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Enhanced dissolved lipid production as a response to the sea surface warming

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ABSTRACT

The temperature increase in oceans reflects on marine ecosystem functioning and surely has consequences on the marine carbon cycle and carbon sequestration. In this study, we examined dissolved lipid, lipid classes and dissolved organic carbon (DOC) production in the northern Adriatic Sea, isolated diatom *Chaetoceros pseudocurvisetus* batch cultures grown in a wide temperature range (10–30 °C) and in contrasting nutrient regimes, phosphorus (P)-depleted and P-replete conditions. Additionally, lipids and DOC were analyzed in the northern Adriatic (NA) in two stations characterized with different P availability, occupied from February to August 2010 that covered a temperature range from 9.3 to 31.1 °C. To gain insight into factors governing lipid and lipid classes' production in the NA, apart from temperature (T), Chlorophyll *a*, phytoplankton community abundance and structure, nutrient concentrations were measured together with hydrographic parameters. We found enhanced accumulation of dissolved lipids, particulary glycolipids, with increasing T, especially during the highest in situ temperature. The effect of T on enhanced dissolved lipid release is much more pronounced under P-deplete conditions indicating that oligotrophic regions might be more vulnerable to T rise. Temperature between 25 and 30 °C is a threshold T range for *C. pseudocurvisetus*, at which a significant part of lipid production is directed toward the dissolved phase. Unlike monocultures, there are multiple factors influencing produced lipid composition, distribution and cycling in the NA that may counteract the T influence. The possible role of enhanced dissolved lipid concentration for carbon sequestration at elevated T is discussed. On the one hand, lipids are buoyant and do not sink, which enhances their retention at the surface layer. In addition, they are surface active, and therefore prone to adsorb on sinking particles, contributing to the C sequestration.

1. Introduction

Marine organic matter (OM) plays a key role in CO_2 sequestration capacity of the oceans. Operationally defined, marine OM is in dissolved and particulate form. Marine dissolved organic matter (DOM) represents one of the largest active pools of organic carbon in the global carbon cycle, constituting > 90% of total marine organic carbon inventories (Hedges, 1992; Kaiser and Benner, 2009). The phytoplankton community and heterotrophic organisms are the main source of OM in the sea (Libes, 2009). The photosynthetic production of DOM by phytoplankton can represent a substantial fraction of total primary production (Nagata, 2000; Pugnetti et al., 2006). There is a broad range of organic compounds freshly released by phytoplankton including carbohydrates, proteins, amino acids, lipids, nucleic acids, and to a lesser extent, other organic molecules involved in numerous metabolic processes (Thornton, 2014). Lipids are an important component of productivity in coastal areas. Lipids are carbon rich, of very high energetic value, thus representing important metabolic fuels. Different lipid molecular structures influence their reactivity. However, the molecular structure is not the only factor relevant for OM reactivity. The fate of OM also depends on environmental conditions (Wakeham and Canuel, 2006). Marine lipid characterization on a molecular level enables their use as good geochemical markers for the identification of OM processes in the sea, sources, and plankton adaptation to different stressors (Bourguet et al., 2009; Christodoulou et al., 2009; Gašparović et al., 2013; Van Mooy et al., 2006).

Carbon uptake and sequestration by the ocean (i.e. the biological pump) is mainly enabled by the export of sinking biogenic particles. OM partition between dissolved and particulate phases is an important factor in determining fate of organic carbon in the ocean (Thornton,

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2014), as it has implications to the organic matter export from the photic zone. Nowadays it is well known that DOC can contribute to the biological pump (Hansell and Carlson, 2001). As DOC does not sink, its export to the deep ocean/sea occurs through the water column overturn, and its incorporation on sinking marine particulate organic matter, POM (Hwang et al., 2006) or onto mineral particles (Wang and Lee, 1993). Marine DOM exhibits a spectrum of reactivity, from very fast turnover of the most bioavailable forms in the surface ocean to long-lived materials circulating within the ocean abyss (Hansell, 2013).

The most abundant components of the deep ocean DOM are carboxyl-rich alicyclic molecules that have structural similarities to lipid classes sterols and hopanoids (Hertkorn et al., 2006). Hwang and Druffel (2003) found that lipid-like material is a significant source of the uncharacterized organic carbon in the ocean. Although lipids in DOM may have an important role, there are few studies on dissolved lipids in the ocean in last 50 years (e.g. Parrish et al., 1988; Gerin and Goutx, 1994; Mannino and Harvey, 1999; Goutx et al., 2009; Marić et al., 2013). Dissolution from the particulate fraction is the main source of dissolved lipids in the marine environment is (Yoshimura et al., 2009). Parrish et al. (1988) measured profiles of dissolved marine lipid classes over the Scotian Slope and the Bedford basin. They found high concentrations of dissolved lipids (29–190 μg/L), with the highest dissolved lipid levels measured in the vicinity of pycnocline and composed primarily of acetone-mobile polar lipids (pigments, glycolipids). Gerin and Goutx (1994) investigated dissolved lipids in the Almeria-Oran frontal system. They found highly variable concentration (9–113 μg/L) and depth distribution. Dissolved lipid peaks were closely related to Chl *a*. Most dissolved lipid peaks were found to include alcohols and/or acetone mobile polar lipids as principal constituents. Mannino and Harvey (1999) suggested that, although lipids comprised a small portion of DOM, the composition of dissolved lipids has the potential to provide information on the source and diagenetic processing. Goutx et al. (2009) examined changes in concentration and composition of Iatroscan-measured dissolved lipids in the Ligurian Sea, NW Mediterranean. Dissolved lipid concentrations in 0–1000 m water column, varied from 5.3 to 48.5 μg/L, with highest concentration found in 0–50 m surface layer that coincides with phytoplankton biomass. Significant correlations between glycolipids and various phytoplankton pigments suggested that picoeucaryote phytoplankton were a major source of dissolved lipids. Marić et al. (2013) analyzed dissolved lipids in the northern Adriatic, and found that their concentration ranged from 10.3 to 70.6 mg/L, comprising 0.8–4.5% of the DOC. The investigated period was characterized by the dominance of glycolipids, phospholipids and free fatty acids in the dissolved fraction.

As marine DOM is a major reservoir of carbon, characterizing factors affecting the production is essential to understand the dynamics of the global carbon cycle. Surface temperatures are predicted to warm by 2–3 °C over the next 100 years (IPCC, 2001). Sea surface temperature data collected in the northern Adriatic Sea, evidenced a general warming through all seasons in the period 1988–1999, with respect to the period 1911–1987 (Russo et al., 2002). Temperature effect on DOM release has generally been overlooked (Thornton, 2014). In this study we performed microcosm incubations, covering the present temperature range of northern Adriatic (NA) (10–30 °C) with different nutrient amendments. This was done to test how temperature rise influences DOM, particularly dissolved lipid and lipid classes production, and how it is superposed on the effect of nutrients' availability. We selected to work with extracellular OM produced during diatom *Chaetoceros pseudocurvisetus* cultures growth, according to criteria that genera *Chaetoceros* are an important phytoplankton component in the NA and are frequently bloom-forming taxa (Bosak et al., 2016). In addition, we set out to investigate how annual temperature variations affect dissolved lipid production in the complex system, as the northern Adriatic area.

