

# Contribution of transparent exopolymeric particles (TEP) to estuarine particulate organic carbon pool

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**ABSTRACT:** Transparent exopolymer particles (TEP) contribute to carbon export and can represent a significant fraction of the carbon pool, most notably in estuarine systems. This study investigates, for the first time, TEP seasonal variability, vertical distribution and contribution to the carbon pool in the Lower St. Lawrence Estuary (LSLE), a highly productive subarctic estuary. TEP variability was investigated on a weekly basis at a fixed station (IML-4) from May to October 2011. TEP remained relatively low ( $15 \mu\text{g GX eq l}^{-1}$ ) during spring, but increased markedly in summer and fall, with surface concentrations reaching up to  $1548 \mu\text{g GX eq l}^{-1}$ . TEP concentrations were positively correlated with the phytoplankton biomass in surface waters. No significant relationship between TEP and other biological and physico-chemical factors was found. TEP-C content represented the second most important contributor to the particulate organic carbon pool after phytoplankton-C (41 and 54%, respectively) in the surface layer. The TEP-C contribution decreased in the cold intermediate and bottom layers over the summer and fall (ranging between 24 and 35%). However, this contribution was particularly high during the spring (>94%) in the cold intermediate and bottom layers, possibly due to the colloidal organic carbon fraction contributing to TEP-C in deep waters. Our results suggest that TEP-C combined with phytoplankton-C are major contributors to the carbon pool and could significantly contribute to the subsequent export of macro-aggregates, and probably contribute to the decrease in oxygen concentration and pH in the bottom layer of the LSLÉ by respiration/remineralisation processes.

**KEY WORDS:** Transparent exopolymeric particles · TEP · Estuary · Phytoplankton · Particulate organic carbon · Bacteria · pH · Nutrients

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## INTRODUCTION

Estuaries represent a small proportion of the total marine surface (0.3%). However, these environments show high physical and biogeochemical complexity mainly related to particulate organic matter inputs from both terrestrial and marine sources. They are very important in terms of carbon fluxes, sequestration and export, and the study of their dynamics is essential for the estimation of global carbon budgets (Cai 2011). These ecosystems were traditionally considered as a net source of CO<sub>2</sub> to the atmosphere, due to their dominant heterotrophic activity (Borges et al.

2005, Hopkinson & Smith 2005). However, it has recently been suggested that estuaries could primarily be a sink for CO<sub>2</sub>, if the total contribution of salt marshes and pelagic contributions are considered, in combination with the rapid advection of carbon in surface waters (Cai 2011). Despite the significant amount of information available, great uncertainty regarding the carbon budget of these environments still exists due to their high variability and our lack of understanding of significant aspects of their dynamics. In particular, the contribution of the transparent exopolymeric particle (TEP) fraction to the carbon pool is poorly understood and has largely been

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ignored in the construction of carbon budgets. TEP are ubiquitous gel particles present in the water column; they play a central role in sinking fluxes and food webs in aquatic ecosystems and are formed abiotically by coagulation and aggregation from dissolved and colloidal precursors (Passow 2002a, Thornton 2002, Verdugo et al. 2004). These precursors are mainly composed of acid polysaccharides (Zhou et al. 1998, Passow et al. 2001, Passow 2002a, Engel et al. 2004, Wetz & Wheeler 2007) and are primarily produced by phytoplankton and bacterioplankton exudation (Grossart et al. 1997, Passow et al. 2001, Passow 2002a,b); they constitute most of the colloidal organic carbon pool in the water column (Mari et al. 2009). Although classified as particles, TEP exhibit gel-like properties, including high flexibility, stickiness and a surface-active nature. These properties enable them to attach to each other and coagulate with other particles (i.e. bacteria, microzooplankton, phytoplankton and detritus) to promote the formation of larger organic aggregates ('marine snow') (Passow et al. 2001, Simon et al. 2002, Wetz et al. 2009, Arnous et al. 2010). Marine snow represents a major contribution to the export of carbon from the surface to the deep ocean (Wurl & Holmes 2008).

Most research on TEPs has focused on their distribution in various marine and lake environments, and few studies have dealt with their concentrations, distribution and contribution to the particulate organic carbon pool in estuarine ecosystems. Estuaries are influenced not only by diurnal variability (i.e. tides) but also by seasonal river runoff and nutrients (Sun et al. 2012). They also present strong gradients in salinity, pH, metals, cations and turbulence that alter the structure of exopolysaccharides (Mari & Burd 1998, Wetz et al. 2009). Therefore, these particles may exhibit wide temporal and spatial variations in their sticking properties within the estuarine domain, which, in turn, may affect the biogeochemical functioning of the system (Mari et al. 2012). The Estuary and Gulf of St. Lawrence form one of the largest estuarine systems on Earth (>230 000 km<sup>2</sup>). However, despite the biogeochemical and ecological importance of TEPs, no information is available about TEP concentrations and their contribution to the particulate organic carbon (POC) pool there. The Lower St. Lawrence Estuary (LSLE) is the zone with the highest primary productivity and organic matter accumulation (Le Fouest et al. 2005). The hydrography of the LSLÉ is very complex and dynamic, with a surface estuarine circulation forced by mixing of seawater with high freshwater runoff being discharged downstream through the St. Lawrence River (average flow:

12 000 m<sup>3</sup> s<sup>-1</sup>) and a deep inflow of oceanic water from the North Atlantic (Laurentian and Esquiman channels, 250 to 500 m depth) landward up the LSLÉ (Savenkoff et al. 2000, Thibodeau et al. 2006). This zone shows the maximum advective surface (0 to 30 m) water seaward transport (0.9 Sv; Savenkoff et al. 2001) of all zones of the system, and plays a significant role in the horizontal transport of particles (Zakardjian et al. 2000). The phytoplankton spring bloom is characterised by high primary production, dominated primarily by large cells, mostly diatoms (Levasseur et al. 1984, Roy et al. 1996, Savenkoff et al. 2000, Vézina et al. 2000). Diatoms are especially well known for excreting copious quantities of polysaccharides during all phases of their growth (Passow & Alldredge 1995, Mari & Kjørboe 1996, Passow 2002b); these polysaccharides can partly be advected seaward or also exported to bottom waters as TEP integrated into marine snow. This process may have contributed to the hypoxia and increasing acidification observed in bottom waters (>250 m) since the 1980s, by fueling the benthic/pelagic respiration of organic carbon (Gilbert et al. 2005, Genovesi et al. 2011, Mucci et al. 2011).

The main goal of this work is to understand the status of TEP in highly dynamic estuaries, considering the LSLÉ as a case study. In this context, our specific research objectives were: (1) to study the seasonal pattern and vertical distribution of TEP and (2) to estimate their contribution to the POC pool.

## MATERIALS AND METHODS

### Study site and sampling strategy

Sampling was carried out at the Rimouski station (IML-4; 48° 40' N, 68° 35' W; ~340 m depth; Fig. 1) in the LSLÉ. This estuary is characterized by a semi-diurnal tidal regime, with a tidal range of about 5.4 and 3.8 m for spring and neap tides, respectively (Fisheries and Ocean Canada). The station was visited weekly from May to October 2011 whenever climatic conditions were favourable, as part of the Atlantic Zone Monitoring Program (AZMP; Therriault et al. 1998). Seawater samples were collected using Niskin bottles (5 l) at 0.5, 5, 10, 15, 20, 25, 35, 50, 100, 200 and 320 m depth and stored in isothermal containers in darkness until returned to the laboratory for processing (<2 h). The parameters measured were: TEP, chlorophyll *a* (chl *a*) and POC concentrations, bacterial abundance (BA), phytoplankton composition and abundance, pH levels and nutrient concentrations. Vertical profiles of salinity,

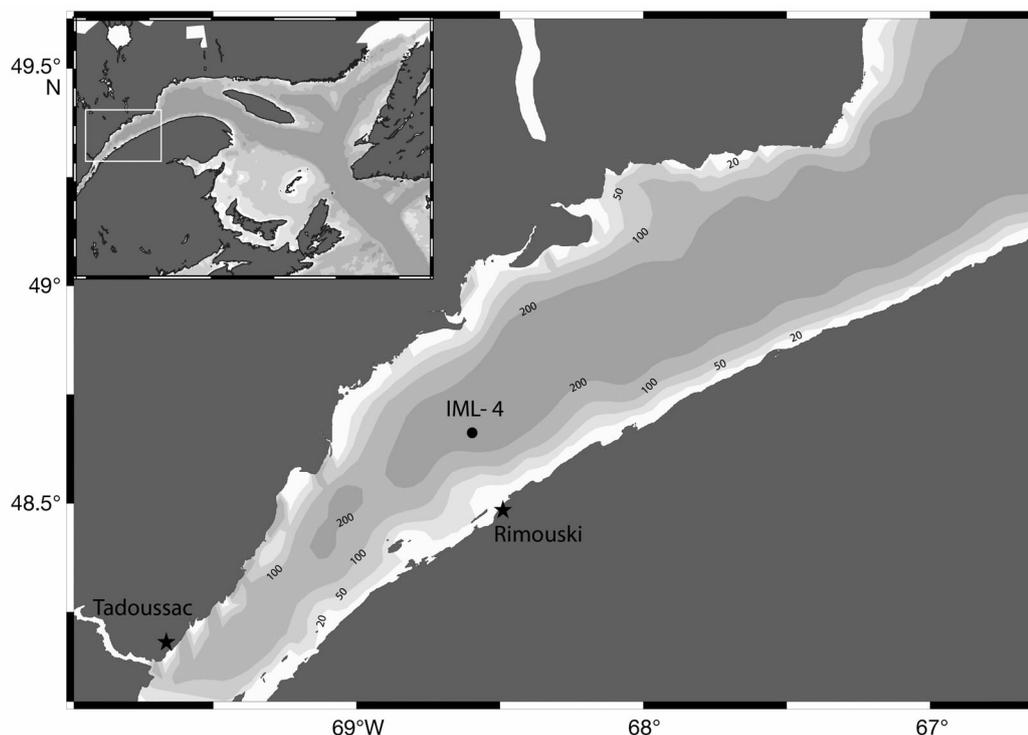


Fig. 1. Location of the Rimouski fixed sampling station (IML-4) in the Lower St. Lawrence Estuary

temperature, fluorescence and oxygen content were measured on each sampling day using a Sea-Bird 19plus CTD; a Secchi disk was used to determine light attenuation in the water column.

### Chemical and biological analyses

TEP concentration was determined colorimetrically following the procedure described by Passow & Allredge (1995). Briefly, 40 to 200 ml samples (depending on particle concentration) were filtered onto 0.2  $\mu\text{m}$  polycarbonate Isopore membrane filters (Merck Millipore) under low vacuum (<10 mbar). Particles retained on filters were stained for <5 s with 500  $\mu\text{l}$  of a 0.02% aqueous solution of Alcian Blue in 0.06% acetic acid (pH 2.5) and were subsequently rinsed with 1 ml of deionised water. Filters were then soaked in 80% sulphuric acid (6 ml) for 2 h and measured spectrometrically at 787 nm, using stained filters without sample as blanks. Alcian Blue absorption was calibrated using a solution of the polysaccharide Gum Xanthan (GX). TEP concentrations were expressed in micrograms of Gum Xanthan equivalents per liter ( $\mu\text{g GX eq l}^{-1}$ ). These values were converted into carbon equivalent units using a natural diatom population conversion factor of 0.75  $\mu\text{g C} (\mu\text{g GX eq l}^{-1})^{-1}$ , according to Engel & Passow (2001). Slopes for diatom species examined in their experiments ranged from 0.51 to 0.88  $\mu\text{g C}$

( $\mu\text{g GX eq l}^{-1}$ )<sup>-1</sup>, due to differences in phytoplankton composition; thus, the TEP-C content values estimated here may be somewhat uncertain.

Water samples for nutrient analyses were pre-filtered through Acrodisc® syringe filters with 0.8  $\mu\text{m}$  Versapor® membranes. The filtrates were stored at  $-80^{\circ}\text{C}$  in acid-cleaned polycarbonate cryogenic vials until subsequent analyses of nitrate plus nitrite ( $\text{NO}_3^- + \text{NO}_2^-$ ), nitrite ( $\text{NO}_2^-$ ), orthophosphate ( $\text{PO}_4^{3-}$ ) and silicic acid ( $\text{Si}(\text{OH})_4$ ) using a Technicon II Auto-analyzer (Mitchell et al. 2002).

Samples for pH measurements were collected on board under bubble-free and no-head-space conditions in 300 ml glass bottles and were processed following Dickson et al. (2007). pH was determined in the laboratory at the Maurice Lamontagne Institute (MLI). The pH was measured with a Perkin Elmer (Lambda 2S) spectrometer and a 10 cm quartz cell using the indicator dye *m*-cresol purple (Sigma-Aldrich) within 1 to 2 h after sampling. Absorbance was measured at 730, 578 and 434 nm before and after dye addition at  $25^{\circ}\text{C}$  (Clayton & Byrne 1993). A similar procedure was carried out before and after each set of sample measurements using a TRIS (tris(hydroxymethyl)-aminomethane) buffer prepared at a practical salinity (S) value of approximately 30 (Millero 1986). Certified Reference Material (CRM) (supplied by Professor Andrew Dickson, Scripps Institution of Oceanography, San Diego, USA) was used for quality control of our pH TRIS buffer. All

measurements were converted to the total proton scale ( $\text{pH}_T$ ) using the measured salinity of each sample and the  $\text{HSO}_4^-$  association constants given by Dickson (1990). Reproducibility and accuracy of our TRIS buffer measurements were on the order of 0.005 pH units or better. The  $\text{pH}_T$  value was then corrected for *in situ* pressure and temperature using the CO2SYS program (Lewis & Wallace 1998), with measured pH and total soluble reactive phosphate and silicate concentrations as input parameters.

Chl *a* was measured using the Parsons et al. (1984) acidification method. Briefly, duplicate samples were filtered onto 25 mm Whatman GF/F filters and extracted overnight in 10 ml of 90% acetone at 4°C in the dark. The extracts were analyzed with a TD-700 Turner Designs fluorometer. Phytoplankton chl *a* data were converted to carbon using a POC:chl *a* ratio of 40 following Levasseur et al. (1992).

Duplicate (250 ml to 1 l) seawater samples were filtered through pre-combusted (450°C, 4 h) Whatman GF/F filters and frozen with silica gel until analysis. The POC filters were then oven dried for 24 h at 50°C prior to analysis. POC concentrations were determined with a CHN elemental analyzer (COSTECH ECS 4010, Costech Analytical). Quantification was based on an external acetanilide standard with a calibration range from 0.025 to 0.080 mg and 0.170 to 0.550 mg for nitrogen and carbon, respectively, and included a blank capsule. Blank capsules and blank filters were also analyzed in every run to confirm the absence of contamination.

Samples for the identification and enumeration of phytoplankton >2  $\mu\text{m}$  were collected at 10 m depth (usually representing the depth of the chlorophyll maximum; DCM). They were preserved in acidic Lugol's solution (Parsons et al. 1984) and stored in the dark until analysis. Phytoplankton cells were identified to the lowest possible taxonomic level using an inverted microscope according to Lund et al. (1958). For each sample, at least 300 cells were counted. The main taxonomic references used to identify the phytoplankton were from Bérard-Therriault et al. (1999).

Samples for heterotrophic bacterial abundance measurements were pre-filtered (200  $\mu\text{m}$  Nitex mesh size) and fixed with glutaraldehyde (Grade I; Sigma) at a final concentration of 0.5% (Marie et al. 2005), placed in the dark at 4°C for 30 min and then frozen at -80°C until flow cytometric analysis. Samples were analyzed using an EPICS ALTRA flow cytometer (Beckman Coulter) equipped with a 488 nm laser (15 mW output) at a flow rate of 60  $\mu\text{l min}^{-1}$ .

Heterotrophic bacteria were analyzed after staining their nucleic acids with SYBR Green I (0.1% final

concentration; Invitrogen Inc.) following Belzile et al. (2008), modified from Lebaron et al. (1998). Then, 600  $\mu\text{l}$  of Tris-EDTA 10 $\times$  buffer pH 8 (Laboratoire MAT) were added to 400  $\mu\text{l}$  of sample in order to maintain an optimum pH during staining with SYBR Green I. Dilution with the buffer also avoided coincidence of several particles in the laser beam and minimized error due to low-volume pipetting. Yellow Fluoresbrite beads of 1  $\mu\text{m}$  (Polysciences) were added in all analyses as an internal standard and allowed to verify that there was no degradation of the side-scatter signal despite the relatively high flow rate used. The green fluorescence of nucleic acid-bound SYBR Green I was measured at  $525 \pm 5$  nm. BA was determined as the sum of the low and high nucleic acid (LNA and HNA) counts. The results showed that this technique was accurate enough, showing a root-mean-squared difference of 4.5% ( $n = 40$ ) between duplicates (Belzile et al. 2008). Bacterial counts were expressed in terms of carbon biomass by using a conversion factor of 14 fg C cell $^{-1}$  (Zubkov et al. 2001).

### Euphotic and mixed layer depth

The euphotic zone depth ( $Z_{\text{eu}}$ , considered as 1% of incident light at the surface), was estimated from the diffuse attenuation coefficient ( $K_d$ ).  $K_d$  was derived from Secchi disk observations, following the empiric relationship of Kirk (1983). The surface mixed layer depth ( $Z_{\text{mix}}$ ) was estimated based on the Brunt-Väisälä frequency ( $N^2$ ).

