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Transient effect of bisphenol A (BPA) and di-(2-ethylhexyl) phthalate (DEHP) on the cosmopolitan marine diatom *Chaetoceros decipiens-lorenzianus*

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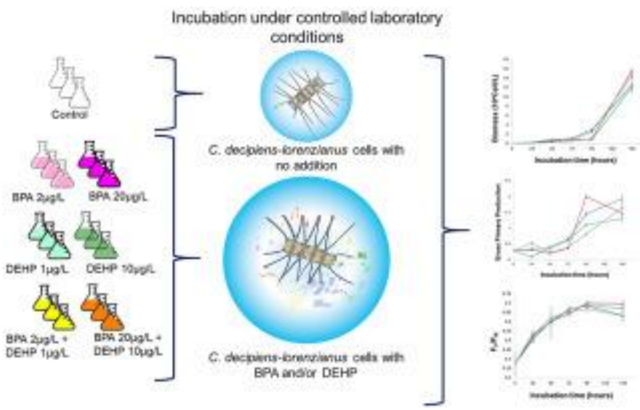
Abstract :

Incubation under controlled laboratory conditions were performed to assess the toxic effects of two plastic derived chemicals, bisphenol A (BPA) and di-(2-ethylhexyl) phthalate (DEHP), on the growth, photosynthetic efficiency and photosynthetic activity of the cosmopolitan diatom Chaetoceros decipiens-lorenzianus. Non-axenic diatom cells were exposed to concentrations of BPA and DEHP (separately and in mixture), mimicking concentrations observed in contaminated marine ecosystems, for seven days. Upon short-term exposure (i.e., during the first 48 h), BPA and DEHP induced a slight but significant stimulation of biomass and photosynthetic activity relative to the control, whereas, no significant impact was observed on the photosynthetic efficiency. Nevertheless, this pattern was transient. The stimulation was followed by a return to control conditions for all treatments at the end of incubation. These results showed that the cosmopolitan diatom Chaetoceros was not impacted by representative in situ concentrations of plastic derivatives, thus confirming its ability to thrive in coastal anthropogenic environments.

Graphical abstract



Highlights

► BPA and DEHP contamination do not significantly impact *C. decipiens-lorenzianus.* ► Slight impacts were observed on the biomass and photosynthetic activity over 48 h. ► *C. decipiens-lorenzianus* might be identified as a tolerant species to BPA and DEHP. ► This diatom might be tolerant to BPA and DEHP at environmental concentrations.

Keywords : *Chaetoceros decipiens- lorenzianus*, bisphenol A, di-(2-ethylhexyl) phthalate, biomass, photosynthetic efficiency and activity

1. Introduction

As marine plastic debris becomes a topic of emerging concern (Galgani et al., 2019; Hahladakis, 2020), scientific interest on the environmental impact of this debris increased remarkably. The number of scientific publications dealing with this topic reached over 37,200 in the last decade (Abalansa et al., 2020; Schmid et al., 2021). In aquatic environments, this plastic debris is accumulated and degraded by various means (e.g., biodegradation, hydrolysis), forming smaller particles called microplastics (Booth et al., 2017; Sharma and Chatterjee, 2017). Furthermore, microplastic fragments release harmful compounds (Hermabessiere et al., 2017), associated with plastic derived compounds or plastic additives, such as phthalic esters, bisphenol, lindane, atrazine and polybrominated diphenyl ethers (Hong et al., 2018; Lithner et al., 2011; Sharma and Chatterjee, 2017).

Among these plastic additives, bisphenol A (BPA) and di-(2-ethylhexyl) phthalate (DEHP) are used extensively on a global basis as plasticizers (Erythropel et al., 2014; Warner and Flaws, 2018). These two man-made compounds are known to act as endocrine disruptor chemicals (Brossa et al., 2005; Oehlmann et al., 2009). Moreover, in the environment, another potential danger is known as the 'cocktail effect' due to the occurrence of pollutants in the mixture (Backhaus et al., 2003; Lloyd-Smith and Immig, 2018; Svingen and Vinggaard, 2016). Several studies reported the occurrence of these compounds in the marine environment at concentrations between 0.1-10 µg/L in estuaries (Careghini et al., 2014; Liu et al., 2010b; Zhang et al., 2018), < 0.1 µg/L in lagoons (Pojana et al., 2007), ≤ 0.1 - 6 µg/L in coastal seawaters (M'Rabet et al., 2019; Paluselli et al., 2017; Sánchez-Avila et al., 2012) and < 0.1 µg/L in ocean (Corrales et al., 2015; Zhang et al., 2019).

As they are associated with Endocrine Disruptor Chemicals (EDCs), studies have highlighted their hazardous impacts on marine organisms (Pojana et al., 2007; Rehman et al., 2017; Windsor et al., 2018). BPA and DEHP potentially affect the physiology, growth, metabolism, and reproduction of fishes (Kim et al., 2011; Saili et al., 2012), crustaceans (Cole et al., 2011; Liu et al., 2009), invertebrates (Canesi et al., 2007; Fromme et al., 2002; Porte et al., 2006), seabirds (Coffin et al., 2019) and marine mammals (Hart et al., 2018; Mathieu-Denoncourt et al., 2015; Net et al., 2015).

Studies on their toxic effect were more focused on aquatic organisms possessing an endocrine system while less attention has been given to those lacking this system, such as primary producers. Most studies focused on the ability of microalgae to bioaccumulate, biodegrade, and transfer these molecules to higher trophic levels with little attention being given to their ecotoxicological impact (Crain et al., 2007; Liu et al., 2010a; Xu et al., 2015). Nevertheless, recent studies highlighted the significant negative impact of these EDCs on the growth and metabolic activity of some phytoplankton species. For example, contamination with DEHP at 10 mg/L, which is much higher than environmental concentrations, showed growth inhibition and physiological disruption associated with oxidative stress in Chlorophyceae *Chlorella vulgaris* (Shen et al., 2019). Similarly, a significant biomass decrease was reported with the dinoflagellate *Cochlodinium polykrikoides,* renamed *Margalefidinium polykrikoides* (Gómez et al., 2017), upon 72 h of exposure to BPA at 68.5 mg/L (Ebenezer and Ki, 2012). Although the ecotoxicological response of phytoplankton to organic pollutants has been considerably investigated (Echeveste et al., 2016; Filimonova et al., 2016; Menezes-sousa et al., 2018), nowadays, and to our knowledge, few studies have assessed the impact of EDCs at realistic environmental concentrations. Interestingly, relevant environmental concentrations of EDCs, resulted in a biomass decrease as well as physiological inhibition, for the potentially toxic dinoflagellate *Alexandrium pacificum* (C. M'Rabet et al., 2018) and diatom *Nitzschia palea* (Debenest et al., 2011).

Among marine phytoplankton, diatoms are a highly productive and diverse group (Norton et al., 1996; Jensen and Moestrup, 1998). Diatoms contribute to ocean biodiversity with 136 genera, with a considerable number of species without precise identification (20,000 - 200,000) (Guiry, 2012; Mann and Vanormelingen, 2013). They are cosmopolitan organisms, occurring in coastal ecosystems, open waters, temperate environments, and polar regions (Deng et al., 2017; Gogorev et al., 2016; Malviya et al., 2016). Diatoms are one of the principal sources of oxygen production in marine environments accounting for up to 40% of total production (Hildebrand, 2008). They are also involved in different biogeochemical cycles, ensuring approximately 20% of CO_2 fixation (Falkowski et al., 1998; Field et al., 1998; Goldman, 1993). Due to their characteristics (cosmopolitan, short generation time and efficient proxy for environmental changes), diatoms are commonly used in ecotoxicological studies (Fourtanier and Kociolek, 1999; Puspitasari et al., 2018; Stevenson and Pan, 2010). They have been classified as bioindicators of aquatic environmental perturbation (Hourmant et al., 2009; Pandey et al., 2017).

