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Do UK coastal and estuarine water samples pose a phototoxic threat?

B.P. Lyons [a,*], F. Goodsir [b], N.G.H. Taylor [a], J.E. Thain [a]

[a] Centre for Environment, Fisheries and Aquaculture Science (Cefas), Barrack Road, Weymouth, Dorset DT4 8UB, United Kingdom
[b] Centre for Environment Fisheries and Aquaculture Science (Cefas), Lowestoft Laboratory, Pakefield Road, Lowestoft, Suffolk, NR33 OHT, UK

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ABSTRACT

Many studies have investigated phototoxicity under controlled laboratory conditions, however, few have actually demonstrated it occurring in environmental samples. Here we report on the potential for UK marine coastal waters to demonstrate phototoxicity when tested using the oyster embryo (*Crassostrea gigas*) bioassay in the presence UV light. Subsurface water, sea surface microlayer samples and subsurface water samples that had been extracted through solid phase extraction (SPE) columns were analysed. Results demonstrated that the majority of samples failed to display any phototoxic potential. However, those collected from Belfast Lough did display an increase in toxicity when bioassays were performed in the presence of UV light when compared to identical samples assayed in the absence of UV light. Analysis of water samples at this location identified known phototoxic PAHs, pyrene and fluoranthene. These findings suggest the need to consider the potential UV light has when determining the toxicity of environmental samples.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous, persistent organic contaminants of marine ecosystems, which enter the environment from a range of sources including the combustion of fossil fuels and industrial effluent (Law et al., 1997; Webster et al., 2007) In general, the majority of PAHs are not directly toxic at concentrations typically found in marine waters (Law et al., 1997). As such, aquatic studies have traditionally focused on their metabolism and subsequent carcinogenic and reproductive impairments (NRCC, 1983; Chen and White, 2004; Lyons et al., 2004). However, there is a growing body of evidence to suggest that the toxic potential of PAHs is enhanced when biota are simultaneously exposed to the ultraviolet (UV) component of sunlight, a phenomenon termed phototoxicity (Arfsten et al., 1996; Diamond, 2003; Fu et al., 2012). For example, laboratory studies have shown that UV light can significantly increase the toxicity of anthracene, fluoranthene and pyrene towards marine invertebrate larvae and embryos (12–50,000× in LC_{50} tests) when compared with parallel tests conducted in the absence of UV light (Pelletier et al., 1997). Similar laboratories studies have identified the phototoxic effects of PAHs in a range of other marine species including diatoms, bivalve embryos, crustaceans and fish embryos (Boese et al., 1997; Lyons et al., 2002; Petersen et al., 2008; Spehar et al., 1999; Wang et al., 2008).

Field studies have confirmed the findings of these laboratory experiments. For example, Lyons et al. (2006) previously demonstrated that seawater samples collected around the Statfjord oil field displayed phototoxicity to Pacific oyster (*Crassostrea gigas*) embryos. This phenomenon has also been detected in a range of other environmental samples including, sediment elutriates (Davenport and Spacie, 1991), storm water runoff (Ireland et al., 1996) and crude oil (Barron et al., 2003: Kirby et al., 2007). However, despite growing evidence, the potential for phototoxicity is not routinely investigated during bioassay directed environmental toxicity testing.

Phototoxicity is thought to primarily occur via photosensitization reactions, whereby the PAH absorbs energy from UV light resulting in the production of reactive oxygen species (ROS) in tissues of exposed organisms (Allred and Giesy, 1985; Fu et al., 2012). PAHs that absorb UV light may have some of their electrons excited to triplet states, which then have the potential to undergo type I or type II photosensitization reactions (Foote, 1991). Both type I and II photosensitizers are efficient at triggering the formation of ROS, which are highly damaging towards biological material within the cell. In such reactions the excited PAH transfers its energy to surrounding oxygen molecules (0_2), generating singlet oxygen ($^1 0_2$), a potent oxidising agent. It is considered that PAHs undergoing type II photosensitization reactions in UV translucent tissues, rather than type I photosensitization or photo oxidation pathways, are the main mode of PAH phototoxicity in aquatic organisms (Arfsten et al., 1996; McCloskey and Oris, 1991). Studies using fish gill cells (Weinstein et al., 1997) and fish cells lines (Choi and Oris, 2000) have supported the concept of ROS mediated toxicity and

* Corresponding author. Tel.: +44 (0)1305 206600; fax: +44 (0)1305 206601.
E-mail address: brett.lyons@cefas.co.uk (B.P. Lyons).

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have demonstrated the mechanism of toxic action is primarily cell membrane damage via lipid peroxidation.

Due to their hydrophobic nature and association with buoyant organic matter PAHs may concentrate at the upper most region of the sea surface (1–1000 μm), termed the sea surface microlayer (SSML) (Hardy et al., 1987a). Studies have indicated that the SSML may pose an increased threat to neustonic marine organisms due to the high levels of contaminants, including PAHs, PCBs, pesticides and heavy metals contained within it (Hardy et al., 1987b; Hardy and Cleary, 1992). SSML samples analysed from Puget Sound had mean total aromatic hydrocarbon concentrations of 132 μg L^{-1}, while concentrations in the water column were generally below the detection limits of the analytical methods used (Hardy et al., 1987a). Similar elevations were also observed in Chesapeake Bay, with SSML aromatic hydrocarbon enrichments 10–100 fold over water column values (Hardy et al., 1990) and in North Western Mediterranean waters (Guitart et al., 2007). Previous studies investigating the biological effects of SSML have detected clear elevations in toxicity, towards both invertebrate and fish embryos, when compared with sub-surface waters (Hardy and Cleary, 1992; McFadzen and Cleary, 1992; Westernhagen et al., 1987).

