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Title: Effects of pyrene on plankton communities under different nutrient conditions

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Abstract

Biomass and nutrient levels of marine systems may affect responses to pollutant stress. This study compared effects at one concentration of pyrene on two pelagic communities that differed in initial biomass due to previous nutrient availability. A mesocosm design was used with randomised replicate bags (n=3) and four treatments. Nutrient enriched and non-enriched communities were exposed to 50 nM pyrene and control in non-enriched and enriched conditions. The addition of nutrients and following pyrene exposure was administered a second time one week after the first addition and exposure.

A nominal concentration 50 nmol L^{-1} pyrene was added to half of the bags in each nutrient treatment under intense stirring. In all the pyrene treated mesocosms, a direct effect was observed in concentrations of chlorophyll a, regardless of nutrient status. The chlorophyll a concentration in the non-enriched community fell to approximately 20 % of control concentrations on day 1 and did not increase above 5 $\mu g\ L^{-1}$ in the remaining period of the experiment. Primary production was significantly lower than (<50 %) control communities in both enriched and non-enriched communities after the first exposure to pyrene.

There were distinct differences in the effects of pyrene between the enriched and non-enriched communities. The effects of pyrene were stronger in the enriched community in all the investigated trophic groups, but at different time points for each of them. Despite the almost double amount of chlorophyll a in the enriched community, concentrations of chlorophyll a decreased to the same levels as the non-enriched community during the first day after pyrene exposure, indicating a more severe effect and quicker rate of decline in the enriched community than in the non-enriched community

The study confirms the assumption of a stronger and more widespread effect of pyrene on nutrient enriched phytoplankton communities. Under the investigated conditions of biomass and nutrient status, there were no dilution effects of pyrene. Diatoms showed to be the least sensitive algae to pyrene exposure in both non-enriched and enriched communities. Indirect effects on zooplankton caused a lower abundance of zooplankton and thereby differences in grazing pressure, which in turn resulted in differences in phytoplankton responses to pyrene exposure between the enriched and non-enriched communities at the end of the experiment. It is imperative to our understanding of contaminant effects on natural communities, that interactions between trophic levels and in relation to abiotic factors are investigated.

Introduction

Pyrene has earlier been shown to have a detrimental effect on planktonic communities at concentrations close to 3.4 nmol l^{-1}(Hjorth et al., 2006), which is the lower Ecotoxicological Assessment Criteria (EAC) recommended by the Oslo Paris Commission (OSPAR), who defines the lower EAC value as a concentration derived for protection of all marine species from chronic effects, including the most sensitive species.

Direct effects were observed on function and structure of three trophic levels of planktonic communities, algae, zooplankton and bacteria. These direct effects led to indirect effects, causing a shift in the function of the pyrene exposed pelagic community that persisted for at least 12 days. Nutrient and substrate limitations were hypothesised to explain the indistinct pyrene effects that were observed on total community function, as opposed to the more evident effects on specific activities, abundance and algal community composition.

In order to assess the extent to which traits such as biomass and nutritional availability influence the effects of PAHs on pelagic communities, the present study was designed to compare effects at one concentration of pyrene on two pelagic communities that differed in initial biomass due to previous nutrient availability.

The focus on a link between sensitivity towards contaminants and nutrient enrichment is not new (Cloern 2001; Koelmans et al., 2001), but specific knowledge of the nature of such relationships in the marine environment is scarce (Sundbäck et al., unpubl.). The effects of insecticides on freshwater pelagic systems are well known, with patterns of indirect effects on phytoplankton communities resulting from direct effects on zooplankton grazers (Wendt-Rasch et al., 2003; Slijkerman et al., 2004). Our studies (Hjorth et al., 2006) suggest that, in the case of pyrene, phytoplankton is the more susceptible, and declines in biomass and/or activity will lead to indirect effects in the bacterial and zooplankton communities.

More available nutrients in a planktonic system may give rise to a rapid increase in phytoplankton biomass, especially at times where nutrients are growth limiting. This can interact with potential effects from a pollutant by dilution (i.e. more organisms per unit pollutant) and the abundant nutrients may also leave the organisms in a better nutritional shape to withstand stress from pollutants (Koelmans et al., 2001). Both interactions would predict less sensitivity in systems with abundant nutrients. Phytoplankton biomass increases as a consequence of more nutrients may also include a change in composition compared to nutrient limited systems (Interlandi 2002). That may in turn result in changes in the response of the community to contaminant exposure. Such direct and indirect effects on function and structure can cause cascading effects to other trophic levels and affect e.g. zooplankton community structure and composition (Preston 2002; Fleeger et al., 2003). To investigate these questions further, a mesocosm design was used with randomized replicate bags (n=3) with four treatments; exposure to 50 nM pyrene and control in non-enriched and enriched conditions. In order to see if the same pattern of response was repeated when applying the same treatment to a previously exposed community, the addition of nutrients and following pyrene exposure was administered a second time one week after the first addition and exposure.

Materials and Methods

The mesocosm experiment was carried out in the Isefjord, Denmark (average depth 5-7 m), for twelve days in April and May 2005. Twelve clear polyethylene cylindrical enclosures (2.5 m deep, 1.25 m in diameter, volume approx. 3 m^3) were filled with ambient fjord water. The pelagic enclosures were attached to a pontoon bridge in the fjord placed 200 m from the shore at a depth of four meters. Temperature in the bags varied between 10 and 15°C, whereas the salinity was

constant at 16 ppt. Sedimentation was avoided by gently pumping water from the bottom of the mesocosm bags to the surface constantly during the experiment with the aid of air pressure.
After two days of acclimatisation, nutrient enriched mesocosms were spiked with nutrients one day prior to each pyrene addition (i.e. the day before day 0 and on day 6). Inorganic nutrients were added as 0.144 mol ammonium (NH_3Cl), 0.009 mol phosphate (NaH_2PO_4) and 0.288 mol silicate (Na_2SiO_3) to obtain a final N:Si:P of 16:32:1.

A nominal concentration 50 nmol L^{-1} pyrene was added to the relevant bags under intense stirring, twice during the experiment on day 0 and on day 7. The addition took place after nightfall to avoid phototoxic effects of pyrene. Pyrene was dissolved in 100 ml acetonitrile (Merck, Darmstadt, Germany). The solvent concentration did not exceed 3 μg L^{-1} in any of the bags. The exposure concentration was chosen on the basis of previous results from mesocosm experiments on natural plankton communities (Hjorth et al., 2006).

Sampling. All variables were measured prior to the addition of pyrene (day 0) and on each day for the next five consecutive days. After day 5, samples were taken every second day until the end of the experiment on day 12. Nutrient samples were also taken immediately before and after spiking with nutrients one day prior to the first pyrene exposure.

