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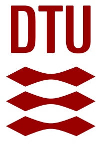

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Pančić, Marina; Köhler, Eva; Paulsen, Maria Lund; Toxværd, Kirstine Underbjerg; Lacroix, Camille; Le Floch, Stéphane; Hjorth, Morten; Nielsen, Torkel Gissel

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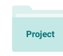

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Effects of oil spill response technologies on marine microorganisms in the high Arctic

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ABSTRACT

We studied how exposure to oil spill response technologies affect marine microorganisms during Arctic winter and spring. Microorganisms were exposed to chemically dispersed oil (DISP), *in situ* burnt oil (ISB), and natural attenuated oil (NATT) in mesocosms from February to May. We subsampled the mesocosms and studied the effects of oil in laboratory incubations as changes in biomass of the major functional groups: bacteria, heterotrophic-nanoflagellates, dinoflagellates, ciliates, pico- and nanophytoplankton, and diatoms over two 14-day periods. In winter, the majority of polycyclic aromatic hydrocarbons (PAHs) remained encapsulated in the ice, and the low concentrations of PAHs in water led to minute changes in biomass of the investigated groups. In spring, however, when the PAHs were partially released from the melting ice, the biomass of many functional groups in DISP and NATT decreased significantly, while the changes in ISB were less pronounced. The overall biomass reduction, as observed in this study, could lead to a disrupted transfer of energy from the primary producers to the higher trophic levels in oil affected areas.

1. Introduction

The area north of the Arctic Circle may contain around 30% of the world's undiscovered gas and 13% of the world's unexplored oil reserves (U.S. Geological Survey, 2009). Recent retreat of the Arctic sea ice due to global warming coupled with rapid technological developments for petroleum exploration have accelerated access to the offshore Arctic regions and, thus, have improved prospects for exploration and production of oil and gas in these areas (Gautier et al., 2009).

Increasing activities in Arctic petroleum development and associated risks of oil spill accidents have led tothe development of several oil spill remediation technologies that could be applied in sub-zero environments (Wilkinson et al., 2017). The response tools considered within the contingency plans for the worst-case scenario in the Arctic include employment of chemical dispersants, *in situ* burning of crude oil, and natural oil weathering. In the event of an oil spill accident, however, urgent decisions must be made on how to minimize the impacts on the environment. First and foremost, the decision process includes assessment of potential impacts on various ecosystem components, for which acute (i.e. immediate) toxicity data or chronic (i.e. long-term) exposure thresholds are used (Olsen et al., 2013). The assessments based on acute toxicity tests, however, may inaccurately estimate the effects of long-term low-dose releases of oil from the sea ice and, consequently, lead to inaccurate predictions of the oil spill effects on the Arctic marine ecosystems. Therefore, chronic exposure studies in combination with acute toxicity data would enable more realistic forecasting of the consequences that oil spills and oil spill response technologies would have on the Arctic marine environments.

Given that more than 90% of all biological processes in the ocean are driven by microorganisms (Hays et al., 2005), a comprehensive understanding of how oil spills and the response technologies would affect these communities is crucial. To date, most of the few studies that

Abbreviations: DISP, chemical dispersion; ISB, *in situ* burning; NATT, natural attenuation; PAH, polycyclic aromatic hydrocarbon; HNF, heterotrophic nanoflagellates

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have examined the effects of oil spills and oil spill response technologies on microbial communities have been conducted in temperate and subtropical environments. Those studies reported an increased abundance of heterotrophic bacteria exposed to different oil types and remediation treatments, indicating that the oil treatments increased the carbon pool that stimulated the growth of some bacteria (Parsons et al., 1984; Linden et al., 1987; Harayama et al., 2004; Koshikawa et al., 2007; Jung et al., 2010; Ortmann et al., 2012). Secondarily, the elevated bacterial growth resulted in increased abundances of bacterivorous heterotrophic nanoflagellates (HNF) (Parsons et al., 1984). The effects of oil spills and oil spill remediation technologies on marine phytoplankton are more equivocal. Some studies reported overall negative effects on picoplankton, nanoplankton, and diatom assemblages (Parsons et al., 1984; Harrison et al., 1986; Linden et al., 1987; Sargian et al., 2005; González et al., 2009), whereas other observed increased growth of haptophytes (*Chrysochromulina* sp.), chrysophytes, prasinophytes, and diatoms (*Chaetoceros* sp., *Nitzschia* sp.) (Oviatt et al., 1982; Vargo et al., 1982; Parsons et al., 1984). Only few studies reported no effect on either phytoplankton diversity or activity (Scott and Glooschenko, 1984). Although oil pollution may lead to higher mortality rates of some phytoplankton groups during first few days, their overall biomass may later increase due to decreased predation pressure (Parsons et al., 1984; Harrison et al., 1986; Linden et al., 1987; Vargo et al., 1982; Sargian et al., 2005; González et al., 2009; Abbriano et al., 2011), because even low concentrations of PAHs can have highly toxic effects on microzooplankton (Almeda et al., 2014). As microzooplankton, such as ciliates and heterotrophic dinoflagellates, are pivotal in marine food webs (Landry and Calbet, 2004), the detrimental effects of oil pollution could disrupt the structure and function of the entire microbial community and, consequently, in the transfer of carbon to higher trophic levels (Ortmann et al., 2012; Almeda et al., 2014).

The toxicity of petroleum products on Arctic microbial communities remains poorly investigated, partly due to operational constraints in the Arctic environment. As yet, few studies on effects of oil spills and the response technologies have been conducted in high-latitudes (e.g., Siron et al., 1993, 1996), with some progress in understanding the bacterial responses to oil pollution there (Delille and Siron, 1993). Because of the low biomass of bacteria and their highly selective biodegradation of oil compounds, in combination with the low evaporation losses at low temperatures, the residence time of the toxic oil compounds may be extended in sub-zero environments (Siron et al., 1993, 1996). Nevertheless, it remains unknown how phytoplankton and microzooplankton respond to oil pollution in the Arctic environment.

The current study was part of a mesocosm campaign conducted in Svalbard in 2015 (Dickins, 2017). The aim of our study was to compare the changes in biomass of the major functional groups of marine microorganisms exposed to various oil spill response technologies. We present results from two incubation microcosm studies with microbial assemblages collected from the mesocosms in March (winter; one month after adding the chemical treatments) and May (spring; three months later), whose biomasses were then assessed over a 14-day period in the controlled laboratory incubation experiments.

2. Materials and methods

2.1. Field site location and mesocosm setup

The mesocosms were deployed in Van Mijenfjorden, Svea on the west coast of Spitsbergen, Svalbard (77°52′13″N 16°44′44″E) (Fig. 1). This industrial fjord with coal mine activities was chosen due to its geographical position that allows the sea ice to persist until late spring, as well as its accessibility by various modes of transportation from Longyearbyen to the site. Eight mesocosms were mounted and anchored in sea ice in February 2015 (Fig. 1). For detailed information regarding the mesocosms see Toxværd et al., (2018). Briefly, large holes were drilled into the ice approx. 800 m offshore and the mesocosms (3-m long by 1.6 m diameter; 6000 L) were lowered into the water in two rows approx. 20–25 m apart. Two control mesocosms were deployed first about 40 m distance from the other mesocosms to avoid contamination during application of chemicals (Fig. 1). Subsequently, chemical treatments were added to the oil mesocosms: 2 L of burnt oil residuals (abbreviated ISB) obtained by burning of 20 L of crude oil *Kobbe* under *in situ* conditions (INERIS, France); a surface slick of 20 L of crude oil *Kobbe* mixed with 1 L of dispersant Finasol® OSR 52 (abbreviated DISP) without any additional mixing energy (Total Fluides, Paris-La Defense, France); and 20 L of crude oil *Kobbe* (abbreviated NATT) (~10 L m^{-2} and oil slick thickness of 0.25 mm) (Toxværd et al., 2018). After treatment, ice was allowed to form naturally in all mesocosms until the samplings for laboratory incubation microcosm studies in March 2015 (winter) and May 2015 (spring).

Water sampling from the mesocosms for incubation microcosm studies was conducted on 26 March and on 14 May 2015. Holes (10 cm diameter) were drilled through the ice in each mesocosm and pipes were inserted into the ice-core holes. The pipes served as a scaffold for the pumps, to prevent contamination from oil in the ice structures, and to avoid disturbing the water column during pumping. The ice-core holes were cleaned of slush and hand-operated pumps with a rigid intake and a flexible outtake were inserted into the pipes. In order to remove any large zooplankton from the pumped water, the outtake of the pumps was covered with a 200-μm mesh filter. From each mesocosm, 20 L of seawater was pumped (approximately 4.6 L min^{-1}) from approximately 10 cm beneath the ice edge into 23-L insulated glass bottles to prevent freezing. The water samples were stored at 1 °C in darkness for < 12 h until being transported to the University Centre in Svalbard, Longyearbyen on 27 March and 15 May 2015.

2.2. Set-up of incubation microcosms

The 20-L water samples from each of the two control mesocosms were pooled, and from the 40-L pool, triplicate 5-L incubation control microcosms prepared and incubated for 14 days (remainder of the pooled water was discarded). The mesocosm water samples from each treatment were pooled to reduce natural variance, should that occur, in the duplicate mesocosms. The same method was applied to the 2 × 20-L water samples obtained from ISB, DISP, and NATT mesocosms. The microcosms were kept at 1 °C, stirred manually at least twice a day, and exposed to 40–50 μmol photons m^{-2} s^{-1} (to simulate ambient solar irradiance of 600 μmol photons m^{-2} s^{-1}, of which < 10% penetrates through ~100 cm thick sea ice; Little et al., 1972) with a light:dark cycle of 16:8 h. The same procedure was applied in March and May. Salinity of the water samples was 33.8 in March and 31.9 in May, as measured with a VWR SympHony SP90M5 salinometer (VWR International, Inc.). The air and water temperatures were measured continuously using HOBO Data Loggers, whereas the light intensity and pH were measured every second day at approximately the same time using LI-COR Biosciences, model LI-1400 Data Logger (Biosciences, Lincoln, NE, USA) and Thermo Electron Corporation, Orion Star Series with a ROSS Ultra combination pH electrode (Thermo Electron Corporation, Beverly, MA, USA), respectively. The pH electrode was calibrated weekly (2-point calibration) using Thermo Electron Corporation, Orion Application Solution buffers of pH 7.0 and 10.0 dilutions.

Subsamples (3 × 60 mL) for measurements of inorganic nutrients were removed from incubation bottles and filtered through 0.2-μm pore Q-Max syringe filters into acid-washed collection bottles on days 0, 4, 8, and 14. The subsamples were stored at −80 °C until analysis. Nitrite and nitrate (NO_2^- + NO_3^-), hereafter refered to simply as nitrate), phosphate (PO_4^{3-}), and silicic acid (H_4SiO_4) were measured on a SmartChem200 wet chemistry analyzer (Unity Scientific, MA, USA), following procedures outlined for NO_3^- + NO_2^- (Wood et al., 1967), for PO_4^{3-} (Murphy and Riley, 1962), and for H_4SiO_4 (Strickland and Parsons (1972). To determine dissolved PAH concentrations (including

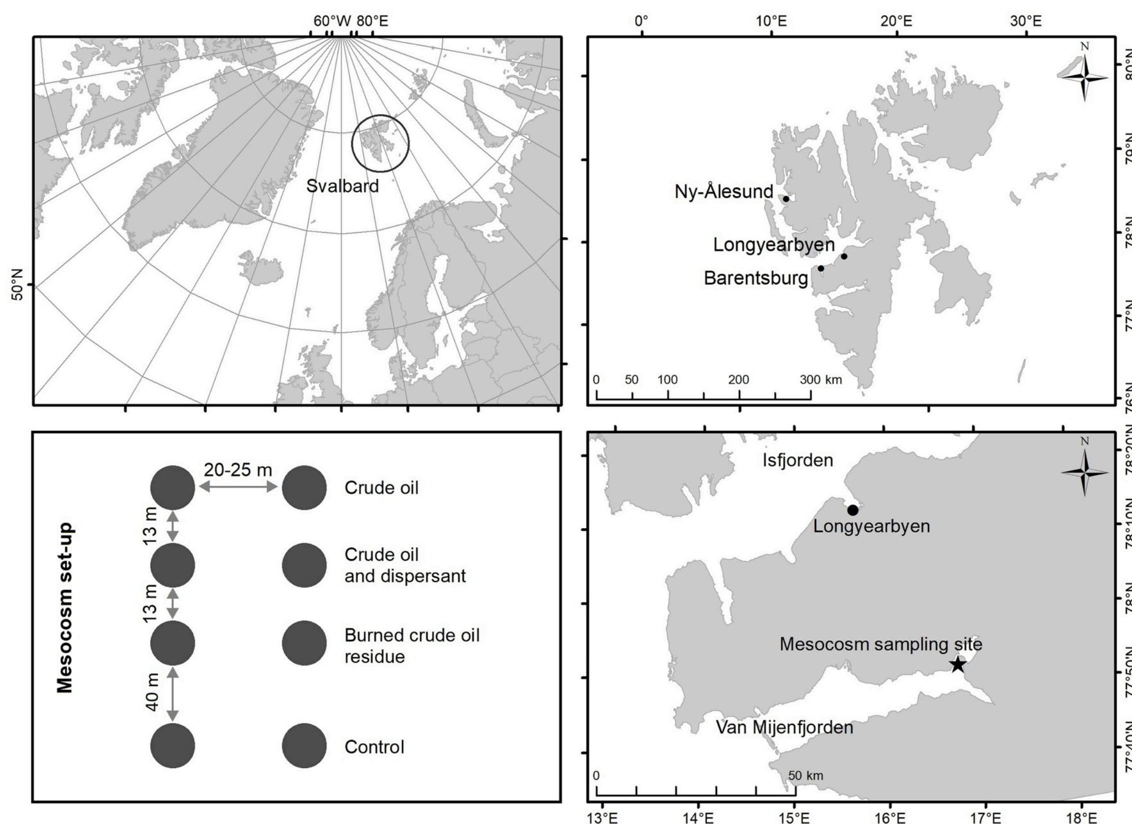

Fig. 1. Top panels show the location maps of the study area; the bottom-right panel shows the mesocosm sampling site in Van Mijenfjorden, Svalbard (black star); bottom-left panel shows a schematic diagram of the mesocosm set-up.

alkylated PAHs), 3 × 100-mL subsamples were collected from incubation bottles on days 0, 8, and 14, and stored at −20 °C. The total concentration of PAHs was determined according to the method described in Toxværd et al. (2018).

2.3. Chlorophyll-a concentration

Subsamples (3 × 100 mL) for measurements of chlorophyll-*a* concentration were taken from incubation bottles on days 0, 4, 8, 12, and 14, and filtered through a 0.7-μm pore size filter. The pigments were extracted in 5 mL of methanol (99.5%) for 24 h in darkness at room temperature. The fluorescence of the extracts was measured before and after addition of 100 μL 0.1 M HCl (37%) with a Turner Designs model 10-AU Fluorometer (Turner Designs, Sunnyvale, CA, USA). The fluorometer was calibrated prior to use with five dilutions of pure chlorophyll-*a* standard (Sigma-Aldrich).

2.4. Biomass measurements

Subsamples were taken to determine the abundances of bacteria, small phytoplankton, and heterotrophic nanoflagellates (HNF) every second day, and diatoms and microzooplankton every fourth day, as described below.

The abundances of bacteria, small phytoplankton, and HNF were estimated using an Attune Acoustic Focusing Cytometer (Applied Biosystems by Life Technologies, CA, USA). The data were analyzed using Attune® Cytometric Software (version 2.1; Life Technologies Corporation, CA, USA). The subsamples were fixed with glutaraldehyde (1% final concentration) for 3 h in the dark at 4 °C, stored at −80 °C until the analysis within 5 months, and thawed immediately before analysis.

The thawed bacterial aliquots (3 × 100 μL) were diluted 10-fold in 0.2-μm filtered TE buffer (Tris 10 mM, EDTA 1 mM, pH 8) and stained with SYBR Green I (Molecular Probes Inc., Eugene, OR, USA) for 10 min at 80 °C in a water bath to provide optimal staining of bacteria (Marie et al., 1999). Afterwards, the aliquots were analyzed at a 100 $\mu L\ min^{-1}$ flow rate on an Attune Acoustic Focusing Cytometer (Applied Biosystems by Life Technologies, CA, USA), with the discriminator set depending on their side scatter (proportional to cell size) and pigment (green and red fluorescence). Fluorescent yellow-green microspheres with diameters of 2-μm (FluoSpheres® Carboxylate-Modified Microspheres, UK) were added to the aliquots as an internal standard. The abundance of bacteria was used for the biomass estimates based on the carbon conversion factor for bacteria (Lee and Fuhrman, 1987) (Appendices A and B).

The small phytoplankton aliquots (3 × 400 μL) were analyzed directly after thawing at a flow rate of 200 $\mu L\ min^{-1}$ on the cytometer with the discriminator set depending on their side scatter (proportional to cell size) and pigment (red and orange fluorescence) (Paulsen et al., 2015).

The thawed HNF aliquots (1.4 mL) were stained with SYBR Green I (Molecular Probes Inc., Eugene, OR, USA) for 4–6 h at 4 °C in the dark. The aliquots were enumerated at a flow rate of 500 $\mu L\ min^{-1}$, and HNF discriminated from autotrophs and bacteria based on their pigment, i.e., red (from Chl-*a*) vs. green (from SYBR Green I) fluorescence. Fluorescent yellow-green microspheres with diameters of 0.5-μm (FluoSpheres® Carboxylate-Modified Microspheres, UK) were added to the aliquots as an internal standard (Zubkov et al., 2007).

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To obtain the size estimates of small phytoplankton and HNF, 15-mL subsamples were additionally filtered through 8-, 5-, 3-, 2-, and 1-μm pore size filters. The size fractionation and the subsequent cell enumeration were implemented in order to assess the percentages of the various phytoplankton and HNF groups within the given size intervals (Zubkov et al., 1998; Paulsen et al., 2015). The flow rates and discriminators for small phytoplankton and HNF were set as described above. The abundances of small phytoplankton and HNF within the different size intervals were converted to the weighted arithmetic mean sizes, and used for the biomass estimates based on the carbon conversion factors (Børsheim and Bratbak, 1987; Søndergaard, 1991) (Appendices A and B).

Microzooplankton (ciliates and dinoflagellates) and diatom subsamples (3 × 110 mL) were fixed with acidified Lugol solution (3% final concentration) and stored in the dark until analysis. The subsamples were settled in Utermöhl sedimentation chambers and examined under 200-times magnification of an inverted microscope (Leica DM IL LED, Leica Microsystems GmbH, Wetzlar, Germany). Based on the gross morphology, ciliates and dinoflagellates were enumerated and divided into 10-μm size classes of equivalent spherical diameter (ESD). The ESD of every specimen was measured and the cellular volume determined using the appropriate geometric shape. The cellular volume was converted to biomass using specific carbon conversion factors (Putt and Stoecker, 1989; Verity and Lagdon, 1984; Menden-Deuer and Lessard, 2000) (Appendices A and B). Loricate and aloricate ciliates, and thecate and athecate dinoflagellates were differentiated.

To summarize the sampling schedule during the two 14day incubation experiments (March and May): pH, temperature, light, bacteria, HNF, and phytoplankton were examined every other day; nutrients, chlorophyll-*a*, and microzooplankton were examined every fourth day; PAHs were examined in the beginning, middle, and end of the experiments.

2.5. Statistical analysis

Repeated measures analysis of variance (rANOVA) was conducted to compare chlorophyll-*a*, pH, nutrients, PAHs, and biomass of organisms among the incubated treatments. If ANOVAs were significant, pairwise comparisons of pooled standard deviations using Benjamini and Hochberg's test of variability were performed. All data were normally distributed (Shapiro-Wilk test) and did not require transformations. The homogeneity of variances was tested using Levene's test. These analyses were performed in RStudio, and the level of significance used was 0.05.

3. Results

3.1. Abiotic factors in winter

In order to compare the effects of exposure to oil spill response technologies on marine microorganisms in winter, the first microcosm incubation experiment was performed in the laboratory with water samples collected from the mesocosms one month after the addition of chemical treatments. During this microcosm experiment, temperature, salinity, light intensity, and pH differed little between the treatments. Although pH increased in all treatments during the experiment (Table 1), no significant differences in pH were found among the treatments (Appendix E). The water temperature remained stable over time, averaging 1.05 ± 0.26 °C. Salinity measured 33.8 and the light intensity at the water surface in the experimental bottles was 55 ± 12 μmol photons m^{-1} s^{-1}.

The concentration of inorganic nutrients (nitrate and phosphate, but not silicate) generally decreased in all treatments during the experiments (Table 1); however, no significant differences were found among the treatments for any of the nutrients (Appendix E). Concentrations of polycyclic aromatic hydrocarbons (PAHs) also decreased over time in all treatments (Table 1) and differed significantly among the treatments ($p < 0.05$). The concentrations of PAHs were significantly higher in DISP and NATT than in the control and ISB ($p < 0.05$), but DISP and NATT did not differ significantly from each other, nor did the control and ISB (Appendix E). Detailed information on the compositions and concentrations of PAHs and alkylated PAHs can be found in Appendix C.

3.2. Abiotic factors in spring

The second laboratory microcosm incubation experiment was performed with water samples collected from the mesocosms in May three months after the addition of chemical treatments. During the microcosm experiment, temperature, salinity, and light intensity differed little among the treatments, averaging 1.04 ± 0.26 °C, 31.9, and 57 ± 8 μmol photons m^{-1} s^{-1}, respectively. The pH levels decreased in all treatments over time (Table 2). Significant differences in pH were found among the treatments ($p < 0.05$) and between all treatments (lower) than in the control. Significant differences were also found among the oil treatments ($p < 0.05$), except for the pair ISB–NATT (Appendix F)

Overall, the concentration of nutrients and PAHs decreased in all treatments during the experiments (Table 2). Nutrient concentrations differed significantly among the treatments ($p < 0.05$). The nitrate concentration was significantly lower in the control group than in DISP and NATT ($p < 0.05$). Significant differences also were found between all other treatment pairs ($p < 0.05$), except between the control and ISB (Appendix F), with the highest concentration of nitrate measured in NATT, followed by DISP, and finally by ISB and the control. Similarly, the phosphate concentration was significantly lower in the control group than in the other groups ($p < 0.05$). Differences were significant between all the other treatment pairs ($p < 0.05$), except between ISB and DISP (Appendix F). The highest concentration of phosphate was measured in NATT, followed by DISP and ISB and, lastly, by the control group. Finally, the concentration of silicate was also significantly higher

Table 1
The average concentrations ± SD of pH, nutrients (nitrate, phosphate, silicate; μmol L^{-1}), and PAH (ng L^{-1}) on Days 0 and 14 in the control, burnt oil (ISB), dispersed oil (DISP), and crude oil (NATT) treatments in winter (March).

Treatment	Day	Concentrations in winter				
		pH	Nitrate	Phosphate	Silicate	PAH
Control	Day 0	7.56 ± 0.00	7.59 ± 0.69	0.55 ± 0.07	4.74 ± 0.24	100 ± 123
	Day 14	7.68 ± 0.03	6.21 ± 0.37	0.32 ± 0.07	4.24 ± 0.59	55 ± 62
ISB	Day 0	7.56 ± 0.00	7.34 ± 0.58	0.40 ± 0.08	3.87 ± 0.19	65 ± 19
	Day 14	7.65 ± 0.02	6.36 ± 0.54	0.36 ± 0.06	4.83 ± 0.67	35 ± 18
DISP	Day 0	7.61 ± 0.00	7.00 ± 0.39	0.45 ± 0.21	4.36 ± 0.56	624 ± 39
	Day 14	7.63 ± 0.02	5.45 ± 0.21	0.29 ± 0.15	4.68 ± 0.98	311 ± 85
NATT	Day 0	7.61 ± 0.00	6.70 ± 0.96	0.48 ± 0.04	4.08 ± 0.22	615 ± 275
	Day 14	7.65 ± 0.01	5.68 ± 0.80	0.35 ± 0.17	4.42 ± 0.31	249 ± 33

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Table 2
The average concentrations ± SD of pH, nutrients (nitrate, phosphate, silicate; μmol L^{-1}), and PAH (ng L^{-1}) on Day 0 and Day 14 in the control, burnt oil (ISB), dispersed oil (DISP), and crude oil (NATT) treatments in spring.

Treatment	Day	Concentrations in spring				
		pH	Nitrate	Phosphate	Silicate	PAH
Control	Day 0	7.65 ± 0.00	5.32 ± 0.96	0.24 ± 0.04	3.40 ± 0.67	90 ± 70
	Day 14	7.58 ± 0.03	4.33 ± 0.36	0.26 ± 0.03	3.18 ± 0.15	27 ± 15
ISB	Day 0	7.63 ± 0.00	5.94 ± 0.66	0.45 ± 0.07	3.88 ± 0.54	261 ± 62
	Day 14	7.49 ± 0.03	4.51 ± 0.11	0.31 ± 0.08	2.88 ± 0.17	59 ± 51
DISP	Day 0	7.60 ± 0.00	6.47 ± 0.13	0.45 ± 0.03	3.76 ± 0.17	20,269 ± 4,219
	Day 14	7.47 ± 0.02	5.19 ± 0.07	0.34 ± 0.01	2.86 ± 0.00	9,519 ± 3,620
NATT	Day 0	7.62 ± 0.00	8.05 ± 0.13	0.66 ± 0.11	5.15 ± 1.08	2,190 ± 1,152
	Day 14	7.50 ± 0.02	7.79 ± 0.28	0.61 ± 0.05	4.02 ± 0.04	1,421 ± 651

in NATT than the other three groups ($p < 0.05$). No significant differences were found between any other treatment combinations (Appendix F).

PAH concentrations differed significantly among the treatments ($p < 0.05$). The concentrations of PAHs in the treatments were much higher in spring than in winter (Table 1). The PAH concentrations were lowest in the control and ISB, which did not differ significantly, but were significantly lower than the PAH concentrations in DISP and NATT ($p < 0.05$). Additionally, the concentrations of PAHs in DISP and NATT also were significantly different ($p < 0.05$) (Table 2; Appendix F). Detailed information on the composition and concentration of PAHs and alkylated PAHs can be found in Appendix D.

3.3. Biomass of microorganisms in winter

Bacteria and heterotrophic nanoflagellates (HNF) exhibited logistic increase in their biomass in all four treatments, with a lag period of 4 days observed in the bacterial population (Fig. 2A, **C**). Biomasses of bacteria and HNF differed significantly among treatments ($p < 0.05$; Table 3). The biomasses of bacteria in the control and DISP were significantly higher than the biomasses in ISB and NATT ($p < 0.05$). The differences in the bacterial biomasses between the control and DISP and between ISB and NATT were not significant. Significantly higher biomass of HNF occurred in the controls than in the other three treatments ($p < 0.05$). The differences in biomass of HNF among ISB, DISP, and NATT were not significant (Appendix E).

Dinoflagellates increased slowly in all treatments (Fig. 2E), with no significant differences in the biomass among groups (Table 3). Ciliate populations did not increase in any treatments (Fig. 2G), with comparable biomasses in all treatments (Table 3; Appendix E).

Photosynthetic picoplankton, nanoplankton, and diatoms exponentially increased in number, with a long (6–8 days) lag period (Fig. 3A, **C**, **E**, **G**); however, biomass differences were not significant among the treatments (Table 3; Appendix E). The observed accumulation of phytoplankton biomass in all treatments was also supported by the increases of chlorophyll-*a* (Fig. 3G).

3.4. Biomass of organisms in spring

The biomass of bacteria increased exponentially at first in all treatments, but after 6–10 days the biomass continually decreased until the end of the experiment (Fig. 2B). Bacterial biomasses differed significantly among the treatments ($p < 0.05$; Table 4). The bacterial biomass in the control group was significantly lower than in the oil treatments ($p < 0.05$). Differences in bacterial biomass were also significant between all the treatment pairs ($p < 0.05$; Appendix F), with the highest biomass found in ISB, followed by NATT and DISP, and finally by the control.

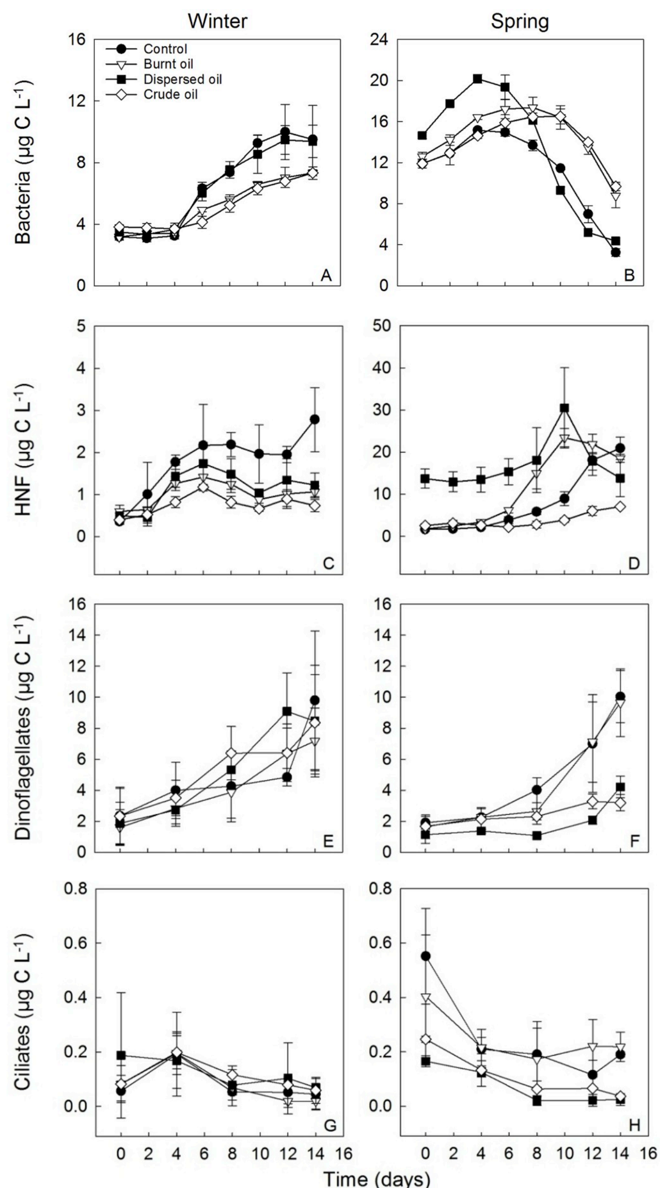

Fig. 2. The mean biomass (μg C L^{-1}) of **(A–B)** bacteria, **(C–D)** HNF, **(E–F)** dinoflagellates, and **(G–H)** ciliates over exposure duration time (days) in the control, burnt oil, dispersed oil, and crude oil treatments in winter (left) and spring (right). Error bars represent ± SD. Note that the Y-axis values may differ.

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Table 3
The overall effects of burnt oil (ISB), dispersed oil (DISP), and crude oil (NATT) treatments on organisms in winter, based on the differences between the computed means of the total biomass of organisms in the treatments versus the control. NS – the biomass of organisms in the particular oil treatment was not significantly different from the control. Negative – the biomass of organisms was lower than the control ($p < 0.05$).

WINTER			
Organisms	ISB	DISP	NATT
Bacteria	Negative	NS	Negative
HNF	Negative	Negative	Negative
Dinoflagellates	NS	NS	NS
Ciliates	NS	NS	NS
Picophytoplankton	NS	NS	NS
Nanophytoplankton	NS	NS	NS
Diatoms	NS	NS	NS

The biomasses of heterotrophic nanoflagellates increased in all four treatments (Fig. 2D), and were significantly different among the groups ($p < 0.05$; Table 4). The biomasses of HNF in the control and ISB were significantly lower than in DISP ($p < 0.05$), but significantly higher than the biomass in NATT ($p < 0.05$). The biomasses in the control and ISB did not differ significantly (Appendix F). The biomass of HNF was highest in DISP, followed by the control and ISB, and finally by NATT.

The biomasses of dinoflagellates differed significantly among the treatments ($p < 0.05$; Fig. 2F; Table 4). The biomasses in the control and ISB were significantly higher than the biomasses in DISP and NATT ($p < 0.05$); however, the biomass differences were not significant in either pair (Appendix F).

Ciliate biomasses decreased during the first four days in all four treatments and then stabilized in the control and ISB, but continued to decrease in DISP and NATT (Fig. 2H). The ciliate biomasses in the control and ISB were significantly higher than those in DISP and NATT ($p < 0.05$). No significant differences in biomass were found between the control and ISB or between DISP and NATT.

The biomasses of picophytoplankton in the control and ISB increased exponentially during the first week, but then decreased until the end of the experimental period (Fig. 3B); however, the biomasses did not differ significantly between the two groups (Table 4). On the other hand, the picophytoplankton populations in DISP and NATT did not increase and differences between the biomass in the two groups were not significant (Table 4). The picophytoplankton biomasses in the control and ISB were significantly higher than those in DISP and NATT ($p < 0.05$; Appendix F).

The biomasses of nanophytoplankton differed significantly among the treatment groups ($p < 0.05$; Fig. 3D; Table 4). The biomass in the control group was significantly higher than those in the oil treatments ($p < 0.05$). Significant differences in the nanophytoplankton biomasses were also observed between all the other treatment pairs ($p < 0.05$), except for DISP–NATT (Appendix F). The highest biomass was found in the control, followed by ISB and, finally, by DISP and NATT.

Diatom biomasses differed significantly between the treatments ($p < 0.05$; Fig. 3F; Table 4). The highest biomass was observed in ISB, which was significantly higher than those in the other three treatments ($p < 0.05$). No significant differences were observed between any other treatment pair (Appendix F). The observed differences in the phytoplankton biomass between the treatments were supported by the chlorophyll-*a* measurements (Fig. 3H).

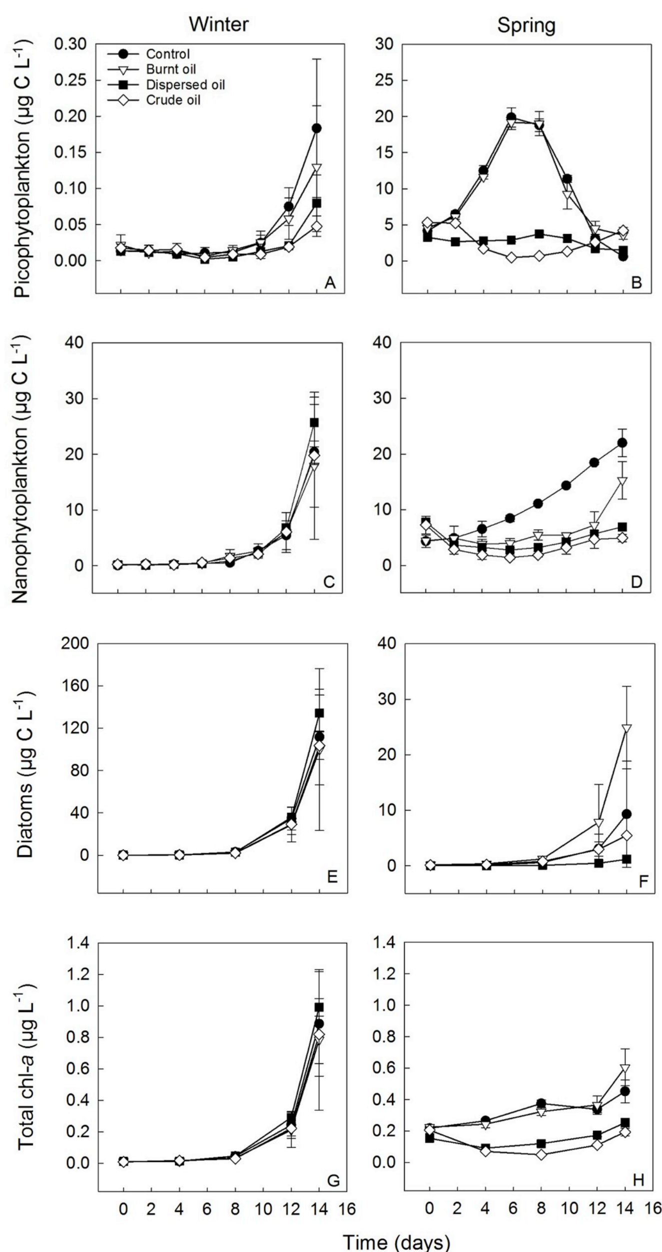

Fig. 3. The mean biomass ($\mu g\ C\ L^{-1}$) of **(A–B)** picophytoplankton, **(C–D)** nanophytoplankton, and **(E–F)** diatoms, and **(G–H)** the chlorophyll-*a* concentration ($\mu g\ L^{-1}$) over exposure duration time (days) in the control, burnt oil, dispersed oil, and crude oil treatments in winter (left) and spring (right). Error bars represent ± SD. Note that the Y-axis values may differ.

4. Discussion

Our study investigated the effects of exposure history to oil and oil residues on the Arctic marine microorganisms. The treatments were chosen to simulate an actual oil spill and subsequent application of different response technologies, specifically, application of chemical dispersant, *in situ* burning of crude oil, and natural attenuation of crude oil. We demonstrated pronounced seasonal differences in the effects of investigated oil spill response technologies on the biomass of the Arctic marine microorganisms. Although the impacts of PAHs in winter were

Table 4
The overall effects of burnt oil (ISB), dispersed oil (DSIP), and crude oil (NATT) treatments on organisms in spring, based on the differences between the computed means of the total biomass of organisms in the treatments versus the control. NS – the biomass of organisms in the particular oil treatment was not significantly different from the control. Negative – the biomass of organisms was lower than the control ($p < 0.05$). Positive – the biomass of organisms was higher than the control ($p < 0.05$).

SPRING			
Organism	ISB	DISP	NATT
Bacteria	Positive	Positive	Positive
HNF	NS	Positive	Negative
Dinoflagellates	NS	Negative	Negative
Ciliates	NS	Negative	Negative
Picophytoplankton	NS	Negative	Negative
Nanophytoplankton	Negative	Negative	Negative
Diatoms	Positive	NS	NS

low (Table 3), the actual effects of oil treatments on the organisms were merely delayed. When sea ice is present, the oil gets encapsulated in the ice shortly after spillage (Fingas and Hollebone, 2003) and the impacts on the pelagic organisms are observed only when the pollutants are released from the melting ice (Table 4). Importantly, during the mesocosm campaign, Van Mijenfjorden was an industrial fjord with a coal mine that for years (1917–1921 and 1925–2016) was exposed to coal dust contamination and several small oil spills in relation to the coal industry (Statsarkivet 1998). Thus, the air- and sea-borne pollution from the coal mine activities may explain the relatively high concentrations of PAHs measured in our control mesocosms (Tables 1 and 2). Consequently, organisms in the fjord may be adapted to this chronic contaminant exposure; therefore, the results of our study may underestimate the impacts of these oil spill remediation technologies on pristine Arctic environments.

Overall, low concentrations of PAHs were measured in the water column in winter (Table 1). Although the concentrations of PAHs in DISP and NATT treatments were low, the amounts were six-times higher than the control and ISB treatments. The low amounts of PAHs in ISB probably resulted from substantial removal of crude oil during the incineration (Appendix C and D) (Potter and Buist, 2010) and rapid encapsulation of the remaining compounds in the ice (Petrich et al., 2013).

Generally, over the course of incubation microcosm experiments, the nutrient concentrations decreased and the pH increased in all four treatments (Table 1), indicating similar levels of microbial activity in the different treatments (Hofslagare et al., 1983; Parsons et al., 1984; Siron et al., 1996). This was supported by the observations of comparable biomasses of dinoflagellates, ciliates (Fig. 2E and G) and phytoplankton (also supported by chlorophyll-*a* measurements; Fig. 3) among the treatments; however, there were some discrepancies in the bacterial and HNF biomasses in the oil groups relative to the control (Fig. 2A and C; Table 3). Our results showed that bacterial biomasses were considerably lower in ISB and NATT treatments than in the control and DISP (Fig. 2A). This could be explained by lower grazing pressure on bacteria in the control and DISP treatments; however, that probably was not true in our case because the biomass of HNF was the highest in the controls (Fig. 2C), suggesting that the bacterial population in the control treatment would have experienced the highest grazing mortality. In addition, because the biomasses of microzooplankton (dinoflagellates and ciliates) were similar in all treatments (Fig. 2E and G) grazing pressure cannot explain the differences between the HNF biomasses in the oil treatments and the control. Thus, our results suggest that even low concentrations of PAHs in the water ($0.6\,\mu g\,L^{-1}$; Table 1) might affect the growth of some bacteria and HNF from the winter populations. This conclusion, however, contrasts with other studies showing enhanced growth of bacteria and, subsequently, HNF in various oil treatments even at PAH concentrations as high as $10\,g\,L^{-1}$ (Delille and Siron, 1993; Jung et al., 2010; Ortmann et al., 2012).

In spring, higher air temperatures and formation of brine channels in the sea ice increased the release of PAHs into the water. Importantly, the concentrations of PAHs measured in the mesocosms in spring did not reflect the total concentration of PAHs introduced into the mesocosms, because most of PAHs were still trapped inside the sea ice. Nevertheless, the PAH concentrations measured in DISP and NATT treatments were up to two orders of magnitude higher than in the control and ISB (Table 2). Consequently, these higher concentrations of PAHs in the DISP and NATT treatments resulted in approximately 50% lower biomass of the microorganisms in the incubation microcosms compared to the control group (Table 4).

The DISP treatment negatively affected biomasses of microzooplankton and small phytoplankton, but stimulated bacteria and HNF populations (Table 4; Figs. 2 and 3). These observations agree with earlier studies, which demonstrated that dispersed oil treatment resulted in enhanced growth of bacteria and HNF but depressed the growth of microzooplankton (Parsons et al., 1984; Ortmann et al., 2012). The NATT treatment negatively affected biomasses of microzooplankton, HNF, and small phytoplankton, but stimulated bacterial growth (Table 4; Figs. 2 and 3). Similarly, bacterial production increased and microzooplankton abundance decreased in the water-soluble fraction of diesel fuel oil treatment, and conversely, production of HNF increased (Koshikawa et al., 2007). Overall, the high numbers of bacteria in oil treatments may imply that some bacteria can grow and utilize oil compounds as substrate, whereas the stimulatory effects of oil on HNF are probably related to indirect effects, specifically increased abundance of bacteria and reduced grazing pressure. Oil compounds can cause the loss of cell mobility (Soto et al., 1975), which could lead to impaired feeding activity of motile microzooplankton and their low production in polluted environments (Fig. 2F and H). Various studies have demonstrated harmful effects of oil compounds on the physiology of phytoplankton cells. For example, both crude oil and chemically dispersed oil negatively affected the cell membrane genes in phytoplankton (Hook and Osborn, 2012). Additionally, oil compounds can accumulate in the cell membrane, subsequently changing its structural and functional properties and irreversibly damaging the cell surface (Sikkema et al., 1995). Moreover, oil compounds also can interfere with photosynthetic processes (González et al., 2009), causing chloroplasts to shrink (Tukaj et al., 1998) or the loss of other pigments (Ozhan et al., 2014 and references therein), which all could affect small phytoplankton growth. Finally, our results indicated that DISP and NATT treatments did not significantly affect diatom numbers (Fig. 3F). González et al. (2009) reported that small diatoms were stimulated by the water-soluble fraction of oil, while large diatoms were negatively affected by high, but not by low, oil concentrations. This could indicate that size of diatoms may be important (Ozhan et al., 2014), with small diatoms being more tolerant to oil pollution than large ones (González et al., 2009). Because we lack data on diatom sizes, and because diatoms in our study were not significantly affected by high concentrations of PAHs, we can speculate that small and possibly more resilient diatoms predominated in the measured biomass.

By contrast, the ISB treatment had no effect on the biomasses of microzooplankton, HNF, and picophytoplankton, but stimulated bacteria and diatom populations (Figs. 2 and 3). Again, the increased abundance of bacteria suggested that some bacteria may be able to degrade and utilize certain oil compounds from the increased carbon

pool in the ISB treatment. Similarly, low concentrations of PAHs were tolerated and even stimulated the growth of diatoms, which agrees with previous observations (González et al., 2009). By contrast, the treatment negatively affected nanophytoplankton (Fig. 3D), indicating that some oil compounds, even at low concentrations, adversely affected biomass of nanophytoplankton. This result, however, contrasts with those from earlier studies, where lower tolerance to oil by picophytoplankton, but not nanophytoplankton, was observed, which was attributed to their smaller size and larger surface to volume ratio (Sargian et al., 2007; Echeveste et al., 2010).

The differences in microbial activity among the treatments were also reflected in differences in nutrients utilized during the experiments. Overall, the concentrations of nutrients decreased in all treatments but stayed significantly higher in DISP and NATT than the control (Table 2). Low chlorophyll-*a* concentrations measured in these treatments could explain low utilization of nutrients (Fig. 3H). Because the lowest nutrient concentrations were measured in the control, the low numbers of organisms in DISP and NATT treatments cannot be explained by the nutrient limitation.

The effects of oil spill response technologies on marine microorganisms are here documented as changes in the structure and biomass of the major functional groups. Our study concludes that application of ISB, DISP and NATT may promote biomasses of bacteria and heterotrophic nanoflagellates, while the use of DISP and NATT probably will have adverse effects on phytoplankton and microzooplankton (Figs. 2 and 3). Due to approximately 50% lower biomass of microorganisms in DISP and NATT and consequently altered structure compared to the controls, disruptions in the transfer of energy from the primary producers to the higher trophic levels may be expected in oil affected areas.

Author contributions

Marina Pančić: developed the protocols, conducted the experiments, analyzed the biological samples, analyzed the data, and wrote the manuscript.

Eva Köhler: conducted the experiments, analyzed the biological samples, discussed the results, and revised the manuscript.

Maria Lund Paulsen: performed the nutrient analysis, assisted with flow cytometry gating, discussed the results, and revised the manuscript.

Kirstine Toxværd: acquired the funding, and revised the manuscript.

Stéphane Le Floch: performed the PAH analysis.

Camille Lacroix: performed the PAH analysis.

Morten Hjorth: developed the project idea, acquired the funding, supervised the project, and revised the manuscript.

Torkel Gissel Nielsen: developed the project idea, acquired the funding, supervised the project, and revised the manuscript.

Author declaration

We wish to draw the attention of the Editor to the following facts that may be considered as potential conflicts of interest and to significant financial contributions to this work:

- The project was co-funded by the International Association of Oil and Gas Producers, Arctic Oil Spill Response Technology – Joint Industry Programme. The role of this funding source was in study design and in the decision to submit the article for publication. The funding source had no involvement in data analysis and data interpretation, or writing the article.
- Some of the authors are employed by private consultancies (K. Toxværd and M. Hjorth at COWI) and private associations (C. Lacroix and S. Le Floch at CEDRE).

We confirm that the manuscript has been read and approved by all named authors and that there are no other people who satisfied the criteria for authorship but are not listed. We confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. We confirm that we have followed the regulations of our institutions concerning intellectual property.

We further confirm that any aspect of the work covered in this manuscript that has involved either experimental animals or human patients has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

We understand that the Corresponding Author is the sole contact for the Editorial process (including Editorial Manager and direct communications with the office). Corresponding Author is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs. We confirm that we have provided a current, correct email address which is accessible by the Corresponding Author and which has been configured to accept email from tgin@aqua.dtu.dk.

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Appendices

Appendix A. The average biomass ± SD (µg C L^{-1}) of dominant functional groups in winter in all treatments in the beginning and in the end of the incubation microcosm experiment. Note that the biomass on Day 0 represents the biomass of organisms acquired from the mesocosms in March, that is, before the organisms were exposed to light in the incubation experiments. Cell volume to carbon conversion factors were obtained from the literature: [1]Menden-Deuer and Lessard (2000): pgC cell^{-1} = 0.760 × volume$^{0.819}$; [2]Putt and Stoecker (1989): 0.19 pgC µm^{-3}; [3]Verity and Lagdon (1984): 0.053 pgC µm^{-3}; [4]Børsheim and Bratbak (1987): 0.22 pgC µm^{-3}; [5]Lee and Fuhrman (1987): 0.02 pgC bacteria^{-1}; [6]Menden-Deuer and Lessard (2000): pgC cell^{-1} = 0.288 × volume$^{0.811}$; [7]Søndergaard (1991): 0.25 pgC µm^{-3}. ESD, equivalent spherical diameter; -, no organisms found

Functional group	ESD (µm)	Biomass (pg C cell^{-1})	Winter biomass (µg C L^{-1})															
			Control				ISB				DISP				NATT			
			Day 0		Day 14		Day 0		Day 14		Day 0		Day 14		Day 0		Day 14	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
[1]Thecate dinoflagellates	< 10	128	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	16.5	439	–	–	0.01	0.00	–	–	–	–	0.01	0.00	–	–	0.01	0.00	0.01	0.00
	25.9	1336	–	–	0.01	0.00	0.00	0.01	0.00	0.01	0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.02
	35.7	2921	0.01	0.02	0.04	0.06	–	–	–	–	–	–	–	–	0.00	0.02	0.01	0.02
	45.6	5316	–	–	–	–	–	–	–	–	0.00	0.04	0.02	0.03	–	–	–	–
	> 50	6684	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
[1]Athecate dinoflagellates	< 10	128	0.42	0.22	6.72	6.37	0.33	0.20	3.58	1.99	0.30	0.21	4.68	2.79	0.29	0.30	4.99	2.10
	16.5	439	0.63	0.38	1.16	0.21	0.47	0.29	1.28	0.79	0.35	0.26	0.98	0.46	0.40	0.35	0.81	0.34
	25.9	1336	0.50	0.40	1.19	0.64	0.31	0.19	0.95	0.35	0.30	0.07	1.05	0.30	0.31	0.06	0.82	0.13
	35.7	2921	0.52	0.61	1.30	0.52	0.26	0.33	0.94	0.61	0.18	0.25	1.12	0.83	0.10	0.74	1.09	0.70
	45.6	5316	0.11	0.09	0.32	0.30	0.11	0.09	0.19	0.03	0.05	0.04	0.43	0.14	0.08	0.15	0.41	0.25
	> 50	6684	0.16	0.17	0.30	0.14	0.11	0.10	0.22	0.15	0.00	0.19	0.18	0.14	0.20	0.14	0.22	0.15
[2]Loricate ciliates	< 10	28	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	16.5	125	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	25.9	486	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	35.7	1263	–	–	0.01	0.01	–	–	–	–	–	–	–	–	0.01	0.00	–	–
	45.6	2622	0.01	0.02	–	–	–	–	–	–	0.00	0.02	0.01	0.02	0.01	0.00	0.03	0.03
	> 50	3469	0.01	0.02	–	–	0.01	0.02	–	–	–	–	0.03	0.03	0.02	0.02	–	–
[3]Aloricate ciliates	< 10	99	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	16.5	448	0.01	0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.00	–	–	0.01	0.01	0.01	0.01
	25.9	1741	0.01	0.01	–	–	0.01	0.01	0.01	0.02	0.19	0.26	0.01	0.01	0.02	0.01	0.02	0.01
	35.7	4527	0.02	0.03	–	–	0.02	0.03	–	–	0.02	0.00	0.02	0.03	0.00	0.06	0.00	0.06
	45.6	9401	–	–	0.05	0.07	–	–	–	–	0.00	0.02	–	–	–	–	–	–
	> 50	12435	–	–	–	–	0.04	0.07	–	–	–	–	–	–	–	–	–	–
[4]HNF	3.4	4.6	0.18	0.03	2.33	0.60	0.22	0.02	0.81	0.13	0.19	0.03	0.89	0.27	0.20	0.04	0.52	0.14
	5.8	22.7	0.18	0.05	0.45	0.18	0.38	0.13	0.25	0.10	0.31	0.03	0.34	0.11	0.20	0.03	0.22	0.04
[5]Bacteria	–	0.02	3.18	0.21	9.51	2.21	3.18	0.18	7.32	0.41	3.49	0.17	9.38	1.05	3.82	0.14	7.33	0.20
[6]Diatoms	39.8	1330	0.08	0.03	111.60	45.27	0.06	0.02	99.81	76.58	0.08	0.07	134.25	17.09	0.11	0.06	103.48	13.05
[7]NanoPP	4.0	7.5	0.07	0.02	1.11	0.36	0.08	0.06	0.70	0.38	0.06	0.02	0.90	0.25	0.10	0.01	0.79	0.02
	9.3	91.2	–	–	19.28	9.65	0.15	0.13	17.22	12.84	0.15	0.13	24.77	3.44	0.15	0.26	18.98	1.58
[7]PicoPP	1.6	0.4	0.01	0.00	0.18	0.10	0.02	0.01	0.13	0.08	0.01	0.00	0.08	0.04	0.02	0.01	0.05	0.01

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Appendix B. *The average biomass ± SD (μg C L^{-1}) of dominant functional groups in spring in all treatments in the beginning and in the end of the incubation microcosm experiment. Note that the biomass on Day 0 represents the biomass of organisms acquired from the mesocosms in May, that is, before the organisms were exposed to light in the incubation experiments. Cell volume to carbon conversion factors were obtained from the literature (see* ***Appendix A****). ESD, equivalent spherical diameter; -, no organisms found*

Functional group	ESD (μm)	Biomass (pg C cell^{-1})	Spring biomass (μg C L^{-1})															
			Control				ISB				DISP				NATT			
			Day 0		Day 14		Day 0		Day 14		Day 0		Day 14		Day 0		Day 14	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
[1]Thecate dinoflagellates	< 10	128	0.01	0.00	–	–	0.01	0.00	0.01	0.01	–	–	3.68	0.70	–	–	–	–
	16.5	439	0.15	0.16	0.10	0.01	0.10	0.06	0.09	0.04	0.00	0.02	0.45	0.01	0.04	0.04	0.07	0.01
	25.9	1336	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.00	0.01	0.04	0.02	0.01	0.01	0.01	0.01
	35.7	2921	–	–	–	–	–	–	–	–	–	–	0.03	0.03	–	–	–	–
	45.6	5316	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	> 50	6684	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
[1]Athecate dinoflagellates	< 10	128	0.39	0.14	8.87	1.96	0.56	0.39	7.60	1.46	0.20	0.23	–	–	0.55	0.34	2.00	0.42
	16.5	439	0.97	0.38	1.41	0.12	0.71	0.27	1.30	0.26	0.17	0.34	0.01	0.01	0.27	0.16	0.88	0.17
	25.9	1336	0.19	0.09	0.14	0.01	0.14	0.08	0.15	0.10	0.01	0.08	–	–	0.05	0.04	0.16	0.05
	35.7	2921	0.01	0.02	0.10	0.06	0.06	0.03	0.04	0.07	0.00	0.02	–	–	0.03	0.04	0.05	0.06
	45.6	5316	0.03	0.04	0.03	0.04	0.05	0.05	0.04	0.06	–	–	–	–	–	–	–	–
	> 50	6684	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	0.04
[2]Loricate ciliates	< 10	28	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	16.5	125	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	25.9	486	–	–	0.01	0.01	–	–	–	–	–	–	–	–	–	–	–	–
	35.7	1263	0.02	0.01	0.02	0.01	–	–	0.02	0.01	0.00	0.01	–	–	0.01	0.02	–	–
	45.6	2622	–	–	–	–	–	–	–	–	0.00	0.02	–	–	–	–	–	–
	> 50	3469	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
[3]Aloricate ciliates	< 10	99	0.04	0.03	–	–	0.03	0.02	0.01	0.01	0.03	0.04	–	–	0.05	0.05	–	–
	16.5	448	0.25	0.14	0.07	0.02	0.23	0.10	0.12	0.03	0.05	0.02	0.01	0.01	0.04	0.01	0.03	0.00
	25.9	1741	0.07	0.02	0.03	0.02	0.09	0.11	0.07	0.02	0.02	0.01	0.02	0.02	0.02	0.06	0.01	0.01
	35.7	4527	0.09	0.00	0.05	0.00	0.05	0.05	–	–	–	–	–	–	0.00	0.03	–	–
	45.6	9401	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	> 50	12435	0.06	0.09	–	–	–	–	–	–	–	–	–	–	–	–	–	–
[4]HNF	3.5	5.0	0.43	0.06	4.17	0.46	0.52	0.11	3.50	0.14	9.10	1.09	4.66	1.18	1.05	0.07	2.18	0.09
	8.4	67.8	1.22	0.35	16.76	2.31	1.24	0.38	15.02	0.86	4.61	1.33	9.15	3.21	1.50	0.37	4.87	0.30
[5]Bacteria	–	0.02	11.91	0.43	3.24	0.38	12.61	0.41	8.77	1.15	14.65	0.31	4.36	0.21	11.90	0.18	9.67	0.41
[6]Diatoms	39.8	1330	0.01	0.02	7.46	9.55	0.17	0.07	24.90	7.44	0.01	0.02	1.16	0.54	0.19	0.21	5.49	3.55
[7]NanoPP	3.9	7.1	3.35	0.87	16.76	1.43	3.57	0.25	11.81	2.00	6.81	0.47	1.90	0.08	5.38	0.71	3.50	0.35
	9.8	107.4	1.00	0.33	5.25	1.70	0.90	0.16	3.50	1.50	0.99	0.56	5.03	0.68	1.88	0.97	1.44	0.87
[7]PicoPP	1.7	0.6	4.15	1.15	0.62	0.16	4.56	0.43	3.59	0.56	3.29	0.17	1.51	0.14	5.32	0.16	4.23	0.40

Appendix C. The average concentration ± SD (ng L^{-1}) of 41 different PAH components in winter in all treatments in the beginning and in the end of the incubation microcosm experiment. NA, standard deviation could not be computed due to insufficient number of iterations (i.e. PAH component measured in one of three replicates). -, PAH component not present, or present in concentrations below detection limit

PAH components	Winter PAH concentrations (ng L^{-1})															
	Control				ISB				DISP				NATT			
	Day 0		Day 14		Day 0		Day 14		Day 0		Day 14		Day 0		Day 14	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Naphthalene	6.9	1.0	7.3	0.6	8.0	2.4	9.0	3.2	68.7	9.6	74.6	6.5	65.2	8.8	87.2	10.0
C1-Naphthalene	15.8	2.7	10.5	1.6	21.1	4.0	16.7	3.4	154.8	15.4	95.0	15.7	145.3	30.8	88.5	9.2
C2-Naphthalene	–	–	–	–	30.5	12.5	21.0	NA	198.8	15.6	83.1	22.4	191.6	94.5	55.2	6.9
C3-Naphthalene	–	–	–	–	–	–	–	–	167.8	6.0	67.1	14.2	160.5	113.3	–	–
Benzothiophene	–	–	–	–	–	–	0.9	NA	1.4	0.6	0.8	NA	1.7	0.3	1.1	0.2
C1-Benzothiophene	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
C2-Benzothiophene	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
C3-Benzothiophene	–	–	–	–	–	–	–	–	–	–	–	–	14.7	5.2	–	–
Biphenyl	–	–	–	–	–	–	–	–	8.2	0.5	5.1	1.0	8.6	3.9	4.4	0.6
Acenaphthylene	0.8	0.2	–	–	–	–	0.6	NA	1.4	0.4	–	–	0.9	0.6	–	–
Acenaphthene	–	–	–	–	–	–	–	–	0.9	0.1	–	–	1.4	NA	0.7	0.0
Fluorene	–	–	–	–	1.3	NA	–	–	3.7	0.2	1.4	0.2	3.7	2.3	1.2	0.1
C1-Fluorene	–	–	–	–	–	–	–	–	6.2	0.2	–	–	10.3	NA	–	–
C2-Fluorene	–	–	–	–	–	–	–	–	–	–	–	–	13.7	NA	–	–
C3-Fluorene	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Phenanthrene	–	–	–	–	–	–	–	–	8.3	0.0	5.5	1.0	6.8	2.5	4.2	0.7
Anthracene	0.5	NA	–	–	–	–	–	–	–	–	–	–	–	–	–	–
C1-Phenan/anthra	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
C2-Phenan/anthra	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
C3-Phenan/anthra	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Dibenzothiophene	0.6	NA	1.0	NA	–	–	–	–	1.4	0.1	0.7	0.1	1.1	0.5	0.7	0.0
C1-Dibenzothiophene	–	–	–	–	–	–	–	–	2.8	0.0	–	–	4.5	NA	–	–
C2-Dibenzothiophene	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
C3-Dibenzothiophene	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Fluoranthene	4.5	0.1	34.2	55.4	3.3	2.0	1.1	0.1	0.8	0.2	1.1	0.2	1.0	0.3	6.9	5.6
Pyrene	1.1	0.6	4.8	NA	0.8	0.1	–	–	0.8	0.1	–	–	0.9	0.4	0.8	0.2
C1-Fluoranthenes/Pyrenes	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
C2-Fluoranthenes/Pyrenes	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
C3-Fluoranthenes/Pyrenes	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Benzo[a]anthracene	13.2	NA	1.3	NA	–	–	1.1	NA	–	–	–	–	0.6	0.0	0.9	NA
Chrysene	24.5	NA	1.3	NA	0.8	NA	0.5	NA	0.5	0.0	–	–	0.6	NA	1.4	NA
C1-Chrysene	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
C2-Chrysene	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
C3-Chrysene	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Benzo[b + k]fluoranthene	22.0	30.1	–	–	0.8	0.0	–	–	–	–	–	–	1.2	NA	–	–
Benzo[e]pyrene	43.6	NA	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Benzo[a]pyrene	8.2	NA	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Perylene	12.1	NA	–	–	–	–	–	–	–	–	–	–	1.2	0.5	1.1	0.2
Indeno[1,2,3-cd]pyrene	16.0	NA	–	–	–	–	–	–	–	–	–	–	4.3	NA	–	–
Dibenz[a,h]anthracene	24.0	NA	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Benzo[g,h,i]perylene	26.4	NA	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Appendix D. The average concentration ± SD (ng L^{-1}) of 41 different PAH components in spring in all treatments in the beginning and in the end of the incubation microcosm experiment. NA, standard deviation could not be computed due to insufficient number of iterations (i.e. PAH component measured in one of three replicates). -, PAH component not present, or present in concentrations below detection limit

PAH components	Spring PAH concentrations (ng L^{-1})															
	Control				ISB				DISP				NATT			
	Day 0		Day 14		Day 0		Day 14		Day 0		Day 14		Day 0		Day 14	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Naphthalene	8.4	2.1	6.4	0.9	12.1	3.7	12.3	5.5	755.0	221.7	17.9	4.2	302.8	144.9	46.5	20.9
C1-Naphthalene	17.1	7.2	12.5	4.6	43.5	12.5	20.8	8.6	2769.0	1488.1	996.4	250.0	465.8	248.8	453.6	183.5
C2-Naphthalene	42.6	18.6	–	–	84.4	16.6	44.9	NA	5763.9	2310.2	3268.8	1373.0	600.2	388.6	433.5	239.5
C3-Naphthalene	50.9	NA	–	–	72.5	18.7	–	–	9433.5	987.1	3914.8	1752.4	608.0	393.6	340.5	166.8
Benzothiophene	–	–	–	–	1.0	NA	–	–	13.9	9.6	12.9	2.2	3.4	1.8	4.6	3.0
C1-Benzothiophene	–	–	–	–	–	–	–	–	–	–	69.0	NA	–	–	–	–
C2-Benzothiophene	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
C3-Benzothiophene	–	–	–	–	13.5	0.0	10.8	NA	–	–	111.3	46.3	38.4	11.5	23.0	6.1
Biphenyl	5.8	1.9	–	–	3.7	0.3	–	–	110.5	71.4	97.1	39.7	18.0	9.4	21.1	8.6
Acenaphthylene	0.5	NA	–	–	1.2	0.5	0.8	NA	11.8	7.1	10.7	4.5	1.7	1.0	2.4	1.7
Acenaphthene	–	–	–	–	0.9	NA	–	–	7.2	4.7	7.2	2.6	1.5	0.4	2.5	1.9
Fluorene	1.6	NA	–	–	2.4	0.6	1.3	NA	80.1	49.0	54.3	16.2	8.9	4.3	6.9	2.4
C1-Fluorene	–	–	–	–	5.4	NA	–	–	161.8	72.7	103.1	28.1	16.9	5.5	10.4	2.2
C2-Fluorene	–	–	–	–	–	–	–	–	208.8	66.9	117.1	32.2	22.4	4.3	13.0	NA
C3-Fluorene	–	–	16.1	NA	–	–	–	–	138.6	41.0	74.2	20.2	23.1	1.7	16.9	NA
Phenanthrene	5.6	NA	–	–	6.2	1.5	5.8	NA	180.0	38.4	165.7	19.4	23.7	4.2	20.8	3.3
Anthracene	–	–	–	–	–	–	–	–	3.8	1.0	3.4	0.2	0.5	0.0	0.5	NA
C1-Phenan/anthra	–	–	–	–	–	–	–	–	247.3	22.7	228.2	25.0	34.7	2.2	27.0	2.9
C2-Phenan/anthra	–	–	–	–	–	–	–	–	165.8	17.1	153.1	16.1	–	–	–	–
C3-Phenan/anthra	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Dibenzothiophene	0.6	0.1	–	–	0.9	0.2	0.7	NA	27.7	2.6	23.5	3.5	3.4	0.9	3.0	0.8
C1-Dibenzothiophene	–	–	–	–	2.8	0.1	–	–	48.6	6.1	40.8	3.9	7.5	0.8	6.4	0.8
C2-Dibenzothiophene	–	–	–	–	–	–	–	–	38.1	3.6	33.0	3.5	6.3	0.2	5.1	0.0
C3-Dibenzothiophene	–	–	–	–	–	–	–	–	16.2	2.0	12.1	2.0	–	–	–	–
Fluoranthene	1.5	0.5	1.4	0.4	1.1	0.2	2.9	2.1	4.1	0.6	10.3	4.0	1.1	0.2	3.6	1.7
Pyrene	0.8	0.2	0.8	0.0	1.2	0.1	1.0	0.1	4.6	0.7	4.0	0.2	1.2	0.1	1.4	0.3
C1-Fluoranthenes/Pyrenes	–	–	–	–	–	–	–	–	29.0	4.7	20.1	2.5	–	–	–	–
C2-Fluoranthenes/Pyrenes	–	–	–	–	–	–	–	–	11.7	2.1	–	–	–	–	–	–
C3-Fluoranthenes/Pyrenes	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Benzo[a]anthracene	0.6	0.1	–	–	–	–	–	–	1.5	0.5	2.1	0.8	0.5	NA	0.9	NA
Chrysene	0.6	NA	–	–	0.6	0.1	–	–	5.0	0.7	5.2	0.9	0.7	0.2	0.9	NA
C1-Chrysene	–	–	–	–	–	–	–	–	6.3	1.7	7.8	0.8	–	–	–	–
C2-Chrysene	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
C3-Chrysene	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Benzo[b+k]fluoranthene	0.6	0.0	–	–	–	–	–	–	1.1	0.1	0.6	NA	–	–	–	–
Benzo[e]pyrene	0.9	NA	–	–	–	–	–	–	1.1	NA	–	–	–	–	–	–
Benzo[a]pyrene	1.1	NA	–	–	–	–	0.7	NA	–	–	1.1	NA	–	–	–	–
Perylene	12.1	15.0	0.9	NA	18.3	2.8	1.1	NA	24.2	10.3	1.3	NA	–	–	–	–
Indeno[1,2,3-cd]pyrene	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Dibenz[a,h]anthracene	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Benzo[g,h,i]perylene	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Appendix E. Results of ANOVAs and pairwise comparisons of pooled standard deviations of pH, phosphate, silicate, nitrate, and PAH concentrations, and biomasses of bacteria, HNF, dinoflagellates, ciliates, pico- and nano-phytoplankton, and diatoms in winter. Significant differences are indicated in bold

Parameters in winter	ANOVA	Pairwise comparisons			
pH	F(3, 8) = 1.488,		Control	ISB	DISP
	P = 0.289	ISB	0.38	–	–
		DISP	0.38	0.82	–
		NATT	0.53	0.73	0.76
Phosphate	F(3, 8) = 0.506,		Control	ISB	DISP
	P = 0.689	ISB	0.80	–	–
		DISP	0.80	0.80	–
		NATT	0.80	0.80	0.80
Silicate	F(3, 8) = 1.580,		Control	ISB	DISP
	P = 0.269	ISB	0.66	–	–
		DISP	0.71	0.51	–
		NATT	0.41	0.51	0.41
Nitrate	F(3, 8) = 1.488,		Control	ISB	DISP
	P = 0.289	ISB	0.88	–	–
		DISP	0.60	0.60	–
		NATT	0.36	0.36	0.60
PAH	F(3, 8) = 46.217,		Control	ISB	DISP
	P = 2.131e-05	ISB	0.60	–	–
		DISP	**7.1e-05**	**7.1e-05**	–
		NATT	**8.7e-05**	**7.1e-05**	0.60
Bacteria	F(3, 8) = 9.415,		Control	ISB	DISP
	P = 0.005	ISB	**0.01**	–	–
		DISP	0.89	**0.01**	–
		NATT	**0.01**	0.89	**0.01**
HNF	F(3, 8) = 8.166,		Control	ISB	DISP
	P = 0.008	ISB	**0.02**	–	–
		DISP	**0.03**	0.54	–
		NATT	**0.009**	0.31	0.15
Dinoflagellates	F(3, 8) = 0.254,		Control	ISB	DISP
	P = 0.856	ISB	0.95	–	–
		DISP	0.95	0.95	–
		NATT	0.95	0.95	0.95
Ciliates	F(3, 8) = 1.802,		Control	ISB	DISP
	P = 0.225	ISB	0.91	–	–
		DISP	0.31	0.31	–
		NATT	0.39	0.39	0.67
Pico-phytoplankton	F(3, 8) = 3.239,		Control	ISB	DISP
	P = 0.082	ISB	0.65	–	–
		DISP	0.15	0.20	–
		NATT	0.15	0.17	0.78
Nano-phytoplankton	F(3, 8) = 0.234,		Control	ISB	DISP
	P = 0.870	ISB	0.96	–	–
		DISP	0.96	0.96	–
		NATT	0.96	0.96	0.96
Diatoms	F(3, 8) = 0.348,		Control	ISB	DISP
	P = 0.792	ISB	0.91	–	–
		DISP	0.91	0.91	–
		NATT	0.91	0.93	0.91

Appendix F. Results of ANOVAs and pairwise comparisons of pooled standard deviations of pH, phosphate, silicate, nitrate, and PAH concentrations, and biomasses of bacteria, HNF, dinoflagellates, ciliates, pico- and nano-phytoplankton, and diatoms in spring. Significant differences are indicated in bold

Parameters in spring	ANOVA	Pairwise comparisons			
pH	F(3, 8) = 55.7,		Control	ISB	DISP
	P = 1.053e-05	ISB	**0.0001**	–	–
		DISP	**8e-06**	**0.002**	–
		NATT	**0.0003**	0.26	**0.0006**
Phosphate	F(3, 8) = 101.14,		Control	ISB	DISP
	P = 1.061e-06	ISB	**0.004**	–	–
		DISP	**0.002**	0.44	–
		NATT	**1.1e-06**	**4.9e-06**	**5.4e-06**
Silicate	F(3, 8) = 1.107,		Control	ISB	DISP
	P = 0.0009	ISB	0.62	–	–
		DISP	0.47	0.62	–
		NATT	**0.002**	**0.002**	**0.002**
Nitrate	F(3, 8) = 276.64,		Control	ISB	DISP
	P = 2.037e-08	ISB	0.13	–	–
		DISP	**0.0002**	**0.0008**	–
		NATT	**2.9e-08**	**2.9e-08**	**1.4e-07**
PAH	F(3, 8) = 210.09,		Control	ISB	DISP
	P = 6.043e-08	ISB	0.91	–	–
		DISP	**8.2e-08**	**8.2e-08**	–
		NATT	**0.05**	**0.05**	**1.2e-07**
Bacteria	F(3, 8) = 73.009,		Control	ISB	DISP
	P = 3.741e-06	ISB	**4.3e-06**	–	–
		DISP	**4.3e-05**	**0.002**	–
		NATT	**9.1e-06**	**0.05**	**0.03**
HNF	F(3, 8) = 21.099,		Control	ISB	DISP
	P = 0.0004	ISB	0.07	–	–
		DISP	**0.002**	**0.02**	–
		NATT	**0.05**	**0.004**	**0.0004**
Dinoflagellates	F(3, 8) = 19.703,		Control	ISB	DISP
	P = 0.0005	ISB	0.46	–	–
		DISP	**0.001**	**0.002**	–
		NATT	**0.002**	**0.004**	0.35
Ciliates	F(3, 8) = 20.075,		Control	ISB	DISP
	P = 0.0004	ISB	0.86	–	–
		DISP	**0.001**	**0.001**	–
		NATT	**0.002**	**0.002**	0.28
Pico-phytoplankton	F(3, 8) = 331.64,		Control	ISB	DISP
	P = 9.931e-09	ISB	0.81	–	–
		DISP	**2.8e-08**	**2.8e-08**	–
		NATT	**2.8e-08**	**2.8e-08**	0.96
Nano-phytoplankton	F(3, 8) = 52.306,		Control	ISB	DISP
	P = 1.336e-05	ISB	**0.0002**	–	–
		DISP	**2.8e-05**	**0.05**	–
		NATT	**1.6e-05**	**0.004**	0.12
Diatoms	F(3, 8) = 7.436,		Control	ISB	DISP
	P = 0.01	ISB	**0.04**	–	–
		DISP	0.24	**0.01**	–
		NATT	0.65	**0.03**	0.37

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