Dynamics of exopolymeric carbon pools in relation with phytoplankton succession along the salinity gradient of a temperate estuary (France)

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Abstract :

In parallel to phytoplankton community dynamics, transparent exopolymeric particles (TEP) and exopolymeric substances (EPS) were investigated along the salinity gradient of a temperate estuary (Seine estuary, Normandy, France) over the course of a year. The phytoplankton community was mainly dominated by marine diatom species (especially *Skeletonema* sp., *Nitzschia* sp., and *Paralia sulcata*) associated with a spring bloom of pico-eukaryotes and the development of *Cryptophyceae* in summer. The decreases in species richness and salinity were correlated along the estuary and a significant exponential relationship between species richness and primary production was identified. Concentrations of TEP and EPS (soluble and bound carbohydrates) are highly dynamic in this estuary and can reach respectively 69 mgC L⁻¹, and 33 mgC L⁻¹. TEP distribution was mainly related to physical factors (hydrodynamics, maximum turbidity zone formation and sediment resuspension) probably produced by stressed or dying phytoplankton, while EPS appeared to be excreted during the phytoplankton spring bloom. Soluble and bound EPS appear to be related to *Skeletonema* sp. and *Cryptophyceae* occurrences. This paper presents the dynamic pattern of these carbon pools, which play an important role in the trophic network and influence the flocculation processes involved in the fate of both organic and inorganic matter.

Graphical abstract

Keywords : Picophytoplankton, Nanophytoplankton, Exopolymeric substances, Species richness

1. Introduction

Estuaries are transitional zones between two aquatic biomes: a freshwater biome in a river and a marine biome along a coast or in a bay. Transitional zones between two biomes are often characterized by pronounced changes in community composition and diversity, and, as such, can be defined as ecotones or ecoclines (Van Der Maarel 1990). An ecotone is defined as a dynamic zone where rapid environmental changes occur and is characterized by unique communities that differ from the communities inhabiting the two adjacent biomes. In contrast, an ecocline is defined as a zone with gradual changes in environmental conditions and where a continuum of assemblages is observed along the gradients. While estuaries have traditionally been defined as ecotones, since the beginning of the 21st century several studies have defined them as ecoclines (Attrill and Rundle 2002; Cortelezzi *et al.* 2007; Muylaert *et al.* 2009). The strong gradients occurring in estuaries (mainly in salinity, turbidity and nutrients) are selective forces of species and drive temporal and spatial successions of phytoplankton. These factors are known to influence both phytoplankton biomass and community structure (Trigueros and Orive 2000; Lionard *et al.* 2008; Muylaert *et al.*

2009; Vigil *et al.* 2009). These types of changes could have a strong impact on the marine system and on the stoichiometry, carbon storage and biogeochemistry (Marinov *et al.* 2010).

The carbon pool in estuaries mainly originates from phytoplanktonic and microphytobenthic primary production (Cloern *et al.* 2014) and is available in the form of cellular biomass and exopolymeric substances (EPS). Excretion of EPS by phytoplankton (Decho 1990; Alldredge *et al.* 1993; Passow and Alldredge 1994) creates a great carbon reserve and is an important component of the carbon budget. These fibrillary polymers may coagulate to form larger particles, called transparent exopolymeric substances (TEP). The abiotic formation of TEP from colloidal precursors depends on environmental conditions such as turbulence, ion density, and the concentration of inorganic colloids, as well as on the types and concentrations of the precursors present (Passow 2002). Some of these substances are degraded by microbial and bacterial respiration, resulting in the release of carbon in the form of CO₂ (Passow 2002). The other part migrates to deep layers as aggregates and, if not re-suspended, is involved in carbon sequestration (Legendre and Rivkin 2002). EPS thus play a major role in aggregation processes, particle sedimentation and carbon fluxes in aquatic ecosystems (e.g. Passow *et al.* 2001; Bhaskar & Bhosle 2005). The production of EPS enables the creation of microenvironments in which cells are protected from rapidly changing environmental conditions, toxins, grazing, and even digestion (Decho 2000). EPS can exist as particulate organic matter in aggregates on particles or dissolved in water. EPS and TEP dynamics are also greatly controlled by the microbial loop which influences enzymatic break down and the conversion of EPS into dissolved organic matter (Bhaskar and Bhosle 2005). The microbial loop forms an important alternative way for organic carbon cycle in the aquatic ecosystems. Moreover, EPS are used as an important source of carbon and energy by numerous organisms in aquatic trophic network (Bhaskar and Bhosle 2005). In estuarine systems, EPS have been shown to account for a large proportion of the colloidal organic carbon pool in the water column (Annane *et al.* 2015) and high concentrations of TEP have been found in the maximum turbidity zone (MTZ) of estuaries

where suspended particle matter (SPM) accumulates (Malpezzi et al. 2013; Morelle et al. 2017). However, although estuaries may present very high concentration of TEP, it is regularly reported that no direct correlation can be found with the phytoplankton dynamics such as the concentration of chlorophyll *a* (chl*a*) or primary productivity (Garcia 2002; Corzo et al. 2005; Klein et al. 2011; Chowdhury et al. 2016).

As in addition to primary production, estuaries are important ecosystems in terms of carbon sequestration and export (Cai 2011), a greater knowledge of EPS dynamics is an absolute prerequisite for a better understanding of carbon fluxes in these transitional zones. However, most research on EPS in estuaries to date has focused on their production by microphytobenthic communities and only a few authors have studied EPS and TEP dynamics in the estuarine water column (Wetz et al. 2009; Annane et al. 2015; Morelle et al. 2017). As the different phytoplankton groups and species do not have the same rates of polysaccharide production, chl*a* concentration, sinking rate or photosynthetic efficiency, spatial and temporal variations in phytoplankton size and community structure could explain the previously described absence of correlation between EPS and phytoplankton dynamics (Garcia 2002; Corzo et al. 2005; Klein et al. 2011; Chowdhury et al. 2016). It is thus important to consider the influence of the succession of different phytoplankton communities in relation with the TEP and EPS distribution in estuaries.

In this context, the aim of this study was to investigate the seasonal dynamics of exopolysaccharides (EPS and TEP), phytoplankton community structure and size class distribution in a temperate estuary (Seine, Normandy, France) in order to study, in addition to physical and chemical variability, how spatial and temporal changes in phytoplankton communities could trigger EPS and TEP dynamics along the salinity gradient.

2. Methods

2.1. Study site

The Seine River and its estuary drains a watershed covering 76,260 km². After Paris, the river flows northwest and drains into the English Channel (Fig. 1). Located 202 km from Paris (the kilometric scale of the Seine River is set at 0 km in the center of Paris), the weir at Poses (Fig. 1) represents the upper limit of the tidal propagation in the Seine estuary. The annual average river discharge at Poses is 436 m³.s⁻¹ with a flood period extending from December to April when the discharge reaches 1 200-2 500 m³.s⁻¹ and a low-flow period with a discharge around 250 m³.s⁻¹ (Data GIP Seine-Aval, 2008; 2011). In the oligohaline part, salinity ranges from 0.5 to 5; in the mesohaline part salinity ranges from 5 to 18; in the polyhaline part from 18 to 30; and in the euhaline part, salinity is above 30. The Seine estuary is a macrotidal estuary, with a tidal amplitude ranging from 3-7 m at Honfleur and 1-2 m at Poses (Fig. 1). The mean residence time in the estuary varies between 17-18 days for a discharge of 200 m³ s⁻¹ at Poses and between 5-7 days for a discharge of 1 000 m³ s⁻¹ (Brenon and Hir 1999; Even *et al.* 2007). The tide in the Seine estuary is characterized by flattening at high tide lasting more than 2 hours due to the deformation of the tidal wave during the propagation at shallow depths (Brenon and Hir 1999; Wang *et al.* 2002). The flow is asymmetric in favor of the flood and this trend increases as the tide propagates up the estuary. Seasonally, water temperature ranges between 25 °C in summer and 7 °C in winter with differences of less than 1 °C along the longitudinal profile and a weak vertical gradient (Data GIP Seine-Aval, 2008; 2011). The turbidity maximum zone (MTZ), containing up to 2 g L⁻¹ of SPM, is most often located between Honfleur and Tancarville, but can move upstream depending on the intensity of the tide and on river discharge. During winter flood events, the MTZ can be flushed out into the Seine Bay (Etcheber *et al.* 2007; Garnier *et al.* 2010).

2.2. Sampling strategy

Sampling was conducted monthly from January to December 2015 onboard the Ifremer ship “Delphy” at eight sampling sites distributed along the salinity gradient (Fig 1) from the euhaline

zone (site 1) to the oligohaline zone (site 8). In order to sample a steady waterbody along the estuary, sampling was performed every month in spring tide conditions (tidal coefficient 90) during daylight and during flattening of the high tide, which, in these conditions, lasts up to three hours. At each sampling site, water was sampled with a pump from the sub-surface (i.e. at a depth of 1 m) and with a Niskin bottle at the water-sediment interface (WSI) (i.e. 1 m above) for analysis of the physical-chemical (SPM, nutrients) and biological parameters (chl *a*, exopolysaccharides and community structure). Vertical salinity profiles (measured using the practical salinity unit; PSU), turbidity (measured using the nephelometric turbidity unit; NTU) and temperature (°C) were recorded at each site with a SBE 19-plusVD CTD (Seabird) from the sub-surface down to 1 m above the water-sediment interface (WSI). In these conditions, the mean sampling depth at the WSI is 13.2 ± 1.4 meters. The physical-chemical parameters are described and interpreted in a previous paper (Morelle et al. 2018) and a slight summary of their dynamics is given in this paper.

2.3. Phytoplankton biomass

Phytoplankton biomass was assessed based on chlorophyll *a* (chl*a*) concentrations. Samples (30-500 ml) were filtered in triplicate through glass-fiber filters (Whatman, GF/F, 0.7µm) to determine the total chl*a* concentration and through polycarbonate filters (Millipore, 10 µm) to determine the phytoplankton biomass of the >10 µm size fraction. The phytoplankton biomass of the <10 µm size fraction was calculated as the difference between the total and >10 µm chl*a* concentrations. Filters were immediately frozen (-20 °C) until analysis. In the laboratory, pigments were extracted in 10 mL of 90% (v/v) acetone at 4 °C for 12 h in the dark. After centrifugation at 2000 g at 4 °C for 10 minutes the concentration of chl*a* with acidification (HCl 0.1 M) was measured on extracts following the fluorometric method of Lorenzen (1966) using a Turner Trilogy fluorometer (Turner Designs, Sunnyvale, California, USA).

2.4. Phytoplankton identification and enumeration

2.4.1. Micro-phytoplankton

At sites 1, 3, 5, and 7: 250 ml of sub-surface water were sampled, preserved in Lugol iodine solution (2% f.c.) and stored at 4 °C in the dark until identification and enumeration of micro-phytoplankton species according to the Utermöhl method (Lund *et al.* 1958). Briefly, 10 ml of sample were left to settle in counting cells for 24 hours. Identification and quantification were then carried out under an inverted microscope with contrast phase optics. Identification was done to the lowest possible taxonomic level. Due to the high turbidity and suspended particles matter in the samples, the specific abundance of cells in an accurate and exhaustive way has proved to be impossible and, therefore, the enumeration of micro-phytoplankton was given only in terms of presence / absence with an estimation of the total abundance of diatoms and dinoflagellates cells.

2.4.2. Pico- and nano-phytoplankton

Water samples (1 ml) were collected in triplicate, fixed with 0.25% (f.c.) of glutaraldehyde, maintained at 4 °C for 15 minutes in the dark before being deep-frozen in liquid nitrogen (Vaulot *et al.* 1989; Olson *et al.* 1993). Back in the laboratory, samples were stored at -80 °C for less than 6 months before analysis by flow cytometry (FCM). Analyses were carried out on a Gallios flow cytometer (Beckman Coulter®) at the FCM facilities of the *structure fédérative* ICORE 146. After being quick thawed and without pre-treatment, pico- and nano-phytoplankton cells were distinguished and enumerated by FCM according to their specific auto-fluorescence and light scatter properties (Marie *et al.* 1999; Pan *et al.* 2005). The forward-angle light scatter (FSC), right-angle light scatter (SSC), and both red (FL4; $\lambda = 695$ nm) and orange (FL3; $\lambda = 620$ nm) fluorescence of each sample were recorded. Fluorescent beads (diameter 1 μ m) (Molecular Probes, Eugene, Oregon) were added as internal standard to all samples. The concentrations of beads were estimated after each

FCM session under epifluorescence microscopy to ensure reliability of this concentration, and all FCM parameters were normalized to it and to fluorescence. *Synechococcus* sp., autotrophic pico-eukaryotic cells and *Cryptophyceae* were distinguished by side-angle light scatter (SSC) versus orange fluorescence (from phycoerythrin) and red fluorescence (from chlorophyll), according to standard protocols (Marie *et al.* 1999; Pan *et al.* 2005). The pico- and nano-phytoplankton was identified and abundance was measured at the different sites from January to September 2015. Due to conservation problems, samples collected from October to December could not be rigorously exploited. The different subpopulations were distinguished based on their fluorescence and size and all cytometric sub-population of each genus have been statistically tested. To our knowledge, these are the first observations of pico- and nano-phytoplankton cells in the Seine estuary.

2.5. Exopolysaccharide analysis

2.5.1. Exopolysaccharides (EPS)

Carbohydrate contents were measured following Dubois's method (Dubois *et al.* 1956; Orvain *et al.* 2014), with glucose as the standard. Briefly, 10 to 50 ml of each sample were filtered onto Whatman GF/F glass fiber filters. The filtrates were considered as colloidal EPS (S-EPS) and stored at -20 °C. In addition to S-EPS, bound EPS (B-EPS) were extracted from the filters. For that purpose, the filters were placed in 15 ml centrifugation tubes with 12 ml of 0.2 µm filtered and sterilized artificial sea water (ASW) and ~1 g of activated cationic resin (Dowex Marathon C, Na⁺; Sigma-Aldrich). The tubes were agitated gently for 1 hour at 4 °C in the dark and then centrifuged at 3000 g at 4 °C for 10 min. The supernatants were collected and stored at -20 °C for further analysis of B-EPS. After the supernatants were thawed at room temperature, high and low molecular weight EPS was extracted by incubating 3 ml of each sample in 7 ml ethanol (70 % f.c.) at -20 °C for 16 hours. The samples were centrifuged at 3000 g at 4 °C for 30 min and the supernatants containing low molecular weight EPS were discarded. The pellet containing high molecular weight EPS was

dried at 50 °C overnight. The dried samples were re-suspended in 3 ml distilled water. To estimate carbohydrate contents, 50 µL of 5% phenol and 250 µL sulfuric acid were added to 50 µL of the extract, and vortexed. After 30 min, absorption was read with a FlexStation plate reader (Molecular Devices) at 485 nm, using glucose as standard for the calibration curve. EPS concentrations were estimated for each site and are expressed in µgGeq.L⁻¹. These concentrations were then converted into carbon using a coefficient of 0.4 corresponding to the carbon mass coefficient in one molecule of glucose.

