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PHOTOINDUCED TOXICITY OF ANTHRACENE IN AQUATIC ORGANISMS: AN ENVIRONMENTAL PERSPECTIVE

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ABSTRACT

The toxicity of anthracene, a common polycyclic aromatic hydrocarbon (PAH), has been assessed in a variety of aquatic organisms under environmentally realistic conditions. In the presence of natural or simulated sunlight anthracene was acutely toxic, at concentrations within aqueous solubility limits, to freshwater zooplankton, insect larvae, and fish. Less than 15 min was required for 50% immobilization of Daphnia pulex at 1.2 μg anthracene/ℓ under natural sunlight (UV-B, 310 +/- 34 nm, = 484 $\mu W/cm^2$). Culicid mosquito larvae were also sensitive to anthracene phototoxicity with a 24 hr LC-50 value of 26.8 μg/ℓ at a solar UV intensity five times less than summer maximum in Michigan. A 96 hr LC-50 value of 11.9 μg anthracene/ℓ was determined for a natural population of juvenile bluegill sunfish at a solar UV intensity equivalent to a depth of 0.6 m in a typical eutrophic north-temperate lake. A freshwater green alga was not adversely affected by the light-anthracene combination. These findings are in contrast to the previously reported non-toxicity of anthracene to aquatic organisms. Evidence exists which suggests that anthracene is only one of many PAH that can cause photoenhanced toxicity, and concentrations of these compounds are expected to increase in surface waters as a result of increased use of fossil-fuel for heat and energy. The potential environmental consequences of increased loading of PAH in freshwater and marine systems are discussed. We conclude that solar UV radiation is an environmental parameter which must be accounted for when assessing the toxicity of PAH to aquatic organisms.

1. INTRODUCTION

Assessing the potential hazards of chemicals in the aquatic environment is a monumental task. Until recently most of the information used for predictive hazard assessment has been obtained from single species toxicity tests conducted in the laboratory. While laboratory toxicity tests have played an important role in the development of hazard assessment, there is a strong need for the implementation of environmental realism in predictive toxicity testing [1]. The concepts of ecological relevance and pollutant realism have been discussed by Blanck and Gustafsson [2]. Pollutant realism is attained when the characteristics of a compound in the natural environment are incorporated into the laboratory test system [1]. In general, standard toxicity tests are insensitive to the complex interactions between an organism and its environment. For instance, standard laboratory toxicity tests generally have

indicated that polycyclic aromatic hydrocarbons (PAH) are not acutely toxic to aquatic organisms within the limits of aqueous solubility [3], and reported acute LC-50 values often exceed maximum solubilities by a factor of 100 to 1000 [4]. Such high concentrations indicate that carrier solvents were used in the tests or that the PAH of interest precipitated or formed micels, possibly altering the solution behavior or the bioavailability of the compound. Additionally, PAH studies in the laboratory are often conducted under specialized lighting to prevent photodegradation of the parent compound. By incorporating ecological realism into test systems, however, we have found that in the presence of natural or simulated sunlight, anthracene, a linear 3-ring PAH, is much more acutely toxic to a variety of aquatic organisms than previously expected, at concentrations well within the limits of solubility (< 35 μg/ℓ).

PAH consist of a class of compounds comprised of two or more fused benzene rings with occasional heteroatom or cyclopentene inclusions in the ring structure, or variously substituted alkyl side chains. Compounds in this class, of environmental importance, range from two-ring napthalene (MW 128.16) to seven-ring coronene (MW 300.36) [4]. PAH occur as natural products in plants and microbes [5]. Some PAH are released from volcanic activity and forest fires [6]. PAH can be formed under anaerobic conditions from quinones and related precursors produced by fungi, plants and animals [4], but evidence for direct synthesis of PAH by plants and animals is inconclusive [4]. Major sources of PAH in surface waters are oil spills, industrial processes, fossil fuel combustion and other pyrolytic processes attributed to human activity [7,8,9,10].

Nearly 230,000 metric tons of PAH enter the world's oceans and surface waters every year [4], and these inputs are expected to increase with increased use of coal as an energy source [11]. Eisenreich et al. [12] determined the major input of organic contaminants in the Great Lakes region to be non point-source atmospheric deposition, and PAH in freshwaters near industrialized areas can be elevated as a result of aerial inputs [6]. Water concentrations of PAH resulting from aerial inputs have been reported to range from 0.05 to 3.0 μg/ℓ [4]. The flux of total PAH and the concentration of 12 individual PAH into Lake Michigan from aerosols were measured, and in southern Lake Michigan, the flux of anthracene to the lake was between 0.9 and 14×10^5 kg/yr in dry deposition and 1.3×10^6 kg/yr in wet flux [13]. These fluxes resulted in considerable increases of PAH in the surface waters of the lake. Anthracene concentrations alone ranged from 0.015 to 0.15 μg/ℓ.