Within the diatom group, the *Chaetoceros* genus is considered as one of the most abundant diatoms in marine phytoplankton (De Luca et al., 2019), with an abundance that can exceed $8 \cdot 10^5$ during phytoplankton bloom Cells/L (Leblanc et al., 2012; Shevchenko et al., 2006). *Chaetoceros decipiens* is a cosmopolitan species, commonly found in coastal waters as well as in the open sea (Balzano et al., 2017; Batistić et al., 2017; Li et al., 2017). In the Mediterranean sea, *C. decipiens* is one of the dominant phytoplankton species (Tas and Hernández-Becerril, 2017; Yahia-Kéfi et al., 2005). For example, in the northeastern Adriatic Sea, *C. decipiens* occurred among the dominant phytoplankton species with $9 \cdot 10^4$ Cells/L (Viličić et al., 2009). Notably, that several studies reported remarkable similarities between *C. decipiens* and *C. lorenzianus*, suggesting the existence of an intermediate form between these two species, the *C. decipiens-lorenzianus* complex (Bosak et al., 2016; Kownacka et al., 2013; Sunesen et al., 2008).

In this study, we aimed to understand the impact of two commonly used plastic additives, BPA and DEHP on the cosmopolitan diatom *C. decipiens-lorenzianus*. Since plastic derived chemicals can have a negative impact on phytoplankton activities, we hypothesized that a representative EDC would provoke growth inhibition and damage of the photosynthetic apparatus expressed through the reduction of photosynthetic efficiency and activity.

2. Material and methods

2.1- Diatom culture

A strain of the *C. decipiens-lorenzianus* complex, an autotrophic diatom species, was isolated from Bizerte Channel (South-Western Mediterranean Sea 37.269˚N, 9.877˚E) in December 2016. This diatom strain was grown in F/2 medium (34.5‰ salinity) enriched with silicates (Guillard and Kilham, 1976). The species was maintained under controlled laboratory condition at 22˚C and illuminated at an irradiance level of approximately 110 µmol photons $/m^2 \cdot s^1$ under a 12:12 light: dark cycle.

2.2. Taxonomic identification of the strain

Taxonomic identification was performed using PCR amplification of a partial large subunit of ribosomal DNA (LSU rDNA, 28S). A polymerase chain reaction (PCR) was carried out using 1 µl of concentrated culture resuspended in distilled water. According to Taq Polymerase (Promega) instructions, amplification of the region D1/D2 was obtained using primers D1R and D3B (Nézan et al., 2012). Sequencing products were run on an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems). The obtained sequence was aligned with 145 other sequences of different putative *Chaetoceros* spp. and two outgroup sequences used by Chen et al (2018) using BioEdit v. 7.0.9.0 software (Hall 1999). Alignment was produced using the MUSCLE v. 3.7 algorithm (Edgar, 2004). The resulting matrix comprised 148 OTU and 650 characters. The evolutionary model and parameters were selected after running Smart Model Selection in PhyML (Lefort et al., 2017). For the present dataset, SMS selected the general time reversible (GTR) model with a gamma correction (G) for among-site rate variation and invariants sites. Maximum likelihood analyses were performed using PhyML version 3.0. (Guindon et al., 2010) and Bayesian analyses were run using Mr Bayes version 3.1.2 (Ronquist and Huelsenbeck, 2003). Bootstrap analysis (1000 replicates) was used for the maximum likelihood (ML) to assess the relative robustness of branches (bootstrap values, bv). Initial Bayesian analyses were run with a GTR model (nst = 6) with rates set to invgamma and nucleotide frequencies set to equal. Each analysis was performed using four Markov chains (MCMC) with two millions cycles for each chain. Trees were saved to a file every 100 cycles and the first 2000 trees were discarded. Therefore, a majority-rule consensus tree was created from the remaining 18000 trees to examine the posterior probabilities (pp) of each clade. The consensus trees were edited using TreeView version 1.6.6. The best ML phylograms were shown with their robustness values for each node (ML, bv / BI, pp).

2.3. Experimental design

Toxicity experiments were performed with non-axenic batch cultures of *C. decipiens-lorenzianus* exposed to BPA and DEHP in 250 mL Erlenmeyer flasks following the protocols of M'Rabet et al (2018). Inoculum of 300 cells/mL were used to start the culture experiments. Different nominal concentrations of BPA and DEHP were added separately to the cultures and in mixture (BPA and DEHP), as follow: BPA at 2 µg/L and 20 µg/L, DEHP at 1 µg/L and 10 µg/L, and mixtures with BPA 2 µg/L + DEHP 1 µg/L and BPA 20 µg/L+ DEHP 10 µg/L.

Incubations were run in triplicate, with respect to the control conditions (no BPA and DEHP addition), for 144h.

2.4. Chemical analyses

Analytical analyses are described in more detail in the supplementary material. BPA and DEHP analysis were performed at the beginning of the experiment (T_0) to validate the nominal concentration added, using LC/MS and SPME coupled with GC/MS (M'Rabet et al., 2019).

2.5. Growth measurement

A 1 mL aliquot of each treatment was fixed with acidic lugol to enumerate cell abundances at 0 (incubation-start), 24, 48, 72, 96 and 144 h (incubation-end), to assess the impact on the short, mid-, and long-term exposure to pollutants. Like all phytoplankton species, *C. decipiens-lorenzianus* has an exponential growth (lag phase, exponential phase, stationary phase and decline phase). In this study, the cellular concentration was checked by inverted microscope counting (Leica 521,234) during the incubation period. For each treatment, the growth rate (GR) was calculated after logarithmic transformation of the cell density. The following equation was applied: $GR = (\ln N_i - \ln N_{i-1}) / (T_i - T_{i-1})$, where N_i and N_{i-1} represent the cell density (cells/mL) at the beginning (T_{i-1}) and end (T_i) of the exponential phase of the incubation period, respectively (Guillard et al., 1973).

2.6. Photosystem II (PSII) efficiency

2.6.1. Quantum yield of the PSII: F_V/F_M measurements

To assess the effect of BPA and DEHP on photosynthetic performance, in vivo chlorophyll fluorescence was measured using a portable Pulse Amplitude Modulation fluorometer PSI-AP-100 (AquaPen- Photon Systems Instruments). The OJIP-transient protocol was applied to calculate F_V/F_M (Strasser and Srivastava, 2000). Before measurement, a subsample of 3 mL of each treatment was dark-adapted for 30 min to inactivate the photosynthetic activity according to the manufacturer's operating manual. The quantum yield of PSII was calculated according to the following equation $F_V/F_M = (F_M - F_0/ F_M)$, where F_0 is the minimum fluorescence in the dark-adapted state, F_M is the maximum fluorescence in the dark-adapted state, and F_V is the maximum variable fluorescence yield.

2.6.2. Light curves and photosynthetic parameters (α, P_{MAX}, and E_K)

To determine the relative electron transport rate versus irradiance (rETR versus E) curves, the LC3 protocol of the Pulse Amplitude Modulation fluorometer PSI-AP-100 (AquaPen- Photon Systems Instruments) was used. A 3 mL subsample of the phytoplankton culture (from each treatment) were submitted to seven actinic light levels from 10 to 1000μmoL/m^2·s^1 (10, 20, 50, 100, 300, 500, and 1000 μmoL/m^2·s^1). The hyperbolic tangent model fitted to rETR versus E curves were obtained as indicated by $rETR= \alpha * E_K * \tanh (E * E_K^{-1})$ (Jassby and Platt, 1976; Silsbe and Kromkamp, 2012). This model was used to estimate the light curve parameters: α (the coefficient of maximal utilization of energy) and E_K (the photon flux density from which the quantum efficiency of photosynthetic activity does not increase proportionally to the light intensity). Then, light saturated P_{MAX} was deduced as $P_{MAX} = \alpha * E_K$.