Depth integrated water samples were taken (2 x 8 L per bag) with a 2.0 m long polyvinyl chloride tube (diameter: 7 cm) in the morning. In total 144 L of water were sampled from each mesocosm bag during the whole experiment, which amounts to ~5 % of the total volume. Sampled water was gently filtered through a 45-μm sieve to collect larger zooplankton, which were used for analyses of abundance. From the filtered water, subsamples of 5 L were taken ashore immediately 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, one hour later and at 24 hour intervals until 96 hours (day 4) during the experiment.

Water from 0.5 m depth was collected in brown glass flasks and warmed to room temperature. A Varian Eclipse fluorescence spectrophotometer was used for measuring fluorescence excitation emission matrices (EEMs). Excitation was recorded from 250 to 350 nm and emission was recorded from 300 to 500 nm in increments of 5 and 2 nm, respectively. Monochromator bandpass was 5 nm. The EEMs were corrected for instrument biases and Raman calibrated as described by Stedmon et al. (2003). Rayleigh scatter effects were removed from the data and a “triangle of zeros” was added to the EEMs. Pyrene was quantified by a PARAFAC analysis performed in MATLAB using ”The N-way Toolbox for MATLAB version 2.11” (Andersson and Bro 2000). PARAFAC is a decomposition method, which can be compared with principal components analysis (PCA). It is a unique model, which can decompose data into chemically meaningful loading vectors as estimates of spectra and concentrations. A good PARAFAC model enables a mathematical kind of chromatography on mixtures and thereby can identify and chemically quantify analytes (Bro 1997). The model was run with three components without any constraints. Sixteen samples with known concentration of pyrene were used for second-order calibration. The detection limit of the assay was 5 nmol l^{-1}.

Nutrients. Thirty millilitres of water from each bag were 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

Chlorophyll a. The phytoplankton biomass was determined as the relative concentration of chlorophyll *a* compared to the controls. Fifty ml samples were vacuum-filtered through a GF/F filter, extracted in 5 ml 96 % ethanol for 24 h and thereafter fluorometrically analyzed (10-AU Turner fluorometer, USA).

Phytoplankton activity. The functional endpoint for the phytoplankton was $H^{14}CO_3^-$ incorporation as an estimate of primary production. From each mesocosm subsample, four replicates of 10 ml were taken with a 10 ml automatic kip dispenser pipette (Schott Duran, Mainz Germany) and transferred to 20 ml glass vials (BN Instruments, Denmark). 2 µCi $H^{14}CO_3$ (1 mCi ml^{-1}, ^{14}C Agency, Hørsholm, Denmark) was added to each sample, and the samples were incubated for 2 hours under cool white light (2x Pope FTD 18W/33, Holland). To test for abiotic ^{14}C adhesion and bacterial incorporation of ^{14}C, two dark samples were run in parallel with each experiment. Immediately after the incubation, 200 µl 1 mol L^{-1} HCl was added to remove non-incorporated ^{14}C in the samples. After 24 h, 10 ml of Insta-gel Plus (Perkin Elmer Life and Analytical Sciences, Inc., Boston USA) was added, and the samples were stored for at least 24 h and at most for one week at room temperature. Finally, the samples were radioassayed 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. Specific primary production was estimated as the total incorporation divided by the amount of chlorophyll a.

Pigment analysis. On days 1, 5 and 10, 100 to 250 ml of water were gently filtered through 25 mm Advantec GF 75 glass fibre filters (Toyo Roshi Kaisha, Japan) which were immediately stored in liquid nitrogen. Filters were subsequently transferred to 2.5 ml methanol, sonicated for 30 sec, and filtered (0.2 µm). One ml of this extract was mixed with 250 µl water immediately prior to analysis. HPLC analyses were performed on a Shimadzu LC 10A system with a Supelcosil C18 column (250 x 4.6 mm, 5 µm) using the method of Wright et al. (1991). Pigments were identified by retention times and absorption spectra identical to those of authentic standards, and quantified against standards purchased from DHI Water & Environment, Hørsholm, Denmark. A matrix-factorization program, CHEMTAX, estimated algal class abundances from concentrations of different pigments (Mackey et al. 1996), by using input matrixes of measured pigment concentrations and pigment ratios for the different algal groups. In all samples a final matrix of the phytoplankton composition, expressed as group-specific contributions to total Chlorophyll a was obtained. CHEMTAX is available from D. J. Mackey at CSIRO Division of Oceanography, Hobart, Australia. As input matrixes we used pigment concentrations in the mesocosm enclosures and pigment ratios of cultures representative of Danish waters (Henriksen et al. 2002).

Bacterial activity. Bacterial community activity was monitored as protein synthesis activity by daily measurements of ^{14}C-labelled leucine incorporation. A modified version of Smith and Azam's (1992) centrifugation (^{14}C)-leucine-incorporation method was applied. From each mesocosm four sub samples of 1 ml were transferred to 2 ml Eppendorf tubes and 50 µl of 4 mmol l^{-1} [^{14}C]-1-leucine (295 mCi $mmol^{-1}$, Amersham, Life Science) were added to achieve a final concentration of 190 nmol l^{-1} in each tube. Blind samples were prepared as 100 µl 100% trichloroacetic acid (TCA) in 1 ml water to estimate any abiotic leucine adhesion and contamination of the leucine solution. All samples was incubated for 60 min, stopped with 100 µl cold 100% TCA and 15 µl of skim milk were added to enhance protein precipitation. The samples were stored at 5 °C until centrifugation and washing steps. The samples were centrifuged twice for 10 minutes (13 000 x g, 4 °C) where the supernatant was discarded and the pellet washed with 1 ml ice cold 100% TCA between centrifugation steps. Finally, the supernatant was removed, 1 ml of Ecoscint A (National Diagnostics, Atlanta USA) 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.

Copepod abundance. Zooplankton were collected from 45 µm filters, through which the daily depth integrated water samples were filtered, and the zooplankton were preserved in formalin (0.4 % final conc.) in brown glass flasks and refrigerated at 5 ºC. Samples were analyzed within two months of sampling. Copepods and copepodites were identified to genus or species level, counted and pooled in one group as an estimate of mesozooplankton.

Statistical analysis. For each functional variable, the data set consisted of daily mean values (n=4) from each mesocosm bag. When testing for differences between means of control and exposed communities (n = 3), a two-factor ANOVA with Dunnett's 2-tailed t-test as a post hoc test was performed after testing for homogeneity of variances (Cochran's test) using SAS® software (version V8.02). In all statistical analyses changes were defined to be significant when $p \leq 0.05$ and marginally significant when $0.05 < p \leq 0.1$.