In outdoor stream microcosm experiments, anthracene caused acute mortality in juvenile bluegill sunfish at 12.7 μg/ℓ [14,15]. This concentration had shown no effects in laboratory fate experiments of similar duration. It was determined that sunlight, specifically the atmospheric ultraviolet (UV) portion of the electromagnetic spectrum, significantly enhanced the toxicity of anthracene in the outdoor microcosm studies. The findings presented in this report are derived from our extended investigations of the photoenhanced toxicity of anthracene in aquatic organisms under laboratory and field conditions. To date, we have studied this interaction in the crustacean, *Daphnia pulex*, larvae of the dipteran, *Aedes aegypti*, natural and hatchery populations of juvenile bluegill sunfish, *Lepomis macrochirus*, and the freshwater green alga, *Chlorella pyrenoidosa*. These findings are discussed with reference to the previously reported nontoxicity of PAH to these organisms, and to the fact that anthracene is probably only one of many PAH which can cause photoenhanced toxicity. We also assess the potential environmental consequences of increased loading of PAH in freshwater and marine systems from difficult to manage, non point-source inputs.

2. MATERIALS AND METHODS

2.1 Laboratory System

Sunlight was approximated in the laboratory using a combination of G.E. Chroma F40C50 white and Westinghouse FS40 ultraviolet fluorescent bulbs. The lights were mounted on a 1.22 X 2.74 m frame on 15.24 cm centers, alternating every other bulb. A 5 mil thick cellulose triacetate (CTA) filter was used to eliminate wavelengths shorter than 285 nm. Light intensity was varied by changing the height of the light bank over the bioassay table or by changing the thickness of the CTA filter. Except where otherwise noted, anthracene (MW 178.23, Sigma grade III, no. A-3885) solutions were obtained from a once-through aqueous elution column, which avoided the use of carrier solvents in the bioassays. Anthracene dissolved in acetone was slowly poured onto a thin layer of silica sand at 0.2% wt/wt, and the solvent was allowed to evaporate in the dark. When dry (24 hr) the sand was packed into a 7.5 X 45 cm glass column and flushed with water for 48 hr to remove loose anthracene crystals. Anthracene eluted from the column, as part of the laboratory water delivery system, at aqueous solubility (ca. 35 µg/ℓ at 22° C) and was diluted to a desired concentration before use.

2.2 Light Measurement

UV-B (310 +/- 34 nm) was quantified using a Macam Photometrics Model UV-103 radiometer equipped with a water-tight Model SD104 cosine-corrected photodiode (Macam Photometrics, Ltd., Livingstone, Scotland). Irradiance was read directly from the meter in units of µWatts/cm^2. Integrated photosynthetically active radiation (PAR) was measured using a LI-COR LI-188 integrating quantum photometer.

2.3 Daphnia Bioassays

Daphnia pulex were collected from a research pond at the Michigan State University Limnological Research Facility and were held in filtered well water. Organisms were fed a maintenance diet of yeast and C. pyrenoidosa. Static acute toxicity bioassays were used to characterize the actinic toxicity of anthracene. To obviate the need to add organic carrier solvents directly to bioassay chambers, a 'shell-coating' technique was employed. Anthracene was combined with 9-^{14}C anthracene (3.3 µCi/µMole, California Bionuclear, radiochemical purity 98%) in HPLC grade acetone to prepare a stock solution having 2.0 µg and 0.14 µCi anthracene per mℓ of acetone. Three nominal anthracene concentrations of 3.0, 9.6 and 30.0 µg/ℓ were achieved by adding 0.3, 2.0, and 6.0 mℓ of the anthracene stock to 300 mℓ beakers, evaporating the acetone to dryness and adding 200 mℓ of filtered well water. Similarly, designated beakers received 6.0 mℓ of acetone without anthracene to serve as 'shell-coated' controls.

Twenty adult D. pulex of approximately the same size and age were placed into each beaker and gently swirled periodically for 24 hr in the dark to allow the anthracene concentrations in the water and the organisms to reach steady state. Actual anthracene concentrations were determined from measured ^{14}C activity in the water and known specific activity.

Exposures were begun 24 hr after the water and organisms were placed in anthracene coated beakers and were conducted outside on clear (UV-B = 484

$\mu W/cm^2$), partly cloudy (UV-B = 278 $\mu W/cm^2$), and cloudy (UV-B = 189 $\mu W/cm^2$) days. Results are reported as the time (min) required to immobilize 50% of the organisms (ET-50). Solar radiation exposures were terminated after 60 minutes, or after greater than 50% immobilization in all anthracene treatments was observed, whichever occurred first.

2.4 Mosquito Bioassays

Mosquito larvae, *Aedes aegypti* (Rockerfeller strain) were reared in enamel pans at 25° C and were fed 15 mℓ of a 1.6% solution of dried liver powder each day. Static bioassays with 15 to 30 four day old (third instar) larvae were conducted in 500 mℓ beakers in the laboratory (sec. 2.1). Larvae were not fed during the bioassays. Larvae were preincubated in test solutions obtained from the anthracene column (sec. 2.1) or dilution water for 24 hrs to allow equilibration of anthracene with the organisms. After preincubation, the test solutions were replaced with fresh solutions of the same anthracene concentration. The larvae were then exposed to simulated sunlight, and percent mortality was recorded after 24 hrs of irradiation. Dark controls were exposed to similar anthracene concentrations in the absence of light. Light controls were exposed to simulated sunlight but were incubated in dilution water containing no anthracene. Larvae molted from 3rd instar to 4th instar during the course of the bioassays. Any larvae that pupated during the exposure period were not counted as part of a bioassay. Anthracene water concentrations were determined directly by reverse-phase HPLC.

