**Organic matter production during late summer-winter period in a temperate sea**

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ABSTRACT

The quantity and quality of fresh organic matter (OM) formed by primary production in relation to phytoplankton community structure was calculated for the late summer–winter 2009/2010 in the northern Adriatic Sea (Mediterranean). Phytoplankton species, as a direct measure of fresh OM, Chlorophyll *a*, the OM pool (DOC and POC) and lipids, including classes, were analyzed. Data for temperature, salinity and nutrients enabled deeper insight into the conditions that promoted fresh OM production and associated processes at two stations of different trophic status. Phytoplankton growth was controlled by bottom regenerated nutrients and to lesser extent by riverine nutrients. The phytoplankton community was mainly dominated by nanoplankton. Species of moderate carbon content *Chaetoceros compressus, Asterionellopsis glacialis, Leptocylindrus danicus* and *Bacteriastrum jadranum* dominated the microplankton fraction. Availability of orthophosphates was the key factor influencing fresh OM production. POC varied from 37-522 μg l-1. Freshphyto POC, *i.e.* carbon fixed in phytoplankton cells, contributed 7–79% to the POC pool. The DOC (890-1560 μg l-1) level decreased during the investigated period. Calculation of fresh DOC, *i.e.* carbon fixed during primary production and released as dissolved OM, revealed it as a minor part (0-2%) of the DOC pool. Lipid concentrations varied from 9.9-55.0 μg l-1 and 20.0-40.2 μg l-1 in the particulate and dissolved fractions, respectively. Nutrient limitation caused increased synthesis of lipids, among which energy reserve lipids triacylglycerols, which are further immobilized for the construction of glycolipids with increasing depletion of orthophosphates.

*Keywords:*organic matter, lipids, phytoplankton, nutrients, northern Adriatic Sea

**1. Introduction**

Organic matter (OM) in the sea is autochthonous, produced by the phytoplankton community and heterotrophic organisms (Libes, 2009), and is usually higher in coastal areas (Wollast, 1991). During primary production produced organic carbon (OC) is mainly directed towards particulate organic carbon (POC), although dissolved organic carbon (DOC) is a substantial fraction of total primary production as well (López-Sandoval et al., 2011). Partitioning of photosynthetic carbon towards proteins, carbohydrates and lipids in marine phytoplankton represents metabolic strategies of phytoplankton in response to changing environmental factors such as irradiance or nutrient concentration, rather than the effect of variations in the species composition of the community (Suárez and Marañón, 2003).

Lipids are an important component of OM in coastal areas (Frka et al., 2011). The quantity and quality of lipids depend on the stage in the life cycle of the primary producers and environmental factors (Zhukova and Aizdaicher, 2001). Characterization of marine lipids on a molecular level enables their use as geochemical markers for the identification of sources, carbon cycling and reactivity of OM. Polar lipids, *i.e.* phosphatidylglycerols and diphosphatidylglycerols (PG), phosphatidylethanolamines (PE) and phosphatidylcholine (PC), are plankton biomembrane structure components. The glycolipids (GL) that are located predominantly in photosynthetic membranes (thylakoids) indicate on presence of autotrophs (Guschina and Harwood, 2009). Triacylglycerols (TG) indicate plankton metabolic reserves. Diacylglycerides (DG), monoacylglycerides (MG) and free fatty acids (FFA) are glyceride breakdown products and characterize degradation level (Goutx et al., 2003).

The northern Adriatic (NA) is a temperate sea, considered as a dilution basin for the major rivers, including the Po River, with an overall cyclonic counter-clockwise circulation (Jeffries and Lee 2007). It is shallow, with an average depth of 35 m (maximum 50 m), characterized by a marked west to east gradient of salinity, nutrient, chlorophyll concentration and phytoplankton abundance (Degobbis et al. 2000, Polimene et al. 2007), which is mainly controlled by wind-induced circulation patterns and discharges of the Po River (Zavatarelli et al. 2000). The cold strong dry northeasterly wind called Bora, characteristic for the fall-winter period, intensifies the southward flowing coastal current along the Italian coast, causes vertical mixing to 20–25 m in the water column, and increases resuspension and southward transport of bottom sediments for the combined effects of currents and waves (Boldrin et al., 2009).

The NA is thought to be one of the most productive regions in the Mediterranean (Harding et al., 1999). But, a general decline of rivers runoff is taking place in the NA, in particular since 2003, together with decreasing discharge of phosphates. This P-depletion promotes oligotrophy in this marine ecosystem (Cozzi and Giani, 2011). Recently a number of papers report on regime shift toward oligotrophy and reduced production in the NA: phytoplankton and microzooplankton abundance, size and community shift (Marić et al., 2012; Mozetič et al., 2012; Monti et al., 2012); temperature, salinity, nutrient input shifts (Solidoro et al., 2009; Djakovac et al., 2012); organic matter pool decrease (Gašparović, 2012).

The majority of OM in the northern Adriatic originates from marine phytoplankton, while the terrestrial contribution is limited to the direct Po River influence (Najdek and Degobbis, 1997; Giani et al., 2005; Boldrin et al., 2005; Tesi et al., 2007). Phytoplankton blooms in the western part of NA occur in February, May, July and September, and the characteristic succession of species was described by Bernardi Aubry et al. (2012). The eastern part of the NA has a similar phytoplankton bloom dynamics (Godrijan et al., 2012), with exception of an autumn bloom which usually occurs in October (Marić et al, 2012).

In the last few years in the NA the phytoplankton autumn bloom was more pronounced than the spring one (Viličić et al., 2009; Mozetič et al. 2012, Marić et al. 2012). Therefore, our objective was to evaluate the amount of newly produced organic matter through phytoplankton carbon contribution to the organic matter pool during the late summer-winter period. We determined phytoplankton composition, abundance and carbon content, as well as measured dissolved and particulate organic carbon (DOC and POC). In order to assess OM quality and the processes regarding its cycle, different lipid classes were measured.