### Statistical analysis

All statistical analyses were carried out using Statistica (Version 7.0) and XLstat (Addinsoft<sup>TM</sup>) software. Interpolation of temporal distributions of the different parameters studied were represented by section scope using the software package Ocean Data View (ODV) 4.5.1 (Schlitzer 2011). The carbon contribution to each water layer from the various particulate fractions (TEP-C, phyto-C, bac-C) was expressed as a percent of POC. ANOVA was carried out to test seasonal variability and depth effects on the studied variables. In order to meet the ANOVA requirements (normality and homoscedascity), data were log transformed ( $\text{Log}_e$ ) and then pooled by season (spring = May to June; summer = July to August; fall = September to October). A Tukey honestly significant difference (HSD) post hoc test was used to compare TEP among the 3 seasons. The Akaike in-

formation criterion (AIC) (Burnham et al. 2011) was used to assess the contribution of different carbon sources (phytoplankton, bacteria and TEP, as independent variables) to the POC pool. The AIC allows for the selection of the best model from a collection of models based on its quality (i.e. minimal information loss), helping to avoid overfitting (use of excess predictor variables). We performed calculations using the log-transformed data for 3 independent variables, subsets of 2 and only 1 variable. AIC computations were performed with the generalized linear models option of the Statistica 10 package, and models were fitted using the general linear models option of the same program. Given the small sample size, a correction to the AIC results (AICc) was applied (Burnham & Anderson 2002). Finally, the AICc values were ranked considering the AICc minimum value, in order to compare the number of models (n) studied (Schloss et al. 2014):

$$\Delta_i = \text{AICc}_i - \text{AICc}_{\min} \text{ (for } i = 1, 2, 3 \dots n \text{)}$$

Statistical analyses, including partial correlation analysis, were performed to assess the potential chemical and biological drivers of TEP distribution, and linear regression analyses were performed from the results of AIC for the best carbon source predictor for POC.

## RESULTS

### Hydrographic conditions

Surface water temperature in 2011 increased from 3.3°C in May to 11.5°C in late June and remained stable until early October (Fig. 2a). The water column was characterized by a 3-layered vertical distribution of temperature, which is typical in the LSLE during the summer period, i.e. the surface layer (SL), cold intermediate layer (CIL) and bottom layer (BL). The depth of these water layers is given in Table 1 according to the criteria proposed by Gilbert & Pettigrew (1997) and Smith (2005) (CIL: temperature  $\leq 3^\circ\text{C}$ ).

Surface water salinity shows the freshwater spring runoff characteristics of the LSLE associated with a neap-tide stratified interval of  $< 25$  PSU at around 0 to 20 m depth until mid-July (Fig. 2b). Salinity increased slightly in surface waters (average: 27 PSU) until the end of the study.

The water column was strongly stratified during the 3 seasons, presenting a well-developed pycnocline between 5 and 32 m (average depth:  $\sim 13$  m)

Table 1. Seasonal depth intervals for each of the water layers identified at the IML-4 station. SL: surface layer; CIL: cold intermediate layer; BL: bottom layer

Water layer	Depth (m)		
	Spring (May–Jun)	Summer (Jul–Aug)	Fall (Sep–Oct)
SL	0–16.33	0–37.4	0–46.66
CIL	16.33–168.5	37.4–147.4	46.66–132.3
BL	168.5–320	147.4–320	132.3–320

(Fig. 2c). The  $Z_{\text{eu}}$  was very stable throughout the seasons (Fig. 2c), varying between 8 and 14 m. In turn, the  $Z_{\text{eu}}/Z_{\text{mix}}$  ratio was close or higher than 1 during almost the whole period of the study, suggesting the existence of favourable conditions for phytoplankton growth in surface waters.

Nitrate concentrations decreased after the begin of the spring bloom in surface waters from  $\sim 12$  to  $0.05 \mu\text{M}$ , in coincidence with algal biomass accumulation episodes (Fig. 2d). Nitrate concentrations increased with depth to  $15 \mu\text{M}$  at  $> 100$  m depth and reached  $25 \mu\text{M}$  below 200 m. Both phosphate and silicate concentrations in surface waters showed the same seasonal patterns as nitrate (not shown here), decreasing from  $\sim 0.5$  and  $\sim 20 \mu\text{M}$ , respectively, to below the detection limit of the method used. In the same way, the phosphate and silicate concentrations increased with depth, with 1.5 and  $20 \mu\text{M}$ , respectively, at around 100 m depth and reaching 2.5 and  $60 \mu\text{M}$ , respectively, below 200 m. Surface waters showed strong nitrogen limitation (Redfield ratio:  $0.4 < \text{N:P ratio} < 5.5$ ) in coincidence with the successive phytoplankton peaks observed during our study. Nutrient concentrations increased in surface waters after the early autumn bloom to  $\sim 13 \mu\text{M}$  for nitrate,  $\sim 1 \mu\text{M}$  for phosphate and  $\sim 17 \mu\text{M}$  for silicate, from mid-September, and remained high until the end of this study.

### Vertical and seasonal distribution of TEP, chl a, BA and POC

The seasonal distribution of chl a in the LSLE was characterized by 4 successive phytoplankton biomass accumulation events, with a major spring bloom in late June reaching an integrated value of  $\sim 547 \mu\text{g chl a m}^{-2}$  and a less pronounced early pulse around 31 May ( $\sim 174 \mu\text{g chl a m}^{-2}$ ), a summer pulse around 21 July ( $\sim 122 \mu\text{g chl a m}^{-2}$ ) and an early autumn bloom around 1 September ( $141 \mu\text{g chl a m}^{-2}$ ) (Fig. 3a).

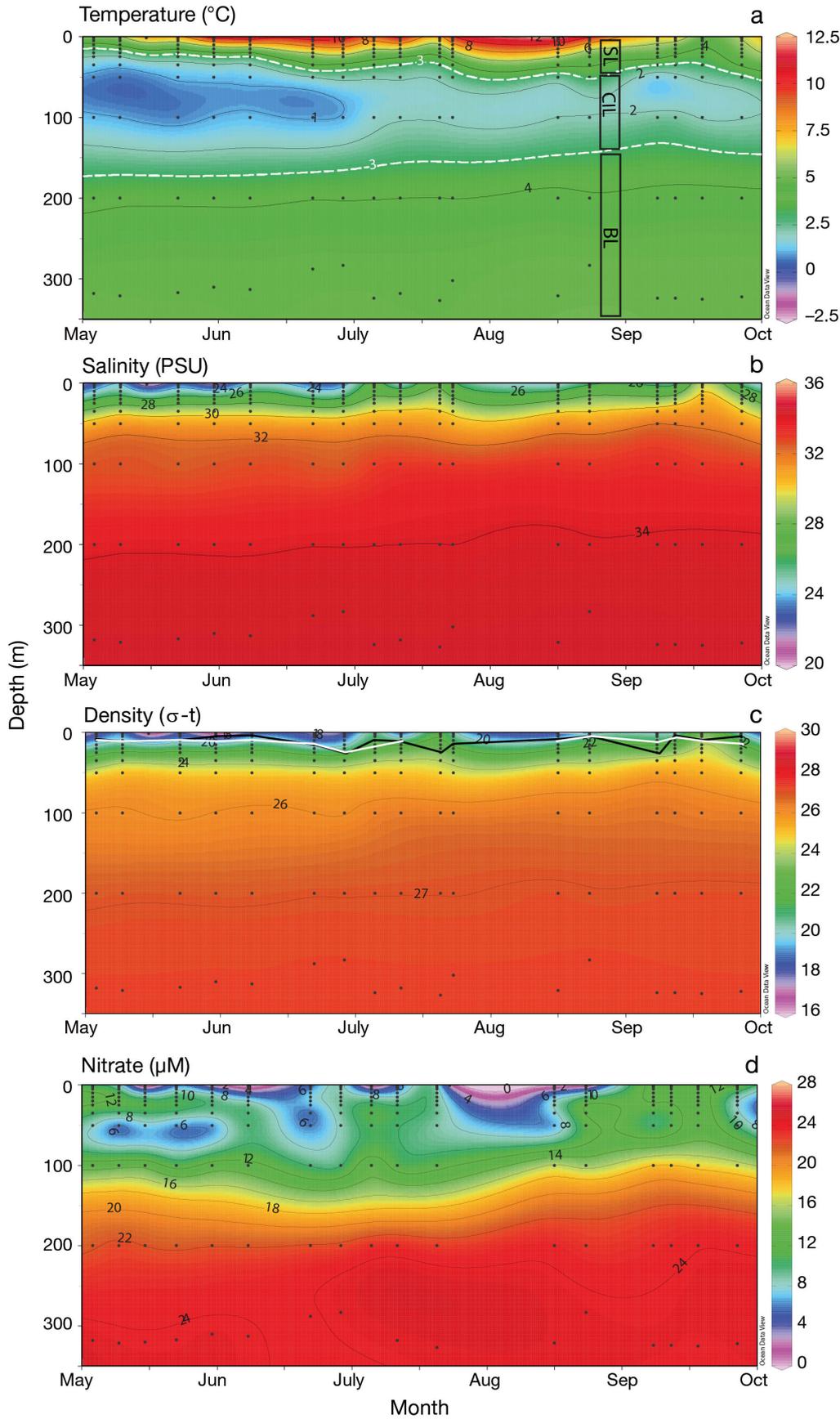


Fig. 2. Vertical and seasonal distributions of: (a) temperature (°C), (b) salinity (PSU), (c) density ( $\sigma$ -t) and (d) nitrates ( $\mu$ M) in the water column at the IML-4 station from May to October 2011. Black line in (c): pycnocline depth; white line in (c): euphotic zone depth ( $Z_{eu}$ ); white dashed lines in (a): CIL limit (temperature  $\leq 3^\circ\text{C}$ ); SL: surface layer; CIL: cold intermediate layer; BL: bottom layer

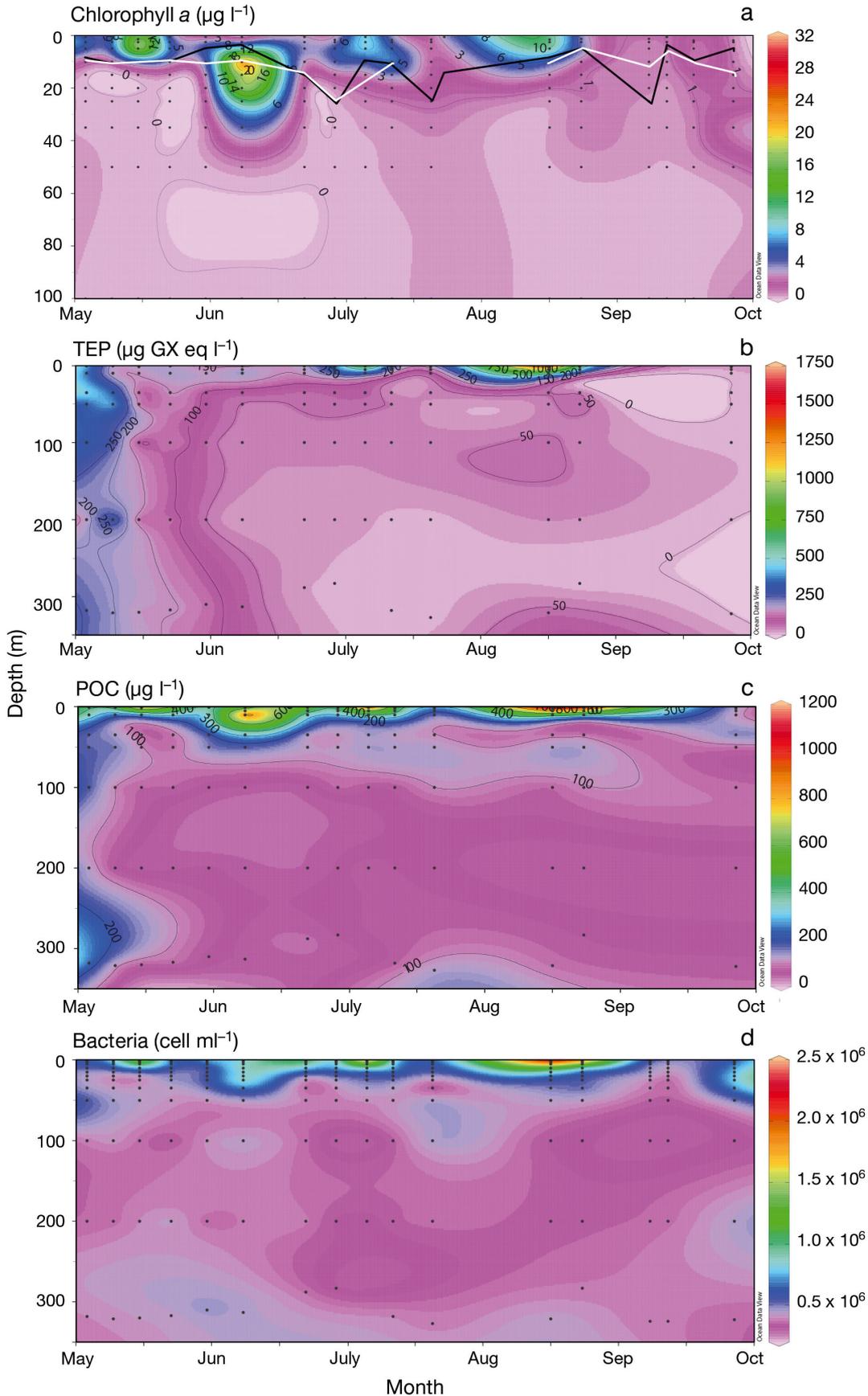


Fig. 3. Vertical and seasonal distributions of: (a) chlorophyll *a* ( $\text{chl } a$ ;  $\mu\text{g l}^{-1}$ ), (b) transparent exopolymeric particles (TEP;  $\mu\text{g GX eq l}^{-1}$ ), (c) particulate organic carbon (POC;  $\mu\text{g l}^{-1}$ ) and (d) bacteria ( $\text{cells ml}^{-1}$ ) in the water column at the IML-4 station. Black line in (a): pycnocline depth; white line in (a): euphotic zone depth ( $Z_{\text{eu}}$ )

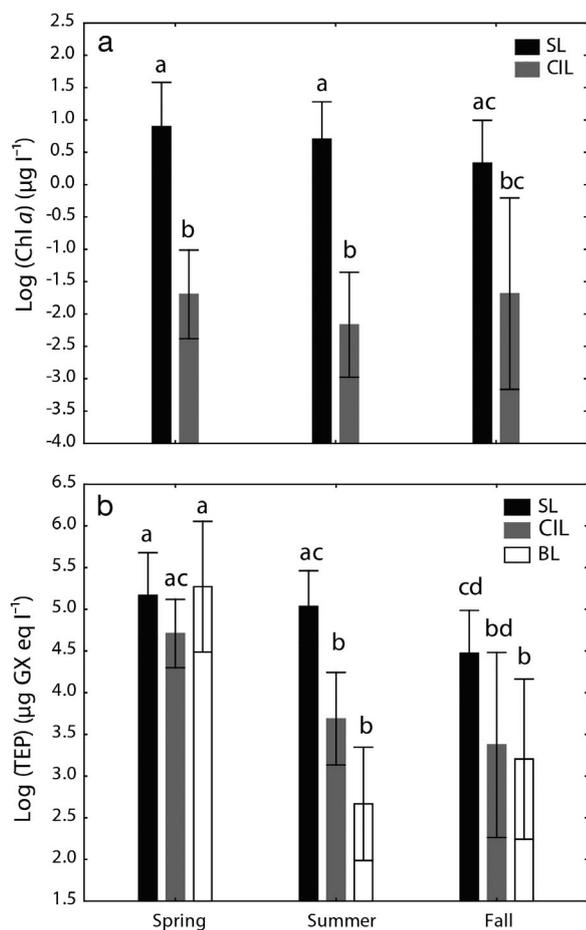


Fig. 4. Seasonal averages ( $\pm$ SE) of log-transformed data: (a) chlorophyll a concentration (chl a;  $\mu\text{g l}^{-1}$ ) and (b) transparent exopolymeric particle concentrations (TEP;  $\mu\text{g XG eq l}^{-1}$ ) corresponding to each water layer (SL: surface layer; CIL: cold intermediate layer; BL: bottom layer) identified at the IML-4 station. Different letters above each bar indicate a significant difference between seasons and layers (Tukey honestly significant difference test:  $\alpha = 0.05$ )

Chl a concentrations did not differ among seasons ( $F_{2,70} = 0.483$ ,  $p = 0.618$ ), but showed significant differences between the SL and CIL ( $F_{2,70} = 48.96$ ,  $p < 0.0001$ ) (Fig. 4a). Surface chl a concentrations were consistent with the scientific buoy IML-4 *in situ* fluorescence record for the same period (St. Lawrence Global Observatory, data not shown), displaying a significant positive correlation between both data series ( $R = 0.68$ ,  $p = 0.007$ ). Phytoplankton assemblages were mainly constituted by diatoms (18 to 96%) and autotrophic flagellates (3 to 63%) during all seasons. Centric diatoms dominated numerically (17 to 96% of the total cell number; data not shown), with *Thalassiosira nordenskiöldii* as the most important species, in particular during the spring bloom, followed in abundance by *Skeletonema costatum*, *T.*

*pacifica*, *Thalassiosira* spp. and *Chaetoceros* sp. In contrast, dinoflagellates (~5%) and cyanobacteria (~0.044%) were minor contributors to the total cell abundance of surface waters.

Overall, TEP concentrations were significantly different among seasons and water layers ( $F_{2,94} = 16.99$ ,  $p < 0.0001$  and  $F_{2,94} = 12.53$ ,  $p < 0.0001$ , respectively) (Fig. 4b). TEP concentrations were significantly higher in the SL, ranging between 15 and 1548 (average: 291)  $\mu\text{g GX eq l}^{-1}$ , corresponding to between 12 and 1161 (average: 218)  $\mu\text{g C l}^{-1}$ . Maximum TEP values were, in general, observed above the pycnocline (between 0 and 10 m depth) after the spring bloom and throughout the summer and peaked in early autumn (by 1 September), simultaneously with the phytoplankton summer pulse and autumn blooms (Fig. 3b). Significant differences between the SL and the other 2 water layers (CIL and BL) were found during summer and fall, whereas no significant difference was observed in TEP concentration between the 3 layers during spring ( $F_{4,94} = 4.005$ ,  $p = 0.004$ ) (Fig. 4b). In addition, significant interaction existed between seasons and the 3 water column layers. These results can be explained by the high TEP concentrations measured in the whole water column during early spring, ranging from 140 to 501 (average: 306)  $\mu\text{g GX eq l}^{-1}$ , corresponding to 105 and 376 (average: 230)  $\mu\text{g C l}^{-1}$ .

For the whole studied period, POC concentrations (Fig. 3c) ranged from 44 to 1052 (average: 228)  $\mu\text{g C l}^{-1}$ . Maximum POC concentrations were observed in the SL, in coincidence with different phytoplankton pulses ( $F_{2,97} = 37.57$ ,  $p < 0.0001$ ). No significant differences were detected among seasons ( $F_{2,97} = 1.23$ ,  $p = 0.294$ ). Relatively high POC concentrations were measured in the whole water column during early spring, ranging from 48 to 409 (average: 197)  $\mu\text{g C l}^{-1}$ . It should be noted that this distribution was consistent with the TEP vertical distribution in the water column during the same period.

BA ranged from  $0.27 \times 10^6$  to  $2 \times 10^6$  cell  $\text{ml}^{-1}$  (average:  $0.57 \times 10^6$  cell  $\text{ml}^{-1}$ ) during this study (Fig. 3d). As observed for chl a and POC, BA did not show significant differences among seasons ( $F_{2,170} = 1.52$ ,  $p = 0.220$ ). However, BA in the SL was significantly higher than that in deeper layers ( $F_{2,170} = 53.48$ ,  $p < 0.0001$ ). Highest BA values were observed in this layer in coincidence with successive phytoplankton peaks.

A marked decrease of pH was observed between the different layers. The ANOVA results showed significant differences between the 3 layers ( $F_{2,73} = 63.98$ ,  $p < 0.0001$ ), with pH values on a total proton scale of  $7.96 \pm 0.02$  in the SL,  $7.84 \pm 0.026$  in the CIL to  $7.58 \pm 0.004$  in the BL. These observations corre-

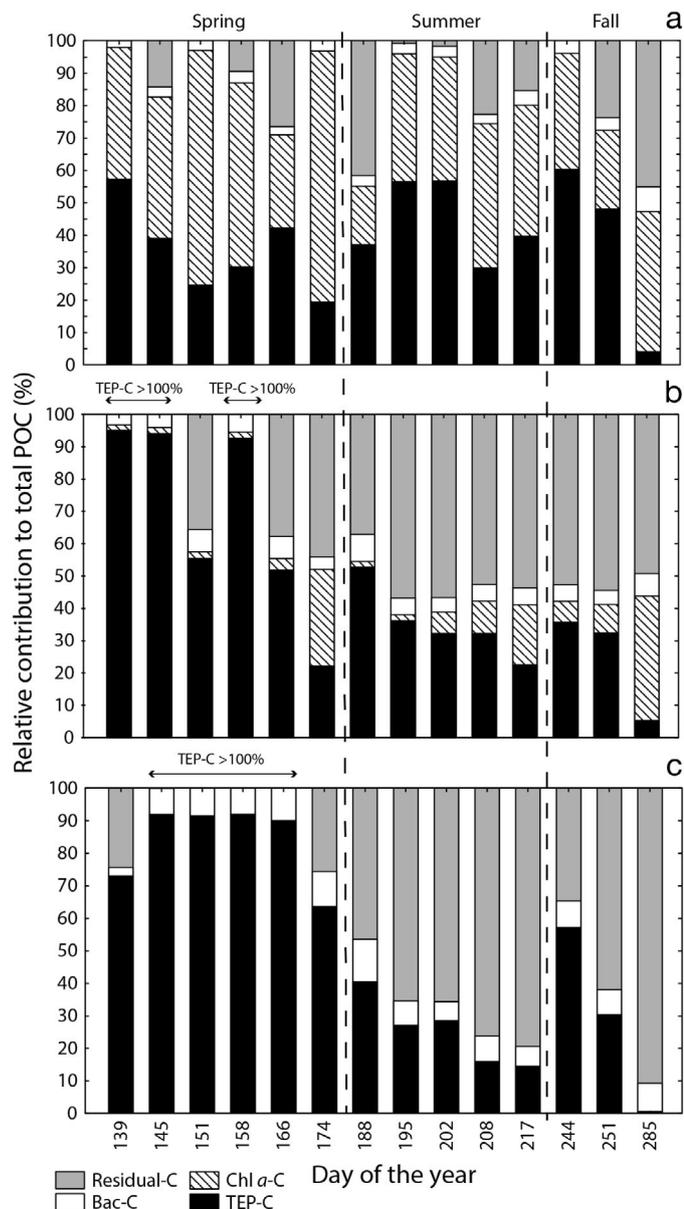


Fig. 5. Relative contributions of carbon from transparent exopolymeric particles (TEP-C), phytoplankton chlorophyll *a* (chl *a*-C), bacteria (bac-C) and other organic matter (residual-C) to total particulate organic carbon (POC) in the 3 water layers identified: (a) surface layer, (b) cold intermediate layer and (c) bottom layer

sponded to a reduction in pH of 0.12 between the SL and CIL, 0.26 between the CIL and BL and a total reduction of 0.38 from the surface to the bottom layer. No significant differences were detected among seasons ( $F_{2,73} = 2.2$ ,  $p = 0.123$ ).

The hypothesis that the main sources of TEP were phytoplankton and bacteria, and that the presence of exopolymeric particles was modulated by nutrient limitation, pH, salinity and temperature, were exam-

ined by multiple regression analysis. Partial correlation coefficients were thus calculated for the 3 layers in the water column with chl *a*, BA, nitrate, phosphate, pH, salinity and temperature as predictors and TEP as the dependent variable. The results indicate that during our study TEP concentrations were mainly related to phytoplankton biomass in the SL (partial correlation coefficient = 0.85,  $p = 0.032$ ). No other significant correlations were detected for the CIL or BL (in the case of the BL, chl *a* was not considered as an independent variable below the lower limit of the CIL, due to the absence of detectable phytoplankton biomass).

### TEP contribution to the POC pool

The relative contributions of TEP-C, chl *a*-C and bac-C to POC for each water layer studied are shown in Fig. 5. The residual portion of POC was considered as residual-C. The main contribution to POC in the SL was represented by TEP-C and chl *a*-C (41 and 54 %, on average, of the POC pool, respectively) for the whole sampling period (Fig. 5a). The standing stock of chl *a*-C decreased slightly from  $4.65 \pm 4.43$  in spring to  $3.69 \pm 1.40$  mg C m<sup>-2</sup> in fall. In contrast, TEP-C increased from  $1.95 \pm 0.99$  in spring to  $5.68 \pm 5.26$  mg C m<sup>-2</sup> in fall (Table 2). TEP-C represented the most important fraction contributing to POC in the CIL (average: 94 %; Fig. 5b) and the BL (average:

Table 2. Carbon standing stock (mg C m<sup>-2</sup>) in the 3 water layers for the 3 seasons studied (average  $\pm$  SD). Chl *a*-C: phytoplankton carbon; TEP-C: transparent exopolymeric particle carbon; bac-C: bacterial carbon; POC: particulate organic carbon

Variable	Spring (May–Jun) (n = 6)	Summer (Jul–Aug) (n = 5)	Fall (Sep–Oct) (n = 3)
<b>Surface layer</b>			
Chl <i>a</i> -C	$4.65 \pm 4.43$	$3.29 \pm 1.39$	$3.69 \pm 1.40$
TEP-C	$1.95 \pm 0.99$	$4.04 \pm 1.75$	$5.68 \pm 5.26$
Bac-C	$0.155 \pm 0.06$	$0.30 \pm 0.04$	$0.51 \pm 0.04$
POC	$5.47 \pm 2.02$	$9.09 \pm 2.43$	$11.23 \pm 4.38$
<b>Cold intermediate layer</b>			
Chl <i>a</i> -C	$1.82 \pm 3.51$	$0.60 \pm 0.50$	$0.79 \pm 0.65$
TEP-C	$18.34 \pm 13.15$	$3.34 \pm 1.77$	$1.31 \pm 0.95$
Bac-C	$0.96 \pm 0.21$	$0.53 \pm 0.24$	$0.26 \pm 0.01$
POC	$20.69 \pm 8.87$	$9.18 \pm 3.08$	$4.99 \pm 0.92$
<b>Bottom layer</b>			
TEP-C	$17.10 \pm 17.12$	$2.19 \pm 0.38$	$3.99 \pm 3.80$
Bac-C	$0.75 \pm 0.20$	$0.769 \pm 0.34$	$1.11 \pm 0.06$
POC	$11.35 \pm 6.67$	$10.51 \pm 6.11$	$13.66 \pm 0.20$