2.5.2. Transparent exopolymeric particles (TEP)

The concentration of TEP was determined using the colorimetric method described by Claquin *et al.* (2008) and adapted from Passow and Alldredge (1995). Briefly, 15 to 50 ml samples were filtered onto 0.4 µm polycarbonate Isopore membrane filters (Millipore) and stored at -20 °C until analysis. Particles retained on the filters were stained with 5 ml of 0.02 % Alcian blue (Sigma) in 0.06% acetic acid (pH 2.5). After centrifugation at 3500 g for 30 min, the supernatant was removed and the filter was rinsed with 5 ml of MilliQ water and centrifuged several times until all excess dye was completely removed from the pellet. After one night of drying in a sterilizer at 50 °C, 6 ml of 80% H₂SO₄ were added and 2 hours later the absorbance of the supernatant was measured at 787 nm using a spectrometer. Alcian blue absorbance was calibrated using a solution of Xanthan Gum (XG). TEP concentrations are expressed in µgXGeq/L. The concentrations were then converted into carbon using a coefficient of 0.7 (Engel and Passow 2001; Claquin *et al.* 2008).

2.6. Data analysis

The plots were performed for each parameter studied by taking the spatial and temporal dynamics into account using SigmaPlot software (v.12.5). Correlations between EPS and the other parameters studied were tested by calculating Spearman's correlation coefficient using SigmaPlot

software (v.12.5). A principal component analysis (PCA) was performed using the “FactoMineR” package in R on data collected from the sub-surface and close to the WSI at all sampling sites in all the studied period.

3. Results

Hydrological parameters

The Seine River flow decreased progressively from January to July 2015 with the maximum values reaching $1240 \text{ m}^3 \text{ s}^{-1}$ in February and May. Between July and November, the flow was around $500 \text{ m}^3 \text{ s}^{-1}$, and the minimum value ($155 \text{ m}^3 \text{ s}^{-1}$) was observed at the beginning of August. An increase in flow was recorded at the end of November. The irradiance level was affected by a typical seasonal trend, with the highest value ($1.13 \times 10^8 \text{ } \mu\text{mol photons m}^{-2} \text{ d}^{-1}$) observed in June. Water temperature also followed a typical seasonal pattern over the course of the year with the highest value ($21.9 \text{ }^\circ\text{C}$) measured in sub-surface water during July and the lowest ($5.1 \text{ }^\circ\text{C}$) measured close to the WSI during February in the oligohaline zone. An inversion of the temperature gradient was observed over the course of the year in sub-surface, with temperature increasing from downstream to upstream in winter, and inversely in summer. Salinity showed a dilution gradient in sub-surface from downstream to upstream. While a similar gradient was observed close to the WSI, it was more disparate and patches of fresh or salty water were observed. The highest salinity value (29.9) was measured close to the WSI at site 2 in November. The highest concentrations of SPM were measured in winter in both sub-surface and bottom waters. In sub-surface waters, the concentrations were often homogeneous along the salinity gradient. Some patches of higher concentration were observed close to the WSI out of the MTZ. The MTZ was located at site 8 from January to February, and at site 5 from November to December, with values higher than 0.1 g L^{-1} in sub-surface. Close to the WSI, SPM concentrations were higher ($> 0.2 \text{ g L}^{-1}$) especially in the MTZ, with a maxima of 2.7 g L^{-1} observed in December at site 8. A correlation was found between SPM concentrations and turbidity

(Person correlation coefficient: 0.58, $p < 0.001$, $n=148$) throughout the salinity gradient and over the entire studied period. Turbidity presented a clear gradient in the sub-surface water with an increase in turbidity values from downstream to upstream and confirmed the position of the MTZ defined with SPM concentrations.

The concentrations of DIN and silicates were higher in sub-surface waters than in close to the WSI throughout the salinity gradient and over the entire studied period. The highest values (up to $482 \mu\text{mol L}^{-1}$ for nitrates and $165.5 \mu\text{mol L}^{-1}$ for silicates) were always observed in the MTZ especially during the flood period. The lowest concentration of DIN ($40.9 \mu\text{mol L}^{-1}$) was measured close to the WSI in August and the lowest concentrations of silicates ($17 \mu\text{mol L}^{-1}$) in May, both at site 2. In sub-surface waters, a clear pattern of dilution was observed for DIN concentrations with a decrease in [DIN] values from upstream to downstream. Close to the WSI, the concentrations of DIN were more disparate along the salinity gradient, despite a dilution being observed from upstream to downstream. The dilution pattern of the silicates was similar to the DIN pattern at both depth (Person correlation coefficient: 0.88, $p < 0.001$, $n=148$). Phosphate concentrations followed a different pattern characterized by weak differences between sub-surface and bottom waters throughout the salinity gradient. The highest values ($> 4 \mu\text{mol L}^{-1}$) were observed upstream close to the WSI at sites 7 & 8 in August, and the lowest value ($0.6 \mu\text{mol L}^{-1}$) was measured close to the WSI at site 2 in May. In March, a patch of freshwater was observed close to the WSI at site 3 (longitude 0.27), characterized by low salinity, high turbidity and high concentrations of nutrients.

3.1. Dynamics of the size fractionated phytoplankton biomass

The total chl a concentrations in the sub-surface layer ranged from 0.2 to $15.9 \mu\text{g L}^{-1}$. A decreasing trend was observed from downstream to upstream from April to October (Fig. 2). Close to the WSI, values ranged between 0.4 to $21.8 \mu\text{g L}^{-1}$ and despite the surprisingly high values

recorded in winter ($> 10 \mu\text{g.L}^{-1}$) in the oligohaline zone, the same gradient was observed as in the sub-surface layer (Fig. 3).

High concentrations of small cells ($<10 \mu\text{m}$) were recorded from June to September in the downstream part, the highest value ($9.6 \mu\text{g L}^{-1}$) being recorded at site 2 in July. Close to the WSI, high concentrations were recorded from May to September with the highest value ($5.05 \mu\text{g L}^{-1}$) recorded at site 4 in June. In winter, the highest value ($6.9 \mu\text{g L}^{-1}$) was recorded in the oligohaline zone (site 8) in December.

High concentrations of large cells ($> 10 \mu\text{m}$) were recorded from April to October, the highest value ($7.63 \mu\text{g L}^{-1}$) being recorded at the limit of the bay at site 1 in July. Close to the WSI, high concentrations were observed from March to September, with the highest value ($6.34 \mu\text{g L}^{-1}$) recorded at site 8 in July. In winter, the highest value ($16.5 \mu\text{g L}^{-1}$) was recorded in the oligohaline zone (site 8) in October.

