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Functional and structural responses of marine plankton food web to pyrene contamination

M. Hjorth[1, 2,*], J. Vester[1], P. Henriksen[1], V. Forbes[2], I. Dahllöf[1]

[1]Department of Marine Ecology, National Environmental Research Institute, Frederiksborgvej 399, 4000 Roskilde, Denmark
[2]Department of Life Sciences and Chemistry, University of Roskilde, Universitetsvej 1, 4000 Roskilde, Denmark

ABSTRACT: The effects of single additions of pyrene (low, medium or high) on a natural marine plankton community are reported in this study. Direct and indirect effects on the function and structure of bacteria, phytoplankton and zooplankton communities were investigated using a food-web approach in a mesocosm. Phytoplankton communities suffered from direct effects of the exposure to pyrene and, after a lag-period of 2 d, so did the bacterial communities exposed to medium and high pyrene concentrations. Effects on the zooplankton community function were not as evident, mainly due to high variation in the measured prey uptake. Abundance of phytoplankton decreased, and the community composition changed in the exposed communities. The total phytoplankton community activity remained stable, resulting in high specific activity (activity per unit chlorophyll *a*), which could be interpreted as functional redundancy. However, we suggest that differences in nutrient availability between exposed and unexposed communities actually caused the indicated functional redundancy, as phytoplankton growth in control communities was nutrient limited during the experiment, whereas nutrient regeneration from the directly affected algae provided the exposed communities with better growth conditions. The system function, which integrates the functions of the 3 trophic levels, was affected at all 3 exposures during the first 5 d of the experiment. The change in system function suggests that PAHs (polyaromatic hydrocarbons) might be an important stress factor for pelagic systems, as a one-time exposure of a single compound changes the development of a pelagic community.

KEY WORDS: Marine plankton food web · Function and structure · Direct and indirect effects · Pyrene · PAH · Mesocosm

INTRODUCTION

Polyaromatic hydrocarbons (PAHs) and their metabolites are some of the most toxic compounds found in the marine environment, and have carcinogenic and mutagenic capabilities (Shaw & Connell 1994, Yu 2002). PAHs in the marine environment originate from diffuse sources, such as atmospheric deposition of combusted fossil fuels, wood, etc., and point sources, such as oil spills in connection with transport and storage or off-shore oil drilling (Menzie et al. 1992, Latimer & Zheng 2003). Pyrene is a suitable model compound in effect studies of PAHs as it is one of the dominant PAHs found in the environment (Verschueren 1983, Prahl et al. 1984) and is used as an indicator of previous oil spills and PAH contamination in marine sediments (Rudnick & Chen 1998, Pastor et al. 2001, Khan & Islam 2005).

PAHs are believed to rapidly transfer into the sediments after introduction to the marine environment (Den Besten et al. 2003). This is especially true for high-molecular-weight PAHs, but also for other PAHs, such as pyrene, since they all have a high affinity to particulate matter. Studies on the biological effects of PAHs have therefore been focused on the benthic environment (Carman et al. 1997, Lotufo 1997, Selck et al. 2003). The few reported measurements of environmental concentrations of pyrene in the pelagic zone range from <0.04 to 5.45 nmol l^{-1} pyrene (Dachs et al. 1997, Law et al. 1997, Rudnick & Chen 1998, Witt 2002, Latimer & Zheng 2003). However, there is evidence of

*Email: moh@dmu.dk

pyrene persisting in the water column for longer periods of time (Yamada et al. 2003), and environmental-fate modelling of PAHs estimates steady-state concentrations from diffuse pollution of up to 5 nmol l^{-1} pyrene in the water column (Khan & Islam 2005). The modelling study concluded that oil spills from point sources, which would lead to higher concentrations than the steady-state concentration, may severely impact planktonic communities and indirectly have implications for the marine food web; therefore, further studies on the impact of PAHs are required.

So far, few studies have been done on marine planktonic ecosystem responses to PAHs. This study investigates if a natural marine plankton system is affected by a single addition of pyrene, where the nominal concentrations of pyrene in this study ranged from 2.5 to 250 nmol l^{-1}. The lower concentration represents the higher limit in the provisional ecotoxicological assessment criteria (0.5 µg l^{-1}, ~2.5 nmol l^{-1}) suggested by the Oslo and Paris Commission (Bignert et al. 2004), as well as the estimated steady-state concentration in the modelling study (Khan & Islam 2005). The highest concentration is below the estimated EC_{50}-value for the marine copepod *Oithona davisae* (Barata et al. 2005), one of the few reported marine copepod single-species tests with pyrene, and well below 300 µg l^{-1} (~1350 nM), which is the criterion set by the USA for pyrene in seawater (Buchman 1999).

The planktonic system in the present study included communities from the 3 trophic levels of phytoplankton, zooplankton and bacteria, representing primary and secondary producers and degraders. Structure and function of bacterial and phytoplankton communities were analysed, as was the function of zooplankton communities, through a series of variables described in the 'Materials and methods' section. Such a detailed food web approach allows analysis of both direct and indirect effects (Preston 2002, Fleeger et al. 2003). Direct effects are defined as those in which changes are related to the direct impact of the toxicant in a concentration-dependent manner. Indirect effects are those where changes at one trophic level, caused by direct effects, have an impact at another trophic level. For example, if a phytoplankton community is reduced due to the direct effects of pyrene, then indirectly its predators (i.e. zooplankton) will be affected due to the reduced food supply, although the zooplankton itself might not be directly affected by pyrene. Assessment of the effects on individual trophic levels may lead to a conclusion of no effects, especially when working with natural systems displaying high variability. But, as subtle changes at one trophic level can have an impact on other levels, the conclusion of no effects may be wrong. Therefore, a measure of the integrated functional response of all trophic levels through a multivariate analysis can reveal subtle effects on many variables, indicating that the impacted system has undergone a different development pattern in time compared to the control system (Dahllöf et al. 1999).

