Biogeosciences Discuss., 11, 15289–15325, 2014
www.biogeosciences-discuss.net/11/15289/2014/
doi:10.5194/bgd-11-15289-2014

Biogeosciences Discussions
Open Access

Size-fractionated dissolved primary production and carbohydrate composition of the coccolithophore *Emiliania huxleyi*

C. Borchard[1,2] **and A. Engel**[1,2]

[1]GEOMAR – Helmholtz Centre for Ocean Research, 24105 Kiel, Germany
[2]Alfred Wegener Institute for Polar & Marine Research, 27570 Bremerhaven, Germany

Received: 7 October 2014 – Accepted: 9 October 2014 – Published: 4 November 2014

Correspondence to: A. Engel (aengel@geomar.de)

Published by Copernicus Publications on behalf of the European Geosciences Union.

Abstract

Extracellular release (ER) by phytoplankton is the major source of fresh dissolved organic carbon (DOC) in marine ecosystems and accompanies primary production during all growth phases. Little is known, so far, on size and composition of released molecules, and to which extent ER occurs passively, by leakage, or actively, by exudation. Here, we report on ER by the widespread and bloom-forming coccolithophore *Emiliania huxleyi* grown under steady state conditions in phosphorus controlled chemostats (N : P = 29, growth rate of $\mu = 0.2\,d^{-1}$). ^{14}C incubations were accomplished to determine primary production (PP), comprised by particulate ($PO^{14}C$) and dissolved organic carbon ($DO^{14}C$), and the concentration and composition of particulate combined carbohydrates (pCCHO), and of high molecular weight (> 1 kDa, HMW) dissolved combined carbohydrates (dCCHO) as major components of ER. Information on size distribution of ER products was obtained by investigating distinct size classes (< 0.40 µm, < 1000 kDa, < 100 kDa and < 10 kDa) of $DO^{14}C$ and HMW-dCCHO. Our results revealed relatively low ER during steady state growth, corresponding to ~ 4.5 % of primary production, and similar ER rates for all size classes. Acidic sugars had a significant share on freshly produced pCCHO as well as on HMW-dCCHO. While pCCHO and the smallest size (< 10 kDa) fraction of HMW-dCCHO exhibited a similar sugar composition, dominated by high percentages of glucose (74–80 Mol %), the composition of HMW-dCCHO size-classes > 10 kDa was significantly different with higher Mol % of arabinose. Mol % of acidic sugars increased and Mol % glucose decreased with increasing size of HMW-dCCHO. We conclude that larger polysaccharides follow different production and release pathways than smaller molecules, potentially serving distinct ecological and biogeochemical functions.

BGD
11, 15289–15325, 2014

1 Introduction

The global ocean inventory of dissolved organic carbon (DOC) is estimated to be in a range of 662–700 Gt (Hansell and Carlson, 1998; Ogawa and Tanoue, 2003). A common classification of marine DOC relies on its reactivity and discriminates between labile (LDOC), semi-labile (SLDOC), semi-refractory (SRDOC), refractory (RDOC) and ultra-refractory (URDOC) DOC with lifetimes of hours to days, weeks to months, month to years, centuries or even millennia (Kirchman, 1993; Carlson and Ducklow, 1995; Anderson and Williams, 1999; Hansell, 2013). Only a small fraction of marine DOC is considered reactive; LDOC (< 0.2 Gt) and SLDOC (6 ± 2 Gt) (Hansell, 2013). In general, these compounds are freshly produced by plankton and represent the major nutritional resource for heterotrophic microorganisms (Cherrier et al., 1996; Amon and Benner, 1996; Amon et al., 2001; Benner, 2002; Azam and Malfatti, 2007; Davis et al., 2009). Especially during the summer season, SLDOC can accumulate in temperate waters, and becomes available for deep convective mixing, contributing to the biological carbon pump (Hopkinson and Vallino, 2005; Hansell et al., 2009). Microbial assimilation of DOC as well as the formation of gel particles, such as transparent exopolymer particles (TEP), lead to a repartitioning of DOC into the particulate organic carbon (POC) pool (Alldredge et al., 1993; Chin et al., 1998; Engel et al., 2004), the sinking of which represents another pathway for carbon export and storage in the ocean. In addition, microbial processing of fresh DOC may result in formation of recalcitrant compounds with longer residence time, also increasing the CO_2 storage potential in the ocean (Jiao and Zheng, 2011). Thus, deeper insights to the origin and quality of DOC in the ocean can greatly abet our ability to quantify carbon and nutrient cycling in the ocean.

The ultimate source of organic carbon in the ocean is primary production, and extracellular release (ER, also referred to as dissolved primary production) of organic carbon is the primary source of fresh DOC, followed by cell lysis (Fuhrman, 1999), grazing (Møller, 2005), enzymatic particle solubilisation (Cho and Azam, 1988; Smith et al., 1992) and sloppy feeding (Copping and Lorenzen, 1980; Nagata, 2000). The major

BGD
11, 15289–15325, 2014

components of phytoplankton ER are high molecular weight (HMW, > 1 kDa) dissolved combined carbohydrates (dCCHO), representing also the largest characterizable fraction of marine dissolved organic matter (DOM); 15–35 % DOC in the surface ocean, 5–10 % DOC in the deep ocean (Benner et al., 1992; Pakulski and Benner, 1994; Biddanda and Benner, 1997; Ogawa and Tanoue, 2003). Composition of HMW-dCCHO in seawater is usually determined on the basis of monomeric sugars after hydrolysis of the polymer chains, and resembles either phytoplankton biomass itself (Pakulski and Benner, 1994; Børsheim et al., 1999) or extracellular CCHO from phytoplankton cultures (Biersmith and Benner, 1998; Aluwihare and Repeta, 1999; Aluwihare et al., 2002). The latter are usually comprised by neutral hexoses, pentoses and deoxysugars like glucose, galactose and mannose, by amino sugars like glucosamine and galactosamine and by uronic acids e.g. galacturonic acid and glucuronic acid (Aluwihare et al., 1997; Biersmith and Benner, 1998; Aluwihare and Repeta, 1999; Engel et al., 2010; Borchard and Engel, 2012).

ER is a normal function of healthy algae cells during all stages of growth (Fogg, 1966; Mague et al., 1980; Bjørnsen, 1988; Borchard and Engel, 2012; Lopez-Sandoval et al., 2011) and can comprise up to 80 % of primary production (Sharp, 1977; Mague, 1980; Fogg, 1983; Bjørnsen, 1988). Two conceptual models have been proposed for phytoplankton ER: (i) the *passive diffusion model* that describes the leakage of smaller molecules from inside the cell to its surrounding environment (Fogg, 1983; Bjørnsen, 1988), and (ii) the *overflow model* that assumes an energy consuming exudation of HMW compounds (Fogg, 1983; Nagata, 2000; Schartau et al., 2007). According to the *passive diffusion model*, DOC crosses the cell membrane independently from PP during day and night, and ER correlates to phytoplankton biomass and cell size. A higher relative contribution of ER to total PP would therefore be expected in communities dominated by small cells due to their higher surface to volume ratio (Bjørnsen, 1988; Kiørboe and Hansen, 1993; Marañón et al., 1996).

Central aspects of the *overflow model* are a dependence of ER on primary production (PP) rates, the absence of ER at night and a high share of HMW substances (Williams,

BGD
11, 15289–15325, 2014

1990 and references therein, Nagata, 2000). Fogg (1966) proposed that photosynthesis and build-up of organic carbon is primarily regulated by irradiance, while cell growth is controlled by the availability of inorganic nutrients. The discharge of photosynthesates, not utilized for cell growth, was suggested to be more energy-efficient than intracellular storage (Wangersky, 1978; Wood and van Valen, 1990). In accordance with the *overflow model*, data from coastal, marine and estuarine systems revealed a linear relationship between PP and ER, and factors influencing PP were suggested to also affect ER (Baines and Pace, 1991). Such effects were shown for light (Zlotnik and Dubinsky, 1989) and later suggested also for CO_2 (Engel, 2002) and temperature (Moran et al., 2006). Under nutrient limitation, however, substantial ER was observed when PP was reduced, leading to higher percentages of extracellular release (PER) (Myklestad et al., 1989; Goldman et al., 1992; Obernosterer and Herndl, 1995; Halewood et al., 2012). Under such conditions, decoupled from PP and biomass, ER becomes difficult to estimate, both in terms of quantity and quality. Moreover, phytoplankton cells display a large physiological plasticity for nutrient requirements, i.e. the nutrient cell quota, which varies with environmental conditions or among different taxonomic groups (Geider and LaRoche, 2002).

Despite their role in marine carbon cycling, processes involved in the production, consumption and remineralisation of extracellular organic matter are little understood and have largely been neglected in biogeochemical models (Flynn et al., 2008; Repeta and Aluwihare, 2006; Hansell et al., 2009; Hansell, 2013). So far, it is not known if extracellular products are mainly released by leakage or by exudation processes, or how much leakage and exudation products differ. We also don't know if and how the physiological status of the cell influences the composition of extracellular products, and whether or not such differences in chemical signatures subsequently affect their microbial cycling, remineralisation rate, or affinity to form gel particles.

In order to improve our understanding on ER, we conducted a chemostat experiment with *E. huxleyi* under fully controlled nutrient supply and growth rate. *Emiliania huxleyi* is a bloom forming cosmopolitan coccolithophore species, and known to pro-

BGD
11, 15289–15325, 2014

duce a methylated, acidic polysaccharide that plays a central role in coccolith formation and agglutination (Fichtinger-Schepmann, 1979; De Jong, 1979). ER by *E. huxleyi* cells was reported earlier (Aluwihare and Repeta, 1999; Biddanda and Benner, 1997; Borchard and Engel, 2012) and carbohydrates were shown to provide a substantial fraction of freshly produced HMW-DOC (35–94 %) (Aluwihare and Repeta, 1999; Biddanda and Benner, 1997).

With our study we wanted (i) to determine ER of DOC and carbohydrates by combining rate measurements for particulate and dissolved primary production with analyses of carbohydrate concentration, and (ii) to characterize monomeric carbohydrate composition in different size classes of DOC in order to elucidate mechanisms of ER. We chose the continuous culture approach, because here cells can be grown under nutrient limitation at steady state biomass. Thus, in a chemostat the increase in extracellular organic matter can primarily be attributed to growing phytoplankton cells and not to cell lysis and decay, processes that co-occur with ER when batch cultures or natural populations become nutrient depleted.

