ASSESSMENT OF THE PHOTOENHANCED TOXICITY OF DILUENT TO THE TIDEWATER SILVERSIDE

Final Report

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December 8, 1998

ABSTRACT

Studies were conducted to determine the interactive toxicity of a water-accommodated fraction of total petroleum hydrocarbons from diluent and solar radiation to an estuarine organism, the tidewater silverside (*Menidia beryllina*). Light treatments for the toxicity tests were based on incident sunlight intensity and spectra measured in the vicinity of an abandoned oil field in California. Juvenile silversides were monitored for survival and growth during a seven-day static-renewal exposure to dilutions of water accommodated fractions (WAF) of diluent collected in the vicinity of the abandoned oil field. Exposure to UV alone was not lethal to the fish. WAF in the absence of UV was toxic at the highest concentration (3.03 mg/L) tested after 96 hours of exposure, whereas at the high UV treatment significant mortality occurred at the highest WAF concentration within 24 hours of exposure and significant mortality was observed at the lowest concentration by day 7 of exposure. Accordingly the 7 day LC50 concentrations ranged from 2.84 mg/L for the reference (control) irradiance treatment; 1.27 mg/L, for the low irradiance treatment; 0.93 mg/L, for the medium irradiance treatment; and 0.51 mg/L, for the high irradiance treatment. Photoenhanced toxicity was also evident through impaired growth, with LOECs for growth declining from 3.03 mg/L under reference irradiance treatments to 0.70 - 1.50 mg/L TPH under UV irradiance. Significant mortality occurred during UV exposure among fish that had been previously exposed to WAF in the absence of irradiance, whereas the toxicity of the WAF was unaffected by UV exposure prior to the toxicity test, thus the mode of action is a photosensitization of the accumulated petroleum residue rather than a photomodification of WAF. Chemical analysis indicates that the WAF is composed primarily of naphthalene, as well as other parent and alkyl homologs for 2- and 3- ring PAH compounds, including substantial concentrations of nitrogen-, oxygen-, or sulfur-substituted heterocyclic compounds that may also be photoenhanced.

INTRODUCTION

Extensive use of petroleum products results in numerous chances for these products to enter aquatic ecosystems. Evaluations of the impact of these perturbations has commonly considered toxicity relative to a range of water quality variables present at the site of contamination. Several investigations have shown the importance of considering solar radiation, particularly ultraviolet (UV) radiation in the assessment of petroleum contamination since UV can significantly increase the toxicity of such products (Newsted and Geisy 1987; Oris and Geisy 1985, 1987; Ankley et al. 1995; Pelletier et al. 1997).

Photoenhancement of toxicity has been found to occur with common components of petroleum, the poly aromatic hydrocarbons (Allred and Geisy 1985). Other studies have demonstrated that the toxicity of whole petroleum was similarly photoenhanced. Scheier and Gominger (1976) found that the toxicity of the water accommodated fraction (WAF) of # 2 fuel oil increased when the WAF was exposed to UV prior to the toxicity test. Recently, Pelletier and coworkers (1997) found a similar photoenhanced toxicity of # 2 fuel oil, Arabian light crude, Fuel oil # 6, and Prudhoe Bay crude oil in the presence of UV light. Studies by Pelletier et al. (1997) and Oris et al. (1990) have indicated that limited UV irradiance is required to induce photoenhanced toxicity. It is particularly important to understand the extent to which UV is effective because water quality, particularly dissolved organic carbon, can significantly limit UV penetration in the water column (Scully and Lean 1994). In the present study we evaluated the toxicity of a diluent over a range of site-relevant UV irradiances. Specifically our objectives were to determine the individual and combined impacts of UV and WAF of diluent at environmentally relevant UV intensities and to determine if photoenhanced toxicity is caused by photoactivation or photosensitization.

TPH was selected as an appropriate measure of toxicity in photoenhanced toxicity tests because: (1) TPH accounts for most constituents in diluent and quantifies the complex mixture of hydrocarbons, rather than accounting for only a small fraction (Stratus Consulting, 1998a); (2) specific components of diluent have not been identified as the single or primary determinants of diluent toxicity (Stratus Consulting, 1998b); (3) the most comprehensive exposure data set at the site is TPH in surface water (Hagler Bailly, 1997); and (4) toxicity thresholds and exposure concentrations were developed using the same analytical chemistry methods, thus field and laboratory TPH values are directly comparable. Additionally, in evaluating the toxicity of complex mixtures of petroleum hydrocarbons, rather than evaluating the toxicity of individual analytes, it is common practice to express exposure as a TPH concentration (e.g., Anderson et al., 1974; Markarian et al., 1995).

MATERIALS AND METHODS

General: Randomized experimental designs were used to expose *Menidia beryllina*, to dilutions of water accommodated fractions (WAF) of diluent collected from an abandoned oil field in the

presence of simulated solar radiation intensities. The organisms were exposed in 7-day static renewal tests to five WAF dilutions and a control treatment which was dilution water with no WAF. Each WAF dilution and control was tested under 4 different simulated solar radiation intensities. Three replicates of each WAF dilution/light intensity were tested. The tests were conducted according to procedures described in Klemm et al. (1994), ASTM (1993), and Little and Fabacher (1996).

The test organism *Menidia beryllina* obtained from Aquatic Indicators (St. Augustine, Florida) were received 48 h before testing and held at 20°C in 20 0/00 saline water prepared with solutions of well water and 40 Fathoms Crystal Sea Salt that had been aged for two weeks. The fish were fed brine shrimp (*Artemia sp.*) daily prior to testing.

WAF Preparation: Diluent was collected from the underground free-product plume at the 5X monitoring well in the abandoned oil field described in Hagler Bailly (1997b). The samples were dewatered and composited, then shipped by overnight courier to the Columbia Environmental Research Center (CERC) in 1 liter amber glass bottles chilled (12-17 °C) with blue ice. The samples were refrigerated at 4° C prior to use. A slow-stir apparatus was used to prepare the water accommodated fraction (WAF) of the diluent (Anderson et al. 1974). A Teflon stirbar and a 20 mm glass tube was placed into a one-liter screw-top glass jar. 800 ml well water was added to the jar then 80 ml of diluent was added gently to the surface of the water. The jar was sealed with the Teflon-lined screw cap and the mixture was slowly stirred to avoid formation of a water diluent emulsion (100 $\pm$ 20 RPMs) for 24 $\pm$ 2 hours in a fume hood at room temperature. A Teflon tube was inserted through the glass tube to siphon off the WAF without disturbing the overlying layer of diluent. The solutions of diluted WAF were adjusted to a salinity of 20 parts per thousand (0/00) daily with appropriate volumes of a 33 0/00 aged brine solution.

Solar Simulation: The toxicity tests were conducted in a solar simulator equipped with cool white, UVB (peak wavelength 313 nm), UVA (peak wavelength 365 nm) fluorescent lamps and halogen flood lamps. Simulator dimensions were approximately 1 meter wide times 2 meters long, with lamps suspended over a water bath of similar dimensions and enclosed with a highly reflective NIST specular aluminum (Little and Fabacher 1996). Water bath temperature was maintained by a recirculating water chiller. The cool white and UV-A fluorescent lamps were controlled by a timer to operate for 14 hours each day. The UVB lamps were activated with a second timer to operate for 4 hours each day. The UV-B photoperiod began five hours after the onset of the white light and UV-A photoperiod. These photoperiods were comparable to an August photoperiod along the central coast of California and were of sufficient length to ensure that the exposed organisms had sufficient irradiance to utilize photorepair mechanisms.

All radiometric measurements were performed with an Optronics Model OL-754 spectroradiometer over a wavelength range of 280 to 700 nm at 1 nm intervals to document the spectral quality and intensity of irradiance treatments during the toxicity tests. The radiometer was calibrated with a NIST-traceable lamp, and radiometer voltage gain and wavelength accuracy were checked during the measurements. The light intensity across the area of simulator

water bath was confirmed by measuring surface irradiance through each filter treatment at 12 locations in the water bath. Underwater irradiance was measured at fixed locations in the simulator using all filter combinations used to generate the test light treatments and to ensure that the output of the simulator lamps remained consistent. The intensity did vary over the 7 day exposure.

The irradiance treatments applied during the toxicity tests were representative of the quality and intensity of natural solar radiation measured in various habitats (intertidal, surf, estuary, marsh ponds) important for estuarine organisms such as *M. beryllina* in the vicinity of the abandoned oil field (Hagler Bailly, 1997). The irradiance treatments were selected on the basis of UV-B intensities because the UV-B wavelengths are most harmful to aquatic organisms. The manipulation of UVB intensities also reduced the intensity of UVA and visible light. Generally, this resulted in an irradiance treatment that approximated irradiance that would occur as sunlight is attenuated in the water column of natural aquatic habitats. The light filtering materials used to generate the irradiance treatments were (1) 0.79 mm thick polycarbonate, (2) 0.79 mm thick UVF polystyrene, (3) 0.39 and 0.13 mm thick cellulose acetate, (4) 0.13 mm thick Mylar, (5) 51% shade cloth and (6) aluminum foil. The materials were selected because of their capacity to filter simulated solar radiation, their stability over time, and their capacity to produce consistent filtering over time. The sides and the top of the exposure chambers were covered with the filtering materials. The nominal simulated solar radiation treatments ranged from a low of 0.12 $\mu w/cm^2$ to a high of 17 $\mu w/cm^2$. The reference light treatment used as a control in the toxicity tests (UVB-0.002 $\mu W/cm^2$; UVA-3.2 $\mu W/cm^2$; visible-247 $\mu W/cm^2$) was the lowest possible irradiance that provided sufficient visible light within the chambers to allow feeding and provided UVB irradiance somewhat lower, and visible irradiance somewhat greater than average office-like lighting (UVB-0.21 $\mu W/cm^2$; UVA-3.2 $\mu W/cm^2$; visible-98 $\mu W/cm^2$) provided in the laboratory. To maintain consistent lighting conditions during the toxicity tests, filter combinations were replaced daily during the test to control for the photolytic degradation of the filtering material.

WAF /UV Exposure Procedures: Randomized experimental designs were used to expose *M. beryllina* in 7-day static renewal tests to 0.63, 1.25, 2.50, 5.0 and 10% dilutions of WAF and a control treatment (dilution water without oil). These exposures were conducted under reference low, medium, and high simulated solar radiation treatment according to procedures described by Klemm et al. (1994). Three replicates of each treatment were tested. On day -1 of the test, the WAF was prepared as described above, and day 0 batch dilutions were prepared with graduated cylinders and volumetric flasks. Ten *Menidia* were exposed to 200 mL of the WAF dilution prepared with 20 parts per thousand (0/00) saline water in 250 mL glass beakers.

On day 0 of the test a subsample of 25 fish were measured (total length), pooled and dried in an oven at 60° C for 24 hours to obtain initial dry weights. To start the test, 10 fish were counted into 30 mL beakers containing 20 0/00 saline water and maintained at 20° C. The fish were then randomly dispensed to the exposure beakers which were placed in a temperature-controlled water bath under the solar simulator. A randomization schematic was used to assign

each treatment replication to a position in the solar simulator water bath. Light filters were placed over the beakers to obtain the desired light intensity and spectra. The exposure beakers and filter covers were labeled with colored tape to identify light treatments, and WAF concentrations were marked on the sides and top of each beaker. The solar simulator was checked daily for lamp function, photocycle intervals, water bath temperature, water bath water level, and recirculating flow.