MATERIALS AND METHODS

Set-up. The mesocosm experiment was carried out in Isefjord, Denmark (average depth 5 to 7 m), for 12 d in June 2004. Twelve clear polyethylene cylindrical enclosures (2.5 m deep, 1.25 m in diameter, volume approx. 3 m^3) were filled with unfiltered ambient fjord water and attached to a pontoon bridge in the fjord 200 m from the shore at a depth of 4 m. Temperature in the bags varied between 10 and 15°C, whereas the salinity was constant at 16 ppt. After 2 d of acclimatisation, nominal concentrations of 0 (solvent only), 2.5, 25 and 250 nmol l^{-1} pyrene were added to the bags (n = 3). Pyrene dissolved in 100 ml acetonitrile (Merck) was slowly added (approx. 2 min) under intense stirring with a steel piston at night, to avoid the phototoxic effects of pyrene. The solvent concentration did not exceed 3 µg l^{-1} in any of the bags. There was no further deliberate mixing of the bags during the experiment.

Sampling. All variables were measured prior to the addition of pyrene (Day 0) and on each day for the next 5 consecutive days. After Day 5, samples were taken every second day until the end of the experiment on Day 12.

Depth-integrated water samples were taken (2 × 8 l bag^{-1}) with a 2.0 m long PVC tube (diameter: 7 cm) in the morning. In total 144 l (~5% of the total bag volume) of water was sampled from each mesocosm bag during the whole experiment. Sampled water was gently filtered through a 45 µm sieve to collect larger zooplankton, which were used for analyses of grazing potential. From the filtered water, subsamples of 5 l were immediately taken ashore in plastic containers for all other analyses, and the remaining 11 l was discarded.

Pyrene analysis. Concentrations of pyrene in the water column were measured immediately after addition, 1 h later and at 24 h intervals until 96 h (Day 4) of the experiment. Water from 0.5 m depth was collected and warmed to room temperature. Fluorescence excitation–emission matrices (EEMs) were measured (Varian Eclipse fluorescence spectrophotometer) according to Stedmon et al. (2003), without pre-concentration of the samples. Pyrene was quantified by a PARAFAC analysis performed in MATLAB using 'The N-way Toolbox for MATLAB Version 2.11' (Andersson & Bro 2000). PARAFAC is a decomposition method, comparable to principal components analysis (PCA) and enables a mathematical kind of chromatography

on mixtures to identify and chemically quantify analytes (Bro 1997). The model was run with 3 components without any constraints. Sixteen samples with known concentrations of pyrene were used for second-order calibration. The model had an explained variance of 99.8%, where the third component described the pyrene concentration ($r^2 = 0.9996$). The detection limit of the assay was 5 nM, and the relative standard deviation at 10 nmol l^{-1} was 8.8%.

Nutrients. From each bag 30 ml of water was kept frozen until analysis of Si, PO_4, NH_3 and NO_3 on a Skalar (Breda, Netherlands) autoanalyser according to Andersen et al. (2004).

Phytoplankton community variables. Phytoplankton biomass was determined as chlorophyll *a*. Samples of 50 ml were vacuum-filtered through a GF/F filter, extracted in 5 ml 96% ethanol for 24 h and thereafter fluorometrically analysed (10-AU Turner fluorometer).

Pigment analysis. A total of 500 to 750 ml of water was gently filtered through 25 mm Advantec GF 75 glass fibre filters (Toyo Roshi Kaisha) and stored in liquid nitrogen. Upon analysis, filters were transferred to 2.5 ml methanol, sonicated for 30 s and filtered (0.2 µm). Of this extract, 1 ml was mixed with 250 µl water immediately prior to HPLC (high-performance liquid chromatography) analysis (Shimazu LC 10A system) on a Supelcosil C18 column (250 × 4.6 mm, 5 µm) according to Wright et al. (1991). Pigments were identified by retention times and absorption spectra identical to those of authentic standards, and were quantified against standards purchased from DHI Water & Environment. Algal class abundances were estimated from pigment concentrations and ratios (Mackey et al. 1996). In all samples a final matrix of the phytoplankton composition was obtained and expressed as group-specific contributions to total chlorophyll *a* (chl *a*). As input matrices we used pigment concentrations in the mesocosm enclosures and pigment ratios of cultures representative of Danish waters (Henriksen et al. 2002).

Phytoplankton activity was measured through $H^{14}CO_3^-$ incorporation as an estimate of primary production. To 4 replicates of 10 ml from each mesocosm 2 µCi $H^{14}CO_3$ (1 mCi ml^{-1}, ^{14}C Agency) was added, and samples were allowed to incubate for 2 h in 20 ml glass vials (BN Instruments) under cool white light (2× Pope FTD 18W/33). Dark samples were run in parallel with each experiment to test for abiotic ^{14}C adhesion and bacterial incorporation of ^{14}C. Incubation was ended with the addition of 200 µl 1 M HCl to remove non-incorporated ^{14}C from the samples. After 24 h, 10 ml of Insta-gel Plus (Perkin Elmer Life and Analytical Sciences, Inc.) was added, and the samples were stored 1 to 7 d at room temperature until analysis in a Beckman LS 1801 scintillation counter. The total incorporation was measured as the amount of radiolabelled carbon, and all the samples were corrected for the amount of abiotic ^{14}C in the dark sample. Mean values of the triplicate control mesocosm bags were set to 100% activity.

Bacterial community variables. Abundance of bacteria was determined on a flow cytometer (Becton Dickinson, FACSCalibur). To a 500 µl sample diluted in 500 µl of 0.2 µm filtered seawater, 10 µl Cybergreen was added, the experiment was run for 1 min at a flow rate of 28 µl min^{-1}, and the data were analysed using Cellquest software (BD Biosciences).

Bacterial community activity was determined as protein synthesis (^{14}C-labelled leucine incorporation) in a modified version of Smith & Azam's (1992) centrifugation (^{14}C)-leucine-incorporation method. Four replicates (1 ml) of each mesocosm were incubated for 1 h with [^{14}C]-1-leucine (295 mCi $mmol^{-1}$, Amersham, Life Science) in 2 ml Eppendorf tubes (final leucine concentration: 190 nmol l^{-1}). Trichloroacetic acid (TCA) was added to blind samples (100 µl 100% TCA ml^{-1}). Incubation was stopped with 100 µl cold 100% TCA, and 15 µl of skim milk were added to enhance protein precipitation. Samples were stored at 5°C until centrifugation, which comprised 2 × 10 min (13 000 × *g*, 4°C); the supernatant was discarded and the pellet washed with 1 ml ice cold 100% TCA in between. Finally, the supernatant was removed, 1 ml of Ecoscint A (National Diagnostics) was added, and the tubes were vortexed. After 24 h of storage at room temperature (18°C), the samples were radioassayed in a Beckman LS 1801 scintillation counter. The mean value of the control mesocosm bags was set to 100% activity.

Zooplankton activity. The grazing activity of the zooplankton community was analysed during the experimental period by measuring ^{14}C labelled prey uptake according to Hjorth et al. (2006).

Statistical analysis. Differences between means of control and exposed communities (n = 3) were tested in an ANOVA for repeated measurements with time as the repeated factor, using SAS software (Version 8.02). Homogeneity of variances was tested with Cochran's test, and if data were not homogeneous they were $\log_{10}$ transformed. Dunnett's 2-tailed *t*-test was used as a post hoc test for comparisons between exposed and control communities, and Tukey's test was used when comparing between all communities (Smith & Mercante 1989). Differences between the integrated community function of the exposed and control communities were analysed by comparing the functional responses in a multivariate analysis using Primer Version 5.0 (Plymouth Routines In Multivariate Ecological Research, PRIMER-E), where similarities were measured as normalised Euclidean distances, followed by ANOVA for repeated measurements. Differences in

phytoplankton community composition, as assessed with pigment analysis, were evaluated using a Bray–Curtis similarity index deduced from normalised means (Bray & Curtis 1957, Clarke & Warwick 1994), followed by ANOVA for repeated measurements.

The percent contribution of phytoplankton species to differences in composition between the treatments and a PCA of the combined functional variables were obtained through the SIMPER and PCA analysis in the PRIMER-E software package. In all statistical analyses changes were defined to be significant at $p < 0.05$ and marginally significant at $0.05 < p < 0.1$.

RESULTS

Pyrene concentrations

Pyrene concentrations decreased below the detection limit after 48 h in all treatments (Fig. 1). Assuming the degradation of pyrene follows first-order reaction kinetics, half-times were 12 and 18 h for 25 and 250 nmol l^{-1} exposed communities during the first 24 h after addition. Within the next 24 h half-times were 7 and 8 h in the same communities. In the control and 2.5 nmol l^{-1} treated mesocosms, pyrene concentrations were never above the detection limit. Reductions in pyrene concentrations over time could be due to both degradation and adsorption to mesocosm walls and particles, but these factors were not further investigated. However, as concentrations measured immediately after the additions were as expected and half-times were longer during the first part of the experiment, it is unlikely that adsorption to mesocosm walls removed pyrene from the water instantly.

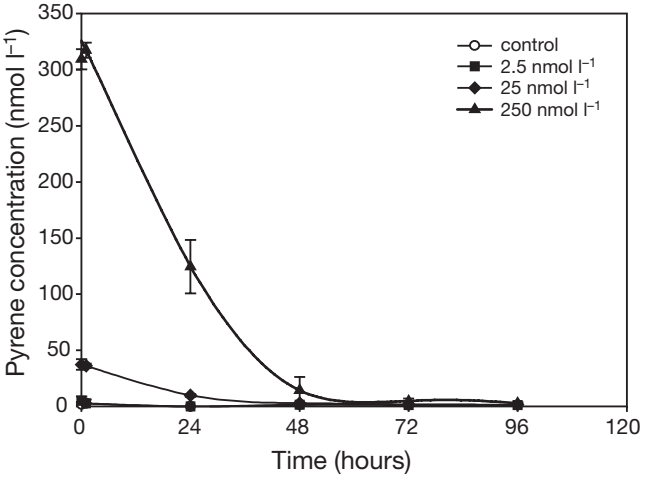

Fig. 1. Concentration of pyrene in the mesocosms after addition of nominal concentrations of 0, 2.5, 25 and 250 nmol l^{-1} on Day 0 (means ± 95% confidence intervals, n = 3)

Nutrients

There were no significant differences in nutrient concentrations between the treatments, mainly due to the concentrations around the detection limit. In all mesocosm bags DIN (dissolved inorganic nitrogen, the sum of NO_3, NH_3 and NO_2) concentrations were <2 µmol l^{-1} during the whole experiment. In the 2.5 and 25 nmol l^{-1} communities, there was indication of the release of DIN during Days 1 and 2, causing a rise in the concentrations of DIN to 0.8 ± 0.3 µmol l^{-1} (SD, n = 3), compared to 0.45 ± 0.15 µmol l^{-1} (SD, n = 3) in the control community. On Day 3, the DIN concentration in the 2.5 nmol l^{-1} community decreased and was not different from the concentrations in the control community during the rest of the experiment (range 0.4 to 0.6 µmol l^{-1}). In the 25 and 250 nmol l^{-1} communities, a release of DIN occurred during the experiment, with elevated concentrations of 1.5 µmol l^{-1} DIN on Day 12 and 1.27 µmol l^{-1} DIN on Day 5, respectively. Silicate concentrations increased in the control community towards the end of the experiment, with concentrations of 1.9 ± 1.1 µmol l^{-1} (SD, n = 3) compared to 1.1 ± 0.79 µmol l^{-1} (SD, n = 3) in the 2.5 nmol l^{-1} community and 0.6 ± 0.51 µmol l^{-1} (SD, n = 3) in the 25 and 250 nmol l^{-1} communities. Concentrations of PO_4 did not differ among treatments, with low levels of PO_4 (<0.05 µmol l^{-1}) during the whole experiment.

