Structural and functional responses of coastal marine phytoplankton communities to PAH mixtures

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Abstract :

The toxicity of polycyclic aromatic hydrocarbons (PAHs) mixtures was evaluated on natural phytoplankton communities sampled from lagoons of Bizerte (South-western Mediterranean Sea) and Thau (North-western Mediterranean Sea). PAHs induced short-term dose and ecosystem-dependant decreases in photosynthetic potential. Chlorophyll a was negatively affected by increasing PAHs concentrations, together with dramatic changes in phytoplankton community composition. Size classes were strongly affected in the Bizerte compare to the Thau lagoon, with a decrease in nano- and microphytoplankton densities compare to picophytoplankton. In both locations, the diatom *Entomoneis paludosa* appeared favoured under PAH exposure as evidenced by increase in cell density, whereas autotrophic flagellates and dinophytes were strongly reduced. Smaller cells were more tolerant to exposure to highest PAHs concentrations, with persistent picophytoplankton carbon biomass at the end of the incubations. Apparent recovery of photosynthetic potential, accompanied with a regrowth of chlorophyll a under the lowest PAH doses, coincided with a significantly altered community composition in both lagoons. Furthermore, sensitivity to PAHs was not related to the phytoplankton cell size, and toxicity-induced modification of top-down control by grazers during the experiment cannot be excluded.

Highlights

► PAHs toxicity was assessed on natural phytoplankton from Thau and Bizerte lagoons. ► Dose-dependent effects on structure and metabolic endpoints are reported. ► Chlorophyll a biomass was more sensitive to PAHs than PSII efficiency. ► Thau lagoon phytoplankton community was more tolerant to PAHs mixture. ► Nano-and microphytoplankton densities decreased dramatically in Bizerte community.

Keywords : PAH mixtures (PAHs), Phytoplankton communities, Mediterranean coastal lagoons, Ecotoxicity

40 In recent decades, toxic pollutants in the environment has increased, particularly in coastal
41 ecosystems, together with concerns about their impacts (Halpern et al. 2008, Doney 2010).
42 Polycyclic aromatic hydrocarbons (PAHs) are considered to be especially hazardous because of
43 their mutagenic and carcinogenic properties (Samanta et al., 2002; Qiao et al., 2006; Sakari et al.,
44 2008). PAHs are present in complex mixtures and mostly introduced into the marine environment
45 *via* different sources (Nizzetto et al., 2008; González et al., 2006). Bizerte and Thau lagoons,
46 located in the Southwestern and Northwestern Mediterranean Sea respectively, are both subject to
47 environmental degradation due to anthropogenic activities (Solidoro et al., 2005). These two
48 lagoons are characterized by the presence of high levels of PAHs in sediments (Lafabrie et al.,
49 2013, Ben Othman et al. 2017). In fact, according to their hydrophobic nature, PAHs tend to be
50 sequestered within sediments (Mille et al., 2007). Besides their impact on benthic communities
51 (Mahmoudi et al., 2005; Louati et al., 2013; Sellami, 2015), PAHs can also cause a negative
52 impact on pelagic species in the water column after resuspension by anthropogenic and natural
53 processes (Roberts, 2012).

Phytoplankton plays an important role in the marine carbon cycle (Echeveste et al., 2010), and exposure to chemical contaminants is therefore prone to result in ecosystem alterations. Numerous studies have assessed the impact of single PAHs on growth (Djomo et al. (2004), photosynthetic processes (Pérez et al., 2010; Ben Othman et al., 2012) of phytoplankton species.

In natural conditions PAHs are present as a mixture and phytoplankton are present in complex and dynamic communities. Thus, the extrapolation of single compound / single species results to natural conditions remains ambiguous and uncertain for two reasons. The first, toxicity could change when considering a natural phytoplankton community including several phyla with different cell sizes. Secondly, the toxic effects and thresholds induced by separate PAHs are not necessarily the same when PAHs are administered simultaneously in a mixture, which is usually the case in the marine environment. In fact, a recent study showed that oceanic phytoplankton communities were strongly affected by PAHs mixtures and it also revealed that complex mixtures of PAHs can be more toxic by three orders than a single PAH in some cases (Echeveste et al., 2010).

The objective of this study was to compare the effects of PAHs mixtures on natural phytoplankton communities sampled from Thau and Bizerte lagoons. Individual compound choice and total mixture concentrations were selected based on the actual contamination of ecosystem sediment, as highlighted by previously documented PAH analyses.

2. Material and Methods

2.1. Study areas

2.1.1. Thau lagoon

The Thau lagoon (43° 24' N-3° 36' E, Fig. 1), is characterized by past industrial activities such as fuel power plant, small industries (foundries), oil refineries, fertilizer production, cement production (La Jeunesse, 2001, Léauté et al., 2008). The PAHs levels in superficial sediments

were up to 8700 $\mu\text{g Kg}^{-1}$ dry weight was reported from the “*Eaux Blanches*” bay (Ben Othman et al. 2017).

2.1.2. Bizerte lagoon

Bizerte Lagoon (37°8'-37°14' N, 9°48'-9°56' E; Fig. 1), is subjected to many anthropogenic pressures including urbanisation, industrial activities (e.g. cement works, metallurgical industry, boatyards). The lagoon is characterized by high levels of PAHs in sediments (up to 1490 $\mu\text{g Kg}^{-1}$ dry weight, Lafabrie et al., 2013).

2.2. Experimental framework

Based on the qualitative and quantitative results of PAHs in sediments from the Bizerte (Lafabrie et al., 2013) and Thau (Ben Othman et al. 2017) lagoons, a stock solutions of each type of PAH were prepared by dissolving pure chemicals in reagent grade dimethylsulfoxide (DMSO, 0.05% v/v) without exceeding the solubility threshold. The stock solutions were then mixed to obtain a "simple" mixture solution containing 8 % (dissolved concentration in w/v compare to the actual PAH concentrations in sediment in w/w) of every type of pure PAH. Finally, this solution (8%) was diluted with DMSO to obtain three intermediate mixtures: 2%, 1% and 0.1% of PAHs. These 8%, 2%, 1% and 0.1% values correspond respectively to a 8/100, 2/100, 1/100 and 0.1/100 ($\mu\text{g/L}$) of the total PAHs present ($\mu\text{g/kg}$ dry weight) in the Bizerte and Thau lagoons sediments. The choice of percentages relative to PAHs present in sediments was arbitrary. The theoretical concentrations corresponding to each mixture are presented in SP1. The following commercially available chemicals (purity >98%) were used: DMSO, anthracene (ANT), pyrene (PYR), benzo(a)pyrene (BAP), benzo(a)anthracene (BAA), chrysene (CHR), fluoranthene (FLU), indenopyrene (IND), naphthalene (NAP), phenanthrene (PHE), benzo(b)fluoranthene (BBF), benzo(g,h,i)perylene (BGP), benzo(k)fluoranthene (BKF). All chemicals were purchased from Sigma-aldrich and from AccuStandard Inc. USA.

Natural phytoplankton communities were sampled from the Thau lagoon (July 2012, at station T) and Bizerte lagoon (November 2012, at station B), and exposed to four PAH mixture concentrations in 4.5 L (final volume) closed microcosms. For every lagoon, seawater was sampled throughout the water column using a Nansen bottle over the entire water column and then filtered through a 200 μm mesh to remove large zooplankton and debris. Initial samples were taken to measure physico-chemical variables (salinity, pH, nutrients and dissolved PAHs) and phytoplankton proprieties (community composition, Chl *a* and Fv/Fm). Sieved water was dispensed into 18 glass microcosms (capacity 5 L, Schott-Duran) to perform six treatments in triplicate: a control (C), a control with DMSO (at 0.05% v/v) (C_{DMSO}) and four contaminated treatments; C1, C2, C3 and C4 which received 0.1%, 1% 2% and 8% of the PAH mixture respectively. The treatment C_{DMSO} was performed to test the toxicity of DMSO solvent to phytoplankton. To maintain exponential phytoplankton growth during the experiment, nutrient solutions were added to all the treatments according to the Redfield ratio of 16N:16Si:1P. All the microcosms were then incubated for 5 days *in situ*. The microcosms were sampled at different periods to measure biotic and abiotic factors.