2.5 Fish Bioassays

For most fish studies, a natural assemblage of juvenile bluegill sunfish (*Lepomis macrochirus*) was used. These fish were collected by seine from Park Lake, Clinton Co., Michigan. Other studies were conducted using *L. macrochirus* obtained from Osage Catfisheries (Osage Beach, MO) or from ByBrook Bass Hatcheries (Ashford, CT). Different populations of fish were kept segregated and were held in large flow-through fiberglass tanks with charcoal filtered, aerated tap-water at 22° C. Fish were held for at least two weeks prior to bioassays on an 18:6 hr light:dark photoperiod under a low pressure sodium lamp (UV fluence negligible) and were fed twice a day with Biodiet-Starter (BioProducts Inc., Worrenton, OR).

Fish were exposed to anthracene in 18.85ℓ glass aquariums in a flow-through system under the laboratory light system (sec. 2.1). Fish were transferred to dosing aquariums 48 hr prior to bioassays for acclimation and to establish an anthracene body burden approximately 80% of the theoretical steady state [16]. Ten fish per aquarium and two aquariums per anthracene concentration were used in all bioassays. Only fish appearing in excellent condition were used. The photoperiod was changed from 18:6 hr light:dark to continuous light in 2 hour per day increments. Fish were not fed for 48 hr prior to, and for the first 96 hr of a bioassay. After 96 hr, fish were fed sparingly every other day for the duration of the test. Fecal and other particulate material was siphoned from the aquariums as needed. Mortality, gross physical damage, and behavioral changes were noted and recorded at least twice daily. A fish was considered dead when no opercular movements could be detected. Results from fish bioassays are reported as median lethal time (LT-50) in units of hours. Anthracene water concentrations were determined as in section 2.4.

2.6 Algal Bioassays

The green alga Chlorella pyrenoidosa was grown in continuous cultures using the EPA-AAP medium of Miller et al. [17] under Sylvania Gro-Lux fluorescent lighting. Primary productivity was measured as a function of ^{14}C-bicarbonate fixation [18,19,20]. Incubation chambers were constructed from 0.5 cm thick Lexan (plexiglas OP-1, UV transparent) or plexiglas OP-2 (UV opaque). There were 6 chambers per incubation box and each chamber held 180 mℓ algal suspension. For bioassays, 19 ℓ glass carboys were 'shell-coated' in a manner similar to section 2.3 with a concentrated stock solution of anthracene in acetone. After all acetone evaporated, 18 liters of EPA-APP medium was added to the carboys and allowed to equilibrate for 24 hr. Algae was added to dosed and undosed carboys at a density of ca. 5 X 10^5 cells/mℓ and allowed to equilibrate for 24 hr in the dark before adding the algal suspension to individual incubation chambers. Immediately prior to exposure to solar radiation 1.0 mℓ of ^{14}C-bicarbonate (1 μCi/mℓ, 0.1 μCi/μg) was added to each chamber. After a three hour incubation, 5.0 mℓ of the algal suspension from each chamber was pipetted into individual 20 mℓ glass scintillation vials containing 100 μl Formalin. Unfixed ^{14}C-bicarbonate was removed by bubbling each vial for 30 min after adding 100 μl of 0.1N HCl. Total ^{14}C-bicarbonate fixation was determined from measured remaining ^{14}C activity in the algal suspension and known specific activity.

Incubations were performed in the laboratory system (sec. 2.1) and in situ on Lake Michigan from the NOAA research vessel Shenehon, 20 km due west of Grand Haven, MI. Field incubations were conducted at a depth of one meter from 0900-1200 hr, during which the UV-B intensity at 1 m increased from 180 to 1125 μW/cm^2. The UV-B intensity during the laboratory incubations was held constant at 130 μW/cm^2. UV transparent chambers transmitted greater than 90% of incident radiation greater than 290 nm. The major proportion of UV attenuation in these chambers was due to reflectance. UV opaque chambers eliminated greater than 80% of all radiation less than or equal to 400 nm.

3. RESULTS

The actinic effects of anthracene were observed only in the presence of UV radiation. Organisms exposed to anthracene under cool-white fluorescent or yellow fluorescent bulbs were not affected. Likewise, organisms exposed to anthracene under natural or simulated sunlight but were shaded from the UV portion of the spectrum by wavelength selective filters were not affected. In addition, anthracene need only be internally present for any toxic effect to occur, yet when anthracene was present in the external medium toxicity occurred more rapidly. Organisms that had attained theoretical steady state anthracene body burdens and were allowed sufficient depuration to eliminate anthracene in the dark were not adversely affected when subsequently exposed to solar radiation. Thus, the pharmacokinetics of anthracene are such that the phototoxic effects are dependent on the anthracene body burden and the exposure history of the organism. These results are in agreement with the findings of Bowling et al. [15].