Phytoplankton community response

Chlorophyll *a*

Chl *a* concentrations decreased on Day 1 in a concentration-dependent response, whereby the the concentration in the 250 nmol l^{-1} community was significantly lower than that in the control community ($p < 0.05$) until Day 5 (Fig. 2). On Day 8 there was no significant difference between exposed and control communities. There was, however, a trend of higher chl *a* concentrations in communities exposed to 250 and 25 nmol l^{-1} pyrene towards the end. Chl *a* concentrations in the 2.5 nmol l^{-1} exposed community developed in a similar way to those in the control community.

Phytoplankton activity

There were no significant differences (ANOVA for repeated measurements) in total phytoplankton activity between the control community and the pyrene-exposed communities during the experiment (Fig. 3a). The data indicated a 25% reduction in activity in the 25 nmol l^{-1} exposed community compared to the control

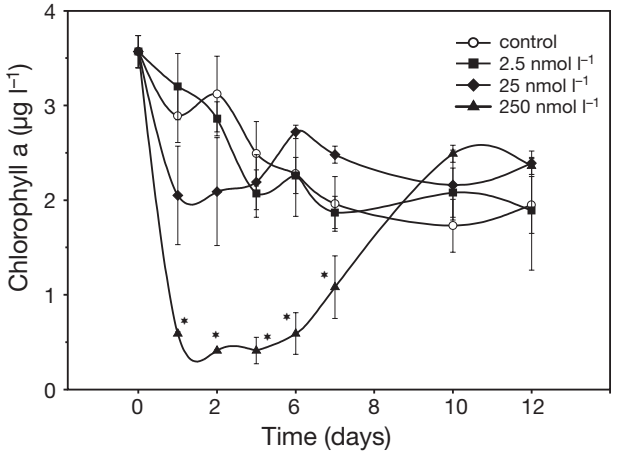

Fig. 2. Changes in chlorophyll *a* concentrations (means ± SD, n = 3) in control, 2.5, 25 and 250 nmol l^{-1} pyrene exposed communities during the 12 d mesocosm experiment. Asterisks mark communities significantly different from control communities

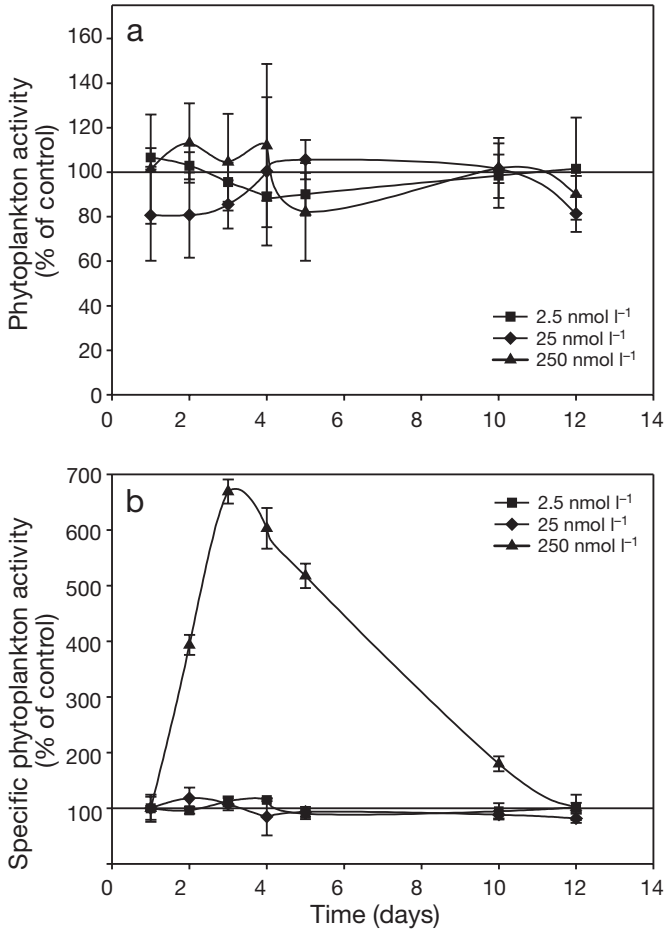

Fig. 3. Phytoplankton community response to pyrene stress as percentage of control (means ± 95% confidence intervals, n = 3): (a) total phytoplankton activity and (b) specific phytoplankton activity

community during the first 2 d after pyrene exposure, although this difference was not statistically significant. Similarly, there were indications of a 13% increase in total phytoplankton activity in the 250 nmol l^{-1} exposed community compared to the control community during the first 4 d.

Specific phytoplankton activity calculated as the total production divided by chl *a* concentration increased significantly ($p < 0.05$) in the 250 nmol l^{-1} exposed community to 670% of the controls on Day 2 (Fig. 3b). Subsequently, the specific activity fell to 200% of the control community on Day 5 and did not reach the level of the control community until Day 10 of the experiment. There were no differences in specific activity of phytoplankton communities exposed to 2.5 and 25 nmol l^{-1} pyrene compared to the control community.

Phytoplankton community composition

Not only did total pigment concentration decline after addition of pyrene, but the composition of dominant pigments deviated significantly ($p < 0.05$, Dunnett) between control and pyrene-exposed communities at almost all time points (Fig. 4). Only the 2.5 nmol l^{-1} community on Days 2 and 3 was not significantly different from the control community. Throughout the experiment 4 to 5 phytoplankton groups made up >75% of the community composition (Table 1). The main difference between the 2.5 nmol l^{-1} and control communities was a reduction in diatoms (Bacillariophyceae) and cryptophytes, and a decrease in prymnesiophytes on Day 1 (Table 1), which remained until Day 12, except for the contribution of prymnesiophytes. Prymnesiophytes were divided into 2 groups according to presence of the pigments 19′-butanoyloxyfucoxanthin

