# Photoenhanced Toxicity of Aqueous Phase and Chemically-Dispersed Weathered Alaska North Slope Crude Oil to Pacific Herring Eggs and Larvae

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**Executive Summary**

Most of the available data on the toxicity and risks of oil and chemically-dispersed oil have been derived from laboratory studies that do not incorporate exposures to the ultraviolet radiation (UV) that occurs in aquatic environments. UV is a component of sunlight, but is not visible to humans. UV contains light energy that can be absorbed by specific components in oil, including PAHs (polycyclic aromatic hydrocarbons). Photoenhanced toxicity has recently been reviewed in an RCAC sponsored study, and is now published in the scientific literature (Barron and Ka'aihue, 2001). The UV that is present in aquatic environments includes UVB (280 to 320 nm) and UVA (320 to 400 nm), where a nanometer (nm; 1 billionth of a meter) is a measure of the specific wavelength of sunlight. Understanding photoenhanced toxicity is important because petroleum and weathered oil is known to be phototoxic, exhibiting a two to greater than 1000 fold increase in toxicity in the presence of UV compared to standard laboratory lighting conditions with fluorescent lights and minimal UV. The photoenhanced toxicity of Alaska North slope crude (ANS) to Alaskan fish species has never been determined, and the potential for photoenhanced toxicity of chemically-dispersed ANS has not been previously evaluated in any species.

This study investigated the photoenhanced toxicity of weathered ANS to eggs and larvae of the Pacific herring (*Clupea pallasi*), and the relative toxicity of chemically-dispersed and aqueous phase oil. Aqueous phase oil is the portion of petroleum that dissolved or accommodated into the water used in toxicity tests. Herring are ecologically and economically important in Prince William Sound and Gulf of Alaska waters, and are known to be sensitive to ANS at concentrations as low as 0.4 ug/L (parts per billion) of total PAHs (tPAH; sum of all individual PAHs that were quantified). Herring were exposed to a series of aqueous phase doses prepared with high energy mixing of ANS with the chemical dispersant Corexit$^{R}$ 9527 either present or absent. Corexit$^{R}$ 9527 is the chemical dispersant stock piled in Prince William Sound for possible use in oil spill responses. Herring eggs (a few days after fertilization) and larvae ( a few days after hatching) were exposed to a combination of oil, dispersant, and UV treatments in the laboratory, with some UV exposures occurring outdoors in sunlight.

Oil exposures occurred at only one life stage of herring: either in embryos or larvae. Following oil exposure, each life stage was exposed to UV treatments: control lighting or UVA (eggs and larvae), or sunlight treatments (only larvae). UV treatments with significant UVB (sunlight, UVA+sunlight) were only performed in the larval experiments because the potential importance of UVB in the photoenhanced toxicity of ANS to herring was not recognized until egg exposures were completed. This work extended the recent work of the National Oceanographic and Atmospheric Administraton's Auke Bay Laboratory on the toxicity of ANS to herring embryos to include an evaluation of the interaction of weathered oil, UV, and chemical dispersant. A separate larval experiment was conducted to discriminate between two possible modes of action of the photoenhanced toxicity of oil, ***photosensitization*** (activation of hydrocarbons that have bioaccumulated in tissue) or ***photomodification*** (photooxidation of hydrocarbons in water); see Barron and Ka'aihue (2001) for additional explanation of phototoxicity mechanisms. Based on prior studies with oil and components of oil, we hypothesized that weathered ANS would have a photosensitization mode of action (phototoxicity after fish first accumulate oil residues).

The interaction of aqueous phase oil, chemical dispersant, and UV resulted in lethal and sublethal effects in Pacific herring larvae and embryos. Oil-only and oil+dispersant treatments exhibited a dose-response relationship between mortality and morbidity and the tPAH concentration in tissue and water (i.e., toxicity increased with increasing concentrations of oil components). Oil alone was acutely toxic to larvae at concentrations below 50 $\mu gXL^{-1}$ tPAH (e.g., 96 LC50 = 37 $\mu gXL^{-1}$ under control lighting), and LC50s and EC50s decreased with time (LC50s and EC50s are median lethal and median lethal concentrations, which are the oil component concentrations that either kill or effect 50% of the test organisms).

Photoenhanced toxicity (a significant increase in toxicity with UV exposure) only occurred when oil was present in larval tissue, and the degree of photoenhanced toxicity increased with increasing UV treatment (UVA+sunlight>sunlight>UVA) and with increasing tPAH concentration in tissue. This means that higher UV and oil exposures both resulted in greater toxicity to herring larvae. The toxicity of chemically-dispersed oil was generally similar to oil-only toxicity at equivalent tPAH concentrations in tissues and in water, with no significant differences in median lethal (LC50s) or effective (EC50s) concentrations between oil-only and oil+dispersant exposures. However, tPAH concentrations in tissue were elevated approximately two times above oil-only exposure, resulting in increased mortality and morbidity-and more rapid mortality in the presence of sunlight. LC50s decreased over time and with increasing UV treatment and EC50s based on impaired swimming in addition to mortality and morbidity were similar to LC50 values. LC50s, EC50s, and lowest observed effect concentrations for oil only and oil+dispersant treatments with larvae were 18-450 fold lower in the UVA+sun treatment compared to control lighting with minimal UV. This means that UV exposure increased the toxicity of oil by 18 to 450 times than is measured under normal laboratory test conditions using flourescent lights which provide minimal UV. UVA-only exposures were less potent than natural sunlight, either because they did not include UVB or because they did not contain the upper wavelengths of UVA present in natural sunlight.

Exposure of herring eggs to oil caused yolk-sac edema, but phototoxic effects were not observed in UVA treatments. Yolk-sac edema is a visible swelling of the yolk sac (remnant of the egg on the underside) of the fish caused by an abnormal accumulation of fluids. UV treatments with significant UVB (sunlight, UVA+sun) were only performed in the larval experiments because the potential importance of UVB in the photoenhanced toxicity of ANS to herring was not recognized until egg exposures were completed. We hypothesize that significant photoenhanced toxicity in herring eggs would occur in the presence of elevated oil residues in eggs and sunlight exposure. Based on tissue residue levels, herring embryos and larvae appeared to have similar sensitivity to oil exposure.

In addition to the dose-response studies discussed above, a comprehensive set of toxicity tests with herring larvae were also performed with oil-only and oil+dispersant exposures to evaluate the mechanism of action of weathered ANS (photomodification vs photosensitization). Understanding the mechanism of action of oil is important in order to better evaluate potential risks from oil spills and dispersant use). In the additional tests, significant toxicity was only observed in larvae that first bioaccumulated oil, then were exposed to sunlight. Consistent with previous studies on the toxicity of individual PAHs and oil to fish and aquatic invertebrates exposed to UV, our results show that exposure to UV increases the toxicity of bioaccumulated PAHs (photosensitization). This indicates that (1) oil residues must be accumulated in organisms

to elicit photoenhanced toxicity, (2) there was no observable toxicity from UV irradiation of oil residues in water, and (3) photoenhanced toxicity could occur from an initial oil exposure (e.g., spill at night) followed by subsequent UV exposure.

The results of this study show that (1) part-per-billion aqueous concentrations of ANS damage or kill herring embryos and larvae, (2) ANS is phototoxic, (3) UV can be a significant and causative factor in the mortality of early life stages of herring exposed to oil and chemically-dispersed oil, and (4) oil low in known phototoxic PAHs can cause significant photoenhanced toxicity. PAH composition in exposure solutions was dominated either by naphthalenes or phenanthrenes, and the composition of PAH accumulated in eggs and larvae was generally similar to that in exposure water. In general, the presence of chemical dispersant appeared to accelerate the movement of PAHs from the whole product oil into the water to form aqueous phase oil and increased the composition of three ring PAHs. The study results also indicated that UVB, in addition to UVA, may be important in causing photoenhanced toxicity. The results also suggest the potential for photoenhanced toxicity as a factor in herring impacts from the Exxon Valdez oil spill because of the high phototoxicity of weathered ANS and measurements of ppb levels of tPAH in the water column during the 1989 spill, the high phototoxicity of weathered ANS, and the limited UV necessary to cause photoenhanced toxicity.

In conclusion, injury and risk to aquatic organisms from an oil spill may be underestimated if based on standard laboratory bioassays and existing toxicity databases that do not account for the phototoxicity of oil. Brief exposure to sunlight (~2.5 hours/day for two days) was sufficient to significantly increase toxicity. Photoenhanced toxicity should be considered in determining the risks and injuries of spilled oil, and in the selection of oil spill responses. We recommend additional research on the relationship between UVB and oil phototoxicity because this area has received only limited investigation, and study results indicate that UVB may be a significant factor in the photoenhanced toxicity of oil. Research should be directed at establishing photoenhanced toxicity thresholds for both UVA and UVB (i.e., minimum UV levels necessary to cause significant toxicity in the presence of bioaccumulated oil residues). We also recommend monitoring light attenuation in the water column to address temporal and spatial variation in ambient UV to pro-actively evaluate potential risks of photoenhanced toxicity in Prince William Sound and associated Gulf of Alaska waters.

**ABSTRACT**

This study investigated the photoenhanced toxicity of weathered Alaska North Slope crude (ANS) to eggs and larvae of the Pacific herring (*Clupea pallasi*), and the photoenhanced toxicity ANS in the presence of the chemical dispersant Corexit$^{R}$ 9527. Oil alone was acutely toxic to larvae at aqueous concentrations below 50 $\mu gXL^{-1}$ tPAH (96 LC50 = 37 $\mu gXL^{-1}$ under control lighting), and LC50s and EC50s (median lethal and effective concentrations) decreased with time. Brief exposure to sunlight (~2.5 hours/day for two days) was sufficient to significantly increase toxicity. Photoenhanced toxicity only occurred when oil was present in larval tissue, and the degree of toxicity increased with increasing ultraviolet radiation (UV) treatment (UVA+sun>sunlight>UVA) and with increasing tPAH concentration in tissue. LC50s, EC50s, and lowest observed effect concentrations (LOECs) for oil-only and oil+dispersant treatments with larvae were 18-450 fold lower in the UVA+sun treatment compared to control lighting with minimal UV. UVA-only exposures were less potent than natural sunlight, either because they did not include UVB or because they did not contain the upper wavelengths of UVA present in natural sunlight. The toxicity of chemically-dispersed oil was generally similar to oil-only toxicity at equivalent tPAH concentrations in tissues and in water, but toxicity occurred sooner in the presence of dispersant and sunlight. Exposure of herring eggs to oil caused yolk-sac edema, but phototoxic effects were not observed in UVA treatments (treatments incorporating UVB were not tested). The results of this study are consistent with the hypothesis that weathered ANS is phototoxic and that UV can be a significant and causative factor in the mortality of early life stages of herring exposed to oil and chemically-dispersed oil. This investigation also indicates that UVB, in addition to UVA, may be important in the photoenhanced toxicity of oil.

