site with a Student's T-Test.

Results

Survival and molting in laboratory culture

Mortality during the laboratory culture period was low for the crabs from the contaminated beach (Sands) with a total of 26 deaths in 6 months (Figure 4). Mortality was relatively higher in the crabs from the control beach (Surf) with 52 deaths total in 6 months. Early mortality of laboratory-held crabs appeared to be primarily related to injuries sustained during collection. The reason for the higher mortality in the control crabs was not clear but may be related to the greater age of these crabs as indicated by their larger size. The mean size (carapace length) of dead crabs did not vary with week in laboratory (Figure 5).

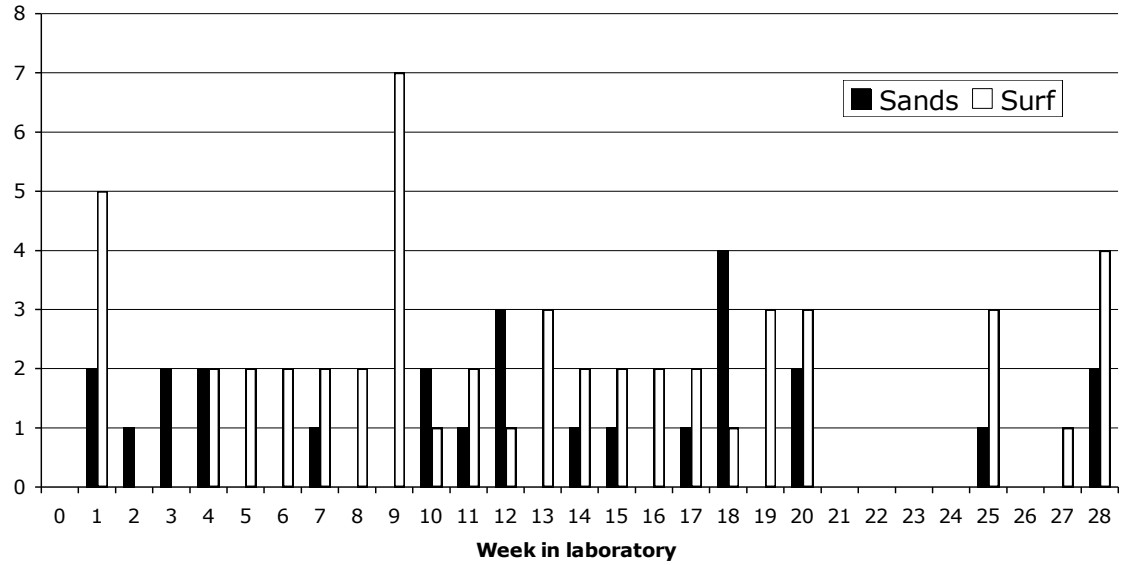

Figure 4. Mortality of sand crabs, *Emerita analoga*, in laboratory culture over time.

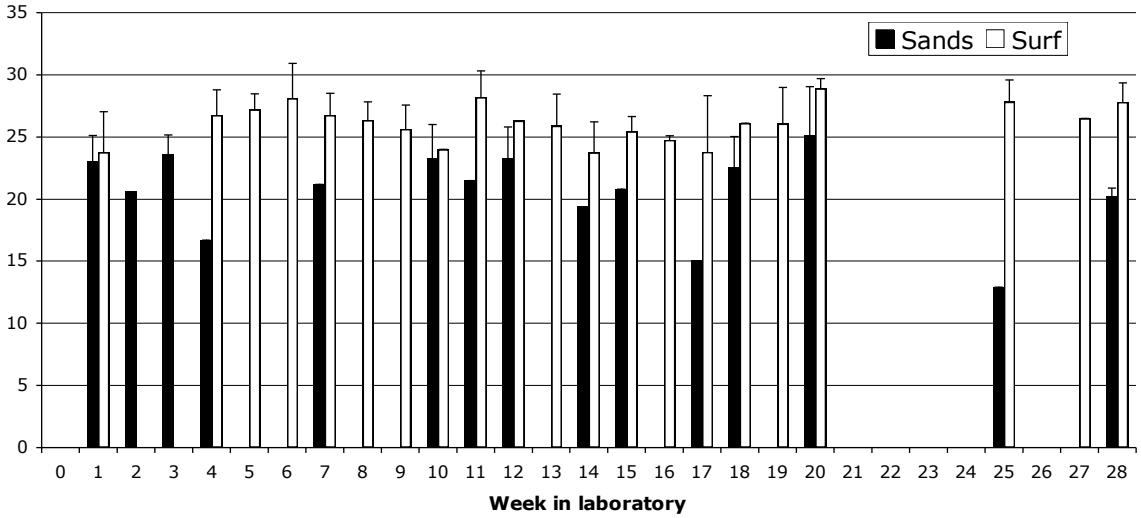

Figure 5. Mean size (carapace length + 1 std. dev) of crabs that died in laboratory culture over time.

