1 *Environmental Pollution*, Volume 239, August 2018, Pages 607–616
2 (<https://doi.org/10.1016/j.envpol.2018.04.079>)

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4 **PAHs sensitivity of picophytoplankton populations in the Red Sea**

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Abstract

In this study, we investigated the *in situ* responses of Red Sea picophytoplankton, the dominant phytoplankton group in the oligotrophic ocean, to two toxic polycyclic aromatic hydrocarbons (PAHs), phenanthrene and pyrene. The experiments were conducted *in situ*, across a latitudinal gradient of the Saudi Arabian Red Sea, an area sensitive to oil pollution. We observed significant adverse effects on the growth and abundance of the picocyanobacteria *Synechococcus* and picoeukaryotes, at all stations sampled. *Prochlorococcus*, which was abundant only in one of the stations, also appeared to be affected. Pyrene was found to be more toxic to phytoplankton, at all stations. In general, picoeukaryotes exhibited a higher sensitivity to PAHs than *Synechococcus*. Populations in the highly oligotrophic Northern region of the Red Sea were more tolerant to PAHs, presumably influenced by the natural selection of more resistant strains of phytoplankton due to the prolonged exposure to PAHs. Toxicity threshold values reported here are higher than those reported for picophytoplankton from other oligotrophic marine waters and exceed by far the natural levels of PAHs in many oceans. Our findings reveal a possible adaptation of picophytoplankton populations to oil-related contaminants, which may clearly influence their spatial distribution patterns in the Red Sea.

Capsule: Sensitivity of picophytoplankton to PAHs was lower in the oligotrophic Northern regions in the Red Sea than mesotrophic Southern Region.

Keywords: PAHs; phenanthrene; pyrene; LC₁₀; *Synechococcus*; Red Sea.

Abbreviations: PAHs, polycyclic aromatic hydrocarbons; μ , specific growth rate; POPs, persistent organic compounds; SSC, side scatter; LC₅₀, 50% lethal concentration; LC₁₀, 10% lethal concentration;

Introduction

Polycyclic aromatic hydrocarbons (PAHs), the most acutely toxic components of crude oil, have been intensively studied as one of the most noxious pollutants of the marine environment (Abdel-Shafy and Mansour, 2016; Alegbeleye et al., 2017). Major sources of dissolved and particulate PAHs in the marine environment include crude oil released by ships during oil seepage and accidental spills, combustion of fossil fuels and related sources of energy, natural fires and domestic or industrial sewage. Other sources worth mentioning are road dust/runoff and long-range atmospheric deposition (Castro-Jiménez et al., 2012). PAHs usually persist in environment as complex mixtures of two or more individual chemicals obtained through various chemical reactions. The low aqueous solubility and high lipophilic characteristics of PAHs accelerate their accumulation in soil, sediments, and oily materials. They can penetrate the tissues of organisms (Agah et al., 2017) and affect vital biological processes through their carcinogenic and mutagenic properties (Witt, 1995).

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. They are vital to the primary ecosystem production and it's functioning at higher trophic levels (Othman et al., 2012). Additionally, phytoplankton serves as the primary route for pollutants to enter the food chain, thereby playing a crucial role in the environmental fate of pollutants such as PAHs. The sensitivity of phytoplankton to contaminated environments is an interesting topic, both from genetic and physiological perspectives. The toxicity of persistent organic compounds (POPs) and PAHs in marine phytoplankton has been extensively studied over the past decade (Almeida et al., 2012; Karacık et al., 2009).

Most studies on the impact of organic pollutants on marine photosynthetic microorganisms (e.g. phytoplankton) have been conducted in nutrient-rich coastal waters (González et al., 2009; Huang et al., 2011; Pérez et al., 2010). There is limited information on the oligotrophic regions of the world's oceans (Echeveste et al., 2010b). Yet, oligotrophic waters dominate the global marine environment, representing almost 70% of the total surface area. Primary production in oligotrophic region is mainly