2.3. Chemical Analyses

2.3.1. PAHs

PAHs were analyzed in all microcosms at the beginning of the experiment and every day in the treatment C3. The PAHs analysis was carried out using a GC-MS method after liquid-liquid extraction as described in Pringault et al. (2016).

2.3.2. Nutrients

For dissolved inorganic nitrogen ($\text{DIN} = \text{NO}_2^- + \text{NO}_3^- + \text{NH}_4^+$), PO_4^{3-} and $\text{Si}(\text{OH})_4$) analyses, subsamples were filtered on polycarbonate filters (0.2 μm) and the filtrates immediately frozen at -20 °C. Nutrients analyses were performed within few hours after room temperature thawing using spectrophotometric methods of Parsons et al. (1984) and Aminot and Krouel (2004).

2.3.3. Chlorophyll *a*

Samples for Chl *a* analysis were filtered on Whatman GF/F filters, and Chl *a* was extracted using 90% acetone for 30 h in the dark at 4°C. Pigment concentrations were measured using the spectrophotometric method according to Ritchie (2006).

2.3.4. Photosynthetic efficiency of PSII

Sub-samples were taken from each microcosm and dark-adapted for 20 min. The maximum quantum efficiency of PSII (Fv/Fm) was analyzed using an Aqua-Pen C (Photon System Instruments, www.psi.cz). The parameters F0, Fv and Fm are used to calculate the potential photosynthetic efficiency Fv/Fm. These parameters have been shown to be useful for determining stress effects on natural phytoplankton communities along environmental gradients (Moor et al., 2006).

2.3.5. Identification and enumeration of phytoplankton

For nanophytoplankton (2-20 µm) and microphytoplankton (20-200 µm), water samples were fixed with Lugol's acid solution (3% final concentration). Abundance was determined under an inverted microscope (Olympus IX70 or CETI; x100 objective). The phytoplankton analysis was performed according to the Utermöhl method (1958, AFNOR Standard 2006). For picophytoplankton enumeration (≤ 2 µm), water samples were fixed with formaldehyde (1% final concentration), frozen in liquid nitrogen (-180°C), and kept at -80°C until analysis. Picophytoplankton was enumerated and analyzed using a FACSCalibur flow cytometer (Becton-Dickinson, San Jose, CA) according to Marc Bouvy et al., 2006.

2.3.6. Estimation of carbon biomasses

The abundances (cells l⁻¹) of the three size fractions of phytoplankton were converted into carbon biomass (µg C l⁻¹) according to Mullin et al. (1966) and Li et al. (1992) for eukaryotic and prokaryotic picophytoplankton respectively.

Regarding the nano-and microphytoplankton, the dimensions and biovolumes were estimated according to Hillebrand et al. (1999). The cell volume for diatoms, dinoflagellates and other classes of flagellates, were converted to cell carbon using the formula according to Menden-Deuer and Lessard (2000) and Hillebrand et al. (1999).

The carbon biomasses of picophytoplankton, diatoms, dinoflagellates and other classes of flagellates were estimated by multiplying calculated cell carbon by measured abundances.

2.3.7. Dose-response evaluation

For the various treatments, initial phytoplankton carbon biomass and carbon biomass after 72 h of incubation were used to calculate the net carbon biomass growth rates of phytoplankton, using the exponential model, according to Sakka Hlaili et al. (2006).

Effective concentrations, EC50s (concentration inducing a response halfway between the control and the maximum toxicity effect) were used to compare available data on the toxicity of PAHs mixture to phytoplankton community and calculated using the REGTOX macro for Excel developed by E. Vindimian (Arzul et al., 2006). Optimal EC50s were selected and 95% confidence limits were given for each calculated value. The time 72 hours was chosen to measure physiological and population EC50 (Gregor et al., 2004; Leboulanger et al., 2006) because phytoplankton growth began to decrease after 96 hours in all treatments, probably due to nutrient depletion.

2.4. Statistical analyses

The Shannon index H (calculated using natural log transformed data) was used to express phytoplankton diversity. Data were processed using PAST 3.14 freeware (Hammer and Harper, 2005). Two-way ANOVAs (time and treatment) were used to test the impact of the different treatments on the biomass and the physiology of phytoplankton communities. When the effect was significant, ANOVA was followed by a Tukey's post hoc test, to identify which concentrations gave significantly different results from the control. The conditions of normality of distribution

(Kolmogorov– Smirnov test) and homogeneity of variance (Bartlett–Box test) were respected. Data were processed using PAST 3.14 freeware (Hammer and Harper, 2005).

3. Results and discussion

3.1. Initial environmental conditions

Salinity (37.34 - 37.80 PSU) and pH (8.12 - 8.13) did not differ significantly between stations B and T (Supplementary material 2 (SM2)). In both stations, total dissolved PAHs in the water column were below detection limits ($<0.01 \mu\text{g l}^{-1}$, SM1). In fact, despite the decrease in the concentration of PAHs in microcosms after their additions compared to theoretical concentrations, the media remained significantly contaminated with PAHs during the experiments.

Seawater from Bizerte lagoon was more nutrient enriched ($\text{NO}_3^- + \text{NO}_2^- + \text{NH}_4^+ = 9.35 \mu\text{M}$; and $\text{PO}_4^{3-} = 0.57 \mu\text{M}$) compared to the Thau lagoon ($\text{NO}_3^- + \text{NO}_2^- + \text{NH}_4^+ = 2.55 \mu\text{M}$; $\text{PO}_4^{3-} = 0.15 \mu\text{M}$). Silicate was more abundant in station T ($13.04 \mu\text{M}$) than station B ($10.18 \mu\text{M}$).

The efficiency of PSII was not different between stations (T: 0.6 ± 0.014 , B: 0.7 ± 0.017). Chl *a* showed similar concentrations for both stations (T: $3.19 \pm 0.01 \mu\text{g l}^{-1}$; B: $3.47 \pm 0.53 \mu\text{g l}^{-1}$).

In contrast to Chl *a*, carbon biomass of phytoplankton was higher at station B ($461.36 \mu\text{g C l}^{-1}$) than in station T ($371.60 \mu\text{g C l}^{-1}$). In the Bizerte Lagoon, the microphytoplankton (20 - 200 μm) dominated the percentage of total biomass (92%, $425.35 \pm 18.13 \mu\text{g C l}^{-1}$, SP2). The nanophytoplankton (2-20 μm : $28.86 \pm 4.36 \mu\text{g C l}^{-1}$) and the picoautotrophs ($<2 \mu\text{m}$: $7.14 \pm 0.33 \mu\text{g C l}^{-1}$) contributed to 6 % and 2% of total biomass, respectively.

In station T, the micro- and nanophytoplankton had almost equal biomasses (186.18 ± 11.92 and $177.02 \pm 39.61 \mu\text{g C l}^{-1}$, respectively). Picophytoplankton, by having an average biomass of $8.38 \pm 0.36 \mu\text{g C l}^{-1}$, contributed weakly to algal biomass (around 2%, SM2). The natural algal community at station B was composed mainly of flagellates (73% of total abundance). Diatoms and dinoflagellates represented 14 and 11% of total phytoplankton abundance respectively, while cyanobacteria and eukaryotic picophytoplankton accounted for 2% (SM2). In station T, natural

phytoplankton consisted almost equally of diatoms (52%) and flagellates (43%) but with a lower abundance of dinoflagellates (5%) (SM2).