Molting occurred primarily in the first 2 months of the experiment for crabs from both the contaminated and the control beaches (Figure 6). The molting peak for the crabs from the contaminated beach occurred earlier (2nd week) in the laboratory culture while the molting peak for the crabs for the control beach was later (4th week). This result is likely related to the smaller size of the animals from the contaminated beach or site-specific food-induced molting synchrony. Molting was greatly reduced in both groups of crabs after the 7th week. This was likely related to the effect of cooler winter seawater temperatures on the molting cycle but may also be associated with the length of time in laboratory culture. The size of molting crabs did not vary over time (Figure 7).

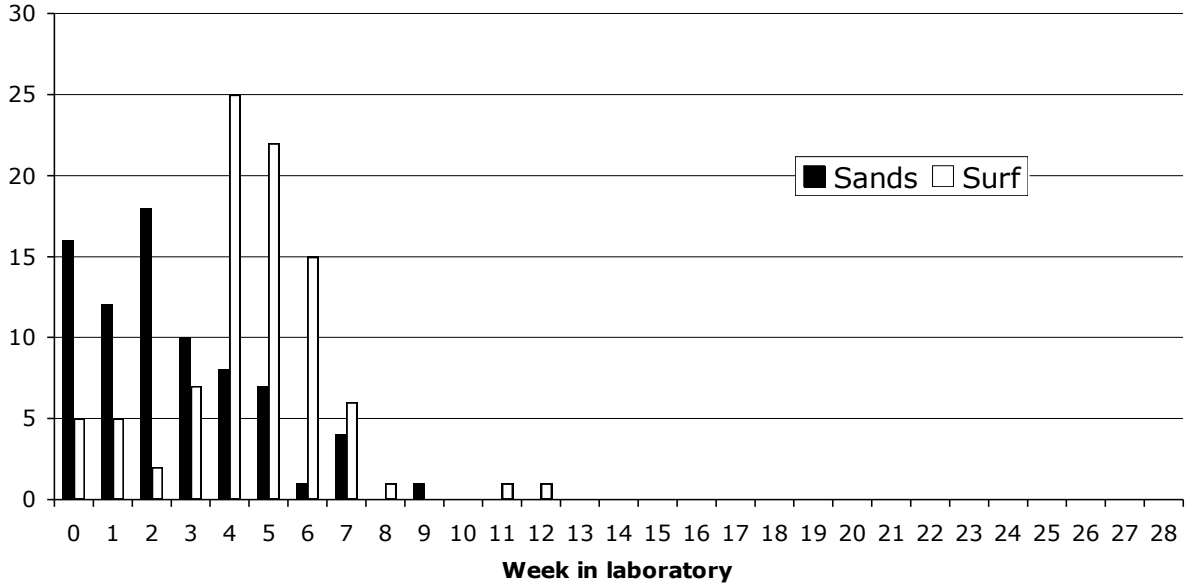

Figure 6. Number of molts for sand crabs, *Emerita analoga,* in laboratory culture over time.