Results

Pyrene. Nominal concentrations of 50 nmol l^{-1} pyrene added to the six pyrene treated mesocosm bags resulted in measured concentrations of 58 ± 18 nmol l^{-1} (95 % confidence limits, n=6) immediately after addition (day 0) as depicted in Figure 1. Two days later (day 2), concentrations were below the detection limit of 5 nmol l^{-1}. On day 7, immediately after the second addition of 50 nmol l^{-1} nominal concentrations, measured concentrations were 79 ± 10 nmol l^{-1} (95 % confidence limits, n=6) and fell below the detection limit after two days, on day 9 (Figure 1).

Development in non-exposed communities

Nutrients. Initial concentrations of nutrients in the mesocosm bags were low (PO_4, Si, NH_3: 0.1-0.3, 0.28-0.3, 0.7-1.0 µM), except for nitrate, which was in the range 18.6-19.2 µM (Figure 2). The first addition of nutrients to six bags, one day before the first pyrene addition, yielded concentration increases of phosphate to 0.5-0.6 µM, of silicate to 4.3-4.6 µM and of ammonia to 7-8 µM. Ammonia concentrations in the enriched control communities decreased to levels of the non-enriched control communities already on day 1, and phosphate concentrations reached the levels of the non-enriched control communities on day 3. On day 7 and 8, there was a release of NH_3 in both the controls. Silicate concentrations in the nutrient-enriched communities stayed above concentrations of the non-enriched control communities until day 5. The second addition of nutrients on day 6 was only reflected in phosphate concentrations, whereas ammonia and silicate concentrations stayed within range of concentrations in the non-enriched control communities.

Chlorophyll a. An increase of chlorophyll a concentration (by a factor of 1.75) was observed in the enriched communities compared to the non-enriched communities on day 0 as an effect of nutrient addition (Figure 3A). The enriched control communities sustained higher chlorophyll a concentrations than the non-enriched control communities throughout the experiment. The non-enriched control communities displayed maximum concentrations on day 1, and the enriched control communities on day 2, after which chlorophyll a concentrations declined to minimum levels of 1.3 and 2.0 μg L^{-1} on day 10 for the non-enriched and enriched communities, respectively.

Phytoplankton activity. Specific primary production of the phytoplankton in the control communities did not deviate significantly between nutrient treatments during the experiment (Figure 3A). From day 0 to day 1, the specific production increased, kept a steady level from day 1-4 after which it declined to a minimum on day 10. Total primary production was higher in the enriched control communities than in the non-enriched but both followed a similar basic pattern, in which an initial increase from day 0 to day 1 was followed by a decrease of total primary production until day 4 (Figure 3B). From day 4 and for the rest of the experiment, total primary production in both the control communities were close to zero.

Phytoplankton structure. Pigment composition analysis revealed a dominance of diatoms, dinoflagellates and cryptophytes in all mesocosm bags during the whole experiment. Ratios between the phytoplankton control communities changed over time but not between nutrient treatments (Table 1). The enriched controls had a 13-14 % dissimilarity in phytoplankton composition compared to the non-enriched controls during the whole of the experiment. The non-enriched control communities consisted approximately of one third of each of the three groups on day 1, which changed to 46 % cryptophytes, 37 % diatoms and only 17 % dinoflagellates on day 10 (Table 1). The enriched control communities consisted of 44 % dinoflagellates, 28 % diatoms and 26 % cryptophytes on day 1, which shifted to a dominance by cryptophytes on day 10, much like the non-enriched control communities.

Bacterial activity. Bacterial activity was not significantly different between the two control communities and showed an initial decrease from day 0 to day 1 followed by a gradual increase during the rest of the experiment.

Copepod abundance. Individuals of *Acartia* spp. and *Centropages hamatus* comprised >90 % of the abundance of adult copepods and copepodites in the two control communities. In both communities the initial numbers were low and increased during the experiment to maximum copepod abundance on day 12 with 57±8 (SD, n=3) and 107±28 (SD, n=3) individuals L^{-1}, in the enriched and non-enriched control communities, respectively (Figure 3C).

Development in exposed communities

Chlorophyll a. In all the pyrene treated mesocosms, a direct effect was observed in concentrations of chlorophyll a, regardless of nutrient status. The chlorophyll a concentration in the non-enriched communities fell to approximately 20 % of control concentrations on day 1 (figure 4A) and did not increase above 5 $\mu g\ L^{-1}$ in the remaining period of the experiment. Chlorophyll a concentrations in the non-enriched communities was significantly different from the control communities until day 5. In the nutrient enriched communities the chlorophyll a concentration were only significantly lower than the control communities on days 1 and 2.

Phytoplankton activity. Primary production was significantly lower than (>50 %) control communities in both enriched and non-enriched communities after the first exposure to pyrene (figure 4B). In the enriched communities, the difference lasted until day 4 and in the non-enriched communities until day 3. The second exposure to pyrene was detectable on day 8 in the non-enriched communities which was significantly lower than the control communities. In the enriched communities, there was a significantly higher production on day 10 than in the control communities. The specific primary production increased above control levels immediately after pyrene exposure on day 1 in the non-enriched communities and reached a maximum of 263 % of the specific primary production in the control communities on day 2 (Figure 4C). The enriched communities also reached a maximum (382 %) on day 2, and the specific primary production in both communities decreased hereafter to control levels on day 4, where it stayed for the remaining period of the experiment.

Phytoplankton structure. The total pigment concentrations showed clear evidence of direct effects of pyrene (Figure 5). Table 2 lists the percentage each algal group contributes to the differences between the pyrene exposed communities and the control communities in the non-enriched and enriched communities. The total average dissimilarity between the non-enriched control communities and the pyrene exposed communities is 37.9 % on day 1 and is significantly different when tested in ANOSIM and stems from diatoms (50 %) and cryptophytes (30.4 %), which increased and decreased, respectively in the pyrene exposed communities compared to the control communities. The dinoflagellates contributed 18.5 % to the dissimilarity, also because their abundance decreased in the pyrene-exposed communities. There were no major differences (14.2 % dissimilarity) between the pyrene-exposed communities and the control communities on day 10, where both were dominated by cryptophytes and diatoms. In the enriched communities, the dissimilarity between the control communities and the exposed persisted throughout the experiment from a dissimilarity of 25 % on day 1 to a 37.2 % dissimilarity on day 10 (table 2). Immediately after the first exposure to pyrene on day 1, the response was similar to the non-enriched communities with increasing diatoms and decreasing cryptophytes and dinoflagellates in the exposed communities compared to the control communities. This pattern was also present on day 10, although with a more equal contribution of all three algal groups to the overall dissimilarity, in contrast to day 1 where the main difference between treatments was due to large increase in diatom abundance in the exposed communities.

