DRAFT FINAL REPORT

MONITORING OF COASTAL CONTAMINANTS

USING SAND CRABS

Prepared for:

Central Coast Regional Water Quality Control Board

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Prepared by:

Jenifer E. Dugan
Marine Science Institute
University of California
Santa Barbara, CA

Gary Ichikawa
and
Mark Stephenson
Marine Pollution Studies Program
California Department of Fish and Game
Moss Landing Marine Laboratories
Moss Landing, CA

David B. Crane, James McCall and Kathleen Regalado
Fish and Wildlife Water Pollution Control Laboratory
California Department of Fish and Game
2005 Nimbus Road
Rancho Cordova, CA

INTRODUCTION

Naturally occurring organisms and entire communities have been used to monitor pollution and can serve as bioindicators. Such organisms and communities also have the potential to be used as indicators of ecosystem recovery from a spill or chronic contamination by pollutants. A range of bioindicator species or sentinel organisms for use in monitoring the level of pollutants present in coastal environments and assessing the recovery of damaged coastal ecosystems need to be established for the California coast. Mussel Watch programs (Farrington et al. 1983, Goldberg et al., 1978, Martin et al. 1988, NOAA 1988, Phillips 1980) have been successfully used to describe the spatial and temporal variability of pollutants in marine and estuarine waters in many regions of the world, including California. However, there are some important limitations to the use of mussels as bioindicators on the California coast, including habitat type and the limited availability of hard substrates in many areas.

Sandy beaches comprise approximately three-quarters of the world's shorelines (Bascom 1980). In southern California, 74%, 93% and 66% of the coasts of Santa Barbara, Ventura and 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 the sandy beaches at Guadalupe, Avila, Santa Ynez River and San Antonio Creek, and Huntington Beach. Beaches are also exposed to contamination from sewage and urban, agricultural and industrial runoff, and many beaches are associated with stream, slough and river mouths.

Exposed sandy beaches in California are inhabited by an abundant and diverse invertebrate macroinfaunal community (Straughan 1982, 1983; Dugan et al. 1995, Dugan et al. 2000a, 2003) which is an important food resource for vertebrate predators including shorebirds, seabirds, marine mammals and fishes. This list of predators includes federally listed species, such as the western snowy plover. In addition, sandy beaches of the region are nesting sites for two endangered bird species: western snowy plovers and least terns. Sandy beaches are also considered extremely valuable as recreational and scenic resources for humans, both residents and tourists (Leatherman 1997).

The use of Mussel Watch programs to monitor pollutants in exposed sandy beach environments is severely limited due to the lack of hard substrate suitable for mussels. A different approach is needed to monitor pollutants along much of the California coastline. The suspension feeding sand crab (*Emerita analoga*), a dominant member of the sandy beach invertebrate community, occurs intertidally on almost every type and length of exposed sandy beach in California. This species reaches abundances exceeding 100,000 individuals per running meter of shoreline and can be easily collected in large numbers over a range of tide conditions.

Populations of *E. analoga* have been used as bioindicators in a few studies (Siegel and Wenner 1984, Wenner 1988) and this species is known to bioaccumulate metals and hydrocarbons (e.g. Burnett 1971, Rossi et al. 1978, Wenner 1988, Dugan and Page unpublished). Tissue loadings of DDT in *E. analoga* were successfully used to describe the distribution of DDT associated with the Whites Point Outfall in the Southern California Bight (Burnett 1971). 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, 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. unpublished). This species has also 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. 1987, Dugan et al. 1991, 1994).

The goal of this study was to collect information that could be used to develop a monitoring program and provide baseline data for evaluating contaminants on sandy beaches using the common sand crab (*Emerita analoga*). This information is needed to assess the potential for this species to be incorporated into the Regional Water Quality Control Board's Central Coast Ambient Monitoring Program (CCAMP) as a way to monitor pollutants in exposed sandy beach environments.

STUDY OBJECTIVES

1) Develop a monitoring program and baseline information (sampling time of year, size class, biomarkers) for contaminants on sandy beaches using the common sand crab, *Emerita analoga*, that can be incorporated into the Regional Water Quality Control Board's Central Coast Ambient Monitoring Program (CCAMP).

2) Determine if there is ongoing petroleum contamination from the Guadalupe oil fields.

3) Determine if there are elevated levels of petroleum in the Guadalupe and Avila areas relative to areas to the north and south in Region 3.

4) Compare contamination levels of petroleum hydrocarbons in sand crabs collected in areas near oil sources, river sources, urban beaches and reference areas.

BIOLOGY OF THE STUDY ANIMAL

Distribution

The anomuran crabs of the super family Hippidae are important components of the macrofaunal communities of exposed tropical and temperate sandy beaches throughout the world (Efford, 1976; Trueman, 1970; Trueman and Ansell, 1969; Haley, 1982; Dugan et al., 1995, Dugan et al. 2000ab, 2003, Lastra et al. 2002). California beaches are inhabited by the suspension-feeding species, *Emerita analoga*, which occurs on exposed sandy beaches of all morphodynamic types on the eastern Pacific coasts of North and South America. The reported North American occurrence of this species spans over 20 degrees of latitude with records on beaches from Kodiak Island, Alaska (58^{o} N) to Magdalena Bay, Baja California (26^{o} N) and in the Gulf of California (Efford 1970, 1976). In South America, this species has been reported on sandy beaches from Salavary, Peru to False Bay, Argentina (Efford 1970, 1976) (note: the Argentina record is a single report from one location just east of Cape Horn). The North American range of *E. analoga* spans Point Conception, an important transition zone between coastal oceanographic regimes and the Oregonian and California marine faunal provinces (Seapy and Littler 1980). Studies to date have found very little genetic variability in populations of this species across its North American range and recent results suggest they may be essentially panmictic (Beckwitt 1985, Barber et al. unpublished,).

Habitat

Emerita analoga is a rapidly burrowing sediment generalist (sensu Alexander et al., 1993) with excellent orientation and swimming abilities that can successfully inhabit the full range of exposed sandy beaches from fully reflective to dissipative morphodynamic states (Dugan et al. 2000b, Jaramillo et al. 2000). This crab is an active tidal migrant and intertidal zonation patterns in this species vary across the tidal cycle, seasonally, and among beaches (Cubit 1969, Fusaro 1980, Jaramillo et al. 2000). Juveniles of *E. analoga* and other hippid crabs generally occupy a notably higher intertidal level than adult crabs (Fusaro 1980; Haley 1982), while adult crabs can occur as deep as the shallow subtidal zone during higher tides, periods of beach erosion, and on some beach types (Jaramillo et al. 2000). These highly mobile crabs generally aggregate in the active swash zone. The ability of *E. analoga* to burrow at similar speeds across a range of sediment grain sizes likely contributes to the success of this species even in the coarse sediments and harsher swash conditions typical of beaches with reflective characteristics (see Dugan and Hubbard, 1996; Dugan et al. 2000b). As such, *E. analoga* may also cope better with temporal changes in sediment grain size, such as those occurring on many intermediate type beaches (Dugan unpublished).

Food habits

Emerita analoga is a suspension feeder that uses the plumose second antennae to sieve particles from the swash water (Efford 1966). This species feeds primarily on phytoplankton which is reflected in carbon and nitrogen stable isotope values (Dugan unpublished). In addition, individual growth rates (molt increments), and life history characteristics of *E. analoga* are significantly correlated with food availability estimated from surf zone chlorophyll concentrations (Dugan 1990, Dugan et al 1994).

Life history

As in many anomuran crabs, *E. analoga* has an extended planktonic larval life of 3-4 months, going through at least 9 molts as a zoea before metamorphosing to a megalopa and settling in the intertidal zone of sandy beaches. The planktonic larvae are the primary dispersal stage of this species, as megalopa and crabs move relatively small distances within the intertidal zone. Recruitment of megalopa to the beach occurs in distinct pulses which maybe associated with shifts in nearshore oceanography. In California, recruitment can occur from February or March though October in many years. Recruitment of this species is most intense in the spring months and tapers through the summer. A smaller late summer/fall pulse of recruitment can occur in some years in California.

Following recruitment to the intertidal zone from the plankton, megalopa of *Emerita analoga* spend approximately one month in the intertidal zone before molting to juvenile crabs. Megalopa are distinguished from small juvenile crabs by their plumose pleopods and short eyestalks. Juvenile crabs grow rapidly in the spring and summer months and can mature in their first season. Crabs live a maximum of 2-3 years and overwintering mortality is very high in some populations. There is marked sexual dimorphism in size in this species (Efford 1967). Male crabs reach much smaller maximum sizes than female crabs and may have decreased survival over winter (Dugan 1990, Dugan et al. 1991, 1994)). This dimorphism is a product of differential growth rates in male and female crabs (Dugan 1990). Female crabs brood eggs/embryos and clutch size (fecundity) is a function of female size (Wenner et al. 1987, Dugan et al. 1991). Multiple clutches can be produced each season, and clutch size varies seasonally (Dugan 1990, Wenner et al. 1987, 1991). The length of the reproductive season varies geographically, ovigerous crabs are generally present from April to October in populations south of Point Conception, but may be present all year in populations living north of that point (Dugan 1990).

The life history of *Emerita analoga*, including size and age at maturity, growth rate, maximum male and female crab size, and survival, varies significantly across the geographic range in California and in South America (Osorio et al. 1967, Dugan 1990, Dugan et al 1991, 1994, Wenner et al. 1993) and on a local scale (Dugan and Hubbard 1996). This variation appears to be a function of variation in growth rates and age at maturity along with increased survival of larger individuals over winter. Molt increments and growth are greater in northern populations of this species. This is likely related to delayed maturity and higher food availability in colder waters.

Ecological role

Populations of *Emerita analoga* are major components of invertebrate macrofaunal communities of exposed sandy beaches in North and South America, often making up the majority of the total intertidal abundance and biomass (Jaramillo and McLachlan 1993, Dugan et al. 1995, 1996, 2000a, 2003). Densities of sand crabs can exceed 100,000 individuals m^{-1} on beaches in Region 3 and elsewhere (Dugan et al. 2003). This species is important as prey for vertebrate predators, such as shorebirds, seabirds, fishes, and marine mammals.

METHODS

Field methods

In cooperation with Vandenberg Air Force Base, Monterey Bay Marine Sanctuary, California State Parks, and the Nature Conservancy, scientists from the California Department of Fish and Game and Marine Science Institute at the University of California at Santa Barbara collected samples of the sand crab, *Emerita analoga* at 19 sandy beach sites in Region 3 (Santa Barbara to Santa Cruz County) (Table 1, Figure 1a-d). Sample areas were selected to include four general categories: reference areas, urban beaches, beaches adjacent to agriculture areas and river mouths, and beaches adjacent to oil sources. Samples of *E. analoga* were collected at a minimum of three beaches fitting each category type (Table 1). At potential point source areas, at least four beach locations were sampled in a spatial array (except for Port San Luis Harbor Beach and Sands Beach). Samples of crabs were collected along a spatial gradient away from