145 %; Fig. 5c) during the spring period, although its contribution decreased over the summer and fall, reaching 35 and 25 %, respectively, in the CIL and 25 and 29 %, respectively, in the BL (Fig. 5b,c). The TEP-C pool reached  $3.34 \pm 1.77$  and  $2.19 \pm 0.38$  mg C m<sup>-2</sup> in the CIL and BL, respectively, during the summer period and  $1.31 \pm 0.95$  and  $3.99 \pm 3.80$  mg C m<sup>-2</sup> in the CIL and BL, respectively, during the fall period. Our estimates of TEP-C in spring were close to or slightly greater than the POC pool in the CIL ( $18.34 \pm 13.15$  compared to  $20.69 \pm 8.87$  mg C m<sup>-2</sup>) as well as in the BL ( $17.10 \pm 17.12$  compared to  $11.35 \pm 6.67$  mg C m<sup>-2</sup>) (Table 2)

Bac-C comprised the lowest fraction contributing to POC, relative to TEP-C and chl *a*-C. On average, the contribution of bac-C to POC increased from 3 % in spring to 5 % in fall in the SL (Fig. 5a), representing a standing stock of  $0.15 \pm 0.06$  to  $0.51 \pm 0.04$  mg C m<sup>-2</sup>. Moreover, the contribution of bac-C to POC increased with depth, reaching an average of 8 % of the POC pool in all seasons in the BL (Fig. 5c), which corresponded to between  $0.75 \pm 0.20$  and  $1.11 \pm 0.06$  mg C m<sup>-2</sup> (Table 2).

Table 3. Models of particulate organic carbon (dependent variable) fitted to the main potential sources of particulate carbon (independent variables — TEP-C: transparent exopolymeric particle carbon; chl *a*-C: phytoplankton carbon; bac-C: bacterial carbon (data are log-transformed averages for each water layer). AIC: Akaike information criterion; AICc: corrected AIC; p: probability; Δ: differences between models in relation to the minimum AIC value; ns: not significant. Models are ranked from the minimum AIC (minimum loss of information) to maximum (maximum loss of information)

Independent variable	AIC	AICc	p	Δ
<b>Surface layer</b>				
(TEP + chl <i>a</i> )-C	-6.624	-5.29	<0.0001	0.00
TEP-C	-4.828	-4.43	<0.0001	0.86
(TEP + chl <i>a</i> + bac)-C	-4.722	-1.72	<0.0001	3.57
(TEP + bac)-C	-2.858	-1.52	<0.0001	3.77
(Chl <i>a</i> )-C	4.335	4.74	<0.05	10.03
(Chl <i>a</i> + bac)-C	5.708	7.04	<0.05	12.33
Bac-C	12.383	12.78	ns	18.07
<b>Cold intermediate layer</b>				
(TEP + chl <i>a</i> )-C	-7.630	-6.30	<0.001	0.00
(TEP + chl <i>a</i> + bac)-C	-5.652	-2.65	<0.001	3.64
(Chl <i>a</i> + bac)-C	-1.321	0.01	<0.05	6.31
Chl <i>a</i> -C	0.009	0.41	<0.05	6.71
Bac-C	0.973	1.37	<0.05	7.67
TEP-C	1.798	2.20	ns	8.49
(TEP + bac)-C	1.971	3.30	ns	9.60
<b>Bottom layer</b>				
Bac-C	22.181	22.58	ns	0.00
(TEP + bac)-C	22.335	22.67	ns	1.09
TEP-C	23.463	22.86	ns	1.28

We explored the relationships between POC and the potential biological sources contributing to the particulate carbon pool at the study site (chl *a*, TEP and BA converted to carbon). AIC was used to decide which variable or sum of variables could best predict POC concentrations, considering log-transformed average data for each of the independent variables for different water column layers. The fitting of POC with these variables showed that the best model included TEP-C and chl *a*-C as descriptors for both the SL and CIL (Table 3). Therefore, we performed a regression analysis of POC on the sum of TEP-C and chl *a*-C, which can explain 83 and 35 % of the POC variance in the SL ( $r = 0.91$ ,  $p < 0.0001$ ) and CIL ( $r = 0.60$ ,  $p < 0.05$ ), respectively. The unexplained fraction of the variance could be attributed to residual carbon (average: ~17 % for the SL and ~49 % for the CIL). Consequently, the sum of TEP-C and chl *a*-C can be considered a good predictor of POC concentration, particularly in the SL (Fig. 6). No significant correlations were found for the BL.

## DISCUSSION

### Hydrological conditions and phytoplankton biomass

Our results show that the hydrological conditions were appropriate for phytoplankton development during almost the whole period of study (from May to October), as suggested by the  $Z_{eu}/Z_{mix}$  ratio values. Successive blooms were observed during late spring, summer and early fall. The development of these blooms can be explained by periodic nutrient inputs during spring tides every other week (Le Fouest et al. 2005) followed by stratified intervals in the water column during neap-tides; this allows phytoplankton biomass accumulation typical of the LSLE (Levasseur et al. 1984, Savenkoff et al. 1997). During our study, the most intense bloom at the Rimouski station occurred around 23 June, an observation consistent with previous reports for the LSLE (Levasseur et al. 1984, Starr et al. 1993, Plourde & Runge 1993, Starr et al. 2004, Plourde et al. 2014).

During these blooms, low nutrient concentrations were observed in surface waters that can be attributed to biological consumption by phytoplankton, along with N limitation as suggested by the observed Redfield ratios. This nutrient limitation may promote exopolymeric exudation by phytoplankton, as reported by several authors (Obernosterer & Herndl 1995, Mari et al. 2001, Beauvais et al. 2003, Engel et al.

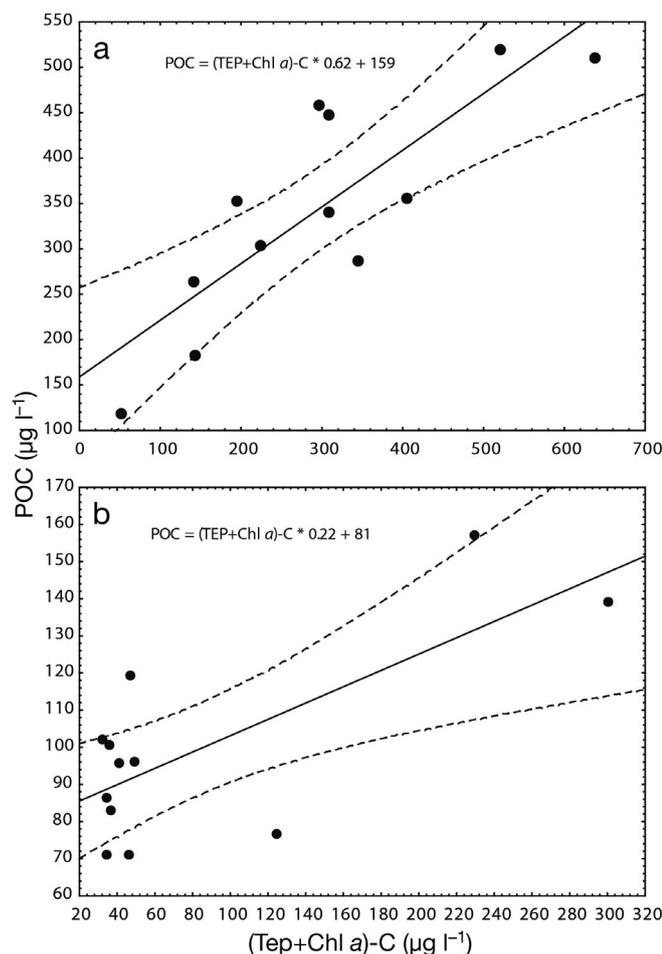


Fig. 6. Relationship between POC and the sum of TEP-C and chl a-C in (a) the surface layer and (b) cold intermediate layer. Dashed lines: 0.95 confidence level. For abbreviations see Fig. 5

2004, Underwood et al. 2004). Although chl a concentrations were higher during spring bloom than in the fall, no significant differences were found between seasons in the SL.