Bacterial activity. In the enriched communities, bacterial incorporation of leucine was significantly higher than the control in the first 3 days after the first exposure of pyrene and again on the 2 days following the second exposure to pyrene (days 8-9)(figure 4D). In the non-enriched communities, only the first exposure of pyrene was detectable in the bacterial activity, which was significantly higher than the control on day 2 and marginally higher on day 3.

Copepod abundance. The mesozooplankton communities in the pyrene exposed communities were, as the control communities, dominated by *Acartia* spp. and *Centropages hamatus*. Abundance of copepods and copepodites in the pyrene exposed communities generally showed a decrease during the course of the experiment compared to the control communities, however the decrease was not statistically significant (Figure 5). There was a trend to a more detrimental effect in the non-enriched communities with greater reductions in abundance compared to the control communities (48 % on day 8) until after day 8, when copepod abundance in the enriched communities started to decrease compared to the control communities.

Discussion

Nutrient enrichment did affect the plankton communities and differentiated nutrient enriched from non-enriched ones. Nutrient additions increased biomass of phytoplankton and total primary production compared to non-enriched communities. Bacterial production was unaffected by the additions of nutrients but increased during the time course of the study in parallel with the decline observed in algal biomass in both enriched and non-enriched communities. Copepod abundance increased over time more in the enriched communities than in the non-enriched communities. These results demonstrate that pyrene stress affects functional and structural variables at several trophic levels in marine plankton, in a similar way as our previous studies showed (Hjorth et al., 2006).

Control communities. The plankton system was nutrient limited from the beginning of the mesocosm experiment. The nutrient additions resulted in two systems with differences in phytoplankton biomass, but with a similar community composition dominated by cryptophytes, dinoflagellates and diatoms in close ratios. The second addition of nutrients did not result in an increased phytoplankton biomass, as did the first addition, most likely due to increased grazing pressure. The abundance of zooplankton was low in the beginning of the experiment in both control communities but as time progressed, zooplankton abundance increased and as a consequence, phytoplankton biomass started to decline on days 3 and 4. Enriched control communities could not sustain as high copepod abundance as in non-enriched control communities, which can be linked to simultaneous high numbers of microzooplankton observed in the enriched controls, known to predate on zooplankton eggs (Tang K.W., personal communication).

Effects of pyrene. Effects of pyrene were stronger in the nutrient-enriched communities in all the investigated trophic groups, but at different time points for each of them. That is supportive of earlier work and recent observations in natural sediments with different nutrient loadings (Larson et al., 2005). Despite the almost double amount of chlorophyll a in the enriched community, concentrations of chlorophyll a decreased to levels of the non-enriched community during the first day after pyrene exposure, indicating a more severe effect and quicker rate of decline in the enriched community than in the non-enriched community. There were no dilution effects to weaken the effects of pyrene at this particular concentration of pyrene and chlorophyll a. The decline of phytoplankton biomass was reflected in the primary production of the phytoplankton, where total community activity decreased by several orders of magnitude (Figure 3B), and stayed at very low levels for the rest of the experiment.

The rapid decline of chlorophyll a after the first pyrene exposure released substrate which led to a high bacterial activity in the pyrene-exposed communities. Bacterial production reached maximum levels of 175 % and 200 % of the control communities on day 2 (figure 4D), in the non-enriched and enriched communities, respectively and was unaffected by the additions of nutrients. The pattern of increased bacterial production parallel with the decline in algal biomass during the time course of the study in both enriched and non-enriched communities was identical to patterns observed in our previous study of pyrene effects (Hjorth et al., 2006) and of an entirely different pollutant (Hjorth et al., 2005).

There were no direct effects of pyrene on zooplankton communities, but indications of indirect effects as a result of decreasing prey because of phytoplankton decline. There were generally more prey in controls (i.e. more chlorophyll a), and thus higher abundances of zooplankton. There were clear indications of significant changes in phytoplankton composition (Figure 6 & table 2) from the addition of nutrients and pyrene. Diatoms seemed to proliferate at the expense of cryptophytes and dinoflagellates, which were more sensitive to pyrene. In the enriched community that pattern was found again on day 10, whereas the non-enriched community showed opposite patterns except for cryptophytes (table 2). The fact that diatoms were the least sensitive to pyrene is consistent with our previous study (Hjorth et al., 2005) but contrary to other findings (Sargian et al. 2005; Nayar et al. 2005).

The second nutrient addition and subsequent pyrene exposure was only visible in the enriched community as a brief increase in chlorophyll a immediately followed by a decrease caused by pyrene exposure. Primary production increased to 150 % of the control on day 10 probably as a result of the second nutrient addition, but no other effects were detectable. The general low biomass and production levels both in the control and exposed communities lead to a poor detection of effects with the methods used and explains the lack of effects from the second nutrient spiking and pyrene exposure on phytoplankton communities. The mesocosms quickly developed into top-down controlled systems, which became stronger as zooplankton biomass increased and phytoplankton decreased. Since zooplankton seemed to be less affected by pyrene and had grazed phytoplankton down to low levels, this could also explain the lack of effect after the second exposure. An alternative hypothesis for the apparent lack of response to the second pyrene exposure could be that the first addition established selection pressure on the community in favor of tolerant species and/or activities. At the time of the second addition, the sensitive components in the community were already removed and a lower response to the second stress is to be expected.

Zooplankton abundance decreased in the exposed communities compared to the controls on day 12 (figure 6) as a reflection of a direct or delayed effect of the second addition of pyrene on zooplankton. Bacterial communities did not show effects from the second exposure either, which can be attributed to the grazing controlled community structure, in which substrate already was recycled and abundant.

The results confirm the assumption of a stronger and more widespread effect of pyrene on nutrient-enriched phytoplankton communities, in the sense that when nutrient and biomass levels are high not only specific activity, community structure and abundance, but also total community function is affected. Effects of pollutants such as pyrene as determined in more complex test systems indeed can be reproduced and a similar pattern of responses on structural and functional variables can be observed even though the experiments are performed at different times with presumably different composition of the communities at start. Interactions between trophic levels, abiotic factors and pollutants from this mesocosm study might easily have been ignored if an extensive array of endpoints has not been applied. Even so, the framework of this study is still general and can be more detailed in terms of the choice of endpoints and organisms investigated and thereby further enhance our understanding of pollutant stress effects on plankton communities. In studies of pollutant effects at the community level, a combination of structural and functional endpoints of the selected trophic levels must be assessed to gain a proper risk evaluation.

