

## UV-photoactivation of polycyclic aromatic hydrocarbons and the sensitivity of sediment-dwelling estuarine organisms

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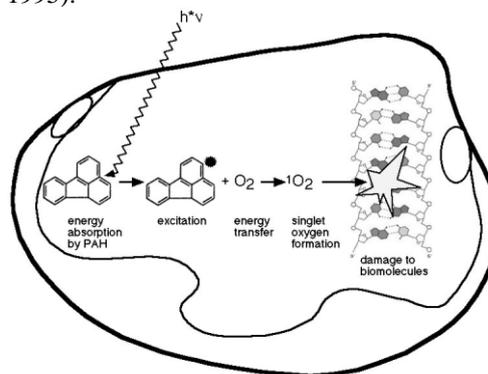
### Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a diverse and ubiquitous group of organic contaminants in terrestrial and aquatic environments. They are of environmental concern since they can accumulate in organism tissues to levels high enough to be toxic. While many PAHs exert only mild narcotic toxicity at environmental concentrations, the toxicity of some PAHs may be greatly exacerbated by UV-photoactivation, a process during which energetic ultraviolet (UV) radiation is absorbed by a PAH molecule and subsequently transferred to molecular oxygen, generating highly reactive singlet oxygen atoms and sometimes even more toxic photoactivation products (Kosian et al. 1998). The aim of this paper is to demonstrate the sensitivity of sediment-dwelling (benthic) invertebrates to UV-PAH photoactivation, and identify a number of protective mechanisms by which animals are able to cope with this potential stressor.

The PAHs found in the environment originate from a variety of sources—both natural and human. PAHs are generally formed during the incomplete combustion and biogeochemical degradation of organic matter (Neff 1979). They also constitute a substantial component of petroleum and coal. Large-scale burning of fossil fuels and vegetation through human activity, various industrial processes (e.g. manufactured gas production, creosote usage and aluminium smelting) and diffuse or accidental releases of petroleum compounds (e.g. via road runoff or oil spills) contribute to high (i.e. parts per million) PAH concentrations in urban aquatic sediments (Burgess et al. in review). Due to their aromatic molecular structure, PAHs are chemically comparatively stable compounds that may persist in the environment for decades. Their breakdown generally occurs by biological or geochemical degradation, or by photolysis (PAHs strongly absorb in the ultraviolet wavelength range). Because of their poor solubility in water (hydrophobicity), PAHs preferably concentrate on sediment or soil particles and accumulate in the lipid-rich tissues of organisms. Once incorporated by organisms, PAHs may exert a variety of toxic effects, including narcosis, genetic mutations or cancer, even without photoactivation by UV radiation.

### UV-phototoxicity

Phototoxicity occurs when UV energy (wavelengths 280-400 nm) excites the conjugated electron orbitals of a PAH molecule and elevates these to a triplet stage (Figure 1). The energy of the excited PAH molecule is subsequently transferred to dissolved oxygen, generating reactive singlet-oxygen, which may destroy tissue and biological macromolecules such as DNA (Newsted and Giesy 1987). Furthermore, photomodification (generally oxidation) of the parent PAH may lead to by-products with increased toxicity (Huang et al. 1995).



**Figure 1:** Schematic of PAH photoactivation in a clam

Not all PAHs are phototoxic. Phototoxicity is determined by structural characteristics of a PAH molecule relating to the energy difference between its highest occupied molecular orbital and the lowest unoccupied molecular orbital, the so-called HOMO-LUMO gap; (Mekenyan et al. 1994, Veith et al. 1995). For example, fluoranthene and pyrene are phototoxic, while phenanthrene and perylene are not.

Numerous studies have demonstrated enhanced PAH toxicity to aquatic animals in the presence of UV radiation. Toxicity can vary by over two orders of magnitude among species (Boese et al. 1997, Spehar et al. 1999), and small, transparent organisms, such as larvae or juveniles, and infaunal animals seem to be particularly UV-PAH sensitive (Pelletier et al. 1997, Weinstein 2001).

Toxicity is a function of PAH dose (i.e. tissue concentration) and UV intensity times duration (Ankley et al. 1997): higher PAH concentrations or prolonged UV exposure tend to increase PAH phototoxicity in a multiplicative manner. Thus, PAH toxicity tests conducted in the laboratory,

under no or fluorescent light (i.e. in absence of UV radiation), may potentially underestimate biological risk.

Animals dwelling in nearshore, intertidal sediments may be at high risk of encountering UV phototoxicity, since they are simultaneously exposed to high PAH concentrations (via sediments) and high UV radiation (e.g. at low tide). On the other hand, many benthic species that are normally buried in sediments (except for protruding feeding or respiratory structures) may be largely shielded from the harmful effects of UV radiation. Analogously, animals living at the sediment surface (epifauna) may have evolved adaptations to abate naturally high UV exposure (e.g. sklerotized body surfaces and pigmentation). We therefore set out to answer the following questions:

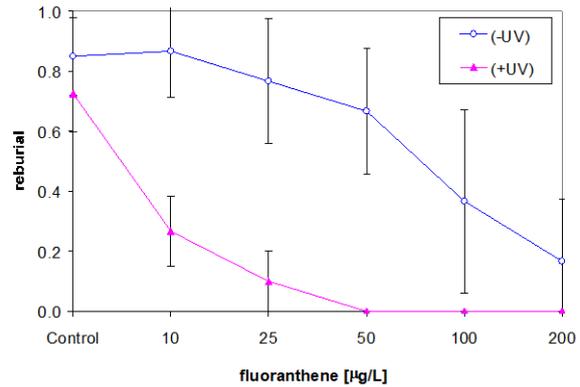
1. How sensitive are infaunal benthic animals to PAH-UV phototoxicity?
2. How effective is burrowing for UV protection?
3. Are epifaunal animals more UV-hardy than infaunal benthos (when exposed directly to UV)?