3.1 Daphnia

Anthracene is extremely photoxic to the crustacean zooplankter Daphnia pulex (Figure 1). Note that the time units for calculated ET-50 values are in minutes whereas toxicity is usually measured in terms of hours or days. Once

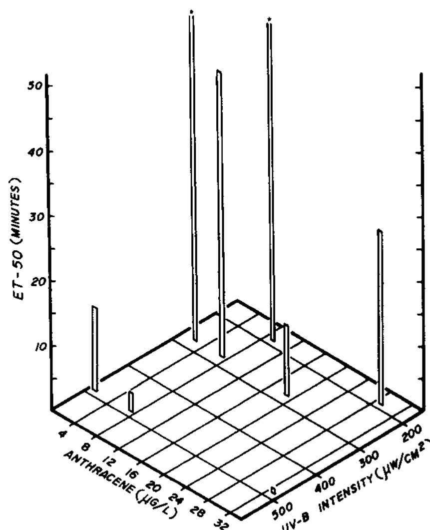

FIGURE 1. Median effect time, ET-50, for the immobilization of Daphnia pulex as a function of natural UV-B (310 $\pm$ 34 nm) intensity and anthracene concentration. Bars open at top, with stars, indicate no organisms immobilized in 60 min.

immobilized by the phototoxic action, no recovery was observed. Control mortality was always less than 10 percent during the exposure, even at the greatest light intensity. At the greatest intensity (484 $\mu W/cm^2$ UV-B) and anthracene concentration (32.7 μg/ℓ), all organisms were immobilized within two minutes, with an ET-50 of 0.86 min. Even at the lowest, more environmentally relevant anthracene concentration (1.2 μg/ℓ), the ET-50 value was still extremely short (14.0 min). Likewise, at lower irradiance levels, ET-50 values were in minutes excepting the lesser anthracene concentrations where no immobilization occurred in 60 minutes (Figure 1).

3.2 Mosquitoes

Culicid mosquito larvae, Aedes aegypti, are more tolerant to anthracene phototoxicity than D. pulex, but are still susceptible to the light-anthracene interaction (Figure 2). There is an apparent threshold level of light intensity for toxicity in A. aegypti, with marked increases in toxicity between 80 and 150 $\mu W/cm^2$ UV-B and again between 150 and 315 $\mu W/cm^2$ (Figure 2). A 24 hr LC-50 of 26.8 μg/ℓ anthracene was calculated for the intermediate light intensity of 150 $\mu W/cm^2$ UV-B. Due to the stepped light-anthracene dose-response and to the lack of partial mortality in tests other than at 150 $\mu W/cm^2$, no other LC-50 values could be calculated. Such a steep dose-response curve, especially across

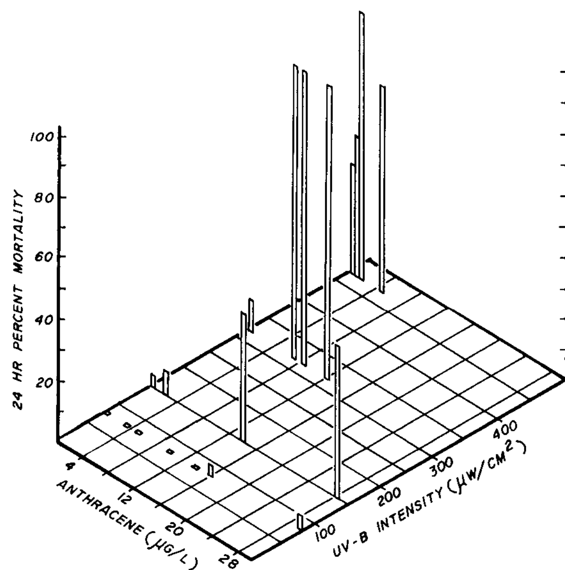

FIGURE 2. Percent mortality of third to fourth instar Aedes aegypti mosquito larvae after 24 hr continuous exposure to simulated sunlight as a function of UV-B (310 $\pm$ 34 nm) intensity and anthracene concentration.

light intensity, indicates a homogeneous population response with no resistance to the toxic interaction of UV radiation and anthracene. At the greatest light intensity (470 μW/cm^2 UV-B), mortality was observed even in the no-anthracene controls, approximating a toxic level of UV radiation alone.

3.3 Fish

Under continuous laboratory illumination, the time to reach 50% mortality (LT-50) for fish was dependent on both UV-B intensity and anthracene concentration (Figure 3). Affected fish showed signs of irritation and hypoxia [21]. Dorsal surfaces became thickened with a creamy white appearance similar to the sunburn in fish described by Bullock [22]. Dead fish exhibited symptoms of asphyxia: open mouth, splayed opercula, and pale gill filaments [23]. In Park Lake fish, the LT-50 values ranged from 38hr at 26.8 μg/ℓ anthracene and 170 μW/cm^2 UV-B to no mortality in 144 hr at the lesser UV intensities and anthracene concentrations.