2 Methods

2.1 Experimental setup

A calcifying strain of *E. huxleyi* (PML B92/11) was grown as continuous culture in two chemostats (~ 9.2 L each) at a constant dilution rate of $D = 0.2\,d^{-1}$. A more detailed description of the chemostat principle and the experimental set-up are given by Borchard et al. (2011), Borchard and Engel (2012), and Engel et al. (2014), respectively. Temperature was set to 14.0 ± 0.1 °C. Irradiance was provided at a 16 h : 8 h light : dark cycle with a photon flux density of 190 µmol photons $m^{-2}\,s^{-1}$ (TL-D Delux Pro, Philips; QSL 100, Biospherical Instruments, Inc.). Nutrient medium was prepared from sterile-filtered (Sartobran P, 0.2 µm capsule, Sartorius) aged natural seawater (NSW) with a salinity of 33, total alkalinity of 2250 µmol kg^{-1} seawater and a pH of 8.24. The seawater was

BGD
11, 15289–15325, 2014

enriched with nutrients according to the f/2 recipe of Guillard and Ryther (Guillard and Ryther, 1962) with final concentrations of 43 µmol L^{-1} NO_3^- and 1.5 µmol L^{-1} PO_4^{3-}. The nutrient medium was treated for 3 h with UV irradiation (Microfloat 1/0, a.c.k. aqua concept GmbH) for sterilisation before the addition of sterile-filtered (0.2 µm, Minisart, Sartorius) f/2-vitamins.

Equilibration of the medium with CO_2 was obtained by constant aeration with 380 and 750 µatm CO_2, respectively. To minimize effects of calcification by *E. huxleyi* on carbonate chemistry in the incubators, TA in the reservoir tank was increased by addition of bicarbonate (LaRoche et al., 2010) resulting in 2460 µmol kg^{-1} seawater. *E. huxleyi* cells were pre-cultured for 30 d at prescribed CO_2 concentrations and temperature conditions in f/2 media in order to avoid short term stress effects on cell physiology. Each chemostat incubator was then inoculated to a final density of ~ 5000 cells mL^{-1}. Cultures were grown in batch mode for 5 d until the constant medium supply was applied at a dilution rate (D) of D = 0.2 d^{-1}. Cells were kept in suspension by gentle mixing at 50 rotations min^{-1}. Here, we report data derived from samplings during steady state growth on experimental day 30, 34, 38, 42 and 44 for ^{14}C rate measurements and on day 38, 42 and 44 for carbohydrate analyses and size fractionations of those and ^{14}C exudation. All samples were taken 3 h after lights on to avoid biases due to physiological variations during the day-night cycle.

2.2 Cell density and chemical analysis

Cell density was determined daily as the mean of three consecutive measurements of 500 µL by an electronic particle counter (Coulter Multisizer III, Beckman Coulter) equipped with a 100 µm aperture. 0.2 µm pre-filtered (Minisart, 2000; Sartorius) NSW with a salinity of 33 was used to dilute the samples 1 : 100. After microscopic inspection, particles with an equivalent spherical diameter in a range of 3.2 to 8.0 µm were identified as *E. huxleyi* cells.

BGD
11, 15289–15325, 2014

Nutrient samples were filtered through 0.2 µm syringe filters (Minisart, Sartorius) and stored frozen at −20 °C until analysis. Measurements of NO_3^-, NO_2^-, NH_4^+ and PO_4^{3-} were made spectrophotometrically after Grasshof et al. (1999) using an Evolution 3 autoanalyzer (Alliance Instruments). Detection limits were 0.3 µmol L^{-1} for N and 0.01 µmol L^{-1} for P.

Primary production and exudation were measured by applying the ^{14}C incubation method according to Steemann Nielsen (Steemann Nielsen, 1952) and Gargas (Gargas, 1975). Triplicate samples (75 mL each) were taken from each chemostat, transferred into cell culture flasks (25 cm^2, Corning®) and spiked with approximately 5 µ Ci $NaHCO_3^-$ (Hartmann Analytics, specific activity 40–60 mCi $mmole^{-1}$). Each triplicate set was incubated for about 4 h at original experimental light and temperature settings, but without aeration. Simultaneously, dark uptake was measured in triplicate from 75 mL samples incubated in the dark. Added activity in the samples was determined by removing a 100 µL aliquot from three dark bottles prior to incubation and transferred to 6 mL liquid scintillation vials in which 200 µL of 2N NaOH were placed. 4 mL liquid scintillation cocktail (Ultima Gold AB) were added before counting. Incubations were stopped by gentle filtration on 0.40 µm polycarbonate filters (Nucleopore) at low vacuum (< 150 mbar) to avoid cell breakage. The filters ($PO^{14}C$) were covered with 250 µL 1 M HCl in order to remove inorganic ^{14}C. After a few seconds they were rinsed with 10 mL filtered seawater. Filters were transferred to 6 mL scintillation vials, 4 mL liquid scintillation cocktail (Ultima Gold AB) were added and samples were stored overnight before being counted in a Packard Tri Carb Liquid Scintillation Counter. Carbon incorporation rates were calculated in accordance to Borchard and Engel (2012).

For the determination of released dissolved organic carbon ($DO^{14}C$), 4 mL of the filtrate were transferred into 20 mL scintillation vials and acidified to pH < 2 by the addition of 100 µL 1 M HCl and left open under the fume hood for 24 h. For size fractionation of $DO^{14}C$, triplicate sets of 10 mL sample were transferred into Macrosep® centrifugal devices with membrane cut off of < 1000 kDa, < 100 kDa and < 10 kDa, respectively. After centrifugation (Heraeus, Megafuge® 1.0 R) for 15 min at 4000 rounds per minute,

BGD
11, 15289–15325, 2014

4 mL sample were transferred into 20 mL liquid scintillation vials. In the following, samples were treated as the whole $DO^{14}C$ samples and after the outgassing of inorganic ^{14}C, 15 mL liquid scintillation cocktail were added. Counting and calculations were accomplished after Borchard and Engel (2012).

Primary Production (PP) was derived from the sum of $PO^{14}C$ and $DO^{14}C$. The percentage of extracellular release (PER) was calculated as $(DO^{14}C/PP) \times 100$.

Total combined carbohydrates (tCCHO) and high molecular weight (HMW; > 1 kDa) *dissolved combined carbohydrates* (dCCHO) were determined by ion chromatography after Engel and Händel (2011). Duplicate samples for HMW-dCCHO were filtered through 0.45 µm syringe-filters (GHP membrane, Acrodisk, Pall Corporation) and stored in combusted (8 h at 500 °C) glass vials at −20 °C. Samples for tCCHO remained unfiltered and were stored identically.

For size fractionation of HMW-dCCHO, 10 mL sample were transferred into Macrosep® centrifugal devices with a molecular weight cut-off (MWCO) of 1000 kDa, 100 kDa and 10 kDa, respectively. After centrifugation (Heraeus, Megafuge® 1.0 R) for 15 min at 4000 rounds per minute, samples were transferred into combusted (8 h at 500 °C) glass vials and stored at −20 °C. Before usage, Macrosep® devices were rinsed twice by centrifugation with ultrapure water to avoid any contamination with carbohydrate compounds in the membrane. Concentrations of CCHO in these blanks were tested to be below the detection limit and did therefore not affect analyses.

Prior to analysis, samples were desalinated by membrane dialysis (1 kDa MWCO, Spectra Por) for 6 h at 0 °C and thereafter hydrolyzed with HCl at a final concentration of 0.8 M for 20 h at 100 °C to yield monomeric CHO. Samples were stored at −20 °C over night and then neutralized by acid evaporation (N_2) at 50 °C. Dried samples were solubilised in ultra pure water before determination of CHO monomers by high performance anion exchange chromatography (HPAEC) coupled with pulsed amperometric detection (PAD) on a Dionex ICS 3000 (Engel and Händel, 2011). A Dionex CarboPac PA10 guard column (2 mm × 50 mm) coupled to a Dionex CarboPac PA10 analytical column (2 mm × 250 mm) was applied for separation of fucose (Fuc), rhamnose (Rha),

BGD
11, 15289–15325, 2014

arabinose (Ara), galactosamine (GalN), glucoseamine (GlcN), galactose (Gal), glucose (Glc), mannose/xylose (Man/Xyl) (quantified together due to co-elution), galacturonic acid (Gal-URA) and glucuronic acid (Glc-URA). Detection limits are 10 nM. *Particulate CCHO* (pCCHO) were derived from subtraction of HMW-dCCHO from tCCHO. Concentrations of CHO are given as µmol carbon per volume of seawater (µmol C L^{-1}) and composition of CCHO is expressed as Mol % CCHO.

Size fractions of DO^{14}C and HMW-dCCHO obtained by Macrosep® centrifugal devices were subtracted from each other in order to present data for each size class. Definitions for size classes are given in Table 1.

For **total alkalinity** (TA), 25 mL of each sample were measured by titrating with 0.05 M HCl until the buffering capacity of the water samples was consumed and all bases of interest were protonated to zero level species. Analysis was accomplished with an automatic titrator (TitroLine® alpha *plus*, SI Analytics) equipped with a sample changer (TW *alpha plus*, SI Analytics) and a piston burette (Titronic® 110 *plus*, SI Analytics). The pH was monitored by a two-point calibrated (buffer solution pH 4.006 and pH 6.865; Applichem, standardised according to DIN 19266) electrode (Schott® Instruments IoLine). The concentration of TA in µmol kg^{-1} seawater was calculated from linear regression of the absolute numbers of protons in solution and the total volume (sample plus HCl) in the range of pH 4 and 3. Determination of the seawater carbonate chemistry was conducted by using the program co2sys (Lewis and Wallace, 1998) with pH (calibrated by the use of reference materials provided by A. Dickson) and TA being the input parameters.

2.3 Data treatment

In order to directly relate daily rates (µmol L^{-1} d^{-1}) and concentrations (µmol L^{-1}), data were converted into each other by applying a growth rate of 0.2 d^{-1}.

