Secretion of sulfated fucans by diatoms may contribute to marine aggregate formation

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Abstract

Microalgae produce copious amounts of structurally diverse polysaccharides, some are bound within cells and cell walls, while others are secreted into the surrounding seawater. A fraction of the secreted polysaccharides assembles into particles promoting aggregation and in turn formation of aggregates increases the export of carbon into the deep ocean via sinking. However, specific polysaccharides contributing to particle formation and carbon export remain unknown. Here, we studied microalgae polysaccharide composition in a system of reduced complexity consisting of lab grown monospecific cultures of the centric diatom species Thalassiosira weissflogii and Chaetoceros socialis. We followed the abundance and dynamics of five specific polysaccharide types in the dissolved and particulate organic matter (DOM and POM) for two weeks. Polysaccharides were detected using monoclonal antibodies (mAbs) specific for β-1,4-mannan, β-1,4-xylan, arabinogalactan, and two fucose-containing sulfated polysaccharide (FCSP) epitopes. Additionally, glycan composition of all samples was analyzed by monosaccharide analysis. The time series revealed polysaccharides partition differently between the dissolved and particulate carbon pools. β-1,4-xylan and β-1,4-mannan were mainly present in POM, possibly as cell wall polymers, while FCSPs were found in both DOM and POM. The data showed that the main glycan component secreted by diatoms was fucose-containing polysaccharide, which accumulated in DOM over time. Roller tank experiments were used to induce aggregate formation finding FCSP transitioned from DOM to POM under aggregating conditions. These results suggest that diatom-secreted FCSPs are involved in the formation of aggregates, which promote the formation of particles, and potentially carbon export.

Microscopic marine phytoplankton cells are responsible for about 40% of the global primary production and are the main source of organic carbon within the ocean (Field et al. 1998). An important fraction of this algal biomass is in the form of polysaccharides. Microalgae produce diverse types of polysaccharides that function as cell wall components and internal energy storage and are therefore part of the particulate organic matter (POM) pool (Becker et al. 2020). In addition, microalgae produce polysaccharides that are secreted into the surrounding seawater and form part of the dissolved organic matter (DOM) pool (Biersmith and Benner 1998; Rossi and De Philippis 2016).

Depending on their chemical structure, certain dissolved and secreted polysaccharides can spontaneously assemble into polymer gels (Chin et al. 1998), known as transparent exopolymer particles (TEP) (Engel et al. 2004). In particular, microalgae (including diatoms and coccolithophores) secrete anionic polysaccharides that can self-assemble by cross-links...
with cations forming TEP (Chin et al. 1998; Zhou et al. 1998; Engel et al. 2004). It is thought that these microscopic particles subsequently aggregate with diatom cells, molecules, other particles, and minerals forming larger aggregates (i.e., marine snow particles) that due to their size and increased density sink out of the surface ocean (Allerdredge and Gotschalk 1988; Iversen and Ploug 2010). Therefore, the small TEP-like particles that promote the formation of larger aggregates may influence carbon export to the deep ocean via the biological carbon pump (Verdugo 2007; Burd and Jackson 2009; Li et al. 2013).

Algal polysaccharides have different physicochemical properties, such as solubility and ionic charge, which determine their capacity to assemble. For instance, previous studies found microalgae secrete polysaccharides enriched in fucose and sulfate that form TEP in surface seawater (Zhou et al. 1998; Vidal-Melgosa et al. 2021). Other polysaccharides, including the intracellular energy storage beta-glucan laminarin, are not secreted and do not form TEP. POM derived from diatoms was found to be enriched in mannose or xylose in several species (Hecky et al. 1973; Gügi et al. 2015; Le Costauëc et al. 2017) and are likely components of the diatom cell wall, which would contribute to carbon export when diatom cells sink (alone or as part of aggregates).

Previously, polysaccharides have been analyzed with measurements of the bulk monomer composition after acid hydrolysis. Hydrolysis into monomers destroys the higher-order structure that encodes essential information about the polysaccharide type, thus limiting our ability to determine their role in aggregate formation and carbon export. Here, we used a bioanalytic approach to identify and follow the dynamics of specific polysaccharides produced by diatoms, including FCSP, xylan, mannann, and arabinogalactan. We combined enzyme-linked immunosorbent assay (ELISA) and highly specific monoclonal antibodies (mAbs) with classical quantitative chromatography to identify and quantify the production of polysaccharides, both in DOM and POM, in laboratory cultures of diatoms. We chose two centric diatoms, Thalassiosira weissflogii (10–20 µm diameter) a frequently studied model species (Gärdes et al. 2011; Seebah et al. 2014) and the chain-forming Chaetoceros socialis (2–15 µm diameter), which was isolated during a North Sea diatom bloom in 2016 and is abundant in temperate, nutrient-rich coastal regions across the globe (Booth et al. 2002; Leblanc et al. 2012; Harrison et al. 2015). DOM and POM samples from the diatom cultures were collected twice per day for 14 d. The objectives of this study were (1) to characterize the distribution of polysaccharides in DOM and POM, (2) to quantify the production of polysaccharides, (3) to monitor total monosaccharides in order to identify any differences in composition over time, and (4) to reveal which types of polysaccharides are present in aggregates. Overall, our data show that there are major differences in polysaccharide composition between the dissolved and particulate pool. Furthermore, roller tank experiments indicate diatom FCSP might be involved in the formation of aggregates. We propose that diatom-secreted FCSP forms TEP-like particles that in turn contribute to aggregate formation.

Materials and methods
Diatom cultures
The centric diatom T. weissflogii was provided by André Scheffel (Potsdam-Golm, Germany) and C. socialis was isolated at station Kabeltonne (54°11.3’N, 7°54.0’E) during the 2016 spring bloom by Tilmann Harder group (University of Bremen, Germany). Both diatom species were cultivated in triplicate batch cultures (non-axenic) with EAWAS media (Harrison et al. 1980) in Flembach flasks at a constant temperature of 15°C, with a 12-h/12-h light/dark cycle, at an irradiance flux density of 140 µmol photon m⁻² s⁻¹. A volume of 1.2 L was incubated with 30 mL culture that had been grown for 7 d and the initial concentration of T. weissflogii and C. socialis after inoculation were (0.91 ± 0.04) × 10⁴ and (1.76 ± 0.12) × 10⁴ cells mL⁻¹, respectively. Diatom abundance was measured by cell counting using a Sedgewick–Rafter chamber. Sampling started from day 0 after inoculation and lasted for 14 d. See sampling scheme in Fig. S1a. Ten milliliters of each of the cultures were harvested every morning (09:00 h) and afternoon (18:00 h) by filtration at 200 mbar on a pre-combusted (450°C for 4 h), 25 mm GF/F glass microfiber filter (Whatman) and the biomass retained on the GF/F was regarded as POM. The filtrate was further filtered with a 0.2-µm polycarbonate (PC) filter (Whatman) and the 0.2 µm filtrate was regarded as DOM, which was stored in a 25 mL precombusted glass vial (450°C for 4 h). All DOM and POM samples were kept at −80°C until further analysis.