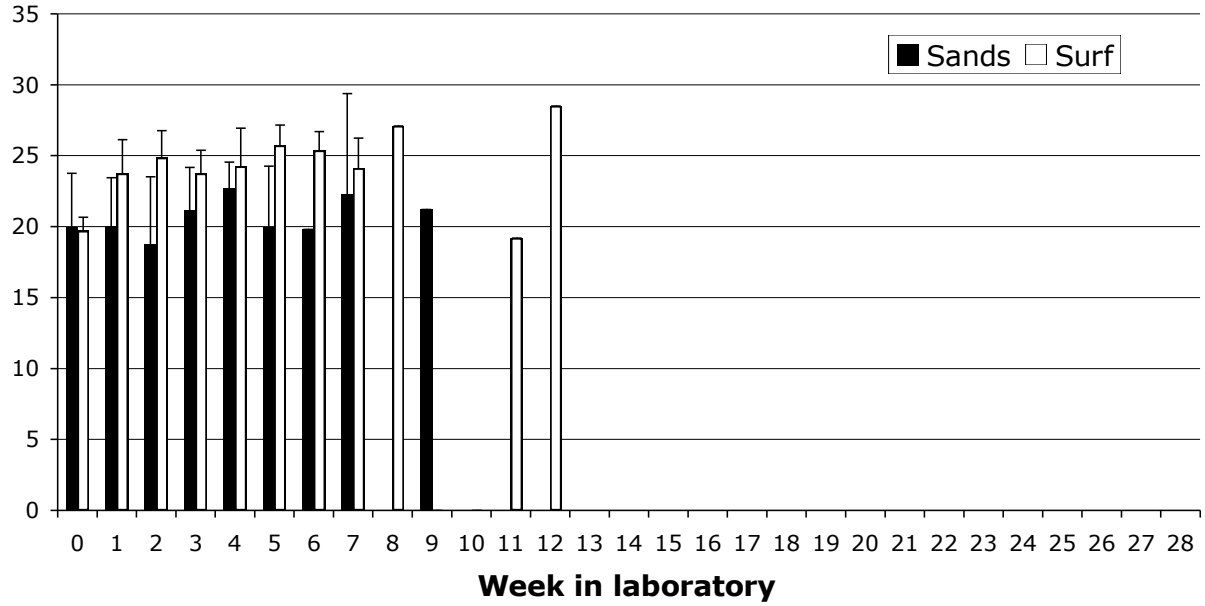

Figure 7. Mean size (carapace length +1 std dev) of molting crabs over time.

PAH analyses

At T-0 the average total PAH (TPAH) concentrations were greater for sand, water and sand crab tissues at the contaminated beach (Sands) than at the control beach (Surf) (Table 2). The average TPAH concentration of swash zone seawater was more than 7 times greater at the contaminated beach than at the control beach. For intertidal sand, the average TPAH concentration at the contaminated beach was more than twice that at the control beach. For sand crabs, the average TPAH concentration was more than 4 times greater at the contaminated beach than at the control beach.

Table 2. Average TPAH Concentration in water, sand and sand crab tissues at T-0 at the contaminated and control beaches.

Site	Contaminated (Sands)	Control (Surf)
Water ppb (ug/L)	0.097	0.013
Sand ppb dry wt (ng/g)	4.37	2.14
Sand crabs ppb dry wt	154	34

Depuration of PAHs in Laboratory Culture

The mean PAH concentrations in sand crab tissues from the contaminated beach (Sands) dropped by more than an order of magnitude by day 16 (T-4) in laboratory culture. The mean PAH concentration of 154 ppb at T-0 had declined to 4 ppb at T-4 (Figure 8). This decline was statistically significant (Repeated Measures ANOVA, $F= 8.474$, $df=8,16$, $p<0.001$). The observed change in tissue concentrations was not linear over time with a 78% decline in mean concentration of PAHs evident by Day 4 (T-2) in the laboratory. Log-transformed data on PAH concentrations illustrate this pattern of decline most clearly (Figure 9).

