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Realistic environmental mixtures of hydrophobic compounds do not alter growth of a marine diatom

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ABSTRACT

In this paper we determine whether a realistic mixture of hydrophobic chemicals affects the growth dynamics of a marine diatom and how this effect compares to the effect of temperature, light regime and nutrient conditions. To do so, we examine the specific growth rate of *Phaeodactylum tricornutum* in a 72 h algal growth inhibition test using a full factorial design with three nutrient regimes, two test temperatures, three light intensities and three chemical exposures. Passive samplers were used to achieve exposure to realistic mixtures of organic chemicals close to ambient concentrations. Nutrient regime, temperature and time interval (24, 48 and 72 h) explained 85% of the observed variability in the experimental data. The variability explained by chemical exposure was about 1%. Overall, ambient concentrations of hydrophobic compounds present in Belgian coastal waters, and for which the passive samplers have affinity, are too low to affect the intrinsic growth rate of *P. tricornutum*.

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1. Introduction

A complex mixture of organic chemicals is present in the Belgian coastal and estuarine waters (Wille et al., 2011; Monteyne et al., 2013) of which the ecotoxicological risk is poorly understood (Claessens et al., 2013). Notwithstanding their low freely dissolved concentrations (Ghekiere et al., 2013), mixtures of organic chemicals can cause ecotoxicological effects in marine ecosystems (e.g. Echeveste et al., 2011; Poulsen et al., 2012) due to their persistency and bioaccumulative potential (Jones and De Voogt, 1999). Especially in coastal and estuarine environments, concentrations of organic chemicals may exceed environmental quality standards (De Laender et al., 2011; Everaert et al., 2015a), suggesting that organic chemicals can pose a risk to marine ecosystems (Halpern et al., 2008; Dachs and Mejanelle, 2010). However, their ecotoxicological effects are not yet quantified (Rockström et al., 2009).

Phytoplankton is the basis of the pelagic food chain (Siegel and Franz, 2010) and alterations in the phytoplankton community can affect the entire ecosystem (Burkiewicz et al., 2005). Therefore, an improved understanding of the ecotoxicological effects of organic chemicals on phytoplankton species is needed. Unfortunately, ecotoxicological data are often based on single-chemical tests and knowledge on the mixture toxicity effect of organic chemicals is limited to simple mixtures (Backhaus et al., 2003). Moreover, test concentrations are often considerably higher than the ambient concentrations. To increase environmental realism of laboratory-based ecotoxicological research, Claessens et al. (2015) used polydimethylsiloxane (PDMS) passive samplers. Passive samplers offer the possibility to test environmentally relevant mixtures of hydrophobic compounds at realistic environmental concentrations (Lohmann et al., 2012). In passive sampling, PDMS sheets are deployed in an aquatic environment and accumulate hydrophobic chemicals (Vrana et al., 2005; Rusina et al., 2007). The uptake process is driven by the passive diffusion of chemical analytes from the matrix that is sampled (e.g. water or sediment) to the passive sampler (where the chemical fugacity is initially low). This process continues until the chemical potential in the sampler equals the chemical potential in the sampled matrix, which is the state typically called ‘equilibrium’ (Claessens et al., 2015). After deployment, loaded PDMS sheets can be used as passive dosing devices to establish constant exposure concentrations in ecotoxicological tests (Claessens et al., 2015) or to analyse the chemical composition of the sampling site (Emelogu et al., 2013; Monteyne et al., 2013). The passive sampling and dosing ability of PDMS samplers has recently been used in ecotoxicological research for chemicals with limited water-solubility (Claessens et al., 2015; Emelogu et al., 2013; Booij et al., 2013). To do so, sheets are first loaded

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with a mixture of organic chemicals, which can be done either passively (deployment in a contaminated aquatic environment) or actively (spiking). Next, sheets are added to a test medium where they release organic chemicals until equilibrium and continuously replenish losses due to volatilization and adsorption (Claessens et al., 2015).

Next to the inclusion of realistic chemical mixtures, the environmental realism of ecotoxicological research would increase by using more environmentally realistic test conditions. For example, changes in test temperature (e.g. Vieira and Guilhermino, 2012), nutrient regimes (e.g. Kong et al., 2010) and light conditions (e.g. Lyons et al., 2002; Wang et al., 2008) considerably alter the ecotoxicological effects of organic chemicals in experimental assessments. The impact of these drivers has often been tested in single-stressor designs, but the combined impact of these drivers on the growth of phytoplankton is poorly studied. In a modelling study using field data from the southern part of the North Sea and the Kattegat region, Everaert et al. (2015b) quantified the relative contribution of four classical drivers (i.e. solar radiation, nutrient concentration, temperature and zooplankton grazing) and one toxicity-related term to phytoplankton growth. By doing so, it was found that natural drivers of phytoplankton growth accounted for more than 90% of the phytoplankton dynamics in both study regions. Indeed, the model-based contribution of hydrophobic chemicals to the phytoplankton dynamics was ca. 1% in Belgian marine waters and ca. 10% in the Kattegat. Despite the model-based study of Everaert et al. (2015b), the combined effects of realistic environmental mixtures of organic chemicals and test conditions on the growth of marine phytoplankton are still poorly understood. Hence, current ecotoxicological data may lack environmental realism.