contributed by picophytoplankton (Agawin et al., 2000), composed mainly of *Synechococcus* sp., *Prochlorococcus* sp. and picoeukaryotes (protists of $\leq 3 \mu\text{m}$ size, composed of a wide diversity of taxonomic groups) (Partensky et al., 1999). The Red Sea is considered to be a poorly explored oligotrophic marine environment, despite its coastal areas of great biological and economic values. However, the over-exploitation of resources and environmental pollution cause great concern. Moreover, human activities such as industrial and urban expansions, petroleum refineries and heavy tanker traffic result in the contamination by oil-related organic compounds. Baseline data on the extent of chronic pollution from these sources in the Red Sea are limited, and the additional impacts of pollutants through atmospheric transport and deposition remain largely underestimated. Significant levels of PAHs have been reported in water and sediment along the Egyptian and Arabian coasts of the Red Sea (Al-Farawati et al., 2009), and a recent study conducted in the Egyptian Red Sea revealed the presence of both pyrogenic and petrogenic PAHs in the water (Abdallah et al., 2015). In addition, high levels of PAHs have been reported in tissues of edible shrimps, mussels and fishes in various regions of the Red Sea (DouAbul et al., 1997; El Nemr et al., 2004; Hussein et al., 2016). Nevertheless, the levels of concentration of PAHs in the Red Sea environment are lower than those in other areas of the world (Salem et al., 2014).

Phenanthrene and pyrene, composed of 3 and 4 aromatic rings, respectively, are the most abundant PAHs found in natural seawater (Echeveste et al., 2011; Nizzetto et al., 2008). Phenanthrene represents a more persistent class of PAHs and has a higher ability to bioaccumulate and lower water solubility. Pyrene has strong phytotoxic effects on algae, affecting nutrient (ammonium, nitrate and silicate) uptake and carbon incorporation in algal communities (Petersen and Dahllof, 2007). Besides their presence in crude oil, phenanthrene and pyrene have widespread industrial use such as the manufacture of resins, pesticides and pigments (Abdel-Shafy and Mansour, 2016). Both phenanthrene and pyrene adversely affect the growth and abundance of oceanic phytoplankton and various threshold levels have been reported from different ocean habitats (Echeveste et al., 2010a).

The response of Red Sea phytoplankton to notorious PAHs and the variations in the sensitivities of its components in physico-chemically diverse environments are almost

completely unexplored. The objective of the present study is to reveal the spatial distribution of PAHs sensitivity of picophytoplankton, conducting on-board experiments across a latitudinal gradient along the Saudi Arabian Red Sea, under natural sunlight. Natural phytoplankton communities are more sensitive to PAHs than cultured strains (Echeveste et al., 2010a), and incubation experiments with natural communities is expected to reveal more relevant toxicity responses at primary producer levels in the marine ecosystem.

Materials and Methods

Study area and sampling stations:

On-board incubation experiments to determine the lethal thresholds of phenanthrene and pyrene on the Red Sea picophytoplankton were conducted during two different research cruises (in October and November, 2016) on RV Thuwal, along Saudi Arabian waters (coordinates 17° 4' N – 41° 27' E to 27° 36' N – 35° 11' E; Figure 1 and Table 1). Samples were collected at 12 locations (S 1 to S 12) across the Northern oligotrophic and Southern mesotrophic regions of the Red Sea. Phenanthrene was tested at all stations whereas pyrene was tested at seven selected stations (Stations 3, 5, 8, 9, 10, 11, & 12). Physicochemical parameters were measured on-site with a conductivity, temperature and density (CTD)-rosette profiler (Table 1).

In order to determine the concentration of chlorophyll *a*, 300 mL of seawater samples, at each sampling station, were filtered on a Whatman GF/F glass fiber filter (0.7 µm pore size), and the chlorophyll *a* was extracted from the retained phytoplankton cells using a 90% acetone solution for approximately 24 hours, at 4°C. The extract was subsequently centrifuged and analyzed using a Turner Trilogy fluorometer (fitted with the non-acidification chlorophyll module) (Turner Designs, Sunnyvale, CA).

Test chemicals and solutions:

Phenanthrene (C₁₄H₁₀, CAS No. 85-01-8) and pyrene (C₁₆H₁₀, CAS No. 129-00-0) stocks were purchased from Sigma Aldrich (Sigma Aldrich, Germany) as standard solutions in Methanol, at respective concentrations of 5000 and 1000 mg L⁻¹. Working stocks for

each PAH (0.5, 1, 10, 50, 100 and 500 mg L⁻¹) were prepared by dilution in HPLC-grade methanol, under controlled conditions to prevent the loss of solvent by evaporation. Stocks were tightly capped and stored in darkness under 4 °C.

