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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