## INTRODUCTION

Traditionally, toxicological studies used to define the hazards of polycyclic aromatic compounds and oil have been conducted in the absence of ultraviolet radiation (UV) (Arfsten et al., 1996). Laboratory studies have demonstrated that the toxicity of specific polycyclic aromatic compounds, oil products, and weathered oil increases 2 to greater than 1000 times in the presence of UV compared to standard laboratory lighting conditions with fluorescent lights and minimal UV (Pelletier et al., 1997; Calfee et al., 1999; Deusterloh et al., in review). Photoenhanced toxicity occurs at the UV wavelengths and intensities that occur in the water column of aquatic environments: UVB (280 to 320 nm) and UVA (320 to 400 nm) (Barron, 2000a). The photoenhanced toxicity of oil in fish and aquatic invertebrates appears to occur through activation of chemical residues that have bioaccumulated in aquatic organisms (termed photosensitization), rather than photomodification of the chemical in water (Little et al., 2000). The specific region of the UV spectrum that activates polycyclic aromatic hydrocarbons (PAH) compounds is considered to be the wavelengths of active UV absorption by the chemical. Current research indicates this includes both UVA and UVB (Huovinen et al., 2001), with UVA possibly the most important because of less rapid attenuation (Diamond et al., 2000).

In a hazard assessment of oil and UV in Alaska waters, Barron and Ka'aihue (2001) concluded that photoenhanced toxicity of spilled oil may occur in Prince William Sound and Gulf of Alaska waters because of the potential for significant levels of oil and UV in the water column. This finding was important because the laboratory studies used to assess the impacts of the *Exxon Valdez* oil spill in Prince William Sound did not account for photoenhanced toxicity, nor do available laboratory databases on the toxicity of oil to Alaskan marine species (e.g., Rice et al., 1976; Marty et al., 1997; Carls et al., 1999; Heintz et al., 1999). Pelletier et al. (1997) showed the Prudhoe Bay crude oil was greater than 100 times more toxic to shrimp and bivalve embryos when tested under UV, and recent studies with two species of marine copepod zooplankton demonstrate that weathered North Slope Crude oil (ANS) is extremely phototoxic (Duesterloh et al., in review).

The interaction of chemical dispersants used in spill response on the photoenhanced toxicity has not been previously investigated. Chemical dispersants break up free product oil into small droplets (e.g., 0.01 to 50 micron) which disperse in the water column. Dispersants generally increase the total concentrations of petroleum compounds (dissolved + particulate oil), but the relative environmental hazards of chemically dispersed and non-chemically dispersed oil are uncertain and are likely spill-specific (DeCola, 1999). To date, laboratory studies determining the toxicity of chemically dispersed oil have only been conducted under standard light conditions (minimal UV). These studies may substantially underestimate the hazard of chemically dispersed oil in the environment because of the potential for photoenhanced toxicity.

The objectives of the current study were to investigate the photoenhanced toxicity of weathered ANS to eggs and larvae of the Pacific herring (*Clupea pallasi*), and to determine the relative toxicity of chemically-dispersed and aqueous phase oil. Herring are ecologically and economically important in Alaska. Both life stages of herring were evaluated because eggs are spawned in the photic zone of near surface tidal areas potentially impacted by oil spills and other sources of PAHs, and larvae develop and grow in these areas. Bioaccumulation of total PAHs (tPAH) was quantified in both eggs and larvae to allow a comparison of exposure and sensitivity

based on tissue residues in addition to aqueous concentrations. This work extends the recent work on the toxicity of ANS to herring embryos (Carls et al., 1999) to include an evaluation of the interaction of weathered oil, UV, and chemical dispersant. An additional objective was to determine if weathered ANS had a photosensitization mode of action (UV activation of bioaccumulated residues) or a photomodification mode of action (UV photooxidation to form more toxic molecules in the water column). Based on prior studies with oil (Little et al., 2000) and components of oil (e.g., Landrum et al., 1987), we hypothesized that weathered ANS would have a photosensitization mode of action.

## MATERIALS AND METHODS

### Experimental Overview

Pacific herring were exposed to a series of aqueous phase doses prepared with high energy mixing of Alaska North Slope crude (ANS) with chemical dispersant either present or absent. Oil exposures occurred at only one life stage: either in embryos or larvae. Following oil exposure, each life stage was exposed to UV treatments: control lighting or UVA (eggs and larvae), or sunlight treatments (only larvae). Toxic concentrations of oil are reported as tPAH concentrations in exposure water and as wet weight concentrations in tissue. Mean dry/wet weight ratios are 0.211 (0.118-0.388) for eggs and 0.087 (0.068-0.108) for larvae. Tests were performed under environmentally realistic conditions of temperature, salinity, oil exposure concentrations, and UV doses.

### Organisms

Reproductively ripe Pacific herring were collected by dip net from a purse seine net in Sitka Sound, Alaska on March 24, 2001. Herring were placed over ice in coolers, transported to the laboratory, and artificially spawned within 7 hours. Gametes from 4 of the 10 females and several males included in the experiment were dissected from fish before transportation, and transported chilled, similar to the methods of Yanagimachi et al. (1992). Eggs were spawned as described by Carls et al. (1999). In brief, eggs from 17 females were spawned onto glass slides (2.5 × 7.6 cm). Fertility was qualitatively assessed the following day, and slides from 10 females with good fertility and egg abundance were selected for study. Slides from each female were randomly distributed among 18 racks, and excess eggs along slide margins and in areas more than one layer deep were removed. Additional eggs were spawned onto 202 μ plankton net to provide eggs for uptake measurements and larval experiments. Laboratory seawater was supplied by a single-pass flow-through system with a -24 m intake in Auke Bay, Alaska. All water was passed through 1 μ polyester filters before use. Hatching larvae were collected every 1 to 2 days (1-3 d for uptake studies) to provide fish of known age for separate larvae experiments. Eggs and larvae were reared at 4.9-6.6EC and 30.1-31.2 ppt. Dissolved oxygen ranged from 89-98% and was not affected by the presence of eggs or larvae.

**Preparation of Dispersed and Aqueous Phase Oil Solution**

Water-accommodated fractions of oil were prepared in 50 L, cone-bottomed fiberglass tanks fitted with 2.5 cm PVC ball valves at the bottom. Tanks were partially filled with 32 L of seawater filtered through a 1 μ polyester filter, which were pre-soaked for 24 h in flowing seawater, rinsed with freshwater, and dried before use. A motor-driven paddle shaft (5.7 cm drywall paddle "Mudslinger", Hyde Mfg. Co.) extended from above the water surface to within approximately 2.5 cm above the tank bottom. Shaft speed was approximately 600 rpm and resulted in vigorous mixing: the vortex extended from the water surface down to the paddle, and a mist formed above the surface. Mixing began a few minutes before addition of oil and dispersant. Separate WAFs were prepared with either oil (300 μL) or oil+dispersant (300 μL oil + 12 μL Corexit 9527; 1:10 to 1:50 ratios are generally recommended). Oil was added near the margin of the vortex with a positive-displacement pipette, followed immediately by dispersant using a gas-tight syringe at the vortex margin. Mixing time was 12 h in all tests followed by a 1 hour separation period. Mixtures were drained from the bottom of the tanks through a clean, pre-soaked 1 μ polyester filter, and a sample of filtered WAF was collected in four liter hydrocarbon-free glass jugs, spiked with a surrogate standard, and extracted immediately. Stock WAF was chilled to testing temperature, then diluted with filtered seawater to achieve each desired oil concentration. For the larval experiments, an additional high WAF concentration was prepared as described above, but using 3000 μL oil and 120 μL dispersant.

**Egg Experiments**

***Exposures***

Herring eggs were exposed to water-accommodated fractions (WAF) of weathered ANS, chemically dispersed WAF, and UV in a 3-factorial experiment. There were 6 oil levels (including controls), 2 dispersant levels (absent, present), and 3 UV treatments (controls, 2.5 hr UVA, 15 hr UVA). Each treatment was conducted in triplicate with approximately 100 eggs per slide in 1 L wide-mouth jars, and included a 96 hr WAF exposure, followed by UV treatment. Eggs from each female were present in each WAF × dispersant combination. Exposure of eggs to WAF began approximately 44 h after fertilization and continued for four days, followed by UV treatment. Racks containing slides with eggs from each female were randomly transferred into 3 L jars containing chilled WAF dilutions and suspended from mobile overhead racks (Carls et al., 1999). Nylon plankton netting that contained additional eggs for determining bioaccumulation was also suspended in each jar. Maximum biomass was estimated as 1.6-3.2 g tissue/L during the first two days and 0.6-0.8 g/L thereafter. Eggs were exposed to hydrocarbons for 4 days with daily WAF renewal, then transferred to clean seawater and randomly allocated to 1 of 3 UV treatments (each replicated 3 times). Each replicate consisted of a glass slide with approximately 100 eggs placed inside a 1 L wide-mouth glass jar. Nets with bioaccumulation eggs were rinsed in seawater and frozen in hydrocarbon-free jars for analysis. Qualitative fertilization success was determined blind 9-11 d after spawning.

***Endpoints***

Hatch timing and success, larval viability, and larval abnormalities were observed daily during peak hatch (which peaked sharply on April 24, 2001) without knowledge of

treatment. Observation frequency was reduced to 2 or 3 day intervals during periods of low hatch, and larvae were collected only if 5 or more were present per jar. Living larvae were assessed for swimming ability and gross morphological deformities, anesthetized with tricaine methanesulfonate, and preserved in 5% phosphate-buffered formalin. Dead larvae were enumerated and discarded. At each observation, the slide with eggs was transferred (within a few seconds) to a jar containing freshly filtered (1 μ) seawater. The swimming ability of live larvae was categorized as effective, ineffective, or incapable. Effective swimmers were active, frequented the water column, and avoided capture. Ineffective swimmers were generally more lethargic, and were more likely to be found on jar bottoms. Incapable larvae were unable to swim in a straight line and were often only capable of spasmodic twitching. Preserved stage 1a and 1b larvae were indiscriminately subsampled blind from each female in each treatment group to score yolk-sac edema, indicated if the anterior margin of the yolk membrane was bounded by an area of clear fluid or irregularities in yolk shape (n = 40 per replicate except where fewer larvae were available). After hatch was complete (as indicated by no viable eggs remaining), any dead embryos were inspected and enumerated.

