PAH exposure and DNA damage in flatfish from southern California bays and harbors, and the Channel Islands

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ABSTRACT - Southern California bays and harbors have been shown to contain high concentrations of a variety of contaminants, including polycyclic aromatic hydrocarbons (PAHs), metals, and pesticides. Conventional monitoring tools do not assess exposure to PAHs in fish, or sublethal effects, which can be more sensitive indicators of stress than traditional methods. This study was conducted to evaluate PAH exposure and DNA damage (a sublethal effect) in flatfish from southern California bays and harbors, and the Channel Islands. California halibut (*Paralichthys californicus*) were collected from eight bays and harbors (Ventura Harbor, Channel Islands Harbor, Marina del Rey, King Harbor, Alamitos Bay, Long Beach Harbor, Newport Bay, San Diego Bay), and a reference site off Camp Pendleton. Pacific sanddab (*Citharichthys sordidus*) were collected near four of the Channel Islands (San Miguel Isle, Santa Cruz Isle, Anacapa Isle, Santa Barbara Isle). PAH metabolites in fish bile were characterized using a semi-quantitative technique that measures fluorescent aromatic compounds (FACs) in fish bile. DNA damage in fish blood cells was assessed by measuring the amount of single-strand breaks in stained DNA using the Comet assay. Among the bays and harbors, average FAC concentrations varied by a factor of three. The concentration of FACs in fish from all bays and harbors was elevated, with average concentrations ranging from three to ten times greater than FACs in reference fish. PAH exposure was elevated in Pacific sanddabs from a Channel Islands station located between Santa Cruz Isle and Anacapa Isle. DNA damage varied by a factor of five among California halibut from bays and harbors, with significant damage occurring in fish from Alamitos Bay. There was a significant association between FAC concentrations and DNA damage in California halibut at Marina del Rey and Ventura Harbor, but not at other locations. While DNA damage and PAH exposure were elevated in fish from bays and harbors, these indicators were not significantly related to sediment contaminant concentrations.

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INTRODUCTION

Fish are important indicators of marine environmental health. The soft-bottom habitat of the Southern California Bight supports a diverse assemblage of demersal fishes (Allen *et al*. 2002). These fish are key members of marine food webs, and are valued by the public as sources of food and recreation. Many species of demersal fish are chronically exposed to environmental contamination through activities such as feeding upon benthic infauna and contact with contaminated sediments or water.

Traditional monitoring methods for demersal fish may not have the sensitivity to assess local contaminant impacts. While measurements of population and assemblage parameters have high ecological relevance, these measures are also strongly affected by environmental variables such as water temperature and prey abundance that can reduce the ability to identify impacts related to contaminant exposure.

An alternative approach for examining impacts on fish is to measure changes at the cellular or biochemical level. The development of numerous biomarker techniques in the last decade has resulted in methods with the potential to assess stress in field-collected fish, and detect effects leading to growth/reproductive impairment (Huggett *et al*. 1992).

One such biomarker is an indicator of exposure to polycyclic aromatic hydrocarbons (PAHs). Exposure to PAHs is a potentially important factor in determining the response of fish to contamination. Many PAHs are toxic, and exposure to this group of compounds has been identified as a risk factor influencing the development of liver lesions in fish

picture

(Myers *et al.* 1998). However, exposure to PAHs cannot be assessed by conventional tissue analyses because these compounds are rapidly metabolized by the liver and secreted into the bile. One biomarker that has been used to quantify the exposure to PAHs in fish is fluorescent aromatic compounds (FACs) in fish bile. Bile FAC concentrations have shown a strong correlation with sediment PAH concentrations (Collier *et al.* 1993). Bile FAC concentrations have been measured as an indication of PAH exposure in fish from southern California and other locations, including Puget Sound, Washington; Galveston Bay, Texas; and Tampa Bay, Florida (Brown and Bay 1999, Varanasi *et al.* 1988, Willett *et al.* 1997, McCain *et al.* 1996).

Another biomarker is an indicator of damage to DNA. DNA damage is a sublethal indicator that can be caused by a variety of environmental contaminants, including metals and PAHs (Shugart 1988). Many contaminants have been shown to cause strand breaks in a dose-dependent manner (Tice 1996). In addition to a linkage with cancer, increases in DNA damage precede or correspond with reduced growth, abnormal development, and reduced survival of adults, embryos, and larvae (Shugart *et al.* 1992, Lee *et al.* 1999, Steinert 1999). The most prevalent type of genetic damage is single-strand breaks in the DNA. By measuring the amount of single-strand breaks, significantly elevated levels of DNA damage have been reported in cells from fish collected at polluted sites, compared to those from reference sites (Pandrangi *et al.* 1995).

