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RESEARCH ARTICLE

Variation in transparent exopolymer particles in relation to biological and chemical factors in two contrasting lake districts

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Abstract In inland waters, transparent exopolymer particles (TEP) can affect carbon export and sequestration in sediments with consequences for lake C budgets. We measured TEP concentration in 32 lakes from two contrasting lake districts covering wide ranges in biological and chemical characteristics. North temperate lakes, located in a wet region, have low to moderate ionic strength and low to high dissolved organic carbon with corresponding variation in color (light absorbance). Mediterranean lakes located in a semiarid region were characterized by high ionic strength and high concentrations of dissolved organic carbon but low color. TEP concentrations were large relative to the living portion of the particulate organic carbon pool in both Mediterranean (36%) and north temperate (33%) lakes. TEP concentrations ranged from 36 to 1,462 μg [as Gum Xanthan equivalents (GX eq)] L^{-1} in north temperate lakes. In the Mediterranean lakes, concentrations were higher that previously reported for other systems and ranged from 66 to 9,038 μg GX eq L^{-1}. TEP concentration was positive and significantly related to chlorophyll *a* (chl *a*) in north temperate lakes and in the entire data set. Although a significant and positive relationship between TEP and chl *a* was also detected in the Mediterranean lakes, bacterial abundance was most strongly related to TEP. In contrast with the positive influence of phytoplankton and bacteria on TEP, there were weaker relationships between TEP and the chemical variables tested. We observed a significant and positive relationship between pH and TEP (for all lakes) but this relationship was indirectly driven by a co-variation of pH with phytoplankton biomass based on multiple regression analysis. For the Mediterranean lakes, the negative (but not significant) trends between TEP and both conductivity and divalent cations suggest thresholds above which TEP will likely be destabilized. Under these conditions, TEP may flocculate or disperse in the water column.

Keywords Transparent exopolymer particles (TEP) · Inland waters · Dissolved organic matter · Carbohydrates

Introduction

Until recently, inland waters were poorly considered in the context of the global carbon cycle (Cole et al. 2007; Tranvik et al. 2010), although significant quantities of terrestrial organic carbon can be stored in their sediments. Tranvik et al. (2010) estimated that the global burial of organic carbon in inland water sediments exceeds organic

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carbon sequestration on the ocean floor. This organic carbon buried in lakes can derive from direct sinking of aquatic organisms or detritus, sedimentation of allochthonous terrestrial matter, and flocculation of dissolved organic matter (DOM). Studies of flocculation in lakes are scarce and mostly performed in boreal sites (von Wachenfeldt et al. 2008, 2009; von Wachenfeldt and Tranvik 2008). These studies indicate that an essential fraction of the settling carbon in these lakes is mediated by conversion from DOM into particulate organic matter (POM) by flocculation processes (10% of the total gross sedimentation). Bacteria are presumed to be active in promoting this flocculation. Polymeric fibrils secreted by bacteria (i.e. extracellular polymeric substances, EPS) are an important fraction of flocculated material (Droppo and Ongley 1994) and these fibrils act as stabilizing factors (von Wachenfeldt et al. 2009).

Consistent with current perspectives on flocculation, Ding et al. (2008) have recently illustrated that DOM assembly to POM can be readily induced by hydrophobic exopolymers released by the bacterium *Sagittulla stellata*. Flocculation is a dynamic process where rates of aggregation and disaggregation depend on local environmental variables such as fluid shear, particle concentration, salinity, dissolved chemicals, pH, temperature and organisms (Lick et al. 1992). Accordingly in an early study, Mulholland (1981) found that the addition of Ca^{+2} and Mg^{+2} to a softwater lake caused an increase in the rate of POM formation as a result of a rapid physicochemical flocculation process. More recently, it has been demonstrated that flocculation of allochthonous DOM is also dependent on pH (von Wachenfeldt et al. 2009).

Transparent exopolymer particles (TEP), which are microscopic organic particles visualized by Alcian Blue staining for acid polysaccharides (i.e. Passow et al. 2001; Passow 2002a), are considered as a planktonic form of extracellular polymeric substances (EPS). TEP are ubiquitous in marine and inland waters and play a significant role in the biogeochemical cycling of elements and structuring the microbial food webs as they constitute a nutrient resource (Worm and Søndergaard 1998; Passow 2002a) and a physical structure for bacteria and for other heterotrophic organisms (e.g. Azam and Malfatti 2007). Therefore, TEP are important components in the turnover, decomposition and sinking flux of both organic and inorganic matter and elements in aquatic ecosystems (Logan et al. 1995; Grossart et al. 1997; Grossart and Simon 1998; Simon et al. 2002). Hence, elevated TEP fluxes are frequently associated with peaks of POM flux to sediments in both marine (Passow et al. 2001; Bar-Zeev et al. 2009) and inland (de Vicente et al. 2009) waters. Despite this apparent relevance of TEP for carbon export to sediments, the few studies of TEP in inland waters (Grossart and Simon 1993; Grossart et al. 1997, 1998; Worm and Søndergaard 1998; Berman and Viner-Mozzini 2001) have mainly focussed on their role as microbial substrate or by product, but not on their contribution in C cycling. Although phytoplankton has been traditionally considered to be the major source of TEP in marine ecosystems, bacteria also directly generate dissolved polysaccharides and TEP and may thereby be a significant source of this material (Heissenberger et al. 1996; Ding et al. 2008; Ortega-Retuerta et al. 2010). Similarly, Berman and Viner-Mozzini (2001) observed that many TEP in the Kinneret Lake appeared be derived from particulate detritus from algae and zooplankton.

In this study, we provide the first extensive analysis on TEP concentrations in inland waters from two contrasting lake districts covering wide ranges in biological and chemical properties. We studied lakes in Michigan (USA) and southern Spain. The Michigan lakes, which we refer to as north temperate lakes, have forested catchments and include systems with both clear and highly color waters. These lakes also tend to be acidic to neutral with low to moderate ionic strength (Reche et al. 1999; Pace and Cole 2002). In contrast, the lakes in southern Spain, which we refer to as Mediterranean lakes, are characterized by high ionic strength, moderate to high pH, high dissolved organic carbon (DOC) concentration and low color because their basins are endorheic with low flushing rates, high evapoconcentration, and high exposure to solar radiation (Pulido-Villena and Reche 2003; Ortega-Retuerta et al. 2007). We determined the significance of TEP in terms of C in comparison to bacteria and phytoplankton in lakes from these two contrasting districts. In addition, we explored the potential influence of biological and chemical factors as drivers of TEP concentrations in the water column.

Methods

Study sites

Twenty-four lakes from Wisconsin and Michigan′s Upper Peninsula located at the University of Notre Dame Environmental Research Center (hereafter north temperate lakes) and eight lakes from southern Spain (hereafter Mediterranean lakes) were selected for this study.

Sampling and analysis

Each lake was sampled once during summers of 2006 (north temperate lakes) and 2007 (Mediterranean lakes). Samples of epilimnetic water from every lake were collected to measure pH, conductivity, selected cations, TEP, dissolved mono (DMCHO) and polysaccharides (DPCHO), dissolved organic carbon (DOC), absorption of DOM (color), chlorophyll *a* (chl *a*) and bacterial abundance (BA).

TEP concentration was determined colorimetrically following Passow and Alldredge (1995). Samples (100–300 mL) were fixed with formaldehyde (1% final concentration) and stored in the dark until analysis. Then, samples were filtered onto 0.4 μm polycarbonate filters (Isopore), stained with Alcian Blue solution, soaked in 80% sulphuric acid (5 mL) for 3 h and measured spectrophotometrically at 787 nm, using stained filters without sample as blanks. Alcian Blue absorption was calibrated using a solution of the polysaccharide Gum Xanthan. TEP concentration was expressed in μg of Gum Xanthan (GX) equivalents per litre (μg GX eq L^{-1}) and in carbon units using the conversion factor of 0.75 μgC μg GX L^{-1} proposed by Engel and Passow (2001).

Samples for DMCHO and DPCHO were filtered through precombusted glass-fiber filters (Whatman GF/F) and stored in sterile polypropylene flasks at −80°C until analysis. DMCHO and DPCHO were analyzed following the ferricyanide reaction before (DMCHO) or after hydrolysis (DPCHO) by oxidation of the free reduced sugars (Myklestad et al. 1997). Total carbohydrate is the sum of DMCHO and DPCHO.

Samples for DOC analysis were collected after filtration through pre-combusted Whatman GF/F filters into pre-combusted 20 mL glass ampoules, acidified with phosphoric acid (final pH ∼2), sealed and stored until analysis. DOC was analyzed using a high-temperature catalytic oxidation on a Shimadzu Total Carbon Analyzer (Shimadzu TOC-5050) for the north temperate samples and TOC-V CSH for the Mediterranean samples.

Water absorbance was measured by filtering lake water through Whatman GF/F glass fibre filters and measuring the filtrate absorbance at 440 nm (A_{440}) (Cuthbert and del Giorgio 1992). Absorption coefficients at 440 nm (a_{440}, m^{-1}) were calculated:

$$a_{440} = \frac{(2.303 \cdot A_{440})}{l}$$

where l is the optical path length in meters and 2.303 is the conversion from natural logarithms. Molar absorption coefficients at 440 nm (ε_{440}, $m^2\ mol^{-1}$) were estimated as a_{440}/DOC.

Chl *a* concentration was measured using the method proposed by Jeffrey and Humphrey (1975). Phytoplankton carbon content was estimated from chl *a* concentration using a conversion factor of 40 μgC μg chl a^{-1} proposed by Banse (1977). Bacteria abundance (BA) was determined by epifluorescence microscopy (Porter and Feig 1980). Water subsamples of 3–5 mL were filtered onto 0.2-μm polycarbonate filters and stained with DAPI (4,6-diamidino-2 phenylindole) to a final concentration of 1 μg mL^{-1}. At least 350 cells in 15 random fields were counted for each sample. BA was converted into carbon units using the conversion factor proposed by Loferer-Krossbacher et al. (1998). We compared the carbon in TEP, bacteria, and phytoplankton and related all three of these constituents to the total POC.

Calcium (Ca), magnesium (Mg) and iron (Fe) were quantified by using inductively coupled plasma mass spectrometry analysis (ICP-MS) for the north temperate lakes and by using atomic absorption spectrometer (Perkin Elmer model. 5100) for the Mediterranean lakes.

Statistical analysis

Statistical analyses were performed using Statistica 6.0 Software (StatSoft Inc 1997) and Excel. For *t* tests, unless otherwise stated, the significance level was set at $p < 0.05$. Regression analyses were performed to assess the potential chemical and biological drivers of TEP concentrations. Data were log transformed to comply with the assumptions of regression analyses.