Differences in carbohydrate composition for the different size fractions were tested by means of analysis of co-variance (two-way ANOVA). Differences as response to

BGD
11, 15289–15325, 2014

CO_2 conditions were tested by means of a *t* test. Statistical significance was accepted for $p < 0.05$. All calculations were performed using the software package Sigma Plot 10.01 (SysStat).

3 Results

3.1 Growth, nutrients and carbonate chemistry

Growth of *E. huxleyi* as well as carbonate and nutrient chemistry during this chemostat experiment are described in more detail in Engel et al. (2014). Briefly, during the steady state period, cell densities were similar in the 380 and 750 treatment and averaged $5.2 \times 10^5 \pm 18.6\,\%$ cells mL^{-1} and $5.1 \times 10^5 \pm 19.7\,\%$ cells mL^{-1}, respectively, over the whole sampling period (Table 1). High variations resulted exclusively from intensive sampling between days 42 and 44. Until day 42 variations did not exceed 11.6 % and biomass production was accepted as balanced growth as a result of controlled nutrient supply. During steady state (days 30–44), both, NO_3^- and PO_4^{3-} concentrations were below the detection limit in both treatments. P-limitation was likely more severe than N-limitation, given a nutrient supply N : P ratio of ~ 29 and indicated also by PON : POP ratios > 16. pCO_2 was calculated from pH and TA and yielded significantly different values between treatments of 337 ± 94 (380) and 623 ± 139 (750) µatm. Time averaged values given here differ slightly from those given by Engel et al. (2014) as the latter used data from replicate chemostats per CO_2 treatment, while only one chemostat per treatment was sampled for the purpose of this study.

3.2 Primary production and exudation

$PO^{14}C$ production of 173 ± 17 and 168 ± 16 µmol C L^{-1} d^{-1} and $DO^{14}C$ production of 8.0 ± 0.7 and 8.2 ± 1.1 µmol C L^{-1} d^{-1} were determined for the 380 and 750 µatm treatment, respectively. Similar $PO^{14}C$ and $DO^{14}C$ production rates in both chemostats are reflected in comparable percentages of extracellular release (PER) of 4.42 ± 0.22 and

BGD
11, 15289–15325, 2014

4.70 ± 0.92 %, respectively. Because production rates of PP, $PO^{14}C$, $DO^{14}C$ and of associated size fractions were not significantly different between the CO_2 treatments (Mann–Whitney Rank sum tests and *t* tests, $n = 5$, $p > 0.69$), data of both CO_2 treatments and repeated samplings are reported as average values ± one SD (Fig. 1, Table 2).

Size fractionated $DO^{14}C$ production ranged between 1.27 ± 0.53 (*medium*) and 2.74 ± 0.88 (*very large*) µmol C L^{-1} d^{-1} (Fig. 1, Table 1). Relative contribution of different size classes to total $DO^{14}C$ was 33.6 ± 9.31 %, (*very large*), 24.6 ± 7.90 % (*large*), 15.9 ± 7.15 % (*medium*) and 25.8 ± 3.55 % (*small*). Thus, total $DO^{14}C$ was comprised by comparable shares of $DO^{14}C$ in these size classes with slightly higher proportions in the *very large* and *small* fractions.

3.3 Combined carbohydrates

Initial HMW-dCCHO concentrations of 7.02 ± 0.15 µmol C L^{-1} were determined in the nutrient seawater (NSW) media. Size fractionation (see Table 1 for definition) of NSW yielded concentrations of 2.39 ± 0.21 (*very large*), 1.31 ± 0.09 (*large*), 1.28 ± 0.09 (*medium*) and 1.95 ± 0.10 *(small)* µmol C L^{-1} (Fig. 3a, left panel). During the experiment, fresh HMW-dCCHO derived from *E. huxleyi* enriched the natural seawater to steady state mean concentrations of 21.9 ± 2.2 (*present day*) and 22.5 ± 2.4 (*high CO_2*) µmol C L^{-1}; not significantly affected by elevated CO_2 (*t* test: $n = 6$, $p = 0.985$). The similarity between both treatments also holds for pCCHO and each size fraction of HMW-dCCHO (*t* tests, $n = 6$, $p > 0.269$), thus CCHO-data are reported as average values for steady state conditions of the *present day* and *high CO_2* treatments in the following (Figs. 2 and 3, Table 2).

Corrected for NSW values, carbohydrate concentration was 119 ± 28 µmol C L^{-1} for tCCHO, 16 ± 2.1 µmol C L^{-1} for fresh HMW-dCCHO and thus 103 ± 27 µmol C L^{-1} for pCCHO (Fig. 2). Hence, 87 ± 3 % of tCCHO were present in the particulate fraction (pCCHO). The size fractionation of HMW-dCCHO yielded 7.07 ± 1.06 (*very large*),

BGD
11, 15289–15325, 2014

3.57 ± 1.21 (*large*), 3.08 ± 1.26 (*medium*) and 3.09 ± 0.92 µmol C L^{-1} (*small*) (Fig. 2, and daily rates given in Table 2), suggesting that freshly produced HMW-dCCHO were primarily comprised by *very large* HMW-dCCHO (46 ± 3 % C). HMW-dCCHO in *large*, *medium*, and *small* contributed 23 ± 6, 20 ± 4 and 20 ± 3 % C, respectively to HMW-dCCHO.

3.4 Carbohydrate composition of exudates

Sugar monomers of three different types comprised the combined carbohydrates (CCHO) determined during the present experiment: Neutral sugars (Fuc, Rha, Ara, Gal, Glc and coeluting Man/Xyl), amino sugars (GalN and GlcN) and uronic acids (Gal-URA and Glc-URA). Various amounts of these monomers were detected in HMW-dCCHO of the initial NSW (Fig. 3b, left panel). Size fractions of HMW-dCCHO in NSW did not show any significant variation in monomeric composition. However, relative to the other size fractions Man/Xyl was slightly enriched in *small*, while a smaller proportion of Fuc was detected in this fraction. No significant differences in monomeric composition of CCHO produced by *E. huxleyi* were determined between the *present day* and *high* CO_2 treatment ($p > 0.05$). Therefore, average values include both treatments in the following (Table 3 and Fig. 3).

ER by *E. huxleyi* led to a clear change in HMW-dCCHO composition of the NSW (Fig. 3b, left panel); primarily caused by Ara being significantly higher ($p < 0.001$) in all size classes, except for *small* (Fig. 3b, left panel). In HMW-dCCHO derived from *Emiliania huxleyi*, Ara and Glc and were the dominant sugars with 46 and 21 Mol %, respectively followed by Gal-URA (11 Mol %), Man/Xyl (10 Mol %), Glc-URA (5.7 Mol %), Gal (4.6 Mol %) and Rha (2.4 Mol %) (Table 3). Proportions of other monomers comprised less than 0.5 mol % and declined in the following order: Fuc > GalN > GlcN (Table 3). In pCCHO, Glc was the most abundant sugar (74 ± 4 Mol %), followed by Rha (6.6 ± 0.8 mol) and Gal-URA (5.2 ± 0.7 mol %). Man/Xyl, Ara and Glc-URA ranged

BGD
11, 15289–15325, 2014

between 3.2 ± 1.7 and 4.2 ± 1.0 mol % while Fuc and the amino sugars GalN and GlcN contributed only a minor fraction (< 0.5 Mol %) to pCCHO (Table 3).

Hence, composition of pCCHO was substantially different from the composition determined for freshly produced HMW-dCCHO ($p < 0.002$), except for the proportions of Gal and Glc-URA. This difference is mainly attributed to a smaller proportion of Glc in the dissolved fraction along with a more than 10 fold higher share of Ara and also higher proportions of Man/Xyl and Gal-URA (Table 3).

Carbohydrate composition of the investigated dCCHO size fractions was significantly different also. Ara was dominant in *very large*, *large*, and *medium* HMW-dCCHO, but not in *the small* fraction in which its contribution was significantly smaller than in all other size classes ($p < 0.01$). Most interestingly, the proportion of Glc increased with decreasing size class, while the proportion of Gal-URA clearly decreased (Fig. 4). In *small,* Glc contribution was 80 ± 12 Mol % and significantly higher than in all other size classes of HMW-dCCHO ($p < 0.002$) (Table 3). Contribution of Glc to *very large* dCCHO was negligible (< 0.5 Mol %). In contrast, Gal-URA contributed 18 Mol % to *very large*, but only 1 Mol % to *small*. Proportions of Gal were also decreasing the smaller the HMW-dCCHO size classes, albeit not as clearly as for Gal-URA. Gal ranged from 6 (very *large*) to < 0.5 Mol % (*small*). Contributions of Rha and Man/Xyl varied among size classes. Mol % of Fuc and both amino sugars, GalN and GlcN were negligible.

4 Discussion

4.1 Particulate and dissolved primary production

Nutrient limitation and low growth rate did not hamper organic carbon production of *Emiliania huxleyi* during the present study. Cell normalized production of $PO^{14}C$ was on average ~ 0.33 pmol C cell^{-1} d^{-1} and well within the range of published values (0.12–0.64 pmol C cell^{-1} d^{-1}; Biddanda and Benner, 1997; Borchard and Engel, 2012). The partitioning of organic carbon between dissolved and particulate pool was

BGD
11, 15289–15325, 2014

shown earlier to be highly influenced by environmental conditions such as light, temperature and nutrient supply (Myklestad and Haug, 1972; Zlotnik and Dubinsky, 1989; Staats et al., 2000; Wetz and Wheeler, 2007). Nutrient depletion, however, seems to be the major factor leading to excess DOC excretion from algae cells to the surrounding environment and was reported from a variety of field and lab experiments (Fogg, 1983; Wood and VanValen, 1990; Smith and Underwood, 2000; Lopez Sandoval, 2010, 2011). ER in the range from 0–80 % was reported over the past decades and only after a long lasting debate primarily concerning methodological constraints (Sharp, 1977; Mague, 1980; Fogg, 1983; Bjørnsen, 1988), it is nowadays accepted, that ER is a normal function of healthy algae cells occurring during all stages of growth. In exponentially growing cells in culture, ER typically ranges between 2 and 10 %, while in natural marine environments ER is generally higher by 10–20 % (see Nagata, 2000 and references therein). Increased percentages of extracellular release (PER) were observed for nutrient limited algae, during the transition period of exponential to stationary growth and during senescence of cultures (Marañón, 2005; Lopez-Sandoval, 2010, 2011; Engel et al., 2013). In chemostats, despite the strict control of nutrient supply and growth rate, cells still grow exponentially. A decoupling of carbon to nutrient metabolism in continuous cultures can occur due to a change in growth rate (e.g. change the inflow of nutrient media) and results in changes in the partitioning between dissolved and particulate carbon pools, as shown with the same *E. huxleyi* strain (B 92/11) by Borchard and Engel (2012). In their study, down-regulation of the growth rate from $\mu = 0.3\,d^{-1}$ to $\mu = 0.1\,d^{-1}$ induced a slight increase in $DO^{14}C$ production, while the $PO^{14}C$ production was significantly minimized, resulting in higher PER. Cells then adapted to the steady state and high PER remained constant. During the present study, growth of *E. huxleyi* was also balanced to the nutrient supply but cells were not exposed to any stress due to nutritional changes. Thus, production of $DO^{14}C$ was not explicitly stimulated by changing experimental conditions, and, albeit constantly P-limited, the cell normalized $DO^{14}C$ production of $\sim 0.015\,pmol\,C\,cell^{-1}\,d^{-1}$ represented a ER of ~ 4.5 %, well within the above mentioned range for non-stressed algae.

BGD
11, 15289–15325, 2014

4.2 Combined carbohydrates production

For cell growth, *E. huxleyi* produced particulate combined carbohydrates in order of $20.7 \pm 5.3\,\mu mol\,C\,L^{-1}\,d^{-1}$ (equivalent to $103.6 \pm 26.7\,\mu mol\,C\,L^{-1}$ at a growth rate of $0.2\,d^{-1}$), representing about 12.5 % of the daily produced $PO^{14}C$ (Table 2). Cell normalized values for pCCHO were $\sim 0.2\,pmol\,C\,cell^{-1}$ and close to values previously given for *E. huxleyi* (e.g. $\sim 0.3\,pmol\,C\,cell^{-1}$, Biddanda and Benner, 1997).

Carbon content of HMW-dCCHO in the NSW was $\sim 7\,\mu mol\,C\,L^{-1}$. In the chemostats, HMW-dCCHO were enriched to an average value of $\sim 22\,\mu mol\,C\,L^{-1}$. The freshly produced $\sim 15\,\mu mol\,C\,L^{-1}$ suggest a HMW-dCCHO content of about 40 % of freshly produced $DO^{14}C$ (Table 2). This is a lower estimate because LMW-DOC would be detected by the ^{14}C-incubation method (Steemann Nielsen, 1952) during the determination of $DO^{14}C$, but would escape the analysis of HMW-dCCHO due to the molecular cut off > 1 kDa during desalinization of seawater samples (Engel and Händel, 2011). In the surface ocean, HMW compounds of dissolved organic matter (DOM) were found to be more abundant (30–35 %) compared to deeper waters (20–25 %) and it was concluded that HMW-DOM inherits a higher reactivity and shorter lifetimes, while LMW-DOM is rather refractory (Amon and Benner, 1996; Ogawa and Tanoue, 2003). Major reaction processes of HMW compounds are heterotrophic degradation (Amon and Benner, 1996; Guo et al., 2002; Aluwihare and Repeta, 1999) and gel particle formation (Mari and Burd, 1998; Leppard, 1995; Passow, 2000, 2002 and references therein). Thus, the HMW-DOM pool is directly linked to processes significant for organic carbon dynamics, nutrient cycling and oxygen consumption in the ocean.

4.2.1 Monomeric composition of CCHO

Natural seawater (NSW) used in the present study to prepare the nutrient media was collected from the North Sea and kept under dark and cool conditions for several months before usage. HMW-dCCHO monosaccharide composition of NSW was dominated by Glc (24 Mol %) and Man/Xyl (24 Mol %). Also high Mol % (~ 10) of Fuc, Gal,

BGD
11, 15289–15325, 2014

Gal-URA and GlcURA were determined, while other monomers were of minor importance (Fig. 3b, left panel). The composition of the aged NSW used here differs from those obtained from the Northwest Atlantic, the Sargasso Sea and the Gulf of Mexico (Aluwihare et al., 1997 and references therein), especially concerning comparably low proportions of Rha and Gal (Fig. 3b, left panel). Differences in carbohydrate composition of the seawater can be explained by seasonal or geographical divergences as well as by storage time of NSW.

Monomeric composition of HMW-dCCHO released by *E. huxleyi* during the present experiment was substantially different from the initial NSW composition (Fig. 3a and b, right panels) and the compositional shift was primarily induced by a profound relative increase in Ara. The HMW-dCCHO and pCCHO derived from *E. huxleyi* during this experiment contained a similar composition as determined earlier for cellular and extracellular carbohydrates derived from this species (De Jong et al., 1979; Fichtinger Schepman et al., 1979; Nanninga et al., 1996; Bilan and Usov, 2001). Cellular pCCHO of *E. huxleyi* differed clearly not only from NSW but also from HMW-dCCHO (Fig. 3b, right panel). This is in accordance with previous studies showing differences between intracellular and extracellular CCHO compositions for various algae (Mague, 1980; Aluwihare, 1999, 2002).

Neutral sugars generally dominated the HMW-dCCHO composition with ~ 83 mol %. These results are consistent with findings by Aluwihare (1999), who report on HMW exudates from *E. huxleyi* being mainly composed by neutral polysaccharides with Ara as the dominant monomer (30 Mol %). However, the fraction of Ara observed during this study is considerably higher than reported for ultrafiltered DOM (> 1 kDa) by Biersmith and Benner (1998), and for HMW-dCCHO sampled during a field study in the Bay of Biscay, when coccolithophores and presumably *E. huxleyi* was the dominating phytoplankton organism (Engel et al., 2012); both studies reported Ara of ~ 3 % Mol.

Apart from well documented species specific differences in CCHO composition (Aluwihare and Repeta, 1999; Myklestad, 1974; Myklestad et al., 1989), variations in the composition of algal extracellular carbohydrates may be related physiological and

BGD
11, 15289–15325, 2014

ecological functions. Although freshly produced DOC is generally a primary substrate for heterotrophic uptake, *E. huxleyi* exudates were shown to exhibit recalcitrant features (Nanninga et al., 1996). Degradation experiments with the diatom *Thalassiosira weissflogii* revealed a special role of Ara in carbohydrate accessibility, as it escaped bacterial degradation over a period of two weeks (Aluwihare and Repeta, 1999). Bacterial cell numbers during the present experiment were relatively high, between 2 and 3×10^6 mL^{-1}, contributing ~ 2 % to particulate organic carbon (POC) and ~ 3 % to DOC (Engel et al., 2014). Assuming a bacterial growth efficiency of 60 % (upper limit, Del Giorgio and Cole, 1998), the bacterial carbon demand could have been about 2 % of POC and 5 % of DOC. Relative to the freshly produced DO^{14}C derived from rate measurements, however, a share of up to 20 % may have been channeled into heterotrophic turn-over. Thus, the HMW-CCHO was potentially subject to bacterial reworking and the high proportions of Ara could have been a result of the selective removal of other monomers. In accordance with the findings of Aluwihare (1999), concentration of Ara in dCCHO remained unchanged during a degradation experiment with the same *E. huxleyi* strain investigated here, while dCCHO were reduced by ~ 60 % (Piontek et al., 2010; J. Piontek, personal communication, 2014). However, we would expect that extensive microbial degradation of larger dCCHO would lead to an increase of Ara Mol % in the *small* size fraction. But this was not observed.

Alternatively, high Mol % Ara and low Mol % Glc may indeed be a characteristic of larger carbohydrate molecules released by *E. huxleyi* that are recalcitrant to microbial decomposition. Assuming these components are bad substrates for microbial utilization, their controlled exudation, if physiologically necessary, may be ecologically advantageous for algal cells that are competing with bacteria for nutrients such as phosphorus. This corroborates earlier findings of DOM produced at P-depletion being more resistant to bacterial degradation (Obernosterer and Herndl, 1995; Puddu, 2003). On the other hand bacteria recycle organic phosphorus and a certain degree of bacterial activity will be advantageous for regenerated productivity of algal cells. So far, little is known on how nutrient limitation affects the composition of algal release products.

BGD
11, 15289–15325, 2014

We suggest that nutrient availability may be one factor responsible for variability in carbohydrate composition observed during various studies (Giroldo et al., 2005; Goldberg et al., 2010; Engel et al., 2013).

Assuming a certain degree of microbial modification, another explanation for the difference of CCHO composition between culture studies, and those observed in natural seawater may be the highly specific linkage between algal release and bacterial community response, proposed by a series of recent studies (Teeling et al., 2012; Taylor et al., 2014; Kabisch et al., 2014). These showed that the release of algal polysaccharides can induce a succession of bacterial communities inhabiting different abilities for enzymes expression related to specific carbohydrate degradation. Because the majority of marine bacteria cannot be kept in culture, bacteria present in this chemostat study, and likely in all culture experiments, represent only a small fraction of the natural diversity. Hence, even if bacteria were present in this study they may have left a different fingerprint on polysaccharide composition than natural communities. Short-term incubation studies with natural bacterial communities may be required to better understand the microbial fingerprint on DOM, specifically polysaccharide degradation. A better understanding of the microbial fingerprint on DOM could also allow for tracing microbial degradation activities in specific environments, such as the ocean's anoxic zones, or the extreme oligotrophic seas.

4.2.2 Size fractionation of CCHO and DOC – considerations on extracellular release

Quantitatively, each $DO^{14}C$ size fraction contributed similar amounts to total $DO^{14}C$ with slightly higher proportions in the *very large* and *small* fraction (Fig. 1 and Table 2). Release rates of HMW-dCCHO were similar for the different size fractions, but highest in the *very large* fraction (Fig. 2, Table 2). On a total basis, ~ 40 % of produced $DO^{14}C$ were characterized as freshly produced HMW-dCCHO (Table 2). Contribution of dCCHO to fresh DOC was lowest in the *small* size fraction (30 %) and highest in the *very large* (60 %) fraction (Table 2). Monomeric composition of different size classes

BGD
11, 15289–15325, 2014

of dCCHO enriched by *E. huxleyi* exudates was profoundly different from those of the aged NSW used as culture media (Fig. 3). In aged NSW, monomers were more evenly distributed among size fractions (Fig. 3b, left panel). In comparison, differences in monomeric composition of size classes in *E. huxleyi* exudates were largely due to changes in Ara, Glc, and Gal-URA. Most remarkably, Ara the dominant monomer in all larger dCCHO size classes, was of minor importance in the *small* dCCHO size fraction and lowest in the particulate fraction (Fig. 3, right panel). This is in accordance with the findings of Biersmith and Benner (1998), who also observed lower Mol % Ara for particulate components of an *E. huxleyi* culture as well as for the cell lysate. In contrast to Ara, Mol % Glc in our study was highest in the particulate and small fraction, relatively small in the *medium* to *large*, and negligible in the*very large* fraction. This also agrees well to earlier findings; Skoog et al. (2008) observed larger Mol % of Glc in LMW-CCHO than in HMW-CCHO, while reporting less Mol % Ara in LMW- than in HMW-CCHO. Thus, differences in size fractions of combined sugar molecules may be one factor responsible for differences in CCHO composition of DOC between study sites.

In general, carbohydrate composition in the smallest size class was similar to cellular *p*CCHO composition, while larger molecules were more distinct (Fig. 3, right panel). The ^{14}C method (Steemann Nielsen, 1952), applied here to measure primary production and ER of organic carbon does not allow distinguishing if DOC is released from the cell passively, i.e. by leakage, or actively by exudation. Leakage is hypothesised to be directly related to biomass and cell size, suggesting a constant value of passive PER. The composition of the *small* size class, and particularly the high share of Glc, resembled the cellular carbohydrate composition (Fig. 3b, right panel). This finding suggests a non-selective, i.e. passive, release of carbohydrates in the smallest size class determined here. Storage glucans in algae are comprised exclusively by Glc in D formation and have a molecular weight of 5–10 kDa. D-Glc was reported as major component of coccolith polysaccharide (CP) of *E. huxleyi* (Fichtinger Schepman, 1979). For chloroplasts in higher plants, porins are described that allow trans-membrane passage of

BGD
11, 15289–15325, 2014

hydrophilic molecules like sugars and amino acids up to a molecular weight of 10 kDa without the use of energy (Flügge and Benz, 1984; Mohr and Schopfer, 1992). The existence of porins in cell membranes of algae is likely but not explicitly reported. If DO^{14}C > 1 and < 10 kDa and associated carbohydrates leak from the cell in accordance to the passive diffusion model, this extracellular release is presumably linear correlated to biomass (*property tax* – Sharp, 1977). For molecules > 10 kDa, however, different mechanisms for the extracellular release are to be expected, since larger molecules can not pass the membrane by diffusion, and CCHO composition clearly differs from intracellular CCHO (Fig. 3b, right panel). If active release, i.e. exudation, follows the overflow model, biomass growth and dissolved primary production might be strongly decoupled (*income tax* – Sharp, 1977). Moreover, exudation requires a series of physiological processes involved in the synthesis, transport and trans-membrane release of exudates. Hence, exudates likely vary in composition. Data obtained during the present study indicate, that components > 10 kDa, rich in Ara and Gal-URA and poor in Glc, are transported actively through the cell membrane. Assembly and coagulation of polymeric precursors has been proposed as mechanism leading to the formation of marine gel particles, such as TEP. Specifically, divalent cation bridging of acidic sugars, such as uronic acids is assumed to be involved in bonding between polysaccharide chains. The release of larger polysaccharides with relatively high Mol % Gal-URA as observed for *E. huxleyi* in this study may be an important first step for high TEP concentrations, observed previously (Engel et al., 2004; Harlay et al., 2009). However, absolute rates of ER were relatively low and apparently insufficient to induce TEP formation during this study. Engel et al. (2014) suggested that acclimations to variations in environmental factors, specifically to changes in nutrient supply, are responsible for excess carbon accumulation inside the cell and for exudation of carbohydrates. Sampling during this study was conducted during the period of steady state growth. This may explain the observed relatively low rates of ER, including potential TEP precursors.

BGD
11, 15289–15325, 2014

5 Conclusions

Carbohydrates of high molecular weight (> 1 kDa) as a product of primary production are released from nutrient limited *E. huxleyi* during steady state growth. Compositional difference between size fractions of combined carbohydrate suggest that dCCHO > 10 kDa are released by active exudation across the cell membrane whereas lower molecular weight carbohydrates (< 10 kDa) can pass the membrane passively by leakage. The underlying mechanism of the release, however, needs to be further elucidated. If the presence of Ara is indeed an indicator for less degradable exudates as suggested by this study or, if Ara degradation requires activities of specific bacterial strains, needs further exploration, i.e. by using axenic cultures or natural bacterioplankton communities. At present our understanding of how microbial processes shape the molecular composition of DOM, specifically of carbohydrates, is still at its infancy. This study suggests that dCCHO composition and size may be valuable indicators of processes related to autotrophy such as primary production and exudation, but may also keep the fingerprint of heterotrophic degradation. A better understanding of compositional changes in dCCHO, as major fraction of semi-labile DOC, may therefore help to unravel carbon cycling and ecosystem dynamics in the ocean.

Acknowledgements. We thank Mascha Wurst, Sonja Endres, Cindy Lee, Carolina Cisternas-Novoa and Tiantian Tang for providing samples from the chemostat experiment, and Nicole Händel, Karin Woudsma, Laura Wischnewksi and Jon Roa for technical support. This research was supported by the Helmholtz Association and is a contribution to the German Research Foundation Collaborative Research Center 754 (DFG SFB754) Programme on Climate–Biogeochemistry Interactions in the Tropical Ocean and to the Chemical Oceanography Program of the US National Science Foundation through the ADAGIO Project.

The service charges for this open access publication have been covered by a Research Centre of the Helmholtz Association.

BGD
11, 15289–15325, 2014

References

Alldredge, A. L., Passow, U., and Logan, B. E.: The abundance and significance of a class of large, transparent organic particles in the ocean, Deep-Sea Res., 40, 1131–1140, 1993.

Aluwihare, L. I., Repeta, D. J., and Chen, R. F.: A major biopolymeric component of dissolved organic carbon in surface seawater, Nature, 387, 166–169, 1997.

Aluwihare, L. I. and Repeta, D. J.: A comparison of the chemical characteristics of oceanic DOM and extracellular DOM produced by marine algae, Mar. Ecol.-Prog. Ser., 186, 105–117, 1999.

Aluwihare, L. I., Repeta, D. J., and Chen, R. F.: Chemical composition and cycling of dissolved organic matter in the Mid-Atlantic Bight, Deep-Sea Res. Pt. II, 49, 4421–4437, 2002.

Amon, R. M. W. and Benner, R.: Bacterial utilization of different size classes of dissolved organic matter, Limnol. Oceanogr., 41, 41–51, 1996.

Amon, R. M. W., Fitznar, H. P., and Benner, R.: Linkages among the bioreactivity, chemical composition, and diagenetic state of marine dissolved organic matter, Limnol. Oceanogr., 46, 287–297, 2001.

Anderson, T. R. and Williams, P. J. L. B.: A one-dimensional model of dissolved organic carbon cycling in the water column incorporating combined biological-photochemical decomposition, Global Biogeochem. Cy., 13, 337–349, doi:10.1029/1999GB900013, 1999.

Azam, F. and Malfatti, F.: Microbial structuring of marine ecosystems, Nat. Rev. Microbiol., 5, 782–791, 2007.

Baines, S. B. and Pace, M. L.: The production of dissolved organic matter by phytoplankton and its importance to bacteria: patterns across marine and freshwater systems, Limnol. Oceanogr., 36, 1078–1090, 1991.

Benner, R.: Chemical composition and reactivity, in: Biogeochemistry of Marine Dissolved Organic Matter, edited by: Hansell, D. A. and Carlson, C. A., Elsevier Science Academic Press, San Diego, 59–90, 2002.

Benner, R., Pakulski, J. D., McCarthy, M., Hedges, J. I., and Hatcher, P. G.: Bulk chemical characteristics of dissolved organic matter in the ocean, Science, 255, 1561–1564, 1992.

Biddanda, B. and Benner, R.: Carbon, nitrogen, and carbohydrate fluxes during the production of particulate and dissolved organic matter by marine phytoplankton, Limnol. Oceanogr., 42, 506–518, 1997.

BGD
11, 15289–15325, 2014

Biersmith, A. and Benner, R.: Carbohydrates in phytoplankton and freshly produced dissolved organic matter, Mar. Chem., 63, 131–144, 1998.
Bilan, M. I. and Usov, A. I.: Polysaccharides of calcareous algae and their effect on the calcification process, Russ. J. Bioorg. Chem., 27, 2–16, 2001.
Bjørnsen, P. K.: Phytoplankton exudation of organic matter: why do healthy cells do it?, Limnol. Oceanogr., 33, 151–154, 1988.
Borchard, C., Borges, A. V., Händel, N., and Engel, A.: Biogeochemical response of *Emiliania huxleyi* (PML B92/11) to elevated CO_2 and temperature under phosphorous limitation: a chemostat study, J. Exp. Mar. Biol. Ecol., 411, 61–71, 2011.
Borchard, C. and Engel, A.: Organic matter exudation by *Emiliania huxleyi* under simulated future ocean conditions, Biogeosciences, 9, 3405–3423, doi:10.5194/bg-9-3405-2012, 2012.
Børsheim, K. Y., Myklestad, S. M., and Sneli, J. A.: Monthly profiles of DOC, mono- and polysaccharides at two locations in the Trondheimsfjord (Norway) during two years, Mar. Chem., 63, 255–272, 1999.
Carlson, C. A. and Ducklow, H. W.: Dissolved organic carbon in the upper ocean of the central equatorial pacific ocean, 1992 – daily and fine-scale vertical variations, Deep-Sea Res. Pt. II, 42, 639–656, 1995.
Cherrier, J., Bauer, J. E., and Druffel, E. R. M.: Utilization and turnover of labile dissolved organic matter by bacterial heterotrophs in eastern north Pacific surface waters, Mar. Ecol.-Prog. Ser., 139, 267–279, 1996.
Chin, W. C., Orellana, M. V., and Verdugo, P.: Spontaneous assembly of marine dissolved organic matter into polymer gels, Nature, 391, 568–572, 1998.
Cho, B. C. and Azam, F.: Major role of bacteria in biogeochemical fluxes in the oceans interior, Nature, 332, 441–443, 1988.
Copping, A. and Lorenzen, A. E.: Carbon budget of a marine phytoplankton-herbivore system with carbon-14 as a tracer, Limnol. Oceanogr., 25, 873–882, 1980.
Davis, J., K. Kaiser, and Benner, R.: Amino acid and amino sugar yields and compositions as indicators of dissolved organic matter diagenesis, Org. Geochem., 40, 343–352, 2009.
De Jong, E., van Rens, L., Westbroek, P., and Bosch, L.: Biocalcification by the marine alga *Emiliania huxleyi* (Lohmann) Kamptner, Eur. J. Biochem., 99, 559–567, 1979.
Del Giorgio, P. A. and Cole, J. J.: Bacterial growth efficiency in natural aquatic ecosystems, Annu. Rev. Ecol. Syst., 29, 503–541, 1998.

BGD
11, 15289–15325, 2014

Engel, A.: Direct relationship between CO_2 uptake and transparent exopolymer particles production in natural phytoplankton, J. Plankton Res., 24, 49–53, 2002.
Engel, A. and Händel, N.: A novel protocol for determining the concentration and composition of sugars in particulate and in high molecular weight dissolved organic matter (HMW-DOM) in seawater, Mar. Chem., 127, 180–191, 2011.
Engel, A., Thoms, S., Riebesell, U., Rochelle-Newall, E., and Zondervan, I.: Polysaccharide aggregation as a potential sink of marine dissolved organic carbon, Nature, 428, 929–932, 2004.
Engel, A., Händel, N., Wohlers, J., Lunau, M., Grossart, H. P., Sommer, U., and Riebesell, U.: Effects of sea surface warming on the production and composition of dissolved organic matter during phytoplankton blooms: results from a mesocosm study, J. Plankton Res., 33, 357–372, doi:10.1093/plankt/fbq122, 2010.
Engel, A., Harlay, J., Piontek, J. und Chou, L.: Contribution of combined carbohydrates to dissolved and particulate organic carbon after the spring bloom in the northern Bay of Biscay (North-Eastern Atlantic Ocean), Cont. Shelf. Res., 45, 42–53, doi:10.1016/j.csr.2012.05.016, 2012.
Engel, A., Borchard, C., Piontek, J., Schulz, K. G., Riebesell, U., and Bellerby, R.: CO_2 increases ^{14}C primary production in an Arctic plankton community, Biogeosciences, 10, 1291–1308, doi:10.5194/bg-10-1291-2013, 2013.
Engel, A., Cisternas Novoa, C., Wurst, M., Endres, S., Tang, T., Schartau, M., and Lee, C.: No detectable effect of CO_2 on elemental stoichiometry of *Emiliania huxleyi* in nutrient-limited, acclimated continuous cultures, Mar. Ecol.-Prog. Ser., 507, 15–30, doi:10.3354/meps10824, 2014.
Fichtinger Schepman, A. M. J., Kamerling, J. P., Vliegenthart, J. F. G., De Jong, E. W., Bosch, L., and Westbroek, P.: Composition of a methylated, acidic polysaccharide associated with coccoliths of *Emiliania huxleyi* (Lohmann) Kampner, Carbohyd. Res., 69, 181–189, 1979.
Flügge, U. I. and Benz, R.: Pore-forming activity in the outer membrane of the chloroplast envelope, FEBS Lett., 169, 85–89, 1984.
Flynn, K., Clark, D., and Xue, Y.: Modeling the release of dissolved organic matter by phytoplankton, J. Phycol., 44, 1171–1187, 2008.
Fogg, G. E.: The extracellular products of algae, Oceanogr. Mar. Biol., 4, 195–212, 1966.
Fogg, G. E.: The ecological significance of extracellular products of phytoplankton photosynthesis, Bot. Mar., 26, 3–14, 1983.

BGD
11, 15289–15325, 2014

Fuhrman, J. A.: Marine viruses and their biogeochemical and ecological effects, Nature, 399, 541–548, 1999.
Gargas, E.: A manual for phytoplankton primary production studies in the Baltic, Balt. Mar. Biolog. Publ., 2, 1–88, 1975.
Geider, R. J. and LaRoche, J.: Redfield revisited: variability of C : N : P in marine microalgae and its biochemical basis, Eur. J. Phycol., 37, 1–17, 2002.
Giroldo, D., Vieira, A. A. H., and Paulsen, B. S.: Extracellular polysaccharides produced by a tropical cryptophyte as a carbon source for natural bacterial populations, Eur. J. Phycol., 40, 241–249, 2005.
Goldberg, S. J., Carlson, C. A., Bock, B., Nelson, N. B., and Siegel, D. A.: Meridional variability in dissolved organic matter stocks and diagenetic state within the euphotic and mesopelagic zone of the North Atlantic subtropical gyre, Mar. Chem., 119, 9–21, 2010.
Goldman, J. C., Hansell, D. A., and Dennett, M. R.: Chemical characterization of three large oceanic diatoms: potential impact on water column chemistry, Mar. Ecol.-Prog. Ser., 88, 257–270, 1992.
Grasshoff, K., Kremeling, K., and Ehrhardt, M.: Methods of Seawater Analysis, 3rd completely revised and extended edition, Wiley-VHC, 1999.
Guillard, R. R. L. and Ryther, J. H.: Studies of marine planktonic diatoms I, Cyclotella nana Hustedt and Detonula confervacea Cleve, Can. J. Microbiol., 8, 229–239, 1962.
Guo, L., Hung, C. C., Santschi, P. H., and Walsh, I. D.: ^{234}Th scavenging and its relationship to acid polysaccharide abundance in the Gulf of Mexico, Mar. Chem. 78, 103–119, 2002.
Halewood, E. R., Carlson, C. A., Brzezinski, M. A., Reed, D. C., and Goodman, J.: Annual cycle of organic matter partitioning and its availability to bacteria across the Santa Barbara Channel continental shelf, Aquat. Microb. Ecol., 67, 189–209, 2012.
Hansell, D. A.: Recalcitrant dissolved organic carbon fractions, Annu. Rev. Mar. Sci., 5, 421–445, 2013.
Hansell, D. A. and Carlson, C. A.: Deep ocean gradients in dissolved organic carbon gradients, Nature, 395, 263–266, 1998.
Hansell, D. A., Carlson, C. A., Repeta, D. J., and Schlitzer, R.: Dissolved organic matter in the ocean a controversy stimulates new insights, Oceanography, 22, 202–211, 2009.
Harlay, J., De Bodt, C., Engel, A., Jansen, S., d'Hoop, Q., Piontek, J., Van Oostende, N., Groom, S., Sabbe, K., and Chou, L.: Abundance and size distribution of transparent exopoly-

BGD
11, 15289–15325, 2014

mer particles (TEP) in a coccolithophorid bloom in the northern Bay of Biscay, Deep-Sea Res. Pt. I, 56, 1251–1265, 2009.
Hopkinson, C. S. and Vallino, J. J.: Efficient export of carbon to the deep ocean through dissolved organic matter, Nature, 433, 142–145, 2005.
Jiao, N. and Zheng, Q.: The microbial carbon pump: from genes to ecosystems, Appl. Environ. Microbiol., 77, 7439–7444, 2011.
Kabisch, A., Otto, A., König, S., Becher, D., Albrecht, D., Schüler, M., Teeling, H., Amann, R. I., and Schweder, T.: Functional characterization of polysaccharide utilization loci in the marine bacteroidetes "Gramella forsetii" KT0803, ISME J., 8, 1492–1502, 2014.
Kiørboe, T. and Hansen, J. L. S.: Phytoplankton aggregate formation: observation of patterns and mechanisms of cell sticking and the significance of exopolymeric material, J. Plankton Res., 15, 993–1018, 1993.
Kirchman, D. L.: Biomass and production of heterotrophic bacterioplankton in the oceanic Sub-Arctic Pacific, Deep-Sea Res. Pt. II, 40, 967–988, 1993.
LaRoche, J., Rost, B., and Engel, A.: Bioassays, batch culture and chemostat experimentation, in: Guide to Best Practices for Ocean Acidification Research and Data Reporting, edited by: Riebesell, U., Fabry, V. J., Hansson, L., and Gattuso, J.-P., Publications Office of the European Union, Luxembourg, 81–94, 2010.
Leppard, G. G.: The characterization of algal and microbial mucilages and their aggregates in aquatic ecosystems, Sci. Total Environ., 165, 103–131, 1995.
Lewis, E. and Wallace, D.: Program Developed for CO_2 System Calculations. ORNL/CDIAC-105, Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, US Department of Energy, Oak Ridge, TN, 1998.
López-Sandoval, D., Marañón, E., Fernández, A., González, J., Gasol, J. M., Lekunberri, I., Varela, M., Calvo-Díaz, A., Morán, X. A., Álvarez- Salgado, X. A., and Figueiras, F. G.: Particulate and dissolved primary production by contrasting phytoplankton assemblages during mesocosm experiments in Ría de Vigo (NW Spain), J. Plankton Res., 32, 1231–1240, 2010.
López-Sandoval, D. C., Fernández, A., and Marañón, E.: Dissolved and particulate primary production along a longitudinal gradient in the Mediterranean Sea, Biogeosciences, 8, 815–825, doi:10.5194/bg-8-815-2011, 2011.
Mague, T. H., Friberg, E., Hughes, D. J., and Morris, I.: Extracellular release of carbon by marine phytoplankton; a physiological approach, Limnol. Oceanogr., 25, 262–279, 1980.

BGD
11, 15289–15325, 2014

Marañón E., Fernandez, E., Harris, R. P., and Harbour, D. S.: Effects of the diatom-*Emiliania huxleyi* succession on photosynthesis, calcification and carbon metabolism by size-fractionated phytoplankton, Hydrobiologia, 317, 189–199, 1996.
Marañón, E., Cermeño, P., and Pérez, V.: Continuity in the photosynthetic production of dissolved organic carbon from eutrophic to oligotrophic waters, Mar. Ecol.-Prog. Ser., 299, 7–17, 2005
Mari, X. and Burd, A.: Seasonal size spectra of transparent exopolymeric particles (TEP) in a coastal sea and comparison with those predicted using coagulation theory, Mar. Ecol.-Prog. Ser., 163, 63–76, 1998.
Mohr, H. and Schopfer, P.: Photosynthese als Funktion der Chloroplasten, in: Pflanzenphysiologie, 4th edn., Springer-Verlag, Berlin Heidelberg New York, ISBN 3-540-54733-9, 139–172, 1992.
Møller, E. F.: Sloppy feeding in marine copepods: prey-size-dependent production of dissolved organic carbon, J. Plankton Res., 27, 27–35, 2005.
Moran, X. A. G., Sebastian, M., Pedros-Alio, C., and Estrada, M.: Response of Southern Ocean phytoplankton and bacterioplankton production to short-term experimental warming, Limnol. Oceanogr., 51, 1791–1800, 2006.
Myklestad, S.: Production of carbohydrates by marine planktonic diatoms I, Comparison of nine different species in culture, J. Exp. Mar. Biol. Ecol., 15, 261–274, 1974.
Myklestad, S., Haug, A., and Larsen, B.: Production of carbohydrates by the marine diatom *Chaetoceros affinis var Willei* (Gran) In Hustedt II: preliminary investigation of the extracellular polysaccharide, J. Exp. Mar. Biol. Ecol., 9, 137–144, 1972.
Myklestad, S., Holm-Hansen, O., Varum, K. M., and Volcani, B. E.: Rate of release of extracellular amino-acids and carbohydrates from the marine diatom *Chaetoceros affinis*, J. Plankton Res., 11, 763–773, 1989.
Nagata, T.: Production mechanisms of dissolved matter, in: Microbial Ecology of the Oceans, 1st edn., edited by: Kirchmann, D. L., Wiley-Liss, New York, 121–152, 2000.
Nanninga, H. J., Ringenaldus, P., and Westbroek, P.: Immunological quantitation of a polysaccharide formed by *Emiliania huxleyi*, J. Marine Syst., 9, 67–74, 1996.
Obernosterer, I. and Herndl, G. J.: Phytoplankton extracellular release and bacterial growth: dependence on inorganic N : P ratio, Mar. Ecol.-Prog. Ser., 116, 247–257, 1995.
Ogawa, H. and Tanoue, E.: Dissolved organic matter in oceanic waters, J. Oceanogr., 59, 129–147, 2003.

BGD
11, 15289–15325, 2014

Pakulski, J. D. and Benner, R.: Abundance and distribution of carbohydrates in the ocean, Limnol. Oceanogr., 39, 930–940, 1994.
Passow, U.: Formation of transparent exopolymer particles, TEP, from dissolved precursor material, Mar. Ecol.-Prog. Ser., 192, 1–11, 2000.
Passow, U.: Production of transparent exopolymer particles (TEP) by phyto- and Bacterioplankton, Mar. Ecol.-Prog. Ser., 236, 1–12, 2002.
Piontek, J., Lunau, M., Händel, N., Borchard, C., Wurst, M., and Engel, A.: Acidification increases microbial polysaccharide degradation in the ocean, Biogeosciences, 7, 1615–1624, doi:10.5194/bg-7-1615-2010, 2010.
Puddu, A., Zoppini, A., Fazi, S., Rosati, M., Amalfitano, S., and Magaletti, E.: Bacterial uptake of DOM released from P-limited phytoplankton, FEMS Microbiol. Ecol., 46, 257–268, 2003.
Repeta, D. J. and Aluwihare, L. I.: High molecular weight dissolved organic carbon cycling as determined by natural abundance radiocarbon measurements of neutral sugars, Limnol. Oceanogr., 51, 1045–1053, 2006.
Schartau, M., Engel, A., Schröter, J., Thoms, S., Völker, C., and Wolf-Gladrow, D.: Modelling carbon overconsumption and the formation of extracellular particulate organic carbon, Biogeosciences, 4, 433–454, doi:10.5194/bg-4-433-2007, 2007.
Sharp, J. H.: Excretion of organic matter by marine phytoplankton – do healthy cells do it?, Limnol. Oceanogr., 22, 381–399, 1977.
Skoog, A., Alldredge, A., Passow, U., Dunne, J., and Murray, J.: Neutral aldoses as source indicators for marine snow, Mar. Chem., 108, 195–206, 2008.
Smith, D. C., Simon, M., Alldredge, A. L., and Azam, F.: Intense hydrolytic enzyme-activity on marine aggregates and implications for rapid particle dissolution, Nature, 359, 139–142, 1992.
Smith, D. J. and Underwood, G. J. C.: The production of extracellular carbohydrates by estuarine benthic diatoms: the effects of growth phase and light and dark treatment, J. Phycol., 36, 321–333, 2000
Staats, N., Stal, L. J., and Mura, L. R.: Exopolysaccharide production by the epipelic diatom *Cylindrotheca closterium*: effects of nutrient conditions, J. Exp. Mar. Biol. Ecol., 249, 13–27, 2000.
Steemann Nielsen, E.: The use of radioactive carbon (^{14}C) for measuring primary production in the sea, J. Cons. Perm. Int. Explor. Mer., 18, 117–140, 1952.

BGD
11, 15289–15325, 2014

Taylor, J. D., Ellis, R., Milazzo, M., Hall-Spencer, J. M., and Cunliffe, M.: Intertidal epilithic bacteria diversity changes along a naturally occurring carbon dioxide and pH gradient, FEMS Microbiol. Ecol., 89, 670–678, 2014.

Teeling, H., Fuchs, B. M., Becher, D., Klockow, C., Gardebrecht, A., Bennke, C. M., Kassabgy, M., Huang, S., Mann, A. J. , Waldmann, J., Weber, M., Klindworth, A., Otto, A., Lange, J., Bernhardt, J., Reinsch, C., Hecker, M., Peplies, J., Bockelmann, F. D., Callies, U., Gerdts, G., Wichels, A., Wiltshire, K. H., Glöckner, F. O., Schweder, T., and Amann, R.: Substrate-controlled succession of marine bacterioplankton populations induced by a phytoplankton bloom, Science, 336, 608–611, 2012.

Wangersky, P. J.: The distribution of particulate organic carbon in the oceans: ecological implications, Int. Rev. Ges. Hydrobio., 63, 567–574, 1978.

Wetz, M. S. and Wheeler, P. A.: Release of organic matter by coastal diatoms, Limnol. Oceanogr., 52, 798–807, 2007.

Williams, P. J. L. B.: The importance of losses during microbial growth: commentary on the physiology, measurement and ecology on the release of dissolved organic matter, Mar. Microb. Food Web., 4, 175–206, 1990.

Wood, M. A. and van Valen, L. M.:Paradox lost? On the release of energy-rich compounds by phytoplankton, Mar. Microb. Food Web., 4, 103–116, 1990.

Zlotnik, I. and Dubinsky, Z.: The effect of light and temperature on DOC excretion by phytoplankton, Limnol. Oceanogr., 34, 831–839, 1989.

BGD
11, 15289–15325, 2014

Table 1. Definition of size classes for fractionated high molecular weight (> 1 kDa) dissolved carbohydrates (HMW-DCCHO) and dissolved organic carbon ($DO^{14}C$).

	HMW-dCCHO	$DO^{14}C$
Total	1 kDa < HMW-dCCHO < 0.45 µm	$DO^{14}C$ < 0.40 µm
Very Large	1000 kDa < HMW-dCCHO < 0.45 µm	1000 kDa < $DO^{14}C$ < 0.40 µm
Large	100 kDa < HMW-dCCHO < 1000 kDa	100 kDa < $DO^{14}C$ < 1000 kDa
medium	10 kDa < HMW-dCCHO < 100 kDa	10 kDa < $DO^{14}C$ < 100 kDa
Small	1 kDa < HMW-dCCHO < 10 kDa	$DO^{14}C$ < 10 kDa

BGD
11, 15289–15325, 2014

Table 2. Size class resolved production rates of fresh organic carbon and of high molecular weight (> 1 kDa) carbohydrates (HMW-CCHO) during the chemostat experiment, as well as contribution of carbon contained in CCHO to primary production (^{14}C) of particulate and of different dissolved organic carbon fractions.

		HMW-CCHO [µmol C L^{-1} d^{-1}] avg. ± 1 sd (n = 6)		^{14}C [µmol C L^{-1} d^{-1}] avg. ± 1 sd (n = 10)		HMW-CCHO: ^{14}C [%]	
Particulate		20.7	5.34	171	15.9	12.5	3.54
Dissolved	Total	3.10	0.41	8.11	0.88	40.0	5.37
	Very Large	1.41	0.21	2.74	0.88	59.7	17.2
	Large	0.71	0.24	2.01	0.72	44.6	24.7
	Medium	0.62	0.25	1.27	0.53	52.9	33.7
	Small	0.62	0.18	2.09	0.32	29.5	9.30

BGD
11, 15289–15325, 2014

Table 3. Freshly produced combined carbohydrates (CCHO) in various size fractions. Average values (bold) and standard deviations in Mol % CCHO are given for samplings during steady state cell growth, $n = 6$. Fuc, GalN and GlcN were always < 0.5 Mol % and are not included.

CCHO [mol %] Size fraction	Rha	Ara	Gal	Glc	Man/Xyl	Gal-URA	Glc-URA
pCCHO	**6.56**	**3.69**	**3.09**	**74.0**	**3.22**	**5.18**	**4.23**
	0.84	0.99	1.45	4.08	1.65	0.68	1.01
HMW-dCCHO	**2.44**	**46.0**	**4.64**	**20.5**	**9.68**	**11.0**	**5.74**
(total)	0.70	3.0	1.95	7.48	2.37	4.40	2.99
very large	**3.46**	**54.2**	**6.34**	**< 0.5**	**9.62**	**18.2**	**8.17**
	1.88	13.3	4.11	–	6.35	5.35	6.11
large	**0.91**	**41.0**	**5.92**	**18.9**	**16.5**	**8.45**	**8.34**
	0.85	24.2	5.10	11.2	18.9	13.3	13.41
medium	**1.71**	**48.8**	**3.54**	**34.9**	**8.20**	**2.96**	**< 0.5**
	1.03	9.41	3.41	19.2	9.88	3.35	–
small	**2.25**	**9.70**	**< 0.5**	**79.8**	**4.03**	**1.13**	**2.64**
	1.54	6.17	–	11.9	3.74	3.35	2.89

BGD
11, 15289–15325, 2014

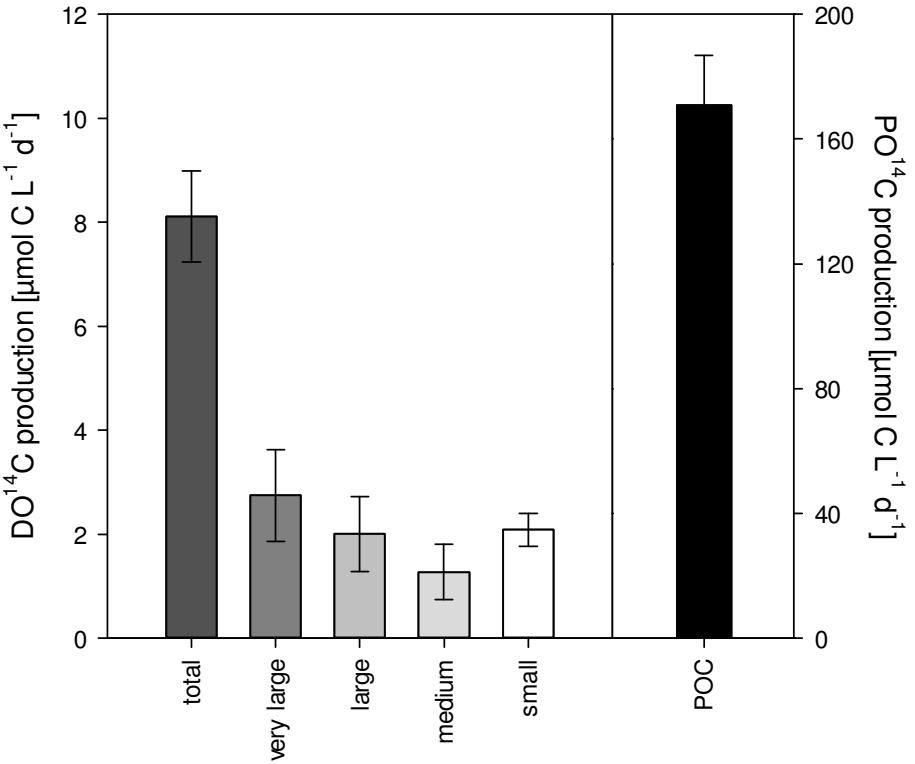

Figure 1. Dissolved ($DO^{14}C$, left) and particulate ($PO^{14}C$, right) primary production [µmol C L^{-1} d^{-1}] of *Emiliania huxleyi*. Daily rates are additionally given for each $DO^{14}C$ size fractions.

BGD
11, 15289–15325, 2014

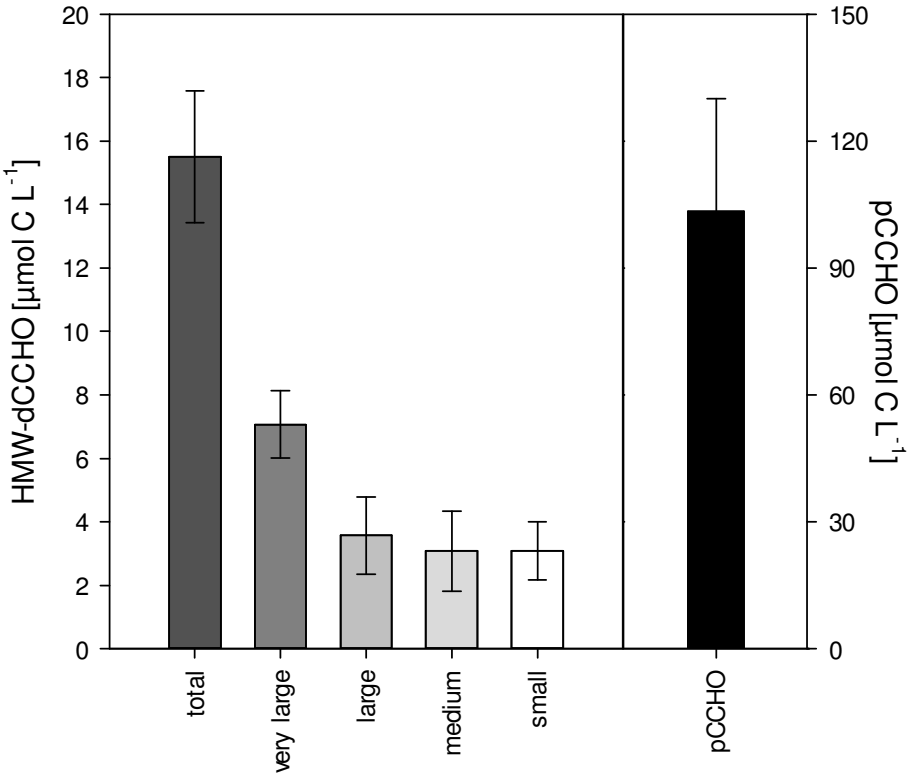

Figure 2. Freshly produced high molecular weight (HMW > 1 kDa) dissolved combined carbohydrates (HMW-dCCHO, left), particulate CCHO (pCCHO, right) [µmol C L^{-1}] derived from *E. huxleyi*. Concentrations are additionally given for each size fraction of HMW-dCCHO.

BGD
11, 15289–15325, 2014

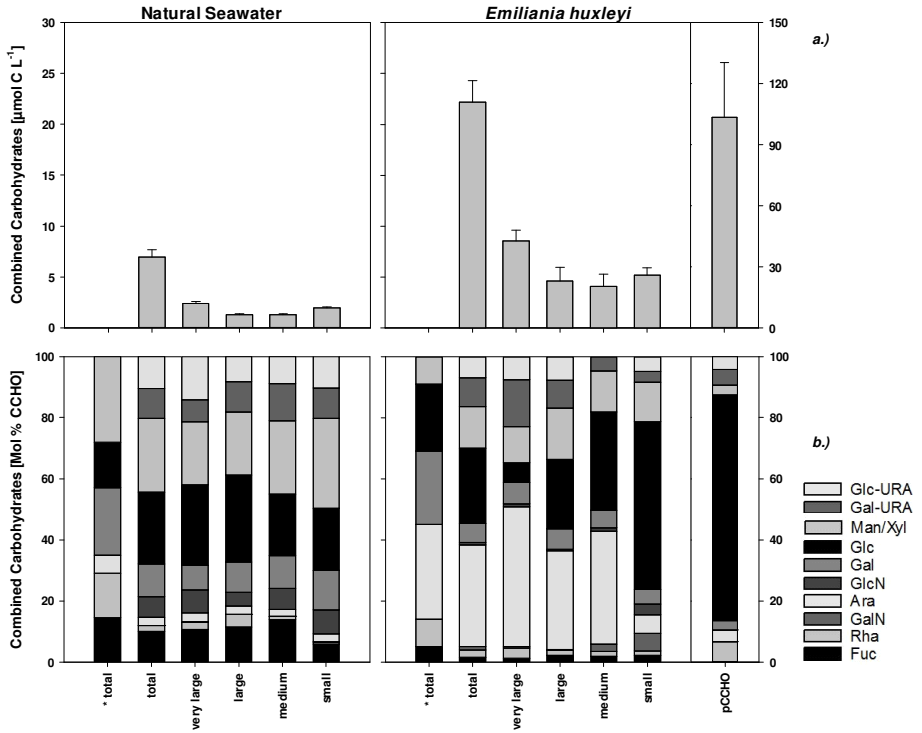

Figure 3. Concentration [µmol C L^{-1}] **(a)** and composition [Mol % CCHO] **(b)** of high molecular weight (> 1 kDa) dissolved combined carbohydrates (HMW-dCCHO). Data are shown for natural seawater used to prepare the experimental culture media (left panels) and composition in natural seawater enriched with freshly produced HMW-dCCHO derived from *E. huxleyi* (rights panels) grown in chemostats.
*: Data for *HMW-dCCHO for Natural seawater* and *E. huxleyi* taken from Aluwihare (1999) for comparison. Here, only neutral carbohydrates are included, since amino- and acidic HMW-dCCHO were not analyzed.

BGD
11, 15289–15325, 2014

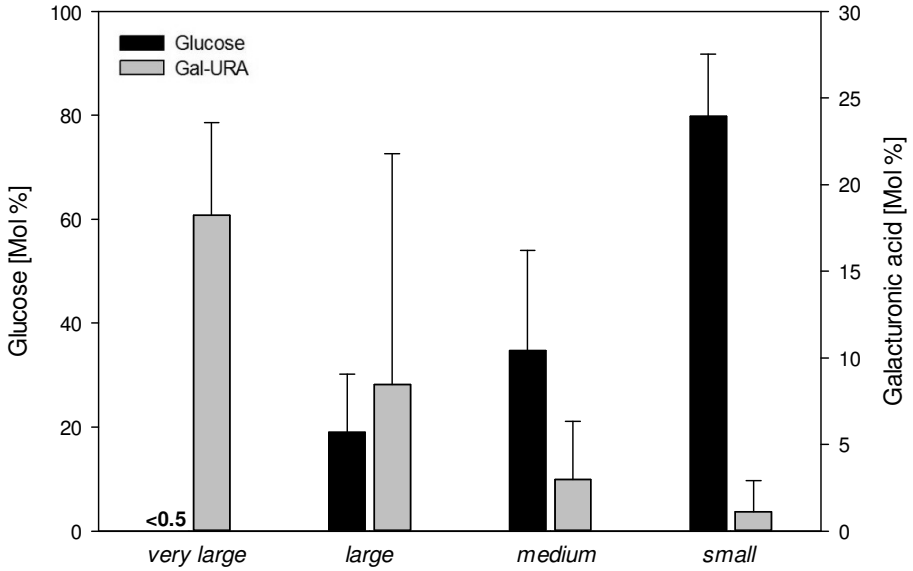

Figure 4. Changes in molar percentages of Glc and Gal-URA in dCCHO with the molecular weight of released dCCHO as defined in Table 1. Bars show average values; error bars ±1 SD, $n = 6$.

BGD
11, 15289–15325, 2014