These biomarkers have had little application in southern California. While FACs have been measured in fish from bays and harbors in southern California, the data represent a limited number of locations (Varanasi *et al.* 1989). Moreover, no single species of fish was collected from the various areas sampled, so comparisons among all locations studied cannot be made. In addition, the data were collected in the mid-1980s, and it is uncertain whether the FAC values measured reflect current levels of PAH exposure.

This project had three objectives. The first objective was to characterize the levels of DNA damage and PAH exposure in fish from southern California bays and harbors. The second objective was to compare these indicators in fish to concentrations of contaminants in sediments. The third objective was to examine the relationship between PAH exposure and a measure of biological effect by comparing concentrations of FACs and DNA single-strand breaks in individual fish.

METHODS

Sampling Design

PAH exposure and DNA damage was assessed in flatfish from southern California bays and harbors, the Channel Islands, and a reference location off Camp Pendleton. Eight geographic areas were selected for sampling in the bays and harbors, including Ventura Harbor, Channel Islands Harbor, Marina del Rey, King Harbor, Los Angeles/Long Beach Harbor,

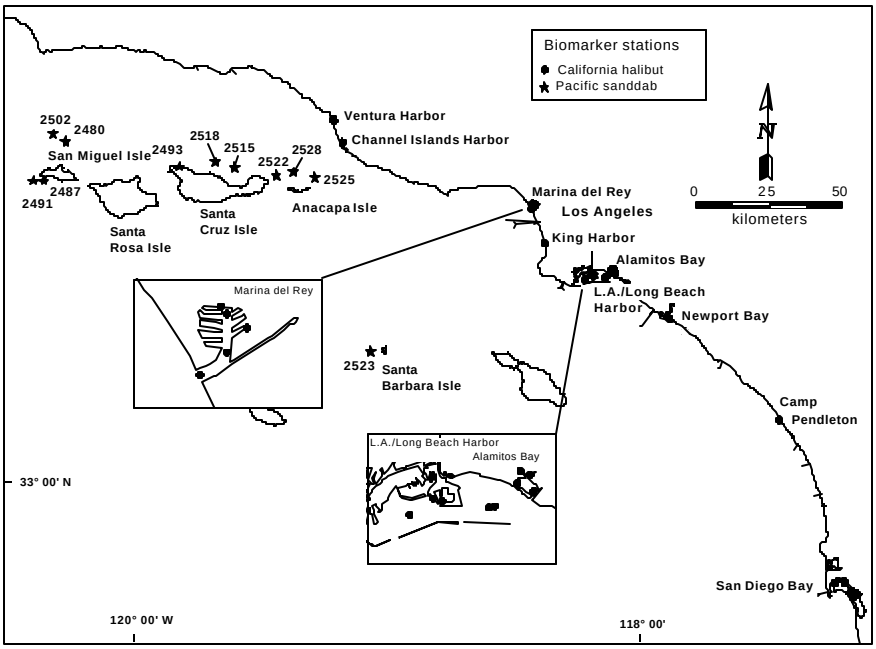

Figure 1. Sampling locations for fluorescent aromatic compounds (FACs) and DNA damage in California halibut and Pacific sanddab. California halibut were collected from bays and harbors, while Pacific sanddabs were caught near the Channel Islands. Additional California halibut were collected from a reference site off Camp Pendleton.

Alamitos Bay, Newport Bay, and San Diego Bay (Figure 1). The Camp Pendleton reference station was located approximately 5 km from the Santa Margarita River, and 8 km from Oceanside Harbor, the nearest discrete sources of contamination. The Channel Islands are located away from large sources of pollution (e.g., point sources and heavy boat traffic).

Selection of the species for biomarker analysis was based on the abundance and distribution of species collected. For the bays and harbors, the species selected was California halibut (*Paralichthys californicus*). For the Channel Islands, the species selected was Pacific sanddab (*Citharichthys sordidus*).

Field Sampling

Juvenile California halibut (< 22 cm standard length) were collected for biomarkers from the bays and harbors between July 31 and September 2, 1998. Fish were collected at multiple stations within each bay and harbor. The total number of stations in this study varied between biomarkers; FACs were successfully measured in fish from 17 stations, and DNA damage from 26 stations. Additional fish were collected from Marina del Rey, Long Beach Harbor, Alamitos Bay, and off Camp Pendleton between August 23 and September 10, 1999. Pacific sanddabs were collected near the Channel Islands between July 28 and September 15, 1998, at depths ranging from 31 m to 202 m. FACs were successfully measured in fish from 11 stations located near San Miguel, Santa Cruz, Anacapa, and Santa Barbara Islands. All fish were captured using an otter trawl. Trawls were conducted for five minutes in the marinas, and ten minutes in the larger industrial harbors. Gall bladders (containing bile for FACs) were removed from the fish with the liver at sea, and frozen on dry ice or in liquid nitrogen. Blood (for measuring the level of DNA damage) was diluted in a 10% dimethyl sulfoxide/phosphate buffered saline solution before freezing. Only fish that were dissected while alive were used for DNA damage analysis. The tissues were stored in the laboratory at –80°C until they were analyzed.

Sediments were collected at many of the bay and harbor stations for contaminant analysis as part of another coordinated study (Noblet *et al.* 2002).

FAC Analysis

Bile was collected by piercing the side of a gall bladder and allowing the contents to flow into a small test tube. The bile was diluted 1:1 with 50% MeOH, then centrifuged to remove debris that would interfere with analysis. When there was insufficient material from individual fish, bile from all fish from a given station was composited for analysis. Concentrations of FACs were determined following the methods of Krahn *et al.* (1986), using reverse-phase high performance liquid chromatography (HPLC) and fluorescence detection. Five µL of the diluted bile supernatant was injected onto the HPLC. Bile proteins and other bile components that could potentially interfere with PAH quantification are water soluble, and were passed through the nonpolar reverse-phase column with a 5 ppm acetic acid solution mobile phase, while the hydrophobic PAHs and metabolites were adsorbed onto the column. The PAH metabolites were then eluted from the column using methanol, and measured using fluorescence detection. Fluorescence of the PAH metabolites was measured at the excitation/emission wavelength pair (380/430 nm) appropriate for PAHs with similar structure as benzo[a]pyrene (BaP) and its metabolites. The higher molecular weight PAHs (products of combustion) and their metabolites tend to fluoresce at the same wavelength, and are semi-quantitated collectively as BaP equivalents. Fluorescent values were converted to ng BaP equivalents using a seven-point BaP standard curve.

Laboratory quality assurance procedures included measuring at least one BaP external standard, a method blank, and a California halibut bile reference sample prior to sample analysis.

DNA Damage Analysis

The blood was diluted 1:5 with ice cold phosphate-buffered saline (PBS), then centrifuged at 600 x g for 2 minutes. The blood cells (contained in the centrifuge pellet) were then embedded in agarose on glass microscope slides. The slides were placed in lysing solution (2.5 M NaCl, 10 mM Tris, 0.1 M EDTA, 1% Triton X-100, 10% DMSO, pH 10) and incubated for 1 h. The slides were next rinsed with distilled water, then transferred to an alkaline solution (300 mM NaOH, 1 mM EDTA) for 15 minutes to denature the DNA. This step relaxes and unwinds the DNA, to help separate the portions that are single stranded from the double-stranded molecule. After DNA unwinding, the slides were electrophoresed at 300 mA, 25 V for 10 minutes. The single-stranded portions of the DNA (resulting from breakage) are smaller, and tend to migrate through the agarose at a

faster rate than the double-stranded molecule; in this way, single-stranded DNA can be separated from the nucleus. The slides were then neutralized with 0.4 M Tris, and fixed in ice cold ethanol for 5 minutes.

For analysis, the DNA was stained with a solution of ethidium bromide in distilled water and covered with a coverslip. Stained slides were analyzed by viewing with an epifluorescent microscope (excitation filter 510-560 nm green light, barrier filter 590 nm) with an attached CCD camera and image analysis software (Komet image analysis system, Kinetic Imaging, Ltd U.K.). The fluorescent "head" or nucleus diameter and the length (μm) of any accompanying trailing DNA "tails" resulting from strand breakage were measured for each nucleus analyzed. Following electrophoresis, the nucleus of intact double-stranded DNA, and the trailing tail of damaged DNA gives the appearance of a comet; hence the term "Comet assay." Measurements were made in five sectors on each slide, counting 5-10 nuclei in each sector by randomly positioning the lens above each sector and counting left to right from the upper left-hand corner of the field of view. Overlapping nuclei or tails were not counted. The image system calculates a large number of quantitative parameters for each nuclei, the most important being the total intensity of each comet (comet optical intensity), the percent of DNA in the tail, and the tail moment (TM), which is the product of the percent of DNA in the tail x the tail length/100. A mean TM value was calculated for each fish sampled.