Temperature in the water bath was recorded daily. The pH, oxygen, and salinity of the batch dilutions were measured once on day 0 of the test. The pH, oxygen, and salinity of twelve randomly selected test beakers were monitored daily during the test following a randomized sample schedule. Fish in each test beaker were fed 1 mL of concentrated *Artemia* daily. The fish were fed at least two hours before the daily renewals of test solutions were performed. The amount of food was reduced proportionately as mortality occurred during the test. Seventy-five percent of the exposure volume in each replicate test beaker was removed daily with a large pipette and replaced with dilutions of fresh WAF. Mortality and fish showing loss of equilibrium were recorded daily. On day 7, the dry weight of fish surviving each treatment was measured.

Photoactivation and Photosensitization Tests: Tests were conducted to determine if UV photoenhanced toxicity was induced through structural changes in the oil (*in vitro* photoactivation) or through oxidation of tissue bound petroleum residues (*in vivo* photosensitization). In tests for photoactivation, duplicate 5% WAF and control saline solutions were exposed to UV for 24 hours prior to testing. Additional WAF and saline solutions were wrapped with foil but held under similar conditions. Following the UV exposures, 10 fish were added to each of the replicate solutions and were exposed for 48 hours under reference lighting conditions. In tests for photosensitization, fish were exposed to control and 5% WAF solutions for 48 hours under reference lighting conditions, then transferred to clean water where they were exposed to the 4 $\mu W/cm^2$ UVB treatment for 48 hours.

WAF Chemistry and Sampling: WAF samples were analyzed for semi-volatiles, expressed as total petroleum hydrocarbon (TPH), and volatiles, expresed as and benzene, toluene, ethylbenzene, and xylene (BTEX). The samples were taken from batch dilutions of new WAF and from the exposure chambers during the toxicity test. Sample volumes ranged from 0.25 to 1.0 L. All samples were gently transferred to pre-cleaned amber glass sample bottles (TPH analysis) or 40 mL volatile organic analysis vials (BTEX analysis) and stored in the dark at 4°C until they were analyzed.

WAF concentrations of 0, 0.63, 1.25, 2.5, 5, and 10% were used in the *Menidia* toxicity test. Initial (newly prepared test solutions, test days 0 and 6) and final (pooled test solution sampled 24 hours after renewal, test days 1 and 7) samples of the 0, 0.63, 1.25, 5, and 10% WAF solution were collected for analysis of TPH. Separate samples were collected from each light treatment (reference , low, medium, high). Initial samples of 10% WAF were sampled once daily on days 0 to 6 to assess variability in TPH concentrations in newly prepared WAF across

preparation days. TPH samples were extracted and analyzed for semi-volatiles using a gas chromatography/mass spectrometry (GC/MS) modified from EPA method 3510 (Stratus Consulting 1998a). The minimum detection limit ranged from 0.05 to 0.2 mg/L TPH, depending on the collected sample volume. Initial and final samples for each WAF and light treatment combination were collected at test end and analyzed for BTEX compounds following EPA method 8260 (Stratus Consulting 1998b). The minimum detection limit was 0.0005 mg/L for each analyte.

Statistical Analysis: Data collected at the end of the exposures (day 7) were analyzed as an irradiance versus WAF factorial arrangement of treatments. The one-tailed Dunnett's test (Dunnett 1955) was used to compare all treatment means. Because of a significant light-WAF interaction term, ANOVAs were performed for each light treatment using its 0 % WAF treatment as a control. ANOVA and the Dunnet's test were used to determine no-observed-effect concentrations (NOECs) and lowest-observed-effect concentrations (LOECs). Arcsine square root transformations were performed on all mortality data before analysis. Daily mortality data were statistically analyzed. The Toxstat^{R} computer program (TOXSTAT^{R} V3.5, 1996), which incorporates control mortality corrections, were used to calculate seven-day LC50 and LC20 values as TPH concentrations within each light treatment. EC50 values were estimated by incorporating one-half of the control weights for *Menidia* into the regression line formula. EC20 values were calculated in a similar manner. All computations were performed using Statistical Analysis System (SAS 1985) computer programs. Confidence intervals were only calculated if regression coefficients were significant (Snedecor and Cochran 1980). ANOVA was used to evaluate the effect of light regime (four treatments), WAF level (six TPH concentrations including the control), and sample time (day 1 versus day 7 final samples) to determine differences in TPH concentrations resulting from exposure of test solutions to the test light treatments (reference, low, medium, high intensities); as well as differences in test concentrations between sample days.

RESULTS

During the 7-day exposure, salinity ranged from 20 to 21.7 0/00; dissolved oxygen ranged from 6.1 to 7.4 mg/L; pH ranged from 7.7 to 8.5; and temperature ranged from 19.5 to 20° C. All measurements were within the range for test acceptability recommended by Klemm et al.(1994).

In evaluating the toxicity of complex mixtures of petroleum hydrocarbons, rather than evaluating the toxicity of individual analytes, it is common practice to express exposure as a TPH concentration (e.g., Anderson et al., 1974; Markarian et al., 1995). Measured TPH test concentrations corresponded to the nominal WAF dilution and ranged from below the detection limit in the control (0. 00) to 3.0 mg/L TPH in the highest treatment level (10% WAF) (Table 1). Measured TPH concentration for the 0.63 % WAF dilution was also below the detection limit

and was estimated as one-half of next highest test concentration. The results of 3-way ANOVA of % WAF, irradiance treatment, and time showed that TPH concentrations significantly increased as percent WAF content increased ($p = 0.0001$). Newly prepared 10% WAF sampled on multiple test days did not vary significantly in TPH concentration ($p = 0.14$, Day 0 sample excluded). The sample day (day 1 versus day 7) did not significantly affect TPH concentrations of test solutions ($p = 0.14$). Light treatment also did not significantly affect TPH concentrations of test solutions ($p = 0.40$) and there were no significant WAF level-light treatment interactions ($p = 0.99$). The results of 2-way ANOVA also indicated that light treatment did not significantly affect TPH concentrations of test solutions ($p = 0.24$). Based on these results, we concluded that TPH concentrations were not affected by the light treatments used in the photoenhanced toxicity tests.

Total BTEX (sum of benzene, toluene, ethylbenzene, and xylenes) concentrations (mg/L) in the Menidia test were less than 0.007 mg/L (Figure 1). At the highest test concentration (10% WAF), the initial concentrations of toluene (0.0016 mg/L), ethylbenzene (0.0007 mg/L), and xylenes (0.0041 mg/L) declined to below 0.0005 mg/L after 24 hours. WAF at 100% contained low concentrations (<10 μg/L per analyte) of n-alkanes (predominantly C12 to C14) and 195 μg/L of total PAHs of which 67% were naphthalenes (25 to 35 μg/L per analyte) WAF also contained C3 to C6 alkyl benzenes and parent- and alkyl-aromatic heterocycles (140 to 6,600 μg/L). Benzene, toluene, ethylbenzene, and xylenes (BTEX) were very low (<0.05 mg/L). The measured heterocycles in each WAF included quinolines, carbazoles, thiophenes, benzothiophenes, and benzofurans (2 to greater than 100 μg/L per analyte). C2 to C4 alkyl phenols (1000 to 3000 μg/L) were the predominant heterocycle analytes in WAF.

Measured UV-B irradiance ranged from 0.002 $\mu W/cm^2$ UV-B in the reference light treatment to 16.6 $\mu W/cm^2$ in the high light treatment (Table 2). The UV-A and visible light intensities in the high light treatment were lower compared to the medium light treatment. This inconsistency resulted from the light filtering properties of the materials used to produce the high light treatment and could not be avoided. Generally, the four light treatments tested approximated the irradiance that would occur as sunlight is attenuated in the water column of natural systems (Figure 2).

***Menidia beryllina* Survival:** ANOVA performed on the mortality data revealed highly significant toxicity of TPH and interactions between TPH treatment, duration of exposure and light treatment (Table 3 and Figure 3). Mortality significantly increased with increasing TPH concentration, increasing UV irradiance, and increasing duration of exposure compared to similar TPH treatments under reference irradiance conditions or when compared with the TPH control treatment within each irradiance treatment. Exposure to TPH under reference irradiance conditions caused significant mortality at the highest TPH concentration (3.03 mg/L) which occurred on Day 4 of exposure (Figure 3a). Under low irradiance conditions (0.12 $\mu W/cm^2$ the 3.03, and 1.5 TPH induced mortality which became significant by the Day 2 of exposure. Under intermediate irradiance conditions (4 $\mu W/cm^2$) concentrations of 0.7 mg/L and higher caused significant mortality which first occurred among fish exposed to 3.03 mg/L on day 1; among fish

exposed to 1.5 mg/L on day 2; and among fish exposed to 0.7 mg/L on day 6 of exposure. Under the high irradiance concentration, significant mortality was observed among all TPH treatments beginning on day 1 at 3.03 mg/L, day 3 at 1.5 mg/L, day 5 at 0.7 and 0.12 mg/L. Within each light treatment a significant increase in the mortality of *M. beryllina* was directly related to TPH concentration and duration of exposure. For example, at day 4 of exposure, a 63 to 73 % mortality occurred among fish exposed to 1.50 mg/L under the medium and high irradiance conditions (Fig 3a). By day 7 of exposure mortality in the 1.5 mg/L treatment was 100% in the high, 95% in the medium, and 60% in the low irradiance treatment, and a 43% mortality occurred among fish exposed to 0.7 mg/L (Fig 3b). Mortality was not significantly affected by the light treatments in the absence of TPH ($p = 0.09$).

***Menidia beryllina* Growth:** Within the reference light treatment, growth of *M. beryllina* exposed to 3.03 mg/L TPH was significantly decreased ($p = 0.001$) compared to control fish (Table 4). Significant reductions in growth were observed for *M. beryllina* exposed to 0.70 mg/L TPH within the low light treatment ($p = 0.0001$), but in the next higher TPH concentration (1.50 mg/L) growth was similar to that of the control fish ($p = 0.925$). In the medium and high irradiance treatments, no significant effects ($p > 0.05$) on growth occurred among *M. beryllina* that survived exposure to concentrations of TPH up to 0.70 mg/L (Figure 4). Growth was not determined for *M. beryllina* exposed to 3.03 mg/L TPH in the low light treatment and to 1.50 and 3.03 mg/L TPH in the medium and high light treatments because survival was significantly reduced. Generally, the test met acceptance criteria for growth of 0.5 mg for the control treatment (Klemm et al. 1994), except for the TPH control treatment within the high light treatment.

***Menidia beryllina* Toxicity Estimates:** Increased irradiance potentiated the effects of TPH on *M. beryllina* mortality. At day 4 of the study, NOEC values for *M. beryllina* mortality ranged from 1.50 mg/L TPH in the reference light treatment to 0.70 mg/L TPH in the high light treatment (Table 5). Effects on mortality were more severe at day 7 and NOEC's ranged from 1.50 mg/L TPH in the reference light treatment to less than 0.12 mg/L TPH in the high light treatment (Table 5). The NOEC for *M. beryllina* exposed to the light treatments in the absence of TPH was 17 $\mu W/cm^2$, the highest intensity tested. Similarly, at day 4 of the study LOEC values decreased from 3.03 mg/L TPH in the reference light treatment to 1.50 mg/L in the high light treatment: at day 7 LOEC values decreased from 3.03 mg/L TPH in the reference light treatment to 1.50 mg/L TPH in the high light treatment (Table 5). The four and seven-day LC50 and LC20 values for *M. beryllina* show that TPH becomes significantly more toxic as irradiance and exposure time increase (Table 5). The LC50 at day 4 ranged from > 3.03 mg/L for the reference irradiance to 1.09 for the high irradiance treatment. At day 7 the LC50 ranged from 2.84 mg/L for the reference irradiance to 0.51 mg/L for the high irradiance treatment.