**2. Methods**

2.1. *Sampling and sample treatment*

Data were collected in the NA, at two stations that are considered hydrodynamically and trophically different: oligotrophic eastern station 107 and mesotrophic western station 101 (Fig. 1), on four cruises in 2009 and 2010: September 23, October 29 and November 18 2009 and January 19, 2010. Samples were collected with 5 l Niskin bottles at five/six depths (surface, 5m, 10m, 20m, 30m and 2 m above bottom).

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

Determination of dissolved inorganic nitrogen (DIN) (calculated as sum of nitrates (NO3), nitrites (NO2), ammonium (NH4)) and orthophosphates (PO4) were made by spectrophotometric methods (Parsons et al. 1984), aboard immediately after sample collection. The absorbance readings for all nutrients were made on Shimadzu UV-Mini 1240 spectrophotometer with 10 cm quartz cuvettes. 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 on a Turner TD–700 fluorometer (Parsons et al. 1984).

2.2. *Dissolved and particulate organic carbon*

For the DOC and POC determination 1.3 l of seawater were filtered on board through 0.7 μm Whatman GF/F filters pre-burned at 450°C/5h. Filtered samples for DOC analysis were collected in duplicates in the 22 ml glass vials combusted 450°C/4h. The samples were preserved with mercury chloride (10 mg l-1) and stored at +4oC in the dark until analysis. After filtration GF/F filters were rinsed with Milli-Q water to remove salts and stored in liquid nitrogen on board and at –80oC in the laboratory until POC analysis.

A model TOC–VCPH (Shimadzu) carbon analyzer with a platinum silica catalyst and a non–dispersive infrared (NDIR) detector for CO2 measurements was used for DOC measurements and calibrated with potassium hydrogen phtalate. Concentration was calculated as an average of three to five replicates. The average instrument and Milli–Q blank correspond to 30 μg C l-1 (n=44) with high reproducibility (1.6%). POC was analyzed by a solid sample module SSM–5000A connected to a Shimadzu TOC–VCPH carbon analyzer calibrated with glucose. POC concentrations were corrected on the basis of blank filter measurements. The average filter blank including the instrument blank corresponds to 5 μg C l-1. The reproducibility obtained for the glucose standard was 3%.

2.3. *Lipids*

For lipid class determination, 3 l of seawater were passed through a 200 μm stainless steel screen to remove microzooplankton and larger particles. Immediately after sampling, seawater was filtered through Whatman GF/F filters pre-burned at 450ºC/5 h. Filters were stored in liquid nitrogen until particulate lipid extraction. Filtrates containing dissolved lipids were stored in dark bottles until extraction by liquid-liquid extraction with dichloromethane (twice at pH 8 and twice at pH 2) that was performed within 24 h. Particulate lipids were extracted by a modified one-phase solvent mixture of dichloromethane-methanol-water procedure (Blight and Dyer 1959). 10 µg of internal standard *n*-hexadecanone were added to each sample before the extraction for the estimation of lipid recovery. Extracts were concentrated by rotary evaporation under a nitrogen atmosphere and stored at -20ºC until analysis.

Lipid classes were separated on Chromarods SIII and quantified with an external calibration using a mixture of standard lipids by a thin-layer chromatograph-flame ionization detector (TLC-FID) Iatroscan Mark-VI (Iatron), using a hydrogen flow of 160 ml min-1 and an air flow of 2000 ml min-1. Details may be found in Penezić et al., 2010. For the analysis 2 µl aliquots of seawater extract in 20–100 µl solution of dichloromethane were spotted on Chromarods with a semiautomatic sample spotter.

2.4. *Phytoplankton*

Phytoplankton samples were collected with Niskin bottles, 200 ml were preserved in 2% (final concentration) formaldehyde neutralized with disodium tetraborate decahidrate and analyzed within one month from sampling. Sub-samples of 50 ml were settled for 40 h and analyzed on 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).

Bio-volume approximation was made from calculations of species cell shapes as proposed by Sun and Liu (2003). Measurements of every identified phytoplankton species were performed on micrographs (acquired by AxioCam MRc5, Zeiss), in the software AxioVision version 4.8.1.0. For each species at least 33 different specimens were measured, and averaged values of cell dimensions were used in the calculation. Carbon content of identified phytoplankton species was obtained from the cell bio-volume using the conversion formulae proposed by Menden–Deuer and Lessard (2000), details are given in Section 2.6 and in Scheme 1.

2.5. *Satellite data analysis*

Data from the MODIS/Aqua instrument were used to assess the variation of Chl *a* concentration in the NA from September 01, 2009 – January 31, 2010. Data were retrieved from the Ocean Biology Processing Group (OBPG) at NASA’s Goddard Space Flight Center as 5 min granules in Level 2 format (processing version 2009.1) and Chl *a* concentrations were derived with the OC3 algorithm (O’Reilly et al., 2000). During investigated period 124 granules were collected. Comparisons with *in-situ* stations were performed by comparing coincident satellite pixels in time (within a 6 hours) and space (within 10 km) with *in-situ* surface measurements, since the main contribution to the water-leaving radiance that reaches the satellite and accounts for satellite derived Chl *a* comes from the surface layer (Gregg and Casey, 2004).

*2.6. Calculations of fresh and transformed organic matter*

According to Fogg´s (1983) observations, in oligotrophic waters 40% of total carbon fixation is released as DOC and 60% as POC. In eutrophic seas this ratio is changed toward much lower carbon fixation with DOC, about 5%, and POC about 95%. Therefore, in mesotrophic waters carbon fixation released as DOC might be taken as % between those two extremes as 23%, with leaving carbon fixation that is released as POC as 77%. Following his results and the experimental results of Pugnetti et al. (2005), who measured very close percentages of primary production that were released as dissolved organic carbon in the northern Adriatic as Fogg (1983) did, we estimated the approximate quantity of “fresh” particulate and dissolved organic matter produced in the NA (Scheme 1). Carbon content in phytoplankton cells represents freshphyto POC. For diatoms freshphyto POC was calculated using conventional formulae: (pgC cell-1) = 0.288xV0.811 and for other phytoplankton groups: (pgC cell-1) = 0.216xV0.939 (Menden–Deuer and Lessard, 2000). Transformed POC was calculated by subtraction of calculated freshphyto POC from measured POC.

Freshphyto DOC is calculated from the freshphyto POC, with relationships for the oligotrophic and mesotrophic stations as: “freshphyto DOC= freshphyto POC x 40%/60%” for station 107 and “freshphyto DOC= freshphyto POC x 23%/77%”, for station 107. Transformed DOC was calculated by subtraction of calculated freshphyto DOC from measured DOC (Scheme 1).

Satellite Chl *a* data served to evaluate freshphyto OM (freshphyto POC+ freshphyto DOC) produced during the investigated period in the uppermost layer. A relationship between satellite Chl *a* and calculated produced freshphyto OM evaluated for the sampling dates and for the surface is confirmed by a linear data fit. The fit is forced to zero intercept because zero Chl *a* implies zero freshphyto OM. The equations of linear fit for the stations 107 and 101 are: “Freshphyto OM=0.0337×Chl *a* ±0.0095 (R=0.83, p=0.0241, n=5)”, and “Freshphyto OM=0.0491×Chl *a* ±0.0099 (R=0.91, p=0.0069, n=5)”, respectively. The calculated freshphyto OM values are plotted in Figs. 6a and b.

Possible contribution of riverine POC to POC pool and riverine DOC to DOC pool was evaluated upon connection between Po River flow (Fig. 2e) and the quantity of riverine POC and DOC exported to the northern Adriatic (equation given in Pettine et al., (1998)), from salinity and by assuming conservative mixing between the fluvial and marine organic matter (Boldrin et al., 2005). Shortly, at zero salinity, i.e. in Po water, content of POC (DOC) is 100% of that calculated by Pettine et al., (1998) equation. Salinities of 37.9 PSU for station 101 and 38.1 PSU for station 107 were taken as salinities with no influence of fresh water where riverine POC (DOC) is 0%. For all others intermediate salinities % of riverine POC (DOC) was calculated taking into account conservative mixing between fresh and sea water.

*2.7. Calculations of fresh and transformed lipids*

Organic carbon in lipids is present in the form of particulate and dissolved lipids. Dissolved lipids were considered as transformed lipids. Particulate lipids are composed of the fresh lipids in the phytoplankton cells (termed freshphyto lipids) and transformed lipids that are consisted of non-living lipids from POM, riverine lipids, and lipids in bacteria and microzooplankton. Freshphyto and transformed lipids in the particulate lipid pool were calculated. Carbon content in the measured lipids was calculated by assuming that the carbon content in lipids is 70%. Therefore measured particulate lipids were transformed by this formula: Lipids (μgC l-1) = measured particulate lipids (μg l-1) × 0.7.

For the calculations of freshphyto lipids we used average % of particulate lipids in POC for our dataset (please see Section 3.6). Thus, 24% contribution of lipid carbon to total phytoplankton carbon was used for oligotrophic station 107 and for mesotrophic station 101the value of 16% was used. Different percentages are reasonable as increased biosynthesis of phytoplankton lipid occurs during oligotrophy (Frka et al., 2011). These percentages were similar to literature data of average percentage of lipids in the phytoplankton cells of 20 ± 7% (Smetacek et al., 1979; Barlow, 1980; Takahashi et al., 1985). Thus the formulas are: “freshphyto lipids (μgC l-1) = Freshphyto POC (μgC l-1) × 0.24” (station 107); and: “Freshphyto lipids (μgC l-1) = Freshphyto POC (μgC l-1) × 0.16” (station 101). Transformed lipids represent the difference between measured particulate lipids and freshphyto lipids: Transformed lipids (μgC l-1) = measured particulate lipids (μgC l-1) − freshphyto lipids (μgC l-1). All the calculations are summarized in Scheme 1.

2.8. *Data analysis*

Data were statistically analyzed using Origin 7 software (Origin Lab). Linear fit and non-parametric Man-Whitney U-Test (because data did not follow normal distribution) were used to analyze similarities between the data.

**3. Results**

3.1. *Environmental conditions*

During the transition from late summer-winter, the thermocline and halocline observed at 10–20 m depth in September diminished until November, after which the water column was mixed (Fig. 2a-d). The low salinity surface layer discharged by the Po River with salinity little higher than 34 PSU spreads east to station 107 in September, and to much lower degree in October, when stations 101 and 107 had salinities around 37 PSU. November and January were characterized by salinities approaching 38 PSU and even higher at 107 in January. In the period from September to January the surface water cooled from 23°C to 12°C and to 10°C at stations 107 and 101, respectively.

3.2. *Nutrients*

Increased nutrient concentrations were observed in the uppermost layer in September (0.07±0.01 μmol l-1 PO4 at station 107 and 0.09±0.02 μmol l-1 PO4 at station 101; 0.5±0.02 μmol l-1 DIN at station 107 and 3.3±0.10 μmol l-1 DIN at station 101) as well as in the bottom layer in September and October (0.19±0.01 μmol l-1 PO4 at station 107 and 0.31±0.02 μmol l-1 PO4 at station 101; 4.3±0.13 μmol l-1 DINat station 107 and 6.9±0.21 μmol l-1 DIN at station 101) (Fig. 3). In November and January nutrients were uniformly distributed through the water column (0.02±0.01 μmol l-1 PO4 at station 107 and 0.05±0.01 μmol l-1 PO4 at station 101; 1.29±0.04 μmol l-1 DIN at station 107 and 1.78±0.05 μmol l-1 DIN at station 101). Depletion of DIN was observed until winter. DIN dropped down from 2.0±0.07 to 1.0±0.03 μmol l-1 at station 107 and from 2.6±0.07 to 1.6±0.04 μmol l-1 at station 101. The average concentration of PO4 also decreased until January, from 0.07±0.01 to 0.02±0.01 μmol l-1 at station 107 and from 0.11±0.02 to 0.06±0.01 μmol l-1 at station 101. In general, higher concentrations of DIN and PO4 were detected at station 101 than at station 107, on average 1.4 and 2.4 times higher, respectively. The N/P ratio was very high during all the period, being on average 62. The two stations were statistically significantly different in orthophosphates (p=0.030) and DIN (p=0.013) concentrations.

3.3. *Phytoplankton*

The general trend showed decrease of phytoplankton abundance with depth, and domination of the nano– over microplankton fraction (Fig 4 a, b, d and e). Exceptions were the domination of microplankton in January at station 107 and in September and November at station 101. Chl *a* concentrations were usually lower than 1 μg l-1, with exceptions in surface waters in September at station 107 and in September, October and November at station 101 (Figs. 4c and f). The two stations were statistically significantly different in measured *in-situ* Chl *a* (p=0.004) concentrations. An enrichment of Chl *a* mean concentration (1.2–2.0 times) was observed at station 101 in comparison to station 107.

The contribution as a percentage of the major phytoplankton groups to the phytoplankton community is shown in Fig. 5a and detailed species list is presented in supplementary Table 1.

At station 101 the September phytoplankton were dominated by up to 40% diatoms of which *Chaetoceros compressus*, *Bacteriastrum* *jadranum* and *Cyclotella* sp. were the most abundant. At station 107 cryptophytes made up 33% of the phytoplankton. The highest diversity and abundance of dinoflagellates was seen at station 107.

In October both stations were dominated by coccolitophorids (35% and 34% at stations 101 and 107, respectively), mainly *Emiliania huxleyi*, *Ophiaster* sp. and *Syracosphaera pulchra*.

The diatom community dominated at station 101 in November, contributing up to 70% of total cells*.* The community was dominated by *Asterionellopsis glacialis*, *Leptocylindrus danicus* and by Chaetoceraceae. Station 107 was characterized by diatoms though a large contribution from cryptophytes (18%) and coccolitophores (20%) was also noted. Diatoms were represented by *A. glacialis* while coccolithophorids by *Rhabdosphaera clavigera* and *Calciosolenia murrayii*.

During January station 107 showed dominance of the diatom *A. glacialis*. At station 101 the community was dominated by coccolitophores (52%), in particular *E. huxleyi*.

The contribution of the major phytoplankton groups to total phytoplankton organic carbon is given in Fig. 5b.

3.4. *Satellite observations of Chlorophyll a*

Satellite derived Chl *a* concentrations were 1.91±4.11 µg l-1 at station 107 and 2.62±3.45 µg l-1 at station 101. Satellite derived Chl *a* concentrations were checked for the significant differences between the two stations. The stations 107 and 101 were significanlly different (P<0.05) in satellite derived Chl *a* concentrations. The highest Chl *a* were found between September and October samplings (Figs. 6 a and b), enhanced by 'clear sky' conditions. *In–situ* Chl *a* and satellite data were compared by performing statistical analysis on logarithmically transformed (base 10) data. Derived statistical parameters show small discrepancies (0.07±0.16 µg l-1) and good correlation (R=0.91, p=0.00035, n=8) between surface *in–situ* and satellite values. Calculated freshphyto OM is graphed as x% of Chl *a* in Fig. 6 and will be discussed later.

3.5. *POC and DOC*

POC comprised a smaller fraction of total organic carbon (TOC=POC+DOC) than DOC at both stations (3.2–25.0%, on average 8.2%). Concentrations of POC ranged from 37 to 522 μg C l-1 (Fig. 7). In general, POC concentrations were 23% higher at station 101 than at station 107. September was characterized by increased POC concentrations in the upper water column while relatively uniform POC concentrations were measured from October to January. A significant positive correlation between POC and phytoplankton carbon was noted for the data from station 107 in September (R=0.95, p=0.0021, n=6), while no significant correlation was found for station 101.

Concentrations of DOC ranged from 890 to 1560 μg C l-1 (Fig. 7). DOC decreased from early autumn until winter. The highest concentrations were observed at the surface in September and October, while a relatively uniform distribution throughout the water column was measured in November and January.

3.6. *Particulate and dissolved lipid classes*

Total particulate lipids varied from 9.9 to 55.0 μg l-1 (Figs. 8a and c). The highest values were measured for surface waters in September at station 101 (39 μg l-1) and at station 107 (53 μg l-1). The rest of the period was characterized by uniform distributions throughout the water column. The lowest concentrations were measured in January; on average 12.7 and 10.8 μg l-1, at stations 107 and 101, respectively. The average contributions of particulate lipids to POC in September, October, November and January were 20.3, 26.6, 35.6 and 14.4% (average 24%) at station 107, and 15.7, 17.1, 21.8 and 8.4% (average 16%) at station 101. Average values were taken for calculations (Scheme 1).

The particulate lipid class composition showed that PG was the largest group, followed by GL and FFA. The temporal and vertical water column profiles of majority lipid classes followed those of the total particulate lipids. The detailed list of all measured particulate lipid class concentrations is given in the Supplement Table 2a.

Contribution of chloroplast GL to total particulate lipids (Fig. 8b and f) was the highest in in surface waters. In January there were significant sub-surface GL maxima. The lowest contribution was found in deeper layers and in November. The phytoplankton storage lipids, TG (Guschina and Harwood, 2009) contributed more to the total lipids at nutrient richer station 101 (Fig. 8c and g). The lowest TG contribution was measured for September samples while the highest was measured for October and November samples. The highest contributions of bacterial phospholipid, PE (Rütters et al., 2002) were recorded in September for the upper water column above thermocline for both stations. The contribution of lipid degradation indices, FFA, 1,2DG, 1,3DG, MG and ALC, to the particulate lipids was considerable during the entire period with values of 23–33% at station 107 and 20–28% at station 101 (Supplementary Table 2a and b).

The concentrations of total dissolved lipids ranged from 10.3 to 70.6 μg l-1 (Figs. 8b and d), with the highest values at station 101 in November. Total dissolved lipids comprised 0.8-4.5% of the DOC. Generally, there was no obvious trend in their distribution throughout the water column. The concentrations of dissolved lipids were greater than particulate lipids during October to January. However during the stratified season in September the upper water column had greater concentrations of particulate lipids.

Detailed results on dissolved lipid classes are presented in Supplementary Table 2b. The investigated period was characterized by the domination of GL, PG and FFA in the dissolved fraction. The majority of lipid classes found in the dissolved form represent non–living organic material. The contribution of lipid degradation products to total dissolved lipids was similar at both stations and was lower in September, contributing on average 12–18%, and higher in January, contributing on average 23 and 28%, at both stations. The lowest contribution of bacterial lipid PE in the dissolved fraction was seen in January.

3.7. *Fresh organic matter production*

The POC pool is composed of carbon fixed in phytoplankton cells by primary production, termed the “freshphyto POC”, and transformed POC that includes non-living POC, riverine POC, and also bacteria and microzooplankton which thrive on phytoplankton produced OM and as such constitute transformed POC pool. Calculated concentrations of freshphyto POC *i.e*. phytoplankton carbon (Supplementary Table 1) were 3–50 μg l-1 at station 107 and 4–111 μg l-1 at station 101 (Figs. 7b and e). The contribution of freshphyto POC to POC pool at station 107 was 9% in September, 7% in October, 42% in November and 37% in January (Fig. 9a). The highest average percentage contributions at station 101 was noted for November (79%), followed by September (29%) and then October and January (7%) (Fig. 9b). Diatoms contributed the most to the freshphyto OC during the investigated period (Fig. 5b). The calculated contribution of riverine POC to POC at station 101 was probably substantial in September (average 30%), lower in October (average 20%), even lower in November (average 15%) and negligible in January. The content of river derived POC was calculated as 32, 35 and 13 % on average in September, October and November, respectively, at station 107.

The DOC pool is composed of transformed DOC including riverine DOC and of carbon fixed during primary production and released as dissolved organic matter representing as thus freshphyto DOC (Scheme 1). The contribution of DOC from viral lysis and sloppy feeding on phytoplankton to freshphyto DOC were not evaluated due to the inability of estimates. The content of river derived DOC was calculated as 0.08 mg l-1, 0.03 mg l-1 and 0.01 mg l-1 in September, October and November for station 101 and 0.08 mg l-1 and 0.02 mg l-1 in September and October for station 107. The contributions of freshphyto and transformed DOC to the DOC pool are presented in Figs. 9c and d. Transformed DOC represented the major DOC fraction. Calculated freshphyto DOC contributed 0-2% to the DOC pool.

The calculated freshphyto OM (freshphyto POC+ freshphyto DOC) produced during the investigated period in the uppermost layer are presented in Figs. 6a and b. The concentration range of freshphyto OM produced at station 107 was 4 to 146 μg l-1, on average 37 μg l-1. At the same time 2.4 times more freshphyto OM was produced at station 101, being 20 to 366 μg l-1, on average 90 μg l-1.

The total particulate lipids consist of lipids from phytoplankton cells (freshphyto lipids) and transformed lipids that are consisted of non-living lipids from POM, riverine lipids, and lipids in bacteria and microzooplankton. The approximate contributions assessed for fresh phytoplankton lipids to total particulate lipids in September, October, November and January at station 107 were 16, 7, 29 and 71%, respectively, and at station 101 were 28, 7, 57 and 13%, respectively (Fig. 9e and f). The calculated average percentage of freshly synthesized lipids in freshphyto OM was between 18.7 and 21.3 at station 107 and between 17.1 and 17.3% at station 101.

**4. Discussion**

4.1. *OM and phytoplankton abundance*

Phytoplankton community and heterotrophic organisms are the main source of OC in the sea (Libes, 2009). Phytoplankton accounts for more than half of the total primary production on Earth and its role in the global carbon cycle is very important. Quantification of organic matter produced during phytoplankton blooms is essential to estimate carbon flow through the ecosystem (Boyd and Trull, 2007). POC accounts only for minor part of total OC, but is an important component of primary production. To gain insight into the level of OM in the NA during late summer-winter, measured POC and DOC concentrations were compared with those from other data from the Mediterranean for the same seasons (Table 1a). Concentrations of POC and DOC measured in this study were comparable to other oligotrophic coastal regions, but were lower than those reported earlier for the NA (Giani et al., 2005).

Lipids are important indicator of the productivity of coastal areas, even though present in low concentrations. Comparison of the concentration ranges of the total particulate lipids from the literature are given in Table 1b. There is a lack of data on both total particulate and dissolved lipids during the autumn–winter period in marine waters. Previous investigations were focused on the more productive periods of spring and summer. The concentrations of total dissolved lipids obtained in the present work fall within values measured for the Ligurian Sea, NW Mediterranean, in the September–October period (Goutx et al. 2009) (Table 1b).

The phytoplankton annual cycle in NA shows maximum values in spring and autumn although it may vary between different areas, reflecting the effect of local environmental factors, such as fresh water discharge, level of anthropogenic eutrophication, urban sewage and particular hydrographic conditions (Cerino et al., 2012). Phytoplankton abundances recorded in this study were lower or in the range with other oligotrophic regions in the Mediterranean Sea (Bode et al., 2005, Totti et al. 2000). The size distribution of phytoplankton assemblages is a major biological factor affecting the functioning of pelagic food-webs and consequently the rate of carbon export from the upper sea to the deep layers (Cerino et al., 2012). Only a few studies consider size classes and carbon production of phytoplankton observable by light microscopy but no connection to different lipids classes, organic matter and other environmental parameters was made so far.

4.2. *Fresh organic matter production*

Investigations of marine organic matter (OM) are becoming more important since carbon capture and sequestration were found to reduce the atmospheric CO2 concentrations. Therefore, studies on OM concentration, production, characterization, cycling and distribution, are necessary. Studies on OM in the NA have included investigations of dissolved organic carbon (DOC) and particulate organic carbon (POC) (Giani et al., 2005), surface active substances and carbohydrates (Gašparović et al., 2011; Tepić et al., 2009). The studies on the primary production in the NA are visibly distinguishing stations directly influenced by the Po River plume and offshore station (Giani et al., 1999; Pugnetti et al., 2005 and 2006). But those papers do not present estimations of the ratio between newly produced and transformed carbon. Therefore this study estimated the percentage of freshphyto and transformed OM (Scheme 1).

Evaluation of freshly produced POC, was made from the carbon content in phytoplankton cells. Freshphyto POC represented a minor component of the POC varying from 3–111 μg l-1 (Figs. 7 and 9). Only in November, at the mesotrophic station 101 the POC pool was dominated (79%) by freshphyto carbon as a consequence of a phytoplankton bloom, with the major contribution of diatoms. In addition to the freshly produced carbon the system contained degraded or transformed carbon. Contribution of non–phytoplankton organisms such as bacteria and microozooplankton to the transformed POC had to be lower. According to La Ferla et al. (2005) the bacterial biomass in POC in the NA accounted for about 1% during the winter period. The microzooplankton carbon in autumn–winter period ranges from 3-12 µg l-1 (Kamburska and Fonda-Umani, 2009). This implies lower carbon contribution from microzooplankton in comparison to phytoplankton.

In coastal systems, terrestrially derived OC is released by river runoff. Our data suggest that freshphyto OM produced in the NA during late summer-autumn may compete in quantity with riverine OM that is reaching the two stations.

The photosynthetic production of DOC by phytoplankton can represent a substantial fraction to total primary production (Nagata, 2000; Pugnetti et al., 2005). Therefore, the possible production of freshphyto DOC during the investigated period was examined. The estimated DOC release, as freshphyto DOC according to our calculation, represented only a minor part of the DOC pool (0–2%). Accumulation of the DOC in the NA occurs when heterotrophic bacteria activity is limited by low P availability (Fonda Umani et al., 2012) which was indeed low during our investigated period.

Calculated total freshphyto OC (Fig. 6) produced in the region showed enrichment of 2.4 times at mesotrophic station 101 relative to oligotrophic station 107. This elevated OM production was expected since phytoplankton abundances and nutrients were higher at station 101. Availability of orthophosphates was the key factor that governed freshphyto OM production, based on the concentrations of PO4 2.4 times higher at station 101 than at 107, and nitrogen concentrations were higher. The importance of the availability of orthophosphates for OM production was expected as the region is continuously under P-limitation (Ivančić et al., 2009).

An estimation on the quantity of freshly synthesized lipids in phytoplankton cells showed this source was a minor portion of measured particulate lipids (7-29%), with an exception in November at station 101 (57%), and in January at station 107 (71%) (Figs.9e and f). Calculations on percentage of freshly synthesized lipids in freshphyto OM, were 18.7-21.3% at station 107, and 17.1-17.3% at station 101. This agrees with lipid production methods based on 14C uptake (Sargent et al., 1985). These authors found that the percentage of total fixed carbon recovered as total lipid was 9-28% in northern Norwegian fjord samples during spring bloom. Suárez and Marañón (2003) found that the relative C incorporation into lipids was <15% in the Cantabrian Sea, the sea that was richer in phosphate than the NA.

4.3. *Northern Adriatic functioning during late summer-winter period*

This late summer–winter period of 2009-2010 differed from the long-term average of flows from the Po River. Decreased flows resulted in decreased nutrients discharged to the NA (Djakovac et al., 2012). The phytoplankton grew in the conditions of phosphorus limitation (DIN/PO4>>22 and PO4<0.1 µmol l–1 (Justić et al. 1995)) and moderate DIN concentrations (on average 2.1 μg l-1). PO4 concentration was always lower than 0.1 µmol l–1 in the upper 20 m depth suggesting strong P-limitation. Nitrogen limitation, DIN<1 µmol l-1 (Justić et al. 1995), was observed in the upper 10 m of the water column in September at both stations.

The phytoplankton growth and consequently freshphyto OM production relied on nutrients from different sources. Specific oceanographic conditions including a highly stratified water column and circulation regime in the late summer allowed the Po freshwater to spread over the NA providing moderate concentration of new nutrients in a thin surface layer (Fig. 3). This nutrient source enabled surface phytoplankton blooms more pronounced at station 101 which is closer to the Po River. While a lower nutrient status in October conditioned elevated abundances of coccolithophorids, a group thriving in oligothrophic conditions (Baumann et al. 2005) (Fig 5a).

Autumn water column overturn dispersed regenerated nutrients from the bottom throughout the whole water column in a period between October and November samplings (Fig. 3). This enabled development of phytoplankton blooms, dominated by diatom species of moderate carbon content, such as *Chaetoceros compressus*, *Asterionellopsis glacialis*, *Leptocylindrus danicus* and *Bacteriastrum jadranum*, which usually reaches its highest cell abundances during the strong freshets of the Po River plume (Godrijan et al., 2012). Higher nutrient levels at station 101 led to higher production of freshphyto OM in comparison to station 107. The modest contribution of GL to total particulate lipids averaged less than 15% for the two stations, confirming that November phytoplankton grew in adequate nutrient conditions. Oppositely, as seen in Frka et al. (2011) nutrient exhaustion promotes higher contribution of GL to the particulate lipid pool.

Abundant phytoplankton community that developed in January at station 107 during low photosynthetically active radiation and low temperature did not relay on riverine nutrients but *in-situ* regenerated nutrients and on those from sediments resuspended from water column mixing. This is due to the main circulation regime of NA that prevails in the late autumn- winter, which drives fresh waters southwards along the Italian coast *i.e.* out from NA. Atmospheric deposition of nutrients through the long rainy period in late November to January (data not shown) might have contributed to the bloom development. Other possible sources of nutrients could be organic nitrogen and phosphorus species, although not to a significant degree according to very low alkaline phosphatase and protease activity in NA during autumn-winter period (Ivančić et al., 2009; Celussi and Del Negro, 2012). Only one difference that was observed between the two stations in January might have promoted the diatom bloom at station 107 and this was the higher temperature measured at station 107 of almost 2°C. The high content of GL in the particulate fraction in January implies that phytoplankton grew on limited nutrient resources. At station 101, close to the Po River, phytoplankton populations were more typical of oligotrophic regions (Godrijan, 2012), *i.e.* nanoflagellates and coccolithophorids mainly *Emiliania huxleyi* contributing 80% to the phytoplankton community (Supplementary table 1b).

Nutritional status has been reflected in the quality of biosynthesized OM. Thus, lower nutrient concentrations at station 107 than at station 101 led to increased accumulation of lipids at station 107, substantially enriching the OM pool. Higher lipid content, up to 50%, was found in the POM in ultra-oligotrophic waters of the east Mediterranean Sea (Yılmaz and Besiktepe 2010). During nutrient exhaustion phytoplankton metabolism has to adapt to this environmental change. The enhanced GL synthesis took place during phytoplankton growth in low nutrient conditions (upper water column, January). Increased TG implies an accumulation of energetic reserves in phytoplankton cells regulated by nutrient availability (Parrish and Wangersky, 1987), appeared to be higher at nutrient richer station 101 and in November when phytoplankton population grew on abundance of nutrients that came from the bottom layer after water column overturn. Khozin-Goldberg et al. (2005) proposed that under nutrient limitation there is TG immobilization for GL synthesis. We propose that the first reaction of phytoplankton upon growing nutrient depletion would be accumulation of TG. Further on, cellular mechanisms use accumulated TG for the construction of more GL that are physiologically more important lipids (Hölzl and Dörmann, 2007). It is to be concluded that this mechanism is dependent on P-availability rather than N-availability, as N-limitation was only observed in September while P-limitation was observed during whole period in the upper water column (Fig. 3).

The largest OM pool determined for September was not dominated by freshphyto POM but rather by transformed OM. The abundant non–living OM pool allowed the development of rich bacterial population in September, concluded from analysis of PE as a tracer. Decreased PE concentrations toward January well agree with generally decreasing bacterial enzyme activity from autumn to winter (Kamburska and Fonda-Umani, 2009). Therefore bacteria did not control the rate of lipid degradation. This is inferred from the domination of GL and the phosphorous containing lipids PG, comprising 54-64% of the dissolved lipid fraction. Dissolved GL and PG are fresher non–living lipids than already re–worked lipid degradation indices (FFA, MG, 1,2– and 1,3-DG) (Supplementary Table 2b). This agrees with the findings of Fonda Umani et al. (2012) that trophic balance in the NA shifts from autumn heterotrophy towards winter autotrophy.

**5. Conclusion**

The late summer–winter phytoplankton succession and consequent OM production in the northern Adriatic Sea is dependent on the two main nutrient sources including the Po River freshet and regular autumn water column overturn bringing regenerated nutrients from the bottom and to some degree on *in-situ* regenerated nutrients.

OM content resembles other oligotrophic coastal regions. Nanoplankton dominated over microplankton. The quality of biosynthesized OM depends on the nutritional status, *i.e.* nutrient limitation supports lipid biosynthesis. During increasing phosphate depletion phytoplankton life-strategy is first to synthesize metabolic reserve lipids, TG, followed by the synthesis of chloroplast lipid GL, at the expense of TG.

While carbon fixed during primary production and released as dissolved OM is calculated to be minor part (0-2%) of DOC pool, freshphyto POC may reach even 79 % of POC pool under favorable conditions, when freshly synthesized lipids in phytoplankton cells are also reaching high percentage of measured particulate lipids. Freshphyto OM produced during late summer-autumn may compete in quantity with riverine OM released to the NA.

Freshphyto organic matter production was higher at the mesotrophic than at oligotrophic part of the northern Adriatic, although general features were also noted: diatoms were the most important phytoplankton group, contributing the most to the freshphyto OM. The whole period was characterized by OM accumulation and reprocessing. Transformed OM dominated over freshphyto OM; dissolved lipids dominated over particulate lipids. Lipid degradation indices contributed significantly to both the particulate and the dissolved lipid pools.

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**Figure captions**

**Fig. 1.** Map of sampling stations in the northern Adriatic Sea.

**Fig. 2.** Vertical profiles of salinity (a and c) and temperature (b and d). Daily mean of the Po River discharge rate (Q) (solid line) and statistical model (1972-2002; dotted line) of discharge rate throughout the time period from September 2009 to January 2010(e). Symbols indicate sampling dates. Data for September (squares), October (circles) and November (triangles) 2009, and January (rhombus) 2010 for stations 107 (a and b) and 101 (c and d).

**Fig. 3.** Vertical distribution of nutrient concentrations at station 107 (a and b) and station 101 (c and d); DIN (a and c), PO4 (b and d). Data for September (squares), October (circles) and November (triangles) 2009, and January (rhombus) 2010.

**Fig. 4.** Vertical profiles of microplankton (a and d) and nanoplankton (b and e) abundances (cell/l) and Chlorophyll *a* (c and f). Data for September (squares), October (circles) and November (triangles) 2009, and January (rhombus) 2010 for stations 107 (a–c) and 101 (d–f).

**Fig. 5.** Relative abundance of the major phytoplankton groups (in %) to the total abundance (a) and contribution of the major phytoplankton groups to total phytoplankton organic carbon (b) in September, October and November 2009, and January 2010 at stations 107 and 101.

**Fig. 6.** Time series (September 2009 to January 2010) of MODIS–derived Chl *a* and calculated freshphyto organic matter (OM), comprising freshphyto POC + freshphyto DOC, produced at stations 107 (a) and 101 (b). Vertical lines indicate sampling dates.

**Fig. 7.** Vertical profiles of POC (a and d), calculated freshphyto POC *i.e*. phytoplankton organic carbon (b and e) and DOC (c and f). Data for September (squares), October (circles) and November (triangles) 2009, and January (rhombus) 2010 for stations 107 (a–c) and 101 (d–f).

**Fig. 8.** Vertical profiles of total particulate lipids (µg l-1) (a and e), percentage of particulate glycolipids (%) (b and f), percentage of particulate triglycerides (%) (c and g) and total dissolved lipids (µg l-1) (d and h) lipids. Data for September (squares), October (circles) and November (triangles) 2009, and January (rhombus) 2010 at stations 107 (a-d) and 101 (e-h).

**Fig. 9.** The contribution of the calculated freshphyto and transformed POC to the POC pool (a and b), freshphyto and transformed DOC to the DOC pool (c and d), and freshphyto and transformed lipids to the particulate lipid pool (e and f) averaged through the water column in September, October and November 2009, and January 2010 at stations 107 (a, c and e) and 101 (b, d and f).

Fig. 1



Fig. 2

Fig. 3



Fig. 4



Fig. 5



Fig. 6



Fig. 7



Fig. 8



Fig. 9



**Scheme 1**. Calculations of freshphyto organic matter production

a)

**FRESHphyto**

(calculated **(1)**)

Carbon (C) contenent in phytoplankton cells.

**TRANSFORMED** (calculated **(2)**)

Non-living POC

Bacteria

Microzooplankton

Riverine POC

**FRESHphyto**

(calculated **(3)**)

C fixed during PP and released as DOC.

**TRANSFORMED** (calculated **(4)**)

Bacteria

Viruses

non-living DOC

Riverine DOC

**Organic carbon (OC) pool**

**DOC pool** (measured)

**POC pool** (measured)

**(1)**Freshphyto POC (diatom C content) (pgC cell-1) = 0.288xV0.811

Freshphyto POC (other phytoplankton C content) (pgC cell-1) = 0.216xV0.939

 **(2)** Transformed POC (μgC l-1) = measured POC pool (μgC l-1) − calculated freshphyto POC (μgC l-1)

**(3)** Input parameter: total carbon fixation released as DOC (Fogg, 1983) in oligotrophic waters was 40% and 23% in mesotrophicwaters:

C fixed during primary production (PP) (μgC l-1) = Freshphyto POC (μgC l-1) + Freshphyto DOC (μgC l-1)

⇒ at oligotrophic station 107: Freshphyto POC (μgC l-1) / Freshphyto DOC (μgC l-1) = 1.50

⇒ at mesotrophic station 101: Freshphyto POC (μgC l-1) / Freshphyto DOC (μgC l-1) = 3.35

 **(4)** Transformed DOC (μgC l-1) = measured DOC (μgC l-1) − calculated freshphyto DOC (μgC l-1)

Details are given in paragraph 2.6.

b)

**TRANSFORMED** (calculated **(7)**)

Lipid C in non-living OM

Lipid C in bacteria and microzooplankton

Riverine lipid C

**FRESH**phyto

(calculated **(6)**)

Lipid C in phytoplankton cells

**Lipid OC pool**

**Particulate lipid pool** (μg l-1)

(measured and recalculated **(5)** to μgC l-1)

**Dissolved lipid pool** (measured)**(8)**

**(5)** Lipids (μgC l-1) = measured particulate lipids (μg l-1) x 0.7

**(6)** Input parameters: lipid content in phytoplankton cells at oligotrophic station 107 was 24% and 16% at mesotrophic station 101. These percentages represent average % of measured particulate lipids to the measured POC for the two stations.

⇒ at oligotrophic station 107: Freshphyto lipids (μgC l-1) = Freshphyto POC (μgC l-1) x 0.24

⇒ at mesotrophic station 101: Freshphyto lipids (μgC l-1) = Freshphyto POC (μgC l-1) x 0.16

**(7)** Transformed lipids (μgC l-1) = measured particulate lipids (μgC l-1) − calculated freshphyto lipids (μgC l-1)

**(8)** Dissolved lipid pool is assumed all to be transformed.

Details are given in paragraph 2.6