Experimental setup

Water samples were collected at a depth of 3 m (referred as surface water) with Niskin bottles attached to the CTD rosette; they were subsequently pre-filtered using a 100 µm filter gauze (CellTrics, Sysmex Partec GmbH, Germany) to eliminate grazing by larger organisms during the incubation. Once filtered, water samples were distributed into pre-cleaned Erlenmeyer's flasks (100 ML with 50 ml test volume), in triplicate, subsequently spiked with phenanthrene/ pyrene in order to obtain final concentrations of 0.5, 1, 10, 50, 100 and 500 µg L⁻¹. The final concentration of methanol in the test flasks was 0.1 %. Controls without any PAH and solvent controls with 0.1 % methanol were also incubated in triplicate. After gentle vortexing to ensure a complete dissolution of the PAHs, test flasks were tightly closed and incubated in an open tank, on deck under natural solar irradiation. A continuous surface seawater running system kept the *in situ* temperature conditions constant and the tanks were covered with neutral mesh to simulate irradiance conditions at 3 m-depth. After 48 h of incubation, the flasks were recovered and the abundance of picophytoplankton was estimated by flow cytometry.

Sampling and analysis

After incubation, 1 mL water sample from each test flask was transferred to a 24-well cell culture plate (Becton Dickinson, France). The abundance of the picocyanobacteria *Prochlorococcus* and *Synechococcus*, and picoeukaryotes was directly estimated using a high performance, absolute counting, bench-top flow cytometer (CyFlow Space, Sysmex Partec GmbH, Germany) equipped with a 488-nm blue solid-state laser, and provided with a CyFlow Space Autoloading Station, controlled by the CyPAD software (Sysmex Partec GmbH, Germany). Data were acquired by triggering on chlorophyll fluorescence (FL3, 675 nm) in log mode and picophytoplankton populations were enumerated following the standard protocols (Marie et al., 1999) based on the characteristic signals in the cytogram of FL2 (590±50 nm, corresponding to the phycoerythrin fluorescence) versus FL3. The populations were further distinguished in a side scatter (SSC) versus

FL3 dot plot. *Synechococcus* was detected by the orange fluorescence signature of phycoerythrin in FL2 versus FL3 dot plots. *Prochlorococcus* showed lower FL3 signal and no FL2 signal and picoeukaryotes showed higher FL3 and SSC signals and lower or no FL2 signals. The abundance of *Prochlorococcus*, *Synechococcus* and picoeukaryotes were obtained as cells mL⁻¹.

Statistical analysis

All values presented here are the means ± 95% confidence intervals. All parameters were compared across the treatments with one-way ANOVA (n = 3; at *p* = 0.05).

Picophytoplankton growth rates were estimated using the variation in cell abundance over time. The specific growth rate (μ , d⁻¹) was calculated using the following formula:

$$\mu = \frac{\text{LN}(\frac{N_2}{N_1})}{(t_2 - t_1)} \quad \text{eq. 1}$$

where N_1 and N_2 are the numbers of cells at time t_1 (initial) and t_2 (final), respectively (Levasseur et al., 1993).

The effect of the PAH treatment on picophytoplankton growth rate was calculated as:

$$\% \text{ inhibition} = \frac{\mu_C - \mu_T}{\mu_C} \times 100 \quad \text{eq. 2}$$

where μ_C is the growth rate in controls, and μ_T the growth rate in PAH treatments.

The lethal concentrations at which 50 % and 10 % declines in population abundance occur (LC₅₀ and LC₁₀, respectively) were estimated using the JMP software (JMP® Pro version 13.1, SAS Institute, Cary, NC, USA) and the following equation, as described by Echeveste et al. (Echeveste et al., 2010b);

$$\text{LC}_{50} = \ln 0.5 / \Omega$$

$$\text{LC}_{10} = \ln 0.9 / \Omega$$

where Ω is the slope of the relationship between the ln of the cell abundance decline at the end of the experimental incubation and the PAHs concentration tested. Representative dose-response curves and fitting statistics are given in the Supplementary Figure S1.

Results

Picophytoplankton distribution and growth

We observed a variation of the picophytoplankton community composition on a North to South latitudinal gradient in the Red Sea, with a gradual increase in *Synechococcus* and picoeukaryotes from Northern to Southern stations. This disparity was directly related to the variability in total Chl *a* concentration (a proxy of phytoplankton biomass) at respective stations (Table 1). *Synechococcus* sp. was found more abundant than picoeukaryotes in all the surface water samples examined (Table 1). However, the cyanobacteria *Prochlorococcus* sp. showed very low abundance (mean $< 1000 \text{ cells mL}^{-1}$) across the stations sampled, except at S8, located in the Central Red Sea.