Conclusions

Nutrient enrichment did affect the plankton communities and differentiated nutrient enriched from non-enriched ones. The effects of pyrene were stronger in the enriched community in all the investigated trophic groups. The magnitude of direct and indirect relative responses to pyrene can be modified by altering nutrient status and algal biomass. Under the investigated conditions of biomass and nutrient status, there were no dilution effects of pyrene. Diatoms were found to be the least sensitive algae to pyrene exposure in both non-enriched and enriched communities. Indirect effects on zooplankton reduced grazing pressure, which resulted in different phytoplankton responses to pyrene exposure between the enriched and non-enriched communities at the end of the experiment. Low levels of biomass and activity at the time of the second nutrient and pyrene addition lead to difficulties in determining small changes in responses. It is imperative to our understanding of contaminant effects on natural communities, that interactions between trophic levels and between chemicals and abiotic factors are investigated.

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Figure legends

Figure 1. Concentration of pyrene in the mesocosms from additions of a 50 nmol L^{-1} on day 0 and on day 7. Concentrations are shown as means ± 95 % confidence intervals (*n*=3).

Figure 2. Nutrient concentrations in control communities during the mesocosm experiment. Concentrations are as shown as means ± SD (n=3).

Figure 3. Development of variables in control communities during the mesocosm experiment. (A) chlorophyll a (bars) and phytoplankton specific activity (lines). (B) shows total community activity of bacteria in squares and algae in circles. Development of copepod abundance is shown in (C). Open bars and symbols are non-enriched communities and closed bars and symbols are enriched communities. Data are shown as means ± SD (n=3).

Figure 4. Development of variables in communities exposed to 50 nM pyrene as % of control during the mesocosm experiment. Chlorophyll a (A), phytoplankton specific activity (B), total community activity of phytoplankton (C) and bacterial production (D). Open circles are non-enriched communities and closed circles are enriched communities. Data are shown as means ± SD (n=3).

Figure 5. Development of copepod abundance in communities exposed to 50 nM pyrene as % of control during the mesocosm experiment. Data are shown as means ± SD (n=3).

Figure 6. Composition of phytoplankton communities in exposed and control communities with and without nutrient enrichment.

	day 1	day 10
phytoplankton group	**Non-enriched control**	
Cryptophytes	35.7	46.3
Dinoflagellates	33.7	16.5
Diatoms	29.7	27.2
	Enriched control	
Cryptophytes	25.5	40.1
Dinoflagellates	44.4	21.7
Diatoms	27.8	37.6

Table 1. Pigment composition of control communities on day 1 and 10 as average abundance in % of total pigment.

	day 1	day 10
	Non-enriched	
average dissimilarity	37.9	14.2
% contribution to dissimilarity		
Cryptophytes	↓ 30.4	↓ 30.3
Dinoflagellates	↓ 18.5	↑ 40.2
Diatoms	↑ 50.0	↓ 20.5
	Enriched	
average dissimilarity	25.0	37.2
% contribution to dissimilarity		
Cryptophytes	↓ 18.0	↓ 29.5
Dinoflagellates	↓ 27.5	↓ 29.9
Diatoms	↑ 50.0	↑ 37.2

Table 2. Percentage contribution of phytoplankton groups to the dissimilarity between control and pyrene-exposed communities on day 1 and 10. ↓ indicates a reduction, and ↑ indicates an increase of the respective groups to the average contribution of dissimilarity between control and exposed communities.

Figure 1.

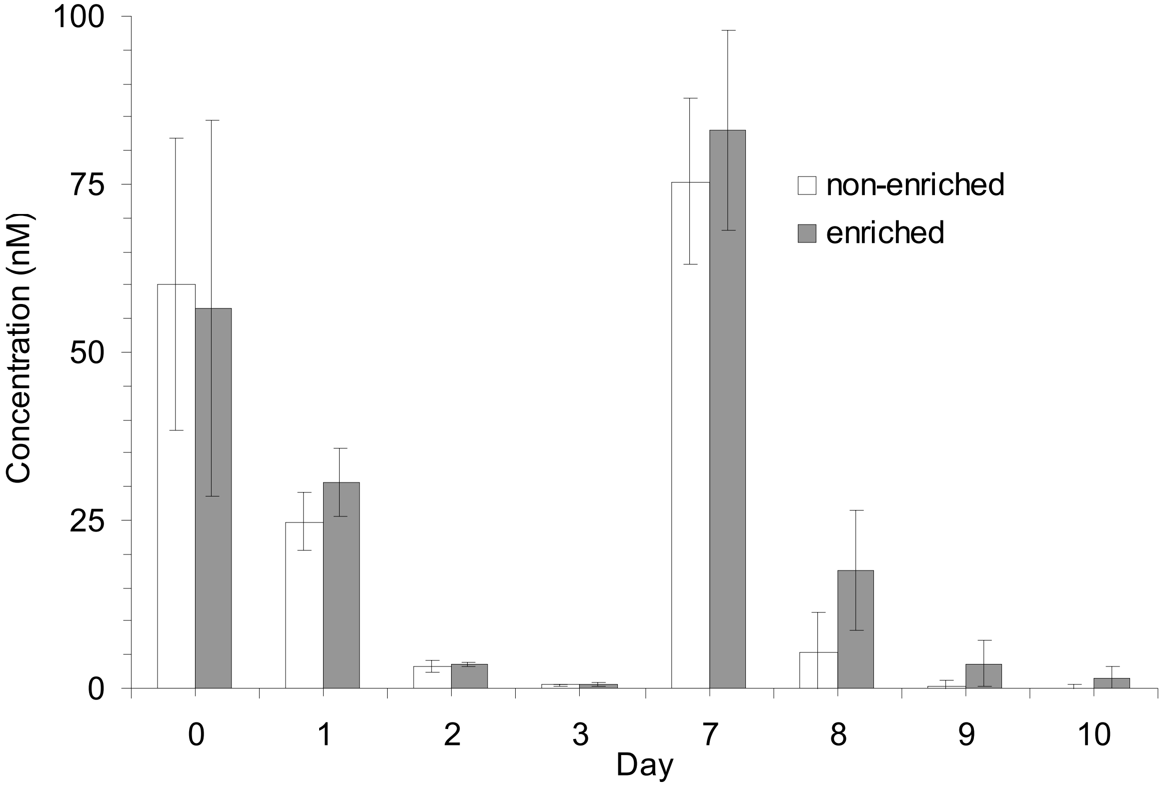
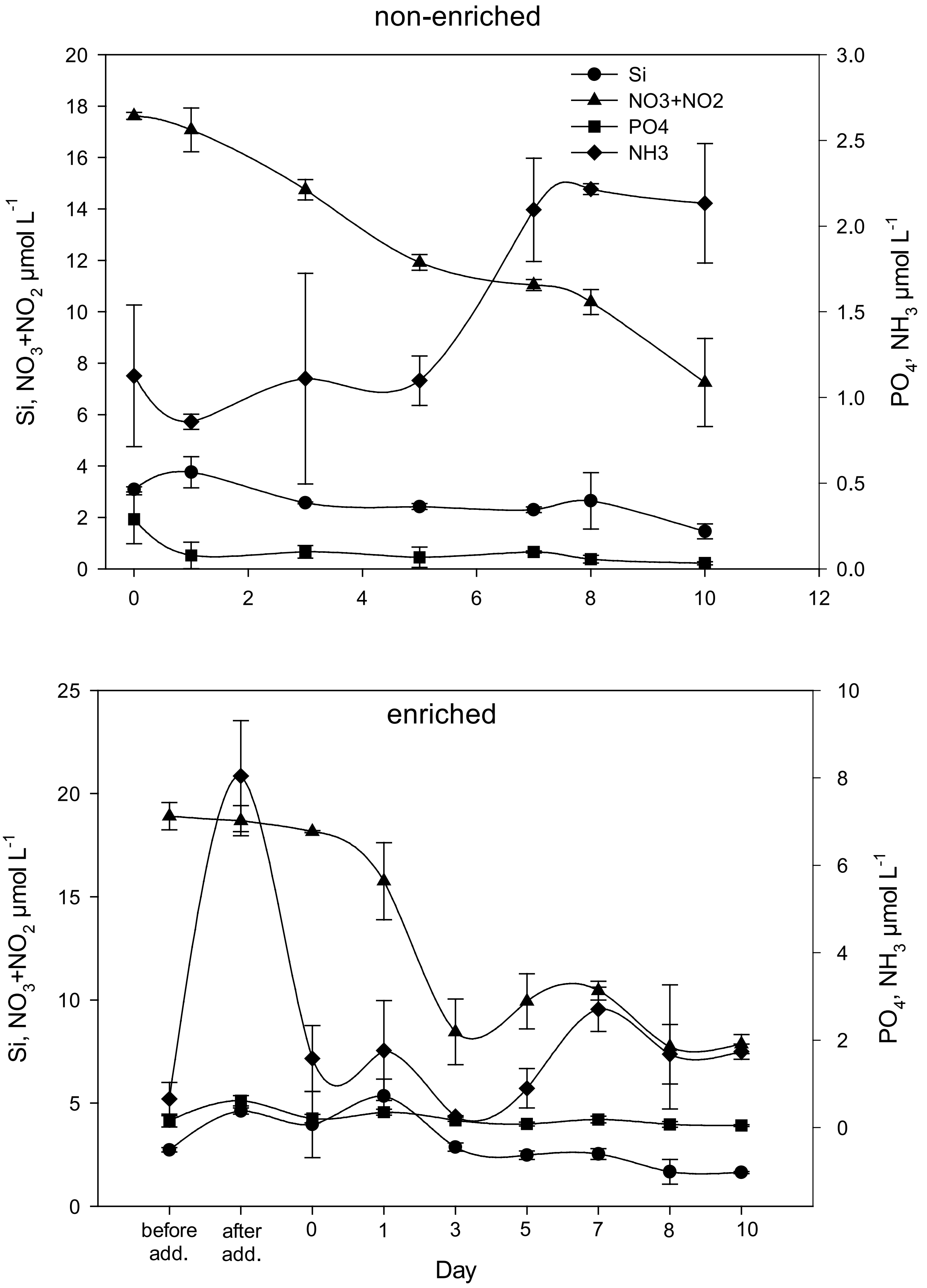

100
75
50
25
0
Concentration (nM)
☐ non-enriched
▨ enriched
0
1
2
3
7
8
9
10
Day

Figure 2.



non-enriched
Si
NO3+NO2
PO4
NH3
Si, NO_3+NO_2 µmol L^{-1}
PO_4, NH_3 µmol L^{-1}
0
2
4
6
8
10
12
14
16
18
20
0.0
0.5
1.0
1.5
2.0
2.5
3.0
0
2
4
6
8
10
12
enriched
0
5
10
15
20
25
0
2
4
6
8
10
before add.
after add.
0
1
3
5
7
8
10
Day

Figure 3.