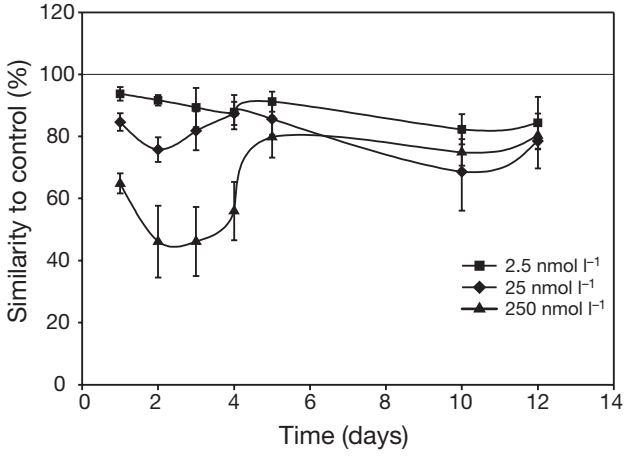

Fig. 4. Phytoplankton community composition as percentage similarity to control (means ± SD, n = 9)

Table 1. Phytoplankton groups that contributed >75% of the dissimilarity between control and pyrene-exposed communities on Days 1, 4 and 12. Data are percent contribution to overall dissimilarity; arrows indicate a reduction or an increase in the respective groups compared to the average contribution of the group in the control. Prymnesiophyceae 1 are larger cells with 19′-hex pigment and Prymnesiophyceae 2 have both 19′-hex and 19′-but pigments and are ca. 2 µm in size

Pyrene conc.	Day 1		Day 4		Day 12	
	Phytoplankton group	%	Phytoplankton group	%	Phytoplankton group	%
250 nM	Prymnesiophyceae 1	24↓	Nostocophyceae	34↑	Bacillariophyceae	24↓
	Bacillariophyceae	19↑	Bacillariophyceae	15↓	Euglenophyceae	21↑
	Cryptophyceae	18↓	Chlorophyceae	15↑	Prasinophyceae 1	13↑
	Chlorophyceae	16↑	Prymnesiophyceae 1	12↓	Prymnesiophyceae 2	11↓
	Sum	77		76		79
25 nM	Cryptophyceae	42↓	Cryptophyceae	25↓	Bacillariophyceae	21↓
	Prymnesiophyceae 1	13↑	Prasinophyceae 2	18↑	Cryptophyceae	19↓
	Prasinophyceae 2	12↑	Prymnesiophyceae 1	15↓	Prasinophyceae 1	13↑
	Bacillariophyceae	7↓	Bacillariophyceae	13↑	Prasinophyceae 2	13↑
	Prymnesiophyceae 2	7↑	Prasinophyceae 1	8↓	Euglenophyceae	12↑
	Sum	81		79		78
2.5 nM	Prymnesiophyceae 1	26↓	Cryptophyceae	30↓	Cryptophyceae	26↓
	Bacillariophyceae	22↓	Bacillariophyceae	19↑	Bacillariophyceae	21↓
	Cryptophyceae	16↓	Prymnesiophyceae 1	18↑	Prasinophyceae 1	12↑
	Chlorophyceae	14↑	Chlorophyceae	11↑	Prymnesiophyceae 2	12↓
			Prasinophyceae 1	11↓	Dinophyceae	8↓
	Sum	78		89		79

(19′-but) and 19′-hexanoyloxyfucoxanthin (19′-hex). Prymnesiophyceae 2 are smaller algae (ca. 2 µm) containing 19′-but and 19′-hex. Prymnesiophyceae 1 are larger sized algae with 19′-hex only. Cryptophytes were also the most affected group in the 25 nmol l^{-1} community on Days 1 and 4, but with a stronger reduction than in the 2.5 nmol l^{-1} community. On Day 4, prasinophytes 2 (prasinophytes without the carotenoid prasinoxanthin) increased, and, on Day 12, the contribution of diatoms had decreased compared to the control community. The difference between the control community and the 250 nmol l^{-1} community on Day 1 was due to a smaller contribution of prymnesiophytes and cryptophytes and a higher contribution of diatoms. On Day 4, the difference between the control community composition and the 250 nmol l^{-1} community was still large, but due to increases in blue-green (Nostophyceae) and green algae (Chlorophyceae). On the last day of the experiment, the major difference between the control and 250 nmol l^{-1} communities was an increased contribution of euglenophytes (21% contribution).

Bacterial community response

Bacterial abundance increased during the first 2 d after pyrene exposure in all mesocosm communities including the control community. Abundance in control and 2.5 nmol l^{-1} communities continued to increase until Day 4. On Day 3, the abundance in the 25 and 250 nmol l^{-1} communities decreased significantly ($p < 0.05$) compared to that in the control community (Fig. 5a). The 25 nmol l^{-1} community had the lowest abundance on Day 3, which was significantly different from the results for both the control and 2.5 nmol l^{-1} communities. One day later, on Day 4, bacterial abundance in the 250 nmol l^{-1} community also decreased to significantly lower levels than that in control and 2.5 nmol l^{-1} communities. Both 25 and 250 nmol l^{-1} communities showed lower values than the control and 2.5 nmol l^{-1} communities on Day 5, but only the 250 nmol l^{-1} community had a significantly lower abundance during the rest of the experiment. Bacterial activity (protein synthesis) in the 250 nmol l^{-1} community was significantly higher than in all other treatments on Day 1 (Fig. 5b), and marginally higher than in the control and 2.5 nmol l^{-1} communities ($p < 0.1$) on Day 2. For the remaining 8 d of the experiment, there were no significant differences in total activity among the bacterial communities in any treatments, except on Day 4 when the 250 nmol l^{-1} community was significantly different from the 2.5 nmol l^{-1} community ($p < 0.05$), and marginally different from the control and 25 nmol l^{-1} communities ($p < 0.1$).