In the on-board incubation experiment, the growth of overall net picophytoplankton community varied across sampling stations (Table 2). Net growth rates of *Synechococcus* estimated over 48 h in the controls were low, showing positive values for the range $0.007 - 0.224 \text{ d}^{-1}$ at 4 stations (stations 1,2,3 and 5), located in the Northern Region of the Red Sea (Table 2). However, picoeukaryotes, showed a positive control net growth in the majority of stations except at stations 4, 6, and 8, where the values were -0.96 , -0.66 and -0.12 , respectively. At most stations, the growth rates of picoeukaryotes were higher than those of *Synechococcus*. Despite the higher abundance of picophytoplankton at Southern stations (Table 1), growth rates in the experimental control flasks were significantly lower (ANOVA, $F = 717$, $df = 11$, $p < 0.05$), compared to those at Northern stations. Presumably, the nutrient-rich water in the Southern Region supported higher abundance of phytoplankton, which was shown by the initial cell abundance and total chlorophyll contents (Table 1).

Effect of PAHs on picophytoplankton growth rates

Cellular growth rate of picoplankton was examined as criterion for ecologically relevant toxicity responses in the natural Red Sea communities. At the higher concentrations, phenanthrene and pyrene led to a decrease of the growth rate of picoplankton population at all stations (Table 2). Higher doses of exposure resulted in negative growth rates, indicating the decline and decay of most populations (Table 2). At the highest concentrations tested, phenanthrene and pyrene resulted in $> 90\%$ inhibition of the

growth rate of picoplankton, more prominently in the Southern Region of the Red Sea basin. Additionally, a stimulatory effect on net *Synechococcus* growth, at lower PAHs concentrations, was observed in the Southern Region. A similar growth increase of the picoeukaryote population was also observed after treatment with phenanthrene at stations 2, 4, 8, 9, 10 and 11 (Table 2). Figure 2 summarizes the percentages of growth rate inhibition of picoplankton after exposure to PAHs. Pyrene resulted in higher rates of inhibition, compared to phenanthrene, for all test stations. Moreover, significant (Student's t-test, $p < 0.01$) reductions in growth were observed at lower doses of PAH; for instance, at $10 \mu\text{g L}^{-1}$, phenanthrene and pyrene respectively led to a growth inhibition of picoplankton, by up to 30 and 60 % respectively. The extent of this inhibition varied randomly across various stations.

Sensitivity of picophytoplankton to PAHs

The two tested PAHs showed direct adverse effects on the picophytoplankton at the end of the incubation, causing a significant reduction of the *Synechococcus* and picoeukaryotes abundances at all stations, as the concentration in PAHs increased (Student's t-test, $p < 0.01$). Final values for the two groups of picoplankton after exposure to PAHs are reported in Tables S1 and S2 in the Supplementary Data. The highest dose of tested phenanthrene caused a reduction of 47 to 93 % and 53 to 95 % in the *Synechococcus* and picoeukaryote populations, respectively. Pyrene, on the other hand, led to more catastrophic impacts with 87 – 98 % and > 99 % reductions in the same populations. The toxicity thresholds of the tested PAHs, expressed as lethal concentrations (LC10 and LC50), are given in Table 3 and Table S3 in the Supplementary Data, respectively. 48 h LC50 values for phenanthrene ranged from $158.03 \mu\text{g L}^{-1}$ to $1066.38 \mu\text{g L}^{-1}$ and $124.82 \mu\text{g L}^{-1}$ to $456.02 \mu\text{g L}^{-1}$ for *Synechococcus* and picoeukaryotes, respectively. Pyrene caused a more drastic reduction, with LC50 values ranging from $84.14 \mu\text{g L}^{-1}$ to $219.84 \mu\text{g L}^{-1}$ and $56.53 \mu\text{g L}^{-1}$ to $105.90 \mu\text{g L}^{-1}$ for *Synechococcus* and picoeukaryotes, respectively. Clearly, picoeukaryotes were significantly more sensitive to the tested PAHs than *Synechococcus*, except to phenanthrene at stations 6 and 12, with maximum EC50 values of $456.02 \mu\text{g L}^{-1}$ and $105.90 \mu\text{g L}^{-1}$ for phenanthrene and pyrene, respectively (Table S3 in the Supplementary Data).

The *Prochlorococcus* population was found to be extremely low, except at station 8, which is situated at the central region of the Red Sea where sensitivity measurements could be performed. At station 8, phenanthrene and pyrene caused significant declines of the *Prochlorococcus* abundance (ANOVA, $F = 5.82$, $df = 7$, $p < 0.05$ and $F = 35.89$, $df = 7$, $p < 0.05$, respectively), with 48 h LC50 values of $570.49 \pm 28 \mu\text{g L}^{-1}$ and $367.91 \pm 22 \mu\text{g L}^{-1}$, respectively. The LC values obtained for *Prochlorococcus* were clearly higher than those for *Synechococcus* and picoeukaryotes at the same station (Table S3 in the Supplementary Data). LC10 values for *Prochlorococcus* ($86.72 \pm 12 \mu\text{g L}^{-1}$ and $55.92 \pm 10 \mu\text{g L}^{-1}$ for phenanthrene and pyrene, respectively) showed a resistance three times higher to PAHs than picoeukaryotes.