2. Materials and methods

2.1. Site description, sampling and basic environmental determinations

The northern Adriatic Sea is biologically the most productive region in the Mediterranean Sea (Harding et al., 1999). The NA is a highly variable, dynamic environment, with close coupling between river-borne nutrients, net productivity and vertical carbon fluxes. The most important source of the nutrients in the region is the Po River and the winter overturn of regenerated nutrients from the bottom layer, which does not exceed 50 m in the entire basin (Degobbis et al., 2000). It is a complex basin, the western part is greatly influenced by the Po River freshwater input, while its eastern part receives highly saline oligotrophic waters from the southern Adriatic. Chemical and biological processes are influenced by the hydrodynamic regime of the system, which changes strongly due to short-term meteorological phenomena that influence the circulation and vertical structure of the water column (Supić and Vilibić, 2006).

We sampled the NA monthly from the research vessel "Vila Velebita", at two stations that are considered hydrodynamically and trophically different: oligotrophic eastern station 107 (mostly depleted in PO_4) and mesotrophic/eutrophic western station 101 (Fig. 1). Seven cruises were made from February to August 2010 covering a temperature range from 10 to 30 °C. Samples were collected at the surface with 5 L Niskin bottles.

Temperature and salinity were measured using a CTD probe (Seabird SBE25, Sea–Bird Electronics Inc., Bellevue, Washington, USA).

Dissolved inorganic nitrogen (DIN) (calculated as sum of nitrates (NO_3), nitrites (NO_2), ammonium (NH_4)) and orthophosphates (PO_4) were determined aboard by spectrophotometric methods (Parsons et al., 1984), immediately after sample collection. The absorbance readings for all nutrients were made on Shimadzu UV-Mini 1240 spectrophotometer with 10 cm quartz cuvettes. Method accuracies for NO_3, NO_2, NH_4, and PO_4 were ± 3%, ± 3%, ± 5%, and ± 3%, respectively, and detection limits 0.05 μmol/L, 0.01 μmol/L, 0.1 μmol/L, and 0.02 μmol/L, respectively.

Subsamples for the determination of Chlorophyll *a* (Chl *a*) were filtered on Whatman GF/C filters. Following a 3 h extraction in 90% acetone (in the dark, with grinding after addition of acetone), Chl *a* concentrations were determined by a Turner TD–700 fluorometer (Parsons et al., 1984).

2.2. Phytoplankton analysis

Phytoplankton samples were collected using Niskin bottles, 200 mL were preserved in 2% (final concentration) formaldehyde neutralized with disodium tetraborate decahydrate and analyzed within one month from sampling. 50 mL sub-samples were settled for 40 h and analyzed by Zeiss Axiovert 200 microscope following Utermöhl method (1958). Total phytoplankton abundances include all species counted in the microphytoplankton (20–200 μm) and nanophytoplankton (2–20 μm) groups (Sieburth et al., 1978).

2.3. Phytoplankton cultures

We set out to investigate temperature dependent variability in the quantity and composition of organic matter released during growth in P-replete (F2 medium, 36 μmol/L PO_4) and P-depleted (F2 medium with PO_4 reduced to 1 μmol/L) conditions. Marine diatom *Chaetoceros pseudocurvisetus* monoclonal culture was selected for the microcosms experiments at 10, 15, 20, 25 and 30 °C. *C. pseudocurvisetus* colony was manually isolated using a micropipette from a net sample collected at the station SJ101 on 30th October 2014. The culture's genetic material is deposited in GenBank under Accession numbers MG385841 (18S DNA) and MG385842 (28S DNA). Batch culture of *C. pseudocurvisetus* was maintained in F2 medium (Guillard, 1975) in sterile VWR® Tissue

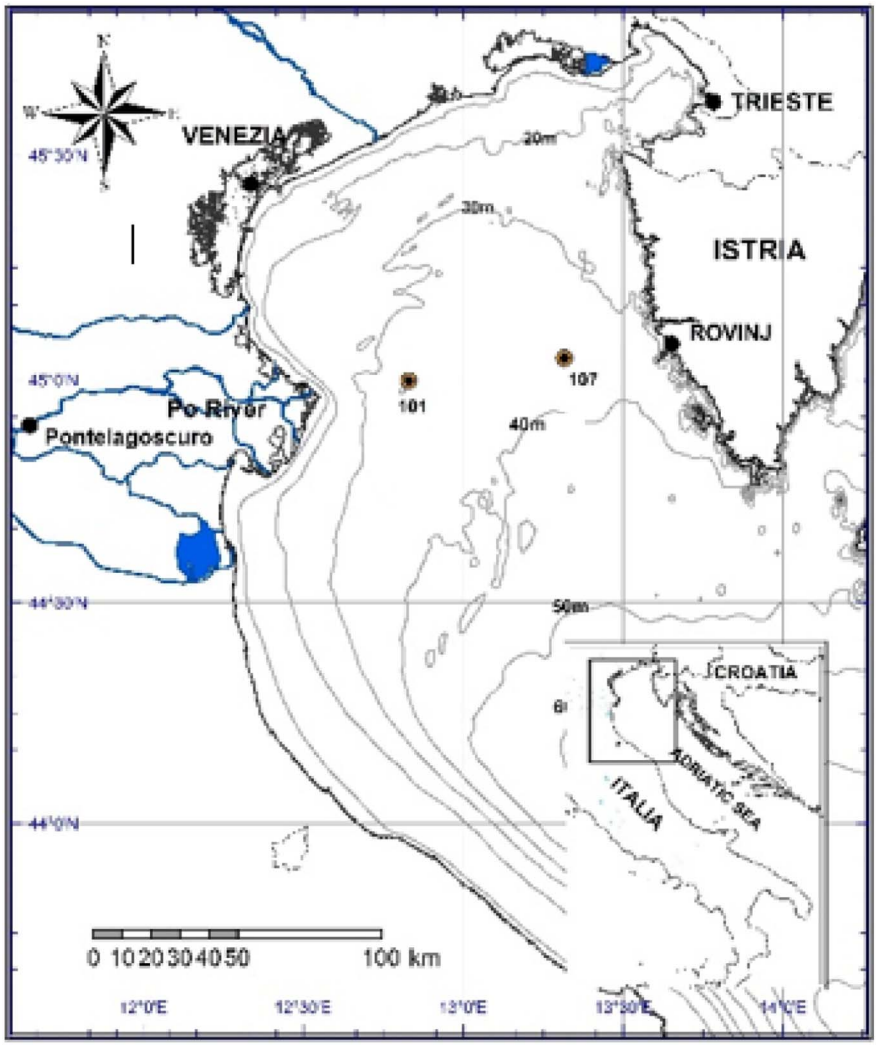

Fig. 1. Study area in the northern Adriatic Sea showing position of stations 107 and 101.

Culture Flasks (VWR, Radnor, Pennsylvania). The media was prepared in NA seawater rested for 2 months in the dark, filtered on sterile 0.22 µm white plain filters (Merck Milipore Ltd.) and boiled in microwave (Keller et al., 1988). Media amendments were added aseptically after sterilization. All experiments were done in duplicate. Cells were pre-conditioned in 50 mL, at experimental temperatures of growth prior to inoculation in 800 mL of medium.

We inoculated 10^5 cells of *C. pseudocurvisetus* in 800 mL of each batch culture medium at the beginning of the experiment. All cultures were grown on a 12/12 light/dark cycle under illumination of 4500 lx. Growth was terminated at onset (third day) of stationary growth phase. The growth phases were determined every second day by cell counting with Fuchs-Rosenthal Chamber hemocytometer under Olympus BX51-P polarizing microscope. Batch cultures were not axenic, however, we took all precautions to avoid further contamination with bacteria. Bacteria were not detected by microscopy, and cultures were not blurry, which would indicate bacterial contamination. During cell counting, we observed a negligible number of dead cells, lacking pigments and cell content. Therefore, analyzed OM is discussed as mainly produced by *C. pseudocurvisetus*. Samples for DOC and lipid analyses were performed from the culturing medium before inoculation and at the onset of stationary phase growth (third day). DOC and dissolved lipid data were obtained by deducing their values measured at the end of cultivation from those measured in the culturing medium.

