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Toxicity of benz(*a*)anthracene and fluoranthene to marine phytoplankton in culture: Does cell size really matter?

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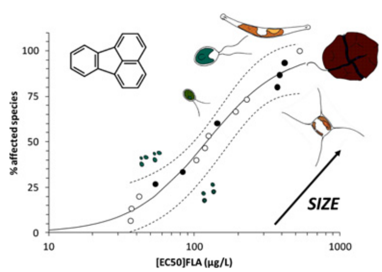
HIGHLIGHTS

- ► Polycyclic aromatic hydrocarbons (PAHs) in the marine environment are a hazardous chemical legacy.
- ► Benz(*a*)anthracene and fluoranthene are toxic to phytoplankton photosynthesis and growth in culture.
- ► Acute (photosynthesis) and chronic (population growth) effects have different thresholds.
- ► Toxicity depends on both the species selected as a model and the compound considered.
- ► Further study of the size/sensitivity relationship is required to draw more general conclusions.

GRAPHICAL ABSTRACT



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ABSTRACT

The toxicity of benz(*a*)anthracene and fluoranthene (polycyclic aromatic hydrocarbons, PAHs) was evaluated on seven species of marine algae in culture belonging to pico-, nano-, and microphytoplankton, exposed to increasing concentrations of up to 2 mg L^{-1}. The short-term (24 h) toxicity was assessed using chlorophyll *a* fluorescence transients, linked to photosynthetic parameters. The maximum quantum yield Fv/Fm was lower at the highest concentrations tested and the toxicity thresholds were species-dependent. For acute effects, fluoranthene was more toxic than benz(*a*)anthracene, with LOECs of 50.6 and 186 $\mu g\,L^{-1}$, respectively. After 72 h exposure, there was a dose-dependent decrease in cell density, fluoranthene being more toxic than benz(*a*)anthracene. The population endpoint at 72 h was affected to a greater extent than the photosynthetic endpoint at 24 h. EC50 was evaluated using the Hill model, and species sensitivity was negatively correlated to cell biovolume. The largest species tested, the dinoflagellate *Alexandrium catenella*, was almost insensitive to either PAH. The population endpoint EC50s for fluoranthene varied from 54 $\mu g\,L^{-1}$ for the picophytoplankton *Picochlorum* sp. to 418 $\mu g\,L^{-1}$ for the larger diatom *Chaetoceros muelleri*. The size/sensitivity relationship is proposed as a useful model when there is a lack of ecotoxicological data on hazardous chemicals, especially in marine microorganisms.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are commonly found and are relatively persistent organic pollutants originating from both natural sources (forest and prairie fires, natural petroleum

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seeps, volcanic eruption, *etc.*) and anthropogenic sources (incomplete combustion of fossil-fuel and organic matter, motor vehicle emissions, industrial activities, *etc.*). PAHs are found in all biotic and abiotic compartments on the Earth (atmosphere, soil and sediments, water column) and in both terrestrial and aquatic organisms [1,2]. PAHs can enter aquatic ecosystems directly from natural or man-made sources, in particular from oil spills and leakages: an estimated 230,000 metric tons of PAHs were released to the aquatic environment worldwide annually during the 1980s [3]. These point-source inputs are combined with the main supply of contamination through atmospheric deposition [4], making PAHs among the most common pollutants present at high concentrations in the aquatic environment. Depending on chemical structure, PAHs are subjected to biodegradation by microorganisms, mainly by fungi, bacteria, cyanobacteria, and microalgae [5]. PAHs are potent carcinogens and mutagens for higher organisms [6], and owing to their toxicity, persistence and accumulation along food chains [7] they are among the most studied contaminants. Recently, De Laender et al. [8] suggested that, compared to other legacy chemicals such as PCBs and DDT which were strictly regulated at national and international level, the PAH level had not decreased in the global environment. These authors suggested that this was due to increasing fossil fuel combustion, renewing concern about the fate and effects of PAHs in ecosystems that could be as significant as the global increase in temperature [8].

Coastal marine ecosystems are often contaminated by PAHs [9,10] and the biota is affected by this pollution. The toxicity and lethality of PAHs have been assessed for a variety of marine organisms such as fish [11,12], zooplankton [13] and amphipods [14]. Phytoplankton, a heterogeneous group of photosynthetic microorganisms thriving in the euphotic zone of aquatic systems, is considered to be the basic fuel for aquatic food webs and determines primary ecosystem production and its functioning at higher trophic levels. As the structure and function of pelagic marine food webs depend on the energy and matter supplied by phytoplankton photosynthesis, it is important to understand how PAHs affect phytoplankton populations [15–18]. Despite this particular concern about the prevalence of PAHs and their effect on aquatic organisms, still not enough information is available on the acute toxicity of individual compounds to phytoplankton relative to other chemicals such as pesticides, although studies have been carried out into bioaccumulation for bioremediation [19,20] and trophic transfer [21]. The EPA ECOTOX database provides information on the ecotoxicity properties derived mainly from experiments on model freshwater species such as *Selenastrum capricornutum*, whereas it has been shown that marine phytoplankton are threatened by PAH toxicity even in the open seas [18]. As pointed out by Sumpter [22], attention should be paid to the effects of "old-fashioned" chemicals such as PAHs, as they can have a significantly adverse effect on aquatic biodiversity.