We observed a spatial distribution of LC10 threshold values of picoplankton along the latitudinal gradient in the Red Sea (Figure 3). However, a consistent trend in the distribution of PAH sensitivities of picoeukaryotes across the latitudinal gradient did not appear very clearly. In the Northern Region of the Red Sea, higher LC10 values were obtained, more evidently in the case of phenanthrene. When comparing LC10 values, we found that pyrene was more toxic than phenanthrene, and that picoeukaryotes were more sensitive to PAHs than *Synechococcus* (Figure 4). For example, with phenanthrene, average LC10 values for *Synechococcus*, in the Northern, Central and Southern Regions were $87.88 \mu\text{g L}^{-1}$, $53.43 \mu\text{g L}^{-1}$ and $40.14 \mu\text{g L}^{-1}$, respectively (Figure 5a). In the case of pyrene, and for similar regions, average LC10 values for *Synechococcus*, were $21.76 \mu\text{g L}^{-1}$, $23.69 \mu\text{g L}^{-1}$ and $16.14 \mu\text{g L}^{-1}$, respectively (Figure 5a). With phenanthrene, LC10 values for picoeukaryotes showed lower in the Central region ($36.24 \mu\text{g L}^{-1}$) than in the Northern and Southern Regions ($43.06 \mu\text{g L}^{-1}$ and $39.32 \mu\text{g L}^{-1}$, respectively). The lowest pyrene LC10 values for picoeukaryotes were recorded in the Northern Region ($9.54 \mu\text{g L}^{-1}$); similar values were obtained in the Central and Southern Regions (12.34 and 12.39 , respectively).

Discussion

Picophytoplankton represents an essential contributor to microorganism biomass and primary productivity in oligotrophic oceanic ecosystems (Buitenhuis et al., 2012; Li, 1994). However, their minute size and high surface-to-volume ratio make these

organisms very vulnerable to toxic pollutants such as heavy metals and organic compounds (Debelius et al., 2010; Echeveste et al., 2010a). These characteristics are ecologically very significant. Indeed, picophytoplankton are the first step in the oceanic food web and plays major role in marine carbon cycle (Agawin et al., 2000). Here, we report significant variations in sensitivity of picoplankton populations along the Red Sea to two widespread PAHs. In general, toxicity of PAHs depends on the size of plankton cells, with the smallest picoplankton being the most sensitive (Echeveste et al., 2010a; Othman et al., 2012). Besides their toxicity, at sub-lethal concentrations, PAHs can be retained in the algal cells and get transferred to organisms at higher trophic levels. This trophic transfer in aquatic ecosystems is an important criterion to assess the ecological risk of PAHs. In addition, the intracellular partitioning of PAHs in algal cells, which determine their trophic transfer to predators, is directly related to the size of their cells (Fan and Reinfelder, 2003). Therefore, the presence of PAHs is a serious issue for the size distribution of primary producers in marine pelagic food web, and causes great concern as PAHs can adversely affect the overall ecosystem functions and energy cycling.

PAHs critically reduce the planktonic photosynthesis on acute exposure, resulting in lower growth of the biomass when chronically exposed (Othman et al., 2012). The reduction in abundance of picoplankton that we observed may be due to a reduced growth rate as well as increased death rates (Echeveste et al., 2010b). Cerezo and Agustí (Cerezo and Agustí, 2015) reported that PAHs delay cell division in picophytoplankton by preventing the synthesis of DNA and lowering the fraction of cells entering mitosis. PAH-induced complex effects on the microbial community food web have been recently evidenced in open oligotrophic oceans (Cerezo and Agustí, 2015). However, at lower concentrations, PAHs can enhance the net growth of phytoplankton populations by cascade effects on food webs, with an acceleration of this effect under low nutrient conditions (Zhu et al., 2012). This influence possibly involves a decrease in the number of sensitive species and an increase in more resistant species, as previously observed with crude oil exposure (Ozhan and Bargu, 2014). A similar stimulation of the growth of picoplankton at sub-lethal PAHs exposures, as we observed in the Red Sea, may also be influenced by the oligotrophic nature of the seawater. We further assume that at certain