2.4. Lipid analysis

In order to determine NA seawater dissolved lipid classes, 3 L of seawater was collected in glass containers and passed through a 200 µm stainless steel screen to remove zooplankton and larger particles. Immediately after sampling, seawater was filtered through 0.7 µm Whatman GF/F filters pre-burned at 450 °C for 5 h. Duplicates of 100 mL culture were filtered on precombusted 0.7 µm Whatman GF/F filters to determine the lipid composition of diatom *C. pseudocurvisetus*. Filtrates containing dissolved lipids were stored in dark bottles until liquid-liquid extraction with dichloromethane (twice at pH 8 and twice at pH 2), which was performed within 24 h for NA samples, and immediately for *C. pseudocurvisetus* samples. Ketone 2–nonadecanone was added as internal standard to each sample to estimate the recoveries in the subsequent steps of sample analysis. The extracts were evaporated to dryness under nitrogen atmosphere and redissolved in 20 to 50 µL dichloromethane, depending on sample concentration.

Lipid classes were determined by thin–layer chromatography–flame ionization detection (TLC–FID; Iatroscan MK–VI, Iatron, Japan). Eighteen lipid classes, which constitute total lipids, may be detected by this technique including hydrocarbons, wax esters and sterol esters (WE/SE herein after termed WE), fatty acid methyl esters (ME), fatty ketones (KET), fatty acid methyl esters, ketones, triacylglycerols (TG), free fatty acids, fatty alcohols, 1,3– and 1,2–diacylglycerols, sterols (ST), pigments (PIG), monoacylglycerols, three glycolipids (GL) (monogalactosyldiacylglycerols, digalactosyldiacylglycerols and

sulfoquinovosyldiacylglycerols), and three phospholipids (PL) (phosphatidylglycerols, phosphatidylethanolamines and phosphatidylcholines). Free fatty acids, fatty alcohols, 1,3– and 1,2–diacylglycerols, monoacylglycerols and fatty acid methyl esters are products of early lipid degradation representing degradation indices (DI). Lipid classes were separated on Chromarods SIII and quantified by an external calibration with standard lipid mixture, with a hydrogen flow of 160 mL/min and air flow of 2000 mL/min. Total lipid concentrations were obtained by summing all lipid classes quantified by TLC-FID. The standard deviation determined from duplicate runs accounted for 0–14% of the relative abundance of lipid classes. A detailed description of the procedure is described in Gašparović et al. (2014, 2015).

2.5. Dissolved organic carbon analysis

One liter of seawater and 50 mL of culture medium were filtered through 0.7 μm Whatman GF/F filters combusted at 450 °C/5 h for the DOC determination. Filtered samples for DOC analysis were collected in duplicates in the 22 mL glass vials combusted 450 °C/4 h. Samples were preserved with mercury chloride (10 mg/L) and stored at +4 °C in the dark until analysis.

A model TOC–VCPH (Shimadzu) carbon analyzer with a platinum silica catalyst and a non–dispersive infrared (NDIR) detector for CO_2 measurements was used for DOC measurements and calibrated with potassium hydrogen phthalate. Concentration was calculated as an average of three replicates. The average instrument and Milli–Q blank correspond to 30 μg C/L with high reproducibility (1.5%).

2.6. Data analysis

Principal component analyses (PCA) was used to elucidate the relationships between temperature, DOM producers (phytoplankton) and DOM including DOC, dissolved lipid and lipid classes for *C. pseudocurvisetus* cultures and NA seawater samples.

3. Results

3.1. Chaetoceros pseudocurvisetus cultures

We determined how and to what extent phytoplankton production of dissolved organic matter (DOM) depends on temperature, and investigated DOC and total dissolved lipids (Lip_diss) including lipid classes. DOM production was examined for early stationary growth phases of cultures of diatom *C. pseudocurvisetus* that were grown under phosphorus deplete (P-depleted) and replete (P-replete) conditions.

Both DOC and Lip_diss concentrations increased in the temperature range from 10 to 30 °C together with their contribution to total organic carbon and total lipids, respectively (Table 1). DOC concentration increased 1.6 times between 10 and 30 °C in both P-depleted and P-replete cultures. The content of DOC to total organic carbon (TOC) (DOC(%)) increased with increasing T 3.2- and 2.6-fold for the P-depleted and P-replete culturing conditions, respectively. Lower Lip_diss concentrations in P-replete cultures, for which concentration of Lip_diss increased 1.9-fold between 10 and 30 °C, were measured. In P-depleted cultures Lip_diss concentrations increased 2.2-fold in the temperature range from 10 to 30 °C. Lipids made higher contribution to DOC at P-depleted than at P-replete conditions, with increasing tendency at higher temperatures (Supplementary materials, Fig. S1).

Concentrations and contributions of particular lipid class to total dissolved lipid concentration are presented in Table 1. Majority of lipid classes concentration, including GL, TG, ST and DI, increased with increasing temperature, apart from PL concentration that did not show temperature dependence for both P-depleted or P-replete cultures. For both growth regimes, the contribution of GL, TG, ST and particularly DI to Lip_diss increased with increasing T, while for PL it was the opposite.

Final *C. pseudocurvisetus* cell number decreased with temperature for both culturing conditions, with highest abundance recorded for 15 °C (Table 1). The concentration of released DOC and dissolved lipids per cell (data are not shown but can be calculated from the Table 1) was the lowest for 15 °C, and substantially highest at 30 °C, for both culturing conditions. In P-depleted conditions 16.6 pg DOC and 12.3 pg lipids (what would roughly be 8.6 pg lipid carbon assuming 70% carbon in lipids) were released per *C. pseudocurvisetus* cell at 15 °C, while in P-replete conditions 17.4 pg DOC and 5.9 pg lipids per cell (what would roughly be 4.1 pg lipid carbon per cell) were calculated. Much more DOC and Lip_diss were released in the cultures grown at 30 °C. In P-depleted conditions 13.9 and 14.6 times more DOC and Lip_diss, respectively, were released per cell at 30 °C (229.6 pg DOC and 125.8 pg lipid C) in comparison to 15 °C. For P-replete conditions the increase in DOM release was lower, 9.6 and 14.2 times more DOC and Lip_diss, respectively, were released per cell at 30 °C (166.1 pg DOC and 84.1 pg lipid C per cell) regarding 15 °C. Generally, more DOC and Lip_diss were excreted per cell in the less favorable growing conditions when less *C. pseudocurvisetus* cells developed (Fig. 2).

Principal component analyses of T and DOM variables of P-depleted and-P replete cultures are presented in Fig. 3. The first two principal components of P-depleted cultures (Fig. 3a) explained 85.1% of the total variability among the 14 variables. For P-replete cultures (Fig. 3b), the first two principal components explained 77.7% of the total variability among the 14 variables. For both culturing conditions T, DOC, DOC(%), three Lip_diss variables (concentration, contribution to total lipids (Lip_diss(%)), as well to DOC (Lip_diss in DOC)), the contribution of GL to the dissolved lipid pool (GL(%)), and the contribution of DI to the dissolved lipid pool (DI(%)) variables predominated in the high negative values of PC1. This indicates their positive correlation with temperature. These variables were inversely related to increase in cell abundances and the contribution of PL to dissolved lipid pool (PL(%)), indicating that increased T leads to lower cell abundances and lower PL content in dissolved OM pool. The GL contribution is mainly on the expense of PL.