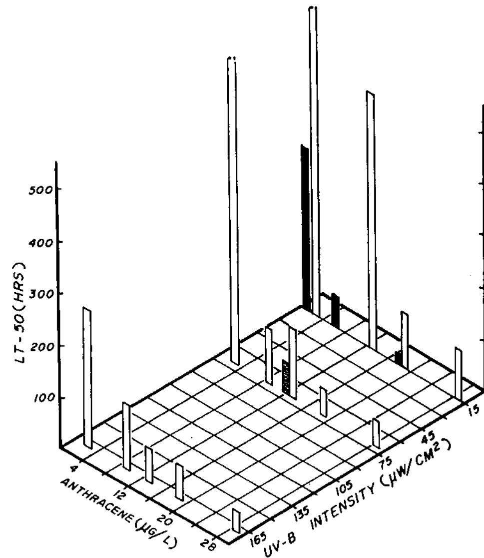

FIGURE 3. Median lethal time, LT-50, of juvenile bluegill sunfish, Lepomis macrochirus, exposed to simulated sunlight (photoperiod = 24:0 hr light:dark) as a function of UV-B (310 ± 34 nm) intensity and anthracene concentration. Bars open at top, with stars, indicate no mortality in 144 hr. Open bars = natural assemblage of juvenile sunfish collected from Park Lake, MI (USA). Black bars and stipled bars = juvenile bluegill sunfish obtained from Osage Catfisheries, MO (USA), and ByBrook Hatchery, CT (USA), respectively.

Calculated ninety-six hour LC-50 values [24] are presented in Table 1. The natural assemblage of juvenile sunfish was not as sensitive to the light-anthracene combination as were the hatchery bluegill sunfish. Bluegill sunfish from Osage Catfisheries were 10 times more sensitive than the Park Lake sunfish (Table 1). Although no LC-50 value could be calculated for ByBrook bluegills, it is apparent from Figure 3 that for at least one anthracene concentration and UV intensity these fish were approximately twice as sensitive than Park Lake fish. LT-50 values were 63 hr and 136 hr for ByBrook and Park Lake fish, respectively, at comparable anthracene concentrations and at the same UV intensity (Figure 3). No direct comparisons between Osage and ByBrook bluegills could be made.

3.4 Algae

The ^{14}C-bicarbonate fixation by the freshwater green alga, Chlorella pyrenoidosa, was not adversely affected by anthracene in the presence of UV radiation in the laboratory or in the field. In fact, under controlled laboratory conditions, there was significantly greater ^{14}C-bicarbonate uptake (α = 0.05) in anthracene treatments (Figure 4). However, there was significant inhibition of carbon fixation due to UV radiation alone.

TABLE 1. 96 hr LC-50 values for juvenile bluegill sunfish exposed to anthracene at different UV-B (310 +/-34 nm) intensities in the laboratory.

FISH	UV-B intensity ($\mu W/cm^2$)	LC-50 ($\mu g/\ell$)	95% Fiducial limits Lower	Upper
Park Lake	14.8	26.47	22.62	34.48
	70.0	18.23	16.14	21.11
	170.0	11.92	10.15	13.40
Osage Hatchery	14.8	2.78	1.94	3.92

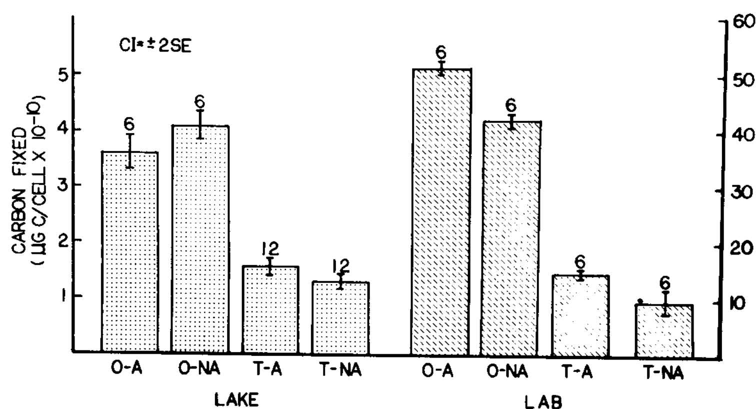

FIGURE 4. ^{14}C-bicarbonate uptake by the green alga, Chlorella pyrenoidosa, exposed to anthracene (A) or not exposed to anthracene (NA) in solar UV opaque (O) or transparent (T) plexiglas chambers after 3 hr incubations under natural (LAKE = 1 m deep, Lake Michigan, 20 km offshore Grand Haven, MI) and simulated (LAB) sunlight.