2.7. Oxygen metabolism

To evaluate the impact of BPA and DEHP on primary production (GPP) and respiration (R), dissolved oxygen evolution was determined using an oxygen microelectrode (Unisense, Denmark) and the light-dark method (Pringault et al., 2007). A 1 mL vial filled with subsamples from each treatment were used to measure net production (NP) during the light phase and respiration (R) during the dark phase. All vials were exposed to controlled light-dark and temperature conditions. GPP was then calculated as GPP =NP + |R|.

2.8. Statistical analyses

Difference between treatments were analyzed by a two-way analysis of variance (ANOVA), considering the incubation time and concentrations of the plastic derived chemicals as independent variables. Data normality and homoscedasticity were checked before the ANOVA test. The growth rate was analyzed by a one-way ANOVA and the Tukey's post hoc test. Differences were considered as significant at $p \leq 0.05$. All statistical analyses were performed using STATGRAPHICS Centurion software (STATGRAPHICS 12.0).

3. Results

3.1. Strain identification

The molecular identification dataset was comprised of 148 LSU and 650 characters. The sequences obtained were aligned with sequences of *Chaetoceros* spp. from GenBank using MUSCLE (EMBL-EBI) software. The ML phylogenetic tree inferred from the LSU rDNA (28S) dataset revealed that the sequence obtained for the Bizerte strain (MW111279) formed a well-supported group, together with accession number KX065227 and EF423436 of *C. decipiens* and *C. lorenzianus*, respectively (ML bootstrap 90). This confirmed that the identified species belonged to the *C. decipiens-lorenzianus* complex (Figure 1).

3.2. Plastic derived chemicals measurement

To confirm the nominal concentrations added to the *Chaetoceros* cultures, dissolved BPA and DEHP were measured at the beginning of incubation. For all treatments, the measured concentrations of dissolved BPA and DEHP were close to the nominal one (Table S1), regardless of the conditions (single compounds or mixture).

3.3. Effect of BPA and DEHP on the growth of *C. decipiens-lorenzianus*

Cell density in the control showed a regular increase from the beginning (an average of 315 Cells/L) until the end of the incubation period (7 days, $1.56 \cdot 10^5$ Cells/L), associated with a growth rate of 0.82 ±0.05 day^{-1}. Upon short-term exposure (i.e., during the first 72h), BPA and DEHP contamination induced a slight but significant increase ($p < 0.05$) in the diatom cell number relative to the control (Figure 2). Simultaneously, growth rates were significantly enhanced ($p < 0.05$), particularly with the presence of DEHP (Table 1). The most pronounced density increase was observed with BPA and DEHP mixture, both at low and high concentrations, with a growth rate of 1.17 ± 0.07 day^{-1} and 1.19 ± 0.02 day^{-1}, respectively, relative to 0.82 ± 0.05 day^{-1} in the control. Short-term cell stimulation was observed, followed by a return to control levels at the end of the incubation period for all treatments (Figure 2).

3.4. Impact of BPA and DEHP on the photosynthetic efficiency

3.4.1. Impact of BPA and DEHP on the quantum yield of PSII

The quantum yield of PSII (F_V/F_M) in *C. decipiens-lorenzianus* from the control treatment showed a continual increase from 0.3 to 0.71 during incubation (Figure 3). F_V/F_M in the contaminated treatments exhibited the same trend as the control conditions. An increase was observed for all treatments from 0.3 to about 0.69 (Figure 3), with no significant differences relative to the control conditions ($p > 0.05$).

3.4.2. Impact of BPA and DEHP on the photosynthetic performances: Light-response-curves (LR-curves) and photosynthetic efficiency parameters

The initial measurement of the Light Response-Curve (LR-curve) of *C. decipiens-lorenzianus* showed an increase and saturation between 300 to 500 µmol photon/ m^{2} $\cdot s^{1}$, followed by clear photo-inhibition above 500 µmol photon/ m^{2} $\cdot s^{1}$. Using the photosynthetic model of Jassby and Platt (1976), characteristic LR-Curve parameters were calculated. The efficiency (α) with which *C. decipiens-lorenzianus* cells harvested the light was about 0.5 (µmol O_2/ mg^{1} Chl $a \cdot h^{1}$)/ (µmol photons/ $m^{2} \cdot s^{1}$) in control conditions. The maximum photosynthetic production (P_{MAX}) was about 236.41 (mg C/ mg Chl $a^{l} \cdot h^{l}$), at an irradiance (E_K) of 117.47 (µmol photon/ m^{2} $\cdot s^{1}$). As observed previously with F_V/F_M, the same pattern was obtained with photosynthetic performance. No changes were observed on the LR-curves for all treatments, with no significant differences between contaminated treatments and controls (Figure S1). Concomitantly, no significant differences were observed on the parameters of the photosynthetic efficiency (α, E_K, P_{MAX}) (Table S3). Alpha values increased with incubation time to an average of 0.71 (µmol O_2/ mg^{1} Chl $a \cdot h^{1}$)/(µmol photons/ $m^{2} \cdot s^{1}$), independent of the contamination conditions (Table S3). This increase of α was followed by an increase of E_k and a decrease of P_{MAX} for all treatments (Table S3).

3.5. Impact of BPA and DEHP on O_2 metabolism

O_2 metabolism was evaluated through the measurement of gross primary production (GPP) and respiration (R). In the control treatment, the photosynthetic activity varied between GPP_{0h} = 0.3 ± 0.1 µmol O_2/ L ·min (R_{0h}= 0.1 ± 0.001 µmol O_2/ L ·min) and GPP_{144h} = 1.46 ± 0.01 µmol O_2/ L ·min (R_{144h}= 0.38 ± 0.02 µmol O_2/ L ·min). As observed previously with the biomass of *C. decipiens-lorenzianus*, GPP in the contaminated treatments showed a slight, but significant ($p < 0.05$) stimulation (Figure 4A-C) within the first 72 h of incubation relative to the control conditions, particularly with BPA at a high concentration (0.6 ±0.1 µmol O_2/ L ·min) relative to the control (0.4 ± 0.04 µmol O_2/ L ·min, Figure 4A). Similarly, GPP stimulation was concomitant to slight but significant ($p < 0.05$) respiration stimulation within the first hours of incubation (Figure 4D-F). Short-term O_2 metabolism stimulation was followed by a return to control levels in all treatments. Overall, no significant differences were observed in the ANOVA test ($p > 0.05$) when the whole incubation time was considered. From GPP values and cell densities, the specific phytoplankton activity was calculated (specific GPP = GPP / Cell density). Considering the total experimental period (7 days), the specific GPP of all treatments did not vary significantly relative to the control (Figure 5).

4. Discussion

Molecular analyses performed on the strain isolated from the Bizerte lagoon (MW111279) formed a common sub-group between *C. decipiens* and *C. lorenzianus* (Figure 1). This signature led us to confirm that the identified strain belongs to the *C. decipiens-lorenzianus* complex. Previous studies reported close similarities between these two diatom species in different areas of the world including the north-eastern Adriatic Sea, the southern Baltic Sea, and the coast of Buenos Aires Province, Argentina (Bosak et al., 2016; Kownacka et al., 2013;

Sunesen et al., 2008), suggesting the existence of an intermediate form of both *C. decipiens* and *C. lorenzianus*.

Despite the high accumulation of plastic debris in the marine environments, BPA and DEHP occur at low concentrations, in the range of 6 µg/L (M'RABET et al., 2019; Paluselli et al., 2017; Sánchez-Avila et al., 2012), close to those used in the present study (Table S1). To assess their toxicity on a representative of marine primary producers, we selected the cosmopolitan diatom *C. decipiens-lorenzianus*, considering its large marine occurrence. This species is commonly observed in coastal ecosystems, as well as in open waters (Bosak et al., 2016; Gogorev et al., 2016). Species belonging to the genus *Chaetoceros* contribute considerably to ocean primary production (Booth et al., 2002). In this study, we isolated strains of *C. decipiens-lorenzianus*, that were grown under non-axenic conditions, as microscopic observation revealed the presence of bacteria associated with the diatom. Moreover, the experimental conditions were chosen to mimic *in situ* conditions as much as possible, where the diatoms are naturally associated to bacteria (Amin et al., 2012; Vincent and Bowler, 2020).

Transient stimulation of *C. decipiens-lorenzianus*: first hypothesis

At realistic environmental concentration of EDCs, the cosmopolitan diatom *C. decipiens-lorenzianus* showed a relative tolerance to BPA and DEHP after 7 days of incubation. In all treatments, a transient slight but significant stimulation of biomass and photosynthetic activity was observed, especially upon DEHP exposure. Two hypotheses can be proposed to explain this significant transient stimulation.

First, the stimulation of biomass and oxygen gross production might be explained by the direct impact of the environmental concentration of EDCs on the photosynthetic apparatus, as recently observed in microalgae cultures. A recent work highlighted that BPA released from small fragments of expanded polystyrene (EPS) at a concentration of 1.5 ng/L provoked a significant stimulation of the photosynthetic efficiency of Chlorophyceae species (Chae et al., 2020). They showed that BPA exposure significantly stimulated the quantum yield of PSII (F_V/F_M), which consequently induced a growth stimulation of several phytoplankton strains (*Dunaliella salina*, *Scenedesmus rubescens*, *Chlorella saccharophila*, and *Stichococcus bacillaris*) (Chae et al., 2020). Furthermore, this would suggest that BPA and DEHP contamination at environmental concentrations can stimulate the photosynthetic apparatus of phytoplanktonic cells, subsequently inducing the stimulation of biomass and photosynthetic activity. Nevertheless, in our study photosynthetic efficiency of PSII, measured through the Fv/Fm ratio, as well as specific activity (Figure 3 and 5), were not significantly impacted by the presence of BPA or DEHP. Consequently, a direct transient effect of EDCs on PSII of *C. decipiens-lorenzianus* cannot explain the short-term stimulation observed for growth and oxygen production.

Transient stimulation of *C. decipiens-lorenzianus*: second hypothesis

The second hypothesis to explain this transient significant stimulation relies on the presence of bacteria in the diatom culture. Recently, Romera-castillo et al (2018) demonstrated that bacterial activity was stimulated in response to phthalate exposure when released from polyethylene (PE) and polypropylene (PP). They observed that PE and PP particles in seawater promoted the release of dissolved organic carbon (DOC), which induced the stimulation of bacterial activity.

In the present study, although DOC was not measured, we speculated that BPA and DEHP promoted the metabolic activity of bacteria associated with *C. decipiens-lorenzianus*, which in turn induced biomass and photosynthetic stimulation. The microbiome associated with the non-axenic diatom might have benefited from diatom-bacterium interactions. In fact, this association can promote synergistic interactions between both compartments, through the availability of nutrients, such as vitamins (B_1 and B_{12}), iron, DOC, and nitrogen (Amin et al., 2012; Seymour et al., 2017). We hypothesized that the short-term observed stimulation of *C. decipiens-lorenzianus* upon EDC exposure at environmental conditions could be the consequence of a cascading effect of BPA and DEHP on its phycosphere, which subsequently induced cell growth and photosynthetic activity stimulation. Furthermore, phytoplankton possess a unique microbiome (Behringer et al., 2018) that could act as a protective barrier (Fouilland et al., 2018; Ramanan et al., 2016) against toxic contaminant effects, ensuring an optimal phytoplankton production. The association between the cosmopolitan *C. decipiens-lorenzianus* and its phycosphere could form an effective consortium able to cope with the toxic effects of BPA and DEHP at realistic environmental concentrations. In addition, the large size of *C. decipiens-lorenzianus* might represent a defense mechanism, relative to other smaller phytoplankton cells. The low cell volume ratio of diatoms reduces the diffusion of organic pollutants through the cell relative to smaller phytoplankton cells, favoring their tolerance to chemical contamination (Ben Othman et al., 2012; Staniszewska et al., 2015).

Situation of the ecotoxicological answer of *C. decipiens-lorenzianus* compared to other species

Previous works studying the ecotoxicological impacts on microalgae have often used higher concentrations of EDCs than those employed in the present study (Table S1). Significant toxic effects of EDCs on phytoplankton were reported for concentrations far above the observed *in situ* concentrations. For example, growth and activity inhibition were observed with diatom species at high BPA concentration of > 0.04 mg/L with *Ditylum brightwelli* and *Navicula incerta*

(Lee et al., 2014; Liu et al., 2010a). Similarly, a high EC50 was measured for the Chlorophyceae *Chorella vulgaris* (6.02 mg/L) with DEHP (Shen et al., 2019) and for the diatom *Chaetoceros muelleri* (EC_{50} at 96 h: 194 mg/L) with diethyl phthalate (Chi et al., 2019). Recent ecotoxicological works studying the impact of EDCs at environmental realistic concentrations have observed contrasting effects depending on the phytoplankton species studied. Exposure to EDCs drastically inhibited the biomass and physiological activity of the toxic dinoflagellate *Alexandrium pacificum* at concentrations of 2 and 20 µg/L BPA and 1 and 10 µg/L DEHP, similar to those used in the present study M'Rabet et al (2018). Similarly, Cunha et al (2019) observed significant inhibition of cell abundance after exposure of a Chlorophyceae *Scenedesmus* sp. to dibutyl phthalate (DBP). They reported an EC_{50} after 48 h of DBP exposure of 41.88 µg/L. In contrast, Chae et al (2020) showed that BPA at 0.0019 µg/L enhanced the photosynthetic activity, as well as the growth of Chlorophyceae species. Thus, these observations indicate that the ecotoxicological impact of EDCs on phytoplankton at relevant environmental concentrations is very likely species dependent.

5. Conclusions

Overall, results of the present study highlighted that in simulating environmental conditions, the cosmopolitan diatom *C. decipiens-lorenzianus* was not significantly impacted by BPA and DEHP contamination, the two most observed EDCs in contaminated marine ecosystems. This apparent tolerance might rely on the heterotrophic compartment associated with the diatom *Chaetoceros*. This laboratory ecotoxicological study confirms what was observed at the community scale, where environmentally relevant EDC contamination promoted the dominance of the genus *Chaetoceros* relative to other phytoplankton species (M'Rabet et al., 2019). Future ecotoxicological studies are required to better understand the possible role of the phycosphere associated with *Chaetoceros* to face EDC contamination.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Tables and figures

Table 1 : Growth rate (/day) of *Chaetoceros decipiens-lorenzianus* during the exposure to BPA and/or DEHP mean of three replicate ± standard deviation (SD), the letters (a–d) indicate homogeneity of different treatments (P < 0.05).