### Methods

We measured acute phototoxicity to six benthic invertebrates in response to varying PAH (fluoranthene) concentrations and sediment thickness. The estuarine species *Potamopyrgus estuarinus* (gastropoda), *Anthopleura aureoradiata* (anthozoa), *Exosphaeroma falcatum* (isopoda), *Chaetocorophium lucasi* (amphipoda) and juvenile *Macomona liliana* and *Austrovenus stutchburyi* (bivalvia) were exposed to fluoranthene (water) concentrations ranging between 10-500 µg/l for 48-96h. Viable animals were transferred to PAH-free seawater and irradiated under a solar simulator for 1-9h (no sediment) or 48 h (sediment treatments). UV intensity was 3.5 W/m<sup>2</sup> (UVB) and 20 W/m<sup>2</sup> (UVA), which is approximately 50-75% of mid-summer levels in New Zealand. (+UV)-exposed treatments were covered with UV-permeable polyethylene foil, while light controls (-UV) were shielded from UV radiation with clear polycarbonate sheets (absorbing wavelengths <400 nm). Sediment thickness was varied from 0-15 mm. Response endpoints measured were reburial (bivalves), escape (amphipods), curl-up (isopods), tentacle retraction (anemones) and turning (snails).

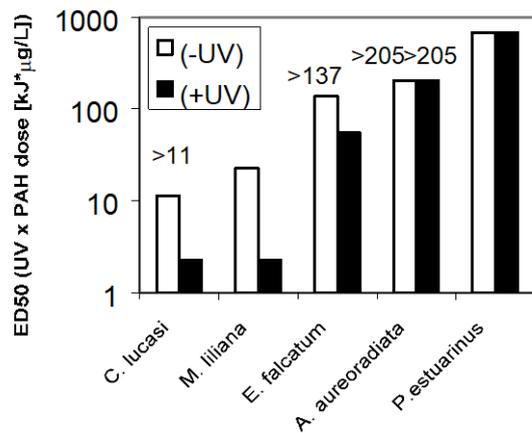
### Results and Discussion

Fluoranthene phototoxicity was five to ten times greater (i.e. EC<sub>50</sub>, the 50% effects concentration, was diminished 5-10x) under UV light compared to UV shielded controls, for bivalves and amphipods (Figure 2).



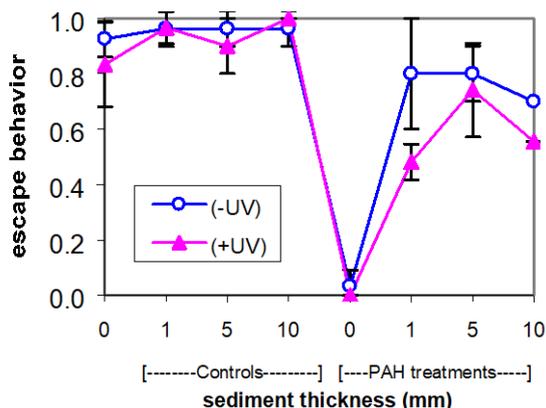
**Figure 2:** Dose-response of the clam *M. liliana* to fluoranthene and UV exposure (1 h).

UV exposure in the absence of sediment lead to nearly complete mortality of bivalves and amphipods, and approximately 30-50% mortality among gastropods and isopods, respectively. Anemones showed no signs of UV phototoxicity (Figure 3), correlating with high concentrations of UV-absorbing mycosporine-like amino acids in their tissues.



**Figure 3:** Effects doses (fluoranthene EC<sub>50</sub> x UV irradiance received) for 5 benthic species. For all treatments with “>”, the selected doses for were too low to produce a 50% effect.

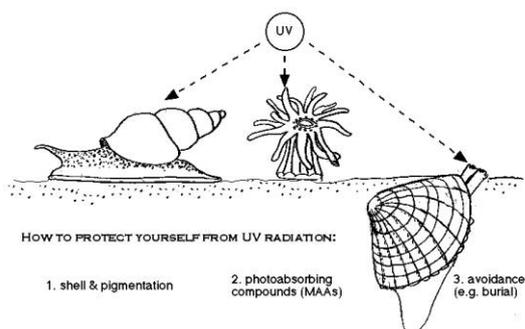
Thin layers of sediment effectively attenuated UVA and UVB radiation by an order of magnitude, and presence of sediment raised animal survival to over 50% for all treatments and species. However, a beneficial effect of sediment presence was also seen for UV-shielded treatments, suggesting that some of the stress encountered by unburied animals was unrelated to UV exposure (Figure 4).



**Figure 4:** Effect of sediment thickness on escape behaviour of amphipods exposed to fluoranthene (10 µg/L) and 48 h UV radiation.

### Conclusions

Infaunal invertebrates, such as *M. liliانا*, *A. stutchburyi* and *C. lucasi*, are highly sensitive to UV activated PAH toxicity. However, they are largely protected from harmful UV effects if they remain buried in sediments. Becoming unburied, or emerging from sediments under unfavourable environmental conditions, poses a risk to these animals. In contrast, epifaunal snails, isopods and anemones endure high PAH and UV doses without noticeable detriment. Their hardiness is probably due to inherent photoprotection, either by pigments and/or an opaque shell/cuticle (in *E. falcatum* and *P. estuarinus*) or UV-absorbing compounds (e.g. mycosporine-like amino acids, MAAs) in *A. aureoradiata*. Thus, depending on individual lifestyle, benthic invertebrate either avoid UV radiation (and PAH photoactivation) or have evolved effective adaptations to shield themselves from it (Figure 5).



**Figure 5:** Different ways by which benthic invertebrates can reduce their UV exposure

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