4. DISCUSSION

The observed acute toxicity of anthracene in the presence of solar radiation is in sharp contrast to the majority of literature toxicity values. Previously, anthracene and related PAH have been widely regarded as not being acutely toxic to aquatic organisms. Acute toxicity values for PAH are consistently in the parts per million (mg/ℓ) range [4], and are 100 to 1000 times greater than the concentrations at which we observed toxicity under ecologically

relevant conditions of UV-irradiation. Direct comparisons of our results with literature toxicity values for anthracene are difficult. For example, Eastmond et al. [25] reported that anthracene is "non-toxic" to Daphnia magna, whereas we observed an ET-50 of 14 min at 1.2 μg anthracene per liter on a sunny day for D. pulex. However, UV irradiated nauplii of the crustacean, Artemia salina were found to be sensitive to a wide range of PAH at molar concentrations similar to those used in our study [26].

No values for anthracene toxicity in mosquitoes are available in the literature. However, larval A. aegypti are known to be photosensitized by polyacetylenes and other thiophene derivatives at small μg/ℓ concentrations [27]. The nontoxic xanthene dye, fluorescein, was found to synergise the toxicity of rose bengal to larval A. triseriatus in sunlight, under fluorescent light, and upon illumination by laser light of 488.0 and 514.5 nm [28]. Exposure to solutions containing parts per million concentrations of the dye erythrosin-B in the presence of visible light caused mortality in larvae of the mosquito, Culex pipiens quinquesfasciatus, comparable to our mosquito studies [29].

Applegate et al. [30] reported a 24 hr "no-effect" anthracene concentration of 5 mg/ℓ for juvenile bluegill sunfish. This value is 190, 274, and 420 times greater than our calculated 96 hr LC-50 values at UV-B intensities of 14.8, 70, and 170 $\mu W/cm^2$, respectively, for the natural assemblage of sunfish (Table 1). Bluegill sunfish from the Osage hatchery were 1800 times more sensitive than the "no-effect" concentration.

The only known studies of anthracene photosensitized toxicity to fish are the studies of Giesy et al. [14] and Bowling et al. [15]. Dunbar [31], however, may have unknowingly reported one of the first observed cases of photoenhanced PAH toxicity in fish. The report describes a high rate of mortality in rainbow trout fingerlings held in "black-asphaltum painted" troughs after two days of exposure to bright sunlight. Direct effects of UV radiation were considered, though no association was made between possible photosensitization by PAH leached into the water and the observed mortality. Kagan et al. [32] has shown that late embryonic stages of the frog, Rana pipiens, that share similar habitats with juvenile sunfish are extremely sensitive to anthracene in the presence of natural sunlight. LC-50 values of 65 μg/ℓ and 25 μg/ℓ for 30 min and 60 min exposures, respectively, were determined. Although solar irradiance was not measured, these toxicity values closely correspond to the results of our studies.

Ecologically relevant UV radiation has been shown to inhibit carbon fixation by phytoplankton [33-37], and the potential for anthracene photosensitization in C. pyrenoidosa was thought to be great. In general, however, phytoplankton have been observed to be very tolerant of exposure to PAH. For instance, under illumination by cool-white fluorescent lights anthracene did not inhibit $^{14}C\text{-}HCO_3$ incorporation [20]. Similar to our studies, Prouse et al. [38] observed a slight stimulation of the growth of marine phytoplankton exposed to low concentrations of oil and concluded that concentrations of oil encountered in polluted sea water could affect the growth of phytoplankton but the effects would be minor and short lived. The studies of Prouse et al. [38] were conducted under laboratory conditions and possible photosensitization was not considered. However, our results suggest that the conclusions of their study are valid.

The autotrophic capability of phytoplankton requires that they be exposed to both photosynthetically active solar radiation and potentially damaging UV radiation. Also, the very nature of the reactions of photosynthesis expose phytoplankton to potentially damaging photo-oxidative chemical reactions. The

carotenoid accessory pigments of algae and photosynthetic bacteria are known to protect these organisms from lethal photo-oxidation caused by their own chlorophyll [39]. In addition, a carotenoidless mutant of the photosynthetic bacterium, Rhodopseudomonas spheroides, is extremely sensitive to exposure to UV radiation [40]. The active response of cells to photosensitizers can procede by both free radical (Type I) and singlet oxygen (Type II) mechanisms [41]. However, the singlet oxygen pathway is much more prevalent [41-47]. β-carotene can protect organisms against both Type I and Type II reactions [48], but it is known to be an extremely effective singlet oxygen quencher [39,49] and has been shown to almost completely inhibit the photosensitization reactions of compounds such as porphyrins in vitro and in vivo [50]. Thus, we would expect the carotenoid containing phytoplankton to be less sensitive to PAH photosensitized toxicity than the zooplankton, insect larvae, or fish.

4.1 Potential Environmental Phototoxins

Photoenhanced toxicity of anthracene in aquatic organisms is probably not an isolated case since many other PAH strongly absorb radiation in the solar UV range, and can be considered potential environmental phototoxins (Table 2). Almost every organic molecule which absorbs radiation in the region of the electromagnetic spectrum from 320 to 900 nm has been proposed as a potential photosensitizing compound [51]. There are several possible reasons for the photosensitizing potential of PAH such as anthracene. These include the great absorbance by PAH in the portion of the solar spectrum that penetrates the atmosphere, high quantum yields of singlet and triplet excited states, and long lifetimes of these excited states [52].