The NOEC and LOEC was 1.50 and 3.03 mg/L TPH, respectively for growth of *M. beryllina* exposed under the reference light treatment. Under the low light treatment the NOEC , and LOEC decreased to 0.24 and 0.70 mg/L TPH, respectively; however, these values are

conservative approximations based on the significant reduction in growth observed at 0.70 mg/L TPH but not at the next higher concentration of 1.50 mg/L TPH. The NOEC for *M. beryllina* growth under the medium and high light treatments was 0.70 mg/L TPH and the LOEC is estimated as a range between the NOEC and the next higher TPH concentration of 1.50 mg/L in which total mortality occurred. The estimated EC20 and EC50 were 3.0 and 8.0 mg/L TPH, respectively, for *M. beryllina* growth in the reference light treatment. The EC20 and EC50 values could not be calculated for the low, medium and high light treatments due to non-significant regression coefficients.

Photosensitized Toxicity: Fish receiving UV exposure in uncontaminated saline water had significant mortality ($p = 0.04$) if they had been previously exposed to 5% WAF (Figure 5). Mortality among fish formerly exposed to WAF but shielded from UV exposure was similar to that of fish that had not been exposed to WAF ($p = 0.778$). Therefore, the photoenhanced toxicity appears to be the result of photosensitization of tissue-bound petroleum.

UV exposure of the test solution prior to use in the fish exposure did not result in significant mortality ($p > 0.05$). Therefore, the photoenhanced toxicity does not appear to be caused by photoactivation of the parent compound in the test solution.

DISCUSSION

The results of this investigation clearly indicate that the toxicity of the water accommodated fraction of diluent was significantly increased in the presence of UV radiation. Under UV conditions, the mortality rates were 12 fold higher as compared to mortality induced by WAF in the absence of UV. Mortality varied with time across treatments with mortality induced at day 1 of exposure by the highest WAF concentration to day 5 of exposure for the lowest WAF concentration under the highest irradiance conditions. Mortality also varied with UV treatment, with significant mortality occurring at the highest WAF treatment on day 1 of exposure compared to mortality occurring on day 4 of exposure under the reference irradiance conditions.

These results are consistent with other studies that have shown oil products and components of petroleum are photoenhanced by UV radiation (Arfsten et al. 1996). For example, the toxicity of an Arabian light crude to *Mysidopsis bahai* doubled in the presence of UV light (Pelletier et al. 1997). The PAH composition of crude oil plays a major role in the photoenhanced toxicity of crude and refined petroleum. The toxicity of the PAH, anthracene, to bluegill increased by 1800 times in the presence of UV (Oris and Geisy 1985) and the toxicity of individual PAH compounds to marine invertebrates increased by over 50,000 times (Pelletier et al. 1997). In the present study, previously documented photo-modifiable PAHs present in WAF such as anthracene, fluoranthene, and pyrene were at very low levels (175 ug/L total PAH concentration) in the undiluted (100%) WAF. WAF prepared from diluent was low in 3 ring and larger PAHs, including known photoactivated chemicals (Stratus Consulting, 1998). Thus TPH was used as the measure of petroleum exposure in photoenhanced toxicity tests because diluent

toxicity was not obviously linked to any specific PAH or total PAH concentration.

Although most investigations of the photoenhanced toxicity of petroleum have focused on a few non-alkylated PAHs such as anthracene, other petroleum components may also contribute to the photoenhanced toxicity of the petroleum. In contrast to unalkylated parent compounds such as anthracene, the alkylated forms are the dominant PAHs in crude oils and many refined products and their water accomodated fractions. QSAR modeling suggests that alkylation will have little effect on photoactivation. (Veith et al. 1995). In addition to PAHs, heterocyclic aromatics and their alkylated homologs are abundant in petroleum and can be photoactivated. Previous studies have identified acridine (Oris and Giesy 1987) and dibenzothiophenes as likely phototoxic compounds (Kosian et al. 1996). Water soluble fractions are likely to be enriched by these compounds because of greater heterocycle solubility. Bowling et al. (1983) found that a non-toxic concentration of anthracene (12 μg/L) was toxic at 0.03 μg/L to bluegill in sunlight. WAFs also contained a large unresolved complex mixture, which may include unidentified petroleum hydrocarbons or heterocycles contributing to the photoenhanced toxicity of the diluent.

The light intensity and spectra applied during the laboratory studies were similar to those measured in habitats adjacent to the abandoned oil field. The lowest UV irradiance applied during this study to cause photoenhanced toxicity was observed in the turbid marsh as well as the eutrophic lagoon habitats (Barron et al. In review). The high UVB intensity of 17 μW/cm^2 was intermediate between a high of 49 μW/cm^2 uW/cm2 observed at a depth of 16 cm measured at the beach and 15.2 μW/cm^2 UVB measured in the estuary at 10 cm. The effects of UV in the absence of WAF was evident in the reduced growth of *M. beryllina* exposed to the high irradiance level of 17 μW/cm^2 UVB. Although this irradiance level was within environmental limits, the test exposure conditions in the laboratory did not allow the fish any shelter or variation in photic conditions, such as wave action, shadows, or cloud cover and thus may have resulted in the reduced growth. The lower UV irradiances applied during this investigation did not impair growth or cause other grossly apparent effects. The intermediate exposure of 4 μW/cm^2 was less than the UV-B at depths of 10 cm at all sites except the shaded vegetated areas. The low UV-B treatment of 0.13 μW/cm^2 was comparable to UVB intensity at depths of 45 cm in the estuary, or approximately 1 % of surface irradiance (Hagler Bailly 1997). Thus it is clear that photoenhancement can occur in a range of habitats and should be of concern even when light penetration is low.

UV radiation at less than 10% of surface irradiance in Lake Michigan was sufficient to photo-enhance anthracene toxicity (Gala 1989). Water quality factors are also important to consider when evaluating the impact of photoenhanced toxicity of petroleum. The toxicity of anthracene was reduced by the presence of humic acids (Oris et al. 1990) presumably because of humic sequestration of the PAH as well as the reduction of UV in the water column. Ireland and Burton (1996) found that photo-induced toxicity of PAHs in storm water runoff at 7.9 μW/cm^2 UVB and 64 μW/cm^2 UVA. Pelletier et al. 1997 found that UV levels as low as 9.7 μW/cm^2 UVA and 3.4 μW/cm^2 UVB were sufficient to induce photoenhanced toxicity of water soluble fractions of # 2 fuel oil, Arabian light crude, Fuel oil # 6, and Prudhoe Bay crude oil and

toxicity of these increased significantly at higher UV fluence (307 $\mu W/cm^2$ UVB; 134 $\mu W/cm^2$ UVA). The fluorescent lighting used for the control conditions of the Pelletier et al. study also photoactivated the petroleum products and caused mortality. This irradiance treatment included UVB levels of 3.4 $\mu W/cm^2$ which was intermediate to the UVB treatments applied during the present study.

Photoenhanced toxicity of contaminants can occur through direct and indirect photomodification. Photooxidation (*in-vitro*) modifies the chemical to a more toxic form as a result of the energy absorbed by the parent compound (Ren et al. 1994: Zepp and Schlozhauer 1979). *In vivo* photosensitization occurs when the chemical (often tissue-bound) passes absorbed energy on to other chemicals forming reactive species such as free radicals (Landrum, et al. 1987; Newsted and Giesy 1987; Boese 1997). The toxicity observed in the present study was consistent with photosensitization mode of action observed for PAH chemicals, for when organisms were exposed to WAF in the absence of UV, then subsequently exposed to UV in clean water, they had significantly greater mortality than those not exposed to UV. In contrast, UV exposures of WAF had no effect on subsequent toxicity of the WAF in the absence of UV. These results suggest that organisms with petroleum residues may be at risk of photomediated toxicity if solar irradiance increases through the reduction of shade or increased water column clarity. A photoenhancement threshold concentration for anthracene residues was found to be 131 $\mu g/kg$ for juvenile bluegill (Oris and Giesy 1985).

A number of factors will influence photoenhanced toxicity in natural habitats. Solar angle associated with time of day, and season, air pollution, clouds, and surface reflection will influence UV irradiance levels. Water quality, especially humic acid concentration, will limit the amount of UV penetrating the water column and may also influence the availability of petroleum to the organism. Chemical concentration is also important, since a threshold concentration is implied. This threshold may vary with species because UV penetration through tissue may depend on pigmentation (Fabacher and Little 1995; Blazer et al. 1997). In studies with frog larvae (*Rana sphenocephala*) which have substantial amounts of dermal pigmentation, we found that the threshold for inducing photoenhanced toxicity of WAF was dependent on a UV exposure more than twice that required to induce toxicity in *M. beryllina* with the same concentration of WAF (Little, et al. in prep).

In conclusion, the results of this study indicate that the toxicity of photoactive compounds can be underestimated if photo-enhanced toxicity is not considered in the assessment of environmental risk. The photoenhanced toxicity of diluent demonstrated for *Menidia beryllina* is in agreement with responses observed for *Mysidopsis bahia* (Cleveland et al. 1998, Report), *Ceriodaphnia dubia* (Hurtubise et al. 1998, Report), and *Rana sphenocephala* (Little, et al. 1998, report) These results also indicate that photoenhanced thresholds vary with species and light intensity. However photoenhance toxicity was observed at environmentally relevant UV irradiances (Hagler Bailly 1997).

ACKNOWLEDGMENTS

This report was prepared through contract with the Department of Fish and Game Office of Spill Prevention and Response. Contract No. FG 4427 OS.

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Table 1. Percent dilution of a water accommodated fraction (WAF) prepared from diluent and corresponding total petroleum hydrocarbon (TPH) concentrations measured during a seven-day static renewal test with *Menidia beryllina.*[a]

Treatment (% WAF dilution)	Measured TPH (mg/L)[b]	Standard deviation	N
0 (control)	0.00	[c]	10
0.63	0.12 [d]	[d]	9
1.25	0.24	0.06	10
2.50	0.70	0.10	10
5.00	1.50	0.27	8
10.00	3.03	0.54	11

[a] Analytical chemistry methods and data are described in detail in Stratus Consulting, 1998b,
[a] Values are means for the duration of the study.
[c] All samples below detection limit.
[d] Estimated as one-half of next highest test concentration. All samples less than 0.14 mg/L TPH.

Table 2. Mean simulated solar irradiance with standard deviation in parentheses measured during exposure of *Menidia beryllina* to dilutions of a water accommodated fraction of diluent.

	Nominal irradiance (μW/cm^2)[a]			Measured irradiance (μW/cm^2)[b]			Filter combinations used to obtain UV-B treatments[b]
Light treatment	UV-B	UV-A	Visible	UV-B	UV-A	Visible	
Reference	0.002	3.0	260.0	0.002 (0.00)	3.17 (0.32)	246.60 (14.89)	Side wraps - one piece of 0.79 mm thick polycarbonate and one piece of 0.13 mm thick mylar : Top covers - two pieces of 0.79 mm thick polycarbonate and one piece of black, meshed shade cloth
Low	0.12	56.0	1664.0	0.13 (0.03)	56.65 (0.44)	1760.25 (40.70)	Side wraps - one piece of thick 0.79 mm thick polycarbonate and one piece of 0.0.13 mm thick mylar: Top covers - four pieces of 0.39 mm thick cellulose acetate
Medium	4.0	260.0	1670.0	3.67 (0.34)	253.00 (10.65)	1655.75 (30.08)	Side wrap - aluminum foil: Top covers - two pieces of 0.39 mm thick cellulose acetate
High	17.0	120.0	1000.0	16.58 (1.07)	131.00 (14.52)	1080.80 (183.40)	Side wraps - one piece of 0.79 mm thick polycarbonate: Top covers - one piece of black, meshed shade cloth

[a] Values represent integrated wavelength-specific intensities as follows: 280-320 nm for UVB, 320-400 nm for UVA, and 400 to 700 nm for visible light. Wavelength integrations were performed with scanning spectroradiometer software.