Here we report on the potential for UV light, of similar intensity encountered by marine organisms inhabiting upper reaches of the water column, to enhance the toxicity of seawater samples. To do this subsurface seawater (either tested directly or following concentration using solid phase extraction columns) and SSML samples were collected from coastal and estuarine locations around the UK and tested using a standardised 48-h oyster embryo (*C. gigas*) bioassay with and without UV light.

2. Material and methods

2.1. Oyster embryo bioassay

Conditioned adult Pacific oysters (*C. gigas*) were obtained from Guernsey Sea Farms Ltd. (UK). In all toxicity tests the water used for controls and chemical/extract dilution series was made using filtered (2 μm) 32–33‰ reference seawater. The oyster embryo bioassay was essentially carried out as described by Thain (1992). Briefly, conditioned oysters were stripped of eggs and sperm and fertilised in filtered seawater. All subsequent manipulations involving sperm, eggs and developing embryos were conducted at 20 ± 2 °C. Fertilisation was conducted within 30 min of obtaining both the eggs and sperm. Desirable embryo concentrations during the fertilisation procedure were considered to be 1000–4000 per mL. After approximately 2 h the embryos had typically reached the 16–32 cell stage. The concentration of viable embryos was assessed using a Sedewick–Rafter counting cell and the appropriate volume added to the test sample to achieve a final concentration of 50 embryos per mL per water sample tested. Lighting regimes used during the study are described in Section 2.2. After 48-h the bioassay tests were terminated by the addition of 0.5 mL 30% neutral buffered formalin. Sub-samples from each test pot were then placed under a microscope and the numbers of normal D-shell larvae counted. Three replicates were used for each water sample tested. Abnormal D-shell larvae displayed deformities generally characterised by 'pinching' around the shell edges, reduced valves, velum enlargement and concave hinges.

2.2. UV lighting: experimental set up and UV light measurements

The oyster embryo bioassays were conducted under both fluorescent (lacking UV) and UV light regimes using a 12-h light and 12-h dark photoperiod. In all experiments sunlight simulation lamps (Phillips Ltd., Cleo 20W) were used to provide artificial sunlight. Lighting regimes were quantified using a spectroradiometer (Glen Spectra Ltd.) and a broad-band radiometer (UV-103, Macam Photometrics). For the phototoxicity tests the UV light intensities were 3.3–6.3 μW/cm^2 (UVB) and 280–456 μW/cm^2 (UVA). Levels of UV light were based on previous field recorded light conditions measured 1 m below the water surface at mid-day during the spring in the north-eastern Gulf of Mexico (McCloskey and Oris, 1993). UVB is attenuated by ozone in the atmosphere and it is estimated that surface UVB irradiance is in the order of 20–200 μW/cm^2 (Preston et al., 1999). Indeed, measurements taken in the middle of the North Sea as part of this study recorded mean levels of 168 ± 32 μW/cm^2 and 3399.7 ± 576 μW/cm^2 for UVB and UVA respectively (June–July, average readings recorded between 14:00 h and 19:00 h). No UVA or UVB was detectable in any of the experiments conducted under laboratory fluorescent lighting.

2.3. SSML and sub-surface water sampling

SSML and sub-surface waters were collected during June/July from a small inflatable boat launched from the RV *Cirolana* (Table 1). Samples were taken from the front of the boat facing into the prevailing wind or current to minimise cross contamination from chemicals potentially leaching off the sampling craft. Methods used to collect microlayer samples have been described in detail previously (Cleary and Stebbing, 1987). For the purposes of this research programme SSML samples were collected by the Garret screen method (Garret, 1965). Sub surface waters were collected by placing a 2.5 L amber winchester sampling bottle under water at arms length (ca. 45 cm depth). The winchester was then opened while under the water to collect a discrete subsurface seawater sample. Samples were kept refrigerated and in the dark prior to testing.

2.4. PAH analysis of surface waters by coupled gas chromatography–mass spectrometry

Seawater samples (2.5 L of sub-surface water) for the determinations of total PAH were collected along side samples taken for

Table 1
Subsurface water, SSML and SPE sampling locations used during this study.

Sample location	Latitude	Longitude	Description of location
Tyne			
Site 1	54. 57 30	01. 38 15	Mid channel River Team confluence
Site 2	54. 59 15	01. 28 36	Mid Channel, Howdon
Site 3	55. 00 30	01. 25 53	Mid channel mouth of River Tyne
SPE sample	55. 01 35	01. 23 85	Offshore Tyne
Tees			
Site 1	54. 36 20	01. 09 38	Mid Channel, Teesport
SPE sample	54. 44 75	01. 08 21	Offshore Tees
Southampton			
Site 1	50. 50 13	01. 19 41	Oil terminal inner channel
Site 2	50. 51 03	01. 19 52	Oil terminal mid channel
Site 3	50. 51 03	01. 18 30	Mouth River Hamble
SPE sample	50. 44 99	01. 24 97	Solent Channel
Falmouth			
Site 1	50. 09 18	05. 03 10	Main docks
Site 2	50. 10 44	05. 03 10	Mouth of Mylor Creek
Site 3	50. 11 31	05. 03 32	Mouth of Restronguet Creek
SPE sample	50. 08 10	05. 02 44	Falmouth Bay
Belfast Lough			
Site 1	54. 36 10	05. 55 11	Mid channel, Largan road bridge
Site 2	54. 37 27	05. 53 30	Mid Victoria channel
Site 3	54. 39 44	05. 51 21	CSEMP[a] 845
SPE sample	54. 42 45	05. 42 46	CSEMP 845

[a] CSEMP: Clean Seas Environmental Monitoring Programme (http://www.bodc.ac.uk/projects/uk/merman/project_overview/).