Medical and biochemical researchers have long recognized that PAH are involved in photosensitization and phototoxic reactions in the skin of mammals [53,54]. Complex mixtures such as coal tar as well as individual PAH have been observed to cause erythema in the presence of UV-A (345-390 nm) and UV-B (285-345 nm) [55]. As early as 1939, Burkhardt [56] reported hypersensitivity and pronounced edema in patients treated with coal tar in the presence of sunlight. The structure-activity relationships of photoreactive PAH have been determined [57-61], and the mechanisms of photosensitized reactions are well characterized [41-47]. There is a need, however, to identify compounds which, due to their environmental mobility and bioavailability, have a great potential to act as phototoxins in the aquatic environment, and to identify environmental parameters which may serve to attenuate or magnify the actinic effects of these compounds.

4.2 Ecological Consequences

For largely unknown reasons, but perhaps due to the lack of instrumentation, aquatic biologists historically have assumed that solar ultraviolet radiation does not penetrate natural waters to significant depth, and have discounted the importance of solar UV in the aquatic environment. Solar UV does penetrate surface waters to a considerable extent and this penetration has been observed by numerous authors [62,63,this study]. The depth of UV penetration is dependent on the productivity and turbidity of a particular body of water [62,64]. For example, in eutrophic Park Lake, MI, 99% of incident UV-B

TABLE 2. Solar Radiation Absorption Characteristics of Selected Polycyclic Organic Compounds [83,84].

Compound	Absorption in Atmospheric Solar Range		
	UV-B (285-345 nm)	UV-A (345-390 nm)	Visible (390-700 nm)
Acridine	*	–	–
Anthracene	*	*	–
Anthranthrene	*	*	*
Benz[a]acridine	*	*	–
Benzo[b]chrysene	*	*	–
Benzo[a]fluoranthene	*	*	*
Benzo[c]fluorene	*	–	–
Benzo[g,h,i]perylene	*	*	*
Benzo[a]pyrene	*	*	*
Benzo[b]naptho (1,2-d) thiophene	*	*	–
Chrysene	*	*	–
Coronene	*	–	–
Dibenz[a,j]acridine	*	*	–
Dibenz[a,c]anthracene	*	*	–
Dibenz[a,j]anthracene	*	*	–
Dibenzo[a,h]pyrene	*	*	*
Fluoranthene	*	*	–
Fluorene	*	–	–
Napthacene	–	*	*
Napthalene	*	–	–
1-Nitropyrene	*	*	*
Phenanthrene	–	–	–
Pyrene	*	*	–

is attenuated in the upper 2 meters (attenuation coefficient (K) = 2.504 m^{-1}), while in offshore Lake Michigan 1% of incident UV-B penetrates to about 10 meters (K = 0.496 m^{-1}). From these measurements, it is evident that solar ultraviolet radiation is present at ecologically significant depths and may play an important interactive role in an environmentally realistic assessment of PAH toxicity.

The impacts of environmental phototoxins in aquatic systems are unknown since research to present has not made explicit tests on an ecosystem-wide basis. It is instructive, however, to examine the direct effects of UV radiation on aquatic organisms since UV exposure is necessary to elicit the phototoxic phenomenon due to PAH. In addition, photosensitized PAH toxicity may be considered to enhance the damaging potential of solar UV to aquatic organisms. It is common to describe this toxic response as the photoenhanced toxicity of PAH in aquatic organisms, but there are no compelling reasons why the converse cannot be true (i.e. PAH enhanced toxicity of UV radiation).

The impact of direct UV irradiation on planktonic organisms has been the focus of many studies. Shrimp larvae, crab larvae, and euphasids are known to be living at or near their UV-B tolerance under current irradiance conditions [65]. These animals have a threshold UV-B tolerance level, below which little or no effect occurs and above which a strong dose/dose-rate response is

observed. Damkaer et al. [65] suggest that near surface waters are environmentally important since many zooplankters have their center of abundance in these strata or are found exclusively there for at least part of their life cycle. These authors calculate that a 20% reduction in global ozone could significally shorten the larval season of these species, and suggest that natural intensities of UV-B have had a selective role in the seasonal adaptation and community structure of zooplankton species. At slightly enhanced UV-B intensities, seasonal or geographical restrictions could occur in shrimp populations if reproductive success is dependent on late season (i.e. summer-fall) larvae [66]. Tolerance of exposure to solar UV of many aquatic microorganisms (e.g. bacteria, yeast, algae, protozoa and arthropods) and current environmental UV intensities are approximately equal, and solar UV has been implicated as being a major ecological factor controlling the distribution of these organisms [67]. It has been suggested that there may be no large reserve of organismal resistance which could cope with altered solar UV exposure, or UV sensitivity, without requiring modification of physiology or behavior [67].