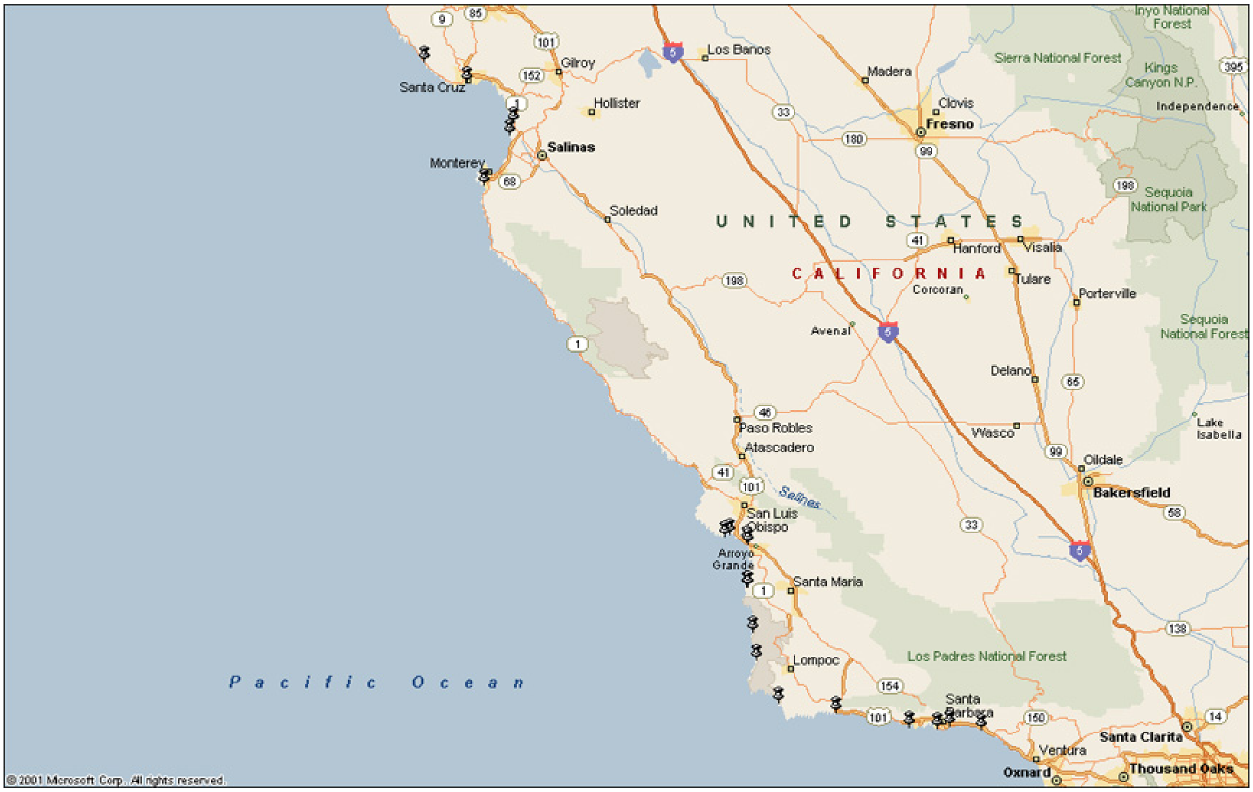

Figure 1a. Map of the study area showing locations of the 19 sampling sites for sand crabs.

Figure 1b. Locations of sampling sites for sand crabs in Monterey and Santa Cruz Counties.

Figure 1c. Locations of sampling sites for *E. analoga* in San Luis Obispo County

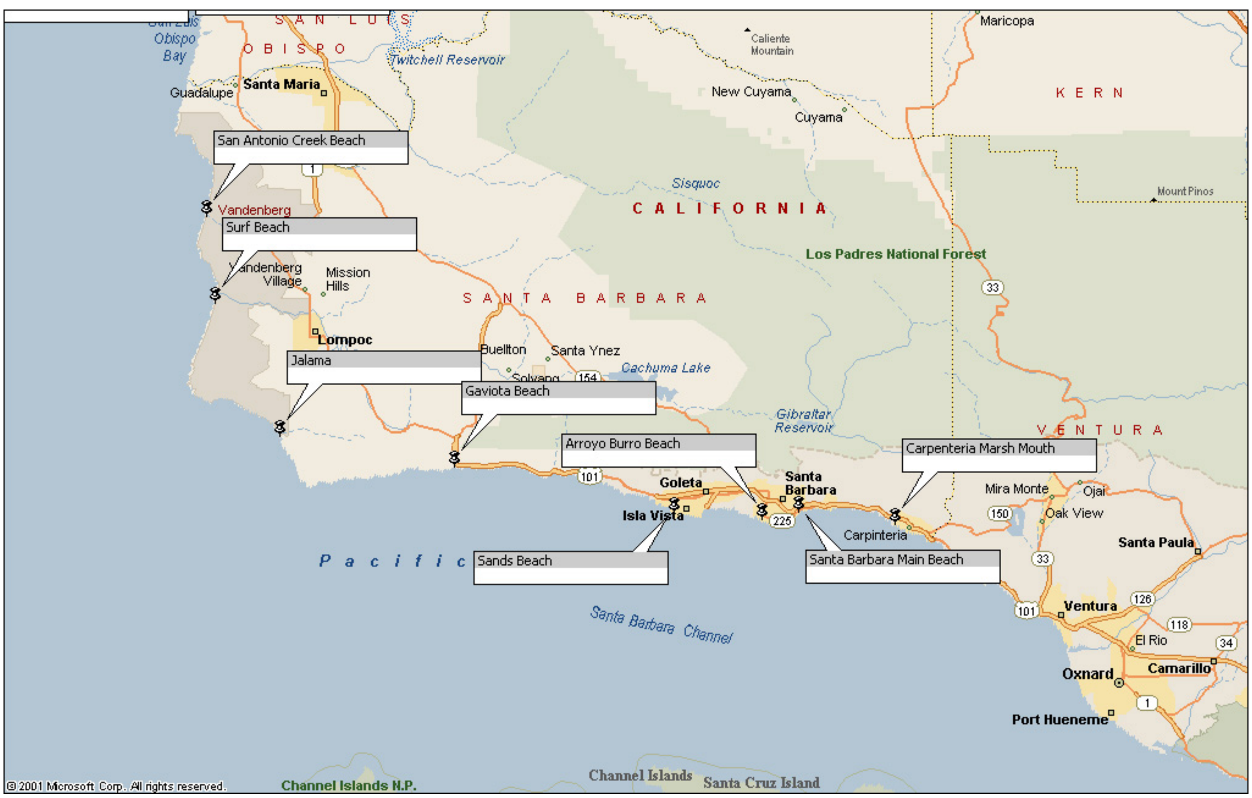

Figure 1d. Locations of sampling sites for sand crabs in Santa Barbara County.

a potential point sources at distances ranging from 10 - 900 meters. Where possible, samples were collected both north and south of a potential point source, such as a river mouth.

In general, two replicates were collected at each sample site. Individuals of *Emerita analoga* were collected using cleaned stainless steel shovels and gloved hands. Latitude and longitude were measured at each sampling location with a GPS. All crabs collected and determined to be of the appropriate size and/or age class were placed in methanol rinsed aluminum foil and then into pre-labeled ziplock^{TM} bags. Bags were placed in ice chests with dry ice and kept frozen until analysis. Both of the replicates collected were analyzed for polynuclear aromatic hydrocarbons (PAH). One of the replicates was additionally analyzed for pesticides and trace elements.

For the purpose of investigating spatial variation in contaminant burdens in *Emerita analoga*, the samples were collected at the majority of sites listed in Table 1 during the summer (July/August) of 2000. To investigate possible seasonal variability in uptake of contaminants by E. *analoga*, samples were collected during the spring (May 2000) summer (July/August 2000) and the winter (February 2001) at Avila Beach and Guadalupe Beach. To investigate contaminant levels present in different life stages of this species, megalopa of *E. analoga* were collected during the spring months (May) and compared to overwintered crabs.

Sample handling and analysis

Sample Handling

A chain-of-custody form was completed and accompanied every sample. The chain-of-custody record contained the following information, as appropriate: project name, sample identification # (unique for each sample), name and signature of field recorder, signatures of persons involved in the chain of possession, inclusive dates and times of possession and sample shipping date and mode. Each shipping container containing samples was accompanied by its own chain of custody record.

Chemical analysis

Each replicate was analyzed for polynuclear aromatic hydrocarbons (PAH). One of the replicates was additionally analyzed for pesticides and trace elements. Analytical methodologies conformed to the State Mussel Watch protocols and quality assurance requirements for PAHs, pesticides, and trace metals. Samples were prepared for pesticide, PCB and PAH analysis using pressurized fluid extraction (Dionex ASE 200) followed by gel permeation chromatography and column chromatography cleanup and fractionation. Sample extracts were analyzed for pesticides and PCBs using Agilent 6890 gas chromatographs each equipped with dual electron capture detectors and dual 60 meter DB5 and DB17 columns (J&W Scientific). Sample extracts were analyzed for PAHs using an Agilent 6890/5973 GC-MSD equipped with a 30 meter DB5MS column (J&W Scientific). In addition, the petroleum products were analyzed for a variety of biomarkers such as terpane, sterane and monoaromatic and triaromatic sterane families. The pesticide and PCB extraction and analysis followed Fish and Game SOP “SO_Tissue”. The PAH extraction followed Fish and Game SOP “PAH Extraction and Cleanup” and PAH quantification followed the Fish and Game protocol #FG-Polycyclic Aromatic Hydrocarbons.

Quality assurance
During the sampling, sample duplicates, trip blanks, and field equipment blanks were collected. Duplicate samples were collected using the same procedures as those used to collect primary samples. Duplicate samples were analyzed for PAH quantification as above. Two trip blanks consisting of a pre-cleaned aluminum foil accompanied each of the sample teams into the field. The two trip blanks were opened for five minutes at a randomly selected sampling site and evaluated for possible incorporation of airborne contaminants. Equipment blanks were prepared by pouring deionized laboratory reagent water over sampling shovels after decontamination following sample collections. The rinse water was collected and analyzed for PAHs with the rest of the samples as described above.

Data analysis
Sample results at sites associated with oil contamination, urban development, river mouth discharge and reference areas were compared. Samples of different age classes and seasons from Guadalupe and Avila were compared statistically using ANOVA. Regression analyses were used to investigate possible spatial trends in tissue burdens of contaminants.

Laboratory results in electronic format including minimum detection limits, probable quantification limits, and QA/QC results were produced for the samples analyzed. These results were delivered in an Excel data format provided by CCAMP to the RWQCB.

RESULTS

A total of 164 samples of sand crabs were collected at 19 sites characterized as having different land use or potential contamination sources as identified in Table 1. We collected sand crab samples at 3 urban beaches, 5 beaches adjacent to agricultural runoff, 7 beaches adjacent to potential oil sources including sites of chronic petroleum spills at Avila and Guadalupe, sites of a recent oil spill on Vandenberg Air Force Base, sites adjacent to areas of natural petroleum seeps, and 4 potential reference beaches (Table 1, Figure 1a-d)). Samples were arrayed spatially from 10 to 1000 meters distance along the shoreline from potential point sources of terrestrial runoff such as river, slough or creek mouths, or a nearby landmark such as a pier or jetty (Table 1). At Avila and Guadalupe Beaches, additional samples of sand crabs were collected in May 2000 and February 2001 for use in comparisons of lifestages and evaluation of seasonal variation.

Petroleum Hydrocarbons

Polycyclic aromatic hydrocarbons or PAHs are ubiquitous in the environment. These contaminants form when organic matter is exposed to high temperatures or burned. For example, a forest fire is a major source of PAHs. PAHs are present in crude oil and are also formed when gasoline and other petroleum products are combusted. PAHs include a great diversity of compounds and two major classes are recognized in contaminant studies. The pyrogenic PAHs are formed by the burning of organic matter and the combustion of both crude and refined petroleum products. These can reach the intertidal through direct wet or dry deposition from the atmosphere and via runoff from street and parking lot surfaces. The

Table 1. Locations, dates, numbers and types of samples of *Emerita analoga* collected for contaminant analyses. (o= areas of oil production, spills, and natural seeps, a= agricultural land use, u = urban land use, r = reference sites)

County/Beach Name	Lat. N Dec. Deg.	Long. W Dec. Deg	Sample date(s)	n	SamplesDistances (m) and landmark	Life Stage/ Reprod. state
Santa Cruz County						
Scott Creek Beach r	37.040	122.239	September 1, 2000	4	325S, 425S (river)	Adult
Santa Cruz Main Beach u	36.961	122.025	September 1, 2000	4	100S, 100N (pier)	Adult (small)
Monterey County						
Elkhorn Slough Mouth a	36.810	121.792	August 11, 2000	5	105S, 246N (jetty)	Adult
			October 12, 13, 2000	4	263S, 131N (jetty)	Adult
Salinas River Mouth a	36.754	121.806	August 19, 2000	9	0, 100S, 200S, 300S (Monterey Dunes Colony)	Adult
Carmel Beach r	36.554	121.940	September 15, 2000	4	0, 100S (river)	Adult
San Luis Obispo County						
Port San Luis Harbor o	35.171	120.849	August 8, 2000	4	332S, 10 N (pier)	Adult
Avila Beach o	35.178	120.735	May 25, 2000	9	0, 30E, 530E, 250W(pier)	Megalopa
			May 25, 2000	8	0, 30E, 530E, 250W (pier) Adult	
			August 9, 2000	9	25E,100E,10W,100W(pier)	Adult (small)
			February 16, 2001	8	25E, 100E, 10W,100W (pier)	Adult
Shell Beach r	35.148	120.656	August 9, 2000	1	300S (point)	Adult
Pismo Beach u	35.138	120.652	August 9, 2000	4	110S, 10 N (pier)	Adult
Guadalupe Beach o/a	34.972	120.627	May 25, 2000	8	20S, 500S, 134N, 634N (river)	Megalopa
			May 25, 2000	8	20S, 500S, 134N, 634N (river)	Adult
			August 7, 2000	12	150N,300N, 450N,750N, 900N (river)	Adult
			February 16, 2001	10	195S, 335S, 200N, 300N (river)	Adult
Santa Maria River Beach o/a	34.970	120.567	August 7, 2000	8	150S, 300S, 450S, 600S (river)	Adult
Santa Barbara County						
San Antonio Creek Beach o	34.795	120.625	August 12, 2000	7	150S, 300S, 150N, 300N (river)	Adult (ovig.)
Surf Beach o	34.690	120.611	August 11, 2000	9	250S, 150N, (river)	Adult (ovig.)
					0, 275S (Surf Station)	Adult (ovig.)
Jalama Beach r	34.512	120.507	August 10, 2000	4	150S, 150N (Trestle)	Adult (ovig.)
Gaviota State Beach r	34.471	120.236	August 10, 2000	4	20E, 150E (pier)	Adult (ovig.)
Sands Beach o	34.410	119.893	August 30, 2000	4	150E, 50W (slough mouth)	Adult (ovig.)
Arroyo Burro Beach o	34.402	119.747	August 29, 2000	7	150E, 300E, 150W, 320W (creek)	Adult (ovig.)
Santa Barbara Main Beachu	34.414	119.696	August 9, 2000	4	280E, 1000E (pier)	Adult (ovig.)
Carpinteria Marsh Mouth a	34.396	119.535	August 9, 2000	6	150E, 300E, 300W (mouth)	Adult (ovig.)
Totals 19 sites	2.6 degrees	2.7 degrees	14 dates	164 samples		2 age classes

pyrogenic PAHs have a wide range of characteristics, but generally are made up of 4- to 7-benzene rings and are higher in molecular weight. These heavier PAHs are less water soluble, less volatile, less readily metabolized and more persistent and they are dominated by parent species of PAHs. The petrogenic PAHs reach the intertidal via leaks and spills of crude and refined petroleum products. These PAHs generally have 2- to 3- ring structure and are lower in molecular weight. The petrogenic PAHs are more water soluble, more volatile, more readily metabolized and less persistent and are dominated by alkylated or substituted species. Ratios of parent to substituted PAHs can be used to help evaluate potential sources of petroleum contamination.

PAHs were detected in every sample of sand crabs at every beach we sampled (Figures 2, 3). Concentrations of total PAHs in sand crab tissues varied nearly 2 orders of magnitude among sand crab samples collected in August-September 2000, ranging from 20 to 2061 ng/g dry weight and 148 to 14419 ng/g ppb lipid weight. Concentrations of total substituted PAHs in sand crab tissues also varied over an order of magnitude among samples, ranging from 34 to 2141 ng/g dry weight and 254 to 14526 ug/g lipid weight (Figures 2,3).

The highest concentrations of petroleum hydrocarbons found in sand crab tissues occurred at beaches adjacent to oil sources including locations of chronic oil leaks and spills, and natural hydrocarbon seep areas in the study region (Figures 2, 3). The greatest concentrations of total PAHs in sand crabs were found in the vicinity of the Santa Maria River (Guadalupe and Santa Maria River) where values for individual samples collected in August ranged from 310 to 2117 ng/g dry weight and 2167 to 14419 ng/g lipid weight. Mean concentrations of total PAHs in samples from the Santa Maria River site located south of the river exceeded 940 ng/g dry weight and 6500 ng/g lipid weight. Mean concentrations of total PAHs exceeding 500 ng/g dry weight and 3400 ng/g lipid weight were also found in sand crab samples collected from beaches at Avila, Port San Luis, Shell Beach and Sands (Figures 2,3). The mean concentration of total PAHs in sand crab samples was 488 ng/g dry weight at Pismo Beach. Sand crabs from the beach at Arroyo Burro also had relatively high tissue concentrations of PAHs (297 ng/g dry weight) (Figures 2, 3). Both Sands and Arroyo Burro beaches are located near areas with natural petroleum seeps offshore. Freshly deposited tarballs and oil were observed on these beaches during sample collections in August 2000.

Low concentrations of PAHs were present in sand crab samples from beaches on Vandenberg Air Force Base that were affected by the 1997 Torch oil spill (San Antonio Creek, Surf) (Figures 2,3). It is expected that the overwintered adult sand crabs collected in summer of 2000 settled on those beaches in spring of 1999, long after the spill.

For some sites, the variance in PAH concentrations in sand crab tissues was high (coefficient of variation > 1) among samples collected within a few hundred meters (e.g. Salinas River, Pismo and Avila). This result could be associated with a variety of factors, including different exposure histories, variation in lipid content, molt stage, and reproductive state, mortality of highly contaminated crabs, and the metabolism of PAHs in crabs. It is not possible to resolve this issue with the present sampling design and the limited understanding of PAH metabolism in sand crabs. This is a high priority area for further study and is critical to interpretation of results on tissue concentrations and developing a monitoring program for this species.

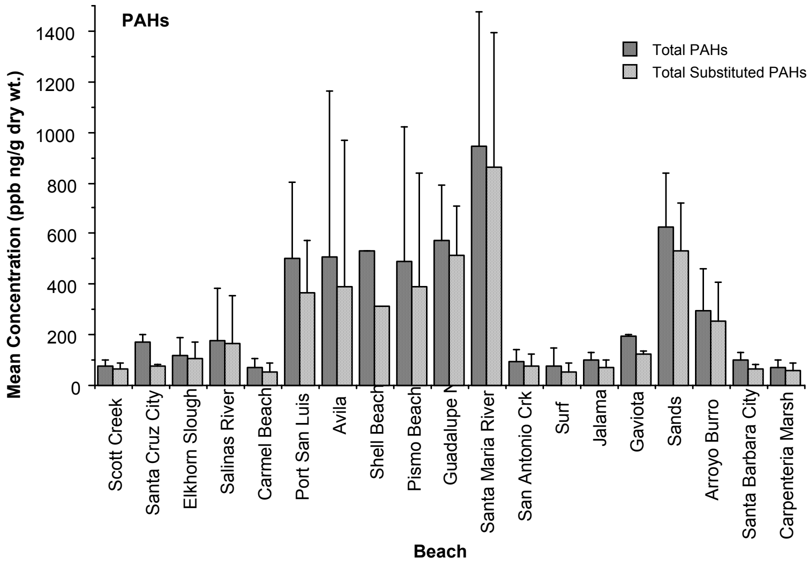

Figure 2. Mean dry wt. concentrations ng/g (+ 1 std. deviation) of total PAHs and total substituted PAHs in tissues of adult sand crabs collected at 19 beaches in August- September 2000.

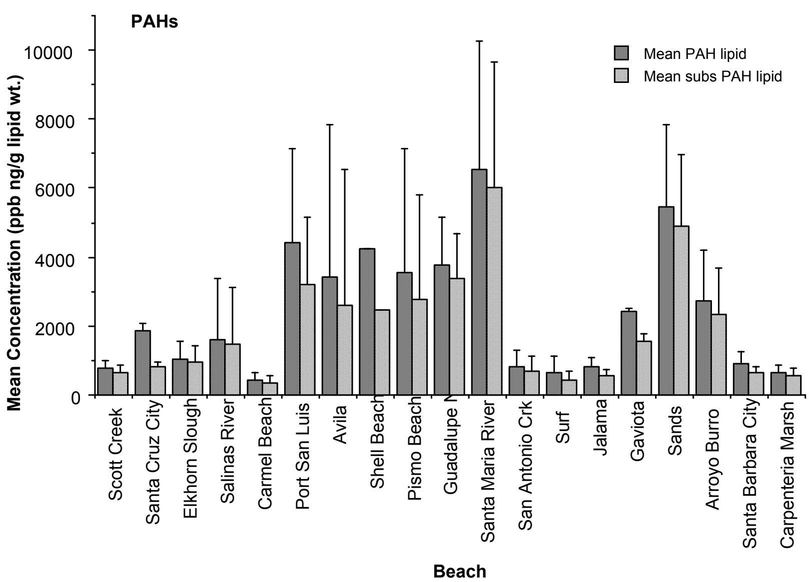

Figure 3. Mean lipid wt. concentrations ng/g (+ 1 std. deviation) of total PAHs and total substituted PAHs in tissues of sand crabs collected at 19 beaches in August- September 2000.

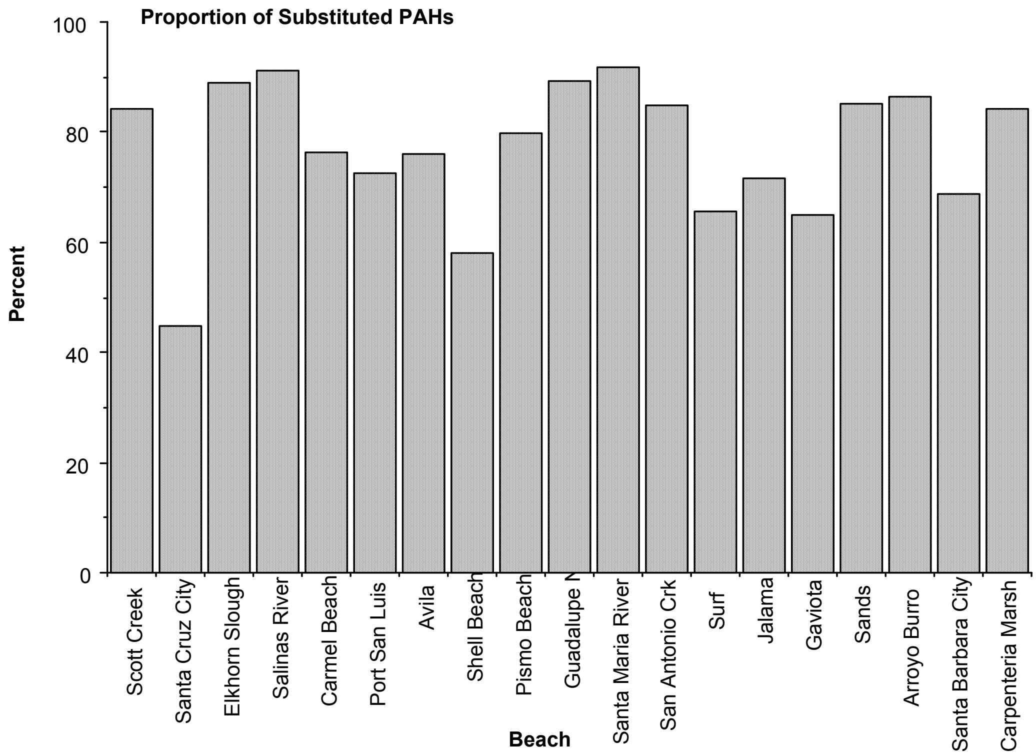

Figure 4. Proportion of substituted PAHs in tissues of adult sand crabs collected at 19 beaches in August-September 2000.

When the proportion of substituted PAHs to total PAHs was plotted (Figure 4), it was evident that the majority of PAHs (70% or more) found in sand crab tissues were substituted PAHs at most of the beaches we sampled. The only beach where substituted PAHs made up less than 50% of the PAHs was Santa Cruz City. Sand crabs in the single sample from Shell Beach also had a relatively low level (<60%) of substituted PAHs. The domination of substituted PAHs observed in sand crab tissues suggests that petrogenic sources such as crude and refined petroleum products (gas, oil or diesel), rather than pyrogenic sources (derived from combustion (smoke, soot, vehicle exhaust) are associated with the PAHs present in the crabs. The indication of petrogenic sources of PAHs suggests that oil leaks, spills and natural seepage are responsible for the high levels of PAHs observed in sand crabs at some sites and for the majority of the PAHs found in sand crabs at all sites.

The concentration of total PAHs did not vary significantly among age classes of sand crabs at Guadalupe (1 way ANOVA, $F= 1.342$, $p = 0.266$, $df = 7$) or at Avila (1 way ANOVA, $F= 0.070$, $p = 0.795$, $df = 7$) in May 2000 (Figure 5). This result suggests that crabs are exposed to and accumulate PAHs within a month of settling in the intertidal zone from the plankton and that sources of PAHs are present and available to intertidal crabs at both of these sites on a more chronic temporal scale than that of winter runoff events or river/stream discharge. This result also points to the need to better understand the metabolism of PAHs in sand crabs.

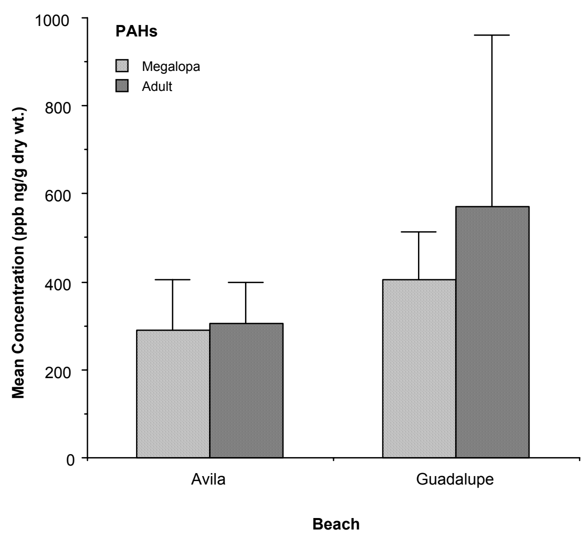

Figure 5. Mean concentrations of total PAHs in newly settled and adult sand crabs at Avila and Guadalupe in May 2000.

Using data from Guadalupe and the Santa Maria River mouth where the highest concentrations of PAHs were present in sand crab tissues, we examined the hypothesis that the concentration of total PAHs in sand crabs varied with distance from the river mouth, a potential source of oil contamination. Since concentrations of total PAHs did not vary with age, both age classes of crabs were included in the regression analyses at Guadalupe for May 2000. We found no relationship between the concentration of total or substituted PAHs in sand crab tissues and the distance from the river mouth for combined data from May and August 2000, and February 2001 (Figures 6,7). In addition, no significant relationships between total PAHs in sand crabs and distance from the river were present in the regressions of the individual data from May and August 2000 and February 2001. This result suggests that exposure of sand crabs to PAHs at Guadalupe does not vary with distance from the river, a potential "point" source (note: the river mouth location moves along the coastline). A combination of factors including nonpoint sources of petroleum, coastal processes, and the movement of sand crabs along the shoreline may contribute to a lack of spatial patterns in tissue loadings of PAHs. These results suggest that although exposure of sand crabs to PAHs was high in the vicinity of the Santa Maria River, the local source may be relatively widespread and is likely associated with the Guadalupe Oil Field.

At Avila, no spatial gradients in total or substituted PAHs were found (Figures 8,9). The high porosity of sandy beaches and coastal dunes means that a constant exchange of water (and potentially contaminants) can occur in the dynamic shallow aquifer present in the intertidal zone of the beach. Sand crabs typically inhabit the intertidal zone where this aquifer reaches the surface and drains across the beach face into the ocean making this a likely source of contaminants at Guadalupe and Avila and other beaches.

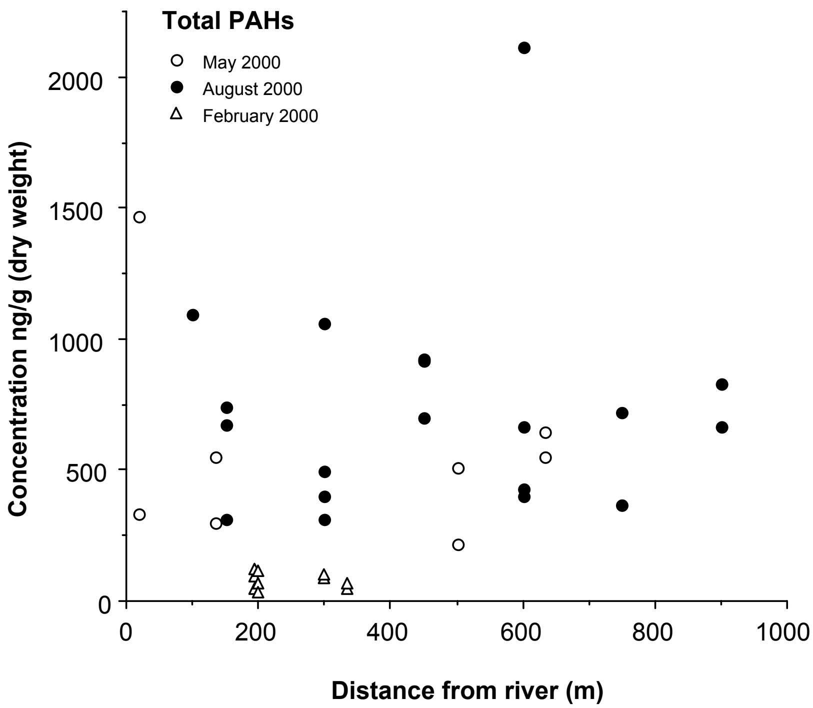

Figure 6. Concentrations of total PAHs in adult sand crabs as a function of distance from the Santa Maria River mouth for samples collected at Guadalupe/Santa Maria River sites on three dates.

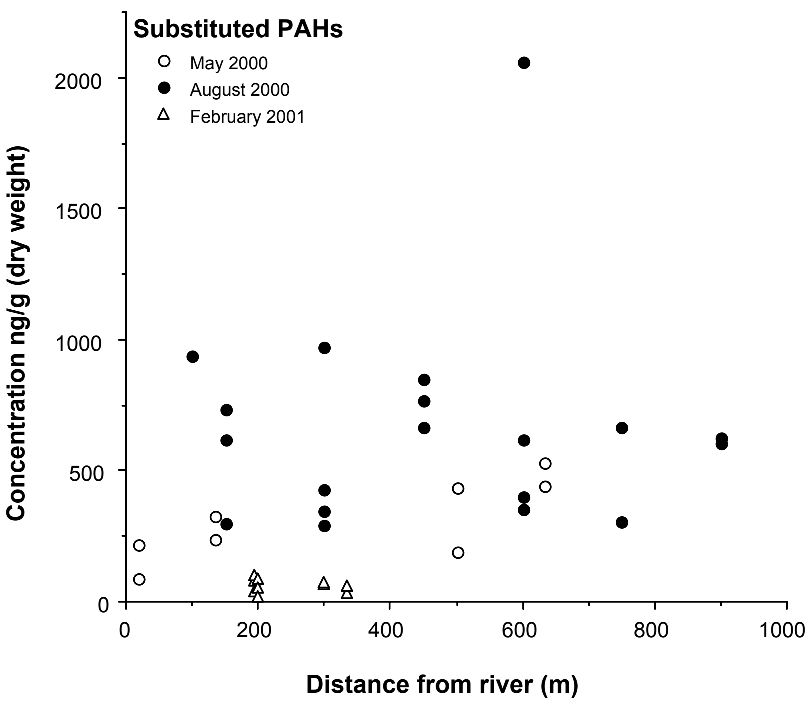

Figure 7. Concentrations of substituted PAHs in adult sand crabs as a function of distance from the Santa Maria River mouth for samples at Guadalupe/Santa Maria River mouth sites collected on three dates.

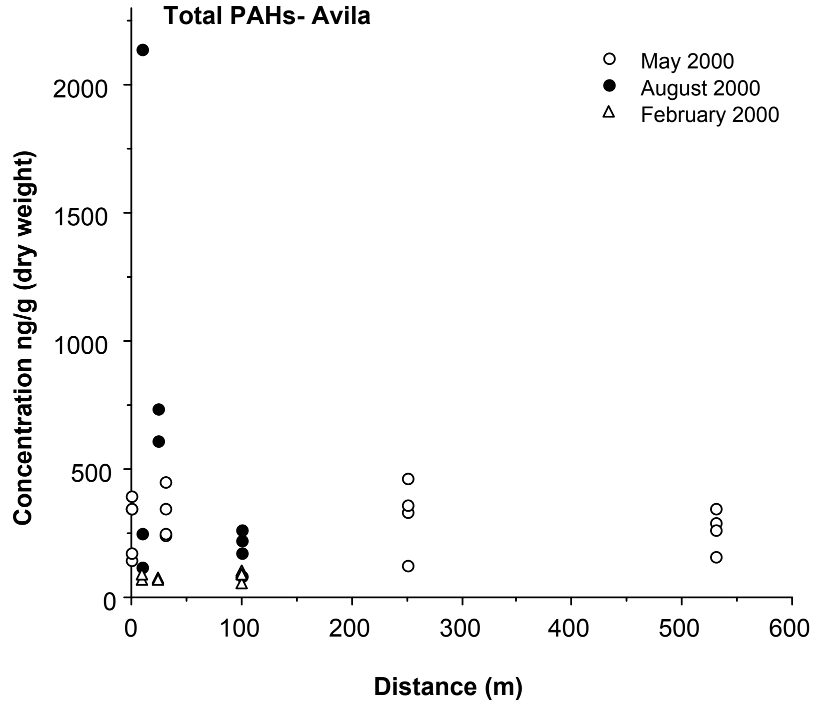

Figure 8. Concentrations of total PAHs in adult sand crabs as a function of distance from the Avila pier for samples collected on three dates.

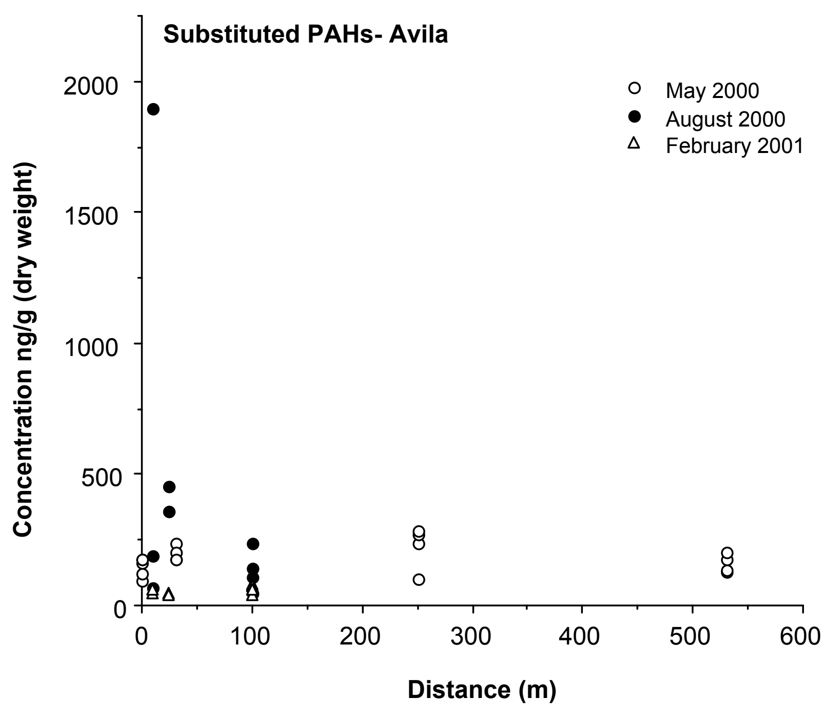

Figure 9. Concentrations of substituted PAHs in adult sand crabs as a function of distance from the Avila pier for samples collected on three dates.

Significant temporal variation in the concentrations of PAHs in sand crab tissues was evident in comparisons of samples from May and August 2000 and February 2001 at Avila and the Guadalupe/Santa Maria River mouth sites(1 way ANOVA, F= 3.04, 13.38, $p < 0.05$, 0.001, respectively). Lower concentrations were present in the spring (May 2000) and winter (February 2001) samples than in the summer for total and substituted PAHs (Figures 10, 11). Factors associated with this variation may include: dilution by runoff, reduced reproduction andmetabolic rates, and variation in molt stage and lipid content of crabs. The lower proportion

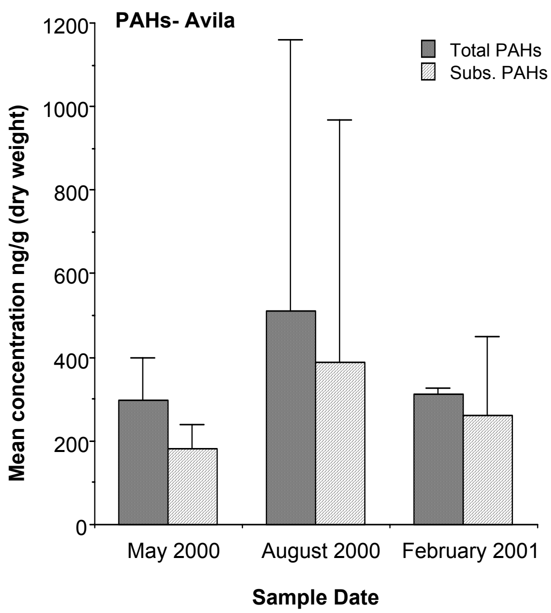

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Figure 10. Temporal variation in mean concentrations of PAHs at Avila (+ 1 std. deviation).

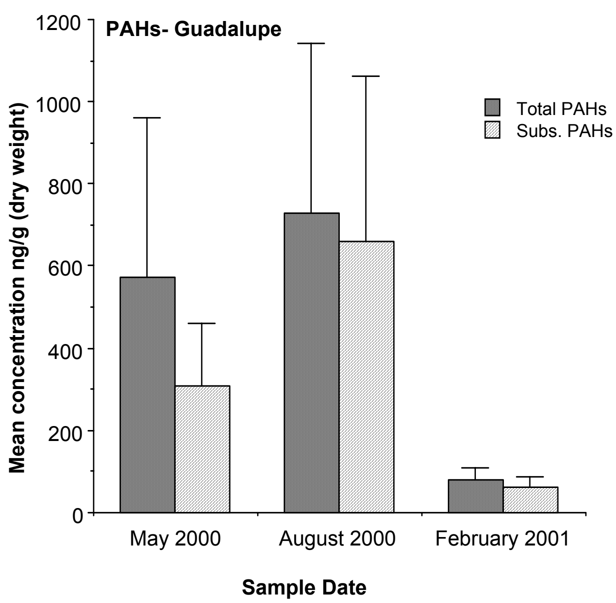


Figure 11. Temporal variation in mean concentrations of PAHs at Guadalupe/Santa Maria River mouth sites (+ 1 std. deviation).

substituted PAHs present in the May 2000 samples are consistent with runoff of pyrogenic PAHs from the watersheds and urban areas.

Pesticides

The highest concentrations of pesticides found in sand crab tissues appear to be linked to past and present agricultural land uses in the study region (Figure 12). The most widespread group of pesticides detected in tissues of sand crabs was the DDTs, a pesticide that was been banned for use in the USA since 1970. This group of pesticides was detected in every sample of sand crabs at every beach we sampled (Figure 12). Concentrations of total DDT's in sand crab tissues varied over an order of magnitude among samples, ranging from 14.7 to 556 ng/g dry weight and 179 to 6488 ng/g lipid weight. The highest concentrations of total DDTs in sand crabs were found in the vicinity of the Santa Maria River (Guadalupe and Santa Maria River sites) where agricultural land use predominates in the watershed. Concentrations of total DDTs exceeding 100 ng/g dry weight and 1700 ug/g lipid weight were also found in sand crabs from beaches near the mouth of Elkhorn Slough. The concentrations of DDTs we found in sand crabs from these two areas in the summer of 2000 are quite similar to those reported by Burnett (1971) for samples collected in these locations in November 1970 and February 1971. This result suggests that comparable amounts of DDT persist and are biologically available 30 years after the use of this pesticide was banned in the USA.

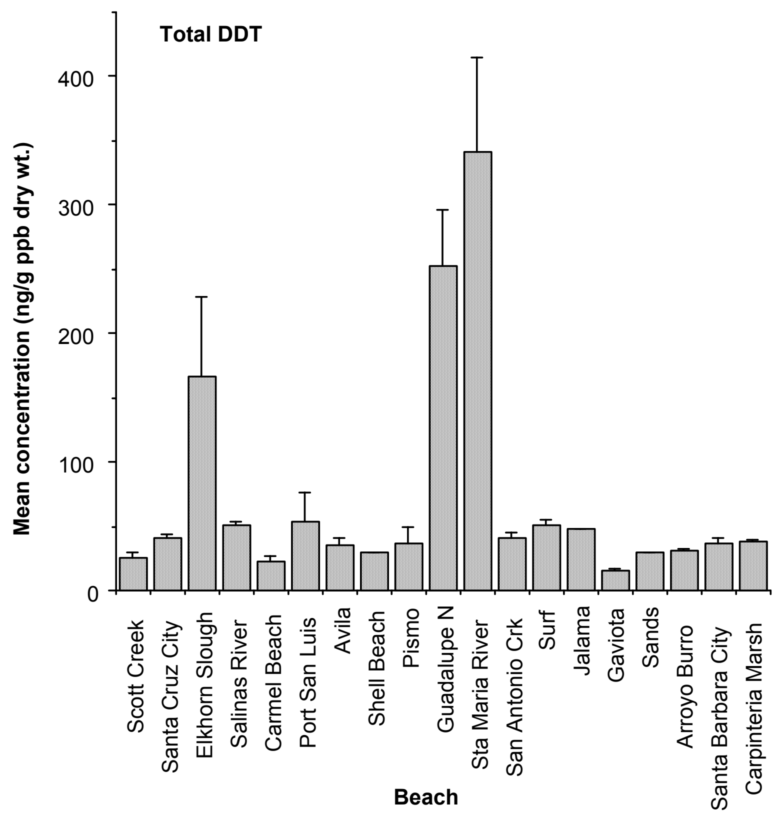


Figure 12. Mean concentrations (+ 1 std. dev) of total DDTs in tissues of sand crabs collected at 19 beaches in August-September 2000.

Although adult crabs had higher mean concentrations of total DDT, concentrations of total DDTs did not vary significantly among age classes of sand crabs at Guadalupe in May 2000 (1 way ANOVA, distance as a covariate, $F= 0.449$, $p= 0.532$, $df = 5$) (Figure 13). This result suggests a fairly constant source of DDTs exists for sand crabs in the vicinity of the Santa Maria River and that surf zone resuspension of sediments bearing DDT may contribute to the tissue levels observed. At Avila, low mean concentrations of DDT occurred in both megalopa and adult crabs in May 2000.

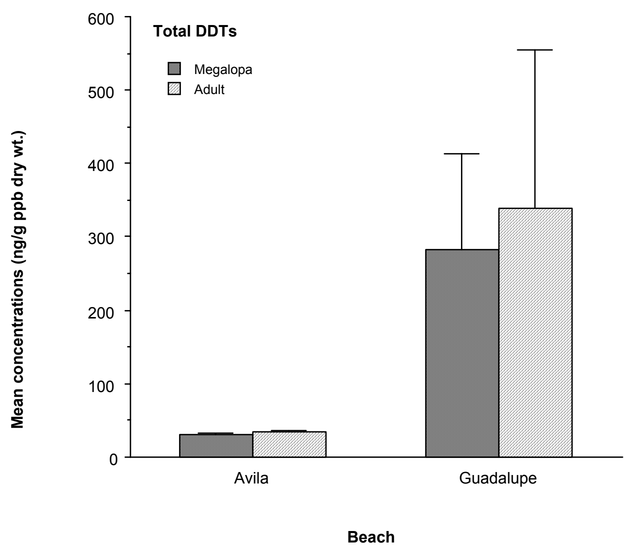


Figure 13. Mean concentrations (+ 1 standard deviation) of total DDT's in tissues of megalopa and adult sand crabs at Avila and Guadalupe in May 2000 (all stations combined).

Using data from Guadalupe and the Santa Maria River mouth, we examined the hypothesis that the concentration of total DDT varies with distance from the river mouth (20 – 900 m) using ordinary least squares regression analyses. Based on the results above, both age classes of crabs were included in the regression analyses. We found a strong and significant negative relationship between the concentration of total DDTs in sand crab tissues and the distance from the river mouth for combined data from May and August 2000, and February 2001 (Figure 14). Statistically significant negative relationships with distance from the river were also present in the data from May and August 2000 ($p< 0.02$ and $p< 0.01$ respectively) and a marginally nonsignificant negative relationship was found for data from February 2001. These results suggests a relatively persistent gradient of exposure to DDTs exists for sand crabs in the vicinity of the Santa Maria River which drains major agricultural areas. This gradient may reflect the nearshore transport and resuspension of sediments contaminated with DDT originating from the Santa Maria River watershed.

At Elkhorn Slough, samples of sand crabs were collected at only two distances from the slough mouth (~100 and ~300 m) on both sampling dates making it impossible to statistically evaluate the results for spatial gradients. No spatial gradient in total DDT concentrations in sand crabs tissues was evident for samples collected at 4 distances from the Salinas River (0-300 m scale). However, total DDT concentrations were relatively low (~50 ppb dry weight) in sand crabs from in this area.

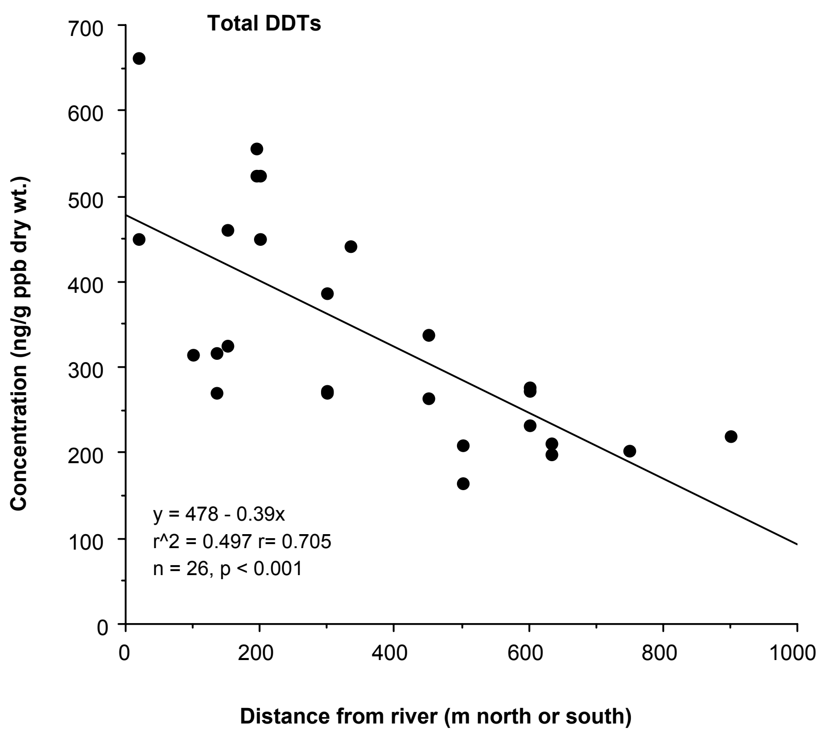


Figure 14. Concentrations of total DDTs in tissues of sand crabs as a function of distance from the Santa Maria River mouth for data for sand crabs from Guadalupe and the Santa Maria River sites from May and August 2000, and February 2001.

Pesticides of several other types were present in sand crab tissues (Table 2). After total DDTs, the most widespread pesticide detected in sand crab tissues was HCB, which occurred in low (1.1 to 26.2 ng/g) but detectable concentrations in sand crabs at 12 sites. Highest concentrations of this pesticide were detected in sand crabs from the Santa Barbara City beach. Oxychlordane was also fairly widespread but detected in low concentrations (4.0 to 10.1 ng/g dry weight) in samples from five sites. Highest levels of this pesticide were detected in sand crabs from the beach at the mouth of Elkhorn Slough. The use of Chlordane on crops has been restricted since 1974 and its use as a termiticide has been severely restricted since 1988.

High levels of two other pesticides were found in sand crabs from beaches near the Santa Maria River, again suggesting a link to agricultural land uses. Moderately high levels of Diazinon (up to 364 ng/g dry weight) were detected in sand crabs from beaches near the Santa Maria River mouth (Guadalupe) in the spring. This pesticide was only detected in overwintered adult crabs at this site and date suggesting a link to runoff associated with winter rainfall. High levels of

Diazinon (629 ng/g dry weight) were also found in one sample of megalopa from Avila in May. Toxaphene (up to 104 ng/g dry weight) was detected in a number of sand crab samples (including megalopa) collected from beaches in the vicinity of the Santa Maria River on all sampling dates (May and August 2000 and February 2001). This result suggests that a fairly constant supply of this pesticide may be available on beaches near the Santa Maria River.

Table 2. Pesticides, other than DDTs, detected in tissues of *Emerita analoga* in this study.

Pesticide	Site	Date(s)	Max. Concentration (ng/g)
Oxychlordane	Elkhorn Slough Mouth	Aug/Oct 2000	10.1
	Santa Cruz City Beach	Aug 2000	4.3
	Carmel	Aug 2000	4.0
	Avila Beach	Feb 2001	4.1
	Santa Barbara City Beach	Aug 2000	7.9
Diazinon	Avila (megalopa only)	May 2000	649.0
	Guadalupe (adult only)	May 2000	364.0
Dieldrin	Guadalupe	May 2000	13.8
		Feb 2001	8.5
	Santa Maria River Mouth	Aug 2000	10.3
HCB	Scott Creek Beach	Aug 2000	1.1
	Santa Cruz City Beach	Aug 2000	2.6
	Elkhorn Slough Mouth	Aug/Oct 2000	1.7
	Port San Luis Harbor	Aug 2000	2.0
	Shell Beach	Aug 2000	6.5
	Pismo Beach	Aug 2000	12.2
	Avila	May 2000	1.0
		Aug 2000	4.2
		Feb 2001	1.9
	Guadalupe	May 2000	1.2
		Aug 2000	1.4
	Santa Maria River Mouth	Aug 2000	1.5
	Sands Beach	Aug 2000	1.1
	Arroyo Burro Beach	Aug 2000	1.7
	Santa Barbara City Beach	Aug 2000	26.2
	Carpinteria Marsh Mouth	Aug 2000	3.3
Toxaphene	Guadalupe	May 2000	90.3
		Feb 2001	95.0
	Santa Maria River	Aug 2000	104.0

PCBs

The presence of PCBs (polychlorinated biphenyls as a total of the congeners) in an intertidal animal is indicative of runoff from industrial land uses. Although use of PCB use began to be phased out in 1971 and a ban on new devices was initiated in 1976, total PCBs were widespread in sand crab tissues in this study and were detected in every sample of sand crabs from the 19 sites (Figure 15). The concentrations of PCBs in tissues of sand crabs varied among sites by over an order of magnitude, ranging from 3.3 to 223 ng/g dry weight and 22.0 to 2247 ng/g lipid weight. The highest concentrations of PCBs were found in sand crabs from beaches near the mouth of Elkhorn Slough (up to 223 ng/g dry weight). Variance was high among samples from that location with nearly 2 fold and up to 8 fold higher concentrations in the samples from south of the slough mouth in August and October 2000 respectively. Concentrations of PCBs exceeded 50 ng/g dry weight in sand crabs from beaches at Port San Luis Harbor and Avila Beach.

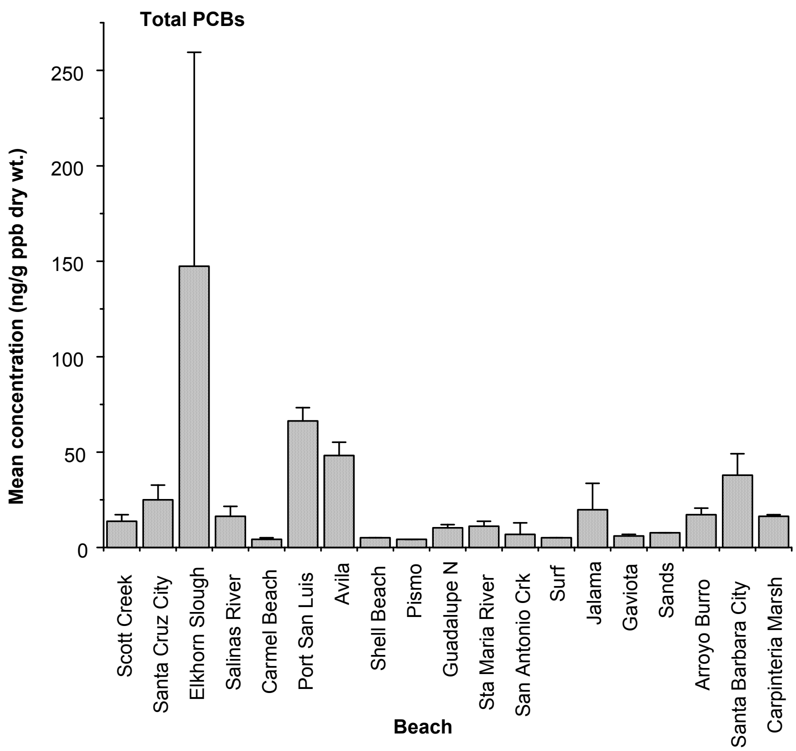


Figure 15. Mean values (+ 1 standard deviation) of concentrations of total PCBs in tissues of adult sand crabs at 19 beaches in August of 2000.

Concentrations of PCBs varied significantly among different life stages of sand crabs (Figure 16). At Avila, where PCB concentrations were higher, statistically significant differences in PCB concentrations were detected between life stages of sand crabs on both a dry and a lipid weight basis (1 way ANOVA, $F = 7.150$, $df = 8$, $p <0.03$ and $F= 16.830$, $df = 8$, $p < 0.005$ respectively). At Guadalupe, dry weight-based concentrations of PCBs were nearly significantly different ($p= 0.07$) among life stages of crabs and lipid weight-based concentrations of PCBs were significantly higher in young of the year than in older sand crabs (1 way ANOVA, F=

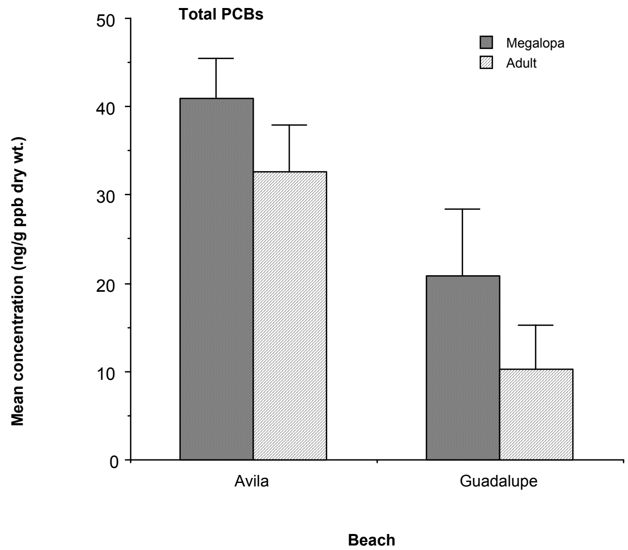


Figure 16. Mean values (+ 1 standard deviation) of concentrations of total PCBs in megalopa and adult sand crabs at Avila and Guadalupe in May 2000.

14.47, df = 6, p <0.01). This could potentially be related to the higher intertidal zone occupied by young of the year sand crabs but it is not possible to resolve this question with these results. It was not possible to statistically evaluate spatial gradients in PCB concentrations in sand crabs at Elkhorn Slough or Port San Luis because samples were collected at only two distances. However, this would be of interest if additional sampling and analyses are conducted.

Trace metals

Trace metals in the tissues of sand crabs could be linked to a variety of land uses and the natural or enhanced erosion of different geologic formations, such as serpentine outcrops and selenium rich rocks and soils. A total of 12 trace metals (silver, aluminum, arsenic, cadmium, chromium, copper, lead, manganese, nickel, selenium, zinc, and mercury) were detected in sand crab tissues. Mercury was only detected in 2 of the samples. Results on the concentrations of the other 11 metals detected in sand crab tissues at each beach are presented in Figure 17 a-k. The majority of the metals we detected were present in relatively low concentrations in sand crab tissues. Aluminum was detected in the highest concentrations in sand crab tissues, reaching 2000 ppm dry weight (Figure 17 b). Aluminum is known to be strongly associated with sediments for bivalve studies and high concentrations may be characteristic of suspension feeder in a sandy habitat. Strong site-specific patterns were present in only a few metals (aluminum, chromium and nickel, Figure 17 b, e, and h) and are likely linked to erosion of local geologic formations, such as serpentine and other rocks. High levels of chromium and nickel reported for coastal sediments from Point Conception, CA to the Hood Canal, WA are associated with the geologic composition of rock formations rather than human activities. No geographic patterns were evident for arsenic, copper, selenium and zinc (Figure 17 c, f, j, and k).

Mercury was detected in sand crab tissues in a single sample at each of two beaches, Santa Cruz City beach (0.142 ppm dry weight) and Pismo Beach (0.185 ppm dry weight). Other hippid crab species, including *Emerita brasiliensis*, *E. portoricensis* and *Hippa cubensis* (Perez 1999) from beaches in Golfo Triste, Venezuela have been reported to contain levels of mercury ranging from 2 to 1449 ppb. This suggests that although sand crabs did not have detectable levels of mercury at most sites in the present study, this species has the potential to accumulate this metal.

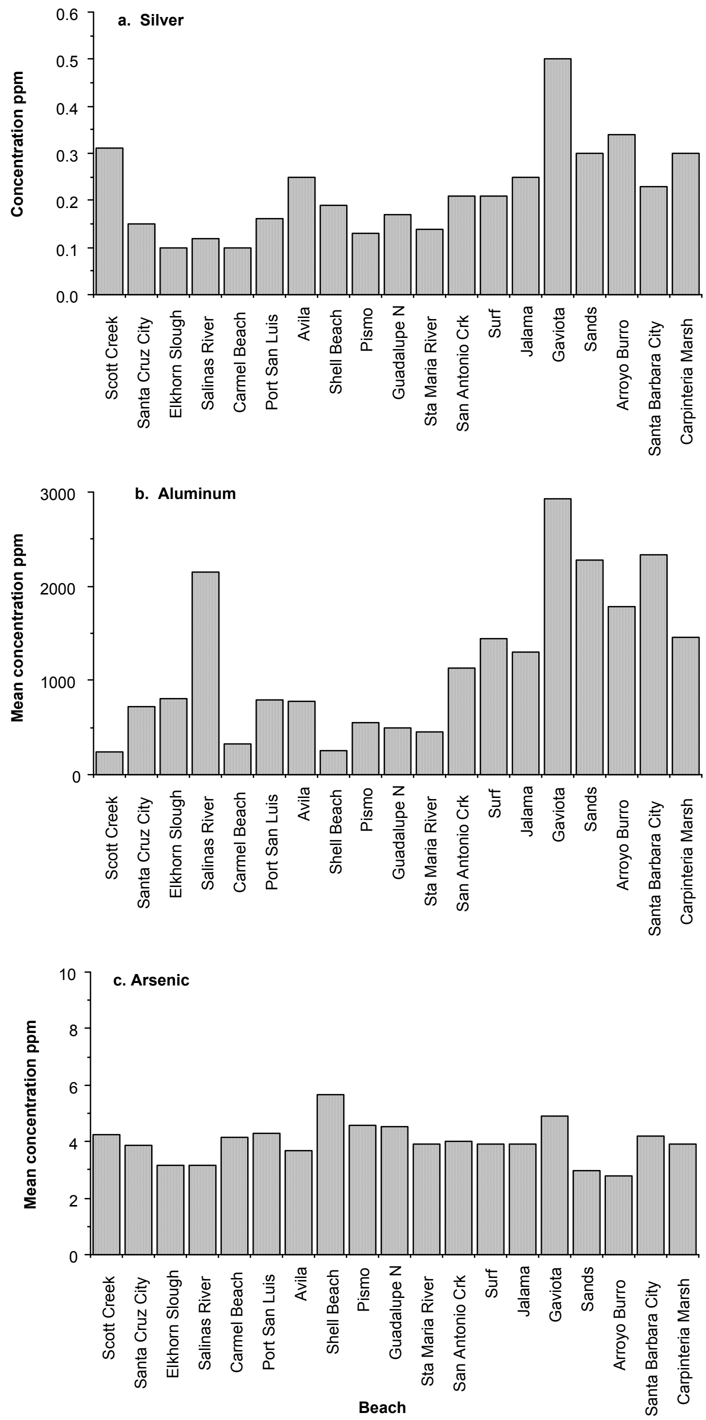


Figure 17 a-c. Mean trace metal concentrations in tissues of adult sand crabs collected at 19 beaches a) silver, b) aluminum, and c) arsenic.

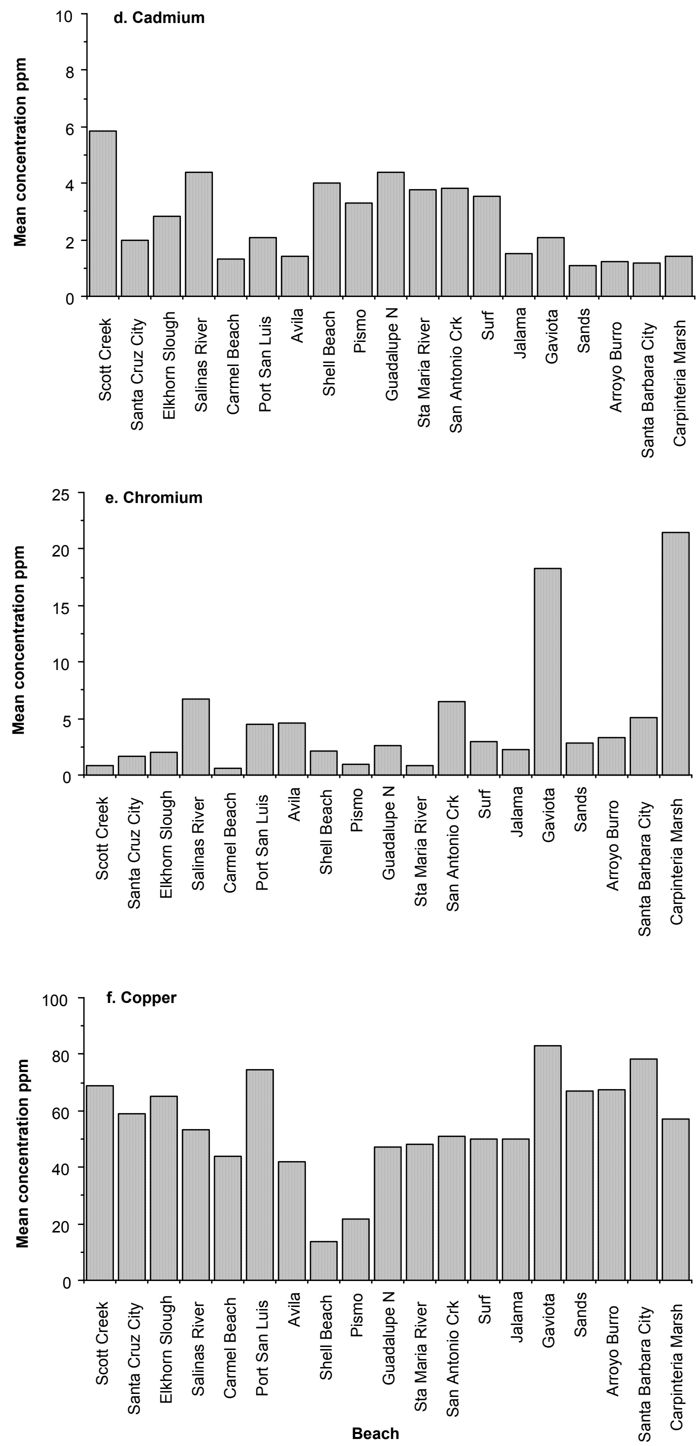


Figure 17 d-f. Mean trace metal concentrations in tissues of adult sand crabs collected at 19 beaches in August/September 2000 d) cadmium, e) chromium, and f) copper.

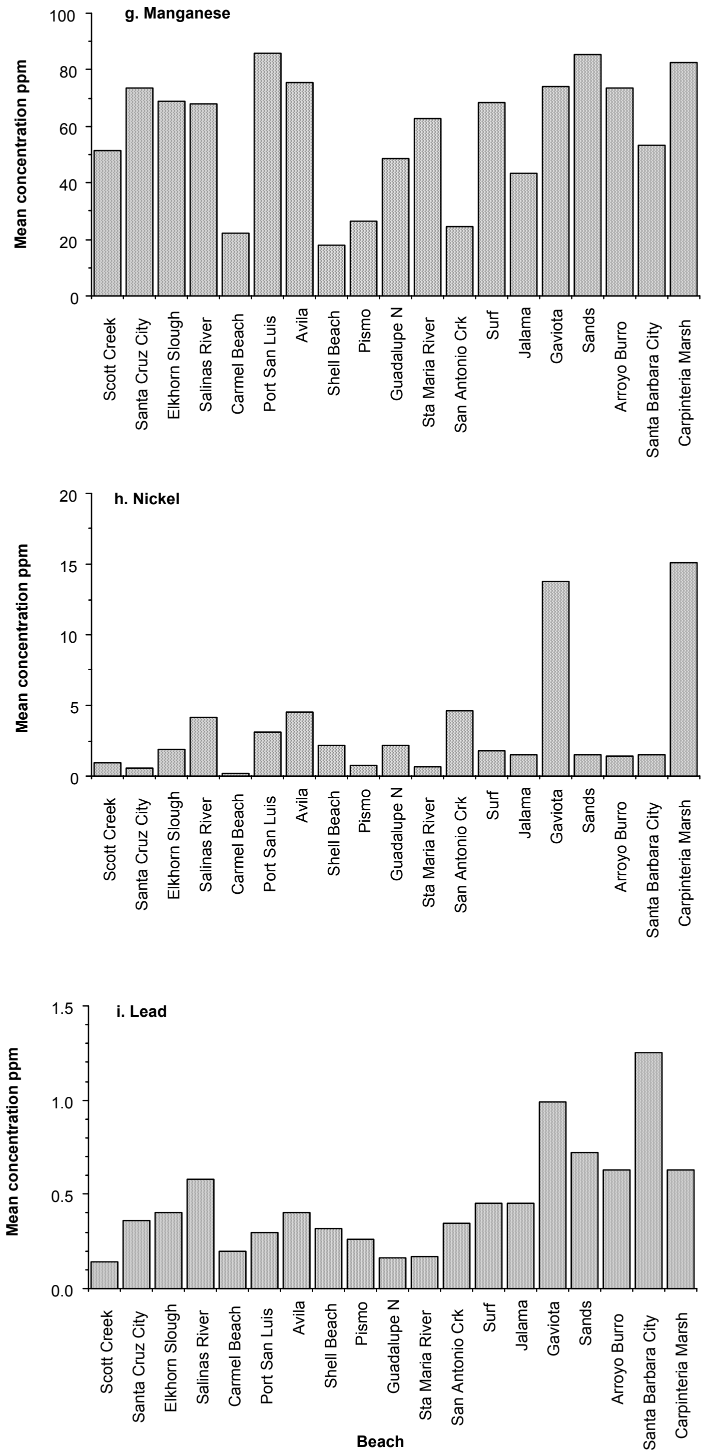
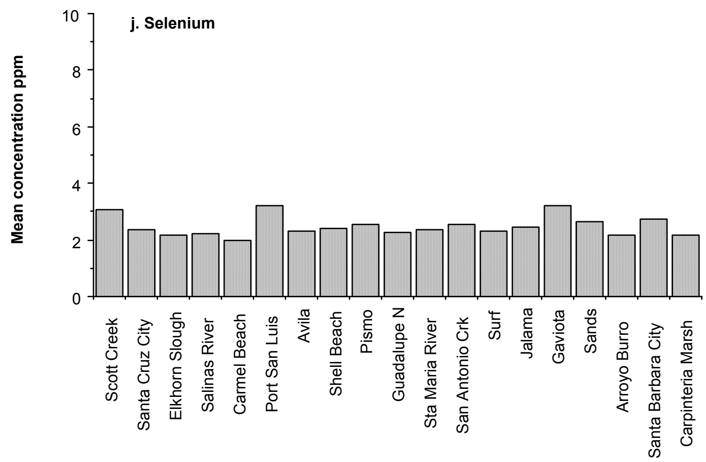


Figure 17 g-i. Mean trace metal concentrations in tissues of adult sand crabs collected at 19 beaches in August/September 2000 g) manganese, h) nickel, and i) lead.



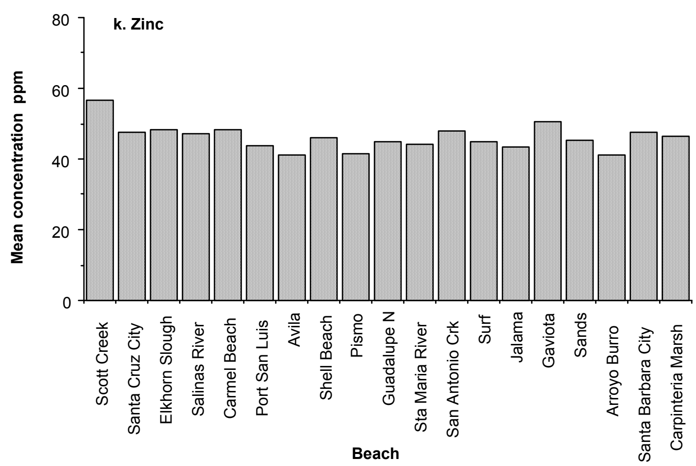


Figure 17 j-k. Mean concentrations of trace metals in tissues of adult sand crabs collected at 19 beaches in August/September 2000 j) selenium and k) zinc.

For samples from Guadalupe and Avila, the age class of sand crab appeared to influence the concentrations of chromium and nickel most strongly (Figure 18 ab). For those two metals, concentrations in older adult crabs were much higher than in tissues of newly settled megalopa (Figure 18 ab). This result also suggests that intertidal organisms maybe exposed to these metals primarily through winter runoff and the erosion of rock formations rich in these trace metals. The concentration of aluminum was also consistently higher in adult crabs than in megalopa which could be related to the difference in length of time the two age classes have been associated with sediments (1 month or less for megalopa and 1+ years for older adult crabs)

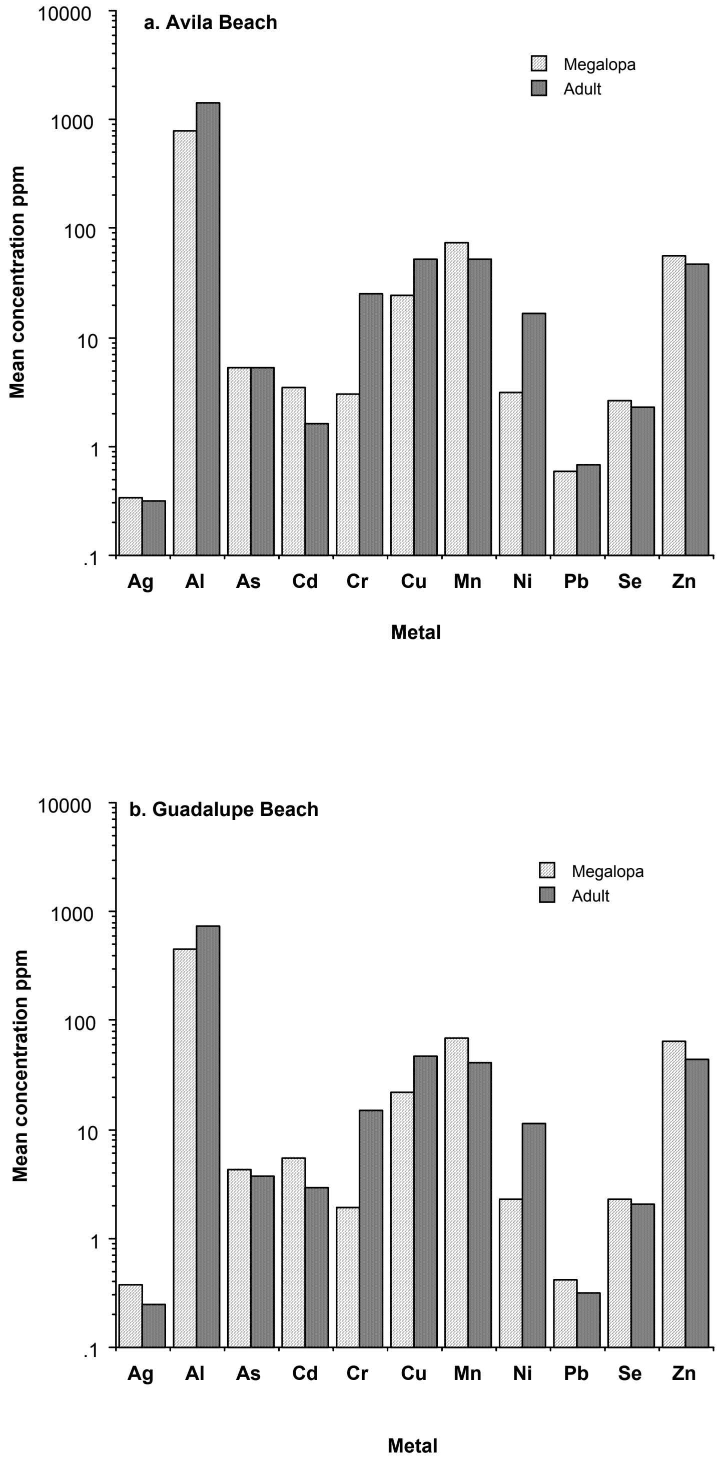
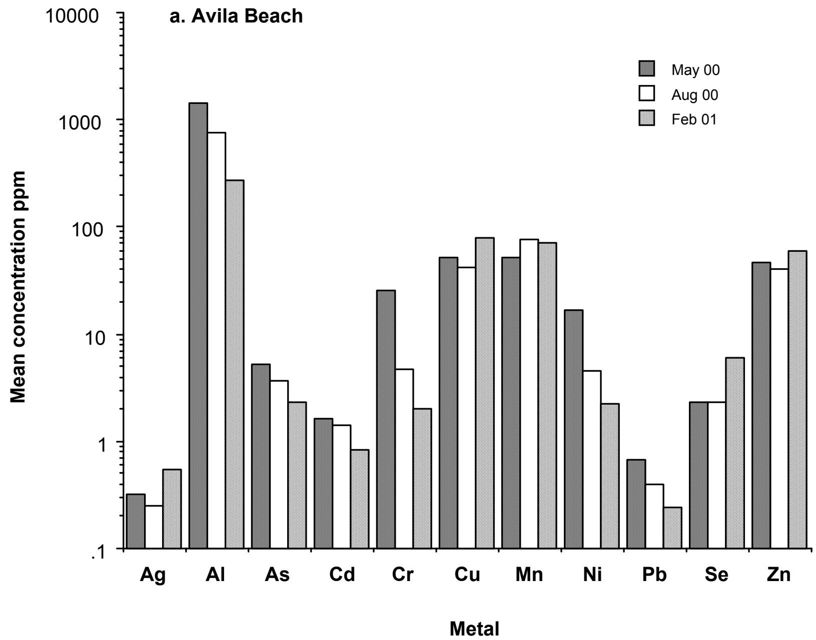


Figure 18 ab. Mean concentrations of 11 trace metals in tissues of young of the year and overwintered adult sand crabs collected at a) Avila and b) Guadalupe in May 2000.

Concentrations of chromium and nickel in sand crab tissues also showed the greatest variation among sampling dates at Avila and Guadalupe (Figure 19 ab). This is also consistent with a link between runoff and the presence of these metals in sand crab tissues.



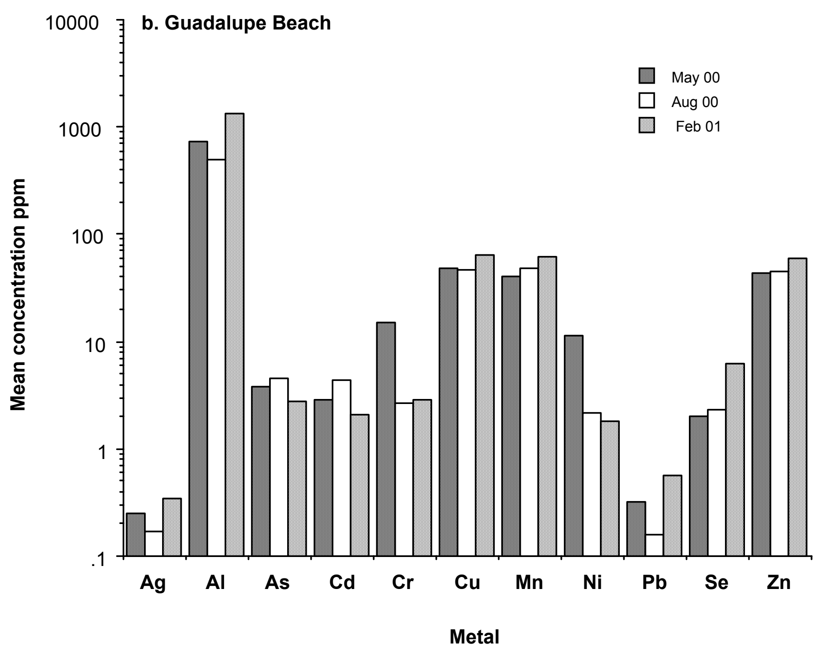


Figure 19 ab. Mean concentrations of 11 trace metals in tissues of adult sand crabs collected at a) Avila and b) Guadalupe for samples collected in May 2000, August 2000 and February 2001.

SUMMARY AND RECOMMENDATIONS:

The results of this study generally support the concept of using sand crabs as a biological indicator for use in long term ambient monitoring of pollutants on the California coast. Tissues of sand crabs collected from 19 coastal beaches spanning a range of land use types contained detectable concentrations of contaminants including petroleum hydrocarbons, chlorinated hydrocarbons (a number of pesticides and polychlorinated biphenyls (PCBs)), and 12 different trace metals. Concentrations of several types of contaminants in sand crab tissues varied over an order of magnitude among the sampling sites. For some contaminants, concentrations in sand crabs varied with distance from potential sources, time of year and age class of the crabs.

Our results suggest that sand crabs could be valuable as biological indicators of pesticides, trace metals, and PCBs in coastal habitats. Analysis of sand crabs collected along appropriate spatial gradients can be used to investigate potential sources of these contaminants as shown by our results from Guadalupe and the Santa Maria River mouth for DDT. Our results suggest that this type of gradient sampling and analysis of sand crabs can be used as part of coastal monitoring programs. Additional sampling along appropriate spatial gradients is recommended, particularly at sites where levels of PCBs and pesticides were high in sand crabs, such as the Elkhorn Slough mouth, Port San Luis and Avila, in the present study.

For most of the contaminants of concern, collection of samples in the summer (August) appeared to provide a reasonable basis for comparisons among sites. At Avila and Guadalupe, where three collections were made (May, August and February) we found little difference among samples collected at three times of year for many of the contaminants analyzed. Variation in precipitation among years could have contributed to low levels of runoff-associated contaminants in February 2001. Resolving relationships between tissue burdens of contaminants in sand crabs, precipitation and runoff will require a considerably longer time series of data collection than was possible in the present study. We suggest that initiating a long term time series of sample collection and analyses at several sites would be helpful in resolving these relationships.

For many of the contaminants analyzed at Avila and Guadalupe, including DDT and PAHs, tissue concentrations of contaminants did not vary significantly between age classes of sand crabs (overwintered female crabs and megalopa). This result suggests that many of these contaminants are readily available in the coastal habitat of sand crabs. Resuspension of contaminated sediments and seepage of contaminated porewater through the porous beach face are two possible mechanisms. Although the number of comparisons was relatively small, this is interesting given the difference in age and microhabitat of the two age classes of this species analyzed. Megalopa have generally inhabited the intertidal sandy beach habitat for 1 month or less while overwintered crabs have been on the beach for a year or more. Megalopa inhabit a higher intertidal zone, while overwintered animals can inhabit a wide range of tidal heights including the shallow subtidal zone.

Megalopa are not consistently available for sample collections throughout the year, appearing in late winter and reaching peak abundance in the spring months. For this reason in particular, we recommend that collections of samples of sand crabs for monitoring of ambient pollutants focus on older overwintered female crabs which are available more consistently through the year.

Rreproductive state (ovigerous or nonovigerous), sex, size, and age class should be used to select animals of consistent types for all samples and these attributes should recorded prior to analyses for contaminants.

Our results for sand crabs from the Santa Maria River and Guadalupe sites indicate that the Guadalupe oil field area remains a significant source of petroleum hydrocarbons to the intertidal biota. We suggest that sand crabs in this area need to be monitored over a sufficient time span (years) to determine if the oil contamination continues to reach the intertidal zone.

The results for petroleum hydrocarbons in sand crabs at Avila and Port San Luis also suggest that petroleum hydrocarbon contamination is still present in the area. Further monitoring of tissue burdens of PAHs in sand crabs is warranted to evaluate if the outlier plume under the Avila Pier and/or other sources continue to release petroleum hydrocarbons to coastal habitats in this area.

Before coastal monitoring programs for petroleum hydrocarbons based on sand crabs can be fully implemented, further research is needed in several areas. Further studies are required on the processes and factors associated with the high variation observed in PAH concentrations among samples of sand crab tissues at several individual sites and at Avila and Guadalupe. It is notable that high variability in tissue loadings of PAHs was primarily observed in older sand crabs from sites where high concentrations of PAHs occurred in crab tissues. Samples of sand crabs from sites where overall concentrations of PAHs in tissues were low did generally not exhibit the high variability in tissue loadings. This result suggests that sand crabs exposed to high levels of PAHs may be experiencing differential mortality or exhibiting metabolic responses to exposure to this contaminant. It is clear from these results that we need to learn more about the metabolism of PAHs in this species and investigate the effects of life history factors such as reproductive state, molt stage, lipid content, nutritional state and age on tissue burdens and metabolism of PAHs. It is also important to assess the potential mortality of different life stages of this species associated with high tissue loadings of PAHs.

Temporal variation in PAH concentrations in sand crab tissues was apparent among samples collected at three times of year for Avila and Guadalupe. This result strongly suggests the need to investigate how the animals interact with potential sources of PAHs such as runoff, beach porewater and ocean sources. Monitoring crabs of specific reproductive or age class types on a finer temporal scale (6 times /year) along appropriate spatial gradients at sites known to be contaminated, such as Avila and Guadalupe, during years with different rainfall patterns and amounts would assist in evaluating these issues.

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