Phytoplankton communities in the both lagoons exhibited the same species diversity, since the index H were similar between the two stations (B: 0.98 bits ind⁻¹, T: 0.95 bits ind⁻¹) (SM2).

3.2. Physico-chemical conditions during incubations

3.2.1. PAH concentrations

In Thau and Bizerte lagoons, daily monitoring of total PAHs in microcosms C3 showed a gradual decline in concentrations from the beginning (Thau= 78.73 µg l⁻¹; Bizerte=13.41 µg l⁻¹) to the end (Thau=25.17 µg l⁻¹; Bizerte= 2.4 µg l⁻¹) of incubation (Fig. 2).

Apart the effect of the volatilization and the adsorption of PAHs, other factors may be responsible for the reduction of PAHs during the incubation. Indeed, photooxidation (Miller et al., 2000; Djomo et al., 2004), biodegradation and bioaccumulation by different organisms (bacteria, phytoplankton and microzooplankton) can also change the fate of PAHs in the environment (Lekunberri et al., 2010; Gomiero et al., 2013).

3.2.2. Nutrient monitoring

At the beginning, nutrient concentrations were high (14.54 -15.42 N µM; 1.72-1.04 P µM; 14.08-14.67 Si µM, respectively in B and T stations), indicating that nutrient conditions were favourable for optimal growth of phytoplankton. Over time, in T station, nutrients showed gradual decreases from the beginning to the end of the experiment in the control (C) and in C1 (Fig. 3 a, b), suggesting active uptake. However, in C2, C3 and C4, the nutrient concentrations remained almost invariable during the incubation (Fig. 3 c, d, e), indicating that plankton growth was disrupted.

A similar trend was also detected during the experiment in Bizerte lagoon. Indeed, in C, C1 and C2, nutrients were significantly consumed by phytoplankton, resulting in their decrease during incubation (Fig. 3 f, g, h). However, in C3 and C4, nutrients remained unused, giving stable concentrations during the incubation (Fig. 3 i, j).

3.3. Chlorophyll *a* concentrations

For the Thau experiment, the Chl *a* concentrations did not significantly differ between C and C_{DMSO} treatments over time ($p>0.05$) (Fig 4 a). This indicated that the DMSO at 0.05% (v/v), used to prepare the PAH mixtures, had no toxic effect on phytoplankton growth during the experiments. In control microcosms (with and without DMSO), chlorophyll increased sharply during the first 48 hours (from 3.04 to 37 $\mu\text{g l}^{-1}$) and then remained stable at around 25 $\mu\text{g l}^{-1}$ (Fig. 4 a). The addition of increasing PAH concentrations was followed by a significant decrease in phytoplankton growth relative to the controls and an inhibition of growth at the higher PAH concentrations (Fig 4 a). The lower level of contamination (C1) induced a significant decrease ($P < 0.05$) in Chl *a* (3.04 - 17 $\mu\text{g Chl } a \text{ l}^{-1}$) compared to the control from the day 0 to day 4. In contrast, the moderate (C2) and the higher concentrations (C3 and C4) were followed by a marked inhibition of phytoplankton growth after a short time and consequently a significant decrease in Chl *a* (Fig 4 a). The EC50 measured for Chl *a* at 72h was 2.04 $\mu\text{g } \Sigma\text{PAHs l}^{-1}$ (SM3).

Similar results were obtained for the Bizerte experiment. Contamination by increasing ΣPAHs concentration also caused a significant ($P < 0.05$) decrease in Chl *a* relative to the control throughout the incubation (Fig 4 b). In control microcosms, Chl *a* increased from the beginning (3.47 $\mu\text{g l}^{-1}$) to the end of the experiment (22 $\mu\text{g l}^{-1}$). A short-term dose- effect was also detected in this experiment. In fact, during the first 48h, the fall of Chl *a* was strongest in the highest contamination level. Moreover, during the rest of the incubation, phytoplankton growth was very low in C2, C3 and C4, where the final Chl *a* concentrations were 5.04, 3.85 and 5.34 $\mu\text{g l}^{-1}$, respectively. Under the highest concentrations of PAH mixtures (C3 and C4), Chl *a* exhibited a renewed growth towards the end of the experiment but was still lower compared to the control. The EC50 measured for Chl *a* at 72 hours was 1.21 $\mu\text{g PAHs cocktail l}^{-1}$ (SM3).

During the Thau experiment, Chl *a* showed a strong increase after 24 hours incubation (from 3.04 to 26.56 $\mu\text{g Chl } a \text{ l}^{-1}$) (Fig 4 a), which was not the case during the Bizerte experiment (Fig 4 b). This increase could probably rely on the fact that the physiological state of the Thau

phytoplankton was more efficient than the community of Bizerte according to the season (spring vs. autumn).

The results of both experiments clearly showed the negative effect of PAH mixtures on the population growth of phytoplankton, which were not limited by nutrients (Fig 3). They also revealed that this effect was dose-dependent, since the decrease of Chl *a* was more pronounced when the level of the contamination increased. These results are in agreement with those of Echeveste et al. (2010), who also demonstrated that phytoplankton was strongly affected by higher concentrations of PAHs. Strong PAH contamination had an inhibitory effect (Bizerte lagoon) and even lethal effect (Thau lagoon) on phytoplankton. Phytoplankton seemed to be able to maintain growth in low and/or moderate contamination (C1 treatments and/or C2). This could be related to a taxonomic change within the community with the disappearance of species susceptible to PAHs and the appearance of resistant species.

3.4. Photosynthetic efficiency of PSII

As observed for Chl *a*, 0.05% (v/v) of DMSO had no significant effect on the PSII efficiency, since similar values of F_v/F_m (average 0.5 ± 0.025) were measured in the treatments C and C_{DMSO} in the experiment conducted in Thau lagoon (Fig 5 a). Other works have also reported the absence of significant DMSO toxicity on phytoplankton at similar concentrations (Djomo et al., 1996, 2004). Under low PAH contamination (treatment C1), no significant change ($P > 0.05$) was observed for F_v/F_m (average value 0.6) compared to the control, suggesting that algal physiology was not affected (Fig 5 a). However, photosynthetic potential sharply decreased during the first 48 h, under the other exposure levels. This reduction was reversible after 72 h in treatments C2 and C3, but not for microcosms C4, in which F_v/F_m exhibited significantly lower values (0.24) relatively to all the other treatments (Fig. 5 a).

During the Bizerte experiment, the treatment C1 had no significant ($P > 0.05$) effect on the F_v/F_m , which was around 0.7. Conversely, the C4 concentration caused the most pronounced decrease in the F_v/F_m after 24h. By the end of the C4 exposure incubation, the values remained

lower than those of the control (Fig. 5 b). The other treatments caused small decreases on Fv/Fm compared to the control (C2: average of 0.6; C3: average of 0.59) (Fig. 5 b). Under treatments C3 and C4, Fv/Fm increased at the end of the experiment, reaching values lower than in the control (Fig. 5 b).

These results revealed that PAH mixtures can also impact the physiology of phytoplankton, since the efficiency of PSII was reduced mainly at elevated concentrations. Contrary to Chl *a*, low exposure to PAH mixtures (C1) did not affect the Fv/Fm during both experiments (Fig. 5 a, b). In addition, the EC50s measured for Fv/Fm ($>75 \mu\text{g l}^{-1}$; $420 \mu\text{g l}^{-1}$ for Bizerte and Thau respectively) were significantly higher than the Chl *a* (0.03 to $2.04 \mu\text{g l}^{-1}$) (SP3). This suggests that growth of phytoplankton was more sensitive to PAH mixtures than photosynthetic activity (SM3).