PAHs levels, the mesozooplankton may also get affected, resulting in a reduction in grazing pressure on picoplankton, subsequently helping the communities to maintain higher growth rates, as previously observed in experiments with natural communities (Cerezo and Agusti, 2015; Echeveste et al., 2011). These sub-lethal impacts results in variation in total biomass and may alter the community composition of phytoplankton. Impact of PAHs on community structure in natural environment is hard to predict due the large number of physico-chemical (temperature, salinity, nutrients concentration and ratios, mixing, light) and biological (grazing, viral infections, sensitivity to other environmental stressors) parameters to account. Nevertheless, it is noteworthy that lower abundance of *Prochlorococcus* was observed in the North Red Sea, which is an oligotrophic system where a higher number of this genus was expected. *Synechococcus* was dominating the populations in the North and we have identified them as most resistant to PAHs, indicating that prolonged exposure to pollutants may have some influence, along with other environmental factors, on the distribution of the picophytoplankton groups. However, further studies are required to test and confirm this hypothesis.

In the Red Sea, we observed significant differences in sensitivity of picoplankton communities between the two PAHs tested. Even though PAHs with higher molecular weights tend to be less toxic than those with lower molecular weights due to their lower solubility, we found that the four-ringed pyrene was more toxic than the three-ringed phenanthrene, when comparing the LC10 values for decay of abundance (Figure 4). In fact, the additional aromatic ring in pyrene can add to its toxicity (Millemann et al., 1984) and as shown previously, the toxicity of PAHs are strongly influenced by multiple factors such as membrane permeability, water solubility, volatility and reactivity potential of hydroxylated radicals with PAHs (Djomo et al., 2004). It was shown that exposure to pollution could force a diversification in the selection of different ecotypes, for a same species, and could result in differences of sensitivity of picocyanobacteria to pollutants, across various oceanic habitats. Echeveste et al. (Echeveste et al., 2010a) found variations in LC values for phenanthrene and pyrene in *Synechococcus*, picoeukaryotes and nanoplankton communities across Mediterranean and Atlantic oceans. We found the lowest LC50 values to be 158.03 $\mu\text{g L}^{-1}$ and 124.82 $\mu\text{g L}^{-1}$ for *Synechococcus* and

picoeukaryotes, respectively (Table S3 in the Supplementary Data). These values are comparable with those reported for picoeukaryotes (158.4 and 137.6 for phenanthrene and pyrene, respectively) in the Mediterranean waters (Echeveste et al., 2010a). However, the higher relative sensitivity of picoeukaryotes observed in the Red Sea is in contradiction with studies performed in the Mediterranean Sea (Echeveste et al., 2010a), in which the sensitivity of picoeukaryotes was found to be almost twenty times lower than that of picocyanobacteria. It is expected that the sensitivity to PAHs varies between strains and geographical locations of the target species, and that the any kind of adaptation of picophytoplankton communities to the constant presence of PAHs in the 'natural background' cannot be ruled out.

Previous studies have shown that the toxic effect of individual PAHs to oceanic phytoplankton was not significant at natural levels (Echeveste et al., 2010a; Echeveste et al., 2010b). In a recent study by Abdallah et al. (Abdallah et al., 2015), the measured levels of phenanthrene and pyrene present in surface water of the Northern Red Sea were $38.41 \text{ ng L}^{-1} - 279 \text{ ng L}^{-1}$ and $75.95 \text{ ng L}^{-1} - 572.82 \text{ ng L}^{-1}$, respectively. Our study reports LC10 threshold levels varying from $18.97 \text{ } \mu\text{g L}^{-1}$ to $162.09 \text{ } \mu\text{g L}^{-1}$ for phenanthrene, and from $8.81 \text{ } \mu\text{g L}^{-1}$ to $33.42 \text{ } \mu\text{g L}^{-1}$ for pyrene. These values are much higher than the natural background levels in the Red Sea water (Abdallah et al., 2015). However, they fall within the range of PAHs detected during major spill events, in the past. For example, during the 1996 North Cape oil spill, values of total PAHs released in the Atlantic ocean reached a maximum of $115 \text{ } \mu\text{g L}^{-1}$ (Reddy and Quinn, 2001), and during the Deep-water Horizon spill, PAHs levels reached up to $189 \text{ } \mu\text{g L}^{-1}$ in the Northern Gulf of Mexico (Diercks et al., 2010). Therefore, the LC10 threshold values reported here indicate a tolerance to acute pollution levels corresponding to spill situations, in which the overall function and productivity of picophytoplankton communities may be greatly affected by toxic PAHs. Considering the broad number of organic pollutants in the marine environment and the potential increase in the amount of pollutants in the surface water layers (Wurl and Obbard, 2004), it is alarming that the pollution reaching marine systems becomes close to threshold levels that adversely affect the phytoplankton communities, due to the formation of complex toxic cocktails of organic compounds (Echeveste et al., 2010b).