The goal of this study was to assess the toxic effect of two PAHs, fluoranthene and benz(*a*)anthracene (see for example Cerniglia [5] for chemical summary), on seven marine phytoplankton species belonging to various phylogenetic groups: the dinoflagellate *Alexandrium catenella*, the diatoms *Phaeodactylum tricornutum* and *Chaetoceros muelleri*, the prymnesiophyte *Isochrysis galbana*, the chlorophyte *Dunaliella tertiolecta* and the trebouxiophytes *Picochlorum* sp. and *Nannochloris* sp. Benz(*a*)anthracene, with 4 rings, is considered to be a human carcinogen and one of the most aggressive PAHs. Fluoranthene, with three rings, is considered to be the most toxic petroleum hydrocarbon for marine biota in the short term and one of the most often noticed PAHs at significant levels in the marine environment. Both benz(*a*)anthracene and fluoranthene are usually found buried in coastal sediment of Mediterranean lagoons where they could persist for many years [23–25], eventually being released into the water column during meteorological events or dredging, especially in shallow environments. The two PAHs selected were assessed for their toxicity at physiological level, using fluorescence transient analysis of the photosystem II (PSII) as a proxy of photosynthetic efficiency, and at population level, using raw *in vivo* chlorophyll *a* (chl *a*) fluorescence as a biomass endpoint. The study compared the sensitivity of various cultured species by analyzing the acute (physiological) and growth (population) toxicity, to provide new data, particularly about the size/related sensitivity of phytoplankton to PAHs.

Table 1
Biovolumes calculated for the seven phytoplankton strains tested in this study.

Species	<α 5%	Biovolume (μm^3)	>α 5%
Nannochloris sp.	3.85	4.68	5.2
Picochlorum sp.	1.85	2.95	3.45
Isochrysis galbana	60	71.5	82
Dunaliella tertiolecta	212	227	242
Chaetoceros muelleri	177	182	187
Phaeodactylum tricornutum	205	211	217
Alexandrium catenella	9650	10,365	12,430

Length, width and shape were determined for 30 individual cells for each species. 95% confidence intervals are given.

2. Materials and methods

2.1. Test algae

Seven species of phytoplankton were studied: *A. catenella* which was isolated from the Thau lagoon (courtesy of Dr. Mohamed Laabir), *C. muelleri* CCAP 1010/3 (courtesy of Dr. Verena Brauer), *Nannochloris* sp. (RCC 240), *Picochlorum* sp. (RCC 484), *Dunaliella tertiolecta* (RCC 6), *I. galbana* (T-iso, equivalent to RCC 0179), *Phaeodactylum tricornutum* (Pt1_8.6). RCC strains were purchased from Roscoff Culture Collection (University Paris 6 and CNRS, Roscoff, France), *I. galbana* and *P. tricornutum* were obtained from aquaculture strains. The standard plankton size classes were represented: *A. catenella* was the largest species, belonging to microphytoplankton, *P. tricornutum*, *C. muelleri*, *D. tertiolecta*, and *I. galbana* belonged to the nanophytoplankton group and *Nannochloris* sp. and *Picochlorum* sp. were picophytoplankton. All cultures were monospecific, from clonal selection, but not axenic.

The calculated cell volume for each strain (Table 1) was based on the apparent diameter for spherical cells (*Picochlorum* sp.), using a rotational ellipsoid for *Nannochloris* sp., *I. galbana*, *D. tertiolecta* and *A. catenella*, a cylinder for *C. muelleri* [26] and a half elliptic prism for *P. tricornutum*. Measurements were taken using a light microscope (Zeiss model AX-10) at ×400 and ×1000 magnification. Digital images were recorded (camera Sony model XCD U-100 CR) and processed using Image J freeware to determine the relevant dimensions for at least 30 individuals of each phytoplankton species.

2.2. Culture and exposure conditions

The cultures were incubated in a thermostatic chamber at 20 °C (±0.5 °C) placed on a rotating tray, using a photoperiod of 12 h light and 12 h dark. Light was supplied by 15 W white fluorescent lamps (Osram Daylight) with a photosynthetically available radiation (PAR) intensity of 120 μmol photons m^{-2} s^{-1} (checked using a spherical quantum mini-recorder MkV/L, AlecElectronics, Japan).

The *A. catenella* strain was maintained in ESAW medium [27], whereas the other species were grown in f/2 medium [28]. The latter was made from a concentrated commercial enrichment solution (Sigma–Aldrich G9903) diluted in 0.2 μm filtered 2-month-old seawater collected 5 nautical miles from Sète Harbor and autoclaved