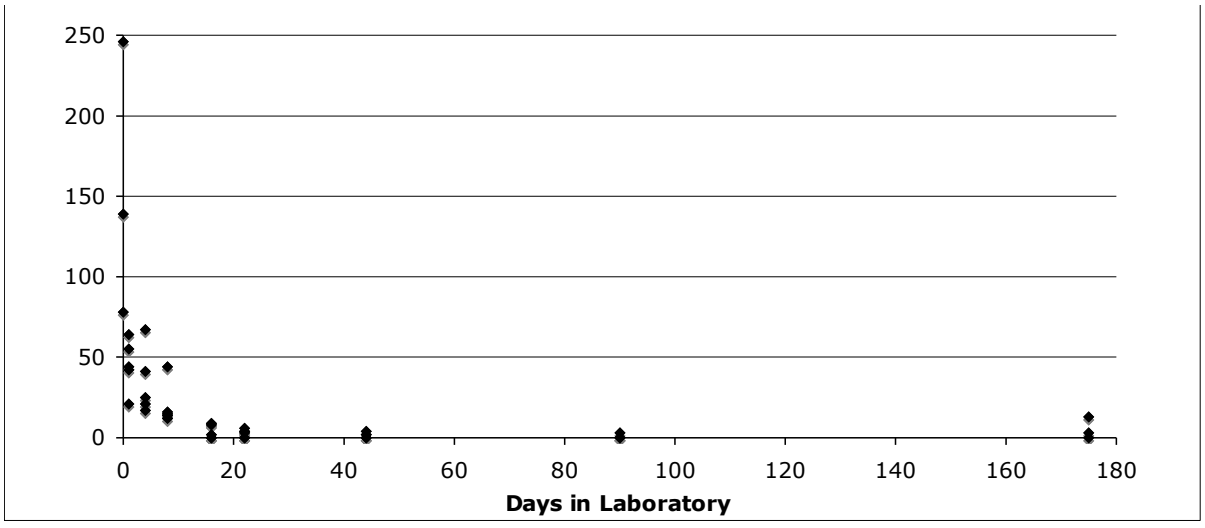

Figure 8. PAH concentrations in tissues of sand crabs from the contaminated beach (Sands) at T-0 and over time (up to 176 days) in clean laboratory culture. (Note: all high outlier values from later samples were removed).

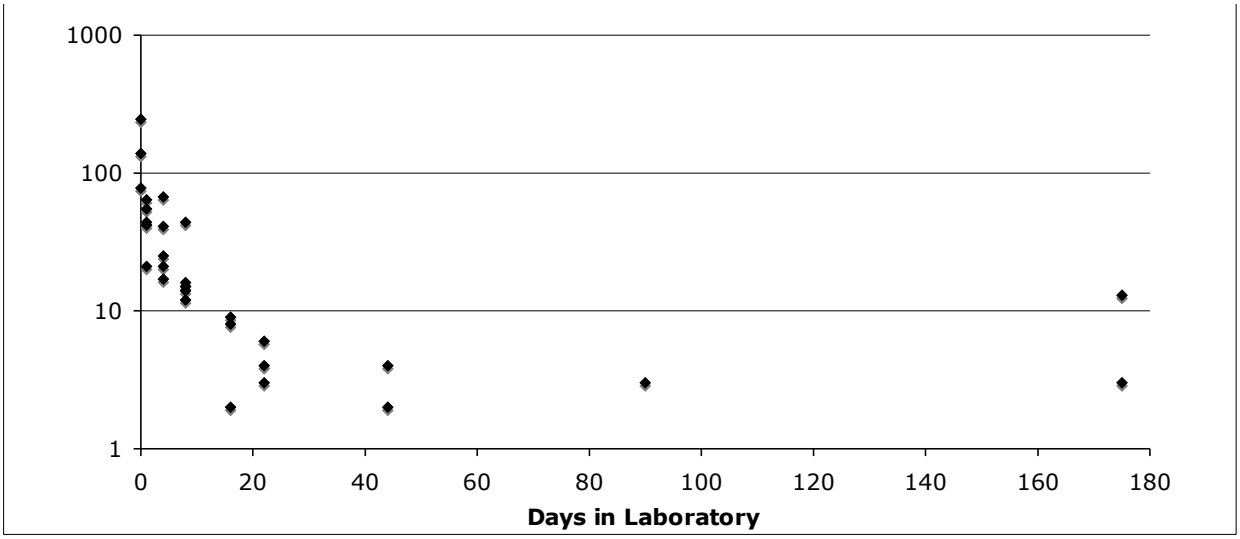

Figure 9. Log-transformed PAH concentrations in tissues of sand crabs from the contaminated beach (Sands) at T-0 and over time (up to 176 days) in clean laboratory culture. (Note: all high outlier values from late samples were removed)

The T ½ of PAH in sand crab tissues estimated by our results in the laboratory was quite short, less than 4 days (Figure 10). This result suggests that PAHs do not accumulate in this intertidal decapod crab.