Several studies found *Synechococcus* to be the dominant cyanophycean genus in the Red Sea (Pearman et al., 2017; Veldhuis and Kraay, 1993). However, pronounced environmental gradients in the Red Sea play a crucial role in the distribution and patterns of picoplankton communities across the basin (Ngugi et al., 2012; Pearman et al., 2017). Water column conditions also play a crucial role on the composition and abundance of phytoplankton communities, and the low turbulence prevailing in the Northern Region (Pearman et al., 2017) favors oligotrophic conditions and communities dominated by picocyanobacteria. The Southern Region of the Red Sea basin, influenced by the inflow of water from the Gulf of Aden and the Arabian Sea (Sofianos and Johns, 2002), is more replete with nutrients and therefore more productive than the Northern Region (Pearman et al., 2016). A similar latitudinal gradient was observed in our study, with the lowest concentrations in Chl *a* at Northern stations and the highest at Southern stations, ranging from 0.11 to 0.89 mg m⁻³.

We found variable growth rates among the different groups of picoplankton studied, depending on the species and the station where samples were collected. The observed values correspond to net growth rates, and in the controls, they are the result of environmental conditions (nutrients, temperature, presence of grazers) and sensitivities of the different populations to those conditions. The availability of nutrients could benefit from a reduction of the pollution-related stress on picophytoplankton communities (Interlandi, 2002; Nayar et al., 2005). Despite its lower biomass and lower nutrient levels, the Northern Region appears more dynamic in terms of *Synechococcus* populations, maintaining higher growth rates. However, this pattern is not seen for picoeukaryotes.

Considering the variability of the physicochemical environment and phytoplankton communities along the latitudinal gradient of the Red Sea, a crucial question is whether or not the sensitivity to PAHs follows any spatial pattern. In most cases, we recorded higher LC10 values in the Northern Region in comparison with those at Southern stations. Our observations lead us conclude that the communities in the more oligotrophic regions of the Red Sea are more resistant to those of the more eutrophic waters, this being the result of complex responses to pollutants (Hjorth et al., 2008). Petroleum-based industrial activities and effluent discharges drastically increased the extent of oil-derived

hydrocarbon pollution in the coastal regions of the Red Sea (Al-Lihaibi and Al-Ghamdy, 1997). The Northern Region of the Saudi Arabian Coast has many petroleum-based industries and the expected background PAHs level in seawater is higher in this region, due to the discharge of effluents. This contaminants might have been a severe challenge to phytoplankton as communities in the North have probably been exposed to higher PAH levels, long enough for natural selection of the appearance of more tolerant species, and subsequently increase the overall resistance of the population to PAH exposure. This may be further explained by the concept of adaptive mutation, in which the mutations produced during the non-lethal selection process release the pressure applied by the pollutant (Cairns, 1998). García-Villada et al. (García - Villada et al., 2002) reported a rare spontaneous pre-selective mutation that ensured the survival of phytoplankton after a drift in the environment due to aquatic pollution. Adaptation to organic pollutants via mutation has been previously reported in cyanobacteria and green algae (Bisen and Shanthy, 1993; Lopez-Rodas et al., 2001). We postulate that the picoplankton communities in the Northern Red Sea have naturally evolved and acquired a higher resistance to PAHs that ensures the survival of their populations in suddenly polluted environments during experimental exposure. The higher genetic variability in natural populations of *Synechococcus* in the Red Sea (Penno et al., 2006) may also guarantee their survival under changing environments. Additionally, higher water temperature towards the South may contribute an increase in toxicity by increasing the water solubility of PAHs (Neff, 1979). However, additional specific investigations are required to further address this assumption.

We demonstrate that the sensitivity of *Synechococcus* to PAHs in the Red Sea is significantly lower than those reported in oligotrophic regions of other oceans (Echeveste et al., 2010a). The LC10 values found for phenanthrene were 9 and 17 folds higher than those reported in Atlantic and Mediterranean waters (Figure 6). Similarly, LC10 values for pyrene were three times less in the Red Sea, which was found to be more oligotrophic, as indicated by the low Chl *a* concentrations of the sampled water. This confirms the higher resistance of Red Sea picoplankton to PAHs contamination. Abdallah et al. (Abdallah et al., 2015) estimated the total amount of PAHs in the Red Sea surface

water to be within the range Σ_{15} 1295 – 4961 ng L⁻¹, a range of values significantly higher than the value of Σ_{29} 8.9 – 197.8 ng L⁻¹ reported in moderate to highly impacted Mediterranean waters (Fourati et al., 2017). Moreover, higher ranges of PAHs (with maximum value (Σ_{38} 5372 – 7927 ng g⁻¹) have been recently reported in highly contaminated lagoons along the Saudi Arabian Coast of the Red Sea (Rasiq et al., 2018). These studies reveal the substantial growing stress of PAHs on Red Sea biota and the lower threshold values (expressed as LC10) reported here are ecologically relevant as a 10 % reduction in the phytoplankton abundance can have significant impacts on the net productivity of the community.