Specific activity of the bacterial communities (activity per bacterial cell) in the 2.5 nmol l^{-1} community was not significantly different from that of the control during the course of the experiment (Fig. 5c). The 25 nmol l^{-1} community showed increased specific

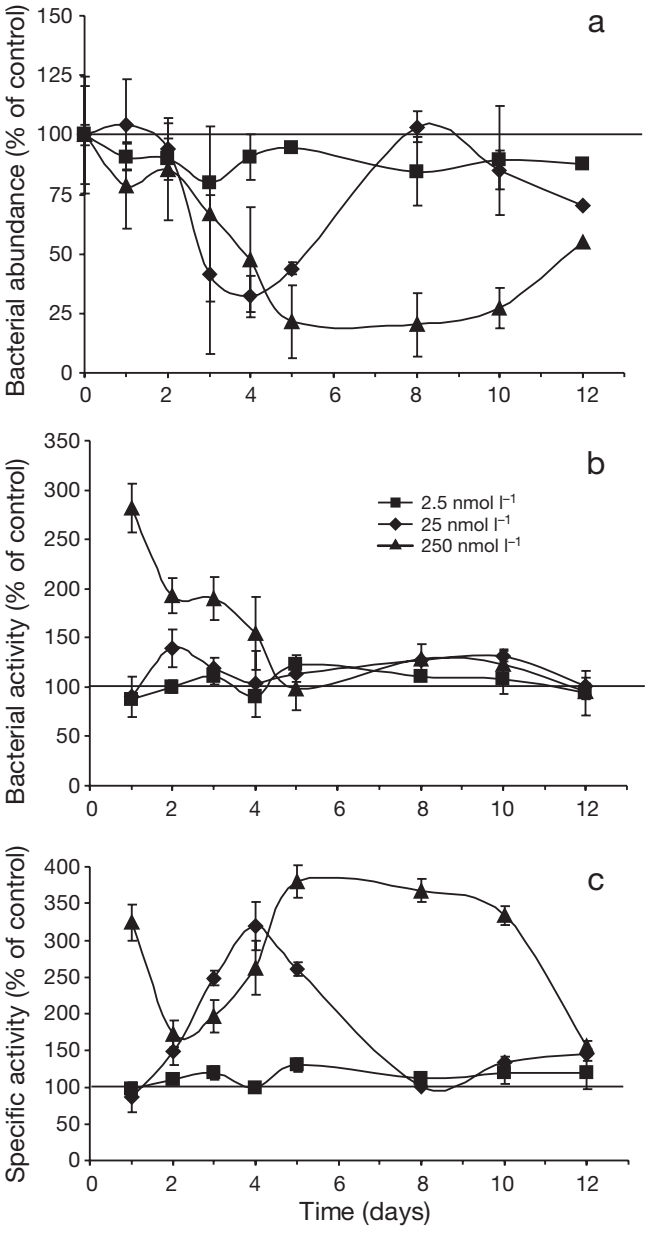

Fig. 5. Bacterial community response to pyrene stress as percentage of control (means ± 95% confidence intervals, n = 3): (a) bacterial abundance, (b) total bacterial activity and (c) specific bacterial activity

activity, reaching 319% of the activity of the control community on Day 4, and, 1 d later, the specific activity in the 250 nmol l^{-1} community increased to a maximum of 380% compared to that of the control community. The high specific activity in the 250 nmol l^{-1} community was maintained until Day 8 for the 250 nmol l^{-1} community. In the 25 nmol l^{-1} community, the specific activity declined as soon as on Day 5 to levels similar to those in the control. The decreases in specific activity for the 25 and 250 nmol l^{-1} communities on Days 5 and 10, respectively, correlated with the decreases in bacterial cell abundances at the same time points.

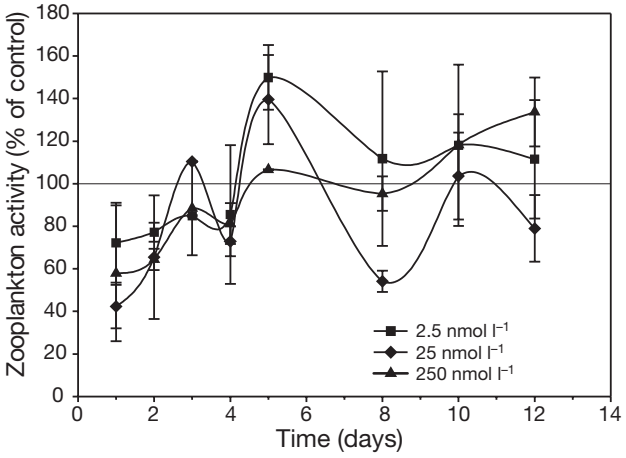

Fig. 6. Zooplankton community response to pyrene stress. Grazing activity is shown as percentage of control (means ± 95% confidence intervals, n = 3)

Zooplankton community response

Zooplankton community grazing was highly variable throughout the experimental period, but with indication of direct effects from Days 1 to 3, where all exposed communities had lower activities compared to the control community (Fig. 6). However, only the values in the 25 nmol l^{-1} community on Day 1 were significantly lower than those in the control community (Dunnett's test, $p < 0.05$). From Day 5 for the rest of the experiment, the mean grazing activity of the 2.5 nmol l^{-1} community showed a tendency towards a positive response compared to the control community, as did the 250 nmol l^{-1} community from Day 8 onwards.

Integrated community function

Integrating the functional variables for the bacterial, phytoplankton and zooplankton communities in a multivariate analysis provided a measure of total pelagic system function relative to each treatment (Fig. 7). The integrated community function was significantly different from the control (Dunnett's test, $p < 0.05$) in the 250 nmol l^{-1} community from the beginning of the experiment (Day 1) until Day 3. On Days 2 and 3 the other exposed communities were also significantly different from the control, whereas on Day 5 the integrated community function of the 2.5 and 25 nmol l^{-1} exposed communities were the ones to be significantly different from the control community.

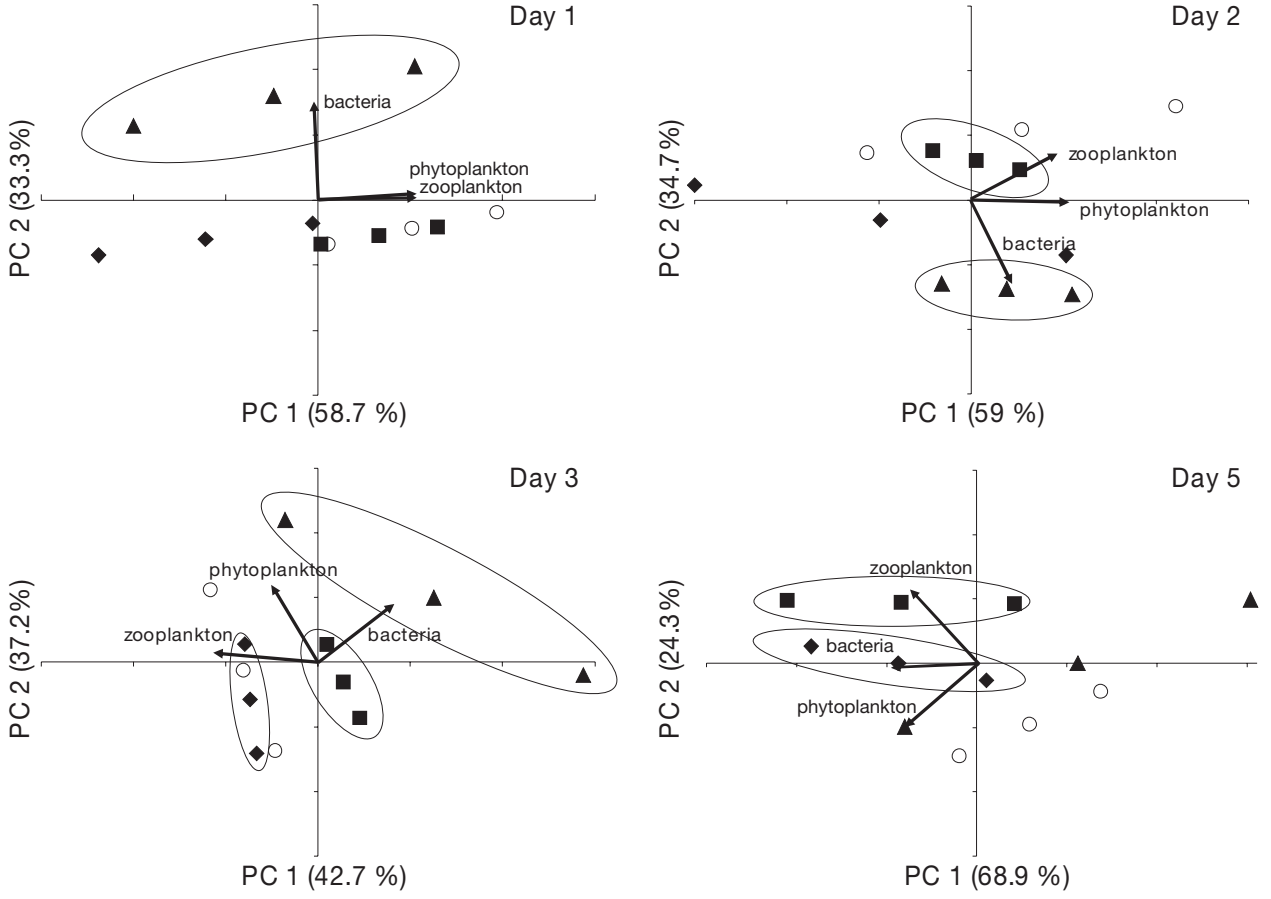

Fig. 7. Plots of principal components (PC) 1 and 2 of the integrated functional response from the days when there were significant differences (encircled symbols). The groups of data points are from the 4 levels of pyrene exposure (O: control; ■: 2.5 nmol l^{-1}; ◆: 25 nmol l^{-1}; ▲: 250 nmol l^{-1}); vectors indicate the correlation scores (scaled × 1.5 to fit) of the functional variables

DISCUSSION

The integrated planktonic community function changed after a single addition of pyrene and remained significantly different from the unexposed control system during the first 3 d of the experiment for 250 nmol l^{-1} pyrene exposures and during Days 3 to 5 for the lower pyrene exposures. Although the responses of the individual trophic levels in the 2.5 nmol l^{-1} community did not differ significantly from the control, the system function was significantly impacted on several occasions during the first 5 d of the experiment. PCA indicates that the differences are mainly due to changes in bacterial activity during the first 3 d, whereas differences on Day 5 may be attributed to zooplankton and/or phytoplankton activity levels (Fig. 7). Changes in phtyoplankton community structure also indicated an impact at the lowest exposure. Since this impact was significantly different from the natural variation in the control communities and since the exposure consisted of a one-time addition of pyrene around the higher limit of the OSPAR environmental assessment criterion, the results do suggest that pyrene has a stronger influence on marine pelagic communities than previously expected.

The integrated community function included both the direct effects of pyrene and the indirect effects that followed from them. We suggest that initial direct effects on phytoplankton, delayed direct effects on bacteria and indirect effects on all trophic levels caused the observed changes in system function, as discussed below.

Direct effects on Day 1 on phytoplankton occurred in all the exposed communities, when both chl *a* concentrations and community composition data are taken into account. Indirect food web effects caused effects on the functional response of the phytoplankton community later in the experiment, but in different ways for the different pyrene concentrations. The total and specific activity of the phytoplankton communities exposed to 2.5 nmol l^{-1} did not deviate significantly from the control community. However, the increase in DIN on Day 1 suggests that there were more

favourable growth conditions in the 2.5 nmol l^{-1} community compared to the control community. The responses from the 25 and 250 nmol l^{-1} pyrene exposed communities represented 2 different patterns. Total community activity in the 25 nmol l^{-1} exposed community was reduced to 75% of the photosynthetic activity of the control community, but the specific activity did not change compared to the control. This suggests that, although parts of the algal community were affected, as shown in the algal composition data, the remaining algae could not immediately utilise regenerated nutrients. From Day 2, the specific bacterial activity increased, which would have released nutrients and resulted in increased algal biomass and total activity by Day 4. However, the specific algal activity did not increase, and it appears that effects on sensitive species were not severe enough to diminish competition or promote more opportunistic algae. A different developmental pattern was observed in the 250 nmol l^{-1} exposed community; there effects were severe enough to cause a ~400% increase in specific algal activity already on Day 1, but again with no changes in total phytoplankton activity. Effects of pyrene were strong enough to eliminate sensitive phytoplankton groups, leaving space and resources to more tolerant and/or opportunistic groups, which responded with high specific activities. The high specific algal activity corresponded to high specific bacterial activity, which occurred already on Day 1 and which led to a rapid regeneration of nutrients from dead algae. Pigment analyses confirmed such a change in exposed communities; diatoms were left to dominate the 250 nmol l^{-1} community together with green algae, whereas prymnesiophytes and cryptophytes declined compared to the control community. This is in contrast to other findings, in which diatoms were found to be more sensitive to a mixture of hydrocarbon compounds than other algal groups (Nayar et al. 2005, Sargian et al. 2005). The discrepancy may be caused by the more complex contamination used in those studies, or to the 'rough' characterisation of phytoplankton communities by the pigment analyses used in this study. In particular, the group identified as diatoms, characterised by fucoxanthin as the major carotenoid, may contain other phytoplankton groups (e.g. prymnesiophytes or raphidophytes) with similar pigment compositions. Unfortunately, no microscopy data were available to verify the development of the diatom community. Towards the end of the mesocosm experiment, blue-green algae increased in the exposed communities, compared to the control community; this observation is consistent with other studies of PAH effects on phytoplankton (Bastian & Toetz 1985, Nayar et al. 2005), in which cyanobacteria have been reported to be more tolerant to PAH exposure.

Structural variables such as chl *a* and algal community composition, together with specific activity of algae and bacteria, were the most significantly affected endpoints, whereas total activity of algae was the least affected. Functional redundancy, whereby functionally equivalent species take over the role of those affected (Walker 1995), may be the cause of this response. However, we suggest that this was not the case in this system, since the exposed and control communities had different growth conditions and the phytoplankton in the control community was nutrient limited. Potential nutrient limitation in a marine system is predictable from inorganic nutrient concentrations and concentration ratio criteria (Dortch & Whitledge 1992). In accordance with such criteria (DIN < 2 µM, N:P < 15), primary production was nitrogen limited throughout the experiment. Nutrient-enriched dilution experiments conducted with samples from control mesocosm bags confirmed this (P. Henriksen pers. comm.). The exposed communities received regenerated nutrients from the breakdown of phytoplankton affected at the time of exposure, which allowed for higher activity. If the control community had received a similar dose of nutrients, their total activity would also have risen and the difference between control and exposed communities would have been larger than seen in this study. This implies that functional effects in a nutrient-limited system are harder to detect and are indirectly compensated for, although effects on structure might be evident.

The bacterial community also responded differently depending on exposure level and time. Bacterial abundance in 2.5 and 250 nmol l^{-1} communities indicated a decrease on Day 1, but was only followed by high total and specific activities in the 250 nmol l^{-1} community. This suggests that initially a direct effect only occurred in the 250 nmol l^{-1} community, which caused a selection for more tolerant/opportunistic species, due to the high amount of substrate available. From Days 2 to 4, the bacterial abundance was reduced significantly in 25 and 250 nmol l^{-1} communities, followed by high specific activity, indicating a delayed effect of pyrene and a selection for tolerant and/or opportunistic species. Bacterial abundances increased again after Day 4, first in the 25 nmol l^{-1} community and, after Day 10, in the 250 nmol l^{-1} community. Increases in bacterial abundance may reflect the released amounts of substrate (dead phytoplankton indicated by a significant decrease in chl *a* concentrations in the 25 and 250 nmol l^{-1} communities), which also has been reported elsewhere (Cretney et al. 1987, Nayar et al. 2005). Total bacterial activity in the exposed communities was similar to that in the control after Day 4, whereas abundance was low and specific activities were high in the 250 nmol l^{-1} community. The high

specific activity of bacteria in the 250 nmol l^{-1} exposed community did not result in an immediate abundance increase, as in the 25 nmol l^{-1} community, but instead the increase occurred first at the end of the experiment. This could be due to a higher level of protein synthesis increasing the cell biomass rather than abundance, or to a higher level of microzooplankton grazing on bacteria.

Grazing by the zooplankton communities decreased significantly to 42% of the control in the 25 nmol l^{-1} community, which is within the range of effects observed in sediment-associated harpacticoid copepods exposed to PAHs (Lotufo 1997). The reduction in total community grazing in all treatments on Days 1 and 2 can be an indirect effect of the reduced zooplankton abundance due to lower food supplies. Since the food reduction was only for a short time and the assay was performed with unlimited food, it cannot be ruled out that the zooplankton were directly affected.

Other studies showed no effects of PAHs on copepod grazing (Carman et al. 1997, and references herein), which was attributed to tolerance variability, within and among species, or to positive indirect effects from changes in phytoplankton (Carman et al. 2000). These possible causes could also be valid in our study, where no severe effects of pyrene exposure were observed on zooplankton community grazing after Day 2.

CONCLUSIONS

In spite of a general perception that PAHs are rapidly removed from the water phase and mostly affect benthic communities, this study provides comprehensive evidence for the effects of pyrene on natural marine plankton communities. Direct and indirect effects were observed on function and structure of 3 trophic levels of planktonic communities. The direct effects on phytoplankton also resulted in indirect effects on bacteria and zooplankton, which caused a shift in the combined functional response of the whole plankton community in the mesocosm experiment. The shift persisted for the first 4 d of the study. Nutrient and substrate limitations in the control community, in contrast to regenerated nutrient inputs in the exposed communities, resulted in less obvious effects of pyrene on total activities, but significant effects on specific activities, abundance of algae and bacteria and algal community composition.

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