Different cell types were analyzed between the samples collected in 1998 and 1999. DNA from both red and white blood cells were analyzed for the samples collected in 1998. However, the samples collected in 1999 (from the Camp Pendleton reference site, and additional sampling in Long Beach Harbor, Alamitos Bay, and Marina del Rey) were stored for approximately one year before they were analyzed for DNA damage, and it appeared the red blood cells had degraded significantly. Therefore, only the white blood cells were used for DNA damage analysis in the samples collected in 1999.

The accuracy and precision of the measurements were assessed by conducting replicate measurements of known reference samples of bird blood consisting of bird white blood cells with little damage as negative (no damage) control cells, and bird red blood cells with extensive DNA strand damage as positive controls. Twenty-one slides were processed through each denaturation/electrophoresis cycle; of these 21 samples (batch), 3 consisted of a mixture of both negative and positive control slides. Slides with cell densities too high to perform image analysis were diluted and re-run. The acceptable mean damage levels of the controls had to fall within the 95% confidence limits previously determined for the reference samples. Any reference values outside of the expected limits would require a procedural review and repeat analysis of that batch of samples.

Data Analysis

Statistical differences in FAC concentrations, and DNA single-strand breaks in fish from the various locations were identified using a one-way analysis of variance (ANOVA). When the assumption of homogeneity of variance was not met, the nonparametric Kruskal-Wallis test was used. The average FAC concentration or tail moment of fish from all stations within each bay and harbor was used to derive a location average. The term "location" identifies the different bay and harbor geographic areas where the fish were collected (e.g., Marina del Rey, Ventura Harbor). Fish from all stations within a location were used to derive the mean value because fish move around, receiving contaminant exposure from various parts of each bay or harbor. A multiple comparison test was used to identify locations with FAC concentrations significantly greater than the Camp Pendleton reference site.

For the Channel Islands, differences in FAC concentrations were analyzed by station. For sites where there was insufficient bile to analyze FACs in individual fish, the bile from all fish was composited for the station.

Cross-indicator comparisons were also made. The relationship between FAC concentrations in bile and DNA damage in blood cells was evaluated using data from the same fish. Comparisons were also made between biomarker response and sediment contaminant concentrations. Because fish move around within each of the bays and harbors, the sediment values used for comparison were the mean concentrations of all sites within each location. All cross-indicator comparisons were made using linear regression.

RESULTS

FAC Concentrations

FAC concentrations in California halibut varied among bay and harbor locations (Figure 2). Concentrations varied by a factor of three, with the lowest average concentration occurring at Ventura Harbor

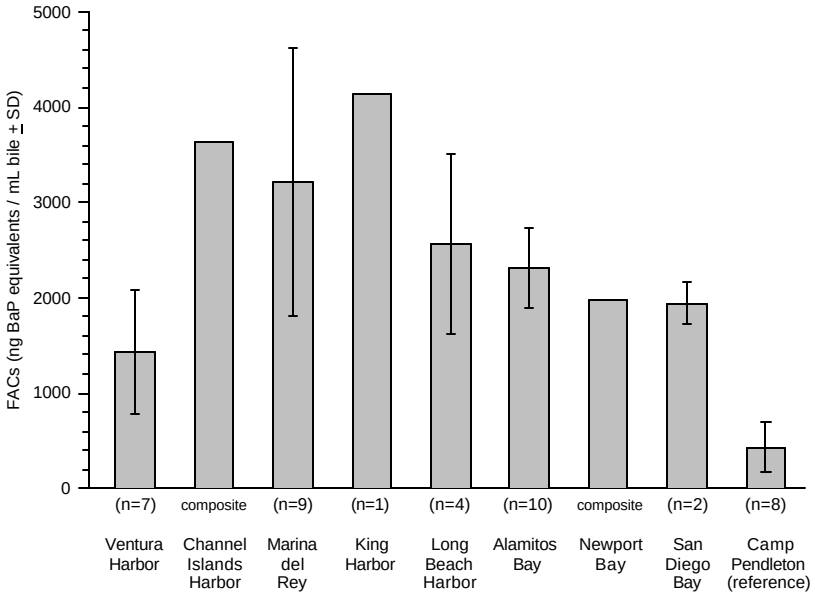

Figure 2. Concentration of fluorescent aromatic compounds (FACs) in California halibut for each bay and harbor location, and the Camp Pendleton reference site. The fish from the Camp Pendleton reference site were collected in 1999, while the fish from the other locations were collected in 1998. n = Number of fish at a given location.

(1424 ng BaP equivalents/mL bile) and the highest concentration at King Harbor (4133 ng BaP equivalents/mL bile).

The FAC concentrations in California halibut from bays and harbors were elevated compared to fish from the Camp Pendleton reference site. Mean concentrations among locations ranged from 3-10 times the reference value. Among the locations that had data for more than one fish, there were significant differences in the concentrations of FACs ($p < 0.001$, Kruskal-Wallis). Fish from Marina del Rey, Long Beach Harbor, and Alamitos Bay had significantly greater FAC values than fish from the Camp Pendleton reference site (Dunn's Method for multiple comparisons), while fish from Ventura Harbor and San Diego Bay were not significantly different from the reference site fish.

FAC concentrations among Pacific sanddab from the Channel Islands varied by a factor of four (Figure 3). Average concentrations ranged from 209 ng BaP equivalents/mL bile in fish from Station 2525 (located northeast of Anacapa Isle), to 862 ng BaP equivalents/mL bile in fish from Station 2522 (located between Santa Cruz and Anacapa Isles). Only 4 of the 11 stations had data from enough fish for statistical analysis: Stations 2491 (west of San Miguel Isle), 2502 (north of San Miguel Isle), 2522 (between Santa Cruz and Anacapa Isles), and 2525 (northeast of Anacapa Isle). The difference in concentrations among Channel Islands stations was statistically significant ($p = 0.03$, Kruskal-Wallis), with fish from Station 2522 having significantly greater FAC concentrations than fish from Stations 2491 and 2525 (Dunn's Method for multiple comparisons). With the exception of fish from Station 2522, average FAC concentrations in Pacific sanddab from most of the Channel Islands stations were comparable to the concentration in California halibut from the Camp Pendleton reference site. Compared to the fish from the bays and harbor areas, all average concentrations found in Pacific sanddab from the Channel Islands were lower than the average concentrations in California halibut from any of the bays and harbors (Figures 2 and 3).

DNA Damage

DNA damage varied by a factor of five among California halibut from bay and harbor locations (Figure 4). The lowest number of strand breaks was detected in fish from King Harbor (TM = 2.0), while the greatest number occurred in fish from LA/Long Beach Harbor (average TM = 10.3). Among the locations that had data for more than one fish, there were no significant differences in the level of DNA damage ($p = 0.12$, Kruskal-Wallis).

DNA damage in fish from bays and harbors collected in 1998 could not be compared to background levels in reference fish because the red blood cells in Camp Pendleton fish had degraded during storage, and the TM values from 1998 were based on both red and white blood cells. However, comparisons among fish collected in 1999 (from the Camp Pendleton reference site, and during additional sampling events in Long Beach Harbor, Alamitos Bay, and Marina del Rey) can be made, since data from the white blood cells were usable. Locations where three or more fish were collected had a significant difference in the level of DNA damage in white blood cells ($p < 0.01$, ANOVA). California halibut from Alamitos Bay had a significantly higher level of DNA

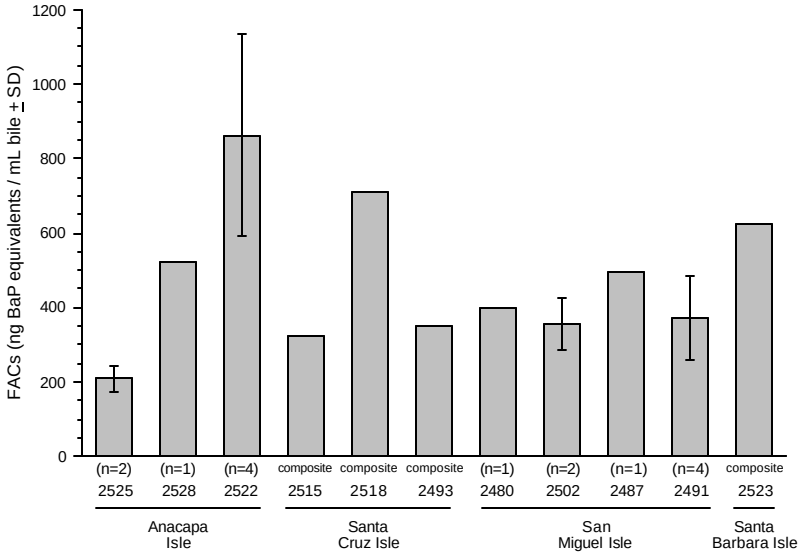

Figure 3. Concentration of bile FACs in Pacific sanddab from each Channel Islands station. n = Number of fish at a given station.

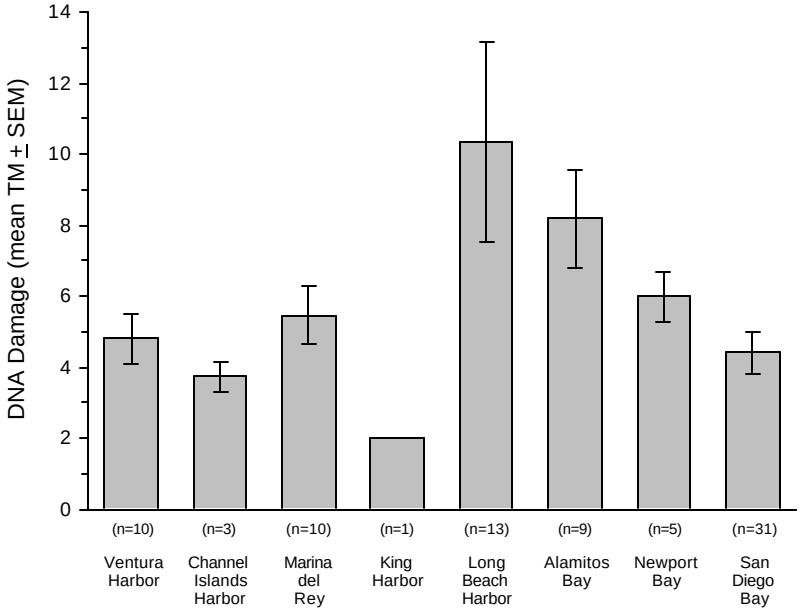

Figure 4. DNA damage in California halibut blood cells for each bay and harbor location from 1998. n = Number of fish at a given location.

damage (TM = 19.3) than fish from the Camp Pendleton reference site (TM = 4.6), while fish from Marina del Rey (TM = 8.9) were not significantly different (Dunnett's Method).

The blood samples for fish from the Channel Islands were compromised. Most of the Pacific sanddab were dead before dissection, and blood samples for DNA damage analysis were unusable. Consequently, the level of DNA damage could not be determined for fish from the Channel Islands.

Biomarker Response versus Sediment Contamination

Six locations had both biomarker and sediment contamination data: Ventura Harbor, Channel Islands Harbor, Marina del Rey, LA/Long Beach Harbor, Newport Bay, and San Diego Bay. Sediments from Alamitos Bay and King Harbor were not analyzed for contaminants. While FAC concentrations were elevated in fish from bays and harbors, there was no significant relationship between the concentration of FACs in fish bile and the concentration of high molecular weight PAHs in sediments ($r^2 < 0.01$), or the total organic carbon normalized concentration of high molecular weight PAHs in sediments ($r^2 = 0.03$). Likewise, there was no significant association between DNA damage and the total concentration of select metals (the sum of Ag, Cd, Cr, Cu, and Pb) ($r^2 < 0.01$), total PCBs ($r^2 = 0.06$), total DDTs ($r^2 = 0.11$), high molecular weight PAHs ($r^2 = 0.50$, $p = 0.11$), or total PAHs ($r^2 = 0.50$, $p = 0.12$).

Sediments from the Channel Islands stations were not analyzed for contaminants during Bight'98. Therefore, the relationship between sediment contaminants and Pacific sanddab biomarkers could not be characterized.