The objective of the present paper is to determine whether a realistic environmental mixture of hydrophobic compounds affects the growth of a marine phytoplankton species, and how this effect compares to the effect of temperature, light regime and nutrient conditions. To this end, we used passive samplers (PDMS sheets) that were deployed close to the harbour of Zeebrugge (Belgium) from December 2013 to March 2014. We performed a full-factorial 72 h algal growth inhibition test with the marine diatom *Phaeodactylum tricornutum* under different environmental conditions (nutrients, temperature and light intensities). Because Echeveste et al. (2010) reported no ecotoxicological effects of exposure to realistic mixtures (and concentrations) of commonly occurring marine microcontaminants, we hypothesised that a realistic mixture of organic chemicals close to environmental concentrations would not affect the growth of *P. tricornutum*.

2. Materials and methods

2.1. Preparation and deployment of passive samplers

Polydimethylsiloxane (PDMS) sheets were used to expose diatoms to a realistic mixture of hydrophobic chemicals as in Claessens et al. (2015). PDMS sheets had a surface area of ca. 100 cm^2, a thickness of 0.5 mm and a mean mass of 3.15 g. Prior to deployment, all sheets were pre-extracted for 100 h in boiling ethyl acetate using a Soxhlet extractor following the procedure of Monteyne et al. (2013). The PDMS sheets were attached to stainless steel cages and deployed in Belgian coastal waters just outside the harbour of Zeebrugge at sampling station MOW1 (51° N 21.644′, 3° E 6.992′) at a depth of 9 m between 10 December 2013 and 27 March 2014. The sheets were exposed in the environment for more than three months, long enough to attain complete equilibrium for low hydrophobic chemicals (Monteyne et al., 2013; Claessens et al., 2015). Details on the oceanographic characteristics at the sampling location are presented in Table S1. After deployment, the PDMS sheets were transported in pre-cleaned closed glass containers at −20 °C, and bio-fouling was removed with soft tissue and deionized water.

2.2. Algal growth inhibition test

P. tricornutum Bohlin strain 1052/1A was obtained from the Culture Collection of Algae and Protozoa (Oban, United Kingdom). The toxicity of organic chemicals was studied in a 72 h growth inhibition test (ISO 10253) using a full factorial design. In the study design following factors were used: three nutrient regimes (14 μmol P L^{-1} & 588 μmol N L^{-1}; 2.8 μmol P L^{-1} & 120 μmol N L^{-1} and 0.7 μmol P L^{-1} & 30 μmol N L^{-1}), 1), two test temperatures (16 °C and 23 °C), three light intensities (10,000 lx; 20,000 lx and 25,000 lx) and three chemical exposures, i.e. a blank control (containing no PDMS sheets), a growth control (containing unloaded PDMS sheets) and the actual chemical exposure (containing loaded PDMS sheets). Three replicates were provided for each unique test condition.

P. tricornutum was maintained in the laboratory according to protocol ISO 10253 (ISO, 2006). Prior to the start of the growth inhibition test, two precultures were grown at both test temperatures used in the growth inhibition test (i.e. 16 °C and 23 °C). Precultures were inoculated with an initial cell concentration of $5.0 * 10^4$ cells mL^{-1}. Erlenmeyer flasks (100 mL) were filled with 50 mL of growth medium (ISO 10253) and PDMS sheets (cut in four equal pieces) were added. After a two-day equilibrium period, which is sufficiently long to equilibrate for the majority of the compounds (Claessens et al., 2015), each flask was inoculated with $1.0 * 10^4$ cells mL^{-1} of the exponentially growing *P. tricornutum* precultures and incubated at the prescribed test temperature and light intensities. Test flasks were shaken manually twice a day. The algal cell density was measured after 24 h, 48 h and 72 h in each test flask using an electronic particle counter (Coulter Counter model DN, Harpenden, Herts, UK). The pH of the test medium was measured at the start and end of the test period (Table S2). All glassware used in the algal growth inhibition test was sterilized in an autoclave at 121 °C for 40 min.

2.3. Statistical analysis

The specific growth rate (μ, day^{-1}) was calculated according to protocol ISO 10253 (ISO, 2006):

$$\mu = \frac{\ln(N_L) - \ln(N_0)}{t_L - t_0} \quad (1)$$

with t_0 the moment of inoculation (day 0), t_L the moment of sampling (day 1, 2 or 3), N_0 the inoculated cell density at t_0 ($1.0 * 10^4$ cells mL^{-1}) and N_L the measured cell density at t_L (cells mL^{-1}). We used a linear regression model to check for differences in the specific growth rates between the full-factorial explored test conditions. A linear regression model, with Y the response variable and X_m the M covariates, is defined as:

$$Y = \alpha + \beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_M X_M + \varepsilon \quad (2)$$

where the parameters α and β_M represent the intercept and the M slopes of the corresponding covariates (Zuur et al., 2009). The residuals ε capture the unexplained variation in the data. The initial model included the day of sampling of the test flasks (further referred to as "sampling day"), the nutrient regime (three conditions), the test temperature (two conditions), the light intensity (three conditions) and the chemical exposure to organic contaminants through PDMS sheets (three conditions) as covariates (Table 1). In addition to these main effects, we also included two way-interactions between the sampling day and the test conditions (model 1; Table 1). We started from the initial model and implemented a hierarchical backward elimination model selection method as in Everaert et al. (2014). The Akaike information criterion (AIC) weighed the model fit against the model complexity (Zuur et al., 2009) and was the primary criterion to select the model with the best fit. The lower the AIC, the better the model fit (Zuur et al., 2009). For

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