Treatment	Growth rate
Control	0.82 ±0.05[a]
BPA 2µg/L	0.85 ±0.02[a]
BPA 20µg/L	0.80 ±0.05[a]
DEHP 1µg/L	1.11 ±0.03[c]
DEHP 10µg/L	0.98 ±0.02[b]
BPA 2µg/L & DEHP 1µg/L	1.17 ±0.07[cd]
BPA 20µg/L & DEHP 10µg/L	1.19 ±0.02[d]

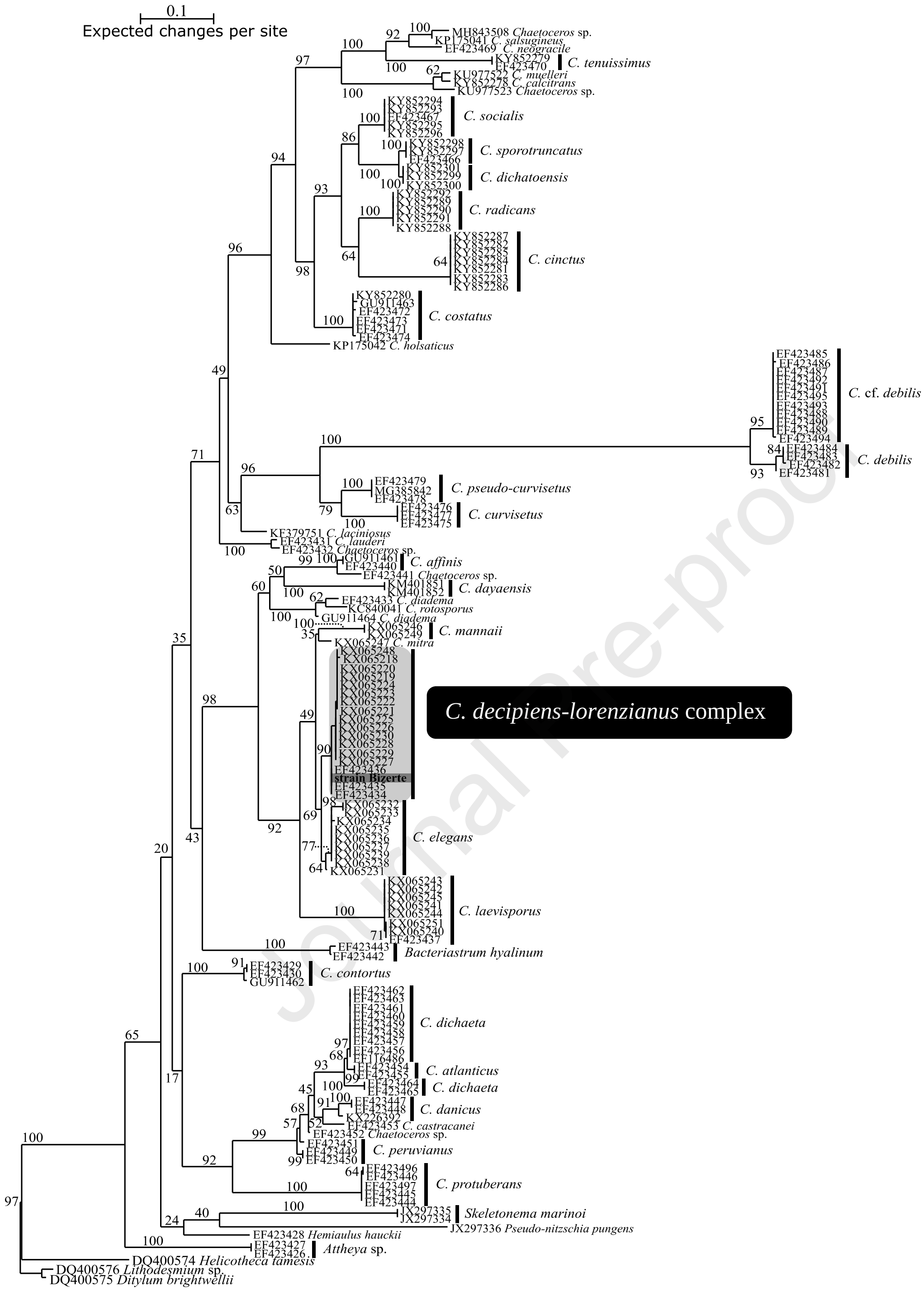

Figure 1: Maximum likelihood (ML) phylogenetic tree based on LSU rDNA sequences (148 sequences/650 characters). The tree was rooted using *Lithodesmium / Ditylum* as out-group

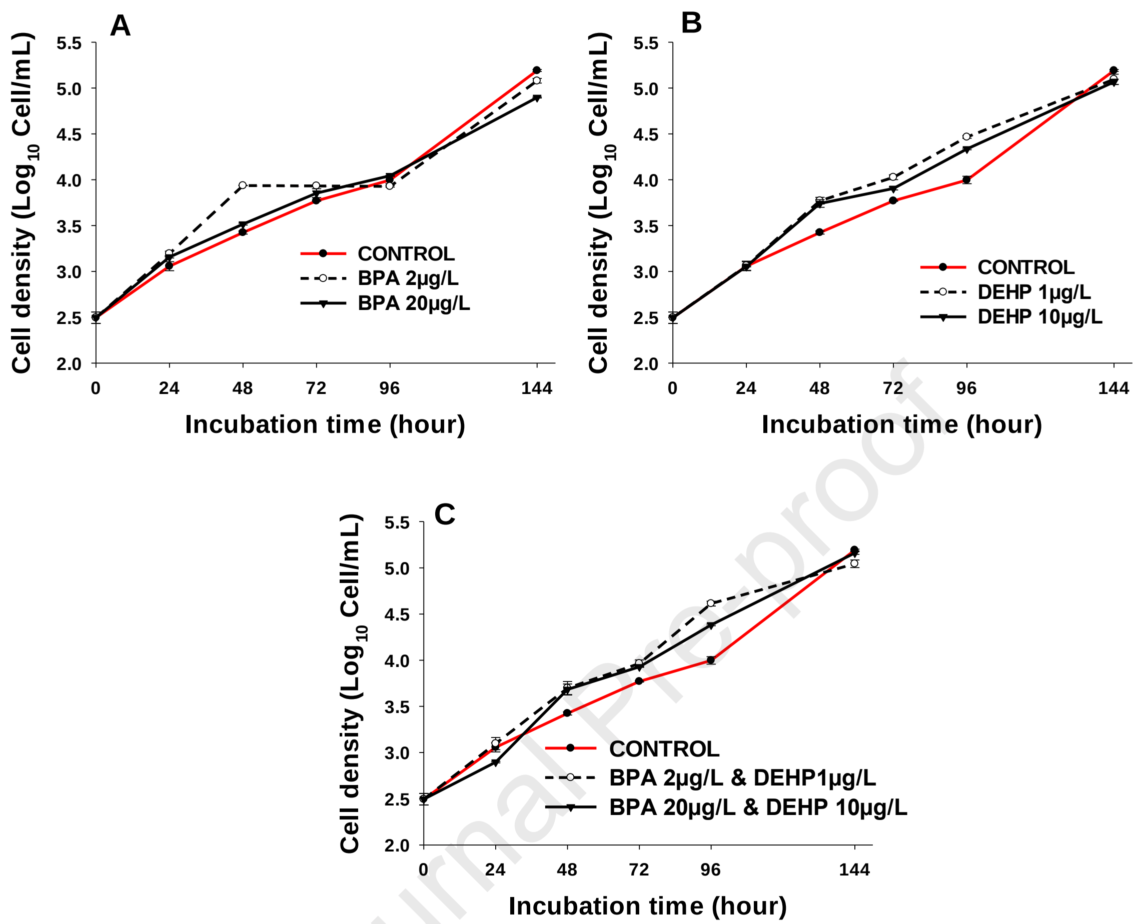

Figure 2: Growth curve of *C. decipiens-lorenzianus* (mean of three replicates ± standard deviation (SD)), expressed as Log_{10} Cell/mL during the exposure to BPA at 2 µg/L and 20 µg/L (panel A), the exposure to DEHP at 1 µg/L and 10 µg/L (panel B), the exposure to the mixture of BPA 2µg/L + DEHP 1µg/L and BPA 20µg/L + DEHP 10µg/L (panel C).

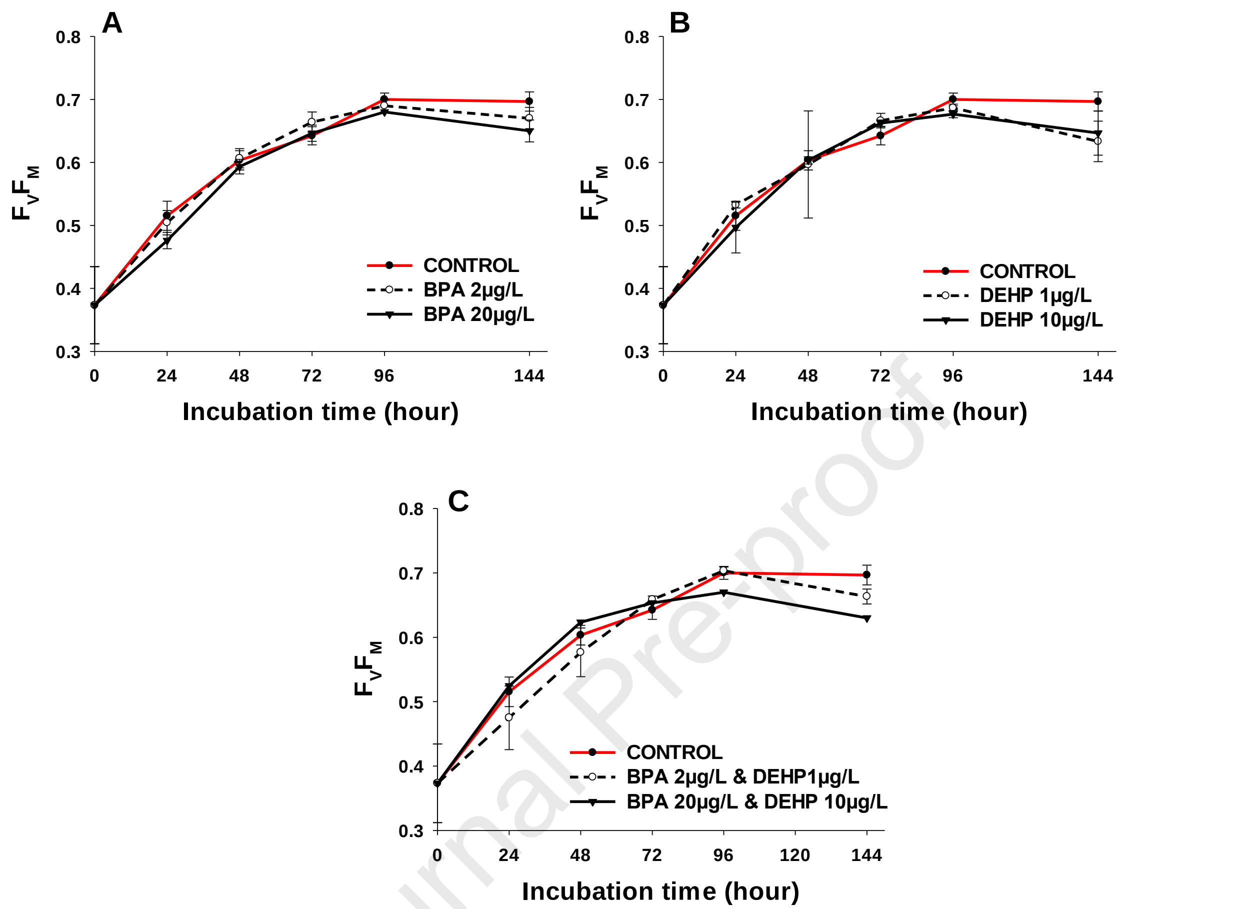

Figure 3: Variation of the maximum quantum yield of PSII (F_V/F_M) during the time incubation (7 days) of *C. decipiens-lorenzianus* to BPA (A), to DEHP (B) and to the mixture of BPA and DEHP (C). The values are expressed of mean of three replicates ± standard deviation (SD)).

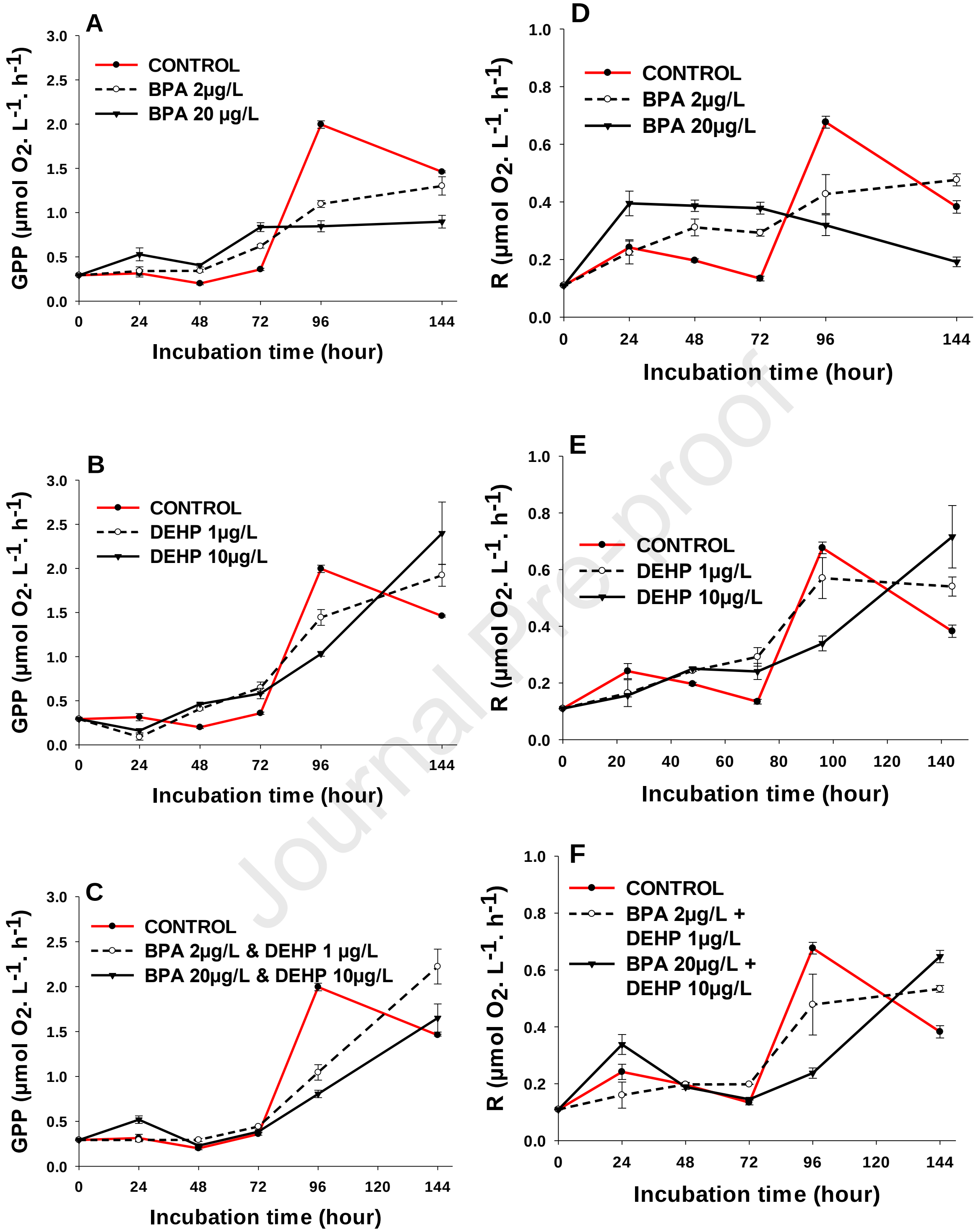

Figure 4: Photosynthetic activity expressed as GPP (Gross Primary Production) and respiration activity expressed as R, during the time exposure of *C. decipiens-lorenzianus* to BPA (A and D), DEHP (B and E) and the mixture of BPA + DEHP (C and F) in the course of the time incubation (7 days) and with respect to the control (red curve).

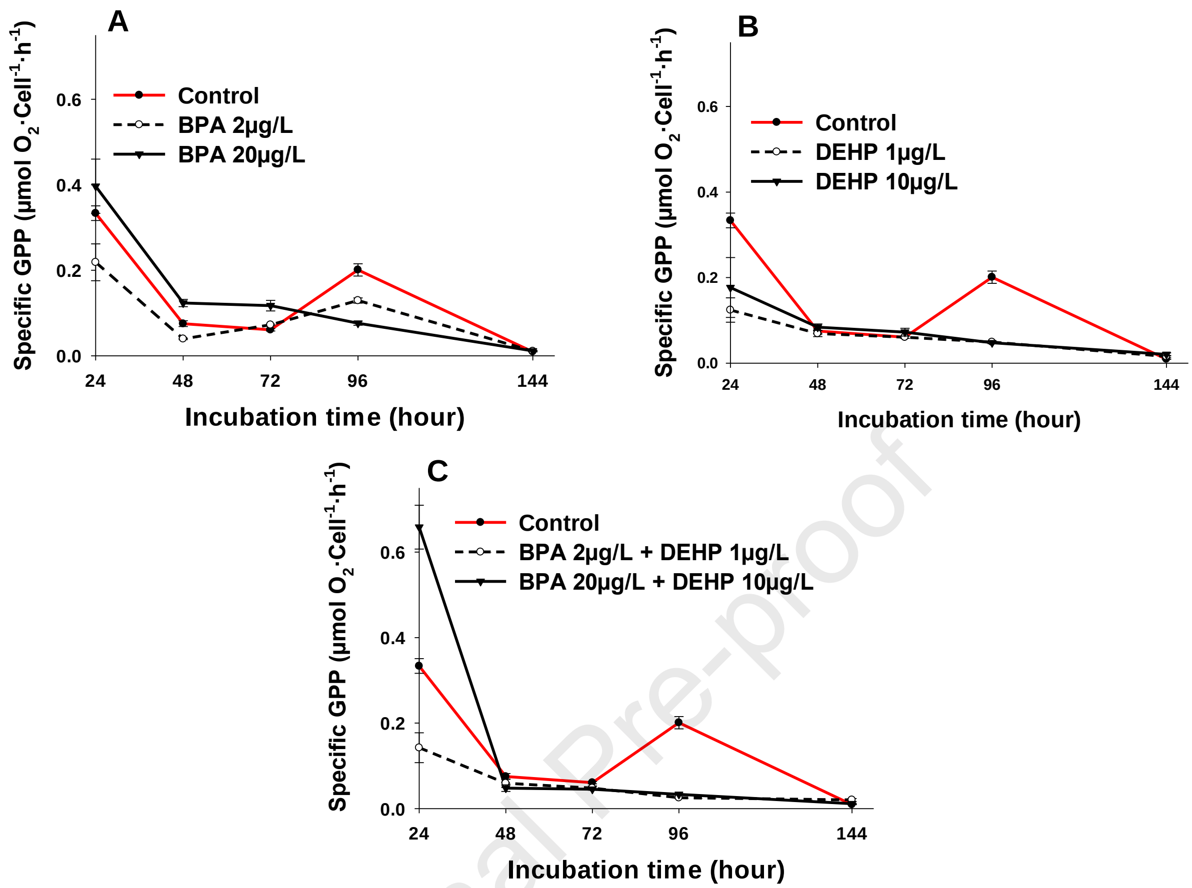

Figure 5: Specific phytoplankton activity (specific GPP), expressed in μmol $O_2 \cdot Cell^{-1} \cdot h^{-1}$ (mean ±S.D) in BPA (A), DEHP (B) and the mixture of BPA and DEHP (C) treatments, over time incubation and with respect to the control (red curve).

Supplementary material

Table S1: Nominal and measured concentrations of BPA and DEHP, expressed in µg/L at the beginning of the experiment

	BPA (µg/L)			DEHP (µg/L)	
Treatment	Nominal concentration	Measured concentration	**Treatment**	Nominal concentration	Measured concentration
Control	0	< 0.3	**Control**	0	0.2 ±0.03
BPA 2 µg/L	2	3.3 ±1.06	**DEHP 1 µg/L**	1	1.2 ±0.35
BPA 20 µg/L	20	20.1 ±2.53	**DEHP 10 µg/L**	10	10.1 ±1.35

BPA and DEHP chemical analysis

DEHP was analyzed using Solid-Phase Micro-Extraction (SPME) coupled with Gas Chromatography/Mass Spectrometry (GC/MS). For the extraction of water samples, 100 µm fiber SPME-PFMS (Supelco) was used. The quality parameters of the SPME were: i) an immersion of the fiber in liquid phase (15 mL of the sample), ii) an incubation temperature of 65 °C, iii) an incubation time of 5 min, iv) agitation 250 rpm and v) 30 min for extraction and 3 min for desorption. DEHP analysis was performed with GC/MS working in electro-ionization impact mode (GC-7890 A; MSD-5975C, Agilent Technologies). An HP5MS-UI column (5% phenyl methyl siloxane, 30 m × 0.25 mm ID × 0.25 µm phase thickness, Agilent Technologies) was used. BPA analysis was performed using Liquid Chromatography/tandem Mass Spectrometry (LC/MSMS) in a negative ionization mode (UPLC Acquity; MSMS-Quattro Premier XE, Waters). The cartridge used was an Acquity UPLC BEH C18 (50 mm × 2.1 mm ID × 1.7 µm granulometry, Waters). Direct injection volume was set at 40 µL. The quality parameters of chromatography were as follows: i) solvent tank A: milliQ-water with 0.5 mM ammonium acetate, ii) solvent tank B: methanol, iii) a mobile phase flow rate of 0.5 mL min-1. BPA and

DEHP were analyzed following the analytical protocols of (Dévier et al., 2013) for DEHP and of (Gagnaire et al., 2009) for BPA (information about the validation parameters in Table S2).