Results

The chemical environment (pH, conductivity and cation concentrations) in the study set of lakes is well contrasted (Table 1). Conductivity values (Mean ± SD) were significantly lower in north temperate (42 ± 44 μS cm^{-1}) relative to the Mediterranean (7,771 ± 7,410 μS cm^{-1}) lakes. Similarly, pH was significantly lower in north temperate (5.52 ± 1.37) than in Mediterranean (8.57 ± 0.54) lakes. In north temperate lakes, characterized by soft waters, Ca and Mg concentrations ranged from 0.2 to 22.6 mg L^{-1} and from 0.14 to 7.4 mg L^{-1}, respectively. In contrast, Mediterranean lakes were characterized by hard waters with Ca and Mg concentrations that ranged from 24.3 to 941.6 mg L^{-1} and from 16.2 to 1,862.3 mg L^{-1}, respectively. Additionally, clear differences in DOC concentrations and in a_{440} were detected between both study groups (Table 1). While DOC concentrations were significantly higher in Mediterranean (34.3 ± 3.1 mg L^{-1}) than in north temperate (9.2 ± 5.0 mg L^{-1}) lakes, a_{440} was much higher in the north temperate systems. Hence, molar absorption coefficients (i.e. *a*440 per unit DOC) were very different between the two lake districts (Table 1).

There were large differences in TEP concentrations among lakes within each district. TEP ranged from 36 to 1,462 (average 256) μg GX eq L^{-1} in north temperate lakes and from 66 to 9,038 (average 1,572) μg GX eq L^{-1} in Mediterranean lakes (Table 1). TEP concentrations were not significantly different between the two groups of lakes.

Table 1 Selected physical, chemical and biological features of the study lakes

Lake	Suface area (ha)	Max depth (m)	TEP (µg GX eq L^{-1})	DMCHO (mg L^{-1})	DPCHO (mg L^{-1})	DOC (mg L^{-1})	a_{440} (m^{-1})	ε_{440} (m^{-2} mol^{-1})	Chl *a* (µg L^{-1})	BA[a] ($\times 10^6$) cell mL^{-1}	Cond (µS cm^{-1})	pH	Ca (mg L^{-1})	Mg (mg L^{-1})	Fe (mg L^{-1})
Santa Olalla, MED	48.4	0.4	1,366	3.3	2.5	74.1	4.8	0.8	246.9	3.4	3,012	9.73	45.3	24.6	0.01
Dulce, MED	78.0	2.5	9,038	4.1	0.9	64.2	9.0	1.7	594.7	8.2	1,291	8.77	24.3	16.1	0.01
Medina, MED	120.0	3.5	1,293	1.3	1.8	28.6	4.0	1.7	37.9	4.4	6,800	8.72	427.1	332.5	0.02
Chica, MED	8.0	8.3	199	1.4	0.8	50.5	2.5	0.6	0.3	2.9	23,600	8.00	901.7	1,862.2	0.15
Grande, MED	9.0	13.0	121	0.4	0.1	12.3	0.8	0.8	1.4	1.4	4,830	8.40	727.0	228.7	0.01
Rincón, MED	3.2	5.4	66	1.1	1.1	16.3	1.7	1.3	12.6	1.6	6,120	8.10	325.30	92.3	0.05
Zoñar, MED	37.0	15.4	321	0.9	1.7	11.8	0.7	0.7	16.9	1.6	2,950	8.30	28.8	42.1	0.15
Amarga, MED	4.0	4.3	172	0.5	0.3	16.4	1.7	1.3	2.5	1.5	13,570	8.50	941.6	720.5	0.18
Bergner, NT	17.8	12.0	53	0.9	0.3	6.4	17.0	31.8	2.9	4.1	11	4.52	0.8	0.3	0.09
Bog Pot, NT	1.8	2.0	1,462	0.8	1.7	12.7	33.7	32.0	17.6	9.2	11	5.10	1.2	0.7	0.84
Bolger, NT	1.1	3.5	159	1.7	0.1	13.5	65.5	58.1	8.8	11.6	31	5.79	4.4	1.4	0.30
Crampton, NT	25.8	15.2	186	1.9	0.6	5.0	12.9	30.8	2.9	1.9	14	5.09	1.1	0.5	0.05
Cranberry, NT	1.3	7.9	59	0.5	0.1	11.5	60.7	63.5	2.9	6.1	14	3.73	0.6	0.3	0.30
E Long, NT	0.1	7.0	36	0.7	1.3	6.9	32.7	56.9	1.5	3.8	15	5.20	1.1	0.5	0.21
Forest Service, NT	0.2	4.9	578	0.7	0.4	5.4	19.8	43.7	5.7	5.2	7	3.78	0.2	0.1	0.09
Hummingbird, NT	0.8	7.6	354	2.2	0.5	18.2	61.4	40.4	5.9	2.6	24	3.82	1.6	0.5	0.55
Inkpot, NT	6.6	5.2	92	0.7	0.2	8.8	21.8	29.7	5.7	5.4	106	7.05	16.0	4.2	0.07
Kickapoo, NT	7.9	2.7	115	0.8	0.1	6.6	31.3	56.8	5.1	3.8	99	6.70	18.4	5.2	0.34
Morris, NT	5.9	6.7	149	1.1	1.7	14.2	80.1	67.9	10.7	4.2	108	6.91	15.9	4.7	0.17
North Gate Bog, NT	0.3	8.0	86	2.9	0.1	20.8	100.8	58.2	3.5	3.2	32	3.56	1.2	0.4	0.58
Paul, NT	1.0	15.0	48	0.4	1.1	4.3	15.3	43.1	3.7	2.5	14	5.56	1.1	0.4	0.13
Peter, NT	2.8	19.6	70	0.5	2.6	5.2	20.7	48.1	2.8	13.2	18	5.84	1.5	0.7	0.04
Plum, NT	91.4	7.3	39	0.3	0.2	5.9	4.5	9.2	18.2	3.4	72	7.20	8.8	2.5	0.04
Raspberry, NT	4.6	6.1	261	0.6	1.5	7.0	19.9	34.0	3.9	2.1	12	4.93	0.9	0.4	0.08
Reddington, NT	1.2	4.9	151	2.1	0.9	18.3	174.9	114.5	10.4	3.6	36	5.54	4.6	1.7	0.42
Roach, NT	45.1	10.0	65	0.2	1.7	2.9	7.4	30.5	2.5	2.6	15	4.51	1.0	0.4	0.07
Tenderfoot, NT	194.2	9.1	69	0.5	2.0	6.6	25.7	46.8	3.2	4.1	103	7.89	14.9	4.0	0.04
Tuesday, NT	0.9	15.0	167	0.8	0.8	7.4	30.4	49.0	4.7	2.1	13	4.88	1.1	0.4	0.06
W Long, NT	–	–	87	0.8	0.7	7.5	32.8	52.7	1.0	3.3	15	5.31	1.1	0.5	0.23
Ward, NT	2.7	8.2	1,339	0.7	0.1	6.7	25.8	46.3	25.7	6.0	156	8.46	22.6	7.4	0.04