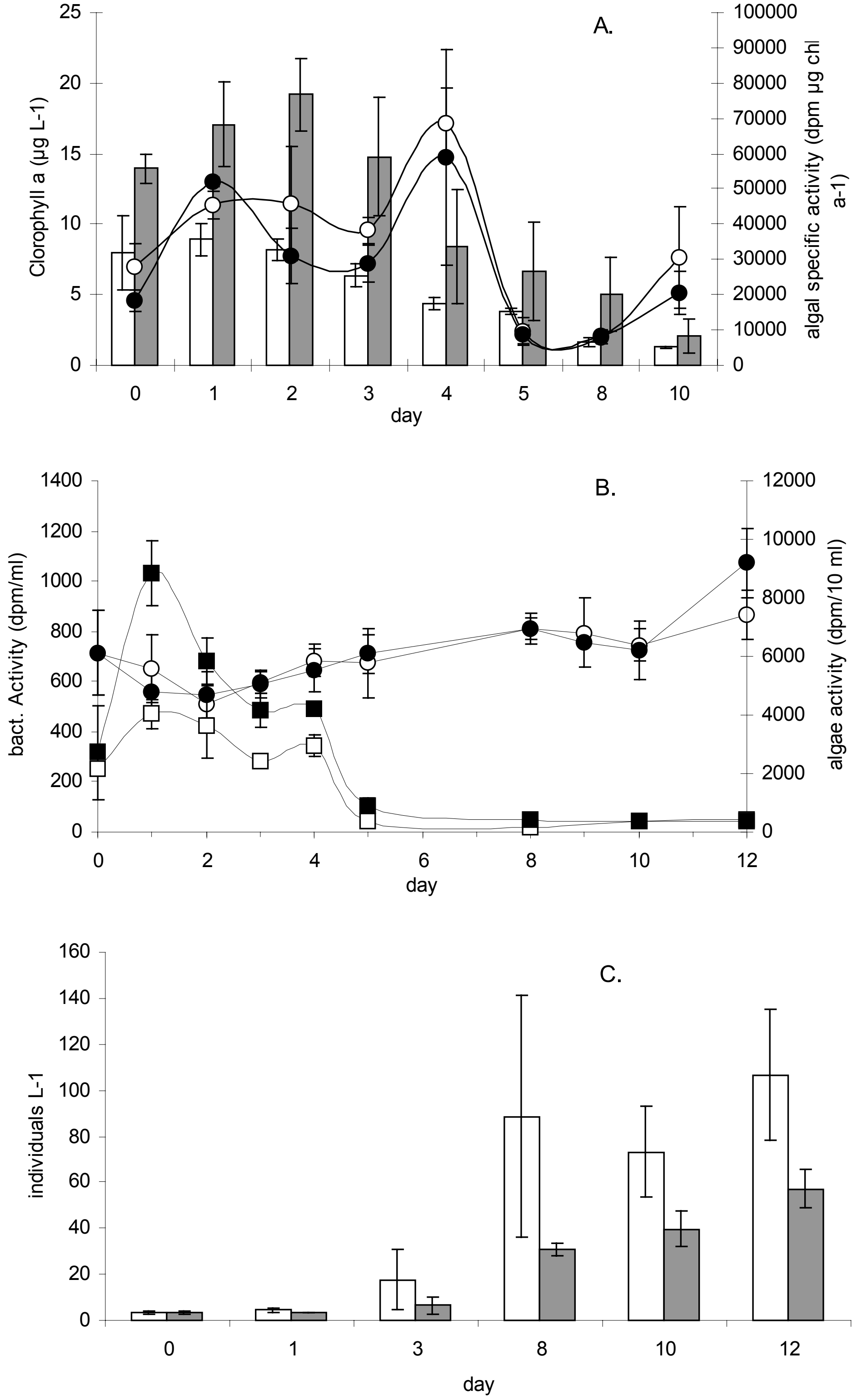

A.
Clorophyll a (µg L-1)
0
5
10
15
20
25
algal specific activity (dpm µg chl a-1)
0
10000
20000
30000
40000
50000
60000
70000
80000
90000
100000
0
1
2
3
4
5
8
10
day
B.
bact. Activity (dpm/ml)
0
200
400
600
800
1000
1200
1400
algae activity (dpm/10 ml)
0
2000
4000
6000
8000
10000
12000
0
2
4
6
8
10
12
day
C.
individuals L-1
0
20
40
60
80
100
120
140
160
0
1
3
8
10
12
day

Figure 4.

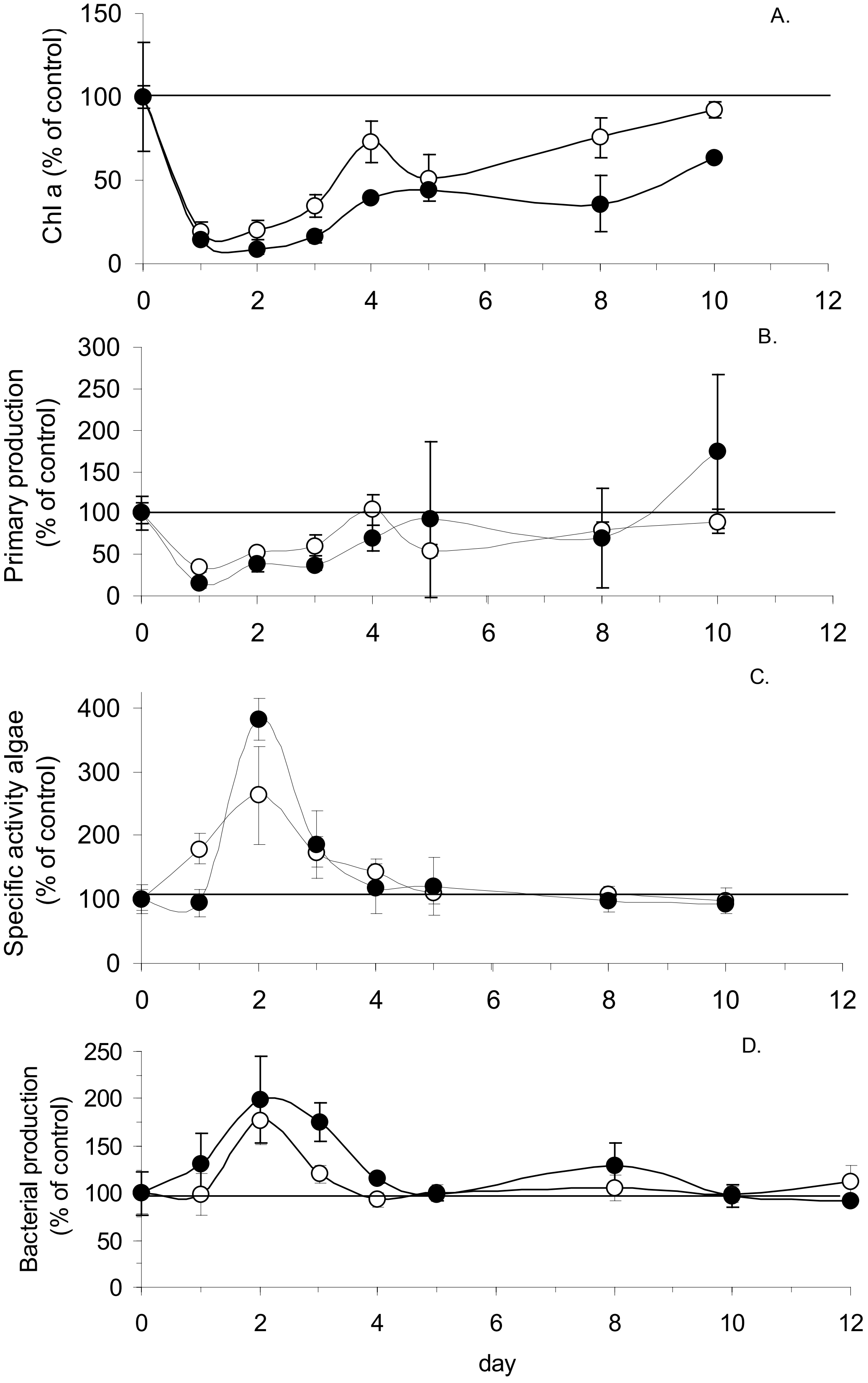

A.
Chl a (% of control)
150
100
50
0
0
2
4
6
8
10
12
B.
Primary production (% of control)
300
250
200
150
100
50
0
0
2
4
6
8
10
12
C.
Specific activity algae (% of control)
400
300
200
100
0
0
2
4
6
8
10
12
D.
Bacterial production (% of control)
250
200
150
100
50
0
0
2
4
6
8
10
12
day