FAC Concentrations versus DNA Damage

There was no significant relationship between concentration of FACs and DNA damage when indicators in fish from all bay and harbor locations were compared ($r^2 = 0.06$) (Figure 5). However, the relationship was stronger between indicators for some locations when data from individual locations were compared. The incidence of DNA damage showed a significant relationship with higher concentrations of bile FACs in fish from Marina del Rey ($r^2 = 0.49$, $p = 0.04$) and Ventura Harbor ($r^2 = 0.56$, $p = 0.05$) (Figure 6). The relationship was not significant in fish from Alamitos Bay ($r^2 = 0.30$, $p =$

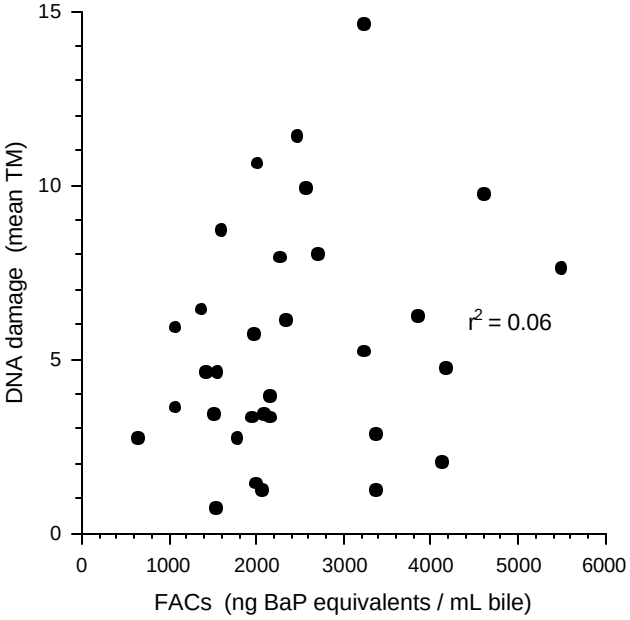

Figure 5. Relationship between bile FACs and DNA damage in California halibut. Data are for all individual fish from bays and harbors that had both types of biomarkers analyzed.

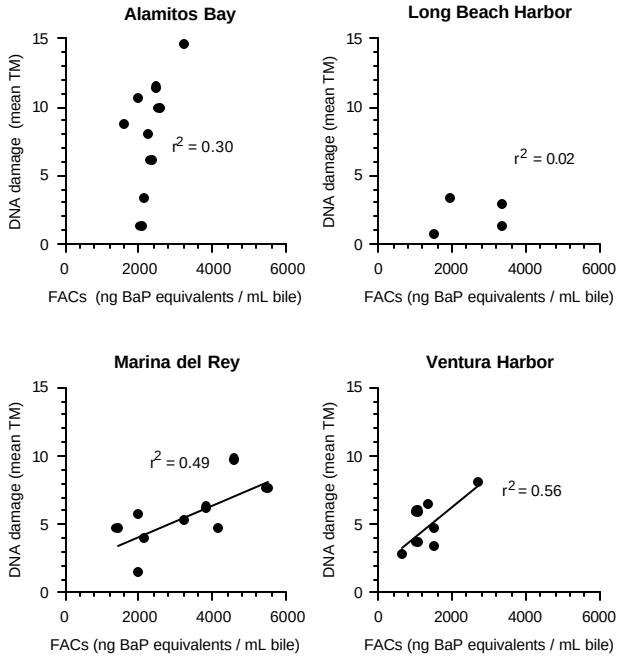

Figure 6. DNA damage and bile FACs in individual California halibut for bay and harbor locations that had data for more than two fish. The relationship between indicators was significant for fish from Marina del Rey and Ventura Harbor ($p \leq 0.05$).

0.13), or Long Beach Harbor ($r^2 = 0.02$, $p = 0.85$). Differences in the relationship between indicators at the various locations is also evident in the slopes of the regression lines (Figure 6).

DISCUSSION

The concentrations of FACs in California halibut from most bay and harbor locations were elevated compared to fish collected off Camp Pendleton, an area containing lower concentrations of sediment PAHs, suggesting that California halibut are exposed to elevated levels of PAHs at most bays and harbors in southern California. This pattern is consistent with the National Oceanic and Atmospheric Administration's (NOAA's) National Benthic Surveillance Project, which found elevated FAC levels in fish collected from urban areas of Puget Sound, San Francisco Bay, Long Beach Harbor, and San Diego Bay (Varanasi *et al.* 1989). For example, the mean concentrations of FACs in white croaker (*Genyonemus lineatus*) collected from Long Beach Harbor and San Diego Bay in the mid-1980s were 2 and 31 times higher, respectively, than the concentration in white croaker from a reference site off Dana Point, California (Varanasi *et al.* 1989). That study also found the mean concentration of FACs in barred sand bass (*Paralabrax nebulifer*) collected at a San Diego Bay station to be over 100 times higher than the concentration in bass from Dana Point (Varanasi *et al.* 1989). The mean concentration of FACs in California halibut from San Diego Bay in the present study was elevated compared to the reference value, but was not found to be significantly different. The statistical power for the current data, however, may not have been sufficient to detect a significant difference, since the FAC data from the San Diego station were comprised of two fish. Moreover, because the FAC data are for a single site within San Diego Bay, they may not be representative of the PAH exposure in other parts of the Bay.

FAC concentrations in Pacific sanddab from most of the Channel Islands stations were equivalent to those in California halibut from the Camp Pendleton reference site, and less than half of the concentrations in California halibut from bay and harbor locations, suggesting that their PAH exposure is low. However, potential differences between species in the uptake, metabolism, and excretion of PAHs preclude interspecies comparisons (Stein *et al.* 1995). With the exception of the FAC concentration in fish

from Station 2522 (862 ng BaP equivalents/mL bile), Pacific sanddab FAC values in this study (209-709 ng BaP equivalents/mL bile) were lower than those found in Pacific sanddab from a reference site off Dana Point in 1997 (741 ng BaP equivalents/mL bile), and much lower than the mean concentration in fish captured near the City of Los Angeles' wastewater outfall system in Santa Monica Bay (1021 ng BaP equivalents/mL bile) (unpublished SCCWRP data). The average FAC concentration at Station 2522 was intermediate between the concentrations at Dana Point and the City of Los Angeles' wastewater outfall system. The source of the PAH exposure in fish from Station 2522 is unknown. Concentrations of FACs were not related to distance from the mainland, or distance from the islands.

Previous studies using laboratory exposures have found agreement between PAH exposure and FACs in fish bile. For example, Varanasi *et al.* (1985) found elevated concentrations of FACs in fish exposed to PAH-contaminated sediments, compared to fish exposed to reference sediments, and Collier and Varanasi (1991) found excellent dose-responses in fish injected with either BaP or a solvent extract of PAH-contaminated sediment. While FACs were elevated in fish from bays and harbors in the present study, FAC concentrations were not related to concentrations of PAHs in sediments. Likewise, levels of DNA damage were not related to concentrations of metals, PAHs, PCBs, DDTs, or chlordanes in sediments. This is not unexpected for areas that have a high variability in concentrations of sediment contaminants. Because fish move around within each location, the exposure they receive is likely to be variable, and not necessarily correlated with contaminant concentrations at discrete sites. Sediment data for Marina del Rey demonstrate how variable PAH concentrations can be over small distances. Concentrations of high molecular weight PAHs in sediments varied by a factor of eight in this marina, ranging from 196-1623 µg/kg dry wt. The results in the present study illustrate the importance of measuring the actual exposure in fish, rather than inferring the exposure from concentrations of PAHs in sediments.

Some laboratory experiments with fish have indicated there is no consistent relationship between FAC concentrations and DNA damage. Exposure of bluegill sunfish (*Lepomis macrochirus*) to BaP in a laboratory experiment leads to an increase in DNA damage in liver cells initially, but a similar level of damage to control fish was observed after 30 d of exposure (Shugart 1988). Theodorakis *et al.* (1992) found consistent FAC concentrations in bluegill sunfish over a 40-week exposure to contaminated sediments, but fluctuations in liver cell DNA damage over this same period. Di Giulio *et al.* (1993) found significant damage in channel catfish (*Ictalurus punctatus*) exposed in the laboratory to contaminated sediments after 28 d, but the damage was much less than it was after 14 d. In the present study, there was a significant association between indicators at Marina del Rey and Ventura Harbor, but not at other locations. Variation in slopes of the regression lines may reflect differences in the types and amounts of contaminants that fish are exposed to at each location, since DNA damage can be caused by a variety of environmental contaminants, in addition to high molecular weight PAHs. Alternatively, the differences in slopes may indicate that the relationship between PAH exposure and DNA damage in circulating blood cells was variable.

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