### Temporal variability of TEP

Our results show an increase of TEP concentrations in the SL at the end of the spring bloom, followed by significant accumulation throughout the summer and a peak in coincidence with the early fall bloom. Measured TEP concentrations in the SL during this study were within the range of average values reported for estuaries and coastal waters (Table 4), but higher than those found in some other marine and freshwater environments (Wurl & Holmes 2008, Bar-Zeev et al. 2009, de Vicente et al. 2009, Harlay et al. 2009, Ortega-Retuerta et al. 2009).

The highest TEP concentrations coincided with the phytoplankton biomass peaks in surface waters. This finding suggests that phytoplankton was the primary source of these compounds, as previously suggested (Alldredge et al. 1993, Passow & Alldredge 1995, Mari & Burd 1998, Passow et al. 2001, Passow 2002b). Phytoplankton biomass was mainly dominated by diatoms ( $R = 0.88$ ,  $p = 0.00015$ ) during the 3 seasons, with nearly 17 to 96% of centric diatoms, as reported by other studies in the same zone (Levasseur et al. 1984, Roy et al. 1996, Lovejoy et al. 2000, Romero et al. 2000, Savenkoff et al. 2000, Vézina et al. 2000, Starr et al. 2004, Plourde et al. 2014). Diatoms are known to be a major source of TEP precursors (Williams 1990, Kiørboe & Hansen 1993, Passow & Alldredge 1994, Mari & Kiørboe 1996, Aluwihare & Repeta 1999, Mari 1999, Passow 2002b). However, their ability to produce these compounds depends on the specific composition of the community (Passow & Alldredge 1994, Passow et al. 2001) and the physiological state of the cells (Passow & Alldredge 1995, Passow 2002b). *Skeletonema costatum*, the dominant species for most of the studied period (except during spring when it was sub-dominant), has been described as a very important producer of TEP (Engel 2000, Beauvais et al. 2003). The biomass accumulation of this species, together with that of other diatom taxa of the genera *Thalassiosira* and *Chaetoceros*, seems to be the main TEP source in our study.

### Vertical distribution of TEP

TEP concentrations followed the autotrophic biomass vertical distribution, with the highest values in surface waters, decreasing sharply to the depth of the pycnocline and more gradually towards the bottom. When data from late May are excluded from our statistical analysis, we found significant differences between the 3 water layers considered, with persistent maximum values in the SL. In the particular case of late May, high TEP concentrations were present in the whole water column, ranging from 105 to 376 (average: 230) µg C l<sup>-1</sup>. In turn, POC concentrations measured were in the range of 48 to 409 (average: 197) µg C l<sup>-1</sup>. Estuarine environments are strongly influenced by freshwater runoff, especially during spring in cold temperate latitudes. During such periods, high loads of particles are transferred from the main river basin to the estuary, including allochthonous TEP (i.e. originating from microbial mats and macroalgal bed exudates; Bhaskar & Bhosle 2005). Under these conditions, TEP should contribute

Table 4. Values reported for transparent exopolymer particles (TEP;  $\mu\text{g GX eq l}^{-1}$ ) and the TEP contribution to the carbon pool for estuarine, marine and freshwater environments (minimum, maximum values are shown, with averages in parentheses). nd: not determined; POC: particulate organic carbon; TOC: total organic carbon; DCM: depth of chlorophyll maximum

Location	Period	Depth (m)	TEP ( $\mu\text{g GX eq l}^{-1}$ )	Carbon	Reference
<b>Marine environments</b>					
Northern Adriatic Sea	Apr 1996	nd	nd	103 % POC	Engel & Passow (2001)
Singapore Strait	Jan–May	Surface	129–1073 (512)	nd	Wurl & Holmes (2008)
Gulf of Aqada	Apr	Profile (5–300 m)	23–228	nd	Bar-Zeev et al. (2009)
E Mediterranean Sea	Sep 2008, Jul 2009	Profile (0–1000)	48–420	63 to >100% POC	Bar-Zeev et al. (2011)
NW Mediterranean Sea	Summer	DCM	nd	22% TOC, 183% POC	Beauvais et al. (2003)
North Atlantic Ocean	May–Jun	Profile (3–120 m)	nd	1.5–68% POC	Harlay et al. (2009)
Southern Ocean	Feb	Profile (0–200 m)	0–49 (15)	18% POC	Ortega-Retuerta et al. (2009)
<b>Freshwater</b>					
Quéntar Reservoir (Spain)	Feb 2004–Mar 2006	5 and 30 m	2–335 (48)	40% POC	de Vicente et al. (2009)
Michigan, lake	Summer	Surface	36–1462 (256)	33% POC	de Vicente et al. (2010)
Mediterranean, lake	Summer	Surface	66–9038 (1572)	36% POC	de Vicente et al. (2010)
<b>Estuaries</b>					
Po River Delta–Rovinj (Italy–Croatia)	Jun 1999–Jul 2002	Profile (0–35 m)	4–14800 (570)	nd	Radić et al. (2006)
Johor Strait Estuary (Singapore)	May 1998–Jul 2000	Surface	281–8026 (2264)	0.1–23% POC	Wurl & Holmes (2008)
Dona Paula Bay	May 1998–Jul 2000	Surface	1.3–149 (57)	0.12–22.8 (6.9)% POC	Bhaskar & Bhosle (2006)
North Carolina's Neuse River estuary	May–Aug	Surface	805–3500 (2300)	16% TOC	Wetz et al. (2009)
Pearl River estuary	Jan	Profile (2–30 m)	89–1587 (828)	2–26% TOC	Sun et al. (2012)
	Aug		522–1727 (989)	5–15% TOC	
Rimouski station (IML-4)	May–Oct	Profile (0.5–320 m)	15–1548 (290)	41–145% POC	This study

to the aggregation of organic and heavy inorganic particles, allowing rapid export to deeper waters. Considering the fast sinking rates of particulate organic matter previously observed in the St. Lawrence Estuary (SLE; 10 to 100  $\text{m d}^{-1}$ ; Larouche & Boyer-Villemare 2010), we hypothesize that such dynamics can explain the relatively high POC and TEP concentrations measured in the whole water column at the beginning of this study. According to Passow et al. (2001), during a field study in the California channel over 2 yr, relative sedimentation rates of TEP at 500 m were comparable to those of POC, suggesting that loss mechanisms were similar for both variables. Actually, few studies have reported results on TEP turnover time as a result of bacterial degradation, which appears to range from hours to months, depending on the chemical composition and age of TEP (Passow 2002a). First results on the degradation processes suggest that some fractions of TEP are rapidly recycled (Aluwihare & Repeta 1999), but evidence is accumulating that other fractions are semi-resistant to microbial degradation or are transformed by bacteria into refractory compounds (Zhou et al. 1998, Ogawa et al. 2001, Passow 2002b, Rochelle-Newall et al. 2010). Consequently, the turnover of

TEP by grazing and microbial degradation needs to be explored further to evaluate the quantitative significance of these particles on carbon export to the bottom and especially on those coming from rivers.

Considering the parameters affecting TEP aggregation and distribution in estuaries, Wetz et al. (2009) and Mari et al. (2012) highlighted the importance of salinity, pH and mixing in processes of aggregation and sedimentation of these particles. Similarly, Sun et al. (2012) indicated that formation and distribution of TEP were largely influenced by the interaction between physical and biogeochemical processes in the Pearl River Estuary. However, these processes vary largely from one estuary to another. As shown above, TEP vertical variability in the SLE seems to be influenced by phytoplankton in the SL, especially by diatoms. In contrast, no significant relationships were found between TEP and the other biological and physico-chemical factors studied in the CIL and BL, which could influence TEP vertical distribution at this station.

We did not detect significant pH differences between seasons. However, a marked vertical pH decrease between water layers was measured. This observation is consistent with the results of Mucci et

al. (2011) for the LSLE. They registered a decrease of pH by 0.2 to 0.3 in bottom waters over the last 75 yr, which is 4 to 6 times greater than what can be attributed to the uptake of anthropogenic CO<sub>2</sub>. These values were related to changes in water masses entering the gulf and, to some extent, to an increase in benthic/pelagic respiration due to the warming of bottom waters (Genovesi et al. 2011, Mucci et al. 2011, Bourgault et al. 2012). pH changes have been shown to modify TEP stability by either swelling or shrinking of TEP particles, as revealed by their volume change (Li & Tanaka 1992, Chin et al. 1998, de Vicente et al. 2010). According to Mari (2008), a decrease in pH by 0.2 units led to a reduction in TEP sticking properties. Wetz et al. (2009) explored the influence of pH changes on TEP formation, and found a positive correlation between pH and TEP. They suggested that this correlation may have been due in part to the covariance of pH with salinity. Similarly, de Vicente et al. (2010) also revealed that pH control on TEP does not appear to be significant or a secondary effect as a consequence of photosynthetic activity. This is consistent with our results, which did not show a significant relationship between TEP concentrations and pH in the water column, despite the decrease in pH value with depth. More research is needed to further understand this relationship using TEP size frequency determined microscopically to explore the possible influence of pH on the size and structure of TEP, as well as chemical composition analyses to identify their origin.