[b] Values based on three replicate measures.

Table 3. Percent cumulative mortality with standard deviations[a] in parentheses for *Menidia beryllina* exposed to total petroleum hydrocarbon (TPH) and varying simulated solar radiation treatments for seven days.

UV-B treatment[b] (μW/cm^2) and TPH concentration (mg/L)[c]	Day of Exposure						
	1	2	3	4	5	6	7
0.002 (μW/cm^2) - Reference	**Cumulative Percent Mortality**						
0	0	0	0	0	3.33 (0.06)	10 (0.10)	10 (0.10)
0.12 [d]	0	0	0	3.33 (0.06)	3.33 (0.06)	3.33 (0.06)	6.67 (0.06)
0.24	0	0	0	0	3.33 (0.06)	3.33 (0.06)	3.33 (0.06)
0.7	0	0	0	0	0	0	0†
1.5	0	0	3.33 (0.06)	3.33 (0.06)	10 (0)	13.33 (0.06)	16.67 (0.06)
3.03	0	0	6.67 (0.06)	13.33† (0.06)	26.67† (0.06)	43.33† (0.06)	56.67† (0.11)
0.12 (μW/cm^2) - Low							
0	0	0	0	3.33 (0.06)	3.33 (0.06)	10 (0)	13.33 (0.06)
0.12	0	3.33 (0.06)	6.67 (0.12)	6.67 (0.12)	6.67 (0.12)	10 (0.10)	10 (0.10)
0.24	0	3.33 (0.06)	3.33 (0.06)	13.33 (0.15)	20† ‡ (0.10)	20‡ (0.10)	20‡ (0.10)
0.7	0	0	6.67 (0.06)	6.67 (0.06)	16.67 (0.15)	16.67 (0.15)	16.67 (0.15)
1.5	0	16.67 † (0.15)	33.33 † (0.32)	33.33 (0.32)	43.33† (0.32)	46.67 (0.31)	60†‡ (0.20)
3.03	3.33 (0.06)	16.67† (0.06)	70† ‡ (0)	100†‡	100†‡	100†‡	100†‡

Table 3 continued

UVB treatment[b] (μW/cm^2) and TPH concentration (mg/L)[c]	Day of exposure						
	1	2	3	4	5	6	7
4.0 (μW/cm^2) - Medium	**Cumulative Percent Mortality**						
0	0	3.33 (0.06)	3.33 (0.06)	3.33 (0.06)	3.33 (0.06)	10 (0)	13.33 (0.15)
0.12	0	0	0	0	0	0	0
0.24	0	3.33 (0.06)	6.67 (0.06)	6.67 (0.06)	6.67 (0.06)	10 (0)	10 (0)
0.7	0	0	0	0	10 (0.10)	13.33 ‡ (0.06)	16.67 ‡ (0.12)
1.5	0	23.33 (0.32)	46.67†‡ (0.31)	73.33 †‡ (0.23)	86.67†‡ (0.12)	90†‡ (0.10)	100†‡ (0)
3.03	20 † (0.10)	70 †‡ (0.52)	100 †‡ (0)	100 † ‡	100†‡	100† ‡	100†‡
17.0 (μW/cm^2) - High							
0	0	6.67 (0.11)	6.67 (0.11)	10 (0.10)	10 (0.10)	13.33 (0.11)	23.33 (0.15)
0.12	0	0	13.33 ‡ (0.06)	23.33 ‡ (0.06)	43.33† ‡ (0.21)	43.33†‡ (0.21)	50†‡ (0.10)
0.24	0	0	10 (0.10)	16.67 ‡ (0.06)	20 ‡ (0)	30 ‡ (0.10)	30 ‡ (0.10)
0.7	0	13.33 ‡ (0.06)	23.33‡ (0.15)	30‡ (0.17)	36.67†‡ (0.15)	43.33†‡ (0.15)	43.33 ‡ (0.15)
1.5	0	20 (0.20)	56.67†‡ (0.15)	63.33†‡ (0.21)	73.33†‡ (0.21)	80†‡ (0.10)	96.67†‡ (0.06)
3.03	43.33†‡ (0.49)	93.33†‡ (0.11)	100†‡ (0)	100†‡	100†‡	100†‡	100†‡

† Denote significant difference from control within each light regime, ($P \leq 0.05$, Dunnetts Test).

‡ Denote significant difference compared to the same WAF treatment of the reference UV-B regime (0.002 μW/cm^2), ($P \leq 0.05$, Dunnetts Test).

[a] Means based on three replicates per treatment.

[b] Values represent integrated wavelength-specific intensities in the range of 280-320 nm for UVB. Wavelength integrations were performed with scanning spectroradiometer software.

[c] TPH concentrations were obtained from dilutions of a water accommodated fraction of a Guadalupe Assessment Site diluent sample.

[d] Measured TPH concentration for the 0.12 TPH treatment was below the detection limit and was estimated as one-half of next highest test concentration.

Table 4. Growth data with standard deviation in parentheses for *Menidia beryllina* exposed to total petroleum hydrocarbons (TPH) and four simulated solar radiation treatments for seven days.

TABLE 4
GROWTH OF *Menidia beryllina*

Nominal Light Treatment	TPH (mg/L)	Replicate	#*Menidia* per replicate (test end)	Mean Weight per Indivdual (mg)*	Mean Total Biomass per Treatment (mg)	Mean Weight Increase (mg)
Standard	0.00	1	10	0.94	4.9 (0.2)	3.9
		2	9	1.0		
		3	8	----		
	0.11	1	9	0.88	4.6 (0.2)	3.6
		2	10	0.94		
		3	9	----		
	0.278	1	9	1.0	5.0 (0.2)	4.0
		2	10	0.96		
		3	10	----		
	0.7	1	10	0.86	4.7 (0.4)	3.7
		2	10	1.0		
		3	10	----		
	1.5	1	8	0.98	4.7 (0.4)	3.7
		2	8	0.88		
		3	9	----		
	3.1	1	10	0.76	3.9† (0.1)	2.9†
		2	3	0.80		
		3	5	----		

* N=5 fish per replicate, survivors were pooled at the end of the exposure and two samples of five fish were randomly selected for weight measurements.

[A] Not determined due to insufficient survival.

† Denote significant ($P \leq 0.05$) difference from control (0.0 mg/L TPH) within each light treatment.

TABLE 4 Cont'd
GROWTH OF *Menidia beryllina*

Nominal Light Treatment	TPH (mg/L)	Replicate	#*Menidia* per replicate (test end)	Mean Weight per Indivdual (mg)*	Mean Total Biomass per Treatment (mg)	Mean Weight Increase (mg)
Low	0.00	1	8	0.94	4.8 (0.1)	3.8
		2	8	0.98		
		3	8	----		
	0.11	1	8	0.66	3.9 (0.8)	2.9
		2	8	0.90		
		3	10	----		
	0.278	1	8	0.78	3.8 (0.1)	2.8
		2	7	0.74		
		3	9	----		
	0.7	1	10	0.68	3.6† (0.3)	2.6†
		2	8	0.76		
		3	7	----		
	1.5	1	4	0.92	4.8 (0.2)	3.8
		2	6	0.98		
		3	2	----		
	3.1	1	0	A	A	A
		2	0	A		
		3	0	A		

* N=5 fish per replicate, survivors were pooled at the end of the exposure and two samples of five fish were randomly selected for weight measurements.

[A] Not determined due to insufficient survival.

† Denote significant ($P \leq 0.05$) difference from control (0.0 mg/L TPH) within each light treatment.

TABLE 4 Cont'd
GROWTH OF *Menidia beryllina*

Nominal Light Treatment	TPH (mg/L)	Replicate	#*Menidia* per replicate (test end)	Mean Weight per Indivdual (mg)*	Mean Total Biomass per Treatment (mg)	Mean Weight Increase (mg)
Medium	0.00	1	8	1.3	5.6 (1.1)	4.6
		2	10	0.96		
		3	10	----		
	0.11	1	10	1.1	5.4 (0.1)	4.4
		2	10	1.1		
		3	9	----		
	0.278	1	9	0.78	4.6 (1.0)	3.6
		2	9	1.1		
		3	9	----		
	0.7	1	8	1.0	5.2 (0.1)	4.2
		2	9	1.0		
		3	7	----		
	1.5	1	0	A	A	A
		2	0	A		
		3	0	A		
	3.1	1	0	A	A	A
		2	0	A		
		3	0	A		

* N=5 fish per replicate, survivors were pooled at the end of the exposure and two samples of five fish were randomly selected for weight measurements.

[A] Not determined due to insufficient survival.

† Denote significant ($P \leq 0.05$) difference from control (0.0 mg/L TPH) within each light treatment.

TABLE 4 Cont'd
SURVIVAL AND GROWTH OF *Menidia beryllina*

Nominal Light Treatment	TPH (mg/L)	Replicate	#*Menidia* per replicate (test end)	Mean Weight per Indivdual (mg)*	Mean Total Biomass per Treatment (mg)	Mean Weight Increase (mg)
High	0.00	1	7	0.60	3.8 (1.1)	2.8
		2	6	0.90		
		3	8	----		
	0.11	1	5	0.70	3.9 (0.4)	2.9
		2	6	0.84		
		3	4	----		
	0.278	1	6	0.76	3.8 (0.0)	2.8
		2	7	0.76		
		3	8	----		
	0.7	1	4	0.82	3.8 (0.4)	2.8
		2	6	0.70		
		3	7	----		
	1.5	1	0	A	A	A
		2	0	A		
		3	0	A		
	3.1	1	0	A	A	A
		2	0	A		
		3	0	A		

* N=5 fish per replicate, survivors were pooled at the end of the exposure and two samples of five fish were randomly selected for weight measurements.

[A] Not determined due to insufficient survival.

† Denote significant ($P \leq 0.05$) difference from control (0.0 mg/L TPH) within each light treatment.

Table 5. No-observed-effect concentrations (NOECs), lowest-observed-effect concentrations (LOECs) for mortality and growth and LC50s (95 % Confidence Intervals in parentheses) for *Menidia beryllina* exposed to dilutions of a water accommodated fraction of the diluent and simulated solar radiation treatments for 7 days.

Nominal light treatments	Mortality NOEC (mg/L TPH)[a]		Mortality LOEC (mg/L TPH)		Dry Weight (mg) NOEC (mg/L TPH)	Dry Weight (mg) LOEC (mg/L TPH)	LC50 (mg/L TPH)		LC20 (mg/L TPH)	
	Day 4	Day 7	Day 4	Day 7	Day 7	Day 7	Day 4	Day 7	Day 4	Day 7
Reference	1.50	1.50	3.03	3.03	1.50	3.03	ND[b]	2.84 (2.35-3.73)	ND[b]	I.55 (1.00-2.00)
Low	1.50	0.70	3.03	1.50	0.24[c]	0.70 [c]	1.77 (1.28-3.10)	1.27 (1.02-1.67)	0.92 (0.28-1.45)	0.47 (0.11-0.71)
Medium	0.70	0.70	1.50	1.50	0.70	>0.70 <1.50	1.20 (0.99-1.44)	0.93 (0.76-1.22)	0.75 (0.41-0.96)	0.55 (0.37-0.71)
High	0.70	<0.12	1.50	1.50	0.70	>0.70 <1.50	1.09 (0.82-1.49)	0.51 (0.24-0.77)	0.14 (-0.38-0.44)	-0.27 (-1.13-0.05)

[a] TPH = total petroleum hydrocarbon.