Dissolved organic carbon (DOC) analysis
Analysis of DOC was performed on the 0.2 µm filtrate. After thawing, samples were transferred to precombusted glass vials (400°C, 4 h), acidified with HCl to pH 2, and diluted with ultra-pure water to yield 10 mL of volume. Quantification was performed after high-temperature catalytic oxidation on a Shimadzu TOC-VPCH total organic carbon analyzer. Accuracy was monitored by replicate analyses of Deep Atlantic Seawater Reference material (DSR, D.A. Hansell, University of Miami), which deviated on average < 5%. Because of limited volume, no sample replicates (technical replicates) were analyzed, and reported concentrations are averages of three replicate cultures after exclusion of obviously contaminated samples (i.e., measured DOC concentration more than two times higher than in the replicates). Deviation of biological replicates after outlier removal was on average 2 ± 3% (range 1–10%).

Particulate organic carbon (POC) analysis
Three filter pieces (diameter 5 mm) were punched out from the 25 mm GF/F filters (containing our POM samples) and were placed in a clean petri dish. After acidification with concentrated HCl vapor for 24 h to remove the inorganic carbon,
filters were dried at 60°C for 24 h using an oven and POC concentrations were measured on an Elemental Analyzer (Elementar). Sulfanilamide was used to make a calibration curve. For each sample, technical triplicates were performed.

**Polysaccharide extraction of POM samples**

GF/F filter pieces (diameter 6 mm) were punched out from the 25 mm GF/F filters (containing our POM samples) and transferred into 1.5 mL Eppendorf tubes. For each filter (one filter piece), polysaccharides were extracted sequentially with 400 μL of the following solvents: MilliQ water, 0.4 M EDTA (pH 8) and 4 M NaOH with 0.1% NaBH4 in a heating block at 600 rpm and 60°C for 1 h. Before each extraction, the content of the tubes was vortexed for about 15 s. After each extraction, samples were spun down at 14,000 × g for 10 min and the supernatants were collected and stored at −20°C. The supernatant from NaOH extraction was neutralized using pure acetic acid until pH test strips showed the pH was about 8.

**Polysaccharide analysis using microarrays and monoclonal antibodies**

Carbohydrate microarray analysis was performed to screen for monoclonal antibodies (mAbs) that recognize polysaccharides produced by the two selected diatom species (see Supplementary Methods for detailed information, Appendix Methods S1). The schematic diagrams of microarray and ELISA are shown in Fig. S1b.

**Selection of mAbs for the time series study**

Five mAbs were selected to analyze the time series samples of this study. The mAbs BAM2 (Torode et al. 2015), BAM3 (Torode et al. 2015), LM11 (McCarty et al. 2005), and LM21 (Marcus et al. 2010) were selected based on the microarray data (Fig. S2). The mAb JIM13 (Knox et al. 1991) was selected based on a previous study, where the epitope recognized by this mAb was detected in samples harvested during a diatom bloom in the North Sea (Vidal-Melgosa et al. 2021).

**Polysaccharide quantification using ELISA and mAbs**

Five polysaccharide epitopes were quantified in DOM and POM samples from the time series study with the five selected specific antibodies (stated above) using ELISA. For the analysis, 50 μL of the POM polysaccharide extracts or 50 μL of the DOM samples were added in wells of a microtiter plate (NUNC Maxisorp, Thermo Fisher Scientific) and 50 μL of phosphate-buffered saline (PBS; 140 mM NaCl, 2.7 mM KCl, 10 mM Na2HPO4, 1.7 mM KH2PO4, pH 7.5) were added in each well thus having 100 μL per well. Plates were sealed and incubated overnight at 4°C. After overnight incubation, ELISA was performed as previously described (Comuault et al. 2014) and absorbance was measured at 450 nm using a SPECTROstarNano absorbance plate reader (BMG Labtech). Polysaccharide concentration was estimated by comparison to calibration curves established with polysaccharide standards applied to the same microtiter plate. Because the mAbs have high specificity and affinity, mAbs binding compared to a no polysaccharide control indicates the presence of structurally defined epitopes. However, the unknown abundance of these epitopes per polymer molecule may differ compared to the polysaccharides that were used as calibration standards and also to develop these mAbs by immunization of rats (McCarty et al. 2005; Marcus et al. 2010; Torode et al. 2015). Therefore, reported concentrations obtained with the ELISA should be regarded as estimates. We report them as polysaccharide standard equivalents (SE) akin to the commonly used quantification of TEP with Alcian blue staining (Thornton et al. 2007; Gärdes et al. 2011).

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\text{SE} = \frac{\text{Standard antibody signal intensity (OD}_{450})}{\text{Sample antibody signal intensity (OD}_{450})} \times \text{Standard concentration}
\]

**Epitope deletion**

To verify the specificity of antibodies, the HMWDOM samples (DOM samples after dialysis at 4°C for 24 h using 1 kDa tubing) and POM extracts were incubated with several carbohydrate-active enzymes (see Appendix Methods S2).

**Quantification of monosaccharides with high-performance anion-exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD)**

For monosaccharide quantification, standard mix ranging from 1–10 to 1000 μg L−1 were used. Standard mix included fucose (Fuc), rhamnose (Rha), galactosamine (GlcN), arabinose (Ara), glucosamine (GlcN), galactose (Gal), glucose (Glc), mannose (Man), xylose (Xyl), gluconic acid (Gul), muramic acid (Mur), glucuronic acid (Glu), and mannuronic acid (Man). Samples were analyzed using a Dionex ICS5000 system equipped with a CarboPac PA10 analytical column (2 × 250 mm) and a CarboPac PA10 guard column (2 × 50 mm). The operation procedure was performed as described in Engel and Händel (2011). See monosaccharides analysis scheme in Fig. S1c.

**Monosaccharide analysis of HMWDOM and POM from diatoms batch cultures**

DOM samples (1 mL) were dialyzed using 1 kDa dialysis tubes at 4°C for 24 h. The DOM samples after dialysis were reported as HMWDOM (molecules > 1 kDa). For HMWDOM, 500 μL of samples were hydrolyzed by adding 500 μL of 2 M HCl and incubated for 24 h at 100°C in a precombusted ampule (450°C for 4 h). For POM, one piece of GF/F filter (diameter 5 mm) was hydrolyzed directly in a precombusted ampule at 100°C with 1 mL of 1 M HCl for 24 h. After hydrolysis, 500 μL of samples were lyophilized and resuspended in 250 μL of MilliQ water. After filtration through a 0.2 μm membrane filter (Costar), 100 μL of the filtrate were subjected to HPAEC-PAD. Arabinose and glucosamine peaks could not be
separated in all samples because they had similar retention times, and were further reported as arabinose + glucosamine. The peaks of mannose and xylose in POM samples did not separate well, and were therefore reported as mannose + xylose.

**Monosaccharide analysis of HMWDOM from aggregate-free water**

The HMWDOM samples were prepared and acid hydrolyzed as described above. Afterward, 500 μL of hydrolysates were dried for 4.5 h at 40°C in a centrifugal vacuum concentrator and resuspended in 110 μL MilliQ water. After centrifugation at a speed of 14,000 × g for 2 min, 100 μL of the supernatant was subjected to HPAEC-PAD analysis.

**Monosaccharide analysis of purified T. weissflogii FCSP**

The monosaccharide composition of seven FCSP fractions after anion exchange chromatography (AEX) and six FCSP fractions after size-exclusion chromatography (SEC) was analyzed. Samples were prepared and analyzed as described in Monosaccharide analysis of HMWDOM from aggregate-free water section, except that samples were not concentrated when resuspended (500 μL samples after acid hydrolysis were dried and resuspended in 500 μL MilliQ).

**Quantification of sulfate**

The samples for sulfate measurement were the same fractions (the fractions after SEC) and were obtained with the same sample preparation as described above in the Monosaccharide analysis of purified FCSP T. weissflogii section. Sulfate determination was performed on a Metrohm 761 ion chromatograph equipped with a Metrosep A Supp 5 column. The mobile phase was 3.2 mM Na₂CO₃ and 1 mM NaHCO₃. The running time was 20 min, and the elution time of sulfate was about 16 min.

**Roller tank experiments**

A volume of 2 liters of diatom cultures was grown in two 1-L Fernbach flasks at 15°C using a 12-h light period at 140 μmol photon m⁻² s⁻¹. After 3 weeks, diatom abundance was measured by cell counting using a Sedgewick-Rafter chamber. Cultures were then diluted with sterile ESAW media to concentrations of 2 × 10⁴ cells mL⁻¹ (T. weissflogii) and 2 × 10⁵ cells mL⁻¹ (C. socialis), followed by incubation in 1.15-L plexiglass cylinders with diameters of 14 cm and depths of 7.47 cm. For each diatom species, two rolling tanks (one experimental tank and one control tank) were prepared. The experimental and control tanks were placed on a rolling table at 15°C for 72 h at three and zero rotations per minute, respectively. To avoid diatom growth, experiments were conducted in darkness. Aggregates and 10 mL of aggregate-free water from rolling tanks were collected after 72 h of incubation. The DOM was produced by filtration of aggregate-free water at 200 mbar on a 0.2 μm PC membrane filter (Whatman). The polysaccharides and monosaccharides in DOM were measured by ELISA (eight technical repetitions) and HPAEC-PAD (three technical repetitions) according to the methods mentioned above. The rolling tank experiment was repeated two times.

**Immunolabeling of aggregates**

Aggregates formed in the roller tanks were individually collected, transferred into glass vials and directly fixed using 2% formaldehyde at 4°C for 12 h (Flintrop et al. 2018). After washing three times with PBS to remove the formaldehyde, aggregate fragments were filtered onto a 0.2 μm PC membrane (Whatman) at 120 mbar. The immunolabeling was performed as previously described (Jackson and Blythe 1993) with some modifications. Briefly, pieces of the membrane were blocked with 1 mL PBS with 5% (w/v) low-fat milk powder (MPBS) for 1 h at room temperature followed by incubation for 1.5 h with 250 μL of one of the probes: BAM2, BAM3, LM11, LM21, and JIM13, all diluted 1 : 5 in MPBS. A membrane incubated with 250 μL MPBS was included as a negative control. After washing three times with PBS, the filters were incubated for 1.5 h in the dark with an anti-rat secondary antibody conjugated to fluorescein isothiocyanate (FITC) (Sigma-Aldrich) diluted 1 : 100 in MPBS. To visualize microbial cells, filters were stained with 4’,6-diamidino-2-phenylindole (DAPI) at 1 μL mL⁻¹ for 10 min at room temperature in the dark. After careful rinsing with MilliQ and 96% ethanol, filters were air-dried and embedded in a mounting medium for microscopic identification. mAb-DAPI-stained filter pieces were imaged with epifluorescence microscopy. A scheme of the experimental design is shown in Fig. S1d.

**FCSP purification by AEX and SEC**

To test if the FCSP was negatively charged, the FCSP from T. weissflogii was purified. For FCSP purification, see Appendix Methods S3.

**Statistical analysis**

To identify the temporal trend of monosaccharides in HMWDOM, we used Pearson’s correlation analysis to group monosaccharides with similar patterns. The correlation coefficients were calculated by SciPy (Jones et al. 2001), a Python-based open-source software ecosystem. Other statistical analyses were carried out by using software GraphPad Prism 7.00. Significant differences among the means were determined by one-way ANOVA. p-Values below 0.05 were considered statistically significant.

**Results**

**Monitoring the growth of diatom cultures**

We monitored diatom cultures of two diatom species over 14 d and found that POC accumulation was tightly related to the increase of cell concentration, while DOC mainly accumulated during the stationary phase (Fig. 1). For both species, the
lag phase lasted for about 1 d, followed by exponential growth until day 5 in *T. weissflogii* and day 4 in *C. socialis*, after which the stationary phase lasted until the end of the experiment on day 14. For *T. weissflogii*, maximum cell concentrations were $19.0 \pm 1.3 \times 10^4$ and for *C. socialis* $68.3 \pm 3.9 \times 10^4$ cells mL$^{-1}$. The observed maximal cell yields were close to the those reported in previous studies of about $17.1-20.8 \times 10^4$ cells mL$^{-1}$ for *T. weissflogii* (*f/2* metal medium) (Ushizaka et al. 2008) and $77.8 \times 10^4$ cells mL$^{-1}$ for *C. socialis* (Myklestad 1974). In this study, organic matter was operationally divided into POM (>0.7 μm) and DOM, with the latter being defined as organic constituents passing through a 0.2-μm pore size filter (Jiao et al. 2010). We measured the concentrations of organic carbon in each of the two pools. DOC accumulated during the stationary phase, and after 14 d reached a maximum of $330 \pm 29$ and $365 \pm 19 \mu$M C/L in *T. weissflogii* and *C. socialis* cultures, respectively (Fig. 1a,b). The accumulation of POC followed a trend similar to diatom cell numbers, and after 14 d reached a maximum of $1712 \pm 101$ and $1486 \pm 96 \mu$M C/L in *T. weissflogii* and *C. socialis* cultures, respectively (Fig. 1c,d).

**Identification of polysaccharides in DOM and POM**

To investigate whether different diatom polysaccharides partition differently between DOM and POM, we used mAbs to identify polysaccharides in both pools and to monitor their abundance over time. First, we screened the polysaccharide composition of *T. weissflogii* and *C. socialis* by carbohydrate microarray analysis to choose several polysaccharide epitopes (i.e., the specific part of the polysaccharide that is recognized by a mAb) for the time series study. Note that microarray analysis uses very low amounts of sample (picoliters) compared to ELISA (microliters), yet for ELISA a concentration step was not required. For the microarray analysis, POM samples from both diatom species as well as DOM samples from *T. weissflogii* were collected from cultures on day 10 after inoculation. The microarray analysis identified fucose-containing sulfated polysaccharide (FCSP), β-1,4-mannan, β-1,3-glucan, xyloglucan, β-1,4-xylan, and alginate (Fig. S2). We chose to focus on the following polysaccharides: FCSP (two distinct epitopes recognized by mAbs BAM2 and BAM3), β-1,4-mannan (mAb LM21), and β-1,4-xylan (mAb LM11) to include polysaccharides found in both pools. In addition, we included arabinogalactan (mAb JIM13) which was not identified in the microarray analysis, but has previously been detected in a North Sea diatom bloom (Vidal-Melgosa et al. 2021).

Once the five polysaccharide epitopes were chosen, their presence and abundance dynamics (using ELISA with the corresponding mAbs) were followed in the DOM and POM pools of the diatom cultures over 14 d (Fig. 2). Polysaccharides from the dissolved fraction were directly analyzed, while polysaccharides present in POM were extracted from the filters. Since polysaccharides have different extractability/solubility, POM samples were sequentially extracted with MilliQ, EDTA,

![Fig. 1](image-url) Changes in cell density and observed dynamics of DOC (a, b) and POC (c, d) concentration during growth of the marine phytoplankton species *T. weissflogii* and *C. socialis*. The study was conducted with three replicate cultures for each diatom species. Each data point is the mean of measurements from the three cultures and error bars are the standard deviation of the mean.
and NaOH to extract water soluble, cation cross-linked, and water insoluble polysaccharides, respectively. Data are presented as polysaccharide equivalents. An overview of mAbs and polysaccharide standards used is given in Table 1. In *T. weissflogii* cultures, the two distinct FCSP epitopes were abundant in both pools (Fig. 2a,b), while β-xylan and β-mannan were primarily present in POM (Fig. 2c,d).

Our data show that FCSP is secreted by diatoms. The concentration of FCSP detected in DOM with BAM2 reached a maximum of 41.1 ± 6.3 mg L⁻¹ after 11 d. A similar pattern, albeit at a lower concentration, was observed in POM with maximum concentrations depending on the extraction solvent being 13.9 ± 3.14 (EDTA), 13.6 ± 1.7 (MilliQ), and 2.2 ± 1.2 mg L⁻¹ (NaOH) (Fig. 2a). In DOM, FCSP was also detected by BAM3 and increased with cell concentration during the exponential phase reaching a maximum of

**Table 1.** Antibodies and polysaccharide standards used in the study.

| Antibody | Epitope | Standard |
|----------|---------|----------|
| BAM2     | Sulfated fucan (fucan) | Fucoidan from *Fucus vesiculosus* (Sigma) |
| BAM3     | Sulfated fucan (fucan) | Fucoidan from *Fucus vesiculosus* (Sigma) |
| LM11     | (1 → 4)-β-D-xylan/ arabinoxylan (xylan) | Wheat Arabinoxylan (Megazyme) |
| LM21     | (1 → 4)-β-D-(galacto)(gluco) mannann (mannan) | Galactomannan (Carob) (Megazyme) |
| JIM13    | Arabinogalactan | None |

Shorter terms used for the polysaccharide epitopes are depicted in bold within parenthesis.

**Fig. 2.** Polysaccharide distribution in DOM and POM. (a–d) Polysaccharides in the DOM and POM of *T. weissflogii*. (e, f) Polysaccharides in the DOM and POM of *C. socialis*. Polysaccharides from POM were sequentially extracted with the solvents MilliQ, EDTA, and NaOH while the polysaccharides from DOM samples were directly analyzed with no previous purification or extraction (see Methods section). Five antibodies (BAM2, BAM3, LM11, LM21, and JIM13) were used to detect five specific polysaccharide structures in POM and DOM using antibody-based ELISA. Data are presented as polysaccharide equivalents. Calibration curves were made using polysaccharide standards (Fig. S3). JIM13 signal intensity in all samples was close to the background signal (data not shown). For xylan and mannan, the NaOH extracts gave irregular signals with high deviation between replicates and data are not shown. The experiment was conducted with three replicates; each data point is the mean of measurements from the three cultures.
48.2 ± 8.2 mg L⁻¹, whereas FCSP (BAM3) detected in *T. weissflogii* POM had a maximum concentration of 55.4 ± 3.9 (EDTA), 18.6 ± 3.1 (MillIQ), and 9.3 ± 2.4 mg L⁻¹ (NaOH) during the stationary phase (Fig. 2b). Binding by BAM2 and BAM3 might result from the presence of two different epitopes within one molecule (i.e., one FCSP that contains the two epitopes recognized by the two antibodies). Alternatively, different types of coexisting FCSPs might be recognized by BAM2 and BAM3.

Xylan and mannin in *T. weissflogii* were primarily detected in POM with lower concentrations in DOM (Fig. 2c,d). The concentration of xylan after 14 d of incubation reached 6.1 ± 1.4 μg L⁻¹ in DOM and in POM 45.3 ± 6.7 (EDTA) and 33.3 ± 3.6 μg L⁻¹ (MillIQ). NaOH extracts gave irregular signals (high deviation between replicates), which probably resulted from the fact that the extracts pH had to be neutralized before adding them into the ELISA plate, which might have affected the solubility of the mannan and xylan polysaccharides (data not shown). Xylan in POM extracted with MilliQ increased until day 14. Overall, the higher concentrations of FCSP in DOM compared to POM are typical for a secreted polysaccharide and consistent with previous observations (Engel et al. 2004), while the presence of xylan and...
Table 2. Concentration of DOC, POC, total monosaccharides (TM), and individual monomers in HMWDOM and POM of *T. weissflogii* and *C. socialis* at the end of sampling (14 d).

| Carbon pool | **T. weissflogii** | | **C. socialis** |
|-------------|-------------------|------------------|------------------|
|             | HMWDOM            | POM              | HMWDOM           | POM              |
|             | DOC (µM C/L) (%)  | DOC (µM C/L) (%) | DOC (µM C/L) (%) | DOC (µM C/L) (%) |
| DOC         | 330±29 100        | 1712±101 100     | 365±19 100       | 1486±96 100      |
| POC         | 0.5 0.2 5.8       | 103.2±2.3 28.3   | 0.6 0.2 4.2      | 114.3±14.5 7.7   |
| TM          | 81.9±1.2 24.8     | 19.3±0.8 5.3     | 32.1±0.8 8.8     | 5.0±0 0.3        |
| Fuc         | 22.8±0.6 6.9      | 0.2±0 0          | 0.4±0.1 0.1      | 3.1±0.3 0.2      |
| Rha         | — —               | 15.2±0.2 4.2    | — —              | 6.6±11.5 4.5     |
| Ara + GlcN  | 1.1±0.2 0.3       | 5.8±0.5 1.6      | 17.1±1.4 4.7     | 13.0±0.6 3.6     |
| Gal         | 14.2±0.1 4.3      | 15.4±1.9 0.9    | 0.2±0 0          | 6.6±11.5 4.5     |
| Glc         | 6.0±0.8 1.8       | 28.6±2.1 1.7    | 15.2±0.2 4.2     | 6.6±11.5 4.5     |
| Man         | 11.0±1.3 3.3      | 104.6±16.3 6.1  | 5.8±0.5 1.6      | 66.2±11.5 4.5    |
| Xyl         | 17.9±0.4 5.4      | 119.4±11.9 7.0  | 17.1±1.4 4.7     | 20.5±2.1 1.4     |
| Man + Xyl   | 119.4±11.9 7.0    | 0.7±0.5 0       | 0.2±0 0          | 0.7±0.1 0.1      |
| GuA         | 0.4±0 0.1         | 5.8±3.8 0       | 0.1 0.1          | 1.1±0.2 0.1      |
| MurA        | 0.5±0.5 0.2       | 15.0±2.1 1.5    | 0.4±0.1 0.1      | 0.3±0 0          |
| GalA        | 3.1±0.1 0.9       | 25.0±3.4 1.5    | 2.3±0.1 0.2      | 0.8±0 0.1        |
| GlcA        | — —               | — —             | — —              | — —              |
| ManA        | — —               | — —             | — —              | — —              |

For abbreviations, see “Materials and methods” section.

Mannan epitopes primarily in POM is consistent with their function as cell wall polysaccharides, whose monomers (xylose and mannose) were previously identified in diatoms (Hecky et al. 1973; Le Costaouëc et al. 2017).

Diatom species synthesize different types of FCSP. Interestingly, FCSP was neither detected by BAM2 nor BAM3 in DOM of *C. socialis* (Fig. 2e,f), despite previous studies showed presence of fucose in exudates of *Chaetoceros* spp. (Haug and Myklestad 1976). However, we detected FCSP in POM of *C. socialis*, although with low concentrations. In the POM extracts obtained with MilliQ and EDTA, FCSP was detected by BAM2 and BAM3 after lag phase. The FCSP concentration detected with BAM3 was higher than that detected with BAM2 especially when POM was extracted with EDTA, which increases the solubility of uronic acid bearing polysaccharides. Differences in the amount of FCSP found with the two mAbs and the different solubility suggest the presence of more than one type of FCSPs. Xylan and mannan epitopes were detected in POM of *C. socialis* but at concentrations close to the detection limit (data not shown).

To further verify the presence of the four polysaccharide epitopes detected by ELISA analysis, we performed epitope deletion with specific enzymes and confirmed the presence of xylan in POM of *T. weissflogii* and *C. socialis* (see Appendix Results S1, Table S2 and Fig. S4).

Monosaccharide composition of polysaccharides in diatom cultures

Since our polysaccharide analysis focused on five epitopes, we analyzed the monosaccharide composition of polysaccharides in HMWDOM and POM to detect major trends occurring in the entire glycan pool (Fig. 3). We identified several monosaccharides and detected different monosaccharide compositions in HMWDOM and POM in both diatom species. In addition, our data show that the relative monosaccharide abundance changed during the time series in HMWDOM, while it remained relatively constant in POM (Fig. 3a).

When looking at the concentrations reached at the end of the incubation (day 14), in *T. weissflogii* HMWDOM the molar concentration of total monosaccharides (sum of all) was 81.9±1.2 µM C/L, which corresponds to 24.8% of the total organic carbon of that pool (DOC), while in POM was 314.8±36.4 µM C/L corresponding to 18.4 mol% of the POC (Table 2). In *C. socialis* HMWDOM, the molar concentration of total monosaccharides increased to 103.2±2.3 µM C/L, 28.3 mol% carbon and in POM to 114.3±14.5 µM C/L, 7.7 mol% carbon. Concentrations of individual monosaccharides compared to DOC and POC are shown in Table 2.

Regarding the relative abundance of monosaccharides as mole percentage (Fig. 3a), in *T. weissflogii* HMWDOM the relative percentage (compared to the total monosaccharides) of...
fucose increased from 1% to 27%, galactose from 2% to 17%, and galacturonic acid from 0% to 6%; while glucose decreased from 42% to 7% and mannose from 28% to 13%. In *C. socialis* HMWDOM, the relative abundance of fucose increased from 1% to 18%, rhamnose from 0% to 31%, galactose from 2% to 14%; while glucose decreased from 30% to 6% and mannose from 39% to 16%. Our data show that an important monomer unique to the exudate of *C. socialis* was rhamnose, a signature monomer in exudates of many *Chaetoceros* spp. (Haug and Myklestad 1976), which was mostly absent in *T. weissflogii*. In regard to POM of both species, the major monosaccharides were glucose, mannose, and xylose (note that mannose and xylose peaks could not be separated in POM samples and their concentrations are presented as the sum of both monosaccharides). Glucose was the most abundant monosaccharide in POM and this is consistent with laminarin (β-1,3-glucan) being the major storage polysaccharide in diatoms (Painter 1983; Alderkamp et al. 2007; Becker et al. 2020).

Fucose is a constituent monosaccharide of FCSPs and was mainly detected in HMWDOM. The relative abundance of fucose in POM was about 5%, thus four times lower than that in HMWDOM (Fig. 3a). Although fucose concentration increased with incubation time in both pools, its concentration in HMWDOM (3.8 ± 0.1 and 3.2 ± 0.1 μM L⁻¹, respectively) was substantially higher than that in POM (1.8 ± 0.2 and 0.7 ± 0 μM L⁻¹, respectively) from both *T. weissflogii* and *C. socialis* (Fig. 3b). As mentioned above, the ratio between different monosaccharides remained rather constant in POM while it changed over time in HMWDOM. Therefore, in order to detect monomers whose abundances are correlated with and are potential components of FCSP, we performed a Pearson’s correlation analysis of the changing monosaccharide composition in HMWDOM (Fig. 3c). In *T. weissflogii* HMWDOM, the
increasing concentration of fucose was most positively correlated with galacturonic acid \( (r = 0.98, n = 16, p = 4.16 \times 10^{-11}) \) and galactose \( (r = 0.93, n = 16, p = 2.26 \times 10^{-7}) \). In \textit{C. socialis} HMWDOM, the increasing concentration of fucose was most positively correlated with galactose \( (r = 0.99, n = 16, p = 1.87 \times 10^{-13}) \) and rhamnose \( (r = 0.98, n = 16, p = 1.91 \times 10^{-11}) \). Fucose, rhamnose, and galactose were previously shown to dominate the exudates of \textit{Chaetoceros} spp. including \textit{C. socialis} (Haug and Myklestad 1976). Our results show positive correlations between these monomers and fucose, suggesting that they might be part of the FCSP structure. In contrast, glucose and mannose were negatively correlated with fucose in both diatom species (Fig. 3c).

**Polysaccharide assembly and particle aggregation**

We next tested if any of the five studied polysaccharide epitopes might be involved in polysaccharide assembly into TEP-like particles and thus promote formation of aggregates. We induced the formation of aggregates by placing diatom cultures in roller tanks, which are an experimental model for aggregation via differential settling by increasing the chance of collision between particles/cells and dissolved molecules (Ziervogel and Forster 2005; Gärdes et al. 2011; Seebah et al. 2014). The tanks were rotated for 72 h, and then the aggregates were individually picked and collected into glass vials. The collected volume, containing the picked aggregates, was then filtered and the material on the filter was analyzed by immunolabeling with the five selected mAbs to visualize if the epitopes were present within aggregates. The five mAbs were used in combination with a secondary antibody coupled to FITC and filters were examined by fluorescence microscopy (Fig. 4).

In \textit{T. weissflogii} aggregates, the FCSP epitope recognized by mAb BAM2 was mainly localized around the diatom cells. In contrast, the polysaccharide epitopes recognized by BAM3 (FCSP) and LM11 (xylan) were concentrated in patches within the aggregates. Mannan (LM21) and arabinogalactan (JIM13) epitopes were not detected, indicating that they were either not present in aggregates or that their concentrations were below the detection limit. Alternatively, epitopes present in diatom cells within aggregates can be covered by other unknown polysaccharides masking the epitopes. In regard to \textit{C. socialis} aggregates, there was no signal detected by any of the two FCSP-specific mAbs (BAM2 and BAM3). These two epitopes were not detected in \textit{C. socialis} DOM. However, the two FCSP-specific mAbs epitopes were detected in \textit{C. socialis} POM (Fig. 2e,f). The absence of mAb signal on the diatom cells buried within the aggregates indicates other polymers masked BAM2 and BAM3 epitopes. There was neither signal for LM21 nor JIM13. From the five mAbs tested, there was only signal for LM11 in confined regions within the \textit{C. socialis} aggregates (Fig. 4).

Based on the microscopy results, we identified that three of the epitopes (recognized by BAM2, BAM3, and LM11) were present in diatom aggregates. To further investigate if these three polysaccharide epitopes might assemble into TEP-like particles and promote the formation of aggregates, we measured polysaccharides in DOM after induced aggregation in roller tanks (rotated for 72 h). To have a control with no aggregate formation, we placed 3-week-old diatom cultures in roller tanks, which are an experimental model for aggregation via differential settling by increasing the chance of collision between particles/cells and dissolved molecules. We measured the polysaccharide content in DOM using ELISA. The tanks were rotated for 72 h, and then the aggregates were individually picked and collected into glass vials. The collected volume, containing the picked aggregates, was then filtered and the material on the filter was analyzed by immunolabeling with the five selected mAbs to visualize if the epitopes were present within aggregates. The five mAbs were used in combination with a secondary antibody coupled to FITC and filters were examined by fluorescence microscopy (Fig. 4).

**Fig. 5.** \textit{T. weissflogii} fucose-containing exo-polysaccharide aggregates in roller tanks. Cultures of diatoms were placed into roller tanks which were kept still or rotated. The abundance of FCSP in DOM and fucose in HMWDOM was measured before and after aggregation with ELISA and HPAEC-PAD. 0: samples from the tanks after 0 h of incubation; N: samples from the tanks with no rotation (no aggregation) after 72 h of incubation; A: samples from the tanks with rotation (aggregation) after 72 h of incubation. (a, b) Decrease of FCSP abundance (mAb BAM2 and BAM3 signal) in \textit{T. weissflogii} DOM during aggregation compared to no aggregation. (c, d) Fucose concentration in HMWDOM measured by acid hydrolysis followed by HPAEC-PAD. For boxplots, the upper, middle, and lower horizontal lines of the square represent upper quartile, median, and lower quartile of a data set, respectively; the whiskers denote the minimum and maximum of a data set. Significance was determined by one-way ANOVA corrected for multiple comparisons with Tukey’s test. ns: not significant, * \( p < 0.05 \), ** \( p < 0.01 \), and *** \( p < 0.001 \). Data correspond to two independent roller tank experiments.
and without rotation, values were close to the detection limit (data not shown); suggesting xylan is not a main player in assembly and/or aggregation. Therefore, our data obtained with ELISA and mAbs pointed toward FCSP as candidate for polymer assembly. To verify the results, *T. weissflorigii* HMWDOM was hydrolyzed with acid and the released fucose was quantified by chromatography. Fucose concentration decreased significantly ($p < 0.01$) after rotation/aggregation (Fig. 5c). When testing the other diatom species, *C. socialis* DOM was not analyzed by ELISA for FCSP content as we did not detect BAM2 and BAM3 epitopes in the dissolved pool (Fig. 2e,f). However, we quantified fucose concentration in *C. socialis* HMWDOM by chromatography and found that fucose concentration decreased, although nonsignificantly, after 72 h of rotation/aggregation compared to the control tank (Fig. 5d). In conclusion, our results from both diatom species suggest that diatom-secreted FCSPs might be involved in polysaccharide assembly and particle formation.

The diatom *T. weissflorigii* produced a negatively charged FCSP rich in fucose and sulfate. As TEP are mainly composed of unknown acidic polysaccharides (Alldredge et al. 1993), we tested if the FCSP secreted by *T. weissflorigii* was negatively charged. FCSP from the supernatant (HMWDOM) of *T. weissflorigii* cultures was separated by AEX. Results showed that FCSP eluted with 1.25 M NaCl (indicated by the BAM2 peak), confirming the anionic nature of FCSP (Fig. S5a). The monosaccharide analysis of seven AEX chromatographic fractions containing the purified FCSP (BAM2 peak) showed that all were rich in fucose (Fig. S5b), confirming the specificity of BAM2 to FCSP. In additional *T. weissflorigii* laboratory cultures, the FCSP in HMWDOM was first separated by AEX, and then further purified by SEC, hydrolyzed with HCl into monomers, followed by quantification of released monosaccharides and sulfate using HPAEC-PAD and HPLC, respectively. The results showed that *T. weissflorigii* FCSP was mainly composed of fucose (27%), galactose (14%), xylose (14%), glucuronic acid (8%), and sulfate (32%) (Fig. S5c).

**Discussion**

In this study, we used a bioanalytic approach to monitor certain polysaccharides in DOM and POM from cultures of two diatom species. It is known that a fraction of secreted algal polysaccharides are involved in the formation of TEP (Zhou et al. 1998; Engel et al. 2004). TEP have been proposed to act as a glue between microalgae cells promoting the formation of aggregates that increase carbon export to the deep ocean (Alldredge and Gotschalk 1988; Iversen and Ploug 2010). Although these glycans have a key role in the global carbon cycle, it is still unclear which ones assemble into particles. Individual polysaccharide structures are difficult to identify within complex organic matter mixtures. Most of the studies on microagal polysaccharides have been focused on their monosaccharide composition (Bernaerts et al. 2018; Gaignard et al. 2019), which provides important information on the building blocks of the glycan pool but no information on linkages or individual polysaccharide structures.

Here, we aimed to detect glycans at a higher molecular resolution by combining ELISA with molecular probes. We monitored five polysaccharide epitopes, including $\beta$-1,4-mannan, $\beta$-1,4-xylan, arabinogalactan, and two FCSP (sulfated fucan) epitopes, following their abundance in diatom monospecific cultures. Four of the five epitopes were present in the diatom cultures (Fig. 2). The epitopes presented different temporal dynamics, different extractability (for the POM fraction), and partitioned differently between DOM and POM. Additionally, in order to have an overall picture of the whole glycan pool, we analyzed the samples by monosaccharide analysis, which revealed that glycan relative composition was more constant in POM than in the DOM, and this was observed for both diatom species (Fig. 3a). Note that our POM fraction corresponds to material retained on a 0.7 $\mu$m filter. Although a 0.7 $\mu$m filtrate is typically considered DOM, which is commonly defined as organic matter that passes through a filter of 0.2–0.7 $\mu$m pore size (Dittmar and Stubbins 2014), our DOM samples correspond to the filtrate of a 0.2 $\mu$m filter. We chose to use the smallest cutoff to remove most bacteria and also to avoid having some TEP (> 0.4 $\mu$m) in our DOM fraction.

Based on the percentage of monosaccharide content that corresponds to the total POC in POM, we identified that the monosaccharides that had the highest contribution to POC were glucose and mannose + xylose in both diatom species (Table 2). Mannose and xylose may be part of cell wall mannan or xylan (or both, as the two monomers could not be separated in the POM monosaccharide analysis and are presented as the sum of both). The high concentrations of glucose in POM most likely originate from the microalgal storage compound laminarin ($\beta$-1,3-glucan), which is highly abundant in diatoms (Becker et al. 2020). In the case of HMWDOM, we found that the monomers that were most abundant and thus had the highest contribution to DOC were fucose and xylose (6.9% and 5.4%) in *T. weissflorigii* and rhamnose and fucose (8.8% and 5.3%) in *C. socialis*. In both diatom species, fucose was one of the main components of DOM, suggesting that FCSP are major diatom-secreted polysaccharides. This correlates with our ELISA data in the case of *T. weissflorigii* DOM, where we found high abundance of FCSP during the 2 weeks experiment (Fig. 2a,b). However, we could not detect FCSP in *C. socialis* DOM with ELISA (Fig. 2e,f). Similarly, we could not detect much xylan (mAb LM11) in DOM from any of the two species, although by monosaccharide analysis a high abundance of xylose (6.9% and 5.4%) in *T. weissflorigii* and rhamnose and fucose (8.8% and 5.3%) in *C. socialis*. In both diatom species, fucose was one of the main components of DOM, suggesting that FCSP are major diatom-secreted polysaccharides. This correlates with our ELISA data in the case of *T. weissflorigii* DOM, where we found high abundance of FCSP during the 2 weeks experiment (Fig. 2a,b). However, we could not detect FCSP in *C. socialis* DOM with ELISA (Fig. 2e,f). Similarly, we could not detect much xylan (mAb LM11) in DOM from any of the two species, although by monosaccharide analysis a high abundance of xylose was detected in their HMWDOM. However, if xylose from the dissolved pool was part of a $\beta$-1,3-linked, or a $\beta$-1,3,1,4-linked xylan, these would not be recognized by LM11. Even if there was xylose as part of a $\beta$-1,4-xylan, specific structural modifications, such as sulfation or additional side chains, would modify the epitope masking it against recognition by the mAb. Therefore, the differences between our
ELISA and monosaccharide analysis results can be explained by the high specificity of mAbs, which recognize one single specific epitope that must be present and accessible at the molecular level.

Although FCSP was detected in \textit{C. socialis} POM by mAbs BAM2 and BAM3, there was no detection of FCSP in DOM. Our monosaccharide data show high abundance of fucose in \textit{C. socialis} DOM (Fig. 3a, b) and thus a FCSP is most likely secreted by this diatom species, which might have specific modifications that are not recognized by mAbs BAM2 and BAM3. It is worth mentioning that FCSPs were only known to be produced by macroalgae and some marine echinoderms, but were recently found to be produced by diatoms (Vidal-Melgosa et al. 2021). The specific structure of diatom fucan is not known. Fucoidan from macroalgae, which differs between \textit{Fucus vesiculosus} and \textit{Laminaria hyperborea} (Kopplin et al. 2018). BAM2 and BAM3 are two mAbs that were recently developed by immunizing rats with polysaccharides out of 16 tested polysaccharides from land plants and algae they only bound to sulfated fucan (Torode et al. 2015); however, the exact structure of their recognized epitopes remains unknown. In conclusion, our data indicate that the two studied diatom species synthesize different types of secreted FCSPs, as FCSP in DOM of \textit{T. weissflogii} was detected by BAM2 and BAM3, while in DOM of \textit{C. socialis} was not detected by either of them (Fig. 2). This is supported by a recent study where FCSP in \textit{C. socialis} DOM was detected by mAb BAM1 (Vidal-Melgosa et al. 2021). The mAb BAM1 is, together with BAM2 and BAM3, directed to sulfated fucan preparations (Torode et al. 2015) but was not used in our ELISA experiment.

The diatom glycans also presented diel changes, with certain monosaccharides changing in abundance during the 12 : 12 h dark–light cycle. This change included glucose in POM from both species as well as galactose and glucuronic acid in HMWDOM from \textit{T. weissflogii}. This suggests that some polysaccharides fluctuated with light, which has been previously observed for the diatom storage glycan laminarin (Becker et al. 2020). Laminarin is made of glucose and in line with the previous observations, we detected that the abundance of glucose in POM was always higher in the afternoon compared to that in the morning (Fig. 3a, Fig. S6), indicating that the diatoms produced the laminarin during the day and used some of it in the dark phase. Changes in laminarin were observed in the POM pool, but Staats et al. (2000) showed that the exopolysaccharide secretion of diatoms is light-dependent (Staats et al. 2000). This raises the question of whether FCSPs were produced in DOM solely due to an overflow metabolism of photosynthesis. Our data do not support this supposition as fucose abundance in DOM did not show a regular change between light and dark phases, but accumulated during the whole incubation period (Fig. 3a, b). Whether FCSP production is influenced by the daily rhythm of light in the environment remains an open question.

Synthesizing the complicated structure of FCSPs requires numerous enzymes and energy. Previous studies demonstrated that FCSPs from brown macroalgae have antibacterial and antiviral properties (Ponce et al. 2003; Shibata et al. 2003; Li et al. 2010), and thus it could be that FCSPs from diatoms have similar properties. Our microscopy experiment showed that \textit{T. weissflogii} cells were surrounded by FCSP (recognized by BAM2) (Fig. 4), which may act as a barrier against pathogens akin to the role of mucins in human gut epithelia cells (Wheeler et al. 2019).

Regarding the FCSPs in DOM, on the basis of FCSPs being main components of diatom exudates and negatively charged, we hypothesize that secreted FCSPs are part of the abovementioned fraction of microalgae-secreted polysaccharides that assemble into TEP-like particles, which promote coagulation and contribute to aggregate formation. Previous studies have indeed shown the formation of TEP by fucose-rich sulfated polysaccharides that were secreted by microalgae (Zhou et al. 1998; Vidal-Melgosa et al. 2021). However, the trends observed in our DOM and POM data (both in ELISA and monosaccharide analyses, Figs. 2, 3) do not present dynamics resembling polymer assembly and/or coagulation. If any or both of those two processes were occurring, we would expect an increase in DOM followed by a decrease that would be accompanied by an increase in POM, due to the DOM to POM transition when TEP is formed (Engel et al. 2004). An increase of FCSP in DOM was detected but not a decrease (Figs. 2a, b, Fig. 3b). An explanation for that could be that cultures were still, i.e., there was no mixing of the diatom cultures through the incubation of the time series experiment, conditions which do not resemble the turbulence and mixing that occur in the ocean. The secretion of unknown dissolved polysaccharides that form TEP has been shown by previous studies, but microalgae cultures/mesocosms were mixed throughout by an airlift (Engel et al. 2004) or via bubbling (Zhou et al. 1998). Thus, our culture conditions were intended to determine the glycan composition and different partitioning in the DOM and POM pool, but were not designed for aggregate formation.

Therefore, we induced aggregation by roller tank experiments. Subsequently, immunolabeling analysis showed the presence of FCSP in \textit{T. weissflogii} aggregates supporting our hypothesis that secreted FCSPs assemble into TEP-like particles, which promote coagulation and contribute to aggregate formation (Fig. 4). The FCSP epitopes detected by BAM2 and BAM3 were not detected in \textit{C. socialis} aggregates, which as described above have been found to contain a FCSP with a structure not recognized by those two mAbs. Furthermore, we investigated the role of FCSP in aggregation by an additional
experiment. As stated above, if FCSP is involved in assembly and/or aggregation, it would decrease in DOM, while increasing in POM. Our results showed that roller tanks promoted aggregation leading to a decrease of FCSP in DOM over time compared to a non-aggregating control (Fig. 5a,b). FCSP therefore appeared to transition from the dissolved to the particulate pool under aggregating conditions. The observed FCSP decrease in DOM was incompatible with bacterial degradation, because FCSPs have a highly complex structure that makes them recalcitrant and difficult to degrade by marine heterotrophs (Sichert et al. 2020) and we had identical diatom cultures in the roller tank controls (the only difference being rotation or no rotation). Similarly, we do not expect that these differences were due to a different FCSP secretion among tanks, as they were kept in dark conditions to minimize new production of glycans. Notably, the finding of FCSP from DOM being incorporated into aggregates was verified with two independent techniques, detection of FCSP by specific mAbs and detection of fucose by chromatography. In conclusion, these data suggest that diatom-secreted FCSPs contribute to the formation of marine aggregates, influencing the ability of the biological pump to export carbon.

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Conflict of Interest

None declared.

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