The vertical migrations of marine and freshwater zooplankton and phytoplankton have been variously interpreted as a maximization of resources such as food or nutrients, photosynthetically active radiation, and avoidance of predation and/or of injurous UV radiation [68,69,70]. In many species of zooplankton, significant negative correlations between incident radiation and the pattern of vertical migration have been observed [71]. In addition, seasonal changes in the vertical distribution and migration patterns correlate significantly with incident radiation, among other factors [71]. In perhaps one of the earliest studies to recognize the ecological importance of solar UV, Klugh [70] addressed the evolutionary significance of vertical migration of marine copepods relative to exposure to damaging UV irradiation. A close relationship was found between the depth of daylight occurrence and the susceptability to UV of these organisms. These results further indicate that the differential sensitivity of organisms to solar UV may play an important role in determining planktonic community structure. More recently, it has been observed that the habitat of a wide variety of planktonic organisms is behaviorally determined by avoidance of solar UV on the basis of differential species sensitivity [72,73,74]. For instance, of the many factors considered to affect the vertical distribution of a protozoan by Barcelo and Calkins [74], including wind, cloud cover, temperature gradients and food gradients, only UV-B and total solar irradiance were significantly correlated with vertical distribution.

The effects of solar UV exposure on marine and freshwater fish have also been examined. Larvae of the northern anchovy are very sensitive to UV-B [75]. Anchovy and other species of clupeiod fish spawn only during seasons when UV-B irradiance is low or utilize habitats where solar UV-B is strongly attenuated (e.g. productive, turbid inshore waters). There are exceptions to the spawning seasonality pattern, such as the pacific mackeral which spawns in June, but these species are more tolerant to UV-B exposure. These seasonal and locational patterns probably evolved with co-occurring periods or areas of optimal food density, but since the food organisms may also exhibit sensitivity to UV irradiation, one should exercise caution in interpreting the evolutionary significance of direct effects of UV irradiance on spawning behavior of fishes [75]. Solar UV has been shown to influence periods of optimal food density, and anchovy, as well as other fish species, are known to be currently existing near their tolerance threshold for UV exposure [76]. Thus, the effect of solar UV on seasonal occurrence and habitat utilization remains an important factor.

Sockeye salmon eggs irradiated with UV-B exhibit a high rate of mortality compared to non-irradiated controls [77]. Hatching among irradiated eggs

occurred approximately 1 month prematurely, and alevins from these eggs suffered significant developmental abnormalities. Decreased hatching success due to solar UV stress could severely alter the population dynamics of fish. The eggs and larvae of fish are not the only life stages that can be deleteriously affected by solar UV exposure. There are many accounts of 'sunburn' in juvenile and adult fishes in the literature [22,31,78,79,80], and it is common knowledge among hatchery workers that juvenile fish are sensitive to exposure to bright sunlight [81].

Worrest et al. [36] investigated the impact of UV-B on estuarine microcosms in one of the few studies of the community level effects of solar UV. Elevated UV-B exposure resulted in altered phytoplankton community structure, lesser community biomass, less total chlorophyl-a concentration, and lesser radiocarbon assimilation. These authors speculated that altered species composition could affect the quality and quantity of food for primary consumption and that organic carbon exchange between trophic levels could be affected. This impact would be significant if organisms selected by UV were of lower nutritional value. In addition, a decrease in size of representative diatoms upon which consumers could graze was observed, thereby possibly increasing the energy allotment required for grazing and reducing the feeding efficiency of the consumers [36]. In another community level study, the possible effects of solar UV on competition among species of coral reef epifauna was examined [82]. It was hypothesized that organisms could gain a selective advantage by developing UV tolerance to avoid competition for space in shaded areas, but that these species would be inferior competitors in the absence of UV since the metabolic burden of maintaining enzyme systems required for UV protection would reduce growth and reproductive potential. This possibility was investigated using two closely related species of sponges, differing mainly in their UV tolerances, and it was found that the shade-adapted species was a better competitor when UV was removed by selective filters, and vice versa when UV was present [82].

From the above discussion, it is apparent that a wide array of aquatic organisms are sensitive to solar UV, and that many are living close to their tolerance threshold or are currently under UV related stress. Any mitigating factor such as increased PAH loading that would either increase the effect of UV or decrease tolerance to UV irradiation of aquatic organisms even slightly could cause significant short and long term ecological consequences through species and size specific mortality, habitat limitations, seasonal tolerance restrictions, reduced reproductive success, or altered energy-flow dynamics.

5. CONCLUSIONS

It has been known for many years that anthracene and other related PAH can act as photosensitizing compounds in the presence of atmospheric solar radiation, but this fact has not been widely recognized as important in the assessment of the environmental hazards of these compounds. Solar UV radiation penetrates to significant depths in natural waters and is an ecological parameter which must be accounted for in an environmentally realistic hazard assessment program. The importance of this consideration is shown in our observed increases, from 100 to > 1000 times literature values, in the toxicity of anthracene to aquatic organisms.

Current environmental concentrations of anthracene are less than our measured acute toxicity thresholds. However, the additive, subtractive, or synergistic properties of natural mixtures of phototoxic PAH are unknown, and chronic effects have not been assessed. Therefore, it is feasible that small

increases of PAH concentrations in surface waters could cause dramatic impacts in aquatic ecosystems, or that aquatic organisms may presently be under considerable phototoxin induced stress.

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