The Surprising high values recorded in winter were attributed rather to freshwater chlorophyll detrital matter than living phytoplankton (Morelle et al. 2018).

3.2. Micro-phytoplankton assemblages

Over the course of the year, 39 distinct taxonomic units of diatoms were observed, 13 distinct taxonomic units of dinoflagellates, and six other taxonomic units of algae, representing a species richness of 58 taxonomic units identified in the Seine estuary. Most were identified at the downstream sites (1 & 3) in spring and summer (Table 1). The high turbidity and the high SPM concentrations in the samples prevented more precise exploitation of this method. The mean species richness per site was 5.56 for diatoms, 1.58 for dinoflagellates, 0.44 for eukaryotes and 7.58 for all taxonomic units taken together. The most frequent diatoms in the 48 samples were *Nitzschia* sp., observed in 32 samples; *Skeletonema* sp., observed in 25 samples; *Paralia sulcata*, observed in 23 samples; and *Rhizosolenia* sp., observed in 22 samples. The most frequent dinoflagellates were

Scripsiella sp., observed in 14 samples and *Prorocentrum* sp., observed in 11 samples (Table 1). Despite being the most frequently observed over the course of the year, the four diatoms species cited earlier were also predominant in some samples (not enumerated) with in winter a majority of *Paralia sulcata* and *Asterionelopsis glacialis*; in spring and summer, a majority of *Skeletonema* sp., *Nitzschia* sp., and *Chaetoceros* sp., and in October, a majority of *Rhizosolenia imbricata*. Some indeterminate centric diatoms were also frequently observed (in 20 samples) from May to December in the oligohaline part of the estuary. The gradient in species richness was positively correlated with the salinity gradient ($p < 0.001$) with an average of 11 different taxonomic units per month observed at site 1, nine at site 3, six at site 5, and four at site 7 (Table 1). Thus, diatoms dominated the community with a mean of 85% of diatom cells per sample.

Per sample, between 400 and 108 400 cells L^{-1} were estimated for diatoms, up to 15 400 cells L^{-1} for dinoflagellates, and up to 6 700 cells L^{-1} for the other eukaryote taxonomic units. Both the lowest and the highest values for diatoms were observed in May, at sites 1 and 7, respectively. The absence of dinoflagellates was often observed in the oligohaline part (sites 5 and 7) while high dinoflagellate abundance was observed in spring and summer in the polyhaline part with the highest value at site 5 in September. Dinoflagellates were more abundant than diatoms in spring and summer (Fig. 2).

3.3. Pico- and nano-phytoplankton communities

Two different populations of Cyanobacteria belonging to the genus *Synechococcus* were observed. These two populations, Syn_1 and Syn_2, could be distinguished from one another by their orange fluorescence, which is linked to their phycoerythrin content: population Syn_1 had higher phycoerythrin content than population Syn_2 (Fig. 4-A). While Syn_1 was observed over the entire study period, Syn_2 was only observed in April and from July to September (data not shown). These two *Synechococcus*-like populations were only observed in the downstream part of the estuary

(Fig.2 & Fig. 3) with the highest abundance (1.5×10^4 cells.L⁻¹ in the sub-surface layer and 2.5×10^4 cells.L⁻¹ close to the WSI) observed in January at site 1.

While two sub-populations of pico-eukaryotes were distinguished, the difference in SSC and FL4 was not significant and consequently only one population of pico-eukaryotes corresponding to the sum of Pic-Euk_1 and Pico-Euk_2 abundances is discussed hereafter (Fig. 4-B). Pico-eukaryote cells were present throughout the year, except in July, and all along the salinity gradient. The highest pico-eukaryote abundance (3.4×10^4 cells.L⁻¹ at both depths) was observed downstream (at sites 1 & 2) in April close to the WSI and in May in the sub-surface layer (Fig. 2 & 3). Pico-eukaryote abundances decreased from downstream to upstream over the course of the year.

Regarding nano-phytoplankton, six different populations of *Cryptophyceae* were identified. These six different populations were distinguished based on their specific fluorescence in the FLA and FL4 channels (Fig. 4-C). The sub-population crypto_3 was present in the Seine estuary throughout the year except in May. The five other sub-populations of *Cryptophyceae* were recorded sporadically along the salinity gradient in spring and summer. Abundances decreased from downstream to upstream over the course of the year. The highest values (3.0×10^4 cells.L⁻¹ in the sub-surface layer and 2.6×10^4 cells.L⁻¹ close to the WSI) were measured downstream at site 2 in June and July (Fig. 2 & 3).

3.4. EPS concentrations

As a function of the different EPS, the S-EPS in the sub-surface layer, ranged between 0 and 39.54 mgGeq L⁻¹ which represents ~73% of the total EPS pool with the highest value recorded at site 5 in June, while B-EPS values ranged between 0.24 and 14.18 mgGeq L⁻¹ representing ~27% of the total EPS pool with the highest value recorded at site 3 in June. High values were recorded from March to July with the highest values recorded in the polyhaline zone (sites 2 to 7) while low values were recorded at all the sites during the rest of the year (Fig. 2). S-EPS and B-EPS were significantly

correlated with the phytoplankton dynamics (F_V/F_M , chl *a*) (Tab. 2). Close to the WSI, S-EPS values ranged between 0 and 74.32 mgGeq L⁻¹ and represented ~ 67.75% of the total EPS pool with the highest value recorded at site 2 in May, while B-EPS values ranged between 0.23 and 17.11 mgGeq L⁻¹ and represented ~32.25% of the total EPS pool with the highest value recorded at site 4 in June. Distribution was similar with high values recorded from March to July in the downstream zone while low values were recorded at all the sites during the rest of the year (Fig. 3). The carbon pool available from EPS in the Seine estuary was between 0.1 and 5.7 mgC L⁻¹ in the sub-surface layer and between 0.1 and 33.2 mgC L⁻¹ close to the WSI.

3.5. TEP concentrations

TEP concentrations ([TEP]) showed high spatial and temporal variability at both depths (Fig. 2). In the sub-surface layer, values ranged between 2.21 and 16.48 mgXGeq L⁻¹ with a mean of 6.32 mgXGeq L⁻¹. The highest values were recorded in February at site 4 and the lowest value was recorded in May at site 1. High values were recorded from January to April and from October to December, while low values were recorded from April to October (Fig. 2). Close to the WSI, values ranged between 3.11 and 98.20 mgXGeq L⁻¹ with a mean of 15.93 mgXGeq L⁻¹ (Fig. 3). The highest value was recorded in December at site 8 and the lowest in May at site 2. Distribution was more variable with some patches. High values (> 20 mgXGeq L⁻¹) were recorded in the low salinity zone (at sites 6 & 8) from September to December and at site 8 in July and August, and low values in summer. At both depths, [TEP] were positively correlated with turbidity and SPM values and negatively correlated with biological parameters (Table 2). The available carbon pool from TEP in the Seine estuary ranged between 1.55 and 11.5 mgC L⁻¹ in the sub-surface layer and between 2.2 and 68.7 mgC L⁻¹ close to the WSI.

3.6. Relationships between biological parameters and environmental variables

Principal component analyses (PCA) were performed on the data set to explore the relationships between biological and abiotic parameters (Fig. 5). The 1st and 2nd components explained 65% of the total inertia for the sub-surface layer and 71% for the bottom layer. The first principal components (PC1; 41% of variance in sub-surface and 52% close to the WSI) formed a typical estuarine axis with parameters related to the inflow of marine waters such as salinity on the left hand side of axis 1, and parameters related to freshwater inputs, such as Si, DIN concentrations or turbidity on the right hand side of axis 1. The second principal component (PC2; 24% of variance in surface and 19% close to the WSI) was strongly influenced by factors related to seasonal changes such as irradiance and temperature. In sub-surface water, chl *a* concentrations were positively correlated with irradiance ($SCC > 0.36$; $p < 0.001$; $n = 96$), temperature ($SCC > 0.43$; $p < 0.001$, $n = 96$) and salinity ($SCC > 0.35$; $p < 0.001$; $n = 96$) while close to the WSI chl *a* concentrations were correlated principally with temperature ($SCC > 0.37$; $p < 0.001$; $n = 48$). The phytoplankton community were positively correlated with the salinity values and negatively with the nutrients concentrations (N, Si). The correlations between TEP and EPS concentrations with the other parameters are presented table 2. The PCA illustrate the high correlation between EPS and irradiance ($SCC > 0.6$; $p < 0.001$; $n = 96$).

4. Discussion

4.1. Phytoplankton taxonomic composition and spatial and temporal dynamics

The stable hydrographical features that prevailed in spring and summer (higher temperatures, higher light availability, lower flow, and lower turbidity than in winter) resulted in denser phytoplankton biomass. The high flow and resulting currents and the reduction in salinity, low light availability with high turbidity in winter drastically reduced population density and production. In this way, the annual cycle of phytoplankton in the Seine estuary is typical of temperate ecosystems.

The dynamics and the high density of phytoplankton in terms of chl *a* coincides with high species richness, high primary production (PP), and high productivity values (Morelle et al. 2018). The Seine estuary thus seems to have a high PP with high species richness in the phytoplankton community ($p < 0.001$; Fig. 6). The richness and the abundance of phytoplankton decreased from downstream to upstream, possibly due to the dominance of marine species in the estuary and the decrease in salinity during the dilution process. Indeed, in the Seine estuary, most of the spatial structure of phytoplankton abundance, composition, or production can easily be linked with the dynamics of the environmental variables, particularly salinity. This hypothesis is in accordance with the large number of phytoplankton originate from high salinity water identified during this study. The Seine estuary thus appears to be mainly inhabited by marine phytoplankton whose species richness and abundance decreases with the salinity gradient. This distribution pattern appears to be closely linked to the ecocline concept proposed by Attrill & Rundle (2002) and already reported in the Schelde estuary (Muylaert *et al.* 2009). The low frequency of freshwater species can be linked to the limits of our sampling strategy, which was applied along the salinity gradient up to the MTZ. It is widely accepted that freshwater species, which are mainly of riverine origin, cannot survive in the MTZ. Thus, a succession of phytoplankton species acclimated to low light are observed in the MTZ (Muylaert *et al.* 2000). These species are able to grow in highly turbid environments by reducing dark respiration rates, and increasing accessory pigments and chlorophyll *a* cell content to increase the efficiency of their light harvesting complex (Muylaert *et al.* 2000). Moreover, in the Seine estuary, the copepod *Eurytemora affinis* (Poppe, 1880) is widely distributed in the oligohaline part of the estuary (up to 2×10^5 ind.m⁻³) and mainly feeds on phytoplankton of river origin (Cailleaud *et al.* 2007).

Thus, if the estuary is represented by a two-ecocline model, the marine ecocline was observed, and the freshwater ecocline is located further upstream beyond the MTZ. This supports the view

expressed by Cloern & Dufford (2005) that the pelagic ecosystem of an estuary is an open system in which immigration and dispersal sustain community diversity.

The dinoflagellate assemblages were characterized by a few species: a spring peak (April-May) was dominated by *Gonyaulax* sp. and a summer peak (July to September) by *Scropsiella* sp., and *Prorocentrum* sp. . Apart from these two peaks, dinoflagellates were sparse in the water column. In the present study, diatoms formed the main component of the phytoplankton population, which could be due to their tolerance of the dynamic environmental conditions and euryhaline nature. Along the estuarine salinity gradient and over the course of the year, phytoplankton in both zones (downstream and upstream) were mainly dominated by *Skeletonema* sp., *Nitzschia* sp., and *Paralia sulcata*. These estuarine assemblages have already been described in the Pearl River estuary (Huang *et al.* 2004). Indeed, *Skeletonema* sp and *Nitzschia* sp are euryhaline and eurythermal species that can grow rapidly under eutrophic conditions (Huang *et al.* 2004). Neither was it not surprising to find *P. sulcata* as a dominant species because this diatom is widely distributed and is often found in temperate brackish to marine planktonic and benthic waters, in both littoral and sublittoral zones (McQuoid and Nordberg 2003). The high vertical mixing and resuspension in estuaries creates conditions that favor the occurrence of *P. sulcata*, especially because this species has a competitive advantage in low light conditions (Hobson and McQuoid 1997). Some other species were often observed in the downstream zone like *Dytilum* sp, *Rhizoslenia* sp, and *Chaetoceros* sp, which are known to be typical species of clear coastal or estuarine waters (Muylaert *et al.* 2000, 2009). It thus appears that the typical estuarine gradients (salinity, turbidity) play a fundamental role in the phytoplankton species community favoring the most competitive species under changes in salinity and light availability.

Because of their high surface to volume ratio, small cells grow much more efficiently than large cells in low light conditions (Kiorboe 1993) and are thus expected to be frequent in turbid systems like estuaries. In our study, no clear differences were found between the size-fractionated

measurements, although observations of micro-phytoplankton revealed the strong presence of large chain-forming diatoms. However, this study also provided observations of pico-nano phytoplankton in the Seine estuary and revealed high abundances of up to 50.10^3 cells.L⁻¹ with a mean of 13.10^3 cells.L⁻¹ over the course of the year. Such high abundances could have a strong impact on the knowledge of the trophic network and on the carbon flow in estuarine systems. Spatially, abundances of pico-nano phytoplankton decreased from downstream to upstream probably due to the decrease in salinity and in light availability, which create conditions that are not optimal for the growth of photosynthetic organisms. A clear seasonal succession was observed for pico-nano phytoplankton with dominance of *Synechococcus* in winter (January to March), then dominance of pico-eukaryotes in spring (April to June), and dominance of *Cryptophyceae* in summer (July to September). The low abundance of *Synechococcus* could be explained by the fact that this cyanobacteria develops preferentially in the upper part of well-lit euphotic zones, which is not the case in an ecosystem as dynamic and turbid as an estuary (Partensky *et al.* 1999). The dominance of pico-eukaryotes in spring is in accordance with the results of previous studies that demonstrated the halotolerant capacities of these organisms (Schapira *et al.* 2010). The dominance of *Cryptophyceae* is not surprising in an estuary given its competitive abilities. Indeed, *Cryptophyceae* thrive in all kinds of marine, brackish, freshwater habitats (Klaveness 1988) and due to an either red or blue phycobiliprotein as a light harvesting complex for photosynthesis *Cryptophyceae* can acclimatize to low intensity light (Hammer *et al.* 2002). The presence of pico- and nano-phytoplankton in the Seine estuary highlighted the significant contribution of these small cells to the biomass and hence to estuarine primary production and should thus be further explored.

4.2.TEP dynamics and distribution in relation with biological, physical and chemical processes

The seasonal and spatial distribution of TEP is in accordance with our previous results at daily scale in the Seine estuary (Morelle *et al.* 2017), which showed that the TEP were inversely correlated with phytoplankton dynamics, and closely linked to resuspension processes. In this study, the TEP concentration was higher close to the WSI (mean of 16 mgXGeq.L⁻¹) than in the sub-surface layer (mean of 6.4 mgXGeq.L⁻¹), and in winter (from October to April: mean of 20 mgXGeq.L⁻¹ close to the WSI and 8 mgXGeq.L⁻¹ in the sub-surface layer) than in summer (from May to September: mean of 4.2 mgXGeq.L⁻¹ at both depths). It is assumed that diatoms are the main source of TEP precursors and that their production ability depends on the composition of the community (Passow and Alldredge 1994; Passow 2002). However, our results differ from those obtained by Annane *et al.* (2015), who showed that *Skeletonema* sp., *Thalassiosira* sp., and *Chaetoceros* sp. seems to be the main source of TEP in the St. Lawrence estuary due to a coincidence between the highest TEP concentrations and the phytoplankton biomass peaks. Some studies have also shown that dinoflagellates as *Lepidodinium*, *Gymnodinium* or *Alexandrium* can produce large amount of TEP (Claquin *et al.* 2008; Villacorte *et al.* 2015). In the present study, these three genera were observed in the Seine estuary throughout the year (Table 1) but their occurrence was not correlated with TEP concentrations. Our results showed that the species most present at high TEP concentrations were *Paralia sulcata*, *Rhizosolenia imbricata* and *Nitzschia* sp.. However, these species were also present during periods with lower TEP concentrations, which allows us to hypothesize that the occurrence of species known to be large producers of TEP is not sufficient to explain the TEP dynamics in an ecosystem as dynamic as an estuary. Especially since the peak of TEP producers can be time-lagged with respect to the peak of TEP due to polysaccharide production processes rates or to diatom stress. This hypothesis is reinforced by the positive relationship we found between [TEP] and [Si] and the negative relationship with diatom abundance (Table 2). In this study, our results rather confirm that, in the Seine estuary, healthy phytoplankton produce less than stressed or dying phytoplankton as already demonstrated in both field and laboratory studies (Liu and

Buskey 2000; Ramaiah *et al.* 2001; Klein *et al.* 2011; Chowdhury *et al.* 2016), confirmed by the negative relationship between [TEP] and F_v/F_m (Table 2). This study also confirms the previously demonstrated strong relationship between TEP and, SPM, turbulence and mixing (Beauvais *et al.* 2006; Malpezzi *et al.* 2013; Morelle *et al.* 2017) which favor TEP production by diatoms but also disruption of aggregates in the water column.

In addition to confirming that TEP dynamics was inversely correlated with phytoplankton dynamics (chl a biomass, production and productivity (data not shown), and species abundance) and positively correlated with SPM and turbidity at a smaller spatial and seasonal scale, this study showed that the TEP dynamics cannot be related to the pico- and nano-phytoplankton populations identified in the Seine estuary. The high TEP concentrations in relation with the sediment resuspension confirm their important role played in the sedimentation processes and the high proportion of the carbon pool available in the form of TEP which, in this study, represented between 1.5 and 69 mgC.L $^{-1}$.

4.3. EPS dynamics and distribution in relation with biological, physical and chemical processes

In contrast to the distribution of TEP, EPS distribution enabled us to make additional observations compared with our previous results at daily scale in the Seine estuary (Morelle *et al.* 2017). Indeed, the daily scale used in the winter and summer season showed values lower than 10 mgGeq.L $^{-1}$ but in this study, an increase in EPS concentrations in the Seine estuary from April to July with values higher than 10 mgGeq.L $^{-1}$ (up to 51.5 mgGeq.L $^{-1}$ in the sub-surface layer and 83 mgGeq.L $^{-1}$ close to the WSI, S-EPS and B-EPS taken together) was observed. Our results revealed a significant negative relationship between [TEP] and [S-EPS] or [B-EPS] (Table 2) suggesting that different factors influence their production. The production of carbohydrates by phytoplankton is known to be highly variable and to depend on the species, growth stage and environmental

conditions (Alldredge 1999; Penna *et al.* 1999). Phosphorus limitation (Alcoverro *et al.* 2000; Staats *et al.* 2000) and, for some species, nitrogen limitation (Granum *et al.* 2002) can cause an increase in photosynthetic extracellular release. In this study, the most frequent species observed during the increase in EPS were *Skeletonema sp.*, present from February to August, and *Nitzschia sp.* present throughout the year. Therefore, *Skeletonema sp.*, which is known to produce large quantities of EPS (Urbani *et al.* 2005) could be responsible for the spring increase in EPS. Moreover, previous studies showed that a reduction in inorganic phosphorus content triggers the production of polysaccharides by different species, particularly *Skeletonema sp.* (Shniukova and Zolotareva 2015). This result is in agreement with a decrease in P concentration from April to July in the downstream zone of the Seine estuary (Morelle *et al.* 2018) reinforced by the negative relationship with P concentration and the positive relationship with diatoms observed for S-EPS or B-EPS concentrations (Table 2). However, the positive relationship between S-EPS and *Cryptophyceae* abundance observed at both depths (Table 2) also suggests a contribution of pico/nano-phytoplankton to the pools of soluble carbohydrates measured. This hypothesis should be tested by an *in vitro* study on the possible production and excretion of EPS by *Cryptophyceae*. The concentrations of EPS measured in this study also confirmed the large proportion of the carbon pool available in the form of EPS which represented up to 30 mgC.L⁻¹ from S-EPS and up to 7 mgC.L⁻¹ from B-EPS.

5. Conclusion

This is the first description of the structure of the phytoplankton community along the salinity gradient of the Seine estuary, and revealed the notable contribution of marine species, thus confirming the ecocline concept. Moreover, the pico/nano-phytoplankton analysis showed in the present study the contribution of smaller size-class (pico- and nano-phytoplankton) to the phytoplankton biomass in the system which suggests a potential contribution of this compartment to primary production. Our results confirm the importance of the TEP and EPS forms in the carbon

pool available for the trophic network, since up to 69 mgC.L⁻¹ from TEP, up to 30 mgC.L⁻¹ from S-EPS, and up to 7 mgC.L⁻¹ from B-EPS were measured. Different dynamics between carbon excreted pools (TEP, EPS) were identified: TEP distribution was mainly related to physical factors (hydrodynamics, MTZ formation and sediment resuspension) and appears to be produced by stressed or dying phytoplankton, while EPS appears to be excreted during the phytoplankton spring bloom. Soluble and bound EPS appears to be related to the occurrence of *Skeletonema sp* and *Cryptophyceae*, but the role of EPS and TEP in microbial loop and trophic transfer remained poorly known. A significant relationship between primary production and species richness was observed in this work, but further investigations are required to propose more general concepts regarding the relationship between community structure and carbon fluxes and

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Figure 1. Map of the Seine estuary (Longitude: 0.2327, latitude: 49.4326 (WGS84) - Normandy, France) showing the study area. Poses is the upper limit of tidal propagation. The sampling transect from site 1 to site 8 followed the salinity gradient from the euhaline zone (Site 1- L: 0.1116, l: 49.4335) to the oligohaline zone (site 8 - L: 0.5149, l: 49.4841). The sites were sampled monthly throughout 2015.

Figure 2. Variations in the parameters in the sub-surface layer (1 m under the surface) of the Seine estuary from January to December, 2015. With total, small cell and large cell chl *a* concentrations ($\mu\text{gchl}a.L^{-1}$); S-EPS, B-EPS (mgG.L^{-1}) and TEP concentrations (mgXGeq.L^{-1}); *Synechococcus*, *Pico-eukaryotes*, *Cryptophyceae*, diatom and dinoflagellate abundance (cells.L^{-1}). The dinoflagellate:diatom abundance ratio is also given. Values were previously smoothed using the Loess non-parametric regression method.

Figure 3. Variations in the parameters close to the water/sediment interface (1 m above the sediment) in the Seine estuary from January to December, 2015. With total, small cell and large cell chl *a* concentrations ($\mu\text{gchl}a.L^{-1}$); S-EPS, B-EPS (mgG.L^{-1}) and TEP concentrations (mgXGeq.L^{-1}); and *Synechococcus*, *Pico-eukaryotes*, and *Cryptophyceae* abundances (cells.L^{-1}). Values were previously smoothed using the Loess non-parametric regression method.

Figure 4. Cytometric signatures of the different populations observed in 2015 along the Seine estuary. A 1 μm self-fluorescent latex marble was added to each sample as a size and fluorescence reference. The graphs show the FL3 median (phycoerythrin), FL4 median (chlorophyll) and SSC median (size). With the two *Synechococcus* populations (A), the two pico-eukaryote populations that were not significantly differentiated (B) and the six *Cryptophyceae* populations (C).

Figure 5. Representation of Principal Component Analysis (PCA) using the abiotic parameters as qualitative variables (Irradiance (J cm^{-2}), flow ($\text{m}^3 \text{s}^{-1}$), temperature ($^{\circ}\text{C}$), salinity (PSU), SPM (g L^{-1}) and nutrients ($\mu\text{mol L}^{-1}$): DIN, P and Si) **and the biological parameters as quantitative variables** (total chl *a* (T.Chl *a*) and chl *a* of small and large cells (S.Chl *a* and L.Chl *a*) in $\mu\text{g L}^{-1}$, Transparent exopolymeric particles (TEP) in mgXGeq L^{-1} , Soluble and Bound exopolymeric substances (S.EPS and B.EPS) concentrations in mgEPS L^{-1} and the abundance of phytoplankton community (*Synechococcus* (Syn), *Cryptophyceae* (crypto), Pico-eukaryotes (Picoeuk), diatoms (Diat.) and dinoflagellates (Dino.) in cells L^{-1}). The sub-surface water was represent in the left panel and the water sediment interface in the right panel.

Figure 6. Primary production as a function of the species richness of the Seine estuary. All the spatial (sites 1, 3, 5 & 7) and temporal (from January to December) data were used in this plot. Species richness was calculated as a function of the microscopic observations made in this study (table 1) and expressed in $\log_{10}+1$. The primary production data ($\text{gC.m}^{-2}.\text{d}^{-1}$) are detailed in Morelle et al. (submitted). The dynamic fit was performed on SigmaPlot 12.5 after 200 iterations of fits and the final equation was $\text{PP} = e^{2.5 \times \text{SR}}$ ($p < 0.001$; $R^2=0.35$).

Table 1. Presence and absence of the diatoms, dinoflagellates and other algal group taxa observed by optic microscopy from January to December 2015 in the sub-surface layer at sites 1, 3, 5 and 7. XXX represent unidentified taxonomic units.