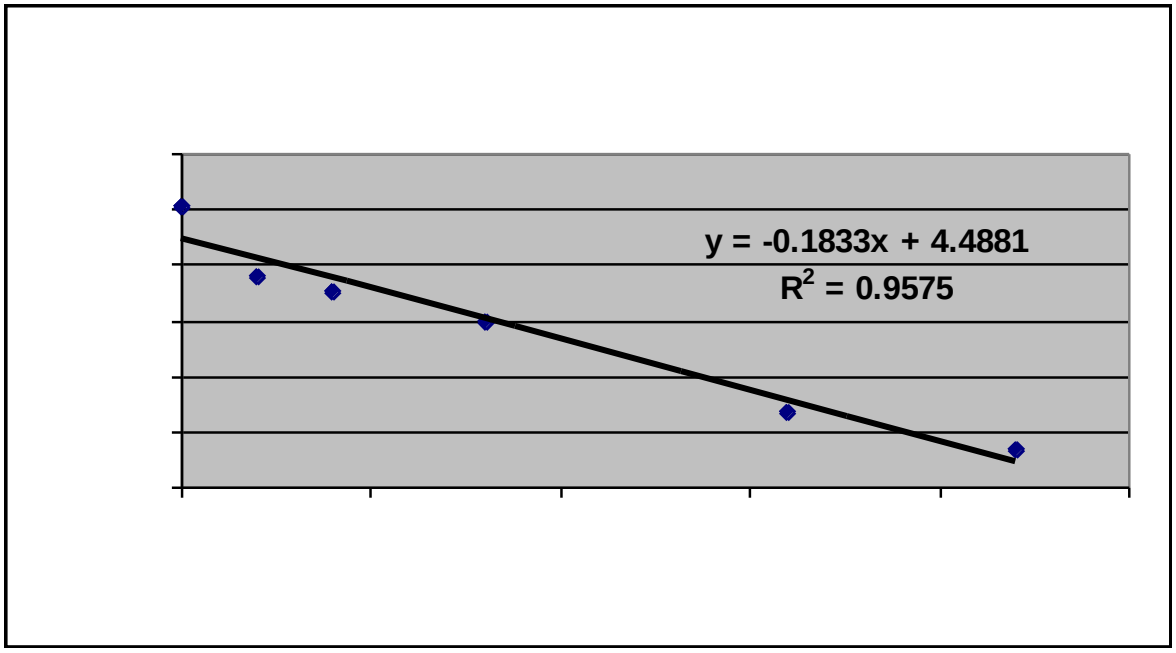

Figure 10. Estimate of the $T_{1/2}$ for PAHs in tissues of sand crabs in laboratory conditions. $T_{1/2}$ = ln(2)/kd = 0.693/0.1833 = 3.78 days, kd = slope = -0.1833

The mean PAH concentrations in sand crab tissues from the control beach (Surf) also dropped to very low levels by day 89 in laboratory culture. The mean PAH concentration of 33.54 ppb at T-0 had declined to 3.75 ppb after 89 days (Figure 11). This difference was also significant ($t = 3.935$, $df = 7$, $p=0.003$). However, it is not possible to determine if the observed change in tissue concentrations was linear over time due to our sampling design in which samples were analyzed from only T-0 and T-7 (final).

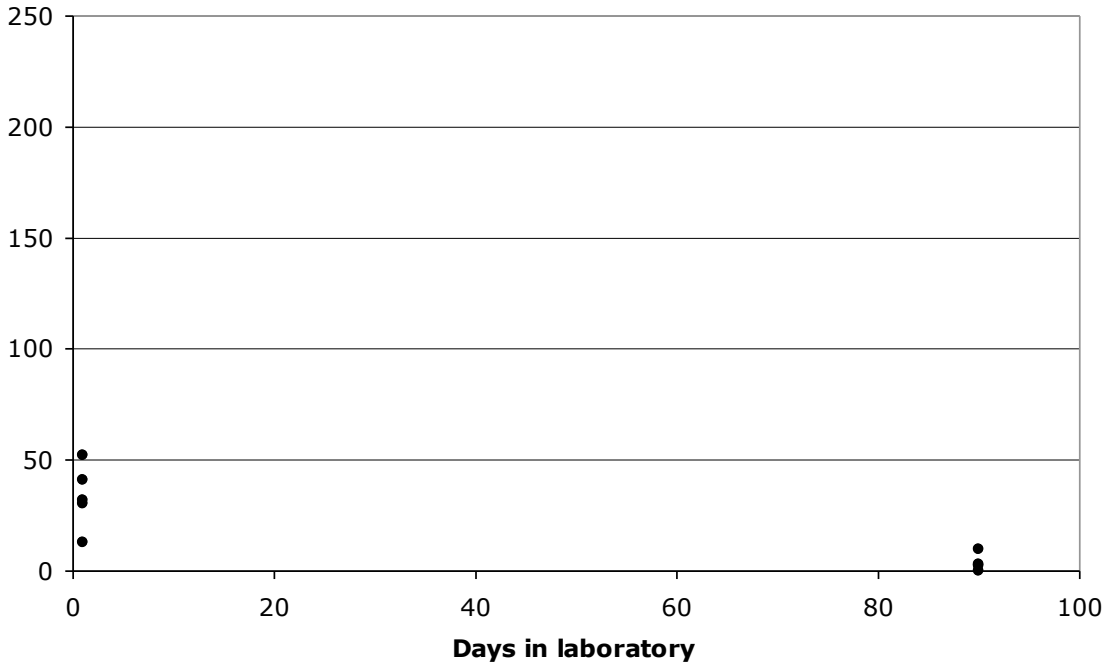

Figure 11. PAH concentrations in tissues of sand crabs from the control beach (Surf) at T-0 and after 89 days (T-7)in clean laboratory culture. (Note: one high outlier value from the 89 day samples were removed)

PAH Profiles and Outlier Values

Late in the course of the experiment ($\geq$44 Days) several unexplained high values for TPAH were found in four of the composited replicate samples from the laboratory cultures of sand crabs. These samples contained a different combination of PAH analytes (Figure 12) than had been found in the T-0 samples suggesting the crabs or samples had been inadvertently exposed to some alternate and unexplained source of contamination prior to analyses. These values were removed prior to statistical analyses. The PAH profiles of these outlier samples exhibited similar patterns (Figure 8) which probably represented creosote, tar or heavily weathered crude oil from the same source. Of interest, the substituted naphthalenes were present in the sample sets from T-0 to T-6, even though they are light end compounds, while the “heavier” substituted phenanthrene/anthracenes were not present after T-3.

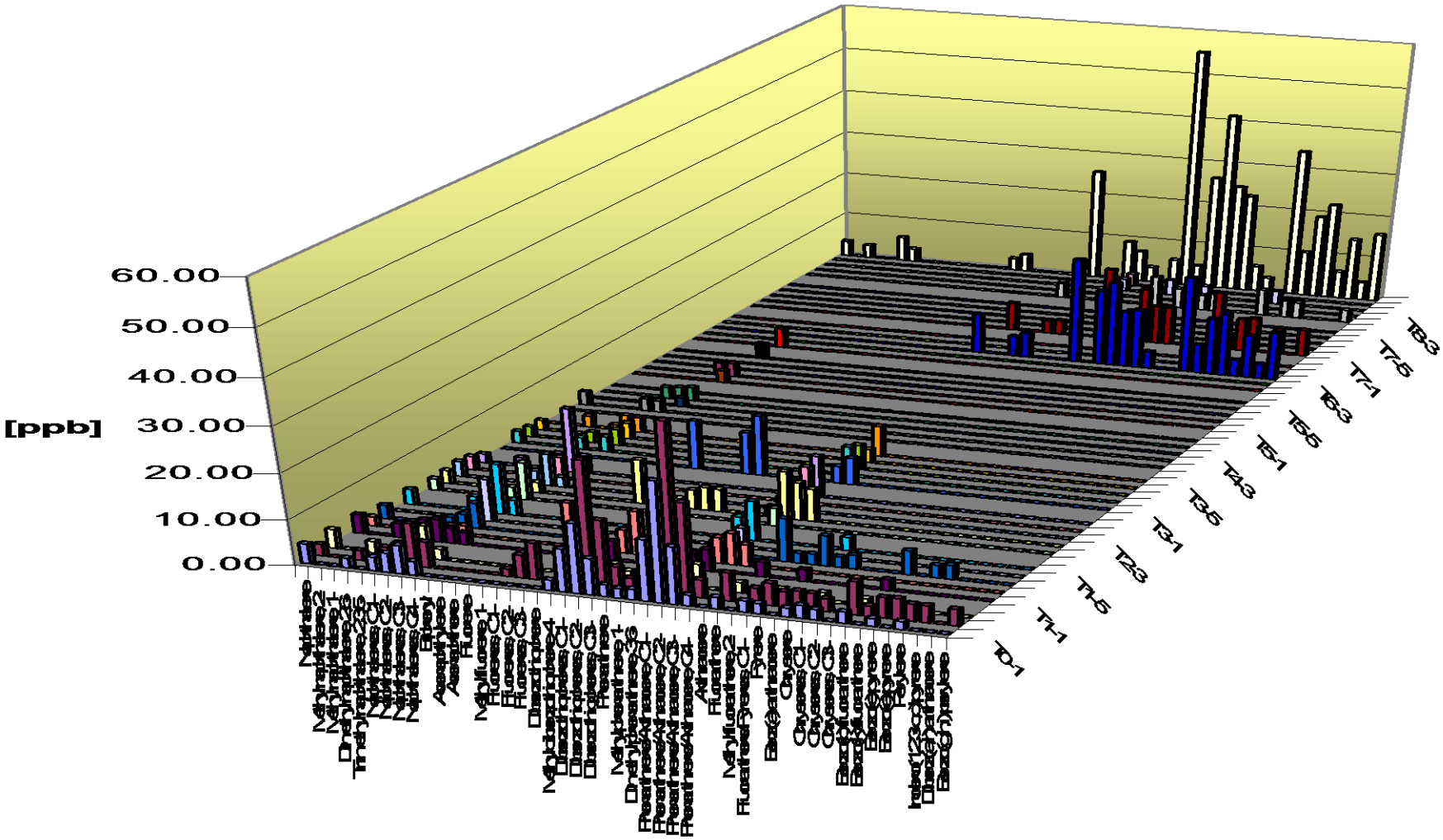

Figure 12. PAH profiles for sand crab tissues from the contaminated beach during the course of the experiment T-0 to T-8. Outlier values of PAHs were detected in samples T6-3, T7-1, T8-1 and T8-5.

Discussion

The short $T_{1/2}$ for *Emerita analoga* in laboratory conditions observed in this study suggests that the detection of high concentrations of PAHs in sand crab tissues collected from sandy beaches, such as has been found in earlier studies (Dugan et al 2005, Entrix 1996), is likely to be associated with exposure to either an existing chronic source or a recent release of petroleum hydrocarbons rather than the result of a long period of bio-accumulation or integration of multiple pulses of contaminants. In combination with the high mobility of sand crabs in the intertidal zone of sandy beaches (Jaramillo et al 2000), the results of our study could also help explain the high variance observed in concentrations of PAHs in the tissues of sand crab in composited samples collected a few meters from one another on a beach observed in an earlier study on this species (see Dugan et al 2005). It also helps to explain 1) the lack of differences observed in PAH tissue burdens of sand crabs of different ages (overwintered adult and newly settled megalopa) and 2) the marked seasonal variation in PAH tissue burdens at contaminated sites, such as Santa Maria River/Guadalupe Dunes and Avila Beach, in that earlier study (Dugan et al 2005).

Our results indicating a rapid loss and short T1/2 (<4 days) for PAH from the tissues of *Emerita analoga* are in general agreement with findings for other marine decapods, including blue crabs, shore crabs, majid crabs, California and Maine lobsters (Lee et al. 1976, Lauren and Rice 1985, Watson et al. 2004). To our knowledge, the present study is the first to provide information on PAH loss in an anomuran decapod, previous studies have been conducted only on brachyuran decapods and astacidean and panulirid lobsters.

Depuration of PAHs by crabs is a complex process that can involve a variety of tissues (e.g gills, antennal gland, hepatopancreas, digestive tract) and active metabolic processes as well as passive processes (e.g. Dam et al 2008, Rewitz et al. 2006) that may be influenced by nutritional and seasonal factors (e.g. Dissanayake et al 2008). Our results suggest that the analysis of biomarkers of xenobiotic metabolism, such as cytochrome P450, may be useful to pursue in this species. Understanding whether PAHs are actively metabolized by sand crabs or more simply diffused across a concentration gradient in an area with high surface area, such as the gills, will require further study as will the determination of the organs and tissues involved in active metabolism of PAHs by sand crabs. However, our study demonstrates that such laboratory studies are feasible for *Emerita analoga* and could be conducted on a much shorter time frame (hours rather than days) than we employed here. Further, because of the potential for this species to be utilized as a biological indicator for the widespread intertidal habitat represented by sandy beaches, such studies may be warranted in the future.

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