3.2. Northern Adriatic

3.2.1. Environmental conditions

We followed DOM dynamics of surface waters at the P richer and P poor stations in the temperate northern Adriatic Sea. During the investigated period, temperature distribution (Fig. 4a) showed a regular sinusoidal annual cycle with minimum in February (9.26 and 9.55 °C at stations 101 and 107, respectively) and maximum in July (31.06 and 28.58 °C at stations 101 and 107, respectively). During the study period, salinity varied within 29.3–37.6 range, and 21.3 and 37.9 at stations 107 and 101, respectively (Fig. 4b). Lower salinities at station 101 coincide with increased Po River flows (data not shown). Spreading of low-salinity surface waters extended to station 107, corresponding to the surface water warming (Lyons et al., 2007) in the period from April to August.

Distribution of nutrients followed the Po River fresh water inflow. DIN concentration (Fig. 4c) was markedly higher at station 101 (1.23–73.69 μmol/L) than at station 107 (0.62–9.00 μmol/L). It peaked in June at station 101 and in July at station 107. Phosphate concentration (Fig. 4d) at station 101 ranged from 0.02 μmol/L to 0.37 μmol/L. The surface water at station 107 was entirely oligotrophic, and phosphate concentration was consistently < 0.02 μmol/L with exception in July when 0.09 μmol/L PO_4 was measured.

Concentrations of Chl *a* (Fig. 5a), used herein as an indicator of phytoplankton biomass, followed that of DIN and PO_4, and peaked in June at station 101 (8.9 μg/L) and in July at station 107 (1.7 μg/L). Phytoplankton abundance (Fig. 5b) was the highest in May (reaching 3.47×10^6 cells/L) at station 101, while the highest phytoplankton abundance at station 107 was recorded in June (0.30×10^6 cells/L). *Chaetoceros* taxa considerably contributed to phytoplankton community (Fig. 5c) with 91.6 and 79.8% in May and June, respectively, at station

Table 1
Temperature (T), average values of DOC and the contribution of DOC to TOC (in parentheses), total dissolved lipids and the contribution of Lip_diss to total lipid (dissolved and particulate) (in parentheses), and concentration and the contribution (in parentheses) of major lipid classes (phospholipids (PL), glycolipids (GL), triacylglycerols (TG), sterols (ST) and degradation indices (DI)) to total dissolved lipids, and cell abundances of *C. pseudocurvisetus* cultures grown in increasing temperatures and phosphorus depleted and replete conditions. The number of cells corresponds to 17, 11, 9, 11 and 10 days of stationary phase onset (growth termination) for temperatures 10, 15, 20, 25 and 30 °C, respectively.

T	DOC	Lip_diss	PL	GL	TG	ST	DI	Cell
°C	μg C/L (%)	μg/L (%)	μg/L (%)					cells/L
P-depleted cultures								
10	440 (12.9)	231.8 (54.5)	106.7 (46.1)	64.8 (28.0)	9.9 (4.8)	4.9 (2.1)	23.2 (11.7)	2.0E + 07 ± 8.1E + 05
15	458 (18.5)	312.2 (58.7)	136.2 (43.6)	83.7 (29.4)	9.0 (3.3)	5.7 (1.9)	43.2 (13.0)	2.8E + 07 ± 5.3E + 06
20	666 (23.2)	373.3 (76.8)	198.4 (53.2)	101.4 (27.0)	8.7 (2.6)	10.4 (1.5)	26.2 (7.0)	1.8E + 07 ± 1.6E + 06
25	626 (20.1)	306.2 (76.2)	132.8 (43.4)	86.5 (28.2)	11.6 (4.3)	7.0 (2.3)	42.6 (13.9)	1.1E + 07 ± 9.8E + 05
30	714 (40.9)	516.7 (76.6)	152.4 (29.5)	154.7 (29.9)	23.4 (5.0)	11.3 (3.8)	149.6 (29.5)	2.9E + 06 ± 3.1E + 05
P-replete cultures								
10	463 (14.2)	197.9 (57.7)	102.8 (51.9)	56.1 (28.3)	6.6 (3.3)	5.2 (2.6)	13.6 (7.3)	1.5E + 07 ± 9.0E + 05
15	649 (15.6)	258.2 (58.7)	93.2 (36.1)	74.7 (28.9)	3.0 (1.2)	7.0 (2.7)	24.6 (13.0)	3.7E + 07 ± 2.6E + 06
20	723 (15.4)	260.1 (71.2)	130.5 (50.2)	82.5 (31.7)	1.6 (0.6)	5.4 (2.1)	16.0 (6.2)	1.6E + 07 ± 2.2E + 06
25	650 (15.6)	364.5 (75.6)	160.4 (44.0)	123.1 (33.8)	12.9 (3.5)	7.7 (2.1)	40.3 (11.0)	1.7E + 07 ± 1.0E + 06
30	758 (37.0)	383.8 (73.8)	111.7 (29.1)	134.6 (35.1)	16.4 (4.3)	14.3 (3.7)	64.6 (16.8)	4.6E + 06 ± 3.4E + 05

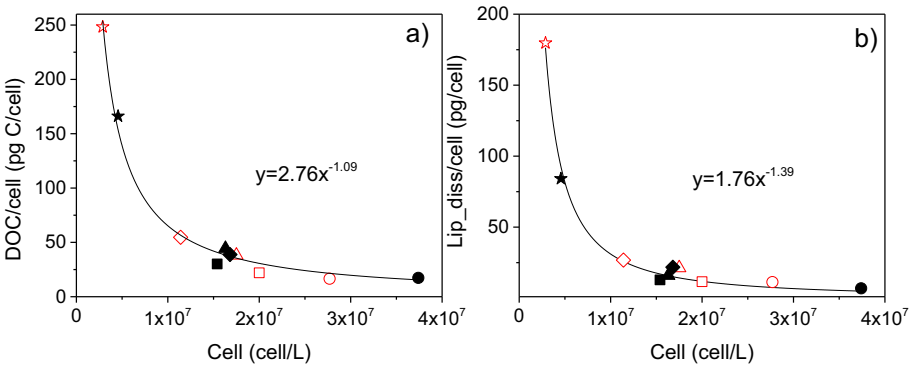

Fig. 2. Relationship between (a) average DOC excreted by *C. pseudocurvisetus* single cell and (b) average total dissolved lipid excreted by *C. pseudocurvisetus* single cell and *C. pseudocurvisetus* concentration for temperatures 10 (squares), 15 (circles), 20 (triangles), 25 (diamonds) and 30 °C (stars) for the P-depleted (open symbols) and P-replete (full symbols) growth conditions.

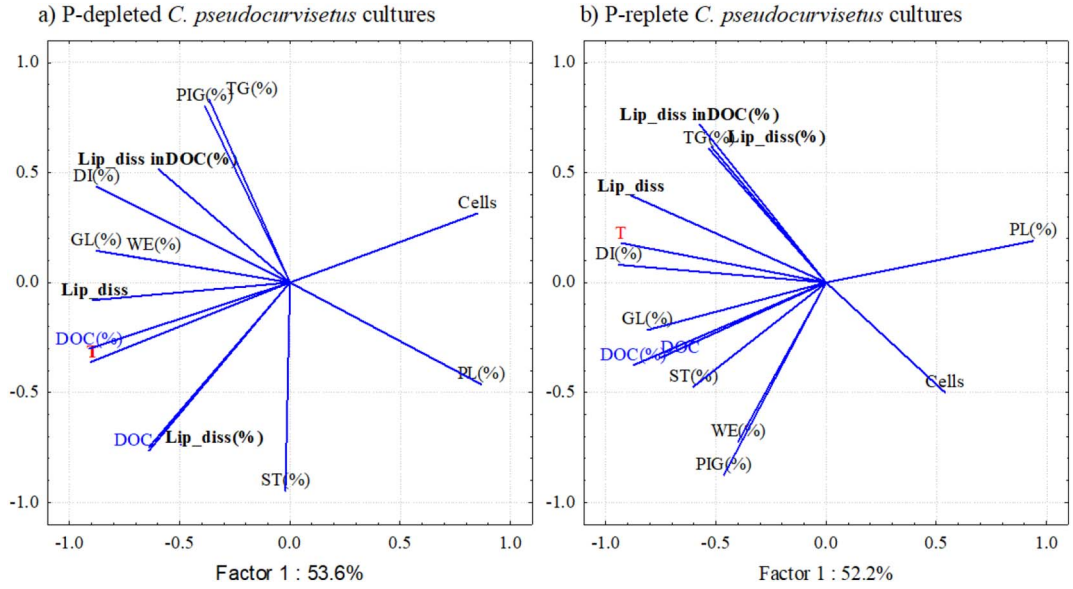

Fig. 3. Principal components analysis (PCA) of the variables: temperature, cell abundances (Cells), DOC, the content of DOC in TOC (DOC(%)), total dissolved lipids (Lip_diss), the content of Lip_diss in total lipids (Lip_diss(%)) and in DOC (Lip_diss in DOC(%)), the content of phospholipids (PL(%PL)), glycolipids (GL(%)), triacylglycerols (TG(%)), sterols (ST(%)), wax/steryl esters (WE(%)), pigments (PIG(%)) and degradation indices (%DI) in Lip_diss for the *C. pseudocurvisetus* cultures grown in P-depleted (a) and P-replete (b) conditions.

101. Meanwhile at station 107 maximum *Chaetoceros* taxa were detected in March and July when it contributed to phytoplankton community with 10.5 and 28.6%, respectively.

3.2.2. Dissolved organic matter production

Temporal distribution of DOC concentration exhibited February minimum (980 and 895 μg C/L at stations 101 and 107, respectively) and maximum in the warmest month of July (2275 and 1903 μg C/L at stations 101 and 107, respectively) (Table 2). DOC accounted for

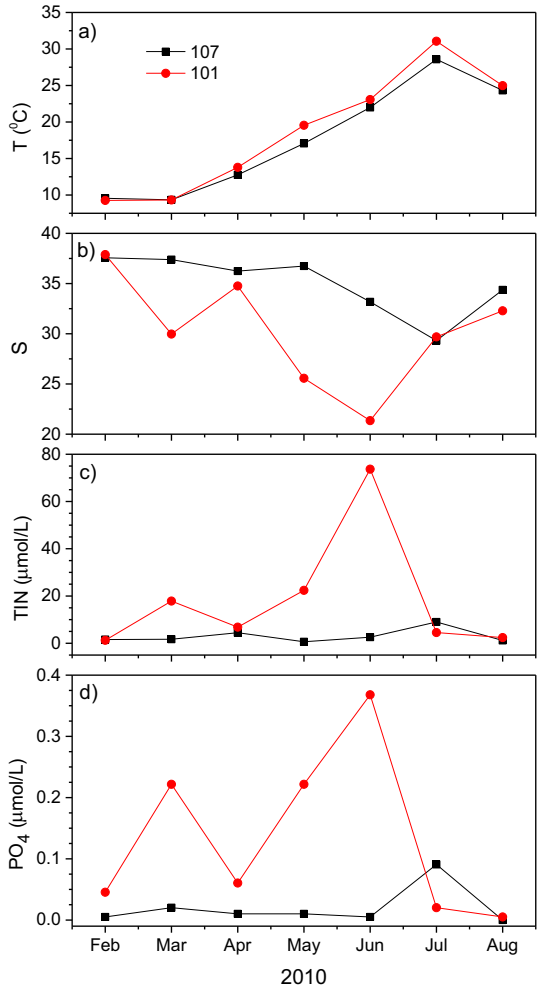

Fig. 4. Temporal distribution of (a) temperature, (b) salinity, (c) total inorganic nitrogen and (d) orthophosphate at stations 101 (circles) and 107 (squares) during the investigation period in 2010.

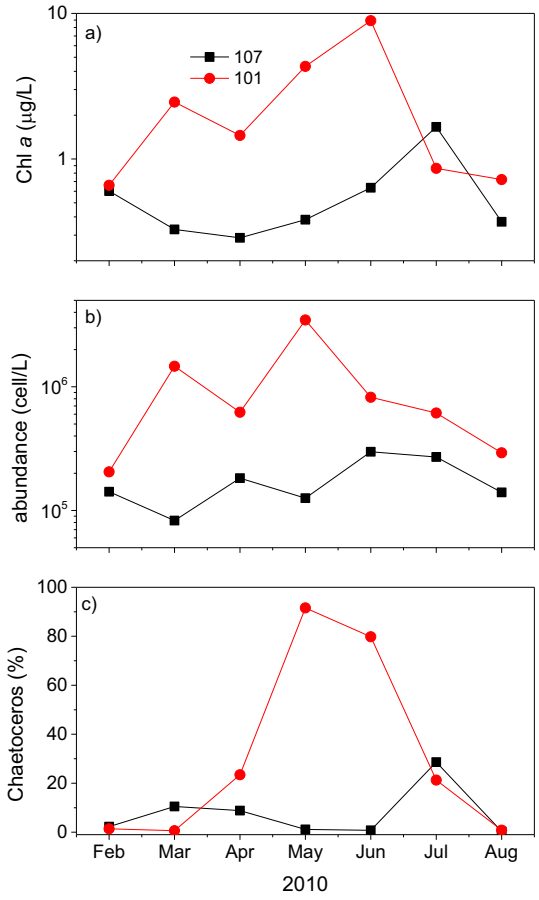

Fig. 5. Temporal distribution of (a) Chlorophyll *a*, (b) total phytoplankton abundance and (c) *Chaetoceros* taxa abundance contribution to total phytoplankton abundance at the mesotrophic station 101 (circles) and oligotrophic station 107 (squares) during the investigation period in 2010.

65–93% of the total organic carbon (TOC) at station 101 (data for particulate organic carbon of both stations are not shown) and 83–94% of the total organic carbon at the station 107 (Table 2). February was an exception, the contribution of DOC to TOC increased toward summer at station 101, while at station 107 trend was opposite, with exception in August (Table 2). Higher Lip_diss concentrations were measured for the oligotrophic, P-depleted station 107, than at mesotrophic station 101. Similarly to DOC, temporal distribution concentrations of Lip_diss increased from winter to summer and peaked in July to values of 45.6 and 50.7 µg/L at stations 101 and 107, respectively (Table 2). General trend of the contribution of Lip_diss to total lipid (dissolved and particulate; data for particulate lipids of both stations are not shown) is not clear. However, if February data were excluded, higher contributions were estimated for summer months (Table 2). Lipids made higher contribution to DOC at station 107 (average 2.0%) than station 101 (average 1.8%) (Supplementary materials, Fig. S2). Generally, more DOC and Lip_diss per Chl *a* was detected for the less favorable plankton growing conditions when lower phytoplankton biomass (determined by Chl *a*) was noted (Fig. 6).

The concentration of the majority of lipid classes, including GL, TG, ST and DI did not show clear relationship with temperature (Table 2). The same was assessed for their contribution to total dissolved lipids (Table 2). These results indicate that other parameters, not only T, define the concentration of DOM in the NA waters.

Evaluating the relationships between temperature and DOM production at NA stations 107 and 101 we performed PCA (Fig. 7). In order to be consistent with *C. pseudocurvisetus* culturing conditions, namely P depleted and P replete conditions, PCA for station 107 was performed only for months when Chl *a* was low (< 1 µg/L), assuming that phytoplankton as DOM producer, grew in P depleted conditions (bolded months in Table 2). Station 101 PCA analysis was performed only for months when Chl *a* was higher than 1 µg/L, assuming that phytoplankton grew in P favorable conditions (bolded months in Table 2).

The first two principal components of stations 107 and 101 (Fig. 7) explained 73.8% and 86.4%, respectively, of the total variability among the 14 variables. T, DOC, PL(%) and GL(%) variables, Lip_diss, and dissolved lipid content in DOC (Lip_diss in DOC(%)) predominated in the positive values of PC1 for station 107. This indicates their positive relationship. Inversely related to these variables were the increase in the content of DOC (DOC(%)), ST (ST(%)), WE (WE(%)), PIG (PIG(%)) and DI (DI(%)) in the lipid pool. All variables regarding Lip_diss (their concentration, contribution to total lipids as well to DOC) had the greatest negative effect on PC2, and were significantly negative correlated to Chl *a* and TG(%). This indicates that at P-depleted conditions more dissolved lipids may be expected for lower phytoplankton biomass as indicated by Chl *a*, and that in such conditions contribution of TG to Lip_diss is decreasing with increased dissolved lipid concentration. The parallel position of variables Chl *a* and TG(%) indicate that in P depleted conditions at station 107, there was higher TG share in total dissolved lipids with higher (but still low) phytoplankton abundance (Chl *a*).

T, DOC, three Lip_diss variables (concentration, contribution to total

Table 2
Temperature (T), DOC and the contribution of DOC to TOC (in parentheses), total dissolved lipids and the contribution of Lip_diss to total lipid (dissolved and particulate) (in parentheses), and concentration and the contribution (in parentheses) of major lipid classes (phospholipids (PL), glycolipids (GL), triacilglycerols (TG), sterols (ST) and degradation indices (DI)) to total dissolved lipids for the northern Adriatic oligotrophic station 107 and the mesotrophic station 101.

Month, 2010	T	DOC	Lip_diss	PL	GL	TG	ST	DI
	˚C	μg C/L (%)	μg/L (%)	μg/L (%)				
Station 107								
Feb	9.55	895 (92.0)	16.3 (50.7)	3.4 (20.7)	3.5 (21.6)	1.1 (7.0)	0.5 (3.1)	6.5 (40.0)
Mar	9.34	1011 (94.0)	15.5 (48.3)	1.8 (11.4)	6.5 (42.2)	0.5 (3.2)	0.6 (4.1)	4.5 (29.2)
Apr	12.76	1109 (91.9)	46.9 (69.5)	10.4 (22.2)	7.2 (15.4)	0.6 (1.3)	3.1 (6.7)	19.9 (42.5)
May	17.06	1119 (89.2)	49.4 (57.5)	7.9 (16.0)	7.4 (15.0)	1.6 (3.2)	2.8 (5.6)	27.5 (55.6)
Jun	21.99	1592 (88.6)	42.5 (58.3)	13.3 (31.3)	18.0 (42.4)	2.8 (6.6)	0.8 (1.9)	6.6 (15.6)
Jul	28.58	1903 (82.7)	50.7 (58.1)	11.7 (23.1)	18.5 (36.4)	3.0 (5.9)	2.5 (5.0)	13.2 (26.0)
Aug	24.32	1630 (92.5)	47.5 (65.7)	14.1 (29.7)	23.0 (48.4)	0.7 (1.4)	0.7 (1.5)	7.5 (15.8)
Station 101								
Feb	9.26	980 (92.7)	21.3 (54.1)	5.3 (24.8)	8.3 (39.1)	0.4 (1.8)	0.7 (3.1)	5.6 (26.2)
Mar	9.32	1299 (65.1)	33.6 (33.6)	4.8 (14.4)	14.6 (43.6)	1.5 (4.5)	0.7 (2.2)	9.8 (29.2)
Apr	13.79	1323 (82.4)	27.2 (31.8)	6.0 (21.9)	9.9 (36.5)	1.5 (5.6)	0.8 (3.1)	8.7 (32.1)
May	19.55	1659 (73.2)	40.1 (43.9)	8.1 (20.3)	16.4 (40.9)	0.4 (1.1)	0.7 (1.8)	12.7 (31.7)
Jun	23.07	1853 (67.6)	91.2 (57.1)	19.7 (21.6)	38.4 (42.1)	3.6 (3.9)	1.5 (1.6)	9.0 (21.9)
Jul	31.06	2275 (88.3)	45.6 (43.0)	13.0 (28.6)	19.5 (42.8)	0.4 (0.8)	1.4 (3.0)	14.8 (32.5)
Aug	24.98	1852 (89.2)	34.5 (48.4)	11.9 (34.6)	15.0 (43.4)	0.3 (0.8)	0.8 (2.2)	4.4 (12.7)

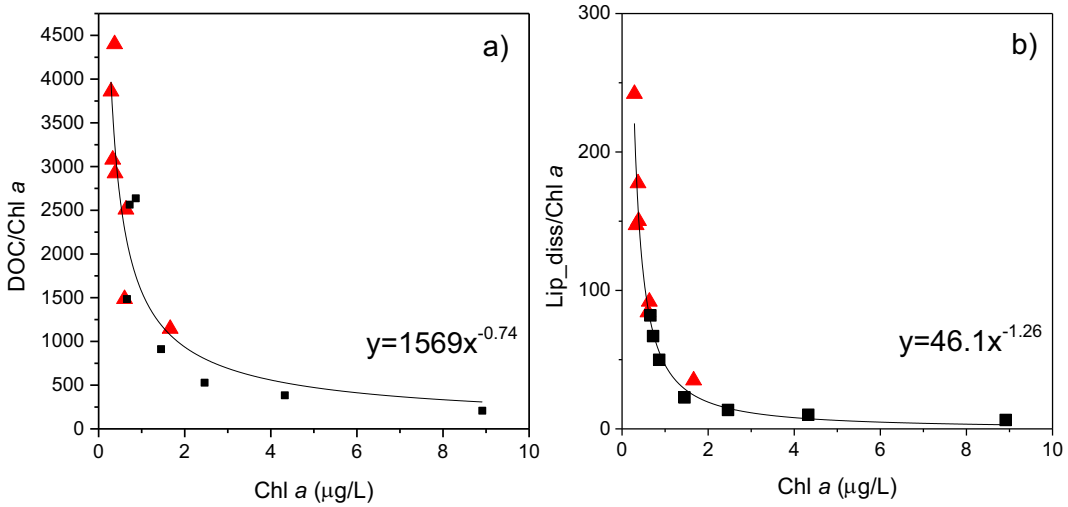

Fig. 6. Relationship between (a) DOC normalized to Chl *a* and (b) total dissolved lipids normalized to Chl *a* vs *Chl a* as an indicator of the phytoplankton biomass for the oligotrophic station 107 (triangles) and the mesotrophic station 101 (squares) in the northern Adriatic Sea.

lipids (Lip_diss(%)) as well to DOC (Lip_diss in DOC)), Chl *a*, PIG(%), and GL(%) variables predominated in the high positive values of PC1 for station 101. This indicates their positive correlation with temperature. Correlation between T and Chl *a* reflects not to their interconnection, but rather to coincidental increase of nutrient concentration at warmer months during 2010 at station 101, and the consequent increase in phytoplankton biomass (c.f. Figs. 4b and c, and 5a). Those variables were inversely related to DOC(%), ST(%) and DI(%), the same as observed for station 107. The increase in the contribution of glycolipids (GL(%)) to the lipid pool at station 101 was inversely related to phospholipid contribution (PL(%)).

4. Discussion

To understand the influence of T on DOM production in seas and oceans, a simple approach is required as a starting point, due to numerous influential parameters and the interplay of their influence on DOM production, its quality, and cycling. Therefore, we started with a simple system by analysing lipid classes and DOC production by diatom *C. pseudocurvisetus* batch cultures grown under different T and nutrient conditions. Furthermore, we investigated lipid classes and DOC from the northern Adriatic covering wide environmental conditions regarding T and nutrient availability. Our data should give insight for the development of models that predict T rise consequences on the biological pump.

Freshly produced DOM is composed of three major biochemical substances, proteins, carbohydrates, and lipids. Phytoplankton is the main lipid source in the oceans as well as in the NA, with heterotrophic bacteria contributing to much lower extent (Gašparović et al., 2013; Frka et al., 2011). Investigations of DOM production by diatoms is important, having in mind that diatoms constitute one of the ecologically most important groups of phytoplankton worldwide, among which *Chaetoceros* is the most abundant and diverse genus (Malviya et al., 2016).

4.1. *Chaetoceros pseudocurvisetus* cultures

DOM (DOC and dissolved lipids) production by *C. pseudocurvisetus* in T range from 10 to 30 ˚C is nonlinear (Fig. 2). The lowest amount of DOM per cell is released by P-depleted and P-replete cultures grown at 15 ˚C. This indicates that at 15 ˚C, energy, carbon and other essential elements are more engaged in the cell reproduction and growth, and

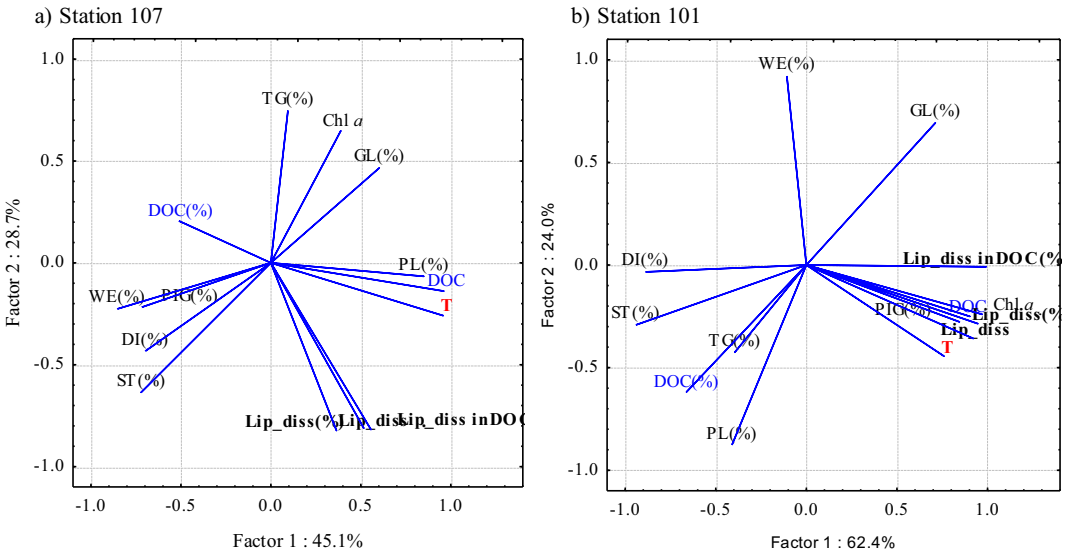

Fig. 7. Principal components analysis (PCA) of: temperature, Chl *a*, DOC, total dissolved lipids (Lip_diss), contribution of dissolved to total lipids (Lip_diss(%)) as well as contribution of dissolved lipids to DOC (Lip_diss in DOC), the contribution of phospholipids (%PL), glycolipids (%GL), triacylglycerols (%TG), sterols (%ST) and degradation indices (%DI) to Lip_diss for the northern Adriatic stations a) 107 and b) 101.

not in DOM release. Temperature rise reflects on *C. pseudocurvisetus* increased DOM release (Fig. 2), together with increased proportion of primary production directed toward DOM, including lipids, production with respect to POM production. An increase in total lipid content generally occurs at higher temperatures for microalgae cultures (Sharma et al., 2012). Increase above the optimal T value probably affects the function of enzymes involved in numerous cellular processes. Consequently, carbon, nitrogen and phosphorus cannot be optimally utilized by cell, but rather are released in the cell surrounding. This is especially pronounced for 30 °C, which is probably above the threshold temperature value, when cellular processes change for *C. pseudocurvisetus* isolated from the NA. Kim et al. (2011) also observed that elevated temperature disproportionately enhances the ratio of DOC to POC production in mesocosm experiment.

Comparing P-depleted and P-replete cultures, we have shown that increased DOC and Lip_diss release per cell is much more pronounced for the cultures growing in P scarcity. Plot of DOC or Lip_diss released per *C. pseudocurvisetus* cell against cell number (as a measure of growth success indicating poor to good growth conditions) (Fig. 2) shows that when *C. pseudocurvisetus* lives in a poor growth conditions (whether elevated temperature or P scarcity) there is generally lower cell abundance and those cells excrete much more DOC and lipids than those living in optimal growth conditions when cell abundance is higher. It is illustrated that there is a combined effect of temperature and nutrient scarcity on the pronounced DOM release. The data show that the temperature above threshold value affects *C. pseudocurvisetus* DOM production more significantly than nutrient limitation. DOC and Lip_diss release can be described by power functions: DOC/cell = $2.76 \times \text{cell}^{-1.09}$ and Lip_diss/cell = $1.76 \times \text{cell}^{-1.39}$, respectively.

Not only DOC and dissolved lipid concentrations rise with increasing T, but also their content in TOC and total lipid pools, respectively. We have observed a very high content of dissolved lipids in DOC, up to 51% for P-depleted cultures grown at 30 °C (Supplementary materials, Fig. S1). Such high content of lipids in DOM might be explained by the fact that DOC, in our dataset, is composed only of fresh DOC. In contrast, most of DOM in the ocean and seas is refractory (Hansell, 2002), and content of dissolved lipids in DOM is much lower (Marić et al., 2013).

For both culturing conditions, content of glycolipids increases proportionally to T increase, while the opposite is observed for phospholipids. Obviously, these two groups of main membrane lipids (Lodish et al., 2004) play significant role in phytoplankton T accommodation. GL are predominantly located in photosynthetic membranes. They not only establish the lipid bilayer into which the photosynthetic complexes are embedded, but GL are also found within photosystems I and II structures (Jordan et al., 2001; Loll et al., 2005). Photosynthesis, which occurs in thylakoids, is the most heat-sensitive cellular function in photosynthetic organisms (Berry and Björkman, 1980). Yang et al. (2006) emphasized that in liposomes, containing only DGDG and/or MGDG, increase the photosystem II thermal stability, whereas phospholipids significantly decrease it. Further on, phosphorus scarcity has an impact on enhanced sulfo-glycolipid accumulation (Van Mooy et al., 2006).

Both, increasing T and P deficiency, cause substantial lipid degradation as concluded by both higher DI concentration and DI content in the dissolved lipid pool for those conditions. Other lipid classes showed T dependence in the whole T range investigated, with much higher proportion at 30 °C (Table 1), which was on the expense of PL. We assume P from PL is reused by alkaline phosphatase activity (Ivančić et al., 2016) for new cells, and that cells probably save P while releasing lipid classes mainly composed of C, H and O. It is to be concluded that the quality of dissolved lipids depends on the lipid producer physiological adaptation to the environmental conditions.

4.2. Northern Adriatic

DOC and Lip_diss concentrations in the NA were highest at the warmest month of July, when content of Lip_diss in total lipids increased. Generally, DOC is accumulating during summer in surface waters of seas and oceans (Børsheim and Myklestad, 1997; Giani et al., 2005; Shen et al., 2016). This DOM accumulation is explained by nutrient limitation of heterotrophic bacteria and subsequent microbial alteration of marine DOC (Shen et al., 2016; Fonda Umani et al., 2012). However, during the warm period in NA bacterial alkaline phosphatase, lipase and protease reach the highest activities (Celussi and Del Negro, 2012). NA is characterized by close bacteria-phytoplankton coupling (e.g. Puddu et al., 1998; Gašparović et al., 2013), and therefore bacteria should have a strong impact on fresh OM reworking. Bacteria modify the carbon cycle in different ways. As bacteria use behavioural and biochemical strategies to acquire organic matter, whether by the expression of enzymes to solubilize particulate organic matter, they

contribute to increased DOM pool, or by direct use of primary produced DOM, which is almost exclusively accessible to heterotrophic bacteria, they contribute to DOM removal from the sea (Azam and Malfatti, 2007).

Here we are pointing that increased temperatures also, usually in summer, likely have consequences on enhanced DOM (DOC and dissolved lipids) production by phytoplankton population. Furthermore, this effect is more pronounced for phosphorus-limited conditions when compared to the P-depleted station 107 with the mesotrophic occasionally P-replete station 101 (Fig. 6). The same we noticed for the batch culture experiments (Fig. 2).

Unlike monocultures, in the northern Adriatic the contribution of lipids to DOC is low (1.1–3.4%) (Supplementary materials, Fig. S2). This is explained by the fact that 90% of marine DOM is refractory and chemically stable (Hansell, 2002), while dissolved lipids represent fresh DOM formed from carbon fixed during primary production and released during life cycle and after cell death (programmed, viral lysis, and sloppy feeding on phytoplankton). Calculation of fresh DOC revealed it as a minor part (0–2%) of the DOC pool in the autumn to winter period in the northern Adriatic (Marić et al., 2013). Higher content of fresh DOC might be expected for the productive period that is investigated in this study.

The difference in the composition of lipid classes in the dissolved fraction is detected between monoculture experiment and the northern Adriatic. Unlike monocultures, there are multiple factors influencing fresh DOM composition, distribution and cycling in the seas that may counteract the T influence. This includes mixed phytoplankton population, phytoplankton species that are adapted to summer temperatures, nutrient input in colder season, changes in light availability, photochemistry and heterotrophy. Increased respiration rates at higher temperature (Vázquez-Domínguez et al., 2007) influence quantity and quality of phytoplankton produced DOM, which are labile compounds with a very short lifetime (Hopkinson et al., 2002). The dissolved lipid composition in the NA is modified by (i) strong bacteria-phytoplankton coupling (Puddu et al., 1998), and (ii) increased heterotrophic bacteria lipase activity during summer (Celussi and Del Negro, 2012). Although *C. pseudocurvisetus* batch cultures were not axenic, we took all precautions to avoid contamination with bacteria and therefore, analyzed lipids are discussed as produced by *C. pseudocurvisetus*. Furthermore, photochemical degradation was omitted by culturing conditions. Therefore, differences between laboratory and real system are expected.

The common feature for the monoculture experiments and NA samples is increased GL content for higher T. This feature is already noticed and explained with the role of GL in achieving thermal stability and prevention/mitigation of photooxidation (Gašparović et al., 2013). It seems that dissolved GL are less prone to degradation than TG, WE, ST and DI whose content in dissolved lipids does not correlated to T or is negatively correlated. Likewise, Tegelaar et al. (1989) reported on resistance of GL in marine sediments to degradation. GL do not possess biologically important phosphorus and nitrogen, and as such are probably less preferred substrate for plankton community. To explain lower susceptibility of GL to photochemical degradation in summer, we may also assume that GL fatty acids are more saturated. This makes sense in context that membrane fluidity is reduced for saturated/less unsaturated compounds (Los and Murata, 2004) which is needed at elevated temperatures.

Rising T would cause sinking of OM to a lesser extent (as DOM does not sink) and less carbon would be sequestered from the atmosphere to ocean/sea sediments (Thornton, 2014). The OM “quality” influences its cycling and capability to sequester carbon. The role of lipids in that process is questionable. On the one hand, lipids are buoyant and do not sink. This might lead to lipid surface remineralization due to prolonged period in surface waters, where they are produced. On the other hand, lipids are surface active, and therefore prone to adsorb on sinking particles, and as such may efficiently contribute to C sequestration. Indeed, Hwang and Druffel (2003) found selective lipid accumulation in the water column. Lipid saturation affects the export of carbon to the deep ocean (Gašparović et al., 2016).

5. Conclusions

From our model experiments on diatom batch cultures *C. pseudocurvisetus*, that were grown in a wide temperature range (10–30 °C) and in P-depleted and P-replete conditions that mimicked conditions in the northern Adriatic, and from the analyses of northern Adriatic samples, we can highlight the following conclusions:

- Temperature rise influences increased DOC and dissolved lipid production in the non-linear manner
- The temperature threshold range above which exponential release of lipids as well as DOC is between 25 and 30 °C for *C. pseudocurvisetus*
- The effect of T on DOC and dissolved lipid release is much more pronounced in phosphorus poor conditions, indicating that oligotrophic regions are more vulnerable to T rise.
- In complex systems, like seas and oceans, unlike the simple and controlled conditions in batch cultures, lipid quantity and quality differ and show different T dependence. Nutrient input in colder season, changes of light availability, mixed phytoplankton population, phytoplankton species that are adapted to summer temperatures, lipid sorption on inorganic and organic particles, photooxidation, and bacterial activity, all influence the dissolved lipid quantity and quality. All these parameters contribute hiding T effect on lipid release.
- Glycolipids are the only lipid class whose content in total lipids increased with rising T for both batch cultures and northern Adriatic samples, indicating their role in phytoplankton T acclimation and longer resistance to degradation in comparison to other classes.
- Rising T would cause lower OM sink (as DOM does not sink), and as such less carbon can be sequestered from the atmosphere to ocean/sea sediments. The role of lipids in that process is questionable: lipids are buoyant, but as very surface active they adsorb on sinking particles and consequently may contribute to the C sequestration.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmarsys.2018.01.006.

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