MED refers to Mediterranean lakes and NT to north temperate lakes. Morphometric features of North temperate lakes and chl *a* values for Bog Plot are taken from Pace and Cole (2002)

[a] Data for NT lakes from year 1996 (Reche, unpublished)

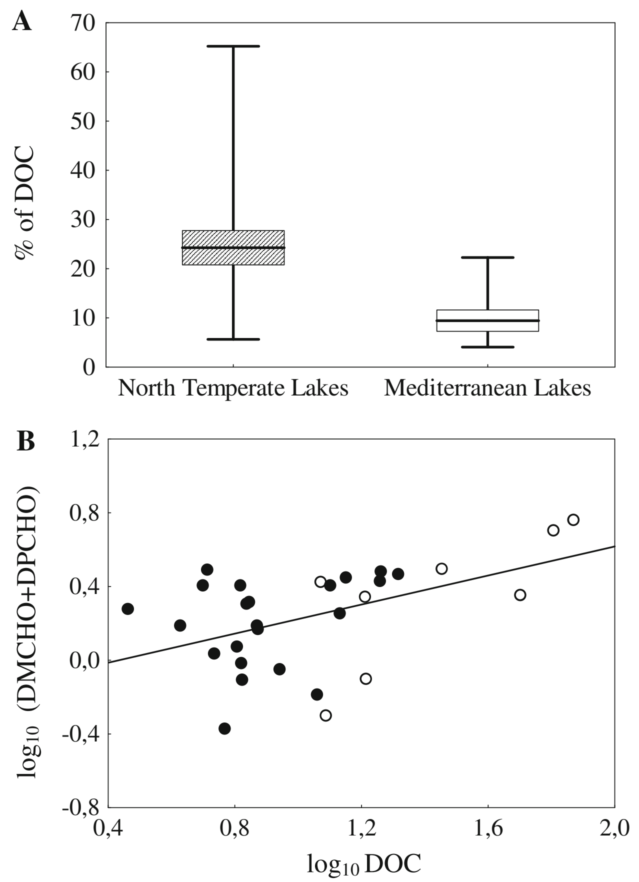

Fig. 1 Relative contribution of dissolved carbohydrates (DMCHO + DPCHO) to the total DOC pool in terms of % of C in Mediterranean and north temperate lakes (**a**). *Boxes* standard errors. *Whiskers* min–max. Scatterplot between total carbohydrates and DOC (log–log) (**b**). *Solid regression line* is shown for significant relationship for the merged data of Mediterranean (*empty circles*) and north temperate lakes (*filled circles*)

Similarly, dissolved carbohydrates (DMCHO + DPCHO) concentrations were not significantly different between Mediterranean and north temperate lakes. Average values were 2.8 mg L^{-1} ± 1.8 and 1.9 mg L^{-1} ± 0.8, respectively. Dissolved carbohydrates accounted, on average, for 9 and 25% of the total DOC pool in Mediterranean and in north temperate lakes, respectively (Fig. 1a). DMCHO accounted for the majority of the dissolved carbohydrates in both Mediterranean (59% ±17) and north temperate (56% ±27) lakes. Dissolved carbohydrates and DOC were positively and significantly related when merging all data ($n = 30$, $r = 0.48$; $p < 0.01$) (Fig. 1b).

Chl *a* concentration ranged from 0.3 to 594.6 µg L^{-1} and from 1.0 to 25.7 µg L^{-1} in Mediterranean and in north temperate lakes, respectively (Table 1). Average chl *a* concentrations did not differ significantly between the two lake districts. BA ranged from 1.4 × 10^6 to 8.2 × 10^6 cells mL^{-1} in Mediterranean lakes and from 1.9 × 10^6 to 13.2 × 10^6 cells mL^{-1} in north temperate lakes (Table 1).