Table S2: DEHP and BPA were quantified by isotopic dilution (Internal standard compound DEHP-d4 and BPA-d16, respectively), the validation parameters for chemical analyses are mentioned in the table below:

Compound	Quantification transition (Collision energy) (m/z)	Quantification confirmation (Collision energy) (m/Z)	Retention time (min)	Dwell (ms)	Internal standard compound	Quantification transition (Collision energy) (m/z)	Rtention time (min)	Dwell (ms)
DEHP	149	167	23.98	100	DEHP-d4	153	23.97	100
BPA	227>212.1 (22)	227>132.9 (25)	1.1	120	BPA-d16	241.2>223.2 (20)	1.09	120

Table S3: Parameters (α, light utilization coefficient; P_{MAX}, maximum gross photosynthesis; E_K, irradiance for the light saturation of photosynthesis) of relative electron transport rate versus irradiance curves. α: Arbitrary unit; P_{MAX}: Arbitrary unit; E_K: µmol photon. $m^{-2}.s^{-1}$. The letters (a–c) indicate homogeneity of different treatments ($P < 0.05$)

photosynthetic performances		Control	BPA 2µg/L	BPA 20µg/L	DEHP 1µg/L	DEHP 10µg/L	BPA 2 µg/L + DEHP 1 µg/L	BPA 20µg/L + DEHP 10µg/L
α (a.u)	4d	0.72 ±0.01^{a}	0.70 ±0.02^{b}	0.72 ±0.01^{a}	0.71 ±0.01^{ab}	0.71 ±0.01^{ab}	0.70 ±0.01^{ab}	0.72 ±0.01^{a}
	7d	0.73 ±0.01^{a}	0.70 ±0.02^{a}	0.70 ±0.02^{a}	0.70 ±0.02^{a}	0.70 ±0.03^{a}	0.70 ±0.02^{a}	0.60 ±0.01^{b}
E (µmol photon $m^{-2}s^{-1}$)	4d	156 ±7.0^{a}	186 ±21.0^{b}	155 ±5.1^{a}	165 ±11.7^{ab}	157 ±11.7^{a}	167 ±21.1^{ab}	164 ±2.4^{ab}
	7d	101 ±3.2^{a}	113 ±10.1^{ab}	122 ±21.4^{ab}	113 ±22.4^{ab}	119 ±12.4^{ab}	136 ±22.4^{b}	139 ±27.5^{b}
P_{Max} (a.u)	4d	112 ±3.7^{a}	129 ±14.0^{a}	111 ±4.2^{a}	117 ±7.5^{a}	112 ±10.2^{a}	118 ±21.9^{a}	118 ±2.4^{a}
	7d	74 ±3.34^{a}	79 ±9.4^{a}	83 ±16.4^{a}	74 ±14.03^{a}	81 ±9.2^{a}	93 ±13.0^{a}	91 ±19.1^{a}

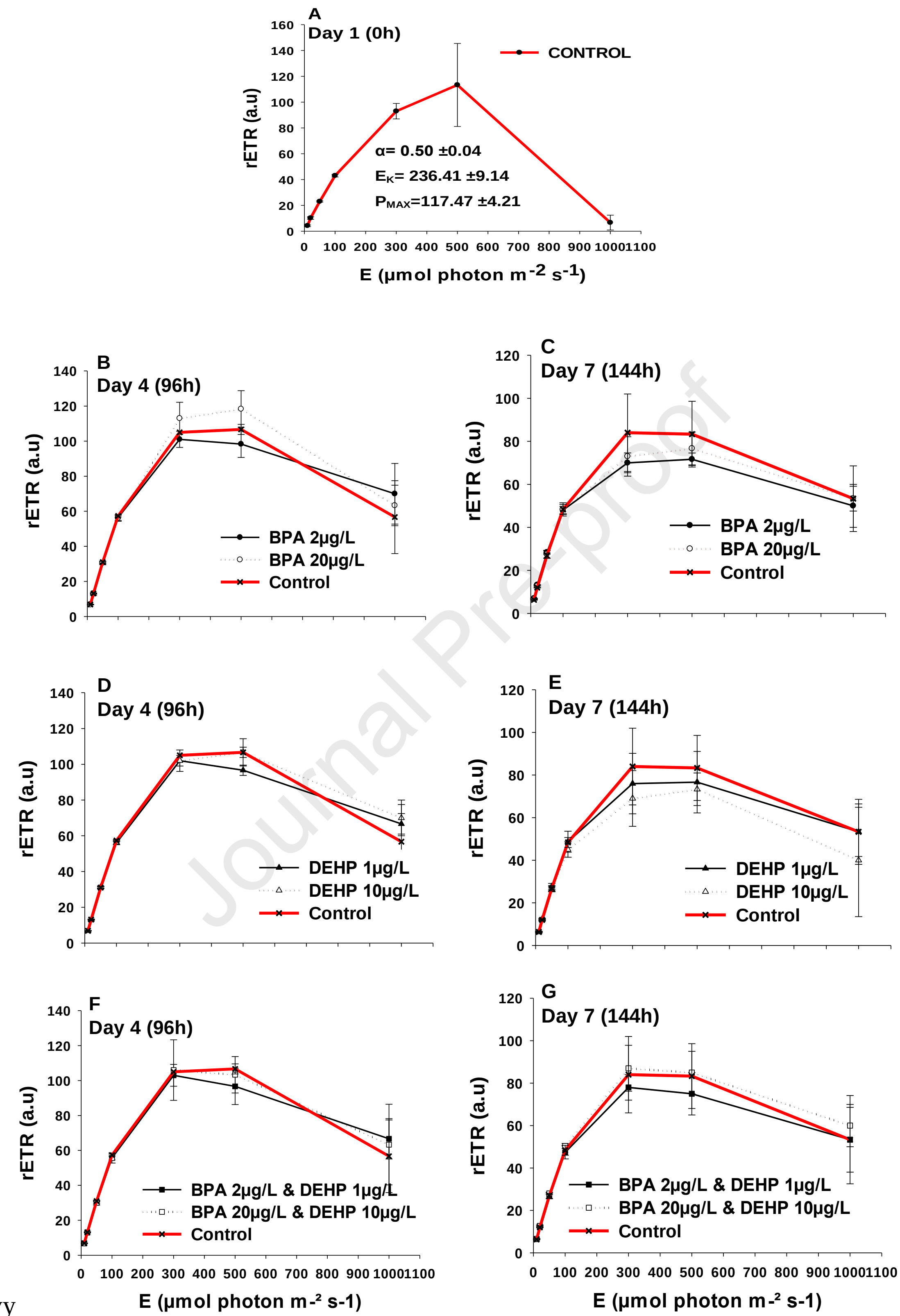

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Figure S1: Relative electron transport rate (rETR)-versus-Irradiance (E) curves for the diatom *C. decipiens-lorenzianus*: (A) at D1-0 h, which corresponds to the start of the experiment, (B) at D4-96 h which corresponds to the exposure of *C. decipiens-*

lorenzianus to BPA, (D) at D4-96 h which corresponds to the exposure of *C. decipiens-lorenzianus* to DEHP, (F) at D4-96 h which corresponds to the exposure of *C. decipiens-lorenzianus* to BPA and DEHP combined, (C) at D7-144 h which corresponds to the exposure of *C. decipiens-lorenzianus* to BPA, (E) at D7-144 h which corresponds to the exposure of *C. decipiens-lorenzianus* to DEHP, (G) at D7-144 h which corresponds to the exposure of *C. decipiens-lorenzianus* to BPA and DEHP combined.

Highlights:

- BPA and DEHP contamination do not significantly impact *C. decipiens-lorenzianus*.
- Slight impacts were observed on the biomass and photosynthetic activity over 48 h.
- *C. decipiens-lorenzianus* might be identified as a tolerant species to BPA and DEHP.
- This diatom might be tolerant to BPA and DEHP at environmental concentrations.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: