



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

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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 *Emiliana huxleyi* grown under steady-state conditions in phosphorus-controlled chemostats (N:P = 29, growth rate of $\mu = 0.2 \text{ d}^{-1}$) at present-day and high-CO₂ concentrations. ¹⁴C incubations were performed to determine primary production (PP), comprised of particulate (PO¹⁴C) and dissolved organic carbon (DO¹⁴C). Concentration and composition of particulate combined carbohydrates (*p*CCHO) and high-molecular-weight (> 1 kDa, HMW) dissolved combined carbohydrates (*d*CCHO) were determined by ion chromatography. Information on size distribution of ER products was obtained by investigating distinct size classes (< 0.4 μm (DO¹⁴C), < 0.45 μm (HMW-*d*CCHO), < 1000, < 100 and < 10 kDa) of DO¹⁴C and HMW-*d*CCHO. 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 *p*CCHO as well as on HMW-*d*CCHO. While *p*CCHO and the smallest size fraction (< 10 kDa) of HMW-*d*CCHO exhibited a similar sugar composition, dominated by high percentage of glucose (74–80 mol%), the composition of HMW-*d*CCHO size classes > 10 kDa was significantly different, with a higher mol % of arabinose. The mol % of acidic sugars increased and that of glucose decreased with increasing size of HMW-*d*CCHO. We conclude that larger polysaccha-

rides follow different production and release pathways than smaller molecules, potentially serving distinct ecological and biogeochemical functions.

1 Introduction

The global ocean inventory of dissolved organic carbon (DOC) is estimated to be in the 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, becoming 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 (TEPs), leads to a repartitioning of DOC into the particulate organic carbon (POC) pool (Allredge 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 carbon dioxide (CO₂) storage potential in the ocean (Jiao and Zheng, 2011). Thus, deeper insights into 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 solubilization (Cho and Azam, 1988; Smith et al., 1992) and sloppy feeding (Copping and Lorenzen, 1980; Nagata, 2000). The major components of phytoplankton ER are high-molecular-weight (HMW, > 1 kDa) dissolved combined carbohydrates (*d*CCHO), representing also the largest characterizable fraction of marine dissolved organic matter (DOM): 15–35 % DOC in the surface ocean and 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-*d*CCHO 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; Børshem 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 of neutral hexoses, pentoses and deoxy sugars 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 primary production (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 PP rates, the absence of ER at night and a high share of HMW substances (Williams, 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 photosynthates 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 also suggested for CO₂ (Engel, 2002) and temperature (Moran et al., 2006). Under nutrient limitation, however, substantial ER was observed when PP was reduced, leading to higher percentage of extracellular release (PER) (Mykkestad 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 remineralization 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 whether extracellular products are mainly released by leakage or by exudation processes, or how much leakage and exudation products differ. We also do not know whether 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, remineralization 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 produce 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).

This study was part of a larger experiment investigating carbon and nutrient cycling under different *p*CO₂ conditions at steady-state growth in *E. huxleyi*. No effect of the CO₂

treatment was observed for elemental stoichiometry of cells as well as for TEP production (Engel et al., 2014). This study focuses on primary production of POC and DOC by *E. huxleyi*, the carbohydrate composition of cells and for the first time on different size fractions of released compounds.

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 a continuous culture in two chemostats (~9.2 L each) at a constant dilution rate of $D = 0.2 \text{ d}^{-1}$. A more detailed description of the chemostat principle and the experimental setup are given by Borchard et al. (2011), respectively. Temperature was set to $14.0 \pm 0.1 \text{ }^\circ\text{C}$. Irradiance was provided at a 16 h/8 h light/dark cycle with a photon flux density of $19 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (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 (TA) of $225 \mu\text{mol kg}^{-1}$ seawater and a pH of 8.24. The seawater was enriched with nutrients according to the $f/2$ recipe of Guillard and Ryther (Guillard and Ryther 1962) with final concentrations of $43 \mu\text{mol L}^{-1} \text{ NO}_3^-$ and $1.5 \mu\text{mol L}^{-1} \text{ PO}_4^{3-}$. The nutrient medium was treated for 3 h with UV irradiation (Microfloat 1/0, a.c.k. aqua concept GmbH) for sterilization before the addition of sterile-filtered (0.2 μm , Minisart, Sartorius) $f/2$ vitamins. Axenic conditions, however, could not be maintained in the 9.2 L chemostats over the long period of time.

Equilibration of the medium with CO_2 was obtained by constant aeration with 380 and 75 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 $2460 \mu\text{mol kg}^{-1}$ seawater. *E. huxleyi* cells were pre-cultured for 30 days 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 $\sim 5000 \text{ cells mL}^{-1}$. Cultures were grown in batch mode

for 5 days until the constant medium supply was applied at a dilution rate (D) of $D = 0.2 \text{ d}^{-1}$. Cells were kept in suspension by gentle mixing at 50 rpm. 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 50 μL by an electronic particle counter (Coulter Multisizer III, Beckman Coulter) equipped with a 10 μm aperture. In order to dilute the samples to 1 : 100, 0.2 μm of pre-filtered (Minisart, 2000; Sartorius) NSW with a salinity of 33 was used. After microscopic inspection, particles with an equivalent spherical diameter in a range of 3.2 to 8 μm were identified as *E. huxleyi* cells.

Nutrient samples were filtered through 0.2 μm syringe filters (Minisart, Sartorius) and stored frozen at $-20 \text{ }^\circ\text{C}$ 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 $0.3 \mu\text{mol L}^{-1}$ for N and $0.01 \mu\text{mol L}^{-1}$ 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 $\mu\text{Ci NaHCO}_3^-$ (Hartmann Analytics, specific activity 40–60 mCi mmol^{-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 10 μL aliquot from three dark bottles prior to incubation and transferred to 6 mL liquid scintillation vials in which 200 μL of 2N NaOH was placed. Four milliliters of liquid scintillation cocktail (Ultima Gold AB) was added before counting. Incubations were stopped by gentle filtration on 0.4 μm polycarbonate filters (Nucleopore) at low vacuum ($< 150 \text{ mbar}$) to avoid cell breakage. The filters (PO^{14}C) were covered with 25 μL of 1 M HCl in order to remove inorganic ^{14}C . After a few seconds they were rinsed with 10 mL of filtered seawater. Filters were transferred to 6 mL scintillation vials, 4 mL of liquid scintillation cocktail (Ultima Gold AB) was 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).

Table 1. Definition of size classes for fractionated high-molecular-weight (HMW, > 1 kDa) dissolved combined carbohydrates (HMW-*d*CCHO) and dissolved organic carbon (DO¹⁴C).

	HMW- <i>d</i> CCHO	DO ¹⁴ C
Total	1 kDa < HMW- <i>d</i> CCHO < 0.45 μm	DO ¹⁴ C < 0.40 μm
Very large	1000 kDa < HMW- <i>d</i> CCHO < 0.45 μm	1000 kDa < DO ¹⁴ C < 0.40 μm
Large	100 kDa < HMW- <i>d</i> CCHO < 1000 kDa	100 kDa < DO ¹⁴ C < 1000 kDa
medium	10 kDa < HMW- <i>d</i> CCHO < 100 kDa	10 kDa < DO ¹⁴ C < 100 kDa
Small	1 kDa < HMW- <i>d</i> CCHO < 10 kDa	DO ¹⁴ C < 10 kDa

For the determination of released dissolved organic carbon (DO¹⁴C), 4 mL of the filtrate was transferred into 20 mL scintillation vials and acidified to pH < 2 by the addition of 10 μL of 1 M HCl and left open under the fume hood for 24 h. For size fractionation of DO¹⁴C, triplicate sets of 10 mL sample were transferred into Macrosep[®] centrifugal devices with a membrane cut-off of < 1000, < 100 and < 10 kDa, respectively. After centrifugation (Heraeus, Megafuge[®] 1.0 R) for 15 min at 4000 rpm, 4 mL of sample was transferred into 20 mL liquid scintillation vials. In the following, samples were treated as the whole DO¹⁴C samples and after the outgassing of inorganic ¹⁴C, 15 mL of liquid scintillation cocktail was added. Counting and calculations were performed following Borchard and Engel (2012).

Primary production (PP) was derived from the sum of PO¹⁴C and DO¹⁴C. The PER was calculated as (DO¹⁴C / PP) × 100.

Total combined carbohydrates (tCCHO) and high-molecular-weight (HMW; > 1 kDa) dissolved combined carbohydrates (*d*CCHO) were determined by ion chromatography after Engel and Händel (2011). Duplicate samples for HMW-*d*CCHO 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 *t*CCHO remained unfiltered and were stored identically.

For size fractionation of HMW-*d*CCHO, 10 mL of sample was transferred into Macrosep[®] centrifugal devices with a molecular weight cut-off (MWCO) of 1000, 100 and 10 kDa, respectively. After centrifugation (Heraeus, Megafuge[®] 1.0 R) for 15 min at 4000 rpm, samples were transferred into combusted (8 h at 500 °C) glass vials and stored at -20 °C. Before usage, Macrosep[®] devices were rinsed twice via 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 therefore did 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 overnight and then neutralized by acid evaporation (N₂) at 50 °C. Dried samples were solubilized in ultrapure 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 × 50 mm) coupled to a Dionex CarboPac PA10 analytical column (2 × 250 mm) was applied for separation of fucose (Fuc), rhamnose (Rha), arabinose (Ara), galactosamine (GalN), glucosamine (GlcN), galactose (Gal), glucose (Glc), mannose/xylose (Man / Xyl) (quantified together due to coelution), galacturonic acid (Gal-URA) and glucuronic acid (Glc-URA). Detection limits are 10 nM. Particulate CCHO (*p*CCHO) was derived from subtraction of HMW-*d*CCHO from *t*CCHO and thus represent carbohydrates in the size fraction > 0.45 μm. Concentrations of CHO are given as μmol carbon per volume of seawater (μmol CL⁻¹) and composition of CCHO is expressed as mol % CCHO.

Size fractions of DO¹⁴C and HMW-*d*CCHO obtained using 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 was 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 done 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, standardized according to DIN 19266) electrode (Schott[®] Instruments Io-Line). 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 using the program CO2SYS (Lewis and Wallace 1998), with pH (calibrated using reference materials provided by A. Dickson) and TA being the input parameters.

2.3 Data treatment

All samplings were performed during the steady-state period of the experiment, when the growth rate (μ) was equal to

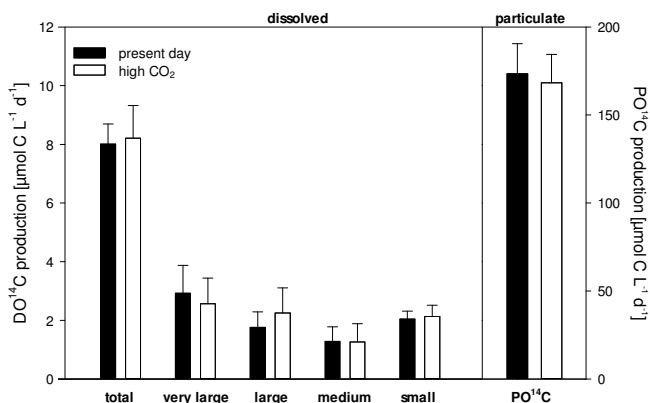


Figure 1. Dissolved (DO¹⁴C, left) and particulate (PO¹⁴C, right) primary production [$\mu\text{mol CL}^{-1} \text{d}^{-1}$] of *Emiliana huxleyi* at present-day (filled bars) and high-CO₂ (open bars) conditions. Daily rates are additionally given for each DO¹⁴C size fraction. Each bar corresponds to the average (\pm standard deviation) of replicate samplings (sampling 1–5, $n = 5$) performed during the steady-state period of the experiment.

the dilution rate (D). The samplings over time thus represent replicates of the same physiological state and values of the respective parameters are given as average \pm standard deviation. Since CO₂ induced no differences between the present-day and high-CO₂ chemostat, they were used as replicate treatments and values are given as mean values with 1 standard deviation if not stated otherwise.

In order to relate daily rates ($\mu\text{mol L}^{-1} \text{d}^{-1}$) directly to concentrations ($\mu\text{mol L}^{-1}$), data were converted into each other by applying a growth rate of 0.2 d^{-1} . For cell-normalized carbon values, concentrations and rates were divided by the cell number.

Differences in carbohydrate composition for the different size fractions were tested by means of analysis of covariance (two-way ANOVA). Differences as a response to CO₂ 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 and biogeochemical composition of *Emiliana huxleyi* as well as carbonate and nutrient chemistry during this chemostat experiment are described in more detail in Engel et al. (2014). Briefly, on day 28 of the experiment, the steady state was reached, with the dilution rate (D) being equal to the growth rate (μ) of *E. huxleyi*. Cell abundances and basic parameters such as particulate organic carbon (POC), nitrogen (PON), phosphorus (POP) and chlorophyll *a* (Chl *a*) remained constant until the end of the experiment, proving

the constant physiological state of *E. huxleyi* (Engel et al., 2014).

During the steady-state period, cell densities were similar in the present-day and high-CO₂ treatment and averaged $5.2 \times 10^8 \pm 18.6$ and $5.1 \times 10^8 \pm 19.7$ cells L^{-1} , respectively. 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 NO₃⁻ and PO₄³⁻ 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 clearly > 16 (Engel et al., 2014). $p\text{CO}_2$ was calculated from pH and TA and yielded significantly different values between treatments of 337 ± 94 (present day) and 623 ± 139 (high CO₂) μatm . Time-averaged values given here differ slightly from those given by Engel et al. (2014), as that study used data from replicate chemostats per CO₂ treatment, while only one chemostat per treatment was sampled for the purpose of this study.

3.2 Primary production and exudation

As determined for cell densities, PO¹⁴C and DO¹⁴C production rates derived from replicate sampling during steady-state growth varied $< 11\%$, confirming the physiological steady state of *E. huxleyi* grown in the chemostats. PO¹⁴C production of 173 ± 17 and 168 ± 16 $\mu\text{mol CL}^{-1} \text{d}^{-1}$ and DO¹⁴C production of 8.0 ± 0.7 and 8.2 ± 1.1 $\mu\text{mol CL}^{-1} \text{d}^{-1}$ were determined for the present-day and high-CO₂ μatm treatment, respectively (Fig. 1). Production rates of PO¹⁴C and DO¹⁴C were not significantly different between the CO₂ treatments (Mann–Whitney rank-sum tests and t tests, $n = 5$, $p > 0.69$) and were thus averaged for both treatments: 171 ± 16 (PO¹⁴C) and 8.1 ± 0.9 $\mu\text{mol CL}^{-1} \text{d}^{-1}$ (DO¹⁴C).

Cell-normalized production of PO¹⁴C and DO¹⁴C during the steady-state period was on average 0.33 ± 0.04 and 0.015 ± 0.002 $\text{pmol C cell}^{-1} \text{d}^{-1}$, respectively for both treatments. Similar PO¹⁴C and DO¹⁴C production rates in both chemostats are reflected in comparable PER of 4.42 ± 0.22 (present day) and $4.70 \pm 0.92\%$ (high CO₂); also, for the size classes of DO¹⁴C, no CO₂ effect was determined (Fig. 1).

Averaged for both treatments, size-fractionated (see Table 1 for definition) DO¹⁴C production ranged between 1.27 ± 0.53 (medium) and 2.74 ± 0.88 $\mu\text{mol CL}^{-1} \text{d}^{-1}$ (very large). Relative contribution of different DO¹⁴C size classes to total DO¹⁴C was 33.6 ± 9.31 (very large), 24.6 ± 7.90 (large), 15.9 ± 7.15 (medium) and $25.8 \pm 3.55\%$ (small). Thus, total DO¹⁴C was comprised of comparable shares of DO¹⁴C in these size classes with slightly higher proportions in the very large fraction.

Table 2. Size class resolved production rates of high molecular weight (> 1 kDa) carbohydrates (HMW-CCHO) and of fresh organic carbon (^{14}C) during the chemostat experiment, as well as contribution of carbon contained in HMW-CCHO to primary production (^{14}C) in particulate matter and in different size fractions of dissolved organic carbon. Values represent averages \pm standard deviation of replicate samplings and both treatments, $n = 6$.

		HMW – CCHO [$\mu\text{mol CL}^{-1} \text{d}^{-1}$] avg \pm sd	^{14}C [$\mu\text{mol CL}^{-1} \text{d}^{-1}$] avg. \pm d	HMW-CCHO : ^{14}C [%] avg. \pm sd
Particulate		20.7 \pm 5.34	171 \pm 15.9	12.5 \pm 3.54
Dissolved	Total	3.10 \pm 0.41	8.11 \pm 0.88	40.0 \pm 5.37
	Very Large	1.41 \pm 0.21	2.74 \pm 0.88	59.7 \pm 17.2
	Large	0.71 \pm 0.24	2.01 \pm 0.72	44.6 \pm 24.7
	Medium	0.62 \pm 0.25	1.27 \pm 0.53	52.9 \pm 33.7
	Small	0.62 \pm 0.18	2.09 \pm 0.32	29.5 \pm 9.30

3.3 Combined carbohydrates

Initial HMW-*d*CCHO concentrations of $7.02 \pm 0.15 \mu\text{mol CL}^{-1}$ were determined in the natural seawater (NSW) media. Corrected for NSW values, carbohydrate concentration during steady-state growth of *Emiliania huxleyi* was 103 ± 28 (present day) and $104 \pm 31 \mu\text{mol CL}^{-1}$ (high CO_2) for *p*CCHO, and 15.2 ± 2.1 (present day) and $15.8 \pm 2.4 \mu\text{mol CL}^{-1}$ (high CO_2) for fresh HMW-*d*CCHO, and hence very similar between the two CO_2 treatments (Fig. 2). Averaged for both treatments, $87 \pm 3\%$ of *t*CCHO was present in the particulate fraction (*p*CCHO). *E. huxleyi* produced *p*CCHO on the order of $104 \pm 27 \mu\text{mol CL}^{-1}$ ($0.20 \pm 0.02 \text{ pmol C cell}^{-1}$), equivalent to $20.7 \pm 5.3 \mu\text{mol CL}^{-1} \text{d}^{-1}$ at a growth rate of 0.2 d^{-1} , representing about 12.5 % of the daily produced PO^{14}C (Table 2). Freshly produced HMW-*d*CCHO was $15 \mu\text{mol CL}^{-1}$ ($0.043 \pm 0.004 \text{ pmol C cell}^{-1}$), equivalent to about 40 % of freshly produced DO^{14}C . Fresh carbohydrate concentrations in various size classes (see Table 1 for definition) also revealed a strong similarity between the present-day and the high- CO_2 treatment (Fig. 2, *t* tests, $n = 6$, $p > 0.269$) and are therefore given as average values in the following. In the different size classes, HMW-*d*CCHO comprised between $29.5 \pm 9.3\%$ (small) and $59.7 \pm 17.2\%$ (very large) of DO^{14}C (Table 2).

HMW-*d*CCHO yielded 7.07 ± 1.06 (very large), 3.57 ± 1.21 (large), 3.08 ± 1.26 (medium) and $3.09 \pm 0.92 \mu\text{mol CL}^{-1}$ (small), suggesting that freshly released HMW-*d*CCHO was primarily comprised of very large HMW-*d*CCHO ($46 \pm 3\%$ C). HMW-*d*CCHO in the large, medium and size fractions contributed 23 ± 6 , 20 ± 4 and $20 \pm 3\%$ C, respectively, to total HMW-*d*CCHO.

Size fractionation of HMW-*d*CCHO in NSW yielded concentrations of 2.39 ± 0.21 (very large), 1.31 ± 0.09 (large), 1.28 ± 0.09 (medium) and $1.95 \pm 0.10 \mu\text{mol CL}^{-1}$ (small) (Fig. 3a, left panel). During the experiment, extracellular release by *E. huxleyi* enriched the NSW to HMW-*d*CCHO

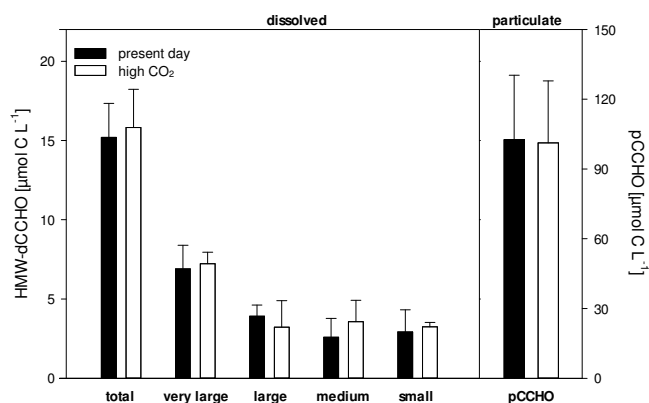


Figure 2. Freshly produced high-molecular-weight (HMW > 1 kDa) dissolved combined carbohydrates (HMW-*d*CCHO, left), and particulate CCHO (*p*CCHO, right) [$\mu\text{mol CL}^{-1}$] derived from *E. huxleyi* under present-day (filled bars) and high- CO_2 (open bars) conditions. Concentrations are additionally given for each size fraction of HMW-*d*CCHO. Each bar corresponds to the average (\pm standard deviation) of replicate samplings (samplings 3–5, $n = 3$) performed during the steady-state period of the experiment.

concentrations of $22.2 \pm 2.1 \mu\text{mol CL}^{-1}$ with size fractions of 8.55 ± 1.08 (very large), 4.64 ± 1.33 (large), 4.09 ± 1.21 (medium) and $5.18 \pm 0.72 \mu\text{mol CL}^{-1}$ (small) (Fig. 3a, right panel).

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-*d*CCHO of the initial NSW used for the present experiment (Fig. 3b, left panel). Size fractions of HMW-*d*CCHO in NSW did not show any significant variation in monomeric composition

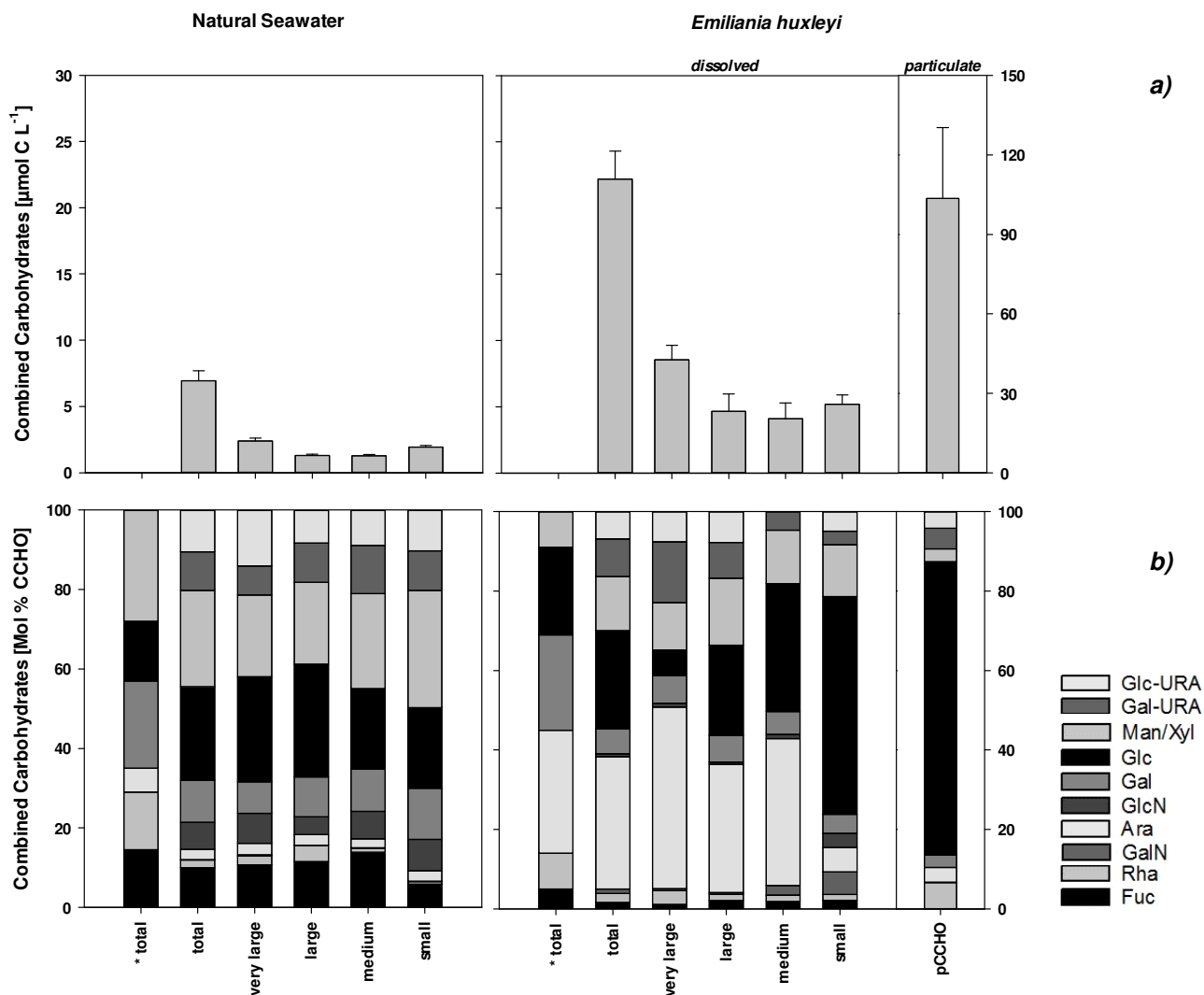


Figure 3. Concentration [$\mu\text{mol CL}^{-1}$] (a) and composition [mol % CCHO] (b) of high-molecular-weight (> 1 kDa) dissolved combined carbohydrates (HMW-*dCCHO*). Data are shown for natural seawater used to prepare the experimental culture media (left panels) and composition in natural seawater enriched with freshly produced HMW-*dCCHO* derived from *E. huxleyi* (rights panels) grown in chemostats. Due to the strong similarity between the present-day and high- CO_2 treatment, both were treated as replicates. Stacked bars show the average of replicate samplings (samplings 3–5, $n = 6$) performed during the steady-state period of the experiment.

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.

($p > 0.462$). However, relative to the other size fractions Man/Xyl was slightly enriched in the small size fraction, while a smaller proportion of Fuc was detected in this fraction. No significant differences in monomeric composition of CCHO produced by *Emiliana huxleyi* were determined between the present-day and high- CO_2 treatment ($p > 0.881$). Therefore, average values are given for replicate sampling during steady-state growth and both treatments in the following (Fig. 3 and Table 3).

ER by *E. huxleyi* led to a clear change in HMW-*dCCHO* composition of the NSW (Fig. 3b), primarily

caused by Ara being significantly higher ($p < 0.001$) in all size classes, except for small (Fig. 3b). In HMW-*dCCHO* derived from *E. huxleyi*, Ara and Glc were the dominant sugars, at 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 and Fig. 3b, right panel). 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 %).

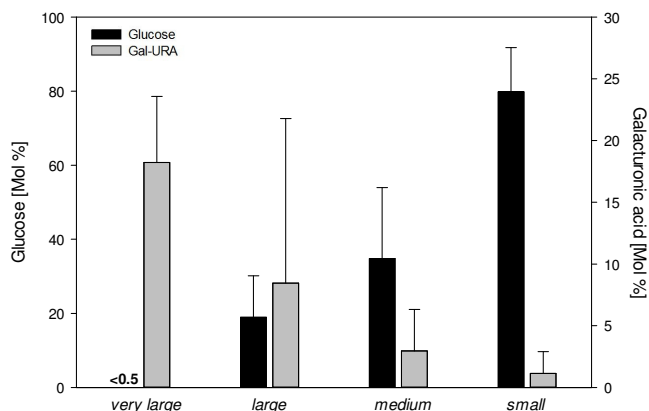


Figure 4. Proportions of glucose (Glc) and galacturonic acid (Gal-URA) in high-molecular-weight (HMW > 1 kDa) dissolved combined carbohydrates (*dCCHO*) of different molecular weight size classes as defined in Table 1. Due to the strong similarity between the present-day and high- CO_2 culture, both were treated as replicates. Bars show the average (\pm standard deviation) of replicate samplings (sampling 3–5, $n = 6$) performed during the steady-state period of the experiment.

Man / Xyl, Ara and Glc-URA ranged 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 also significantly different ($p < 0.002$). 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 the small fraction, Glc contribution was 80 ± 12 mol % and significantly higher than in all other size classes of HMW-*dCCHO* ($p < 0.002$) (Table 3). The contribution of Glc to very large *dCCHO* was negligible (<0.5 mol %). In contrast, Gal-URA contributed 18 mol % to the very large fraction, but only 1 mol % to the small fraction. Proportions of Gal also decreased with the smaller 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 as well as 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 shown earlier to be highly influenced by environmental conditions such as light, temperature and nutrient supply (Mykkestad 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). Extracellular release (ER) in the range of 0–80 % has been reported over the past decades, and only after a long-lasting debate primarily concerning methodological constraints (Sharp, 1977; Mague, 1980; Fogg, 1983; Bjørnsen, 1988) has it become accepted today 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). A relatively constant PER of 20 % was reported for field samples over different ecosystems covering oligotrophic and eutrophic regions (Marañón et al., 2005). Increased PER (up to 37 %), however, were observed for nutrient-limited algae during the transition period of exponential to stationary growth and during senescence of natural phytoplankton communities (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 ~ 0.015 pmol C cell $^{-1}$ d $^{-1}$ represented

Table 3. Freshly produced combined carbohydrates (CCHO) in various size fractions. Average values (bold) and standard deviations (non-bold) in mol % CCHO are given for replicate samplings and both treatments, $n = 6$. 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
<i>p</i> CCHO	6.56 0.84	3.69 0.99	3.09 1.45	74.0 4.08	3.22 1.65	5.18 0.68	4.23 1.01
HMW- <i>d</i> CCHO	2.44 0.70	46.0 3.0	4.64 1.95	20.5 7.48	9.68 2.37	11.0 4.40	5.74 2.99
(total)							
Very large	3.46 1.88	54.2 13.3	6.34 4.11	< 0.5 –	9.62 6.35	18.2 5.35	8.17 6.11
Large	0.91 0.85	41.0 24.2	5.92 5.10	18.9 11.2	16.5 18.9	8.45 13.3	8.34 13.41
Medium	1.71 1.03	48.8 9.41	3.54 3.41	34.9 19.2	8.20 9.88	2.96 3.35	< 0.5 –
Small	2.25 1.54	9.70 6.17	< 0.5 –	79.8 11.9	4.03 3.74	1.13 3.35	2.64 2.89

*p*CCHO: particulate combined carbohydrates; HMW-*d*CCHO: high-molecular-weight (> 1 kDa, HMW) dissolved combined carbohydrates; Rha: rhamnose; Ara: arabinose; Gal: galactose; Glc: glucose; Man / Xyl: coeluting mannose and xylose; Gal-URA: galacturonic acid; Glc-URA: glucuronic acid.

an ER of ~ 4.5 %, well within the abovementioned range for non-stressed algae. Full acclimation to environmental conditions during steady-state growth may also explain the absence of a CO_2 effect on primary production and exudation during this study, and shows that *E. huxleyi* is in principle capable of acclimating to different CO_2 concentrations. Engel et al. (2014) suggested that exudation may be more sensitive to changes in $p\text{CO}_2$ during transient growth phase, such as towards the end of phytoplankton blooms, when cells become nutrient limited. Indeed, significant responses of ER to changes in $p\text{CO}_2$ have mainly been reported for phytoplankton blooms (Engel et al., 2013), batch and semi-continuous cultures (Thornton, 2009; Barcelos and Ramos, 2014), or when growing conditions changed during chemostat studies (Borchard and Engel 2012).

4.2 Combined carbohydrate production

HMW-*d*CCHO freshly produced by *C. huxleyi* during steady-state growth represented about 40 % of freshly produced DO^{14}C (Table 2). This is a lower estimate because low-molecular-weight DOC (< 1 kDa, LMW) would be detected with the ^{14}C -incubation method (Steemann Nielsen, 1952) during the determination of DO^{14}C , but would escape the analysis of HMW-*d*CCHO due to the molecular cut-off > 1 kDa during desalination 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; Passow, 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. Assembly and coagulation of polymeric precursors has been proposed as a mechanism leading to the formation of marine gel particles, such as TEPs. 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 responses 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.

4.2.1 Monomeric composition of CCHO

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-*d*CCHO monosaccharide composition of NSW was dominated by Glc (24 mol %)

and Man/Xyl (24 mol %). Also, high mole percentage (~ 10) of Fuc, Gal, Gal-URA and Glc-URA were determined, while other monomers were of minor importance (Fig. 3b, left panel). The composition of the aged NSW used here differs from what was 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-*d*CCHO released by *E. huxleyi* during the present experiment was substantially different from the initial NSW composition (Fig. 3b) and the compositional shift was primarily induced by a profound relative increase in Ara. The HMW-*d*CCHO and *p*CCHO 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 *p*CCHO of *E. huxleyi* clearly differed from not only NSW but also HMW-*d*CCHO (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-*d*CCHO composition with ~ 83 mol %. These results are consistent with findings by Aluwihare (1999), who report on HMW exudates from *E. huxleyi* being mainly composed of 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 ultra-filtered DOM (> 1 kDa) by Biersmith and Benner (1998), who also investigated non-axenic *E. huxleyi* as a batch culture, and for HMW-*d*CCHO sampled during a field study in the Bay of Biscay where 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; Mykkestad, 1974; Mykkestad et al., 1989), variations in the composition of algal extracellular carbohydrates may be related to physiological and 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 2 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 DO¹⁴C derived from rate measurements, however, a share of up to 20 % may have been channeled into heterotrophic turnover. This means that PER would be underestimated by 20 % at most. The HMW-CCHO was thus to some extent subject to bacterial reworking, and the high proportions of Ara may be a result of the selective removal of other monomers. In accordance with the findings of Aluwihare (1999), concentration of Ara in *d*CCHO remained unchanged during a degradation experiment with the same *E. huxleyi* strain investigated here, while *d*CCHO was reduced by ~ 60 % (Piontek et al., 2010; J. Piontek, personal communication, 2014). However, we would expect extensive microbial degradation of larger *d*CCHO to lead to an increase in Ara mol % in the small size fraction. However, 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. 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 in a series of recent studies (Teeling et al., 2012; Taylor et al., 2014; Kabisch et al., 2014). These studies showed that the release of algal polysaccharides can induce a succession of bacterial communities inhabiting different abilities for enzyme 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. The bacteria present in this study 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 degrada-

dation 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 fraction (Fig. 1 and Table 2). Release rates of HMW-*d*CCHO 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 was characterized as freshly produced HMW-*d*CCHO (Table 2). Contribution of *d*CCHO to fresh DOC was lowest in the small size fraction (30 %) and highest in the very large fraction (60 %) (Table 2). Monomeric composition of different size classes of *d*CCHO 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 *d*CCHO size classes, was of minor importance in the small *d*CCHO 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 % of Ara for particulate components of an *E. huxleyi* culture as well as for the cell lysate. In contrast to Ara, mol % of Glc in our study was highest in the particulate and small fraction, relatively small in the medium to large fraction, 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 % of Ara in LMW-CCHO 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 for distinguishing whether DOC is released from the cell passively (i.e., by leakage) or actively (i.e., by exudation). Leakage is hypothesized 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 of Glc in D formation and have a molecular weight of 5–10 kDa. D-Glc was reported as a 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 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 $\text{DO}^{14}\text{C} > 1$ 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 cannot 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.

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 differences between size fractions of combined carbohydrate suggest that *d*CCHO > 10 kDa is 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. To unravel whether the presence of Ara is indeed an indicator of less degradable exudates, as suggested by this study, or whether Ara degradation requires activities of specific bacterial assemblages, further exploration is needed, i.e., by using axenic phytoplankton cultures combined with the addition of natural bacterioplankton communities. At present our understanding of how microbial processes shape the molecular composition of DOM, specifically of carbohydrates, is still in its infancy. This study suggests that *d*CCHO composition and size may be valuable indicators of processes related to autotrophy such as primary production and exudation but that they may also keep the fingerprint of heterotrophic degradation. A better understanding of compositional changes in *d*CCHO, as a major fraction of semi-labile DOC, may therefore help to unravel carbon cycling and ecosystem dynamics in the ocean.

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