[b] ND = not determined due lack of sufficient mortality at day four.

[c] Conservative estimate based on a significant reduction in growth occurring at 0.70 mg/L TPH but not at 1.50 mg/L TPH.

Figure Legends

Figure 1. Concentrations of PAHS in the 100% water accommodated fraction (WAF) of the diluent. "Total Other" represent total PAHS without perylene minus total naphthalene. PAHS not present in the 100% WAF include C-3 and C-4 phenanthrenes and anthracenes and C-1 through C-4 chrysenes. PAHS present in the 100% WAF at concentrations less than the lower calibration limit of the analytical method included biphenyl, acenaphthylene, and acenaphthene, C-1 through C-3 fluorenes, C-1 and C-2 phenanthrenesand anthracenes, C-0 through C-4 dibenzothiophenes, fluoranthene, pyrene, C-1 fluoranthenes and pyrenes, benzo(a) anthracene, chrysene, benzo(b) and benzo(k) fluoranthene, benzo(e) and benzo(a) pyrene, perylene, indeno (1,2,3-c,d) pyrene, dibenzo(a,h) anthracene, benzo(g,h,i) perylene and 1-methylphenanthrene. Figure from companion report, Cleveland, et. al. 1998, "Photoenhanced Toxicity of a Diluent to *Mysidopsis bahia.*"

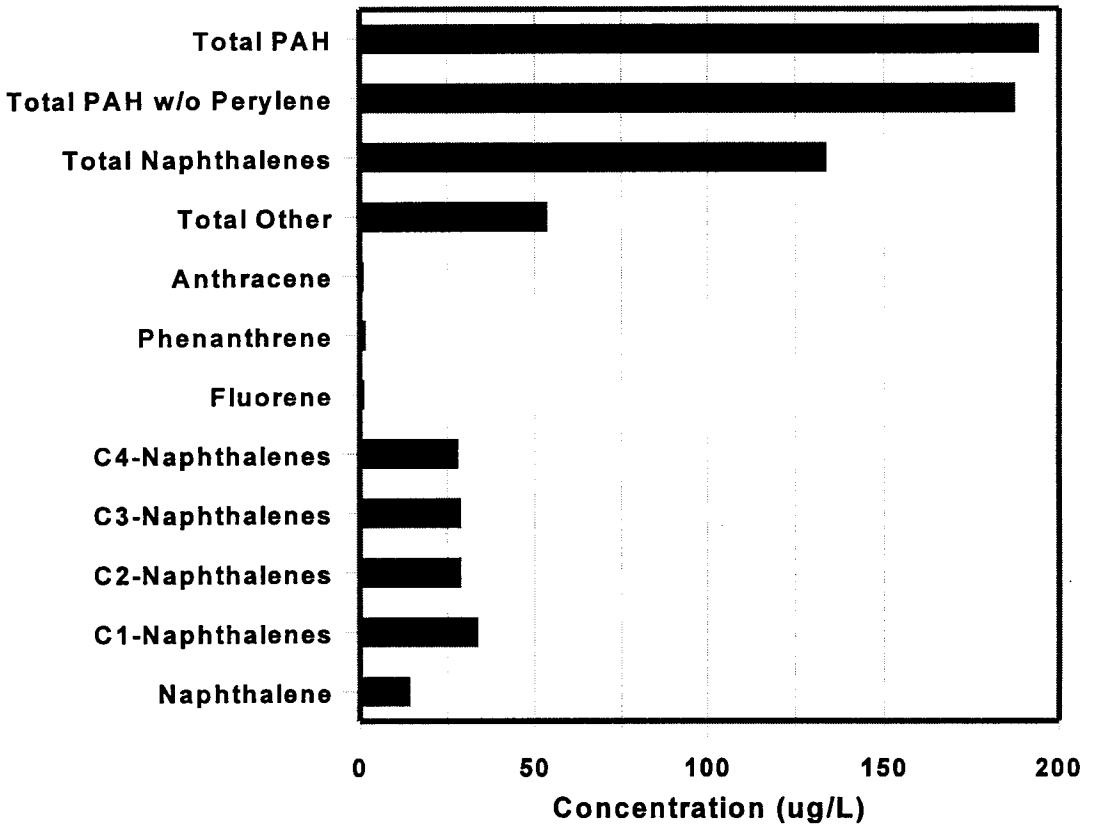
Figure 2. Measured ultraviolet radiation at 10 cm subsurface in an estuary adjacent to an abandoned oil field and measured simulated UV irradiances applied during laboratory studies. Shown as $\mu W/cm^2$ per nm from 280 to 400 nm.

Figure 3. Average percent mortality among *Menidia beryllina* exposed to the water accommodated fraction of diluent, shown as total petroleum hydrocarbons (TPH) under four simulated solar irradiance conditions. (N=30)
a) Response at 96 hours of exposure.
b) Response at 168 hours of exposure.

Figure 4. Average weight (grams) of surviving *Menidia beryllina* larvae following a seven day exposure to the water accommodated fraction of diluent under four simulated solar irradiance conditions.

Figure 5a. Photoactivation Test: Average percent mortality among *Menidia beryllina* exposed to a 5 % dilution of the water accommodated fraction of diluent that was previously exposed to simulated solar radiation (N=30).

Figure 5b. Photosensitization Test: Average percent mortality among *Menidia beryllina* exposed to a 5 % dilution of the water accommodated fraction of diluent for 48 hours prior to simulated solar radiation exposure in clean water (N=30).


Total PAH
Total PAH w/o Perylene
Total Naphthalenes
Total Other
Anthracene
Phenanthrene
Fluorene
C4-Naphthalenes
C3-Naphthalenes
C2-Naphthalenes
C1-Naphthalenes
Naphthalene
0
50
100
150
200
Concentration (ug/L)

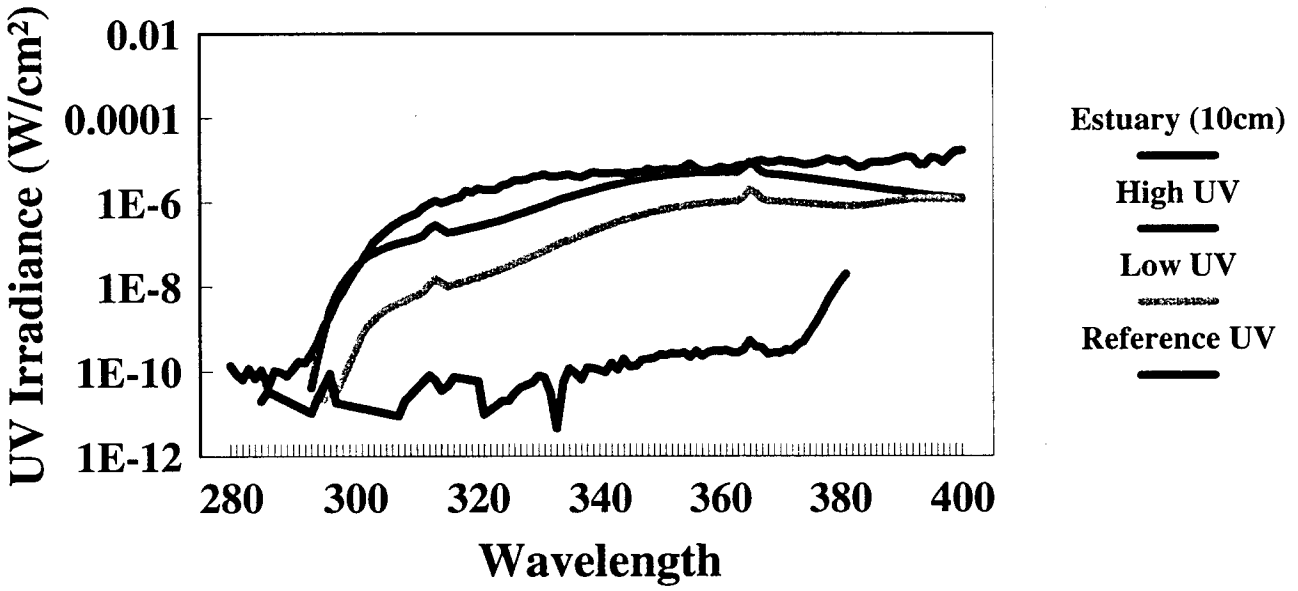

UV Irradiance (W/cm²)
0.01
0.0001
1E-6
1E-8
1E-10
1E-12
280
300
320
340
360
380
400
Wavelength
Estuary (10cm)
High UV
Low UV
Reference UV

Figure 2.

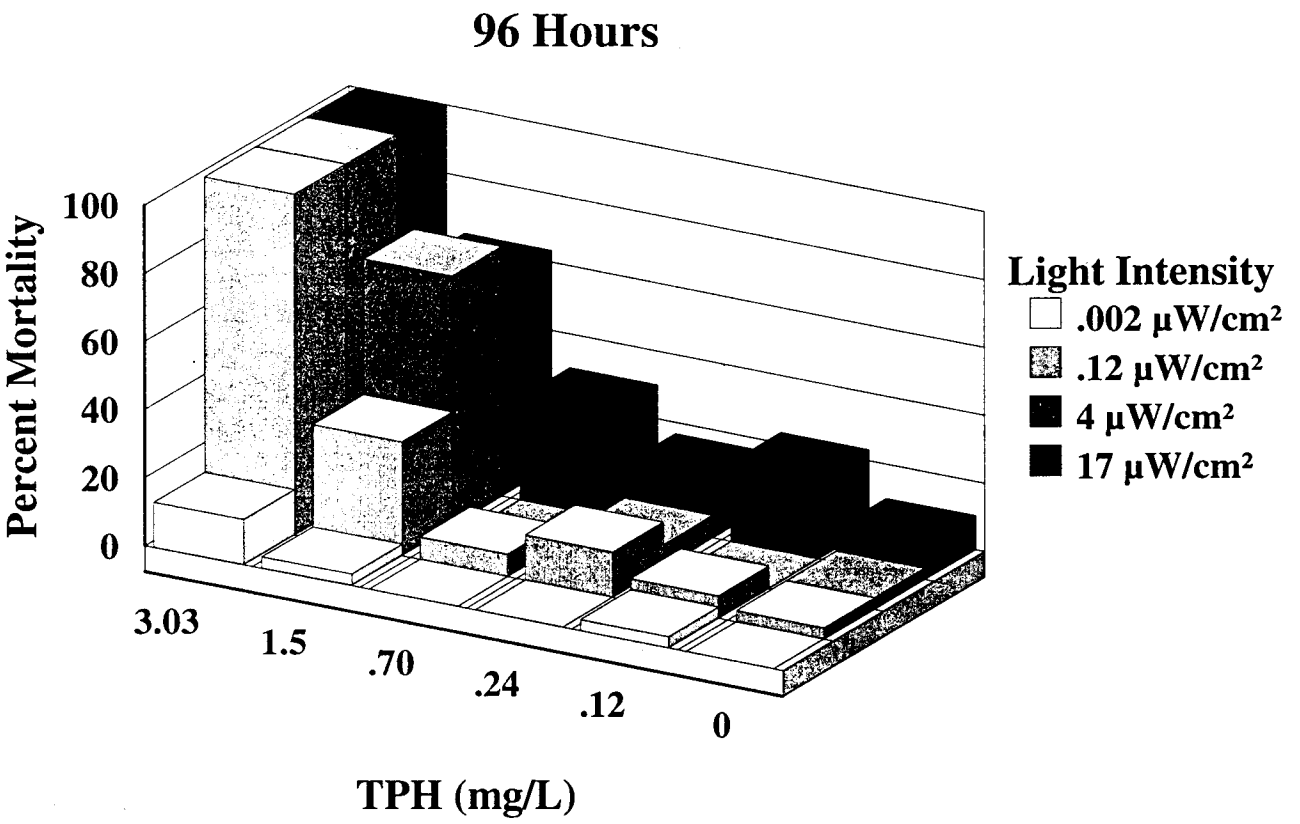
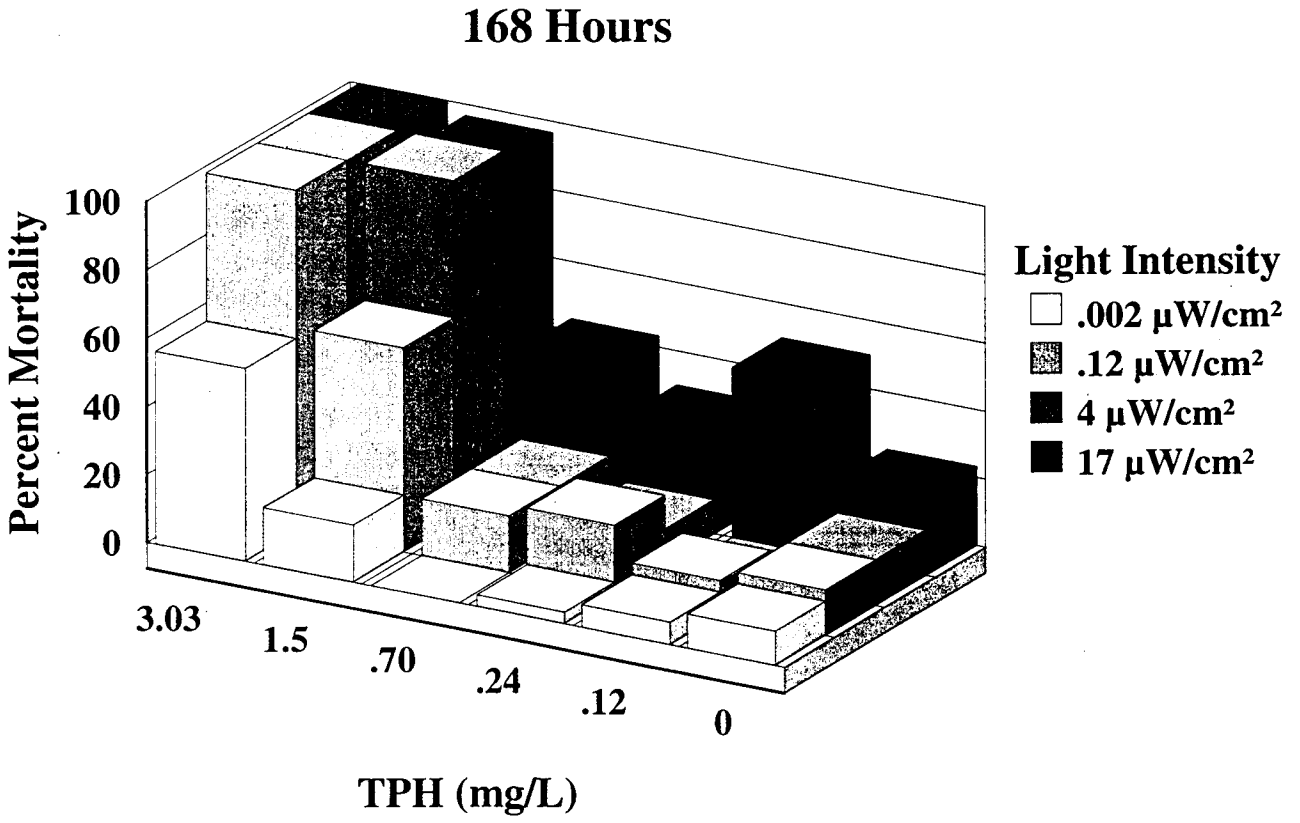

96 Hours
Percent Mortality
100
80
60
40
20
0
3.03
1.5
.70
.24
.12
0
TPH (mg/L)
Light Intensity
.002 µW/cm²
.12 µW/cm²
4 µW/cm²
17 µW/cm²

Figure 3a.


168 Hours
Percent Mortality
100
80
60
40
20
0
3.03
1.5
.70
.24
.12
0
TPH (mg/L)
Light Intensity
.002 µW/cm²
.12 µW/cm²
4 µW/cm²
17 µW/cm²

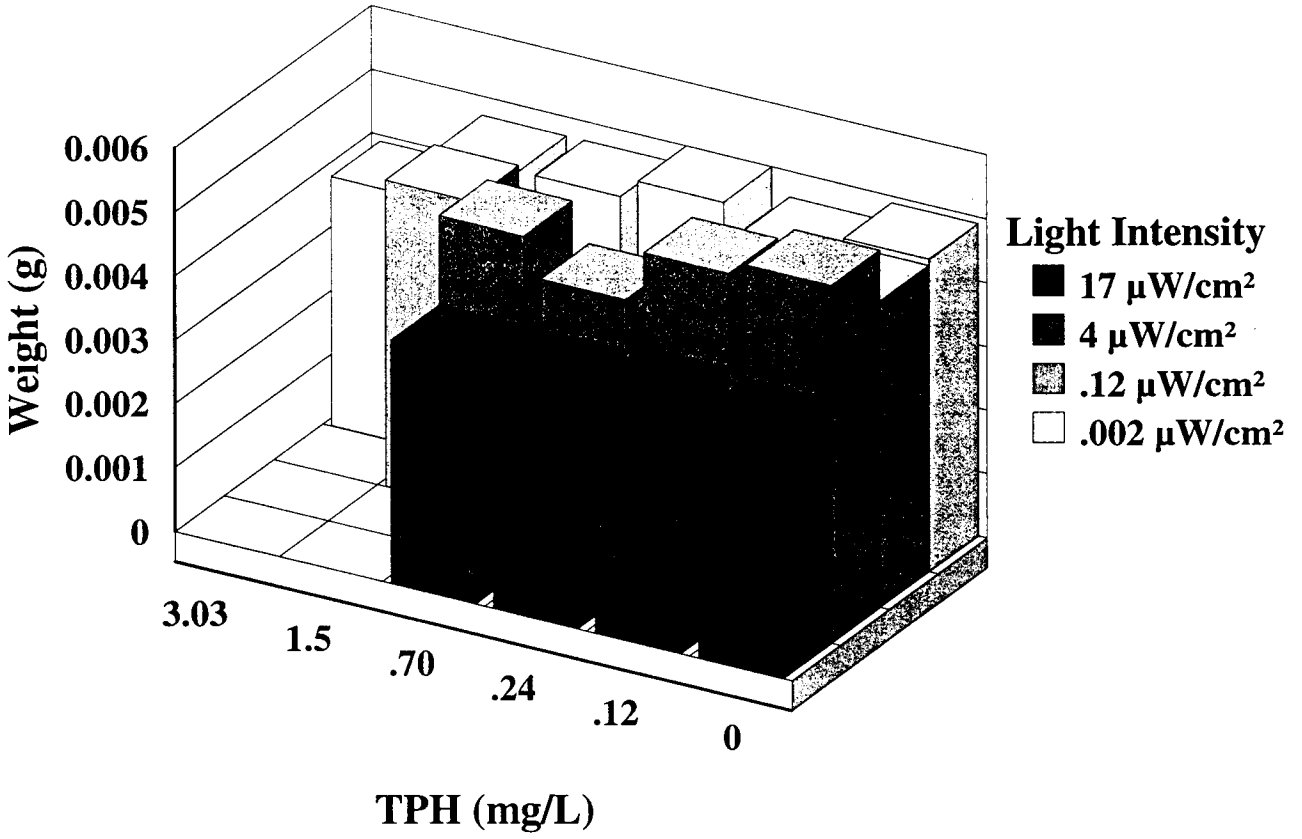

0.006
0.005
0.004
0.003
0.002
0.001
0
Weight (g)
3.03
1.5
.70
.24
.12
0
TPH (mg/L)
Light Intensity
17 µW/cm²
4 µW/cm²
.12 µW/cm²
.002 µW/cm²

Figure 4.

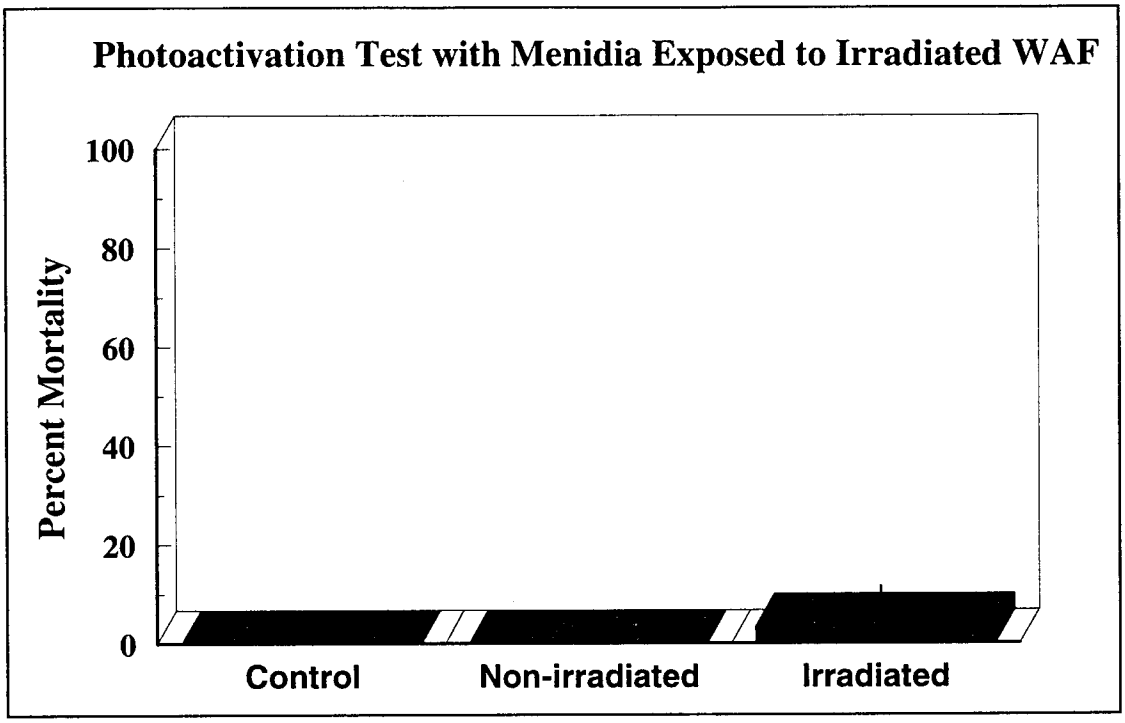
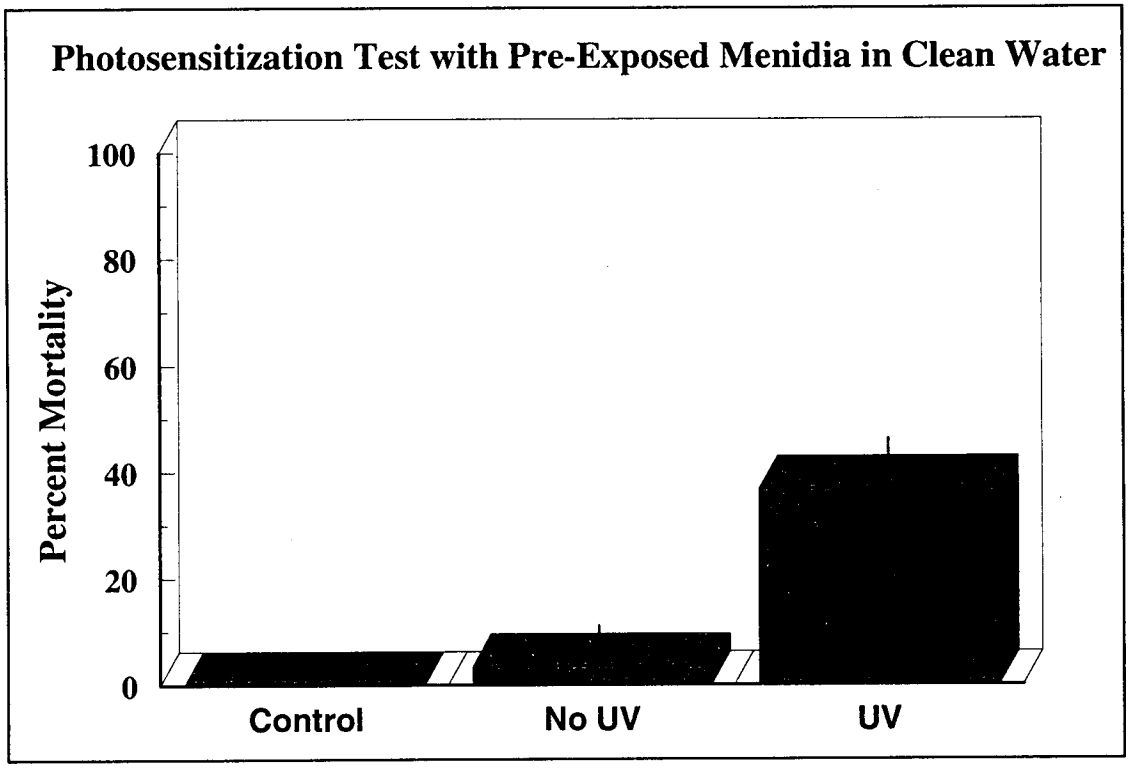

Photoactivation Test with Menidia Exposed to Irradiated WAF
Percent Mortality
100
80
60
40
20
0
Control
Non-irradiated
Irradiated

Figure 5a.


Photosensitization Test with Pre-Exposed Menidia in Clean Water
Percent Mortality
100
80
60
40
20
0
Control
No UV
UV

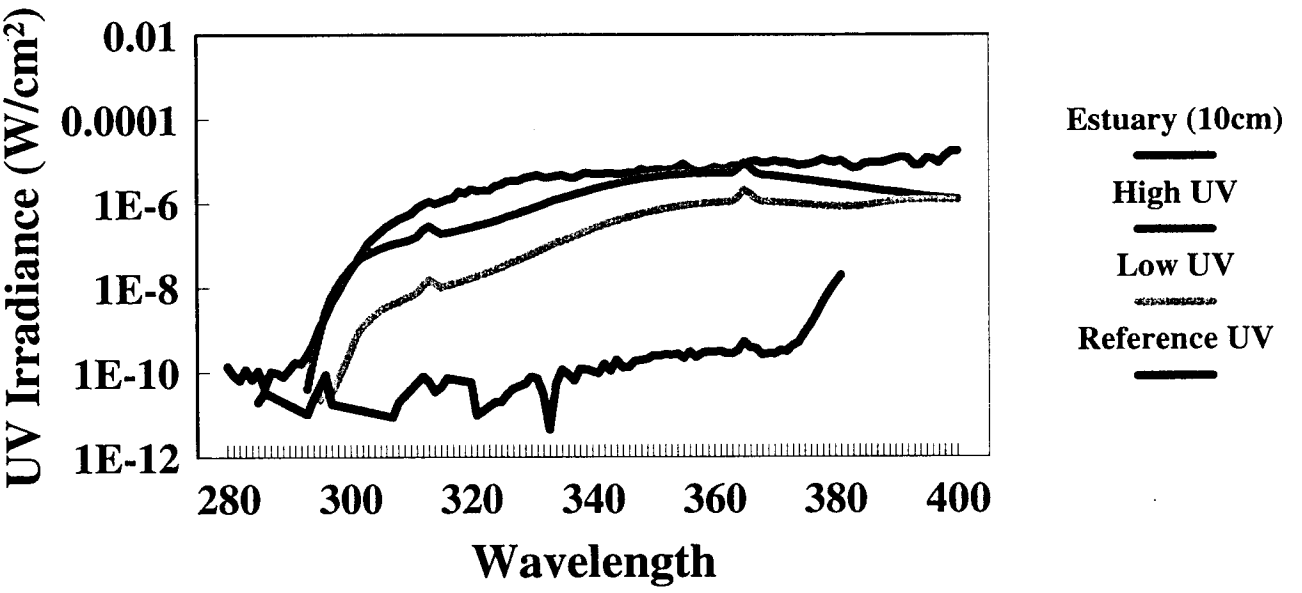

UV Irradiance (W/cm²)
0.01
0.0001
1E-6
1E-8
1E-10
1E-12
280
300
320
340
360
380
400
Wavelength
Estuary (10cm)
High UV
Low UV
Reference UV

Figure 2.

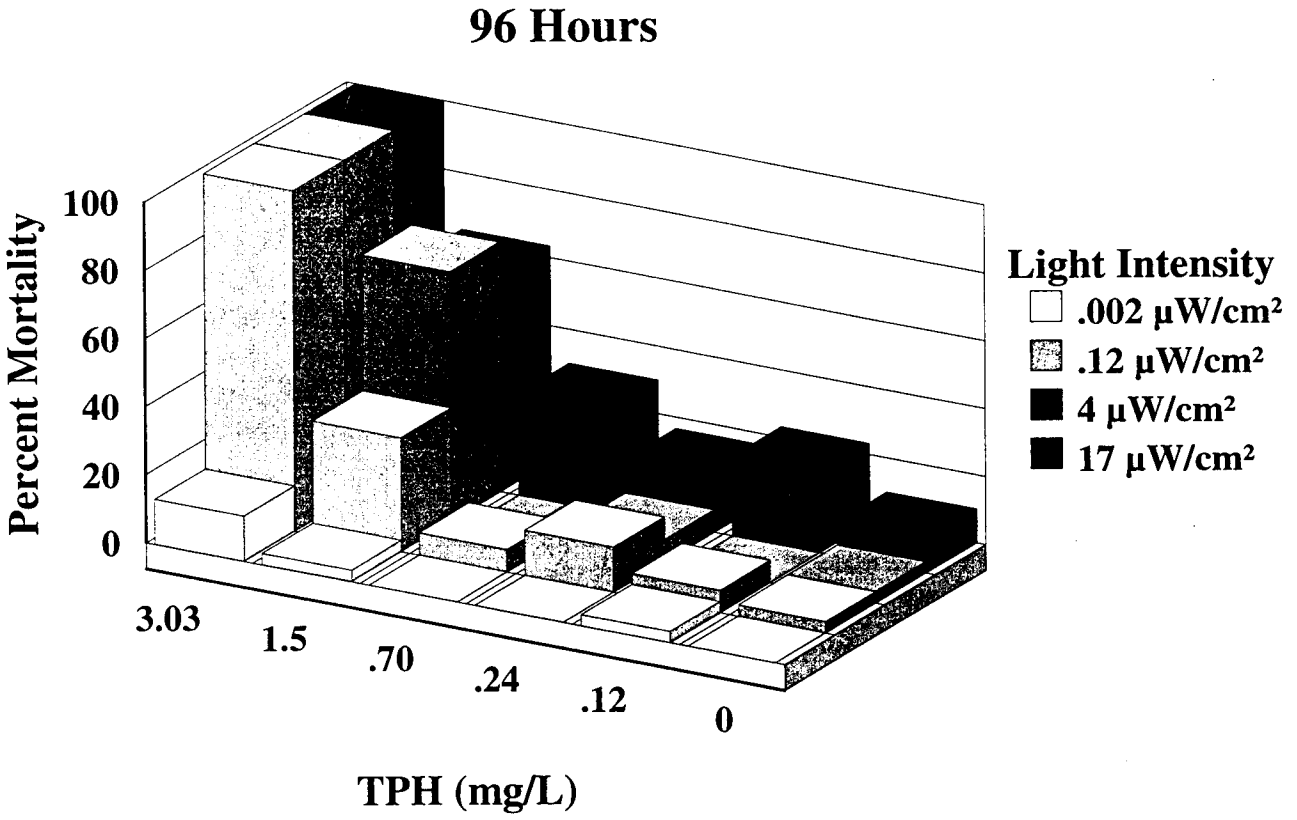
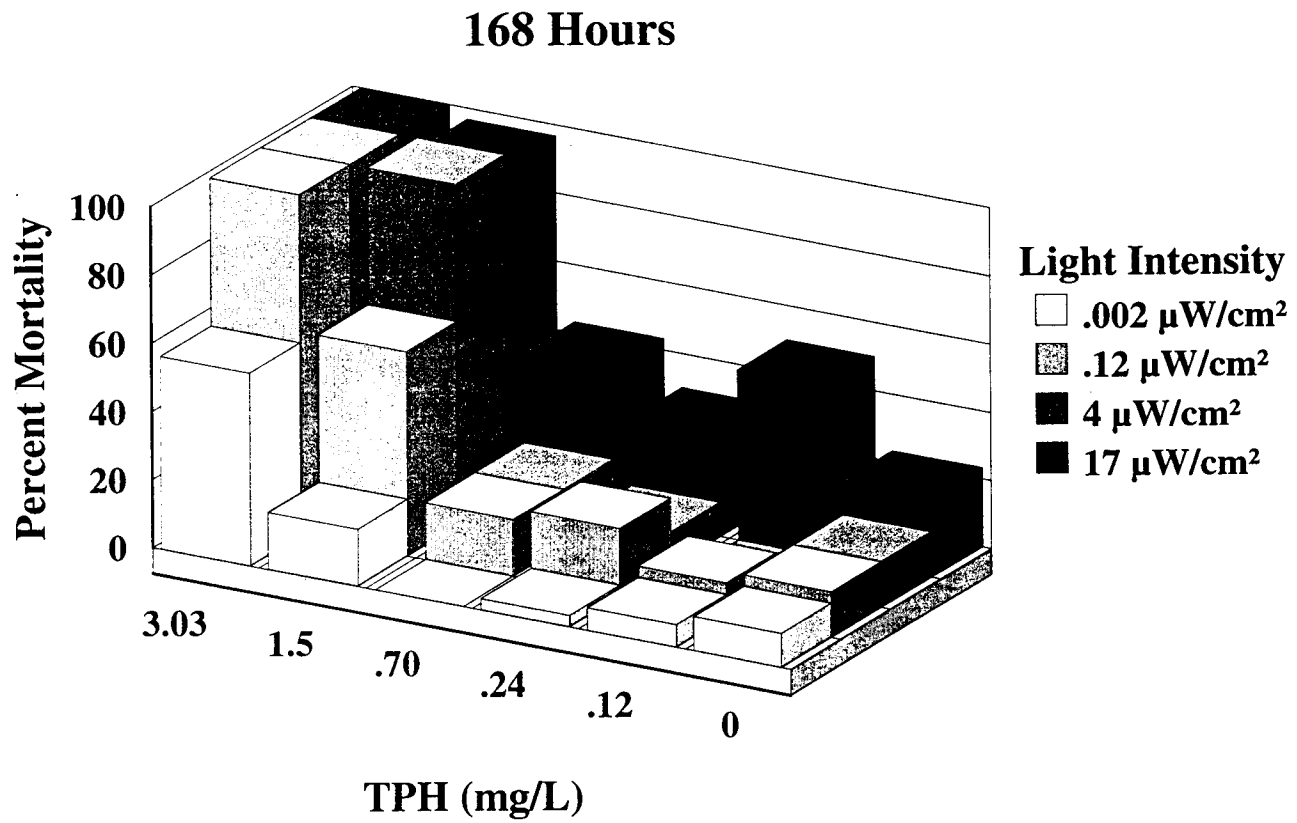

96 Hours
Percent Mortality
100
80
60
40
20
0
3.03
1.5
.70
.24
.12
0
TPH (mg/L)
Light Intensity
.002 µW/cm²
.12 µW/cm²
4 µW/cm²
17 µW/cm²

Figure 3a.


168 Hours
Percent Mortality
100
80
60
40
20
0
3.03
1.5
.70
.24
.12
0
TPH (mg/L)
Light Intensity
.002 µW/cm²
.12 µW/cm²
4 µW/cm²
17 µW/cm²

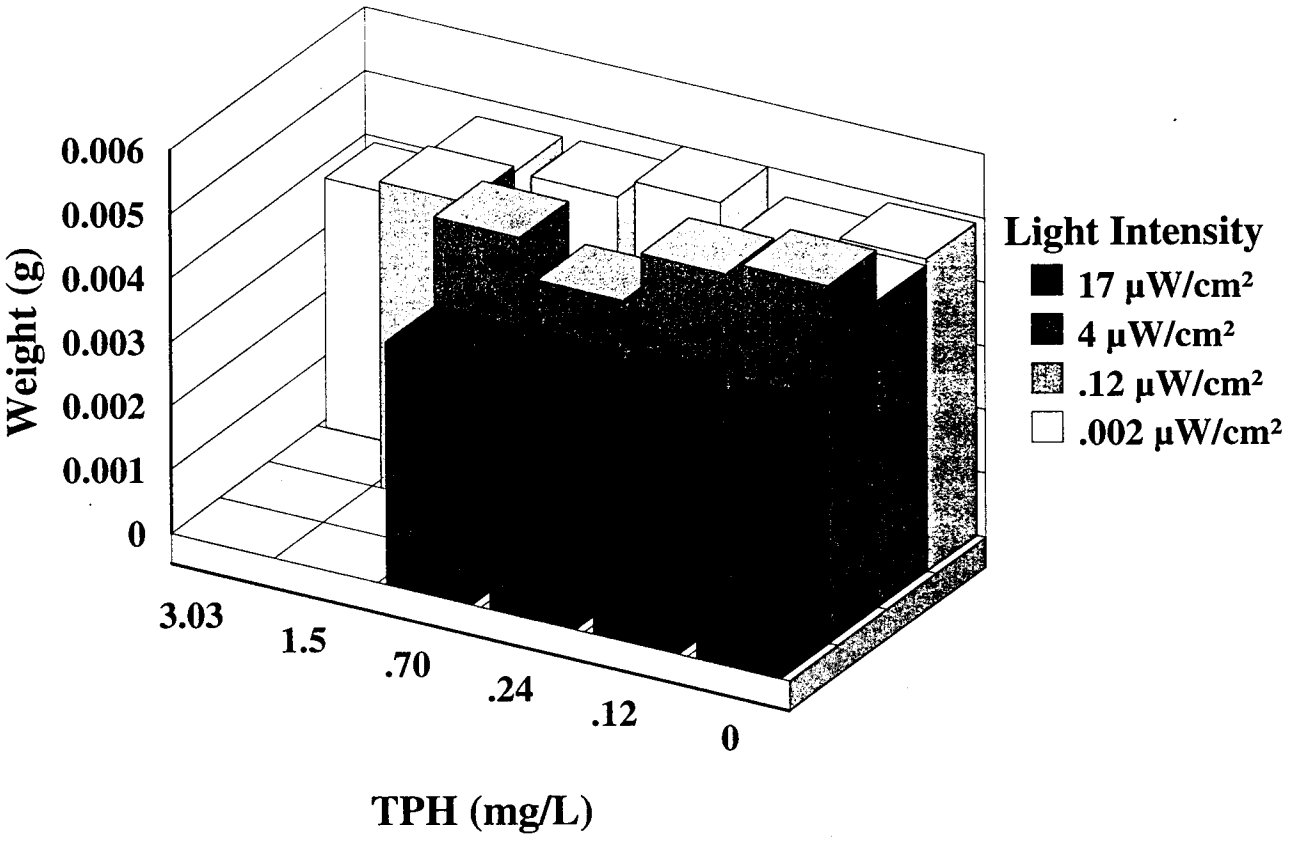

0.006
0.005
0.004
0.003
0.002
0.001
0
Weight (g)
3.03
1.5
.70
.24
.12
0
TPH (mg/L)
Light Intensity
17 µW/cm²
4 µW/cm²
.12 µW/cm²
.002 µW/cm²

Figure 4.

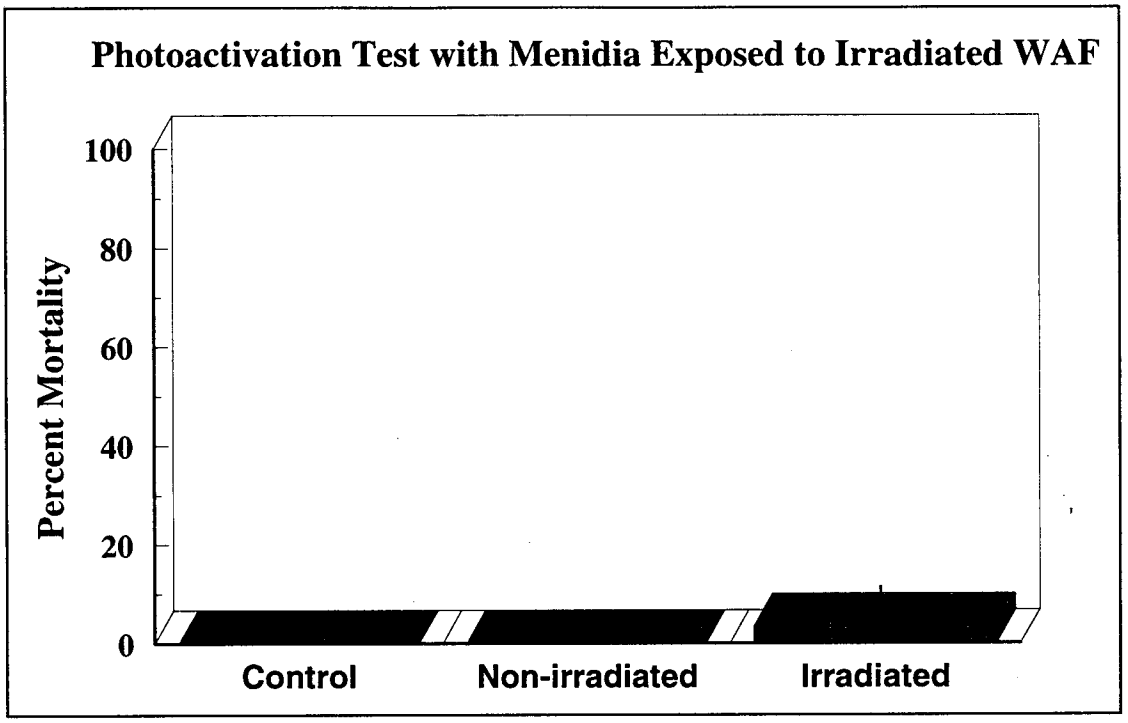
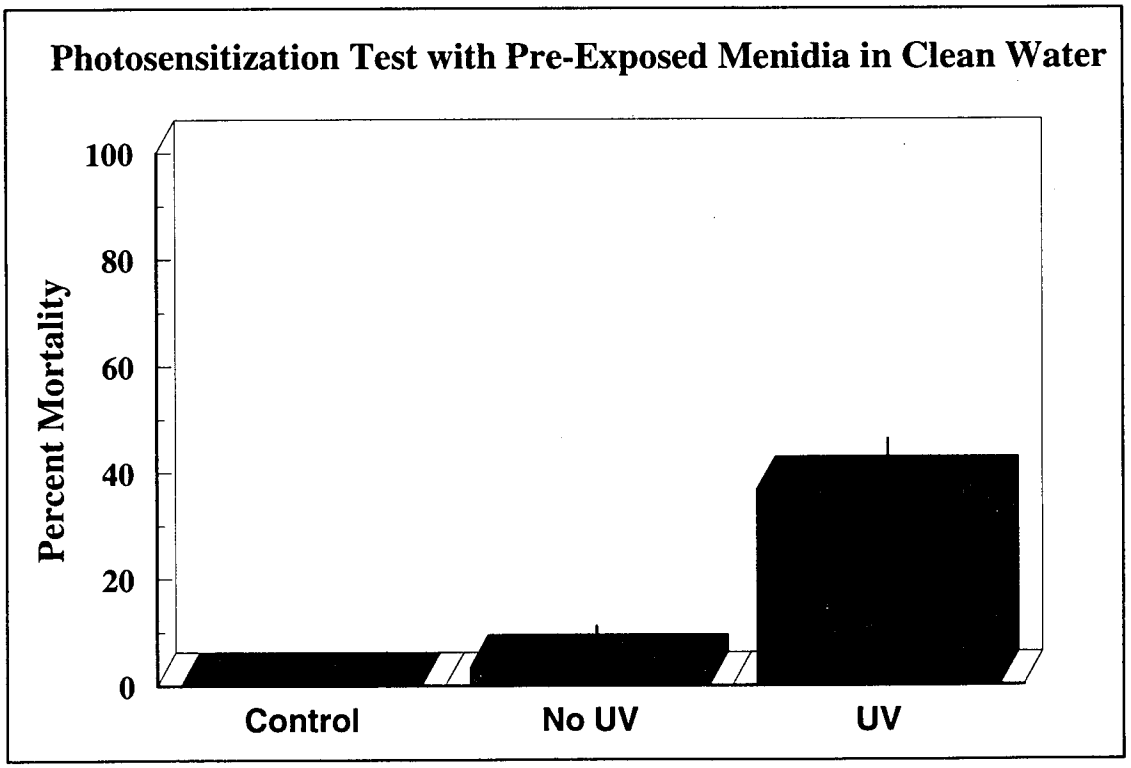

Photoactivation Test with Menidia Exposed to Irradiated WAF
Percent Mortality
100
80
60
40
20
0
Control
Non-irradiated
Irradiated

Figure 5a.


Photosensitization Test with Pre-Exposed Menidia in Clean Water
Percent Mortality
100
80
60
40
20
0
Control
No UV
UV