It is well known that bacteria also produce TEP or their colloidal precursors (Passow 2002b, Radić et al. 2006, Ortega-Retuerta et al. 2010) and, simultaneously, can degrade and/or transform TEP (Radić et al. 2006). In the context of the LSLE, it should also be noted that Piontek et al. (2010) showed that the degradation of polysaccharides by bacterial extracellular enzymes was significantly accelerated during experimental simulation of ocean acidification at pH levels similar to those measured in bottom waters in our study area. However, our results from PCMA did not show a significant relationship between TEP and free-living bacteria in any water layer, which is consistent with some previous field studies (i.e. Passow & Alldredge 1994, Ramaiah et al. 2000, Bhaskar & Bhosle 2008, de Vicente et al. 2009). The importance of bacteria in TEP production, and notably its uptake as a suitable carbon source, is a matter of debate, as both field and laboratory observations are inconsistent, probably due to highly spatial and temporal variability in these processes (Bhaskar & Bhosle 2008) and the underestimated role of attached bacte-

ria (Bar-Zeev et al. 2011, Lapoussière et al. 2011). Therefore, we need to explore the bacterial activity and abundance of both free-living bacteria and bacteria attached to TEP particles in the water column for a better understanding of the formation and degradation of TEP in the SLE.

TEP dynamics in estuarine systems are complex, since the physical, chemical and biological mechanisms involved in their production/decomposition present a high level of spatio-temporal variability. According to the conceptual model of processes regulating TEP distribution proposed by Mari et al. (2012), there is a front of aggregation defined as an 'aggregation web' that seems to occur between salinities ranging from 10 to 15 PSU. Their study suggests that the physical and chemical reactivity of TEP along estuaries may result in a succession of recycling areas, corresponding to <10 PSU, and enhanced aggregation/sedimentation processes and export-dominated areas of >10 PSU. This process could promote the formation of dense aggregates likely to be exported to the bottom. In contrast, particles may remain suspended in the water column, leading to a system dominated by recycling in low-salinity areas of the estuary. Without information on TEP and particle fluxes along the entire St. Lawrence system, this model cannot be tested. Nevertheless, our results suggest the dominance of export from April to May, mostly related to allochthonous particulate matter inputs in the high-salinity portion of the SLE; these results are consistent with the conceptual model. Further investigations are crucially needed throughout the entire St. Lawrence Estuary, especially focused on whether these processes contribute to hypoxia and increasing acidification in the bottom waters of the SLE (Gilbert et al. 2005, Genovesi et al. 2011, Mucci et al. 2011).

#### **TEP contribution to the organic carbon pool/ carbon budget**

The present study suggests that the contribution of TEP accounts for an important portion of the carbon pool in the LSLE. Indeed, TEP-C represented the second most important fraction of carbon after phytoplankton-C—both accounting for 41 and 54%, on average, of POC, respectively, during the whole sampling period in the SL. As observed by Romero-Ibarra & Silverberg (2011) the main carbon contributor during the bloom period was phytoplankton, especially centric diatoms. The phytoplankton-C biomass in the SL was nearly constant for all seasons ( $4.65 \pm 4.43$  in

spring to  $3.69 \pm 1.40 \text{ mg C m}^{-2}$  in fall). This result contrasts with the TEP-C standing stock, which significantly increased in the same layer from  $1.95 \pm 0.99$  in spring to  $5.68 \pm 5.26 \text{ mg C m}^{-2}$  in fall (Table 2).

The TEP-C pools measured in both the CIL and BL were high during the spring, representing ~94 and 145 % of POC, respectively (Fig. 5b,c). However, TEP-C presented a more realistic contribution to POC in the CIL and BL during summer and fall, representing between 25 and 35 %. Similar results have been reported by Bar-Zeev et al. (2011), who registered a very high contribution of TEP-C (>100%) as a percentage of the POC at 1000 m depth in the eastern Mediterranean Sea. Furthermore, Beauvais et al. (2003) calculated a TEP-C of up to 183 % of the total standing stock of POC at the DCM in the northwestern Mediterranean Sea. They suggested that either TEP carbon content calculated from freshly formed TEP produced in the laboratory from diatom batch cultures and natural communities naturally overestimates the occurring TEP-C, since the relationship between TEP and carbon content is species specific (Engel & Passow 2001). Or, POC was underestimated, since the pore size used for filtration is larger than that used for TEP (0.2  $\mu\text{m}$  for TEP vs. 0.7  $\mu\text{m}$  for POC). Indeed, several authors have suggested that filters like those used in our work (0.2  $\mu\text{m}$ ) are the most efficient for collecting TEP (Passow & Alldredge 1995, Passow 2002a, Bhaskar & Bhosle 2008, Wetz et al. 2009, Sun et al. 2012). Passow & Alldredge (1995) observed that >50 % of TEP were lost when 0.6  $\mu\text{m}$  filters were used instead of 0.2  $\mu\text{m}$  filters. Sun et al. (2012), studying the Pearl River Estuary (China), found a difference of 42 % of TEP concentrations when using 0.2  $\mu\text{m}$  instead of 0.4  $\mu\text{m}$  filters. TEP are characterized as particulate material, ranging in length continuously from submicron (colloidal) scales (Wells & Goldberg 1992) to macroscopic particles (marine snow) that can reach a few centimeters in size (Alldredge & Silver 1988). Although, the colloidal fraction is very dynamic, involving a high level of aggregation (Wells 1998). As reported by Bar-Zeev et al. (2011) using microscopical visualization of TEP in deep water, TEP presented newly formed aggregates with amorphous shapes, including different particle sizes. This point highlights the importance of the colloidal organic carbon fraction below 200 m depth that can contribute to TEP-C, as also observed by Kepkay (2000) and Bar-Zeev et al. (2011). Despite these methodological limitations in deeper waters, our AIC analysis results showed that the sum of TEP-C and chl *a*-C presented the best fit, and this combination is considered to be a good predictor of POC, which suggests that overestimation of

TEP-C during this study was generally not significant (Table 3, Fig. 6). Thus, our results suggest a direct relationship between TEP-C and phytoplankton-C as the main carbon contributors to the POC pool in SL waters.

Our findings concerning TEP-C are consistent with previous work conducted in the Gulf of St. Lawrence, where it has been reported that the contribution of marine snow to the total C flux is frequently >60 % in sediment traps deployed at 150 m (Romero et al. 2000, Romero-Ibarra & Silverberg 2011). However, these studies do not include the presence of TEP. The relative contribution of TEP-C to POC decreased over the summer and fall reaching 35 and 25 %, respectively, in the CIL and 24 and 29 % in the BL, respectively. Similar contributions to the carbon pool have already been reported for other estuarine environments, with TEP contributing 6.9 % of POC (Bhaskar & Bhosle 2006), 16 % of total organic carbon (TOC) in North Carolina's Neuse River Estuary (Wetz et al. 2009) and 15 % in the Pearl River Estuary in China (Sun et al. 2012). On the other hand, de Vicente et al. (2009, 2010) found a TEP-C contribution of 33 to 40 % of POC in lake environments. Our estimations of residual-C (51 % in the CIL and 62 % in the BL) are consistent with previous results from the Gulf of St. Lawrence, where it has been found that detritus represents ~51 to 56 % of POC (Savenkoff et al. 2000, Vézina et al. 2000), mostly constituted by detrital organic matter (i.e. dead cells and zooplankton fecal pellets; Romero-Ibarra & Silverberg 2011). This represents new evidence of the importance of the TEP-C linked to marine snow previously estimated in sediment traps, highlighting the importance of TEP in relation to the organic carbon pool, not only in surface, but also in deep waters.

The bacterial-C standing stock did not change markedly among seasons and represented the lowest fraction of POC during our study. On average the contribution of bacterial-C to POC increased from ~3 % of POC in spring to ~5 % in fall in the SL. This contribution increased with depth, reaching ~8 % of POC among all seasons in the BL. These results agree with the observations of Savenkoff et al. (2000) in the Gulf of St. Lawrence, with bacterial-C constituting ~2 % of the POC in winter–spring to 4 % in summer–fall. Our results suggest that TEP-C combined with phytoplankton-C biomass could contribute significantly to the subsequent export of macro-aggregates to the bottom, and could probably contribute to the decrease in oxygen concentration and pH in the BL of the LSLE by respiration/remineralisation processes.

This study provides the first semi-quantitative estimate of the contribution of TEP-C to the POC in the LSLE ecosystem. A characterization of TEP in terms of particle size distribution is nevertheless needed for more complete knowledge of the aggregation processes in this ecosystem. Further research is also needed on TEP spatial and temporal variability, as well as its contribution to POC export and advection, integrating both the upper and lower portions of the St. Lawrence Estuary, as well as the gulf. This is essential for the estimation of the carbon budget in this complex system, as well as to understand the contribution of highly dynamic and productive marginal marine areas to the global carbon balance of the ocean.

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