Conclusions

We report the first study of *in situ* responses of primary producers in the Red Sea to two PAHs: phenanthrene and pyrene. Investigation of the sensitivity of picoplankton to PAHs is essential to understand the future consequences of oil pollution on the functioning of Red Sea ecosystem. The present study reinforces the theory of pre-selective adaptation of the picophytoplankton, as a mechanism behind the evolution of its resistance to PAHs, in marine environments. Considering that the majority of PAHs entering the Red Sea organisms originates from heavy ship traffic and spillages (DouAbul et al., 1997) and from possible sources of industrial anthropogenic inputs into the Northern Arabian Coast, PAHs and their toxic impacts have been exerting selective pressures on the phytoplankton community, constraining them to evolve and adopt a certain degree of resistance to pollutants in their natural habitats. Based on our results, we also postulate that PAHs induced reduction in growth and abundance can have serious impacts on the overall primary productivity, and the understanding the extent of such effect is essential to address the actual ecological impacts of oil pollution in marine environments. Future studies with a higher number of samples, including those from extreme environments, will give a more comprehensive understanding of the sensitivity distribution across unexplored regions of the Red Sea basin. The current methodology has limitations in terms of spatial and temporal replication, however, the results reported herein provide a baseline for the picoplankton responses to toxic PAHs in the Red Sea and further

extensive research is recommended to elucidate the spatial pattern of these responses and the factors contributing to it, in the oligotrophic/mesotrophic waters of the Red Sea.

Acknowledgements

This work was supported by the King Abdullah University of Science and Technology (KAUST). We are grateful to the Coastal and Marine Resources Core Lab (CMOR), KAUST and the R/V Thuwal crew for their help during the sampling procedures.

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Figure legends

Fig. 1. Map of the Red Sea showing locations of the sampling stations.

Fig. 2. Percentage of growth rate inhibition for natural Red Sea *Synechococcus* and picoeukaryotes populations exposed to phenanthrene and pyrene. Each box represents collective data from all stations.

Fig. 3. Spatial distribution of the sensitivity of Red Sea natural picophytoplankton populations (expressed as LC10 threshold values) for (A) *Synechococcus*–Phenanthrene, (B) Picoeukaryotes–Phenanthrene, (C) *Synechococcus*–Pyrene, (D) Picoeukaryotes–Pyrene. The size of the bullets represents the magnitude of LC10 values and the corresponding range is given in the legend.

Fig. 4. Comparison of sensitivities of *Synechococcus* and picoeukaryotes (as LC10 values) to phenanthrene and pyrene, in the Red Sea.

Fig. 5. Distribution of phenanthrene and pyrene sensitivities of (A) *Synechococcus* populations, and (B) picoeukaryotes populations, in various zones in the Red Sea parallel to the chlorophyll *a* distribution (MODIS Chl *a* (mg m^{-3}) annual composite for 2003–2015). The mean LC10 threshold values are represented as bars (not to scale).

Fig. 6. Comparison of estimated PAHs sensitivities (mean LC10 values) of natural *Synechococcus* populations in various oligotrophic oceanic regions. Data for Atlantic and Mediterranean Seas are adapted from Echeveste et al. (Echeveste et al., 2010a).

Table legends

Table 1. Sampling date and location of the experiment, PAHs tested (at each station), sea surface temperature (SST), salinity, total chlorophyll *a* concentration and picoplankton abundance recorded along the Red Sea.

Table 2. Mean growth rates (μ , d^{-1}) of *Synechococcus* and picoeukaryote populations from surface water in controls and after exposure to phenanthrene and pyrene doses (at the various stations).

Table 3. 48 h LC10 values for phenanthrene and pyrene to decay in abundance of *Synechococcus* and picoeukaryotes at various stations. Values shown as mean $\pm$ SD.

Figure 1:

Figure 2:

Figure 3:

Figure 4:

775 **Figure 5A:**
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Figure 5B:

Figure 6: