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Effects of scrubber washwater discharge on microplankton in the Baltic Sea

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ABSTRACT

In 2020, the global cap of maximum allowable sulphur content in marine fuel will be reduced from the current 3.5% to 0.5%. Another way to reduce the sulphur emissions is to install a seawater scrubber that cleans exhausts but instead release acidic water containing nutrients and contaminants back to the marine environment. In the current study, scrubber washwater was tested on a Baltic Sea microplankton community. A significant increase in chlorophyll *a*, particulate organic phosphorus (POP), carbon (POC) and nitrogen (PON) were observed when the community was exposed to 10% scrubber washwater for 13 days as compared to the control. A laboratory experiment with the filamentous cyanobacteria *Nodularia spumigena* and the chain-forming diatom *Melosira* cf. *arctica* showed negative responses in photosynthetic activity (EC10 = 8.6% for *N. spumigena*) and increased primary productivity (EC10 = 5.5% for *M.* cf. *arctica*), implying species-specific responses to scrubber washwater discharge.

1. Introduction

Emissions of acidifying gases such as sulphur oxides (SO_X) and particulate matter (PM) from shipping are substantial on local, regional and global scales, and are associated with vast societal costs in terms of affected human health as well as environmental impact (Corbett and Fischbeck, 1997; Corbett et al., 2007; Schröder et al., 2017). To reduce the impact from shipping, the International Maritime Organization (IMO) has adopted a convention which gradually decreases the maximum allowed sulphur content in marine fuel on a global basis. Even stricter regulation applies in regions adopted as "Sulphur Emission Control Areas" (SECAs), where the highest allowed sulphur content in fuels is 0.1% since 2015. From 2020, the global cap of sulphur content in fuel outside of SECAs will be reduced from the current 3.5% to 0.5%. Driven by economic incitements, an alternative to switching to expensive low-sulphur fuel is to install an exhaust gas cleaning system (EGCS) and continue to use the cheaper Heavy Fuel Oil (HFO).

It exists several different exhaust gas cleaning technologies based on dry or wet scrubbing. Dry scrubbing uses granulates of calcium hydroxide in the de-sulphurization process of the exhaust gases. However, the dominating technique in shipping is wet scrubbers that reduces the emissions of SO_X to the atmosphere by washing the exhausts in seawater. Wet scrubbers can be operated in two different modes, closed-loop or open-loop. In closed loop mode, seawater is mixed with NaOH and recirculated. However, bleed-off water is produced, typically 0.1–0.3 m^3/MWh. In open loop mode, the exhausts are led through a fine spray of seawater in which the SO_X, mainly present as SO_2, are hydrated to sulphurous acid, (H_2SO_3) which in turn can be ionized to bisulphite (HSO_3^-) and sulphite (SO_3^{2-}) ions. This process implies release of two proton (H^+) ions per SO_2 molecule, resulting in large volumes of acidified seawater (typically 45m^3/MWh of ~pH 3) (Ülpre and Eames, 2014). In open-loop mode, the acidic seawater is continuously discharged into the marine environment (typically 13,000 m^3/day for a medium sized, 12 MW, "roll on roll off" (RoRo) ship). The number of scrubber installations are rapidly increasing and a survey conducted by the exhaust gas cleaning system association (EGCSA) showed the number of ships with scrubbers installed or on order to be 983 as of May 2018 (EGCSA, 2018). This development was also forecasted by Lindstad and Eskeland (2016) who in their cost-benefit analysis pointed out that the dominant response to the global sulphur cap of 0.5% by the year 2020 would be the use of scrubbers.

Despite increasing use of scrubbers, little is known about potential impact of the discharged washwater on the marine environment. The use of open loop scrubbers implies that exhaust substances conventionally deposited on the sea surface, are more efficiently and intensively transferred to the marine environment at more local scale, generating temporary higher concentrations of pollutants, eutrophying and acidifying substances. Deposition of ships' emission of SO_X and NO_X on the sea surface, has been estimated to cause regional pH reduction in the same order of magnitude as CO_2-driven ocean acidification (Hassellöv et al., 2013). However, acidified waters (elevated CO_2 levels) itself seems to be having less effect on natural Baltic microplanktonic communities (Olofsson et al., 2018; Wulff et al., 2018). It should be noted, however, that carbonation (increased *p*CO2) and acidification (decreased pH)

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could have different and independent effects (Shi et al., 2019). In a recent study on three planktonic diatoms, Shi et al. (2019) tested combined and separate effects of carbonation and acidification. Overall, positive effects or no effects on growth rate were observed for carbonation and acidification, respectively or combined.

At present, IMO has developed washwater discharge criteria with respect to acidity, polycyclic aromatic hydrocarbons (PAHs), nitrate content, as well as turbidity. However, there is a complete lack of discharge criteria for metals. This is unfortunate since studies have reported elevated copper and zinc concentrations in scrubber washwater as high as 260 μg/L and 537 μg/L, respectively (Hufnagl et al., 2005; Kjølholt et al., 2012; Turner et al., 2017). As emphasized in several reports on scrubbers, the origin of these metals in the scrubber washwater is still unknown (Hufnagl et al., 2005; Kjølholt et al., 2012). Potential metal sources may include combustion of fuel and lubricants, use of impressed current cathodic protection (ICCP) systems in the sea chest and release of metals from the piping material of the seawater cooling system. In a recent study by Koski et al. (2017), the authors found high concentration of copper both in the scrubber inlet and in the effluent water, indicating that copper may originate from releases of copper from antifouling paints or due to pipe constructions rather than the scrubbing process itself.

Large scale discharge of acidic washwater containing high concentrations of metals may pose a significant risk to the marine environment, since the low pH has a strong effect on metal speciation and induces a shift towards the ionic, more bioavailable, fraction of metals (Millero et al., 2009). This is of particular concern in semi-enclosed environment such as harbours, which generally also receive a high load of metals from ships coated with copper-based antifouling paints. Given IMOs convention to reduce the sulphur content in marine fuel, and the resulting increased interest in the use of seawater scrubber, there is an urgent need to assess the impact of scrubber washwater discharge on the marine environment. This is primarily important for coastal areas and water bodies with low water exchange such as estuaries and e.g. the Baltic Sea. The Baltic Sea, which was designated a particularly sensitive sea area (PSSA) by IMO in 2005, is considered particularly vulnerable to pollution due to its unique combination of low biodiversity, limited water exchange and low salinity. The microplanktonic community in the Baltic Sea varies throughout the year. The spring bloom is dominated by diatoms (Wasmund et al., 1998), and the summer bloom is dominated by the filamentous diazotrophic cyanobacteria *Nodularia spumigena* and *Aphanizomenon* sp. (Finni et al., 2001). The drivers that seems to be affecting the Baltic Sea the most are eutrophication (nitrogen and phosphorus) and increased water temperature, as they stimulate and shift the community structure (Hällfors et al., 2013; Suikkanen et al., 2013). Information on the effects of metals on Baltic Sea microplankton is lacking. However, marine cyanobacteria have been classified as sensitive to copper pollution (Moffett et al., 1997), and diatoms have also been reported to be affected both morphologically and physiologically (Masmoudi et al., 2013). Studies using the Baltic Sea macroalgae *Fucus vesiculosus* (Andersson and Kautsky, 1996) and *Ceramium tenuicorne* (Ytreberg et al., 2010) showed that low concentrations of copper (2.5 μg/L) inhibited germination in *F. vesiculosus,* and dimidiated growth (6.4 μg/L) in *C. tenuicorne*.

Microplankton species and communities may be affected by scrubber washwater discharge as these groups of organisms will be exposed directly to the discharge of open-loop scrubber washwater. The aim of the current study was to assess how scrubber washwater discharge affects microplankton species (laboratory experiments) as well as a natural community of pelagic microplankton originating from the Baltic Sea (field experiment).

2. Material and methods

2.1. Production of scrubber washwater discharge

Seawater collected in the Baltic Sea at Askö laboratory in March 2016, was used to produce scrubber washwater. The scrubbing process was conducted at an engine lab at the Department of Mechanics and Maritime Sciences, Chalmers University of Technology. The lab is equipped with a four-cylinder 100 kW engine from Volvo Penta and a scrubber unit made of stainless steel holding a length of 50 cm and a diameter of 40 cm. A marine gas oil (MGO) with 1.0% sulphur was used in the experiment. The exhausts were fed from the engine to the scrubber unit, through an isokinetic heated probe kept at 250 °C, using a vacuum pump. A sub stream of the gas flow was sampled before and after the scrubber unit and analysed continuously with respect to SO_2, NO_X, CO, CO_2, and O_2 using a Fuji Electric, ZRE Gas Analyser. The engine exhaust flow rate was monitored using a mass flow meter (TSI, Mass Flowmeter 4000 Series) and was kept at 15 Litre-Per-Minute (LPM) throughout the production of scrubber washwater. The seawater was pumped to the scrubber unit through 7 different nozzles (BETE, MicroWhirl MW105) to create an adequate mist. The water flow rate was kept at 3.3 LPM yielding a water-to-air ratio of 0.22. The pH of the discharged scrubber washwater was 2.8 (Aquatrode Plus Pt1000, Metrohm). The scrubber washwater was collected in 5 L borosilicate bottles and stored dark prior to the experiment. Subsamples were collected and analysed for metal concentrations as described in Section 2.3.4.

2.2. Ecotoxicological experiments

The scrubber washwater was used in a mesocosm study, as well as in two laboratory experiments, to assess potential effects on a microplankton community and microplankton species, respectively.

2.2.1. Mesocosm experiment

To study effects of scrubber washwater on a microplankton community, scrubber washwater was added to mesocosms with natural microplankton communities. Two treatments, 1% and 10% concentration of scrubber washwater were used, to be compared with one untreated control. The experiment was conducted during a 13-day period, starting 7th of April 2016 at Askö Laboratory (58°49′N, 17°38′E) located in the Baltic Sea. Surface seawater (depth 0–2 m) was collected from station B1 (58°48′28 N, 17°37′60 E) using rinsed 10 L plastic buckets. Larger grazers were removed by gently sieving the water through 250 μm mesh. The whole water mass was stored in a 5 m^3 clean container. In addition, microplankton was collected and concentrated through a plankton net (mesh size 25 μm).

The experiment was conducted in transparent polyethylene bags rinsed and filled with in total 50 L of water. In total 15 bags were used; 5 controls, 5 with 1% scrubber washwater and 5 with 10% scrubber washwater. All bags were first gently filled with 45 L of natural 250 μm filtered seawater. To increase the microplankton density, 200 mL of the concentrated microplankton slurry was added to each bag. For the 1% and 10% treatments, 0.5 and 5 L of scrubber washwater was added, respectively. To obtain similar microplankton density in each treatment, filtered seawater (0.2 μm) was added to the controls (5 L) and to the 1% treatments (4.5 L). The containers were covered with plastic mesh to obtain a photosynthetically active radiation (PAR 400–700 nm) corresponding to a water depth of ca 2 m at the sampling site, B1. Temperature was recorded during the entire experiment via loggers (HOBO Pendant, Onset Computer Corporation, USA). Sampling for physical/chemical parameters (pH, total alkalinity (A_T) and inorganic nutrients) and biological parameters (biovolume, chlorophyll *a* (Chl *a*), particulate organic carbon (POC), particulate organic nitrogen (PON), particulate organic phosphorus (POP), bacterial abundance and productivity) were performed initially (day 0), at day 7 and at day 13 by tapping water via acid-cleaned silicone hoses to borosilicate bottles and pre-cleaned plastic beakers.

2.2.2. Laboratory experiments

Two microplankton species isolated from the Baltic Sea were used;

the filamentous cyanobacteria *Nodularia spumigena* (KAC 12 Kalmarsund, C. Esplund 1997) and the centric chain-forming diatom *Melosira* cf. *arctica* (Askö, Maria Karlberg 2014). In the experiment, five different treatments ranging from 1 to 25% scrubber washwater were used and compared to a control (five replicates per treatment and control). For every replicate, a total water volume of 200 mL was used. Enriched filtered (0.2 µm) seawater (f/2 medium (Guillard, 1975)) was used in the diatom experiment. For *N. spumigena*, f/4 medium was used. The seawater was collected from Askö Laboratory (pH 7.52 and salinity 6 in GF/F (Whatman) filtered seawater). The experiments were conducted in 200 mL cell culture flasks (TC Flask T75, Susp.,Vent. Cap, Sarstedt AG & Co, Nümbrecht, Germany) for 6 days at constant temperature of 15 °C with a light/dark cycle of 16 h/8 h (PAR 70–90 µmol photons $m^{-2} s^{-1}$). The initial cell concentrations were $1.9*10^8$ cells/L (*N. spumigena)* and $4.63*10^7$ cells/L (*M.* cf. *arctica)*. Samples for A_T and pH were collected and measured in every culture flask at the beginning of the experiment while inorganic nutrients were measured at the end of the experiments. Photosynthetic activity, primary productivity and biovolumes were measured at the beginning and end of the experiments.

2.3. Chemical analyses

2.3.1. pH

In the mesocosm experiment, pH was measured in the total scale (pH_T) using spetrophotometry according to the method described in (Clayton and Byrne, 1993). A 2 mM *m*-Cresol Purple sodium salt solution (Aldrich, lot MKBC2604V), dissolved in MilliQ water, was used as indicator. Subsamples for pH_T were collected at day 0, day 7 and day 13 from every replicate by gently overfilling 100 mL borosilicate bottles leaving no headspace. The bottles were subsequently stored in a pre-heated water bath at a temperature of 25 °C prior to analysis. The samples were, within 1 h after sampling, measured on four wavelengths (434 nm, 488 nm, 578 nm and 730 nm) using a spectrophotometer (Helios Zeta UV-VIS, Thermo Scientific). Each sample, with a volume of 3 mL, was analysed without the addition of indicator (auto zero) and with the addition of 20, 40, 60, 80 and 100 µL of the indicator, respectively. The pH_T was calculated for every standard addition of the indicator, according to the method described in Clayton and Byrne (1993). The sample pH was the intercept from a linear regression of pH_T vs. added indicator volume. During the laboratory experiments, a laboratory pH meter (827 pH lab Metrohm AG, Herisau, Switzerland) was used instead of the spectrophotometric method, and the measurements were conducted at day 0 on all replicates. The pH meter was calibrated using pH buffer solution (Metrohm AG, Herisau, Switzerland, batch no: 00470418) and pH 9 buffer solution (VWR BDH Prolabo Titrinorm buffer solutions promotion, batch no: 07B210519) and the bottles were tempered to 25 °C before measurements.

2.3.2. Total alkalinity

In both the mesocosm and laboratory experiment, A_T was measured potentiometrically within 24 h after sampling using an automatic alkalinity titration system (888 Titrando with Aquatrode Plus Pt1000, Metrohm AG, Herisau, Switzerland). Tempered (25 °C) 100 mL borosilicate bottles were used for A_T analyses. The measurements were conducted on all replicates at day 0, day 7 and day 13 (mesocosm experiment) and at day 0 (laboratory experiment). The accuracy of the method was determined daily with a certified reference material (CRM) analyses (n = 6). The CRM (batch 137) was provided by A. G. Dickson, Scripps Institute of Oceanography, CA, USA. A_T was calculated with MATLAB according to the method described in Ulfsbo et al. (2015) and the results were corrected using the CRM measurements.

2.3.3. Dissolved inorganic nutrients

For the mesocosm experiment, samples for dissolved inorganic nutrients were collected at day 0, day 7 and day 13 from all replicates, filtered (0.2 µm) and stored frozen (−20 °C) before analyses. For the laboratory experiment, sampling was only performed at the end of the experiment (day 6). The filtrate was analysed for inorganic nutrients (sum NO_2 + NO_3 in this paper referred to as nitrate, NH_4, $Si(OH)_4$, PO_4) using colorimetric determination (Grasshoff et al., 1999) at Sven Lovén Centre for Marine Sciences, Kristineberg, University of Gothenburg, Sweden.

2.3.4. Metals

Samples for metal analysis were collected from the scrubber washwater and from the Baltic Sea water used as medium in the mesocosm study. Water samples were filtered through acid-cleaned 0.45 µm filters (Supor, PALL) and collected in acid-cleaned 250 mL polypropylene bottles. Dissolved metal analysis was performed by ALS Scandinavia AB, Sweden, using inductively coupled plasma sector field mass spectrometry (ICP-SFMS) according to EPA method 200.8 rev5.4 (1994) and SS EN ISO 17294-1 (2006).

2.4. Biological parameters

2.4.1. Photosynthetic activity

Photosynthetic parameters of photosystem II were measured at days 7 and 13 (mesocosm experiment) and at days 0 and 6 (laboratory experiment), using pulse amplitude modulation (PAM) fluorometry. Measurements were conducted using a Water PAM connected to a Water ED (mesocosm experiment and *N. spumigena*) or a Water ED/B (*M.* cf. *arctica*) unit (Walz Mess- und Regeltechnik, Germany), with a saturation pulse of ~5000–8900 µmol photons $m^{-2} s^{-1}$ for 0.6–0.8 s. Software used was WinControl 2.08 and 3.25. Effective quantum yield ($F_{v'}/F_m$) was calculated in light adapted samples (subsample collected in a 10 mL vial and measured directly). Maximum quantum yield (F_v/F_m) was measured after 45 min (day 6) to 1 h (day 13) of dark adaptation (subsample collected in a 10 mL vial and stored covered in Al foil in water bath holding same temperature as the treatment). $F_{v'}/F_m$ was measured in both the laboratory and the field experiment whereas F_v/F_m was measured in the field experiment only.

2.4.2. Phytoplankton biovolume

Samples for biovolume measurements were fixed in Lugol's iodine solution (final concentration 2%). The samples were stored in darkness at room temperature and analysed within 4 months. For the laboratory experiment, subsamples were transferred into a Sedgewick rafter chamber and examined with an Axiovert 40 CFL inverted microscope (Zeiss Germany) or an Olympus CK 2 inverted microscope (Olympus Corporation). For the field experiment, phytoplankton species relative occurrence was estimated in each replicate following the procedure described in Hasle (1978). The Lugol preserved samples were shaken several times to homogenise distribution of cells and then transferred to 20 mL Utermöhl sedimentation chambers. Cell enumeration was made under Zeiss Axiovert 135 inverted microscope. Species relative occurrence was quantified according the following scale: present (few cells), common (< 50% of the total cell density) and very common (> 50% of the total cell density).

In the laboratory experiment, the chain or filament length of *M.* cf. *arctica* and *N. spumigena* were measured. For the first sample in each treatment, both the length, width and number of cells of the first 30 filaments were measure to calculate the average cell length and width for that treatment. The average filament width and the total filament length were used to calculate the biovolume, assuming a cylindrical shape.

2.4.3. Chlorophyll a

Chl *a* was analysed in the field experiment. After sampling, 0.35–1 L of well mixed sample was gently filtered onto GF/F filters (Whatman). The filtration was performed in dim light and the filters were immediately frozen in liquid nitrogen (−196 °C), transferred to a low-

temperature freezer (−85 °C) and stored for up to 6 months prior to analysis. For extraction, 1.5 mL 100% MeOH was added, the extract was ultrasonicated for 45 s (Vibra-cell sonicating probe, 3 mm), and subsequently filtered through a 0.45 µm nylon filter. The filtrates were analysed for photosynthetic pigment concentrations using high performance liquid chromatography (HPLC) (Shimadzu UV/VIS Photodiode array detector SPD-M20A) equipped with a C18-kolonn (Alltech Alltima C18, 3 µm, 150 × 4.6 mm) according to the method described by Wright and Jeffrey (1997). Here we show Chl *a* concentrations in µg/L.

2.4.4. Primary productivity

In the laboratory experiment, total primary productivity was measured using incubation with radioactive bicarbonate ($H^{14}CO_3^-$), as described by Nielsen and Bresta (1984) and modified according to Torstensson et al. (2013). 10 mL of well mixed sample was added to 20 mL glass scintillation vials. After addition of 150 µl (3 µCi) $H^{14}CO_3^-$ (Lot: 1904214, PerkinElmer, Waltham, MA, USA) all samples were incubated for 1 h in 15 °C and irradiance 100–110 µmol photons m^{-2} s^{-1}. The incubation was terminated by adding 200 µL of formaldehyde (37%). Two drops of 1 M HCL was then added to each vial to lower the pH below 2. To remove all remaining non-particulate $H^{14}CO_3^-$, the vials were bubbled with nitrogen gas for 1 h. Finally, 10 mL of liquid scintillation cocktail Insta-Gel Plus (PerkinElmer, Inc., Waltham, MA, USA) was added to each vial and the vials were then shaken until the cocktail gelled. The samples were stored in room temperature and darkness until analysed with a liquid scintillation counter (Packard Tri-Carb 2900TR Liquid Scintillation Analyzer). The software QuantaSmart (PerkinElmer, Inc., Waltham, MA, USA) was used to analyse the result. For each treatment, an additional sample was taken for dark incubation, which was used to correct for dark uptake of $H^{14}CO_3^-$. Dark samples were wrapped in Al foil and incubated together with the other samples.

2.4.5. Bacterial abundance and productivity

Bacterial abundance was measured in all replicates in the field experiment using flow cytometry, according to Torstensson et al. (2015), following Gasol and del Giorgio (2000). In total, 1.5 mL of sample (n = 1) was fixed in 1% glutaraldehyde (final concentration) and stored in −85 °C until analysis. After thawing, the samples were spiked with SYBR Green 1 Nucleic Acid Gel Stain (Invitrogen) and stored in darkness for 10 min. Bacterial abundance was analysed using FACSCalibur flow cytometer (BD Biosciences) and CountBright absolute counting beads (Invitrogen) were used as internal standards. Bacterial productivity was measured in all replicates in the field experiment and analysed using ^{3}H-thymidine incorporation as described in Torstensson et al. (2015). The samples were incubated in the seawater filled containers for 1 h at a temperature of 4.5–5.5 °C (day 0), 6.0–7.0 °C (day 6 and day 13) after which the bacterial carbon productivity (BCP) was calculated using $1.4*10^{18}$ cells $mole^{-1}$ thymidine incorporated (HELCOM, 2017) and 20 fg C $cell^{-1}$ (Lee and Fuhrman, 1987). The concentration of ^{3}H-isotope was at an acceptable level as assessed by saturation curves. Cell-specific productivity was calculated.

2.4.6. POC, PON and POP

Samples for POC, PON and POP were analysed for the field experiment. A volume of 0.75–1 L water from every replicate was filtered onto a pre-combusted (400 °C for 4 h) GF/F filter (Whatman). Filters for POP analyses were washed with 0.1 M HCl followed by a Milli-Q rinse prior to combustion. Filter blanks were prepared by filtering the corresponding volume of Milli-Q. The filter blanks were used to subtract background concentrations. The filters were left to dry at room temperature before being analysed for POP by Tvärminne Zoological Station, Finland, according to the method described in Solórzano and Sharp (1980). Filters for POC and PON were immediately frozen at 20 °C, and prior to analysis dried in an oven at 60 °C for 75 h, ground into a fine powder (MM301, Retsch) and analysed in an elemental analyser (EA 1108 CHNS-O, Fisons Instruments) applying 2,5-bis(5-tert-butyl-bensoaxzol-2-yl)thiophen as the internal standard (provided by Säntis Analytical AG, Teufen, Switzerland).

2.5. Statistical analyses

Differences in biological and chemical parameters between scrubber washwater treatments and controls were assessed using IBM SPSS Statistics (version 24). Before statistical analysis all data were checked for normal distribution (determined with Shapiro-Wilk test). Equality of variances of the different variables were determined using Cochran's C test or Levene's test (unequal sample size). If the variance was not homogenous, a log transformation was performed. If the variance was homogenous after log-transformation the logged dataset was used performing a one-way ANOVA, otherwise a Welch's ANOVA was performed using the non-log-transformed data. One-way ANOVA or Welch's ANOVA were used to determine if there were statistically significant differences in response between the three independent groups (controls, 1% scrubber and 10% scrubber). To control for type I error Holm-Bonferroni corrections were made for the biological parameters (photosynthetic activity ($F_{v'}/F_m$, F_v/F_m), Chl *a*, POC, POP, PON, bacterial abundance and cell specific bacterial production) and chemical (pH and A_T) parameters, respectively. The family wise error rate was set to α level 0.05. As the biological and chemical analyses at the start of the experiment (d0) where performed to check that all treatments had similar initial concentrations/activity while the d7 and d13 analyses were performed to analyse any biological and chemical response due to scrubber washwater exposure, the type 1 corrections were made for the data set d0 (biological tests = 9, chemical tests = 2) and for the combined data set d7 and d13 (biological test = 18, chemical test = 4), respectively.

To check for significant differences between the control and treatments, the post hoc tests Tukey's test (ANOVA) and Dunnet's T3 procedure (Welch's ANOVA), were used. In the only case where the conditions for parametric tests were not met (POP day 0), a non-parametric test was performed (Kruskal-Wallis H test). A maximum of one outlier was removed from the measurements of alkalinity, pH, bacterial abundance and cell-specific bacterial productivity due to unrealistic values (higher response/concentration than could have been possible in theory). For these datasets with unbalanced sample sizes, Welch's ANOVA was used.

The scrubber washwater concentration at which the laboratory investigated species expressed a 10% and 50% reduction in $F_{v'}/F_m$ and primary productivity (EC_{50} and EC_{10}) was calculated using the program REGTOXEV6.xls http://www.normalesup.org/~vindimian/en_download.html. The program calculates EC-values together with corresponding 95% confidence intervals by optimizing the curve fit in successive iterations.

3. Results

3.1. Mesocosm experiment

3.1.1. Chemical parameters

The mean temperature of the water was 6.4 °C ± 2.5 during the experimental period, following a diurnal variation. The acid addition from scrubber washwater lowered pH and A_T compared to control and was monitored during the experiment (Table 1). The 1% scrubber washwater (Scr) and 10% Scr treatment had both significantly lower pH and alkalinity as compared to the control after 0, 7 and 13 days of exposure. A slight increase in pH_T was observed with time, for both the controls and the treatments (Table 1). For example, the controls (ambient sea water) showed pH_T of 7.82 (s.d. = 0.1) at day 0 and increased to 8.0 (s.d. = 0.02) at day 13. Contrary to pH_T, no increase in A_T was observed with increasing exposure time for either the controls or the treatments.

Table 1
pH, alkalinity (μmol/L) and dissolved inorganic nutrient concentrations (μmol/L) in the controls (ctrl), treatments with 1% scrubber washwater (1% Scr) and treatments with 10% scrubber washwater (10% Scr). n = 5, except for NH_4^+ 1% Scr day 0, alkalinity ctrl day 7, and pH S10% day 7, where n = 4. *All values were below the detection limit (< 0.02 μmol/L).

Sampling day	Treatment	pH	Alkalinity	NO_3	NH_4	$Si(OH)_4$	HPO_4
Day 0	ctrl	7.82 ± 0.11	1558 ± 4.86	0.36 ± 0.04	0.67 ± 0.05	38.23 ± 6.26	0.47 ± 0.07
	1% Scr	7.65 ± 0.02	1524 ± 2.53	0.84 ± 0.33	0.69 ± 0.04	43.07 ± 4.04	0.56 ± 0.07
	10% Scr	6.59 ± 0.06	1162 ± 4.79	4.32 ± 1.32	0.92 ± 0.11	36.09 ± 10.80	0.47 ± 0.17
Day 7	Ctrl	8.04 ± 0.02	1561 ± 5.73	0.30 ± 0.09	0.54 ± 0.15	35.31 ± 17.55	0.23 ± 0.14
	1% Scr	7.89 ± 0.03	1522 ± 5.92	0.49 ± 0.05	0.63 ± 0.11	35.87 ± 5.13	0.20 ± 0.03
	10% Scr	6.76 ± 0.00	1141 ± 3.65	1.95 ± 0.13	0.78 ± 0.06	37.75 ± 3.17	0.11 ± 0.02
Day 13	Ctrl	8.03 ± 0.02	1564 ± 4.66	0.27 ± 0.03	0.73 ± 0.06	38.85 ± 4.68	0.20 ± 0.02
	1% Scr	7.89 ± 0.02	1522 ± 2.48	0.36 ± 0.04	0.61 ± 0.06	31.97 ± 4.69	0.13 ± 0.03
	10% Scr	6.97 ± 0.03	1147 ± 5.29	1.24 ± 0.17	0.74 ± 0.05	28.75 ± 3.66	< 0.02*

The analyses of inorganic nutrients at the start of the experiment (day 0) showed nitrate to be on average > 10 times higher in the 10% Scr treatment as compared to the control (Table 1). Contrary, the silicon and phosphorus species showed rather similar concentrations between treatments and control. After 6 and 13 days of exposure, nitrate and phosphate were reduced in both scrubber treatments compared to the initial start concentrations. The concentrations of Al, Cr, Cu, Ni and Zn were between 3 and 19 times higher in the scrubbed washwater as compared to the ambient water used as inlet water (Table 2).

3.1.2. Biological parameters

No effect of scrubber washwater on the microplankton species composition was detected. At day 0, the community was dominated by the dinoflagellates *Peridiniella catenata* and *Gymnodinium* sp. (data shown in supporting information). At day 6, *P. catenata* still dominated in all samples together with the diatom *Thalassiosira nordenskioeldii*, flagellates and ciliates < 10 μm. However, no effect was seen between the scrubber washwater treatments and the controls. At day 13, the community was dominated by *P. catenata,* the diatoms *T. nordenskioeldii* and *Chaetoceros* sp. as well as by flagellates and ciliates < 10 μm. As for day 6, no effects were observed when comparing the scrubber washwater treatments and the controls.

The concentration of POP, POC and PON were significantly higher in the 10% scrubber washwater treatment compared to the control after 13 days of exposure (Fig. 1A-C). A similar pattern was observed for Chl *a*, where a significant higher concentration was found in the 10% scrubber washwater treatment as compared to the control after 7 and 13 days of exposure (Fig. 2).

No significant effects on the photosynthetic parameters of photosystem II ($F_{v'}/F_m$, F_v/F_m,) were recorded (data not shown).

The bacterial biomass was similar in all treatments and control at the start of the experiment (Fig. 3). However, at day 6 the 10% scrubber washwater treatment showed a significantly higher bacterial biomass compared to the control ($p = 4*10^{-6}$). This effect was not observed at the end of the experiment.

The cell-specific bacterial productivity and the bacterial carbon production was significantly higher in 10% Scr after 6 days exposure compared to the control (Fig. 4 and Fig. 5). Contrary, the initial productivity in the 10% Scr treatment was significantly lower at day 0 as compared to the control. Due to the high variation in the controls, no significant effect was recorded between control and 10% Scr after 13 days exposure.

Table 2
Composition of scrubber washwater used in the experiment. Inlet water was collected from the research station Askö, Baltic Sea.

		Scrubber inlet	Scrubber outlet
Ca	mg/L	93.4	94.6
Fe	mg/L	< 0.004	0.0526
K	mg/L	75.1	75.8
Mg	mg/L	237	241
Na	mg/L	1910	1920
Si	mg/L	0.188	0.195
As	μg/L	0.704	0.697
Al	μg/L	10.1	29.9
Ba	μg/L	19.1	17.8
Cd	μg/L	< 0.05	< 0.05
Co	μg/L	< 0.05	< 0.05
Cr	μg/L	< 0.1	5.55
Cu	μg/L	0.838	16
Hg	μg/L	< 0.002	< 0.002
Mn	μg/L	0.342	1.77
Mo	μg/L	1.69	2.37
Ni	μg/L	0.985	3.62
P	μg/L	< 40	< 40
Pb	μg/L	< 0.3	0.749
Sr	μg/L	1380	1400
V	μg/L	0.186	0.296
Zn	μg/L	3.65	29.1
S	mg/L	171	249

3.2. Laboratory experiments

The primary productivity of *Melosira* cf. *arctica* increased with increasing scrubber washwater concentrations (Table 3), the EC10 was calculated as 9.8% at day 0 and as 5.5% at the end of the experiment. However, no effect was observed on $F_{v'}/F_m$ at either the start or end of the experiment and, thus, no EC-values could be determined. For *N. spumigena,* the addition of scrubber washwater had a positive effect on $F_{v'}/F_m$ at the start of the experiment (EC10 = 3.5%) (Table 3). In contrast, at the end of the experiment, the scrubber washwater showed a negative impact on the $F_{v'}/F_m$ (EC10 = 8.6%). As for *M.* cf. *arctica*, the initial (day 0) primary productivity of *N. spumigena* increased with increasing scrubber washwater concentration (EC10 = 14.7%). However, no dose-response pattern was observed at the end of the experiment and, thus, no EC-values could be determined (data shown in detail in Supporting information). Chemical parameters (pH, alkalinity and concentrations of nutrients) in the laboratory experiments are shown in Supporting information.

4. Discussion

An unexpected result in the experiment was the high uptake of NO_x from the exhausts in the scrubber. Hence the addition of scrubber washwater to the treatments implied an addition of inorganic nitrogen corresponding to 4.32 ± 1.32 μmol/L in the 10% scrubber washwater (Table 2). The uptake is still well within the limits set in the guidelines for scrubber washwater discharge (nitrate concentration < 60 mg/L or 968 μmol/L) (IMO, 2015). Even though a scrubber washwater concentration of 10% is not to be expected in ports and ship lanes, the additional load of nitrate from shipping may have considerable effects on the growth of pelagic microplankton, especially in eutrophicated environments such as the Baltic Sea.

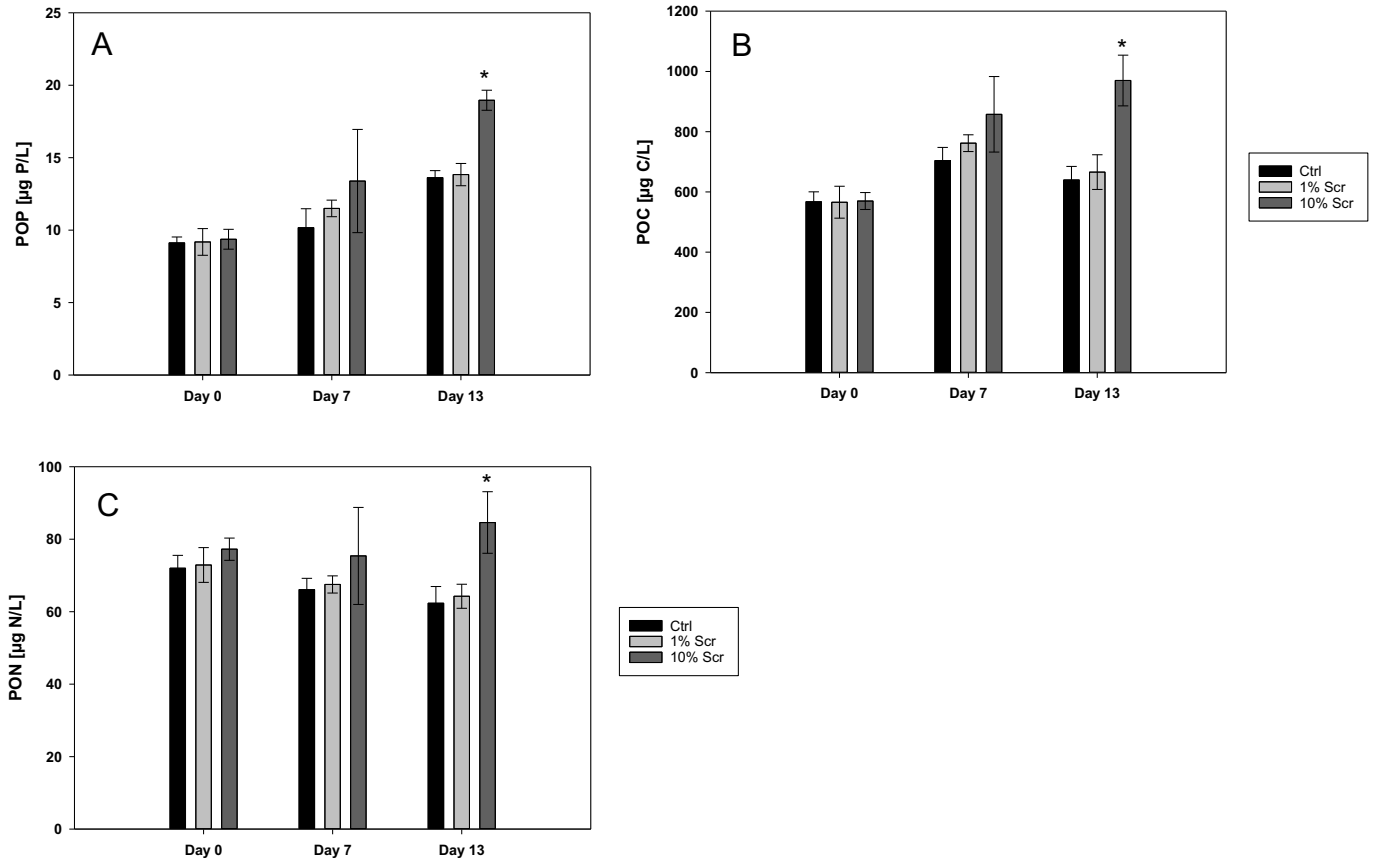

Fig. 1. Average concentrations of particulate organic phosphorous (POP) (A), particulate organic carbon (POC) (B) and particulate organic nitrogen (PON) (C) in the control (Ctrl), 1% scrubber treatment (1% Scr) and 10% scrubber treatment (10% Scr) after 0, 7 and 13 days of exposure. Error bars represent standard deviations (n = 5). Asterix (*) represent significant difference between treatment and control (after H−B correction at a familywise error rate of alfa = 0.05).

The main results of the field experiment showed that increasing concentration of scrubber washwater led to increased Chl *a*, POC, PON, POP, bacterial biomass, bacterial productivity and cell-specific bacterial productivity. The effects could be explained by stimulated algal growth due to the uptake of NO_X in the water during the scrubber process, resulting in increased nitrate concentrations. The filamentous cyanobacteria in our study are diazotrophic and have a competitive advantage over other phytoplankton when nitrogen sources in the water are limited. In the present study, the concentrations of nitrate were higher in the 10% treatment (4.3 µM) compared to the control (0.36 µM) at the start of the experiment. After 7 and 13 days of exposure, nitrate, silicate and phosphate were reduced in both scrubber washwater treatments compared to initial concentrations, indicating an increased uptake of nutrients. The increase in algal density also had an impact on the bacterial biomass which after 7 days showed a significant increase in the 10% scrubber washwater treatment compared to the control. One explanation could be an increased cell lysis releasing labile dissolved organic matter which has been shown to be an important food source for heterotrophic bacteria (Brussaard et al., 1995). Pulsed addition of sulphuric acid to freshwater plankton communities have also shown to increase bacterial biomass and metabolic processes, likely due to reduced gracing of bacteria by zooplankton and protozoans (Thomas

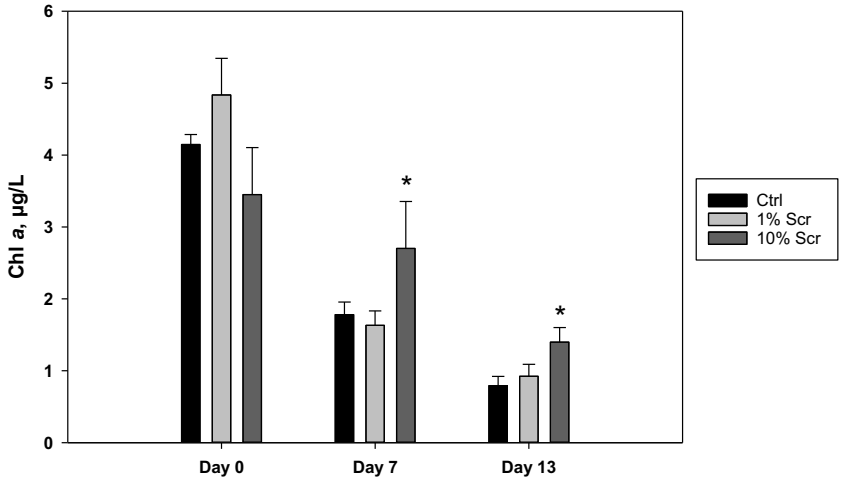

Fig. 2. Chl *a* concentrations (µg/L) in the control (Ctrl), 1% scrubber treatment (1% Scr) and 10% scrubber treatment (10% Scr) after 0, 7 and 13 days of exposure. Error bars represent standard deviations (n = 5). Asterix (*) represent significant difference between treatment and control (after H−B correction at a familywise error rate of alfa = 0.05).

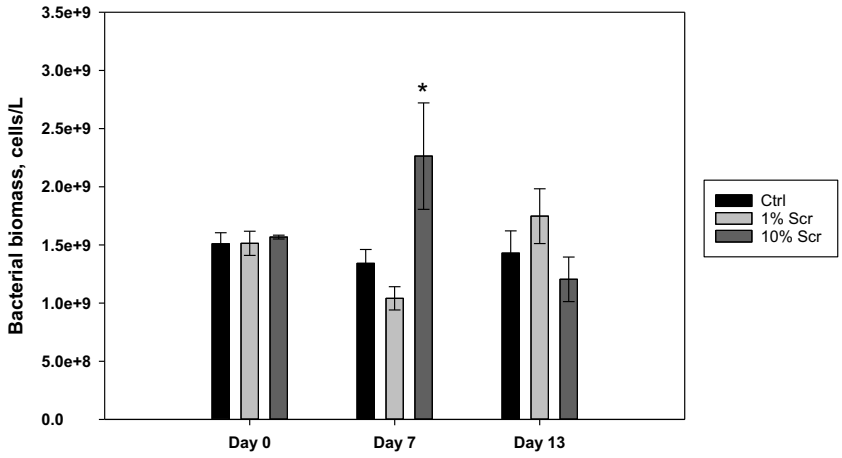

Fig. 3. Bacterial biomass (cells/L) in the control (Ctrl), 1% scrubber treatment (1% Scr) and 10% scrubber treatment (10% Scr) after 0, 7 and 13 days of exposure. Error bars represent standard deviations (n = 5). Asterix (*) represent significant difference between treatment and control (after H−B correction at a familywise error rate of alfa = 0.05).

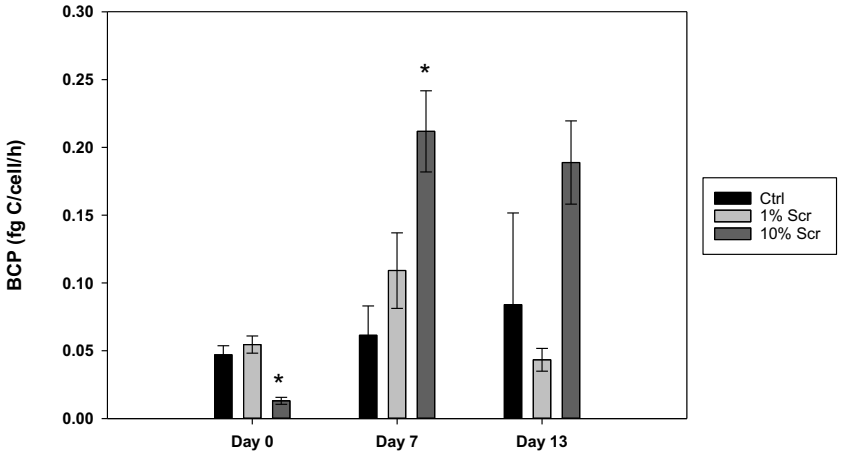

Fig. 4. Cell-specific bacterial carbon productivity (BCP) in the control (Ctrl), 1% scrubber treatment (1% Scr) and 10% scrubber treatment (10% Scr) after 0, 7 and 13 days of exposure. Error bars represent standard deviations (n = 5). Asterix (*) represent significant difference between treatment and control (after H−B correction at a familywise error rate of alfa = 0.05).

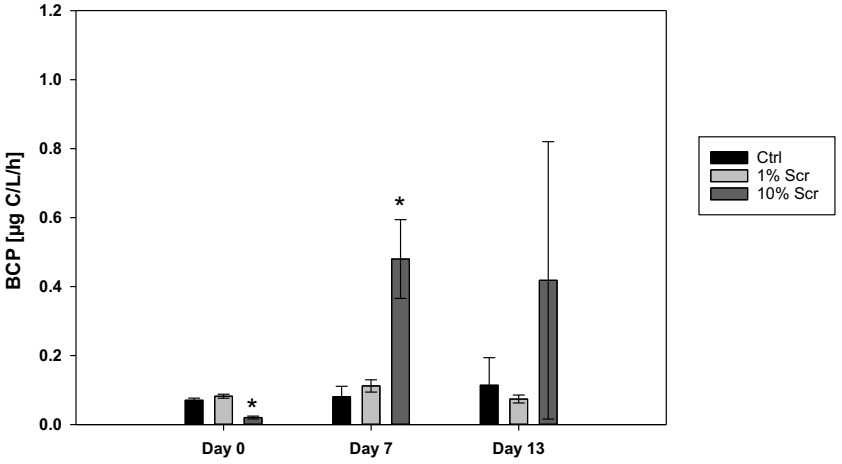

Fig. 5. Bacterial carbon production (BCP) per litre in the control (Ctrl), 1% scrubber treatment (1% Scr) and 10% scrubber treatment (10% Scr) after 0, 7 and 13 days of exposure. Error bars represent standard deviations (n = 5). Asterix (*) represent significant difference between treatment and control (after H−B correction at a familywise error rate of alfa = 0.05).

Table 3
Modelled effect concentrations (EC_x) with the corresponding 95% confidence intervals (CI), from scrubber washwater exposure to *Nodularia spumigena* and *Melosira* cf. *arctica*.

Species	Time	End point	Response	EC50	95% CI	EC10	95% CI
M. cf. *arctica*	Day 0	Primary productivity	Increase	26.9[a]	9.6–91	9.8	5.8–22.1
M. cf. *arctica*	Day 6	Primary productivity	Increase	18.3	9.0–181	5.5	3.5–22.9
N. spumigena	Day 0	Primary productivity	Increase	35.1[a]	9.0–238	14.7	5.3–30.1
N. spumigena	Day 0	$F_{v'}/F_m$	Increase	7.9	4.9–68.6	3.5	1.2–8.3
N. spumigena	Day 6	$F_{v'}/F_m$	Decrease	38.9[a]	29.1–59.6	8.6	4.1–14.0

[a] Indicate an extrapolated value.

James, 1991). Another possible explanation for the increase in bacterial productivity could be linked to the increased black carbon (BC) concentrations in the scrubber washwater. For example, Malits et al. (2015) recorded BC to stimulate heterotrophic bacterial productivity by serving as a carbon source to bacteria or by reducing the impact of viral lysis in seawater spiked with black carbon concentrations of 2000 µg/L. BC was not analysed in the current study, but Fridell and Salo (2016) reported a 90% capture of BC in open loop scrubbers (corresponding to a capture of 9.8 mg/kWh) when operating with a discharge rate of 47 m3/MWh. That capture would result in a theoretic BC concentration of 218 mg/L in the discharged scrubber washwater.

The biotic effects in the mesocosm study are consistent with those observed in the complementary laboratory study where scrubber washwater stimulated primary productivity of *M.* cf. *arctica* (EC10 = 9.81% and 5.51% at the start and end of the experiment, respectively) and *N. spumigena* (EC10 = 14.71% start of the experiment, no effect observed at the end of the experiment). For *N. spumigena*, adverse effects on $F_{v'}/F_m$ was recorded at day 0, EC10 = 3.5% scrubber washwater (95% CI = 1.2–8.3%). No such response was recorded for *M.* cf. *arctica*, implying species-specific responses to scrubber washwater discharge. However, potential adverse effects of metals in the scrubber washwater may have been masked as f/2 and f/4 was used as culture media (Guillard, 1975), containing the chelating agent EDTA.

The increased inorganic nutrient concentration in the scrubber washwater may explain the field experiment results, but as similar effects were seen in the laboratory experiment where no nutrient depletion was observed, also other substances present in the scrubber washwater may have influenced both the field study and the laboratory results.

The very few studies on scrubber washwater effects on organisms have shown both adverse and stimulating responses. For example, Koski et al. (2017) reported an increase in cell growth by the microalgae *Rhodomonas* sp. after 11 days exposure in 10% scrubber washwater compared to a control. For the copepod *Acartia tonsa*, a 10% scrubber washwater concentration resulted in a significant acute effect (mortality) compared to the control (Koski et al., 2017). In the present study, it should, however, be declared openly that a concentration of scrubber washwater of 10% is not currently a realistic scenario for the Baltic Sea. For example, US EPA calculated the discharge dilution of cruise ships moving between 9 and 17 knots to range between 200.000:1 and 640.000:1 (US.EPA, 2002). However, as the numbers of installed scrubbers on ships are increasing rapidly, discharge pulses of acidic water from ships to the marine environment will increase. Compared to long term acidification effects, where the communities will adapt over time, pulsed acidification may actually be more challenging to the microbial communities, both with respect to metabolism and densities. Temporal repeated pH reductions, from pulsed additions of sulphuric acid, have been shown to affect freshwater plankton communities significantly in terms of increased bacterial densities. The effect was suggested to be explained through reduced grazing pressure on bacteria from zooplankton and/or protozoans (Thomas James, 1991), and the results motivates further studies on marine and brackish plankton communities to assess effects of pulsed additions of scrubber washwater. It should be noted that a potential effect of grazers cannot be ruled out in our scrubber washwater experiments. In this experiment, no zooplankton was quantified, only screened initially. Small-sized zooplankton and larval stages could have passed the 250 µm mesh. In addition, eggs could have developed into larvae over the experimental period. However, in an earlier study in the same area using the same methodology, no treatment effects of grazers were found (data unpublished).

At present, there is a lack of scientific knowledge on how scrubber washwater discharge may affect the marine environment. For example, the very few studies that have characterized scrubber washwater have shown a wide range in both pH, metal and nitrate concentrations (e.g. Turner et al. (2017), Endres et al. (2018)). Thus, as the scrubber washwater itself will contain a mixture of different pressures (acidifying substances, nutrients and contaminants) it is a challenge to predict impacts on the marine environments. As shown by Turner et al. (2017), a medium-sized RoRo vessel equipped with a 12 MW main engine and an open-loop scrubber could discharge more copper and zinc to the surface water than what is leached from the ship's antifouling coating. The ecological impact of scrubber washwater discharges may be higher in the Baltic Sea which already receive a high load of pollutants from other anthropogenic sources. Due to its semi-enclosed character, concentrations of pollutants are found to be considerable higher in the Baltic Sea as compared to many other sea areas, and long-term scrubber washwater discharge may accelerate the accumulation of pollutants in the Baltic Sea. For example, Swedish environmental monitoring program has shown 20 of 36 assessed coastal water bodies to hold copper concentration exceeding the environmental quality standard (1,45 µg/L) (VISS, 2019). The situation is similar for Zinc, where 33 out of 47 coastal water bodies had concentration exceeding the environmental quality standard (1,1 µg/L) (VISS, 2019). Hence, an acceleration in scrubber installations on ships and a potential new large source of copper and zinc to Baltic Sea is alarming.

5. Conclusion

In this study, scrubber washwater was produced and its effects on a Baltic microplankton community was investigated. The main result showed scrubber washwater to stimulate the microplankton community; shown in increased Chl *a* concentrations as well as POP, POC and PON concentrations in the 10% scrubber washwater treatment. The result could be explained by elevated concentration of nitrate in the scrubber washwater. As scrubber washwater is a complex mixture of hazardous substances (metals and PAHs), acidifying substances and nutrients it is a difficult to predict environmental effects due to large-scale use of open-loop scrubbers. Hence, more ecotoxicological research on various scrubber washwaters, including effluent waters collected from ships is recommended.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2019.05.023.

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