Figure 5

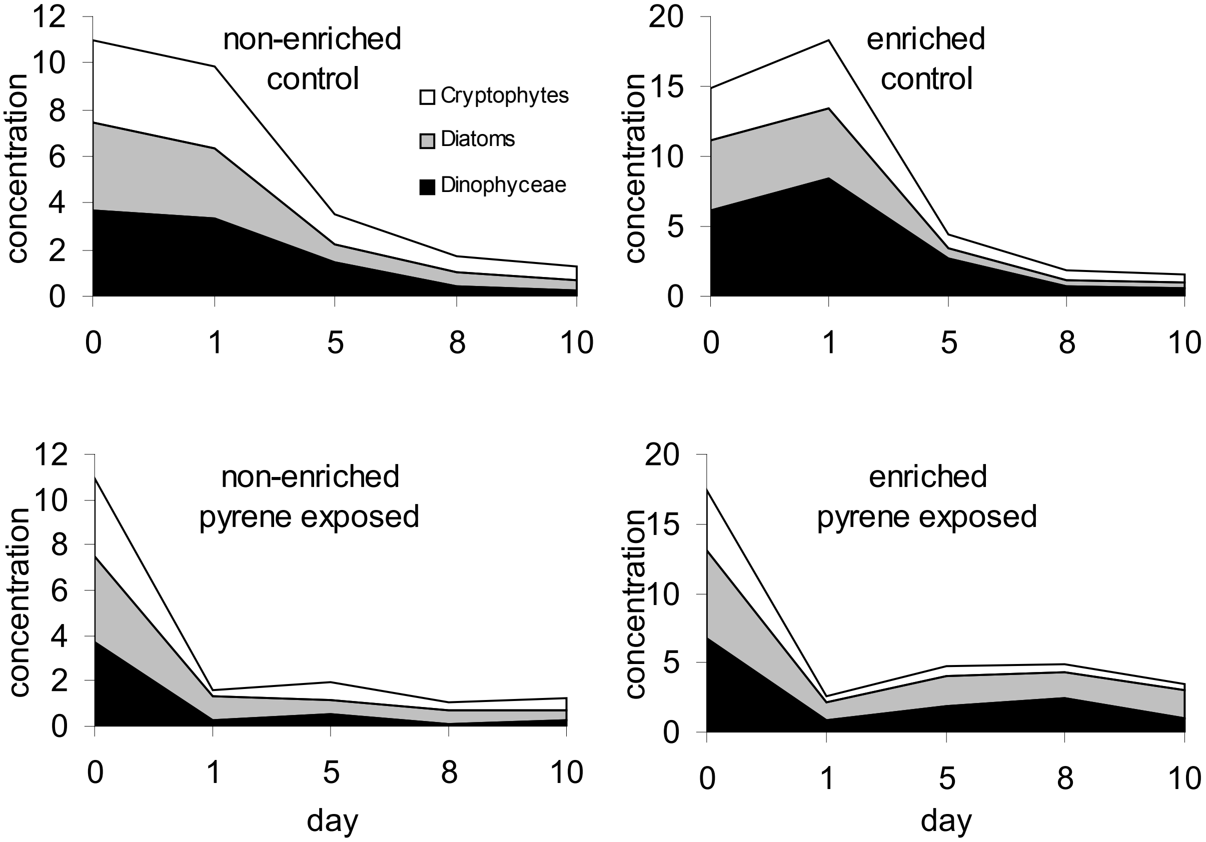
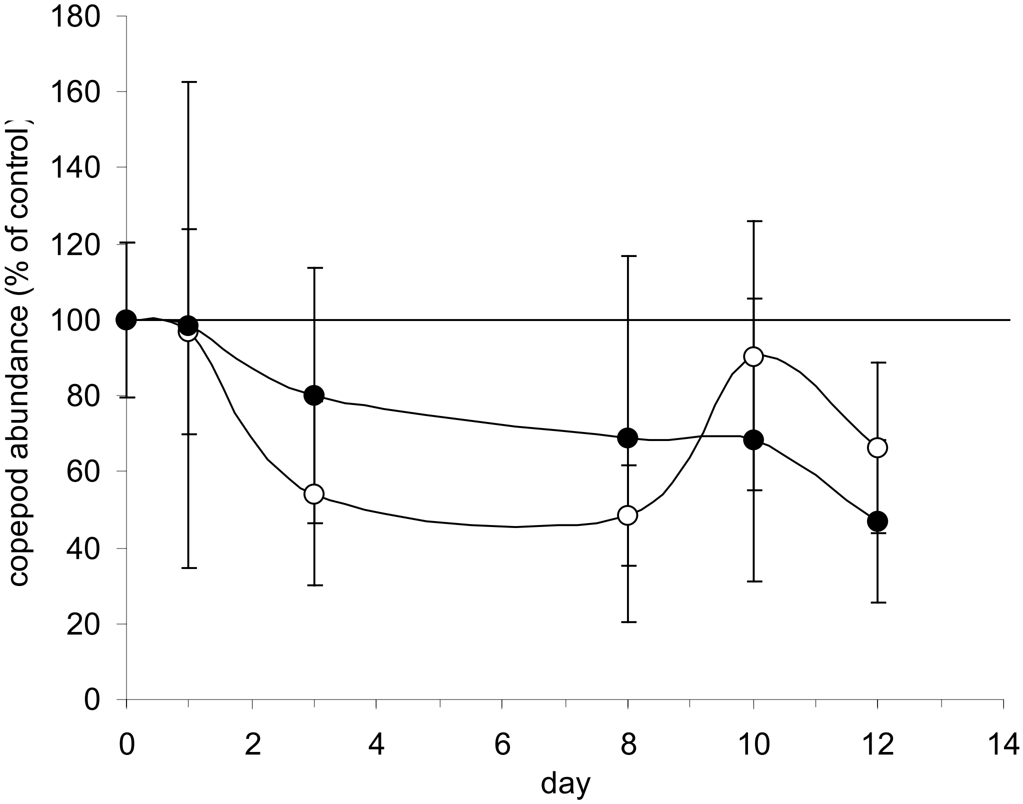

non-enriched
control
☐ Cryptophytes
▨ Diatoms
■ Dinophyceae
enriched
control
non-enriched
pyrene exposed
enriched
pyrene exposed
concentration
concentration
concentration
concentration
12
10
8
6
4
2
0
20
15
10
5
0
0
1
5
8
10
day
day

Figure 6.



180
160
140
120
100
80
60
40
20
0
copepod abundance (% of control)
0
2
4
6
8
10
12
14
day