In addition, the results showed that the community of Thau was more resistant than the Bizerte community and this on the physiological and population level, since the EC50 values were lower for Bizerte than Thau (SM3).

When we compare the responses of phytoplankton communities to PAH contamination at medium and high levels in both experiments, we detect an acute effect on growth comparing to the physiology over the short term (24 h) (Fig 4,5). This was also reported by Pérez et al. (2010), who showed that the growth rate of natural phytoplankton exposed to fuel oil was more sensitive than the Fv/Fm parameter.

3.5. Micro-nano and pico-phytoplankton

During both experiments, the three size classes of phytoplankton maintained growth in the control treatment. This indicated a non-limiting nutrient status (Fig 6). During the Thau experiment, the microphytoplankton showed a 17-fold increase in carbon biomass (from 186 to $3223 \mu\text{g C l}^{-1}$) in the controls (Fig 6.a). Concentrations C1 and C2 did not produce significant effects in the first 24 h of exposure, then microphytoplankton was stimulated under C1 treatment with a biomass increase from 776 (at 24 h) to $7129 \mu\text{g C l}^{-1}$ (at 120 h), this latter being higher than the control. Gonzalez et al. (2009) showed that larger diatoms (which constitute the fraction of

microphytoplankton) were stimulated by low concentrations of PAHs in microcosms. However, in treatment C2, biomass of microphytoplankton increased from 24 h ($832 \mu\text{g C l}^{-1}$) to 120 h ($1342 \mu\text{g C l}^{-1}$) but remained lower than in the control. For C3 and C4 treatments, a dose-response was reported after 24 h, with a significant decrease, and in inhibition of biomass at the end of incubation (Fig 6.a). For nanophytoplankton, a 20-fold increase in the control microcosms was measured, ranging from 177 (0 h) to $3669 \mu\text{g C l}^{-1}$ (Fig 6 b). In the other treatments, biomass was lower compared to the control after 24 h, in a dose-dependent pattern. After that, the nanophytoplankton in C1 treatment showed almost constant biomass towards the end of the incubation. But in microcosms C2, C3 and C4, the nanophytoplankton exhibited a drop in its biomass (C2: $13.77 \mu\text{g C l}^{-1}$) and even completely disappeared in microcosms C3 and C4, where higher doses of PAH mixtures were applied. The picophytoplankton showed a net growth in control microcosms from the beginning to the end of incubation (from 8.38 to $30.70 \mu\text{g C l}^{-1}$, Fig 6.c). In C1 treatment, there was a significant decrease from 24 h ($23 \mu\text{g C l}^{-1}$) and then towards the end of the experiment ($16.81 \mu\text{g C l}^{-1}$). In the other treatments, a dose-response was noted after 24 h (C2: $1.36 \mu\text{g C l}^{-1}$; C3: $0.1 \mu\text{g C l}^{-1}$; C4: $0.9 \mu\text{g C l}^{-1}$). Moreover, this decrease compared to the control continued to the end of the experiment and was more pronounced in the two most contaminated microcosms C3 ($0.29 \mu\text{g C l}^{-1}$) and C4 ($0.5 \mu\text{g C l}^{-1}$).

During the Bizerte experiment, the microphytoplankton was characterized by continued growth during the incubation in untreated microcosms. The biomass increased from $425.35 \mu\text{g C l}^{-1}$ (at 0 h) to $2317.58 \mu\text{g C l}^{-1}$ (at 120 h). Unlike in the Thau lagoon, microalgae in microcosms C1 showed a decrease from 1561.21 (at 24h) to $326.25 \mu\text{g C l}^{-1}$ compared to the control to the end of the experiment (Fig 6 d). For the other treatments (C2, C3 and C4), a significant decrease of these algae was observed in the short term, and was dose-dependent (Fig 6 d). At the end of the incubation, the C2 contaminated microphytoplankton had a lower biomass than the control (ranging from $566.91 \mu\text{g C l}^{-1}$ to $254 \mu\text{g C l}^{-1}$, respectively from 0 h to 120 h). However, in the

C3 and C4, these large producers disappeared earlier (from 72 h, Fig. 6d) than those of station T (after 120 h of incubation Fig. 6a).

The evolution of nanophytoplankton was almost the same as that of microphytoplankton. In fact, in the control, the nanophytoplankton showed a proliferation over time (from 28.86 to 495.67 $\mu\text{g C l}^{-1}$, Fig. 6 e). C1 contamination had no effect on these cells, except in the last day, when their biomass (190.95 $\mu\text{g C l}^{-1}$) decreased compared to the control. The other concentrations, C2, C3 and C4, induced a dose-dependent effect on biomass, and was almost lethal after 72 h for the two higher doses of PAHs mixtures (C3 and C4). This lethality was also observed early (from 72 hours; Fig. 6e) compared to the Thau experiment (at 120 h; Fig. 6b). Unlike the micro- and nanophytoplankton, the picophytoplankton maintained the same biomass (7.14 to 10.26 $\mu\text{g C l}^{-1}$) in the controls during the days of the experiment (Fig. 6f). The response of these small cells to C1 contamination was opposite to that detected during the experiment in the Thau lagoon (Fig. 6c), since they were stimulated by this treatment (Fig. 6f). The C2 treatment did not induce a significant effect up to 72 h, but in turn caused a twofold reduction in picophytoplankton biomass at the end of experiment. Under the highest concentrations (C3 and C4), a rapid decrease of picophytoplankton (C3: 3.96 $\mu\text{g C l}^{-1}$; C4: 3.37 $\mu\text{g C l}^{-1}$) was observed at 24 h. However, at 120 h, this size group was stimulated in the C4 treatment, since its biomass (18.84 $\mu\text{g C l}^{-1}$) exceeded the control (10.26 $\mu\text{g C l}^{-1}$). This little renewal of picophytoplankton (in C3 and C4) may explain the increase of Chl *a* (Fig. 4b) and Fv/Fm (Fig. 5b) detected at end of the experiment in the Bizerte lagoon. The tolerance of picophytoplankton to PAHs was already highlighted by some authors (Hjorth et al., 2007). It was reported that high levels of PAHs can eventually stimulate, after five days of contamination, the growth of cyanobacteria (Nayar et al. 2005).

3.6. Phytoplankton production rates

Addition of PAHs mixtures resulted in significant changes in phytoplankton production rates. These rates decreased in the different treatments compared to the control and this reduction was dose-dependent for both experiments (SM4). The most pronounced inhibition of production was

observed for the Bizerte community. This could reflect a higher sensitivity of phytoplankton in the Bizerte compared to Thau lagoon. In the latter, the highest tolerance of the producers community could be related to the higher level of PAH contamination in Thau sediments compared to those of the Bizerte lagoon (Ben Othman et al. 2017). Therefore, phytoplankton from the Thau lagoon could have developed adaptive mechanisms to tolerate the heavy pollution with PAHs in their environment. Such an adaptation could also explain the resistance of micro- and nanophytoplankton to the highest PAH exposures (C3 and C4) up to 120 h in Thau lagoon (Fig. 6a, b), whereas the equivalent populations were eliminated earlier (at 72 h) for the Bizerte lagoon experiment (Fig. 6d, e).

3.7. Taxonomic composition

3.7.1. Diatoms

At station T, the initial diatom community was dominated by *Skeletonema costatum* (87.5 %, SM5). After 24 h of incubation, this species continuously dominated the diatom community in the various microcosms, contributing to 83 - 98% of total diatoms cells (SM5). After 72 h incubation, *S. costatum* remained dominant (84 - 98 %) in all treatments, except in C1 (32%, SP5), where *Leptocylindrus danicus* became the prevailing species (50%, SP5). Moreover, in the C2 treatment, the large species, *Entomoneis paludosa* (biovolume = 7850 μm^3), appeared with a 7% of contribution (SM5). This species was the only diatom to persist until the end of incubation in C3 treatment (SM5). Under heavy contamination (C3 and C4), all diatoms disappeared. Several studies have shown that diatoms are tolerant to low concentrations of PAH mixtures; nevertheless, their sensitivity increased with the highest concentrations (Nayar et al., 2005; Gonzales et al., 2009). Other studies reported that the silicates present in the frustules of diatoms were involved in retention of PAHs (Karydis et al., 1979) which may cause alteration of the cell membrane. As a consequence, PAHs penetration into the cytoplasm was favoured, resulting in growth reduction (Bate et al., 1985). Other works have demonstrated a down-regulation of genes (*sil3*), which play

an important role in the formation of the silica shell, after exposure to PAH mixtures (Bopp and Lettieri 2007).

During the Bizerte experiment, the natural community of diatoms was highly dominated by the genus *Chaetoceros* spp. (90%) (SM6). After 24 h, *Chaetoceros* spp. remained dominant in all treatments, especially in C4, where they accounted for 81% of total diatoms (SP6). The species *Asterionellopsis glacialis* became more abundant (15.9 and 23%) in C1, C2 and C3 but completely disappeared in microcosms C4. As reported for the Thau experiment, the large diatom *Entomoneis paludosa* grew well under C1 and C2 treatments. At 72 h, in C1, diatoms were represented especially by *Chaetoceros* spp. (52%), whereas in the microcosms C2, *Cylindrotheca closterium* (11%), *Entomoneis paludosa* (18%) and *Navicula* spp. (22%) were dominant. However, under higher levels of PAHs contamination (C3 and C4 treatments), all diatoms disappeared (SM6). At the end of the experiment, the composition of diatoms in the control remained almost unchanged with a significant presence of *Thalassiosira* spp. (30%), and *Chaetoceros* spp. (42%). In microcosms C1, the most abundant species was *Chaetoceros* spp. (30%), whereas in the microcosms C2, *Entomoneis paludosa* (32%) and *Asterionellopsis glacialis* (28%) constituted the largest percentage of diatoms (SM6).

3.7.2. Dinoflagellates

The initial dinoflagellate community present in Thau lagoon was composed of 65% by species belonging to the genus *Gymnodinium* (SM7). After 24 h of incubation, these dinoflagellates remained dominant (73%) in the control and the remaining fraction was made up by *Alexandrium tamarense* (SM7). After 72 h of exposure, the dinoflagellates composition remained almost similar with the dominance of *Gymnodinium* spp. in the control (51%) and *Prorocentrum* spp. in all contaminated microcosms (C1: 61%, C2: 70%, C3: 66%, C4: 53%) (SM7). After 120 h, C2, C3 and C4 were lethal to dinoflagellates, which were able to maintain growth only under the lowest contamination (C1). In these microcosms, the most resistant dinoflagellates seemed to be *Prorocentrum* species (*P. spp.* (C1: 14%) and *P. gracile* (C1: 50%)) but also *Gymnodinium* spp.

(18.5%) and *Gyrodinium* spp. (15%). These latter species contributed the most to dinoflagellate community in the control (39%, SM7).

The initial composition of dinoflagellates in the Bizerte lagoon experiment were represented by several species especially *Protoperidinium* (12%), *Prorocentrum minimum* (10%) (SM8). After 24 h, species of *Prorocentrum* (especially *P. minimum*), *Gymnodinium*, *Ceratium* and *Protoperidinium* were the most abundant in C1, C2, C3 and C4 treatments (SM8). Species of the genus *Alexandrium* were observed in microcosms C1, C3 and C4. After 72 h, the growth of dinoflagellates was completely inhibited by C3 and C4 contamination, but they were able to maintain their growth after the C1 and C2 additions and this in case of *Gymnodinium* species (*G. pygmaeum* (C1: 34.4%) , *Gymnodinium* sp. (C2: 23.6%) and *Prorocentrum minimum* (SM8). At the end of the experiment, the dinoflagellates were observed only in the microcosms C1 and they were represented by four species (*Prorocentrum minimum* (18.3%), *Oblea* sp. (12.7%), *Gyrodinium* sp. (36.5%) and *Gymnodinium* sp. (32.5%)) (SM4). After 120 h incubation, dinoflagellates disappeared completely under C2 treatment, this was contrary to what has been observed for diatoms.

3.7.3. Flagellates

Flagellates (< 5 µm) formed 60% of the natural community of station T. The flagellates larger than 5 µm were represented by many species such as *Chromulina* sp. (2%), *Chrysochromulina* sp. (3%), *Dunaliella tertiolecta* (2%), *Hillea fusiformis* (7%), *Micromonas* sp. (6%), *Ochromonas* sp. (7%) and *Rhodomonas* sp. (5%) (SM5). After 24 h, the small flagellates (< 5 µm) remained dominant in the different treatments, except for the C4 treatment which completely inhibited the growth of flagellates from the first day of treatment (SM9). At 72 and 120 h, C3 and C4 contamination was lethal for all flagellates. Moreover, flagellates < 5 µm remained dominant in the control (64% at 72h and 24% at 120h) and the C1 (97% at 72h and 47% at 120h) and C2 (100% at 72 and 120h) treatments. The exception was *Plagioselmis* sp., which remained abundant, after 120 h of incubation in the control microcosms (50%) and C1 (50%) (SM9).

As observed for the station T, flagellates <5 µm were dominate from the beginning to the end of the experiment in the majority of treatments except in C2 at 24 h, where *Dinobryon* sp. (31%), *Dunaliella tertiolecta*, (16%), *Eutreptia* sp. (16%) and *Heterosigma* sp. (23%) were the most abundant (SP10). The disappearance of flagellates in the most contaminated microcosms (C3 and C4) after 72h and 120 h was also noted (SM10). Generally, we noted that flagellates from Bizerte and Thau lagoons did not show a difference in sensitivity. Indeed, this group was very sensitive to PAHs, especially after five days incubation in highly contaminated treatments C3 and C4. In fact, small flagellates (<5 µm) was the most growing phytoplankton group compared to the other. Also, at the end of the incubations, results reported a significant presence of picophytoplankton in C3 and C4 treatments, with the complete absence of other taxonomic groups. The proliferation of small phototrophic (picophytoplankton and other flagellated phytoplankton <5 µm) under heavy contamination could be related to a decrease in top-down control during the experiment from grazers like ciliates and heterotrophic flagellates (Sakka Hlaili et al., 2007; Stenuite, 2009). Indeed, recent studies have shown that these consumers can be affected by contaminants (Gomiero, 2013).

3.8.MDS analysis

The various responses of different groups, classes or species to PAH additions were observed throughout the experiments (Fig. 7). These observations were statistically confirmed by the MDS analysis.

For the Thau experiment, dissimilarity between the communities in C1 and the other treatments (C, C2, C3 and C4) was observed (Fig. 7a). This dissimilarity was approximately 36% (Data not shown). At 72 h, the dissimilarity (about 87 %, data not shown) was much more pronounced between the two groups (C1 Treatment and the group of C, C2 , C3 and C4) (Fig. 7b). Towards the end of the experiment (120 h), the three treatments C, C1 and C2 showed a highly significant dissimilarity among them (100% between C1 and C2) (Fig. 7c). A SIMPER analysis was performed to highlight the causative species of this dissimilarity (Data not shown). At 24 h,

the species *Skeletonema costatum*, *Hillea fusiformis* and *Pseudo-nitzschia* sp. caused the dissimilarity between the C1 treatment and control. At 72 h and 120 h, *Skeletonema costatum* and *Prorocentrum gracile* were the only species that induced this dissimilarity. Note that the species *Skeletonema costatum* was less abundant in the C1 treatment compared to the others. This was in disaccord with several works, showing that *S. costatum* is the most tolerant species of petroleum hydrocarbons in temperate waters (Gonzales et al., 2009). Other authors have also found that in low concentrations of PAHs, the diatom *S. costatum* was tolerant (Nayar et al., 2005).

For Bizerte's experiment, significant dispersion was also observed in the taxonomic structure of phytoplankton among all microcosms, reaching 50% to 75 % (Data not shown) at 24h (Fig. 7d). This dissimilarity remained almost the same (58.87 - 81 %; Data not shown) among the three treatments C, C1 and C2 from 72h (Fig. 7e) to 120 h (Fig. 7f) of incubation. SIMPER analysis revealed that *Gymnodinium pygmaeum* and *Pyramimonas* sp. were the most dominant species in C1 treatment at 24 h and caused the dissimilarity with the control treatment (Data not shown). However, other species were the causative of difference from the control at 72 h (*Pyramimonas* sp., *Thalassiosira* sp. and *Chaetoceros similis*) and at 120 h (*Eucampia* sp., *Thalassiosira pacifica*, *Entomoneis paludosa* , *Asterionellopsis glacialis* and *Dactyliosolen fragilissimus*). For the C2 treatment, several species (*Prorocentrum minimum*, *Protoperidinium minitum*, *Chaetoceros debilis*, *Dinobryon* sp. , *Gyrodinium* sp. and *Chaetoceros similis*) were dominant and responsible for the dissimilarity with the control at 24 h but others (*Hillea fusiformis*, *Entomoneis paludosa*, *Navicula transitans*, *Cylindrotheca closterium*, *Skeletonema costatum* and *Asterionellopsis glacialis*) were involved in the dissimilarity at 72 h. At the end of the experiment, the two species *Entomoneis paludosa* and *Asterionellopsis glacialis* were responsible for this effect. At 24 h, the heavily contaminated communities were dissimilar from those in control because of *Prorocentrum minimum*, *Protoperidinium cerasus*, *Oblea* sp., *Alexandrium catenella* , and *Navicula transitans* in C3 treatment and *Chaetoceros tenuissimus*, *C. debilis* and *C. danicus* in C4 contamination (Data not shown).

The results have clearly shown that in the two experiments, there was a reorganization and change in structure of the phytoplankton species composition and this depended of the dose and duration of exposure. This change due to the PAH contamination can induce the disappearance of susceptible species and the occurrence of others resistant species, such as the larger diatom *Entomoneis paludosa*, which showed a high resistance to PAHs when compared with other species in both ecosystems (SM5 and SM6).

4. Conclusion

The contamination experiments showed that physiology, population and growth of phytoplankton as well as its taxonomic composition were affected negatively by PAH mixtures and that the responses of endpoints were dose-dependent. Moreover, Chl *a* was more sensitive to PAH mixtures than the efficiency of PSII, since Chl *a* can potentially detect the toxicity of contaminants whose target is different from that of photosynthesis. Furthermore, it seems that the community of Thau lagoon was more tolerant to the PAH mixtures than that of Bizerte lagoon. In parallel, dramatic changes in phytoplankton communities compositions were also observed. Phytoplankton size classes from both lagoons showed various responses to PAH contamination. The nano- and microphytoplankton from Thau were not affected, whereas a decrease in those classes was observed in the Bizerte experiment. In both experiments, the large diatom *Entomoneis paludosa* was stimulated by the presence of PAH mixtures. Furthermore, autotrophic flagellates and dinoflagellates were negatively impacted but smaller cells including picophytoplankton were more tolerant to PAH.

The experiments conducted here provide important information on the impact of organic pollutants to marine phytoplankton. Moreover, these data provide insight into the fate of the marine phytoplankton after a petroleum processing and seepage/accidental oil spills, which may contain significant concentrations of PAHs (Yamada et al 2003).

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683 Figures captions

684 Fig. 1. Sampling stations in Thau (station T) and Bizerte (station B) lagoons.

685 Fig. 2. Daily changes in concentrations of total PAHs in the treatment C3 (contamination with 2%
686 of PAH cocktail) during the experiment conducted in the Thau (full circles) and Bizerte
687 (empty circles) lagoons.

688 Fig.3. Nutriments concentrations in different treatments [control (C) and PAH contaminations
689 (C1, C2, C3 and C4)] during several periods of the experiment conducted in the Thau and
690 Bizerte lagoons (Mean $\pm$ SD).

691 Fig.4. Daily evolution of Chl *a* concentrations in different treatments [control (C) and PAH
692 contaminations (C1, C2, C3 and C4)] during the experiment conducted in the Thau (a) and
693 Bizerte (b) lagoons (Mean $\pm$ SD)

694 Fig.5. Daily evolution of the efficiency of PSII (Fv/Fm) in different treatments [control (C) and
695 PAH contaminations (C1, C2, C3 and C4)] during the experiment conducted in the Thau
696 (a) and Bizerte (b) lagoons (Mean $\pm$ SD)

697 Fig.6. Temporal evolution of phytoplankton biomasses in different treatments [control (C) and
698 PAH contaminations (C1, C2, C3 and C4)] during the experiment conducted in the Thau
699 (a) and Bizerte (b) lagoons (Mean $\pm$ SD).

700 Fig. 7. Non-metric MDS ordination of the algal species biomass data from the control (C) and
701 PAH contaminations (C1, C2, C3 and C4) during experiments conducted in Thau and
702 Bizerte lagoons.

703

704

Fig. 1

Fig. 2

711

Fig. 3

713 Fig. 4

Fig. 5

Fig. 6

Fig. 7

Supplementary Material

Table SM1. Nominal and measured concentrations of the four PAHs cocktails ($\mu\text{g l}^{-1}$) used during the contaminated experiments in the lagoons of Bizerte (station B) and Thau (station T).

	Station B		Station T	
	Theoretical	Measured	Theoretical	Measured
Treatment C4 (8% of PAHs)	119	75.11	696	441.62
Treatment C3 (2% of PAHs)	30	13.41	174	78.73
Treatment C2 (1% of PAHs)	15	4.17	87	25.01
Treatment C1 (0.1% of PAHs)	1.5	0.31	8.7	1.83

Table SM2. Environmental conditions for each experiment carried out in the lagoons of Thau and Bizerte (DIN = $\text{NO}_3^- + \text{NO}_2^- + \text{NH}_4^+$)

	Station B	Station T
Salinity (PSU)	37.80	37.34
pH	8.12	8.13
PO_4^{-3} (μM)	0.57	0.15
DIN (μM)	9.35	2.55
Si(OH)_4 (μM)	10.18	13.04
ΣPAHs ($\mu\text{g l}^{-1}$)	<0.01	<0.01
Chl <i>a</i> ($\mu\text{g l}^{-1}$)	3.47 ± 0.53	3.19 ± 0.01
Fv/Fm	0.7 ± 0.017	0.6 ± 0.014
Carbon Biomasse ($\mu\text{g C l}^{-1}$)		
Microphytoplankton	425.35 ± 18.13	186.18 ± 11.92
Nanophytoplankton	28.86 ± 4.36	177.02 ± 39.61
Picophytoplankton	7.14 ± 0.33	8.38 ± 0.36
Taxonomic composition (%)		
Diatoms	14	52
Dinoflagellats	11	5
Other flagellats	73	43
Cyanobacteria	1	0
Eukaryote picophytoplankton	1	0
The Shannon index <i>H</i> (bits ind⁻¹)	0.98	0.95

Table SM3. Population (Chl *a*) and physiological (Fv/Fm) toxicity parameter for PAHs cocktails on marine natural phytoplankton after 72 h exposure.

	<∞5%	EC50 ([ΣPAH] µg l ⁻¹)	>∞5%
Chl <i>a</i>			
Station B	0,03	1,21	4,23
Station T	1,40	2,04	2,98
Fv/Fm			
Station B	-	> 75	-
Station T	414	420	434

EC50 are the values calculated by Regtox, corrected from measured PAHs cocktail concentrations; –, not determined.

Table SM4: Total phytoplankton production rates ($\mu\text{g C l}^{-1} \text{ h}^{-1}$) measured in the different treatments along the experiments conducted in the lagoons of Bizerte (B station) and Thau (T station)

	Station B	Station T
Treatments		
C	8.38	15.85
C1	4.69	14.44
C2	0	2.56
C3	0	0
C4	0	0

Table SM5: Diatoms species composition (expressed as % of total cell counts in the group) in initial sampling (IS) and at 24h, 72h and 120h in control (C) and in the other treatments (C1, C2, C3 and C4) at station T. Empty cells: taxa not found in the sample.

Species	IS	24 h					72 h					120 h				
		C	C1	C2	C3	C4	C	C1	C2	C3	C4	C	C1	C2	C3	C4
<i>Asterionellopsis glacialis</i>			1,45				12,9					0	2,65	2,65		
<i>Chaetoceros pseudobrevis</i>	0,25	0,14			0,27						0,68					
<i>Chaetoceros sp.</i>										0,49	0,42					
<i>Cyclotella sp.</i>	2,35	0,19	1,48	1,8	2,64	2,07	0,35		5,17	5,05	2,18					
<i>Cylindrotheca closterium</i>	1,38	0,43					0,25				1,68	0,21	4,95	4,95		
<i>Entomoneis paludosa</i>	0,32								6,82						100	
<i>Fragilaria sp.</i>									0,79				2,65	2,65		
<i>Fragilaria striatula</i>	0,25			0,6			2,58									
<i>Fragilariopsis atlantica</i>	0,25															
<i>Fragilariopsis cylindrus</i>		0,28														
<i>Fragilariopsis sp.</i>							0,28									
<i>Leptocylindrus danicus</i>	0,87	0,28			0,69		49						63,1	63,1		
<i>Leptocylindrus minimus</i>	0,44															
<i>Licmophora sp.</i>							3,21									
<i>Navicula sp.</i>									0,71							
<i>Navicula transitans</i>												0,21				
<i>Nitzschia longissima</i>	0,56				0,27		0,14				0,84					
<i>Nitzschia rectilonga</i>	0,22						0,13									
<i>Nitzschia sp.</i>	0,87	0,43	2,96			0,46	0,14		0,36				4,54	4,54		
<i>Pleurosigma strigosum</i>			1,48													
<i>Pseudo-Nitzschia delicatissima</i>	1,53		2,9													
<i>Pseudo-Nitzschia pungens</i>	0,25															
<i>Pseudo-Nitzschia pseudodelicatissima</i>	1,6	0,37				0,36	0,28									
<i>Pseudo-Nitzschia seriata</i>		0,14														
<i>Pseudo-Nitzschia sp.</i>	1,31		5,93	3,38	1,35	2,09			1,81	0,38						
<i>Skeletonema costatum</i>	87,5	97,7	83,8	94,2	94,8	95	98,4	32,4	84,3	94,1	94,2	99,6	22,2	22,2		

Table SM6: Diatoms species composition (expressed as % of total cell counts in the group) in initial sampling (IS) and at 24h, 72h and 120h in control (C) and in the other treatments (C1, C2, C3 and C4) at station B. Empty cells: taxa not found in the sample.

Species	IS	24 h					72 h					120 h				
		C	C1	C2	C3	C4	C	C1	C2	C3	C4	C	C1	C2	C3	C4
<i>Achanthes longipes</i>														1,66		
<i>Amphora</i> sp.								2,92	2,92							
<i>Asterionellopsis glacialis</i>	6,34	22,6	22	15,9	17,6		12,6	5,84	14,6			1,66	8,65	27,5		
<i>Chaetoceros</i> spp	85,2	59,4	54,3	43	52,9	81,5	36,9	52,8	3,38			42,1	29,8	5,96		
<i>Cyclotella</i> sp.														1,66		
<i>Cylindrotheca closterium</i>	3,19	4,46	2,78	7,47	8,82	4,21			11					3,04		
<i>Dactyliosolen fragilissimus</i>							1,46					3,6	10,1			
<i>Entomoneis paludosa</i>			5,28	7,47			1,46	6,33	18,2			1	9,08	31,5		
<i>Entomoneis</i> sp.		2,29				2,74	0,9					4,32				
<i>Eucampia</i> sp.													16,6			
<i>Fragilaria</i> sp.			2,78											1,66		
<i>Fragilaria striatula</i>						1,14										
<i>Fragillariopsis</i> sp.		2,29				0			2,92					1,25		
<i>Guinardia striata</i>		4,46				0,97	1,8									
<i>Leptocylindrus danicus</i>			7,29				5,41					4,68				
<i>Licmophora gracilis</i>							0,9							3,04		
<i>Licmophora</i> sp.				5,3					5,84							
<i>Lyrella clavata</i>									0					1,66		
<i>Navicula</i> spp.			2,78	14,9	20,6	1,14	2,93		22,3			0,5	1,39	5,54		
<i>Nitzschia pellucida</i>														1,25		
<i>Nitzschia rectilonga</i>							0,9									
<i>Nitzschia</i> sp.		4,46				2,07	5,85	5,84	4,2			1,98	1,82	3,88		
<i>Pleurosigma strigosum</i>			2,78	5,91			1,8	2,92	0			1	4,38			
<i>Pseudo-nitzschia</i> spp	5,28					6,22	3,61	2,92	5,84			4,84				
<i>Skeletonema costatum</i>									8,76			4,66				
<i>Thalassiosira</i> spp.							23,4	20,5				29,7	18,2	10,4		

Table SM7: Dinoflagellates species composition (expressed as % of total cell counts in the group) in initial sampling (IS) and at 24h, 72h and 120h in control (C) and in the other treatments (C1, C2, C3 and C4) at station T. Empty cells: taxa not found in the sample.

Species	24 h						72 h					120 h				
	IS	C	C1	C2	C3	C4	C	C1	C2	C3	C4	C	C1	C2	C3	C4
<i>Alexandrium catenella</i>	2,51				12,4											
<i>Alexandrium tamarense</i>		26,7														
<i>Ceratium furca</i>				8,19												
<i>Eutreptiella sp.</i>	4,97															
<i>Gonyaulax sp.</i>				8,19												
<i>Gymnodinium pygmaeum</i>	2,51						27,7									
<i>Gymnodinium roseostigma</i>	2,51															
<i>Gymnodinium sp.</i>	60	73,3	24,5			34,4	50,8	7,1					18,5			
<i>Gyrodinium flagellare</i>	2,51															
<i>Gyrodinium sp</i>	2,51				19,3			19,8			37,4	39,2	14,8			
<i>Gyrodinium spirale</i>	2,51															
<i>Prorocentrum gracile</i>	2,51		33,6	49,2	35,9	28,3	8,85	61	70	65,8	46,3	30,4	49,5			
<i>Prorocentrum minimum</i>	5,03		16,8	24,6	22,8	28,3	12,7	5,44	19,2	15,5	16,3	21,5	14			
<i>Prorocentrum sp.</i>	4,97															
<i>Protoperidinium bipes</i>			12,9	9,87												
<i>Protoperidinium caudicans</i>									10,8	18,6						
<i>Protoperidinium cerasus</i>								1,61								
<i>Protoperidinium granii</i>	4,97											8,85				
<i>Protoperidinium minutum</i>	2,49															
<i>Protoperidinium ovatum</i>			12,2					1,61					1,07			
<i>Protoperidinium pallidum</i>						8,93										
<i>Protoperidinium sp</i>					9,66											
<i>Scipsiella sp.</i>							3,44						2,13			

Table SM8: Dinoflagellates species composition (expressed as % of total cell counts in the group) in initial sampling (IS) and at 24h, 72h and 120h in control (C) and in the other treatments (C1, C2, C3 and C4) at station B. Empty cells: taxa not found in the sample.

Species	IS	24 h					72 h					120 h				
		C	C1	C2	C3	C4	C	C1	C2	C3	C4	C	C1	C2	C3	C4
<i>Alexandrium catenella</i>					8,7	2,41		9,3								
<i>Alexandrium sp.</i>			5,61													
<i>Ceratium furca</i>	4,19	15,2	11,8	11,5		4,54	9,28	9,3				7,14				
<i>Gonyaulax sp</i>	3,16		2,71		6,52	4,28		8,6								
<i>Gymnodinium mikimotoi</i>	13,4	16				2,14										
<i>Gymnodinium pygmaeum</i>		7,2	23		6,52		15,8	34,4	17,4			17,9				
<i>Gymnodinium sp</i>	28,4	7,2	8,68	9,32		20,8	27,8		23,6			14,3	32,5			
<i>Gymnodinium subroseum</i>	4,19															
<i>Gyrodinium heterostriatum</i>							5,46									
<i>Gyrodinium fusiformis</i>	3,16		4,87				12,8									
<i>Gyrodinium metum</i>							10,9					7,14				
<i>Gyrodinium sp</i>	9,47	11,5	17,4	20,2	8,7	18,2	4,28					28,6	36,5			
<i>Gyrodinium spirale</i>	8,39	7,2														
<i>Oblea sp.</i>			2,2		8,7								12,7			
<i>Prorocentrum compressum</i>		4,01														
<i>Prorocentrum micans</i>								9,3	18,1							
<i>Prorocentrum minimum</i>	10,3	15,2	23,8	33,2	34,8	28,6	9,28	29,1	23,6			14,3	18,3			
<i>Protoperidinium cerasus</i>	6,08	11,5			26,1		4,28					10,7				
<i>Protoperidinium conicum</i>									17,4							
<i>Protoperidinium minutum</i>				25,8												
<i>Protoperidinium ovatum</i>	6,08					8,57										
<i>Protoperidinium sp</i>						10,5										
<i>Scrippsiella trochoidea</i>		5,07														
<i>Scripsiella sp</i>	3,16															

Table SM9: Others flagellates species composition (expressed as % of total cell counts in the group) in initial sampling (IS) and at 24h, 72h and 120h in control (C) and in the other treatments (C1, C2, C3 and C4) at station T. Empty cells: taxa not found in the sample.

Species	IS	24 h					72 h					120 h				
		C	C1	C2	C3	C4	C	C1	C2	C3	C4	C	C1	C2	C3	C4
<i>Chlamydomonas sp.</i>	1,97		0,33									0,26				
<i>Chromulina sp.</i>	3,29	2,93														
<i>Chrysochromulina sp.</i>		7,36					3,89					3,83				
<i>Corymbellus sp.</i>												0,65				
<i>Cymbomonas sp.</i>	1,97		0,64									0,65	0,16			
<i>Dunaniella tertiolecta</i>		1,89	0,32				1,14					1,47				
<i>Euglena sp.</i>	0,66		0,64					0,21								
<i>Eutreptiella sp.</i>	57,9											0,09				
Flagellats <5µm	0,66	58	93,8	57,1	100	0	63,8	96,6	100			24,2	47	100		
<i>Hemiselmis sp.</i>	0,54															
<i>Heterosigma sp.</i>	7,02		0,33				2,28	0,38				2,03				
<i>Hillea fusiformis</i>		4,1	1,57				5,7	0,96				2,77	1,33			
<i>Imantonia sp.</i>	0,54						4,56					0,65				
<i>Isochrysis galbana</i>	0,81		0,64				1,14					3,9				
<i>Mamiella sp.</i>	4,59															
<i>Micromonas sp.</i>	0,54						1,14					5,19				
<i>Nephroselmis sp.</i>							1,14									
Non identified flagellats	7,4			42,9								2,08				
<i>Ochromonas sp.</i>	4,27															
<i>Plagioselmis sp.</i>		8,79					2,6					50	50			
<i>Prymnesium sp.</i>	0,66						1,14									
<i>Pyramimonas sp.</i>	4,53	4,63	0,67				5,19	0,53				1,95	1,53			
<i>Rhodomonas sp.</i>	2,63		1					1,27				0,3				
<i>Teleaulax sp.</i>		12,3					5,19									
<i>Tetraselmis sp.</i>							1,14									

Table SM10: Others flagellates species composition (expressed as % of total cell counts in the group) in initial sampling (IS) and at 24h, 72h and 120h in control (C) and in the other treatments (C1, C2, C3 and C4) at station B. Empty cells: taxa not found in the sample.

Species	IS	24 h					72 h					120 h				
		C	C1	C2	C3	C4	C	C1	C2	C3	C4	C	C1	C2	C3	C4
<i>Chlamydomonas sp.</i>	0,86	1,33	0,65				3,16					11,1	4,55			
<i>Chorymbellus sp.</i>							0,25									
<i>Chroomonas sp.</i>							0,25	0,9								
<i>Chrysochromulina sp.</i>	0,94	2,89				8,02										
<i>Cymbomonas sp.</i>			0,91			8,02		0,34				1,23		0,7		
<i>Dictyocha sp.</i>														0,35		
<i>Dinobryon sp.</i>			0,65	31	13,3			0,69	0,92					0,94		
<i>Dunaniella sp.</i>						12,3								0		
<i>Dunaniella tertiolecta</i>				15,5			1,52					2,05		1,76		
<i>Euglena sp.</i>							0,25							0		
<i>Eutreptia sp.</i>	1,42	0,39	0,71	15,5									0,44	0,35		
Flagellés <5µm	69,2	83,2	59,2		25,8	71,6	77,5	81,8	92,2			68,3	84,9	88,7		
<i>Heterosigma sp.</i>		0,39	0	22,6			0,58	0,34	0,46			1,53	0,44	1,41		
<i>Hillea fusiformis</i>	15	6,91	5,81		12,9		5,39	1,03	3,8			1,02	2,18	2,66		
<i>Imantonia sp.</i>	2,36	0,53					1,12		0,46			1,06		0		
<i>Isochrysis galbana</i>		1,08					0,76					0,51		0,35		
<i>Mamiella sp.</i>							0,25							0,7		
<i>Menosiga sp.</i>								0,86								
<i>Meringosphaera sp.</i>			0,65													
<i>Micromonas sp.</i>												6,07				
<i>Nephroselmis sp.</i>			0,65				1,01					0,66	0,96			
Non identified flagellats			5,48		34,8			1,14					0,87	1,06		
<i>Pseudopedinella sp.</i>	0,41						0,51					1,02				
<i>Pyramimonas sp.</i>	8,32	2,92	24,8	15,5	13,3		3,92	12,9	1,24			4,4	4,27	0,55		
<i>Resultor sp.</i>							1,12									
<i>Rhodomonas sp.</i>	1,45		0,46				0,25		0,92				1,44	0,47		
<i>Telaecaulax sp.</i>							1,49									
<i>Tetraselmis sp.</i>		0,39					0,69					0,51				