**Larval Experiments**

Herring larvae were exposed to WAF of weathered ANS or WAF+dispersant, and UV in a 3 factorial experiment. There were four oil levels (including controls), 2 dispersant levels (absent, present) and four UV treatments including controls. Each treatment was conducted in triplicate with approximately 40 larvae in 1 L wide-mouth glass jars; biomass loading was < 0.1 g/L. Larvae (1 to 2 day old) were exposed to WAF for 24 hours, followed by UV treatment. Numbers of live, moribund, dead, and swimming larvae were enumerated daily for 8 days following UV treatment. The total number present was determined at the end of testing to calculate percent responses. Bioaccumulation of petroleum hydrocarbons was determined in separate groups of 1-3 day old larvae (approximately 2100 larvae/jar) at three dose levels (control, two highest WAF treatments) in 3 L jars for 24 hours.

**Laboratory UV Exposures**

***Exposure system***

The laboratory UV exposure system consisted of paired tanks (225 cm × 55 cm) with black bottoms and reflective sides (aluminum foil, dull side out). Fluorescent lamps were positioned approximately 60 cm above tank bottoms. Exact positioning of the light fixtures above the tank was determined in preliminary measurements to minimize variation of UV radiation within the exposure system. Each tank was covered with a thin cracked-crystal style light panels as light diffusers and subdivided into a UVA chamber (130 × 55 cm) and a control light chamber (65 × 55 cm) by an approximately 30 cm black plastic partition consisting of 2 layers of black plastic and aluminum foil lining. Each UVA chamber was illuminated with two UVA lamps (GE 48” F40BLB black light bulb) and two Chroma 50 lamps (GE 48” F40C50) oriented longitudinally. Spectral output of the UVA lamps was approximately 320 to 420, with maximum intensity at 340 to 380 nm (Fig. 1). Control light chambers were illuminated with two Chroma 50 lamps, which provided visible light plus minimal UV. Flowing seawater provided temperature control in each chamber.

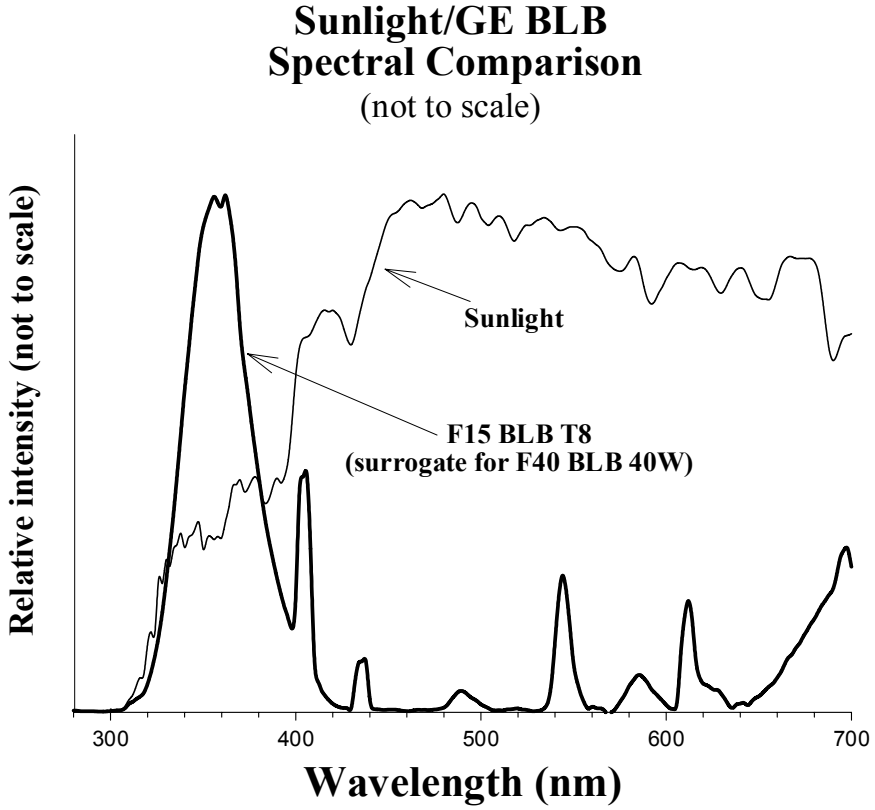


Figure 1. Spectral output of a UVA lamp compared to example solar radiation spectrum of sunlight.

***Light measurements***

A broad wavelength radiometer (Macam UV 203, Macam Photometrics, Scotland) was used to measure visible light (400 to 700 nm, square wave response) and ultraviolet radiation (UVA: 320 to 400 nm, max response at ~365 nm; UVB: 280 to 320 nm, maximum response at ~315 nm). Light intensity was measured with the detector positioned at the bottom of a test jar filled with filtered seawater. Light intensity was measured in multiple positions in each light chamber and at multiple times to capture the variability associated with tank position and measurement time. For both egg and larval experiments, light intensities for the control light exposures ranged from 10-16 $\mu WXcm^{-2}$ UVA, 0.2-0.6 $\mu WXcm^{-2}$ UVB, and 380-640 $\mu WXcm^{-2}$ visible light. Light intensities in the UVA exposures ranged from 470-650 $\mu WXcm^{-2}$ UVA, 0.2-0.5 $\mu WXcm^{-2}$ UVB, and 370-560 $\mu WXcm^{-2}$ visible light. Ambient UV in the laboratory was very low, with light intensities ranging from 0.2-1.0 $\mu WXcm^{-2}$ UVA, 0.001-0.02 $\mu WXcm^{-2}$ UVB, and 29-130 $\mu WXcm^{-2}$ visible light.

***UV treatments***

Egg UV treatments were initiated in clean seawater within 1 hour of the termination of WAF exposure; larval UV treatments began after approximately 24 hours of WAF

exposure without transferring the fish to clean seawater. Larvae were not transferred to clean water to avoid potential injury. Eggs and larvae were exposed to control light or UVA for 15 hours, and a separate group of eggs were exposed to UVA for 2.5 h. UV doses ($\mu WXhXcm^{-2}$) were computed from the specific light intensities measured during each exposure multiplied by the duration of light exposure. All treatments occurred in temperature controlled tanks. Test jars were placed under ambient lighting in the laboratory when not being exposed to UV treatments.

**Sunlight Exposures**

Sunlight exposures were only performed in the larval experiments because the potential importance of UVB in photoenhanced toxicity was not recognized until egg exposures were completed. The sunlight exposure system consisted of a temperature controlled 1.0 m diameter × 19 cm high aluminum-lined basin filled with filtered laboratory seawater. Ambient sunlight exposures were conducted on two sequential days in Juneau, Alaska, and ranged from 1.6 to 2.8 hours, with the duration of exposure adjusted to the ambient UVB. Light intensities were measured every 15 to 30 minutes depending on the variation in ambient conditions, with the radiometer detector positioned at the bottom of a test jar filled with filtered seawater. The environmental conditions ranged from complete to partial clouds, solar disk visible to not visible, haze, and occasional rain. Average light intensities during ambient sunlight exposures ranged from 570 to 1250 $\mu WXcm^{-2}$ UVA, 7.0 to 14 $\mu WXcm^{-2}$ UVB, and 4850 to 11,600 $\mu WXcm^{-2}$ visible. UV doses ($\mu WXhXcm^{-2}$) were computed from the average light intensity measured during each exposure multiplied by the duration of light exposure. Table 1 summarizes the light treatments used in the larval experiments.

Table 1. Range of UV and visible light doses ($\mu WXhXcm^{-2}$) in each larval light treatment.

| Light Treatment | UVA Dose | UVB Dose | Visible Light Dose |
|---|---|---|---|
| Control Light | 126 - 165 | 3.3 - 5.2 | 6,640 - 7,860 |
| UVA | 5,260 - 7,020 | 3.4 - 6.1 | 6,900 - 7,360 |
| Sunlight | 2,160 - 3,600 | 19 - 41 | 22,300 - 26,300 |
| UVA+sunlight | 7,420 - 10,600 | 23 - 47 | 28,400 - 33,600 |

**Photomodification/Photosensitization Experiment**

A separate larval experiment was conducted to discriminate between two possible modes of action of the photoenhanced toxicity of oil, photosensitization (activation of hydrocarbons in tissue) or photomodification (photooxidation of hydrocarbons in water). These experiments were performed similarly to the main larval experiment. Five treatment combinations of WAF (larval exposure to WAF either before or after UV exposure) and UV (sunlight present or absent) were evaluated for each dispersant treatment group (dispersant present or absent): (1) no WAF in water or larvae, no sunlight (2) no WAF in water or larvae, 4 hr sunlight, (3) WAF in water and larvae (24

hour pre-exposure to WAF), no sunlight, (4) WAF irradiated 4 h with sunlight, then larvae with no prior WAF exposure were added, and (5) no WAF in water, WAF in larvae, 4 hr sunlight. Aqueous tPAH concentrations in WAF exposures were 8.0 $\mu gXL^{-1}$ for oil-only treatments and 9.0 $\mu gXL^{-1}$ for oil+dispersant treatments. UV exposures occurred in sunlight (4 hr, 1350 $\mu WXcm^{-2}$ UVA, 14 $\mu WXcm^{-2}$ UVB, and 14,900 $\mu WXcm^{-2}$ visible) with mostly sun, some haze, and solar disk always visible. No sunlight exposures were conducted under ambient laboratory lighting of 0.59 $\mu WXcm^{-2}$ UVA, 0.009 $\mu WXcm^{-2}$ UVB, and 79 $\mu WXcm^{-2}$ visible.

**Chemical Measurements**

Water and tissue samples were extracted with dichloromethane after addition of six internal standards. Isolation and purification of calibrated and uncalibrated compounds was completed by silica gel/alumina column chromatography followed by size-exclusion high-pressure liquid chromatography (HPLC) and fractionation; seawater samples were not fractionated by HPLC. Extracts of PAH were separated and analyzed by gas chromatography equipped with a mass selective detector. Calibrated PAH were identified by retention time and two mass fragment ions characteristic of each PAH and quantified using a five point calibration curve. Uncalibrated PAH homologs (which included alkyl-substituted isomers of naphthalene, fluorene, dibenzothiophene, phenanthrene, and chrysene) were identified by retention time and the presence of a single characteristic mass fragment ion. Uncalibrated PAH were quantified by using calibration curves of their respective parent homologs. Experimentally determined method detection limits (MDL) depended on sample weights, and generally were 1 ng/g in tissue, and 1 to 8 ng/L in water. Concentrations below MDL were treated as 0. Tissue concentrations are reported on a wet-weight basis, but wet to dry weight ratios were measured by dehydrating 1 g wet samples for 24 h at 60EC and weighing the remaining mass. The accuracy of the hydrocarbon analyses was about ±15% based on comparison with National Institute of Standards and Technology values, and precision expressed as coefficient of variation was usually less than about 20%, depending on the PAH. Total PAH concentrations were calculated by summing concentrations of individual PAH. Relative PAH concentrations were calculated as the ratio of PAH concentration to the TPAH concentration.

**Data Analyses**

Data were analyzed with multi-factor analysis of variance (ANOVA) (SAS, 1996), regressions, logit analysis (Berkson, 1957), or Spearman-Karber analysis (Hamilton et al., 1977) as necessary. Where overall ANOVA factors were significant, treatment groups were compared to controls with *a priori* multiple comparisons and the acceptance criterion for statistical significance ($\forall$) was 0.05 divided by the number of comparisons to determine no (NOEC) and lowest (LOEC) observed effect concentrations. Percentage data were arc-sine transformed before ANOVA, and corrected for small *n* (Snedecor and Cochran, 1982), but the same general conclusions were reached with untransformed data. Treatment responses were corrected for corresponding control response (Abbott, 1925) before calculating median lethal (LC50) and median effective (EC50) concentrations. Complete analysis required two models (logit and

Spearman-Karber). Estimates were averaged where both were present (44% of all estimates) to avoid pseudoreplication in the final analysis.

Percent response was based on the total number of larvae present in a given treatment at the beginning of the test in larval experiments, but calculations were more complex in egg experiments. In egg experiments, independent measures of embryo response were percent hatch, percent moribund plus dead, percent with spinal abnormalities, percent effective swimmers, and yolk-sac edema. Percentages of eggs that hatched were based on the initial number of eggs determined before hatch. Hatched larvae were categorized as live, moribund (not moving), or dead (tissue necrosis evident), and percentages were based on the total number of larvae enumerated during hatch including dead embryos remaining on slides. Percentages of effective swimmers were based on the number of live larvae: percentages of larvae with spinal aberrations were based on the total of live and moribund larvae. Larvae were not inspected for morphological damage.

The extent of PAH dissolution in the exposure WAFs was evaluated by calculating the ratio of phytane and total PAH (phyt::PAH). Phytane is a branched aliphatic hydrocarbon of very low solubility and is used here as a proxy for the amount of whole oil in oil-phase droplets. The PAH are more soluble. The phyt:tPAH is 0.12 for unweathered ANS crude oil, and values of the ratio decrease as water extracts of oil become enriched with dissolved PAH in addition to any PAH associated with dispersed, whole oil droplets.

## RESULTS

### Effects on Larvae

#### *Oil-only effects*

Water accommodated fractions of weathered ANS were directly toxic to herring larvae and exhibited a dose-response relationship of increasing mortality and morbidity with both increasing tPAH in water and tPAH residues in tissue (Fig. 2). Under control lighting, the 96 hr LOEC for larvae was greater than the highest tested concentration in oil-only exposures (86.5 $\mu gXL^{-1}$ tPAH; tissue residue of 34.8 $\mu gXg^{-1}$ tPAH), although mortality and morbidity were substantially elevated above no oil controls (bottom panel; Fig. 2). Median lethal concentrations (LC50s) decreased over time ($P < 0.001$; Fig. 3). For example, LC50s in the oil-only control light treatment (only significant visible light) decreased from 37 $\mu gXL^{-1}$ at 4 days to 26 $\mu gXL^{-1}$ at 8 days. Median effective concentrations (EC50s), based on impaired swimming in addition to mortality and morbidity, were similar to LC50 values (Fig. 3).

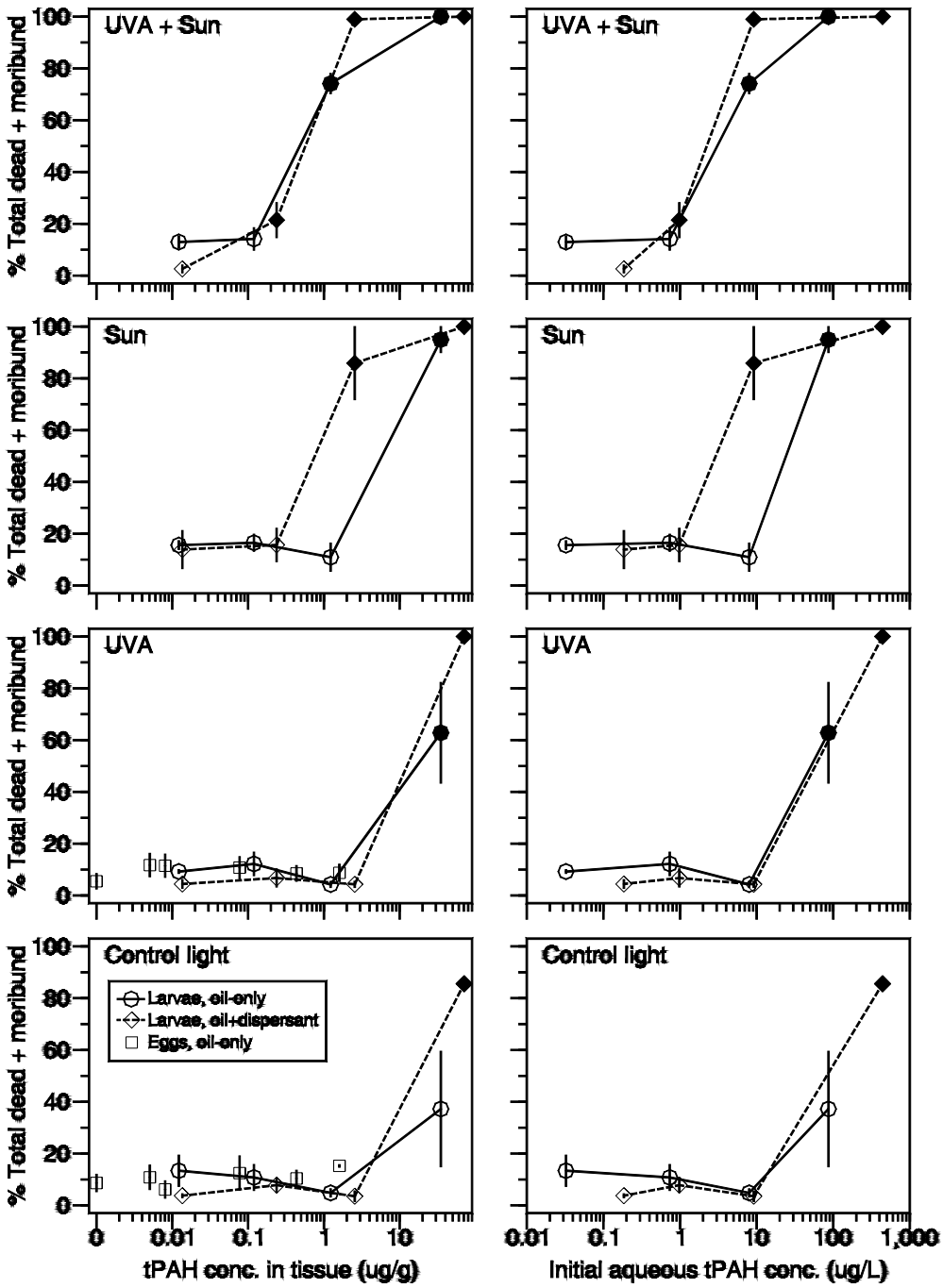


Figure 2. Mean percent dead and moribund (±SE) herring larvae exposed to water accommodated fractions of Alaska North Slope crude oil (circles) or oil+dispersant (diamonds). Solid symbols indicate significant differences from the controls ($P \leq 0.05$). Each panel shows one of four light treatments. Left panels show response vs tPAH in tissue ($ngXg^{-1}$ ww), and right panels show response vs tPAH in water.

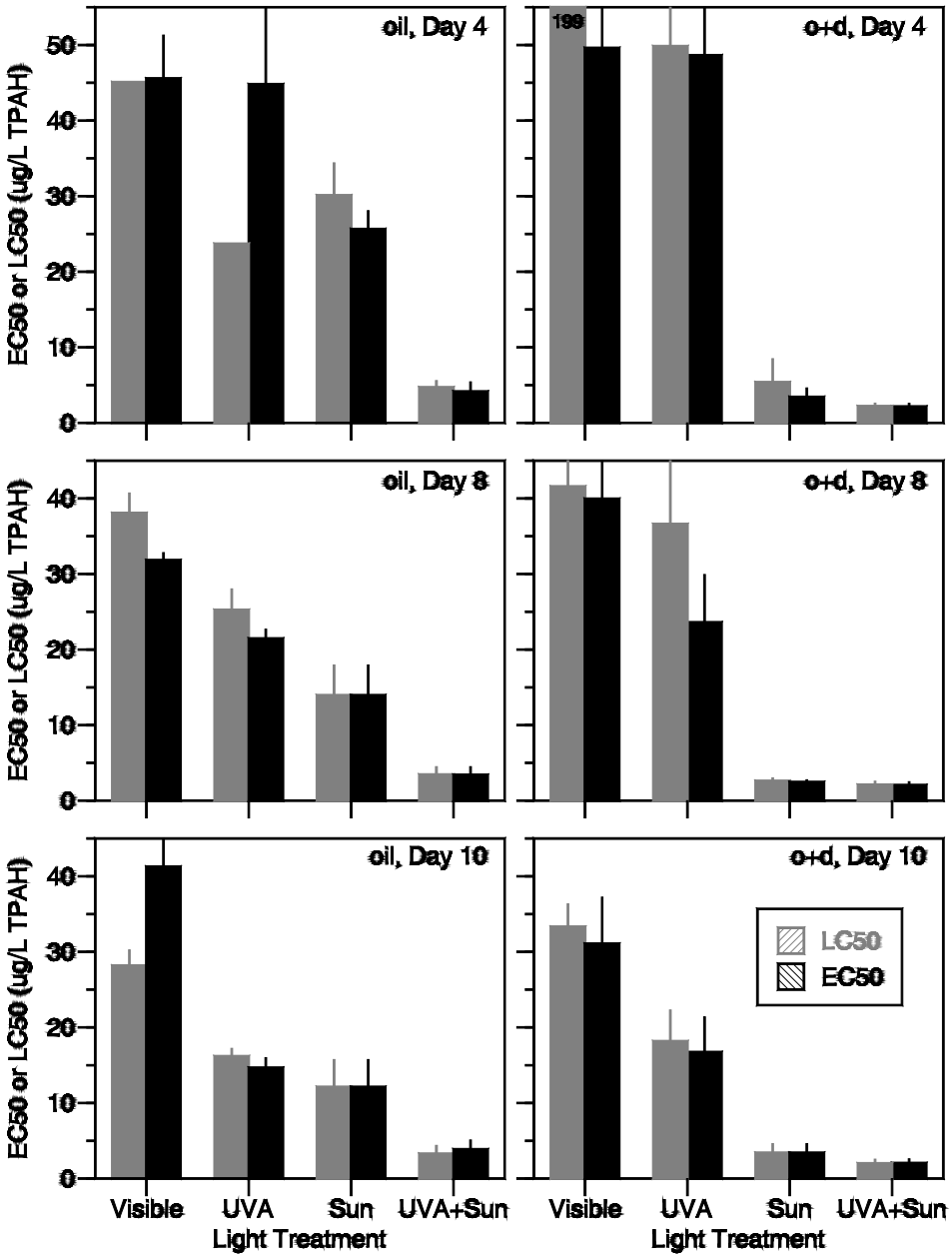


Figure 3. Mean (±SE) median lethal concentrations (LC50; grey bars) and median effective concentrations (EC50; impaired mobility plus death; black bars) for herring larvae exposed to water accommodated fractions of Alaska North Slope crude oil (oil) or oil+dispersant (o+d) for 4, 8, and 10 days. Larvae were exposed to one of four light treatments; visible is the control light treatment (negligible UV). Asterisks indicate significant differences from the corresponding control ($P \leq 0.05$).

***UV+oil effects***

The toxicity of ANS was enhanced by exposure to UV, and the degree of photoenhanced toxicity increased with increasing UV treatment (UVA+sun>sunlight>UVA) and with increasing tPAH concentration in tissue. In the UVA treatment, the percentage of dead+moribund larvae at 86.5 $\mu gXL^{-1}$ tPAH (63%) was significantly elevated and nearly twice that in the corresponding control light treatment (Fig 2). Similarly, UVA exposure also significantly lowered 8 and 10 day LC50s and EC50s to approximately 50% of the control light treatment (Fig. 3). Sunlight, which had approximately 50% of the UVA and 6 times more UVB than the UVA treatment, caused significantly greater photoenhanced toxicity than UVA alone (Fig. 2). For example, in the sunlight treatment there was greater than 90% mortality and morbidity in larvae exposed for 4 days to 87 ug/L, 63% affected in the UVA treatment, and 37% affected under control light. The combination of UVA and sunlight caused the greatest enhancement of ANS toxicity. LC50s, EC50s, and LOECs were 18-450 fold lower in the UVA+sun treatment compared to control lighting with minimal UV. In the highest UV treatment (UVA+sunlight), the 96 hr NOEC in the highest UV treatment were 0.7 $\mu gXL^{-1}$ and 0.1 $\mu gXg^{-1}$ tPAH in oil-only exposures.

***Oil+dispersant effects***

As in oil-only exposures, oil+dispersant treatment exhibited a dose-response relationship between mortality and morbidity and the tPAH concentration in tissue and water (Fig. 3). Under control lighting, the toxicity of chemically-dispersed oil was similar to oil-only toxicity at equivalent tPAH concentrations in tissues and in water (Figs. 2 and 3). The 96 hr NOECs in the oil+dispersant treatment were 9.2 $\mu gXL^{-1}$ and 2.5 $\mu gXg^{-1}$, and 96 hr LOECs were 440 $\mu gXL^{-1}$ and 70.7 $\mu gXg^{-1}$ tPAH (Fig. 2). Mortality in dispersant controls (0.4 and 4 $uLXL^{-1}$ Corexit 9527) was not significantly greater than mortality in water-only controls in any light treatment ($0.658 \# P \# 0.935$) and averaged 10% ± 4% across all light treatments at 4 $uLXL^{-1}$ after 8 days exposure.

***UV+oil+dispersant effects***

Also consistent with oil-only results, UV treatment resulted in substantial photoenhanced toxicity. UVA caused the least enhancement of oil+dispersant toxicity and UVA+sunlight caused the greatest increase in mortality and morbidity above the control light treatment (Fig. 2). There were no significant differences in LC50s or EC50's between oil-only and oil+dispersant exposures (Fig. 3). However, tPAH concentrations in tissue were elevated approximately two times above oil-only exposure, resulting in increased mortality and morbidity (Fig. 2) and more rapid mortality in sunlight and UVA+sunlight treatments (data not shown). In the highest UV treatment (UVA+sunlight), the 96 hr NOECs in the highest UV treatment were 0.2 $\mu gXL^{-1}$ and 0.01 $\mu gXg^{-1}$ in oil+dispersant exposures (Fig. 2).

**WAF Chemistry**

The methods used to prepare the exposure WAFs resulted in different PAH compositions depending on the amount of oil added initially and on whether chemical dispersant present. The WAF prepared from 3,000 uL oil without dispersant resulted in a PAH composition only slightly different than whole ANS crude oil (Fig. 4a and 4d), with a phyt:tPAH of 0.041 and a total PAH concentration of 87 ug/L. This WAF was

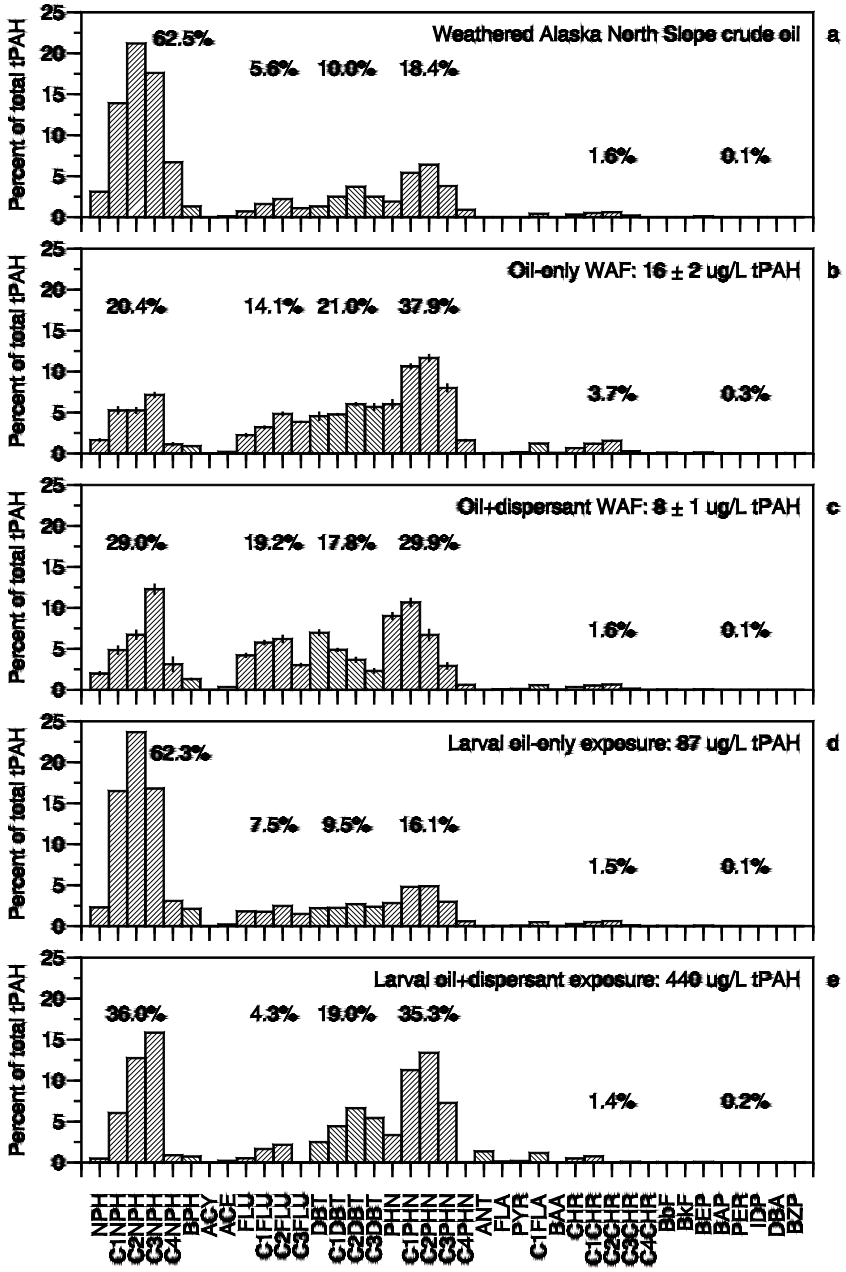


Figure 4. Composition of PAHs in weathered Alaska North Slope crude oil (ANS; top panel) and water accommodated fractions (WAF) of oily-only or oil+dispersant treatments (lower panels). Treatment conditions and tPAH concentration are designated on each panel. Percentages in each figure panel designate the composition of naphthalenes (NPH to C4NPH), fluorenes (FLU to C3FLU), dibenzothiophenes (DBT to C3DBT), phenanthrenes (PHN to C4PHN), chrysenes (CHR to C4CHR), and five ring PAHs (BbF to BZP), respectively.

dominated by the naphthalenes, like whole ANS crude oil. Addition of dispersant to the 3,000 uL oil preparation dramatically altered the PAH composition, and increased the tPAH concentration to 440 ug/L. The phyt:tPAH was 0.021 and contributions from the naphthalene and phenanthrene homologs were nearly equal, indicating much greater dissolution of three-ring PAH than when dispersant was absent (Fig. 4d and 4e). Some losses to the atmosphere of the lowest molecular weight PAH were also evident with addition of dispersant to these high-oil treatments

In contrast to the 3,000 uL oil treatments, the presence of chemical dispersant during WAF preparation had less effect on WAF chemistry prepared from 300 uL oil. Despite an overall similarity among PAH homologs, differences within homologous groups between oil-only and chemically dispersed oil were apparent (Fig. 4b and 4c) and the phyt:tPAH was significantly higher without dispersant (0.070 ± 0.006) than with dispersant (0.051 ± 0.006, $P = 0.050$). Both 300 uL preparations had more losses of naphthalene homologs, with the phenanthrene homologs dominant or equal, when compared with the 3,000 uL oil preparations. Dispersant promoted dissolution of the less substituted, more soluble alkyl homologs (including the naphthalenes, which subsequently volatilized).

**Bioaccumulation of tPAH**

The composition of PAHs bioaccumulated in larvae and eggs was generally similar to the composition of the exposure water (Fig. 5), and concentrations of tPAH in tissue were highly correlated with aqueous tPAH concentrations ($0.92 \# r^2 \# 0.98$, log-log transform; Fig. 6). Eggs accumulated less tPAH from oil and oil+dispersant WAF in 4 days than larvae did in 1 day, resulting in significantly lower tPAH concentrations in tissue at similar aqueous exposure concentrations (Fig. 5). Differences between bioaccumulation in oil-only and oil+dispersant exposures were not significant ($P > 0.1$) and across WAF treatments the mean bioconcentration factor (BCF) in larvae was 249 ± 59 ($n = 4$) and 76 ± 17 ($n = 2$), in eggs. Eggs in clean seawater depurated 19-32% of the accumulated tPAH after 15.5 hours.

**Effects on Herring Embryos**

Exposure of herring eggs to oil significantly increased the incidence of yolk-sac edema, but other responses did not differ statistically, and exposure to UVA did not increase toxicity (Fig. 7). Under control lighting, mean incidence of edema (52%, $n$=3) in the highest oil-only treatment (17 $\mu gXL^{-1}$) was significantly greater than in controls (8%), and similar trends toward increased edema at higher TPAH concentrations were evident in both UVA treatments. Because exposure to UVA clearly did not increase the incidence of edema ($P = 0.877$), all light treatments were combined in the final analysis; edema incidence (34%, $n$ =8) was significantly elevated at 17 $\mu gXL^{-1}$ ($P = 0.002$). Edema effects observed in the oil-only treatments were consistent with observations by Carls et al. (1999) of increased yolk-sac edema in 4-day exposures ($15 \# n \# 21$). Similar to Carls et al. (1999), the 96 hr NOEC for yolk-sac edema under control lighting was 0.4

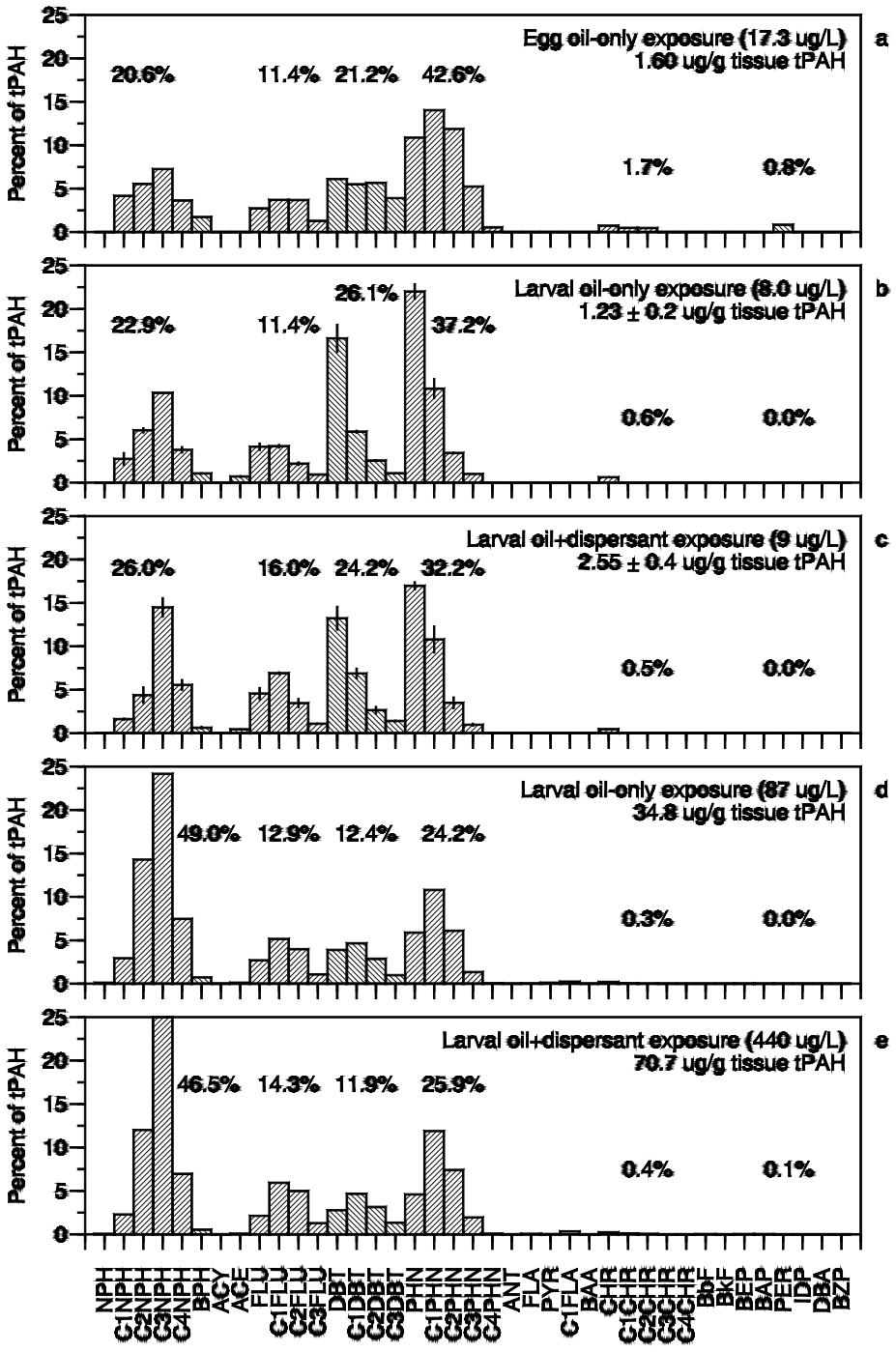


Figure 5. Composition of PAHs in herring eggs (top panel) and larvae (lower panels) exposed to the water accommodated fractions of weathered Alaska North Slope crude oil. Treatment conditions and tPAH concentrations in tissues ($ngXg^{-1}$ ww) are designated on each panel. See Fig. 4 for explanation of percentages and PAH identifications.

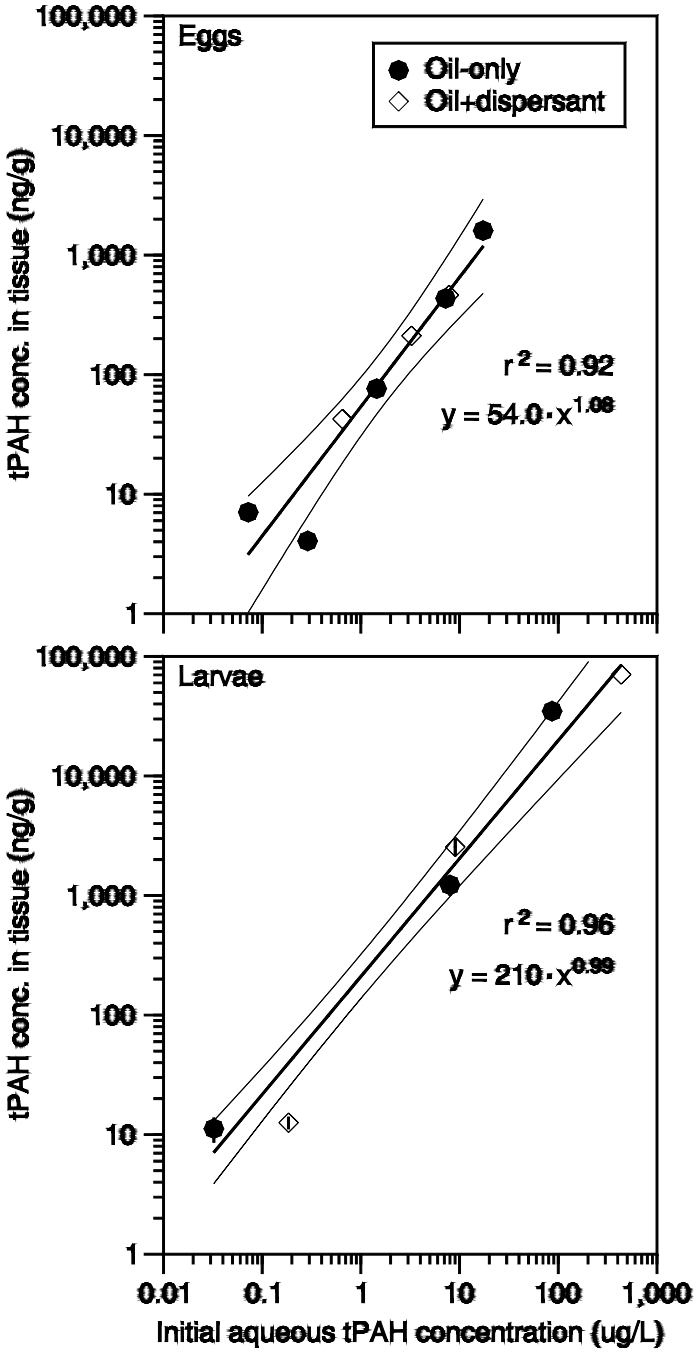


Figure 6. Relationship between tPAH (ng/g ww) in herring eggs (96 h exposure; top panel) and larvae (24 hour exposure; bottom panel) exposed to water accommodated fractions of oil or oil+dispersant. Best fit regressions with 95% confidence bounds are shown.

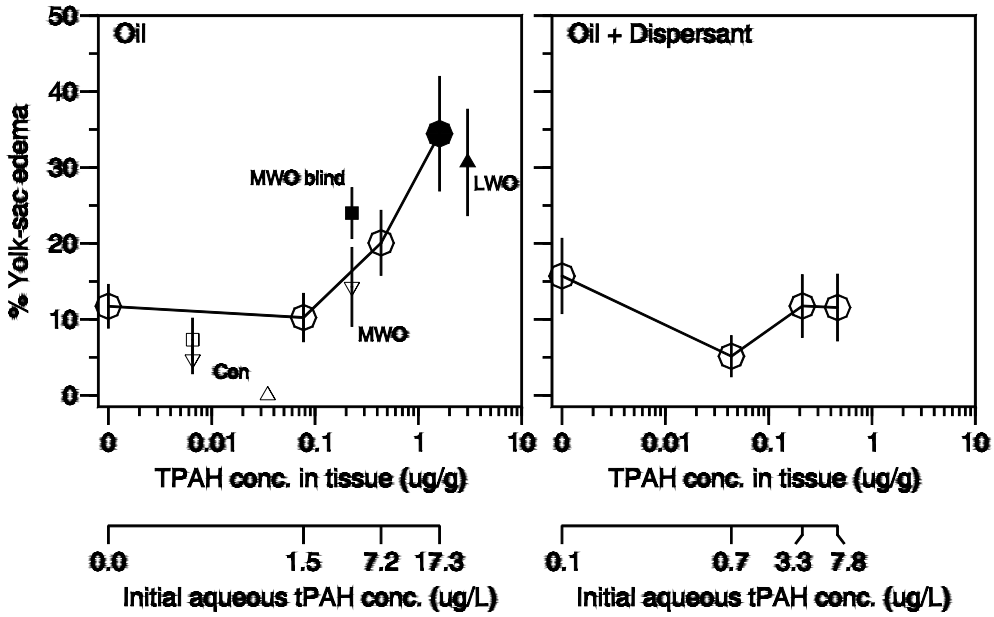


Figure 7. Mean percentage (±SE) of yolk-sac edema in herring embryos (circles) to exposed 4 days to water-accommodated fractions of oil or oil+dispersant vs tPAH concentrations in eggs (ngXg$^{-1}$ wet weight) and exposure water. Triangles show the results of Carls et al. (1999) 4-day egg exposures to less-weathered (LWO) and more weathered (MWO) oil using a different WAF preparation method. Solid symbols indicate significant differences from the controls ($P \leq 0.05$).

µgXg$^{-1}$ tPAH. There were other general trends of increasing adverse biological responses of embryos (hatching, mortality, spine condition, and swimming) with increasing oil exposure, but values in the highest oil treatments were never significantly different than in controls (data not illustrated). No significant embryo effects were observed in the oil+dispersant treatments, but corresponding tPAH concentrations (maximum 7.8 ± 0.9 µgXL$^{-1}$, $n = 4$) were lower than in oil-only WAF (maximum 17.3 ± 1.2 µgXL$^{-1}$, $n = 4$).

**Photomodification**

In tests designed to discriminate between photosensitization and photomodification, exposure to sunlight caused significant larval mortality and morbidity only when oil was present in larval tissue (Fig. 8). When larvae were pre-exposed to oil, sunlight treatment caused 100% larval mortality within 3-5 days in both oil-only and oil+dispersant treatments. There was no significant mortality in any other treatment (2 to 12% mean mortality and morbidity; Fig. 8).

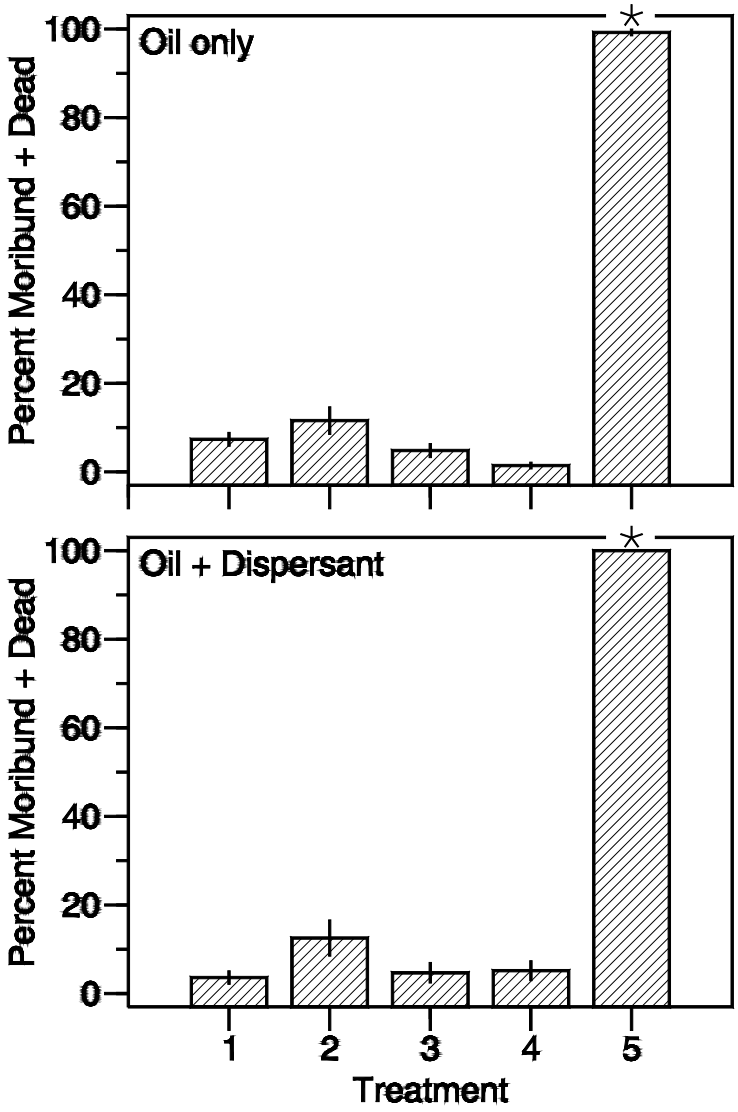


Figure 8. Mean (±SE) percentage of dead and moribund herring larvae exposed to water accommodated fractions (WAF) of North Slope crude oil or oil+dispersant at 4 days from initiation of oil exposure. Larvae were exposed to one of five light and oil exposure regimes: (1) no WAF in water or fish, no sunlight (2) no WAF in water or larvae, then 4 hr sunlight, (3) WAF in water and fish (24 hour pre-exposure to WAF), no sunlight, (4) WAF in 4 h sunlight, then added fish with no WAF pre-exposure, and (5) no WAF in water, WAF in fish, then 4 hr sunlight.

## DISCUSSION

The interaction of aqueous phase oil, chemical dispersant (Corexit 9527), and UV radiation resulted in lethal and sublethal effects in Pacific herring larvae and embryos. ANS was strongly phototoxic to herring larvae and photoenhanced toxicity only occurred when oil residues were present in tissues, rather than through photomodification. Brief exposure to sunlight (~2.5 hours/day for two days) was sufficient to significantly increase toxicity to herring larvae, and was more potent than laboratory exposures using only UVA. LC50s, EC50s, and LOECs in larvae were 1.5-48 fold lower in the sun treatment compared to control lighting with minimal UV. In the sunlight+UVA, LC50s, EC50s, and LOECs in larvae were 18-450 fold lower in than under control lighting with minimal UV. Photoenhanced toxicity was not observed in eggs, but only control and UVA exposures were utilized and accumulated residues were lower than in larvae. The toxicity of chemically-dispersed oil was generally similar to oil-only toxicity at equivalent tPAH concentrations in tissues and in water, but toxicity occurred sooner in the presence of dispersant and sunlight. The chemical dispersant appeared to accelerate PAH dissolution into the aqueous phase. These results are consistent with the hypothesis that weathered ANS is phototoxic and that UV can be a significant and causative factor in the mortality of early-life stages of herring exposed to oil and chemically-dispersed oil.

### Phototoxic Compounds in Oil

The study results are also consistent with previous observations that oil low in known phototoxic PAHs can cause significant photoenhanced toxicity (Pelletier et al., 1997; Little et al., 2000; Calfee et al., 2000; Deusterloh et al., in review). Based on quantitative structure activity relationship modeling (e.g., Veith et al., 1995), the phototoxic compounds in oil are likely specific 3 to 5 ring polycyclic aromatic compounds containing either carbon (i.e., PAHs) or heteroatom substitutions within the conjugated rings (i.e., heterocycles). The weathered ANS and WAFs prepared from ANS had a low composition of known phototoxic PAHs, including anthracene, flouranthene, pyrene, benzo(a)anthracene, and benzo(a)pyrene. Dibenzothiophene, chrysene and alkyl homologs of the known phototoxic PAHs present in ANS are also expected to be phototoxic based on QSAR modeling (Mekenyan et al., 1994; Kosian et al., 1998; Veith et al., 1995), but these compounds were also low in WAFs. To date, only a limited number of potential phototoxic compounds that occur in oil have been characterized in single compound tests. For example, heterocyclic aromatic compounds (e.g., acridine) that are known to be both phototoxic and to occur in oil are not quantified in the typical PAH analyses used in oil science (Barron et al., 1999). In the absence of definitive information on the specific phototoxic components of oil, we recommend tPAH as the best available surrogate for quantifying oil exposures. A polyaromatic structure is necessary to confer phototoxic properties, and because of similar or greater water solubility and partitioning, concentrations of phototoxic PAHs and heterocycles are likely to co-vary with tPAH.

**Mechanism of Oil Phototoxicity**

Previous studies on the toxicity of individual PAHs to fish and aquatic invertebrates exposed to UV have shown that PAHs primarily act through a photosensitization mechanism, where bioaccumulated PAHs rather than aqueous phase PAHs are activated by UV (Landrum et al., 1987). The photoenhanced toxicity of a weathered middle distillate oil also appeared to act through a photosensitization, rather photomodification of aqueous phase oil (Little et al., 2000). In the current study, a comprehensive set of toxicity tests with herring larvae were performed with oil-only and oil+dispersant exposures to evaluate the mechanism of action of weathered ANS. In these tests, significant toxicity was only observed in larvae that first bioaccumulated oil, then were exposed to sunlight. There was no significant toxicity in the photomodification test, where aqueous phase oil or oil+dispersant was exposed to sunlight for 4 hours prior to organism exposures.

**Light Spectra Causing Photoenhanced Toxicity**

The majority of photoenhanced toxicity studies with PAHs and all previous studies with oil have been performed with simultaneous UVA and UVB exposures, thus the relative contribution of UV regions of the light spectrum has been unknown (Barron et al., 2000; Barron and Ka'aihue, 2001). We included light treatments with only UVA because of recent research demonstrating that several PAHs that are components of oil were photoactivated in the absence of UVB (Diamond et al., 2000). We also used limited sunlight treatments to provide environmentally realistic UVB exposures. Significantly greater toxicity was observed in sunlight-only exposures than in UVA-only tests, even under marginal environmental lighting conditions (rain, clouds, haze, intermittent solar disk visibility) and low doses of UV (UVA: 2200-3600 uW*hr/$cm^2$ ($\mu$WXhXcm$^{-2}$); UVB: 19-41 uW*hr/$cm^2$ ($\mu$WXhXcm$^{-2}$). The sunlight+UVA treatment had nearly identical UVB and approximately 50% more UVA than the sunlight only treatment, and resulted in significantly enhanced toxicity. However, sunlight has a greater representation of higher UV wavelengths that may be important in PAH photoactivation (Diamond et al., 2000). Huovinen et al. (2001) showed that increasing UVB exposure increased the toxicity of the photoactive PAHs pyrene and anthracene, but had no effect on phenanthrene toxicity, which was not expected to be phototoxic based on its structural conformation (Mekenyan et al., 1994). These results are consistent with UVB photoactivation of pyrene and anthracene, and support the observations in herring larvae that UVB may be important in determining the magnitude of photoenhanced toxicity. Additional research is needed on the interaction of UVA and UVB because estimation of PAH risks using only levels of UVA (e.g., Diamond et al., 2000) may not be predictive of environmental phototoxicity. Additionally, the possibility that UVB increases tissue susceptibility to damage by photoactivated PAH should be considered in future studies.

**Life Stage Sensitivity**

Based on tissue residue levels, herring embryos and larvae appeared to have similar sensitivity to oil exposure. For example, embryos developed elevated yolk-sac edema at tPAH concentrations of 1.6 $\mu$gXg$^{-1}$ tPAH (oil-only) but no effects on mortality or morbidity was observed. Consistent with this observation, mortality and morbidity were not elevated in experiments with larvae at 1.2 $\mu$gXg$^{-1}$ tPAH. The apparent lower

sensitivity of eggs than larvae based on aqueous concentrations of oil is explained by lower tPAH bioaccumulation by eggs (4-day BCF = 76) than by larvae (1-day BCF = 249). UVA exposure in the absence of UVB had no effect on embryo survival or yolk-sac edema at the highest tested oil concentrations. UV treatments with significant UVB (sunlight, UVA+sun) were only performed in the larval experiments because the potential importance of UVB in the photoenhanced toxicity of ANS to herring was not recognized until egg exposures were completed. We hypothesize that significant photoenhanced toxicity in herring eggs would occur in the presence of elevated oil residues in eggs and sunlight exposure.

Polycyclic aromatic compounds are likely the toxic components of weathered ANS because of their known toxicity (Livingstone, 2001) and the observed dose-response relationships in both eggs and herring in the current study. Egg results were consistent with previous observations by Carls et al. (1999) using WAF prepared by passing water through an oiled gravel column instead of the high energy WAF prepared in the current study. In the column WAF experiments, Carls et al. (1999) observed significantly elevated yolk-sac edema at tissue residues at 0.4 $\mu g X g^{-1}$ in exposures to more weathered oil. Mortality, morbidity, yolk-sac edema, and other sublethal effects increased with the duration of embryo exposure and accumulated tPAH, and the effects were more severe with more weathered oil (Carls et al., 1999). This consistency of results indicates that petroleum compounds were responsible for toxicity rather than products of microbial degradation because of the short WAF preparation period in the current study. Alkanes and the unresolved complex mixture present in WAF generally did not accumulate in embryo and larval tissue. Additionally, a polyaromatic structure is necessary to confer phototoxic properties (Mekenyan et al., 1994) and photoenhanced toxicity studies with larvae clearly indicated a causal relationship between tPAH exposure and UV treatment.

**WAF Chemistry**

The differences in PAH composition between the WAF preparations are caused by the interacting effects of dispersant and oil volume on the surface to volume ratio $(S:V)_{oil}$ of the oil under conditions of constant mixing energy. The $(S:V)_{oil}$ is simply the amount of surface area a unit of mass of oil has. The ratio increases linearly with decreasing radius of oil droplets. The ratio is the single most important factor governing the rates at which soluble components such as PAH dissolve into aqueous solution (Short and Heintz, 1997). In the high-oil (3,000 uL) WAF preparation without chemical dispersant, oil droplets created by the mixing process may coalesce relatively easily compared with the other preparations, leading to a relatively low $(S:V)_{oil}$ , and hence to comparatively slow PAH dissolution rates, resulting in a PAH composition almost like crude oil. Addition of dispersant promotes formation of smaller oil droplets, leading to a much higher $(S:V)_{oil}$ and hence to faster PAH dissolution rates, resulting in a higher tPAH concentration and to greater losses of the most volatile PAH to the atmosphere.

In the low-oil (300 uL) WAF preparations, the smaller amount of oil added intrinsically decreased the probability of droplet coalescence by simple dilution, leading to a lower $(S:V)_{oil}$ and attendant effects on PAH composition. The addition of dispersant had less effect on PAH composition most likely because of greater dilution by water. The dispersant was added after the oil was added in the mixing process, simulating conditions more likely in the field. But only 12 uL of dispersant was added to 32 L of

seawater in the 300 ug oil WAF preparations, compared to ten times that amount in the 3,000 ug oil preparations, so the amount of dispersant added in the low-oil preparations was likely to be correspondingly less effective. However, dispersant did increase the proportion dissolved PAH as indicated by the lower phyt:tPAH when dispersant was present, and increased the proportions of the less-substituted PAH homologues. The increased concentrations of PAH in true solution caused by dispersant likely led to more rapid bioaccumulation of PAH by larvae, which might account for the more rapid appearance of toxic effects in these larvae when exposed to sunlight.

**Phototoxicity of Chemically Dispersed Oil**

The photoenhanced toxicity of chemically dispersed oil was assessed using Corexit$^{R}$ 9527, which is the principle chemical dispersant stockpiled in Prince William Sound, Alaska, for oil spill response. We assessed the photoenhanced toxicity of ANS dispersed with Corexit 9527 because chemical dispersants may enhance the solubolization and bioavailability of the phototoxic components of spilled oil. For example, Corexit 9527 elevated the concentration of phenanthrene in both the aqueous phase of ANS and in algae exposed to oil+dispersant (Wolfe et al., 1999). Corexit 9527 contains nonionic and anionic surfactants and the solvent (ethylene glycol monobutyl ether) (Wolfe et al., 1999). These components are not expected to be phototoxic based on structural considerations (Vieth et al., 1995). Corexit 9527 was not directly toxic to eggs (0.4 uLXL$^{-1}$) or larvae (4 uLXL$^{-1}$) at the highest concentrations tested. Other studies have shown very low direct toxicity of Corexit 9527 to saltwater fish when no oil is present, with 2 to 4 day LC50s greater than 10,000 uLXL$^{-1}$ (e.g., Fucik et al., 1995; Behr-Andres et al., 1999).

Increasing UV exposure significantly elevated mortality and morbidity, and within a light treatment oil-only and oil+dispersant LC50 and EC50 values were generally similar. In contrast, NOECs and LOECs for oil+dispersants were lower in sunlight and sunlight+UVA treatments, and toxicity occurred sooner in the presence of dispersant and sunlight. Greater uptake of tPAH by larvae in the oil+dispersant treatments (e.g., 2.6 ugXg$^{-1}$ at 9 uLXL$^{-1}$) than oil-only treatments (e.g., 1.2 ugXg$^{-1}$ at 8 uLXL$^{-1}$) may explain a more rapid toxic response, rather than differential absorption of PAHs because PAH composition in water and tissue was similar in these two treatment groups. In the highest oil treatments, tPAH concentrations in dispersed WAF were 5 × greater than in oil-only WAF (469 ugXL$^{-1}$ versus 92 ugXL$^{-1}$), but at lower oil concentrations aqueous tPAH concentrations were about the same for dispersed-oil and oil-only WAF (9.2 ugXL$^{-1}$ and 8.0 ugXL$^{-1}$). Other factors besides initial oil-water ratios played an important role in determining aqueous tPAH concentrations, including mixing energy, mixing time, and atmospheric coupling (Carls et al., unpublished).

**Conclusions**

This research supports an increasing body of evidence that oil is toxic to aquatic organisms at extremely low PAH concentrations (Marty et al. 1997; Carls et al. 1999; Barron et al., 1999; Heintz et al. 1999; 2000; Rice et al., 2001) and that exposure to UV can significantly increase the toxicity of oil (e.g., Pelletier et al., 1997; Little et al., 2000; Calfee et al., 2000). In the current study, WAF was acutely lethal when prepared with only 0.01 to 0.1 g/L applied oil, resulting in ppb concentrations of tPAH. While

other hydrocarbons (alkanes, UCM) also entered treatment water, they did not accumulate in embryo or larval tissue. Previous studies have demonstrated that embryonic exposures of herring and pink salmon to weathered ANS at 0.4-5 $\mu gXL^{-1}$ tPAH caused malformations, genetic damage, mortality, decreased size, and impaired swimming in herring and salmon larvae, and reduced the marine survival of pink salmon (Carls et al., 1999; Marty et al., 1997; Heintz et al., 2000). The observation of significant photoenhanced toxicity for weathered ANS is in agreement with phototoxicity studies of fresh crude oils and refined fuels, including Prudhoe Bay crude (Pelletier et al., 2001), and a weathered middle distillate oil (Calfee et al., 2000; Little et al., 2000). Additionally, Duesterloh et al. (in review) have shown that weathered ANS is extremely phototoxic to two species of marine calanoid zooplankton. Along with the current work, these studies support the conclusions of Barron and Ka'aihue (2001) that photoenhanced toxicity of spilled oil may occur in Alaskan waters. Existing laboratory studies and toxicity databases on the toxicity of chemically dispersed oil to aquatic organisms (e.g., Fucik et al., 1995, DeCola, 1999; Behr-Andres et al., 1999) do not assess photoenhanced toxicity, and thus may substantially underestimate the hazard of chemically dispersed oil in the environment (Barron and Ka'aihue, 2001).

Herring appear to be extremely vulnerable to photoenhanced toxicity because both the eggs and larvae are translucent (allowing UV to penetrate deeply) and inhabit the photic zone of the water column. Laboratory studies, including those used to develop the toxicity database for Alaskan species (Rice et al., 1976), were performed with minimal exposure to UV. Thus injury and risk to aquatic organisms from an oil spill may be underestimated if based on standard laboratory bioassays and existing toxicity databases. The results of this study also suggest the potential for photoenhanced toxicity as a factor in herring impacts from the Exxon Valdez oil spill because of ppb levels of tPAH in the water column during the 1989 spill (Short and Harris, 1996), the high phototoxicity of weathered ANS and limited UV required, and potential the potential for sufficient UV exposures in the water column in Prince William Sound (Barron and Ka'aihue, 2001).

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