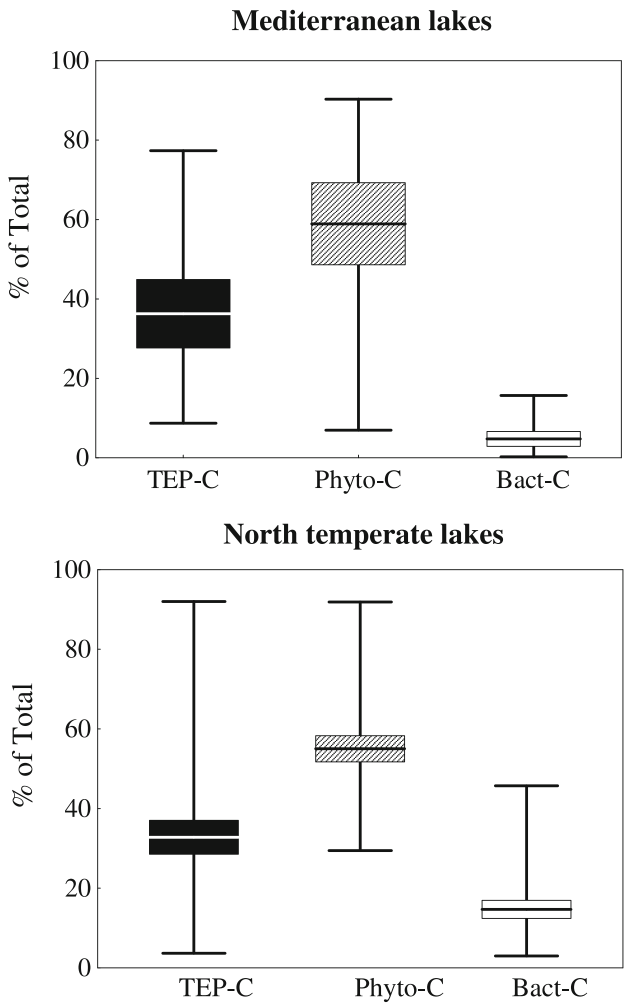

Fig. 2 Relative contribution of TEP-C, Phyto-C and Bact-C (%) to sum of three pools (= "% of Total" on *y*-axis). *Boxes* standard errors. *Whiskers* min–max

Comparing TEP, phytoplankton and BA in terms of carbon, on average, phytoplankton C comprised the most important pool accounting for 59% in the Mediterranean and 53% in the north temperate lakes of the sum of carbon for these three pools. TEP-C represented the second most important fraction accounting for 36 and 33% of this particulate matter in Mediterranean and in north temperate lakes, respectively (Fig. 2). Note also that there was considerable variability of TEP within the same lake district (Fig. 2).

To assess possible drivers of TEP concentration in the water column, we analyzed the relationships between TEP concentration and different chemical and biological variables using linear regression (Table 2). TEP and dissolved carbohydrates (DMCHO + DPCHO) were significant and positively related only when merging all data ($r = 0.42$; $p < 0.05$; Fig. 3a). TEP concentration was significantly related to chl *a* both in Mediterranean lakes ($r = 0.81$; $p < 0.05$) and in north temperate lakes ($r = 0.44$;

Table 2 Results of the regression analyses performed between TEP and different variables

	Indep Var	Lakes	Equations	r^2	p Level	n
TEP	DMCHO + DPCHO	Mediterranean	$y = 1.35x + 2.19$	0.49	ns	8
		North temp	$y = 0.22x + 2.08$	0.01	ns	22
		All data	$y = 0.85x + 2.06$	0.18	<0.05	30
TEP	Chl *a*	Mediterranean	$y = 0.51x + 2.06$	0.66	<0.05	8
		North temp	$y = -0.59x + 1.68$	0.25	<0.05	22
		All data	$y = 0.60x + 1.76$	0.52	<0.001	30
TEP	BA	Mediterranean	$y = 2.34x - 12.37$	0.85	<0.005	8
		North temp	$y = 0.37x - 0.35$	0.04	ns	22
		All data	$y = 0.56x - 1.43$	0.06	ns	30
TEP	pH	Mediterranean	$y = 0.80x - 4.20$	0.37	ns	8
		North temp	$y = 0.01x + 2.07$	0.00	ns	22
		All data	$y = 0.12x + 1.52$	0.14	<0.05	30
TEP	Cond	Mediterranean	$y = -1.14x + 6.90$	0.41	0.08	8
		North temp	$y = -0.004x + 2.13$	0.00	ns	22
		All data	$y = 0.16x + 1.94$	0.10	ns	30
TEP	Ca + Mg	Mediterranean	$y = -0.65x + 4.29$	0.41	ns	8
		North temp	$y = 0.09x + 2.00$	0.02	ns	22
		All data	$y = 0.16x + 2.04$	0.10	ns	30
TEP	Fe	Mediterranean	$y = -0.79x + 1.53$	0.36	ns	8
		North temp	$y = -0.15x + 1.92$	0.03	ns	22
		All data	$y = -0.54x + 1.67$	0.25	<0.01	30

All variables were $\log_{10}$ transformed

$p < 0.05$) and also merging all data ($r = 0.68$; $p < 0.001$; Fig. 3b). The slopes of the different regression lines between TEP and chl *a* were not significantly different. TEP and BA were significant and positively related only in Mediterranean lakes ($r = 0.92$; $p < 0.001$; Fig. 3c). To explore the relative contribution of phytoplankton and bacteria in determining TEP concentration in the Mediterranean lakes, we calculated their corresponding partial coefficients from a multiple regression analysis with chl *a* and BA as the independent variables (Table 3). The results indicated that BA, instead of chl *a*, was most strongly related to TEP concentration in Mediterranean lakes. By contrast, no significant relationships between BA and TEP were detected for north temperate lakes or when merging all data.

TEP concentrations were significantly related to Fe concentrations and to pH merging all data (Table 2; Fig. 4a). To test if pH exerted a direct effect on TEP concentration or if the effect was mediated by phytoplankton activity, we calculated the partial coefficients between TEP and pH and chl *a* (Table 3). The results indicated that chl *a* was most strongly related to TEP concentration.

No significant effect of Fe on TEP was found in the individual lake district; although an inverse pattern emerged when all data were considered (Table 2). No significant linear correlations were found between TEP concentrations and conductivity in Mediterranean, north temperate lakes or when merging all data (Table 2). However, in the Mediterranean lakes (>1,200 µS cm^{-1}) an increase in conductivity appeared to induce a decrease in TEP concentration into the water column (Fig. 4b). Like conductivity, when divalent cations ($Ca^{2+} + Mg^{2+}$) are higher than 24.3 mg $Ca^{2+}L^{-1}$ and than 16.1 mg Mg^{2+} L^{-1} (Mediterranean lakes) an inverse trend emerged (Fig. 4c).

Table 3 Results of partial coefficients obtained in multiple regression analysis

	Dependent Var	Independent Var	Partial coefficient	p Level
Mediterranean lakes	TEP	Chl *a*	0.35	ns
		BA	0.68	<0.05
All lakes	TEP	Chl *a*	0.66	<0.05
		pH	0.13	ns

Discussion

This study demonstrates that TEP in terms of carbon has a similar magnitude to phytoplankton and bacterial biomass in Mediterranean (36%) and north temperate (33%) lakes.

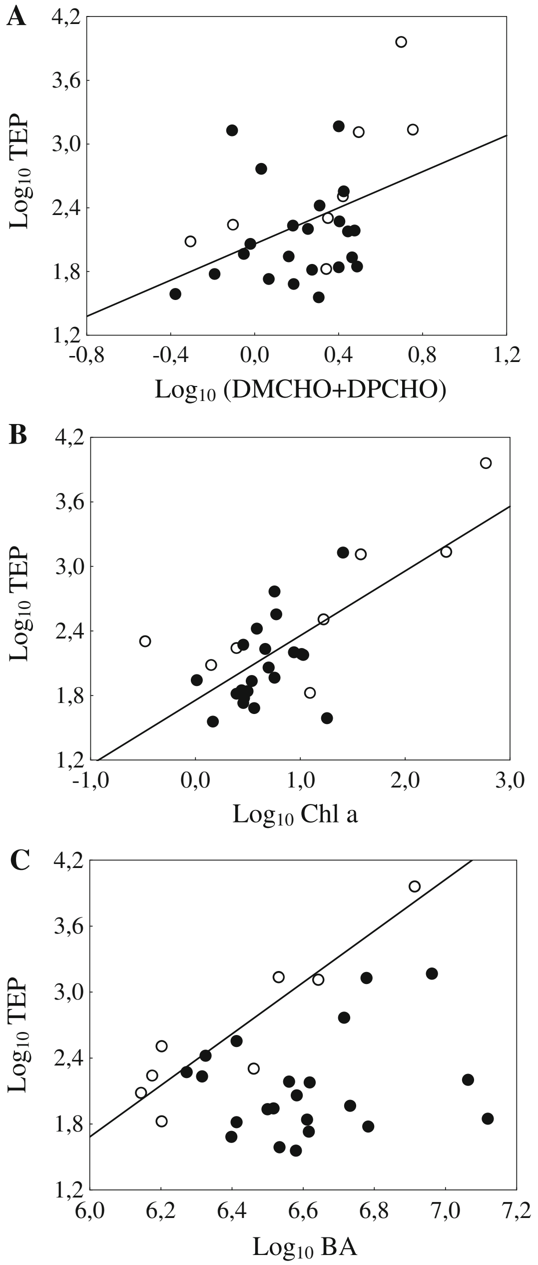

Fig. 3 Relationships between TEP concentrations and DMCHO + DPCHO, chl *a* and BA in Mediterranean (*empty circles*) and north temperate lakes (*filled circles*). *Solid regression lines* are shown where the relationship is significant considering all study lakes (**a**, **b**) and only Mediterranean lakes (**c**) ($p < 0.05$)

This significant contribution of TEP to the POM pool emphasizes its potential role in promoting C export to sediments (de Vicente et al. 2009). TEP as sticky polymers have been suggested to be important stabilizing factors for flocs (Droppo and Ongley 1994); therefore, understanding of the biotic and chemical drivers of TEP formation and stability in the water column is essential for determining the factors that regulate C storage in the sediments of inland waters.

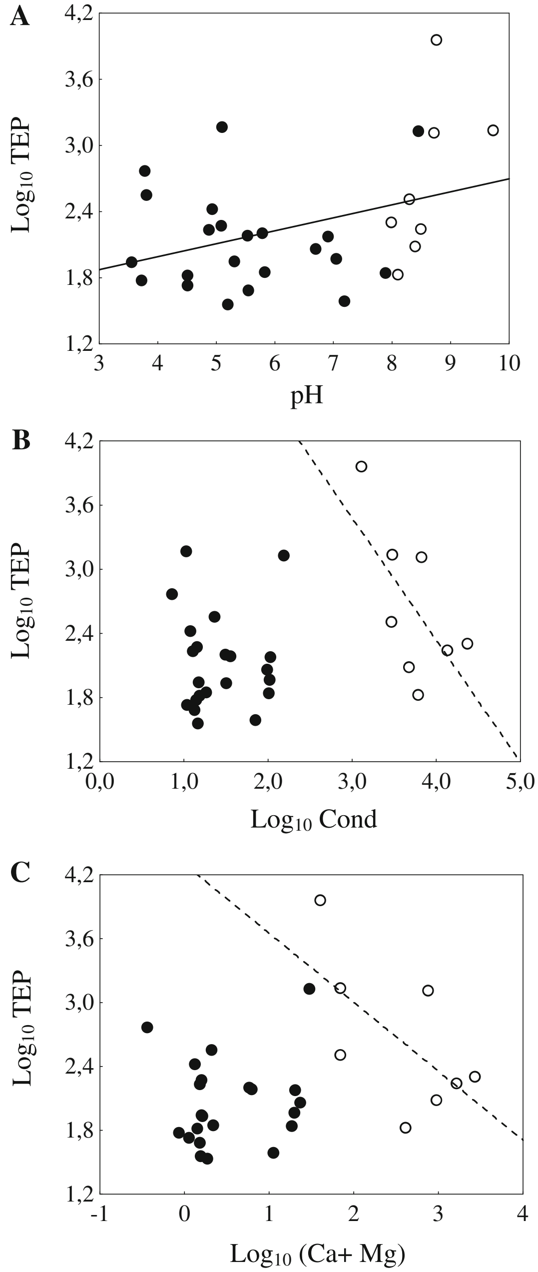

Fig. 4 Scatterplots between TEP concentrations and pH, conductivity and sum of divalent and trivalent cations in Mediterranean (*empty circles*) and north temperate lakes (*filled circles*). *Solid regression lines* are shown for significant relationships considering all study lakes (**a**) while *dashed lines* are shown for non-significant relationship in the Mediterranean lakes (**b**, **c**)

TEP concentrations in north temperate lakes were in the range of those previously reported in the literature for freshwater ecosystems (Berman and Viner-Mozzini 2001) while Mediterranean lakes had much higher TEP concentrations. Previous researchers have noted generally higher values of TEP in inland water relative to marine systems (Passow 2002a; Ortega-Retuerta et al. 2009a; Passow, personal communication). The higher concentrations in inland waters is, however, unexpected because several physico-chemical factors responsible for enhancing TEP coagulation differ between marine and inland waters and in theory marine conditions are more favourable for TEP formation. These factors include the reduction in electrostatic repulsion between marine particles of equal charge (Fletcher 1991), the increase in stickiness of marine particles (Kepkay 1994) and the role of cations, which are higher with salinity in stabilizing TEP formation (Passow 2002a). However, TEP formation is strikingly complex as biological drivers such as phytoplankton and bacteria are also involved. Further, TEP concentrations are generally much more variable among inland waters (36–9,038 μg GX eq L^{-1}, this study) than among marine waters (undetectable-509 μg GX eq L^{-1}, Passow 2002a; Passow personal communication).

The formation of TEP from acidic polysaccharides has been experimentally demonstrated in several studies (Engel 2004; Mopper et al. 1995; Passow 2000). The significant positive relationship between TEP and total dissolved carbohydrates (DMCHO + DPCHO) when merging all Mediterranean and north temperate lakes was in agreement with the role of dissolved carbohydrates as major TEP precursors. DMCHO and DPCHO concentrations measured in this study were at least one order of magnitude higher than those recorded in marine waters (Myklestad et al. 1997; Pakulski and Benner 1994; Ortega-Retuerta et al. 2009a) and about twofold higher than in Lake Biwa (Hayakawa 2004) and in Lake Constance (Hanisch et al. 1996). The contribution of total dissolved carbohydrates to the DOC was similar to that observed in inland waters by Hayakawa (2004) and Jørgensen (2009). A higher contribution of dissolved carbohydrates to the total DOC pool was found in north temperate lakes (25%) than in Mediterranean lakes (9%), likely related to the higher water residence time of Mediterranean lakes. The longer residence times of these systems allow for greater bacterial processing of this labile (carbohydrates) organic carbon (Ortega-Retuerta et al. 2007) and hence lower relative concentrations.

There is an ample evidence that both phytoplankton and bacterioplankton can release high amounts of TEP (Kiørboe and Hansen 1993; Passow and Alledregde 1994; Heissenberger et al. 1996; Passow 2002a, b). Indeed, recently Ortega-Retuerta et al. (2010) documented direct release of TEP by marine bacteria using re-growth cultures in laboratory experiments. Unlike previous observations at seasonal scale within a system (Berman and Viner-Mozzini 2001; de Vicente et al. 2009); in this extensive study, chl *a* exerted a major influence on TEP concentration. Additionally, it is striking that the slope of the regression line between TEP and chl *a* concentrations was similar when comparing Mediterranean (0.51 μg GX μg chl a^{-1}; this study) and north temperate (0.59 μg GX μg chl a^{-1}; this study) lakes. Indeed, when comparing our data including time-average values of those reported previously in the literature for inland waters (Quentar reservoir; de Vicente et al. 2009 and Lake Kinneret, Berman and Viner-Mozzini 2001), the relationship also had a similar slope (0.59 μg GX μg chl a^{-1}; Fig. 5). Furthermore, while data to make a comparison for marine waters are scarce, the slope of the regression between chl *a* and TEP was quite similar to inland waters (0.55 μg GX μg chl a^{-1}; Fig. 5).

Apart from phytoplankton, bacteria are capable of producing TEP or their precursors (Passow 2002b; Grossart et al. 2006; Radic et al. 2006; Ortega-Retuerta et al. 2010), and therefore their abundance may explain some of the variation in TEP. But at the same time, they can degrade and/or transform TEP (i.e. Radic et al. 2006). In this study, we found BA was positive and significantly related to TEP only in the Mediterranean lakes and no significant relationship was observed for north temperate lakes. Other studies have found no relationship between bacterial abundance and TEP (i.e. Passow and Alldredge 1994). Therefore, the lack of consistency in the relationships between TEP and BA across diverse inland and marine waters highlights the complexity of TEP-bacteria link. In this study, the higher partial coefficient obtained for the relationship between TEP and BA than for TEP and chl *a* in Mediterranean lakes points out the relevance of bacteria in controlling TEP formation in this particular lake district.

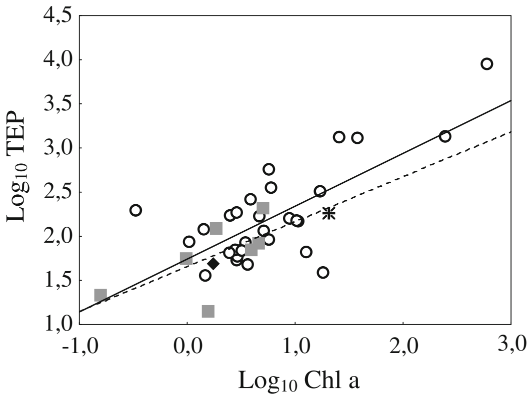

Fig. 5 Relationships between TEP concentrations and chl *a* in inland and marine waters. North temperate and Mediterranean lakes (this study) are represented by *empty circles*, Quentar reservoir by a diamond (spatiotemporal average), Lake Kinneret by an *asterisk* (spatiotemporal average) and marine waters by *grey squares* (spatiotemporal averages). *Solid regression line* is shown for a significant relationship considering all data for inland waters, while a *dashed line* is shown for nonsignificant relationship considering all data for marine waters. Data were obtained as follows: this study (Table 1), Lake Kinneret from Berman and Viner-Mozzini (2001), Quentar reservoir from de Vicente et al. (2009) and marine waters from U. Passow (personal communication), Corzo et al. (2005) and Bhaskar and Bhosle (2008)

The higher incident solar radiation in the Mediterranean in comparison to north temperate region could promote the release of TEP by bacteria as a solar protection screen, since TEP effectively absorb in the ultraviolet range (Ortega-Retuerta et al. 2009b).

TEP are a heterogeneous group of particles that also exhibit properties of gels (Passow 2002a). According to Verdugo et al. (2004), the polymer matrix results from spontaneous or induced assembly of polymer chains (Doi and Edwards 1984); which occurs when the interchain distance allows polymers to interact by chemical (covalent) or physical (i.e. electrostatics interactions, hydrogen bonding, van der Waals forces) bonds. Ultimately, assembly can also depend on pH, ionic composition and dielectric properties of the solvent (Verdugo et al. 2004). Therefore, small changes in ambient pH or in ionic strength can trigger dramatic shifts in TEP stability into the water column.

In this study, a significant positive relationship between pH and TEP concentrations appeared in the merged data (Fig. 4a). It is striking that TEP was positive and linearly related to pH, whereas the relation with conductivity and cations was much more complex. To explore the relationship of pH from biological processes, partial coefficients between TEP and pH and chl *a* were calculated revealing that chl *a* was strongest related to TEP concentration. Therefore, in this study pH control on TEP appears to be secondary as a consequence of photosynthetic activity.

Ionic strength also represents a major factor in the flocculation process. Indeed, Mulholland (1981) noted that POC formation increased dramatically with salinity; which is in agreement with the observation that an increase in salinity decreases electrostatic repulsion between particles of equal charge (Fletcher 1991). In our study, no significant linear relationship between conductivity and TEP concentrations was observed considering all the study lakes. However, interesting differences in the trends between TEP and conductivity emerged depending on the range of conductivity values. In the Mediterranean lakes, higher conductivity was associated with lower TEP concentrations.

An extensive literature exists about the crucial role of divalent cations stabilizing gels (e.g. TEP) (Mulholland 1981; Chin et al. 1998). The negative trend between TEP and divalent cations depending on the range of divalent cations (Fig. 4c) may indicate the existence of threshold values in divalent cations concentration where TEP destabilization or flocculation would occur. However, our results are only suggestive of a threshold and not conclusive. Since these relationships were not statistically significant, testing for and establishing a threshold value for TEP destabilization and/or flocculation in the water column linked to ionic environment will require further research.

Even in the absence of DOM and/or biological activity, Fe chemistry in water is complex and Fe is very insoluble at natural pH values (Maranger and Pullin 2002). However, several studies have reported a direct contribution of trivalent metals to the coagulation of dissolved organic substances by acting as aggregating agents (Mulholland 1981). In fact, von Wachenfeldt et al. (2008) observed that the formation of POM increased with the loss of total Fe from the water column, implying that Fe settled together with POM, and supporting the idea that Fe oxides co-precipitate(s) with humic substances (Tipping and Woof 1983; Liang and Morgan 1990). In this study, we also observed a negative pattern between TEP and total Fe when all data were merged (Table 2), suggesting that the presence of Fe might contribute to sedimentation of TEP from the epilimnion.

This study provides a first quantification of TEP concentration in inland waters covering a wide range of chemical and biological characteristics. Here, we noted that TEP-C was a high proportion (>30%) of the combined C in TEP, phytoplankton and bacteria. Based on the positive relationship of TEP with chl *a*, we can conclude that TEP in inland waters are primarily driven by their direct biological sources. However, their persistence in the water column may also be affected by the chemical environment, where high concentrations of divalent cations or high conductivity values could induce losses of TEP from the water column due to their polymer dispersion or flocculation.

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