Month	January	February	March	April	May	June	July	August	September	October	November	December	
Sites	1 3 5 7	1 3 5 7	1 3 5 7	1 3 5 7	1 3 5 7	1 3 5 7	1 3 5 7	1 3 5 7	1 3 5 7	1 3 5 7	1 3 5 7	1 3 5 7	SUM
Diatoms													
Biddulphiales	1 - - 1	- - - 1	1 1 - -	1 - 1 1	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	8
Thalassiosiraceae sp.	- - - -	- - - -	- 1 - -	- - - -	- - - -	- - - -	- - - -	- - - -	- 1 - -	- - - -	- - - -	- - - -	2
asterionella sp.	- - - -	- - - -	1 1 - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	2
Cerataulina	- - - -	- - - -	- - - -	- - - -	- - - -	- 1 - -	1 - - -	- - - -	- - - -	- - - -	- - - -	- - - -	2
Chaetoceros	1 - 1 -	- - - -	- - - -	- - - -	1 - - -	1 1 - -	- - - -	1 1 1 -	1 - - -	1 1 1 -	1 1 - -	- - - -	14
Detonula sp	- - - -	- - - -	- - - -	- 1 - -	- 1 - -	- 1 - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	3
Ditylum sp.	- - - -	- - - -	1 - - -	1 1 - -	1 - - -	1 1 - -	- 1 1 -	1 1 - -	- - - -	1 - - -	- - - -	- - - -	11
Lauderia sp	1 - - -	- - - -	1 - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	2
Leptocylindrus sp	- - - -	- 1 - -	- - - -	- - - -	- - - -	- - - -	1 1 - -	- 1 - -	- 1 - -	- - - -	- - - -	- - - -	2
Licmophara sp.	- - - -	- - - -	- 1 - -	- - - -	- - - -	- - 1 -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	2
Naviculaceae sp.	1 - 1 -	- - 1 -	- 1 - -	- - - -	- - - -	- - - -	1 - 1 -	- - - -	1 1 - -	- - - -	1 - - -	1 - - -	10
Nitzschia sp.	1 1 1 -	- 1 1 1	- 1 1 -	1 1 1 -	1 1 1 -	1 1 1 -	1 1 1 1	1 1 1 1	1 - - -	1 1 - -	1 1 1 1	- - - -	32
Odontella sp.	- - - -	- - - -	- - - -	- 1 - -	- - - -	1 - - -	- - - -	1 - - -	- - - -	1 - - -	1 1 1 -	1 - - -	8
Coscinodiscus sp.	- - - -	- - - -	- - - -	- - 1 -	- - - -	1 - - -	- - - -	1 - - -	- - - -	- - - -	- - - -	- - - -	1
Actinopterychus sp	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- 1 - -	- - - -	- - - -	1
Plagiogramma sp.	- - - -	- - - -	- - - -	1 - - -	- - - -	1 - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	2
Pleurosigma sp	- - - -	- 1 1 -	1 - - 1	- - - -	1 1 - -	- - 1 1	- 1 1 -	1 - - -	- - - -	- - - -	1 - - -	- - - -	12
Gyrosigma sp	- - - -	- 1 1 -	1 - - 1	- - - -	1 1 - -	- - 1 1	- 1 1 -	1 - - -	- - - -	- - - -	1 - - -	- - - -	12
Pseudonitzschia sp	- - - -	- - - -	- - - -	- - - -	- - - -	1 1 - 1	- - - -	- 1 - -	- - - -	1 - - -	- - - -	1 - - -	6
Raphoneis sp.	- - - -	- - - 1	- - - -	- - 1 -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	2
Skeletonema sp.	- - - -	1 1 1 -	1 1 1 1	1 1 1 -	1 1 - -	1 1 1 1	1 1 - -	1 1 1 1	1 - - -	1 - - -	1 - - -	- - - -	25
Guinardia delicatula	- - - -	- 1 - -	- - - -	1 1 - -	1 1 - -	- 1 - -	- - - -	- 1 - -	1 - 1 -	- - - -	- 1 - -	- - - -	7
Guinardia flaccida	- - - -	- - - -	- - - -	1 - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	1
Lauderia annulata	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- 1 - -	- - - -	- - - -	- - - -	- - - -	- - - -	1
Dactylosolen fragilissima	- - - -	- - - -	- - - -	- - - -	- - - -	1 - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	1
Lithodismium undulatum	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	1 - - -	- - - -	1 1 - -	- - - -	- - - -	- - - -	3
Paralia sulcata	1 1 1 1	- 1 1 1	1 - - -	- 1 1 -	- - - -	1 1 - 1	1 - - -	- - 1 -	1 - 1 -	- 1 - -	1 1 1 -	1 1 - -	23
Rhizosolenia imbricata	- - - -	- - 1 -	1 1 - -	1 1 - -	1 1 - -	1 1 - -	1 - 1 -	- - 1 -	- - - -	1 1 1 -	1 1 1 1	- 1 - -	20
Rhizosolenia setigera	- - 1 -	- - - -	- - - -	- - - -	- - - -	1 - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	2
Thalasionema nitzschoides	- - - -	- - - -	- - - -	1 - - -	- - 1 -	1 - - -	1 - - -	- 1 - -	- 1 - -	- - - -	1 1 - -	- 1 - -	9
Thalassiosira levanderi	- - - -	- 1 - -	- - - -	- - - -	- - - -	- - - -	- - - -	- 1 - -	- - - -	- - - -	- - - -	- - - -	1
Thalassiosira gravida	- - - -	- - - -	- 1 - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	1
Thalassiosira rotula	- - - -	- - - -	- 1 - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	1
Euclampia zodiacus	- - - -	- - - -	- - - -	- 1 - -	- - - -	1 1 - -	- - - -	- - - -	1 - - -	- - - -	- 1 - -	- - - -	4
Meuniera membranacea	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	1 - - -	- - - -	- - - -	1 - - -	- - - -	1
Brockmanniella brockmannii	- - - -	- - - -	- - - -	- - - -	- - - -	1 - - -	- - - -	- - - -	- - - -	- - - -	- 1 - -	- - - -	1
Asterionellopsis glacialis	- - 1 1	- 1 1 1	- - - -	- - 1 -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	6
Bacillaria paxillifera	- - - -	1 - - 1	- - - -	- - - -	- - - -	- - - -	- - - -	- 1 - -	- - - -	- - - -	- - - -	- - - -	3
XXX	- - - -	- - - -	- - - -	- - - -	- - 1 -	- 1 1 1	- - 1 -	1 - 1 1	1 - - 1	- 1 1 1	- 1 1 1	1 1 1 1	20
SUM	6 2 5 2	1 7 7 7	10 7 5 5	8 7 6 2	8 6 4 1	11 13 5 9	8 7 6 2	9 7 4 5	9 6 2 1	7 6 3 1	11 8 5 3	5 6 1 1	5.56
Dinoflagellates													
Protoperidinium sp	- - - -	- 1 - -	- - - -	- - - -	- - - -	- 1 - -	1 - - -	- - 1 -	- - 1 -	- 1 - -	- - - -	- - - -	6
Peridinium sp	- - - -	- 1 - -	- - - -	- - - -	- - - -	- 1 - -	1 - - -	- - 1 -	- - 1 -	- 1 - -	- - - -	- - - -	6
Scropsiella sp.	- - - -	- - - -	- - - -	- - - -	1 1 - -	- 1 - -	1 1 1 -	- 1 1 1	1 1 1 -	1 - - -	1 - - -	- - - -	14
Gonyaulax sp.	- - - -	- - - -	- - - -	- 1 - -	1 1 - -	1 1 - -	- 1 - -	- 1 - -	- 1 1 -	- - - -	- - - -	- - - -	9
Alexandrium sp.	- - - -	1 - - -	- - - -	- - - -	- - - -	- - - -	1 - - -	- 1 - -	- - - -	- - - -	- - - -	- - - -	3
Prorocentrum sp	- - - -	- - - -	- - - -	1 - - -	- - - -	1 - - -	1 1 1 -	- 1 1 1	1 1 1 -	- - - -	- - - -	- - - -	11
Gymnodiniaceae	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	1 - - -	- - 1 1	1 1 - -	- - - -	- - - -	- - - -	5
Gyrodinium sp	- - - -	- - - -	1 - - -	- - - -	- - - -	- - - -	1 1 - -	- - - -	1 - - -	- - - -	- - - -	- - - -	4
Akashiwo sp	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	1 1 1 -	- - - -	- 1 1 -	- - - -	- - - -	- - - -	5
Torodinium sp	1 - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	1 - - -	1 - - -	- - - -	- - - -	- - - -	3
Dinophysis sp	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	1 - - -	- - - -	- - - -	- - - -	1
Heterocapsa triquetra	- - - -	- - - -	- - - -	- - - -	1 1 - -	- - - -	1 - - -	- - - -	- - - -	- - - -	- - - -	- - - -	3
XXX	- - - -	- - - -	- - - -	- - 1 -	- - 0 0	- - 0 0	1 - - -	- 1 1 -	- - 1 -	- - - -	- - - -	1 - - -	6
SUM	1 0 0 0	1 2 0 0	1 0 0 0	1 1 0 1	3 3 0 0	2 4 0 0	10 5 3 0	1 3 8 3	6 5 7 0	1 2 0 0	1 0 0 0	1 0 0 0	1.58
Other Algal groups													
Euglenaceae	1 - - -	1 - - -	- - - -	1 1 - -	1 1 - -	- - - -	- - - -	- - - -	- - - -	- - - -	1 1 - -	- - - -	8
Scenedesmus sp	- - - -	- - - -	- - - -	- - - -	- - 1 -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	1
ulothrix	- - - -	- - - -	- - - -	- - - -	- - - -	1 - - 1	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	2
Dictyocha sp	- - - -	- 1 - -	1 - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- 1 - -	- - - -	3
Pediastrum sp	- - - -	- - - -	- - - -	- - 1 -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	1
Ciliates	- - - -	- 1 1 -	- - - -	- - - -	- - - -	- - 1 -	- 0 0 0 0	- - - -	- - 1 -	- 1 - 1	- - - -	- - - -	6
SUM	1 0 0 0	1 2 1 0	1 0 0 0	1 1 1 0	1 1 1 0	1 0 0 2	0 0 0 0	0 0 0 0	0 0 1 0	0 1 0 1	1 1 0 0	0 1 0 0	0.44
TOTAL SUM	8 2 5 2	3 11 7 7	12 7 5 5	10 9 7 3	17 10 5 1	14 17 5 11	18 12 9 2	10 10 12 8	15 11 10 1	8 9 3 2	13 9 5 3	6 7 1 1	7.88

Table 1. Presence and absence of the diatoms, dinoflagellates and other algal group taxa observed by optic microscopy from January to December 2015 in the sub-surface layer at sites 1, 3, 5 and 7. XXX represent unidentified taxonomic units.

Month Sites	January 1 3 5 7	February 1 3 5 7	March 1 3 5 7	April 1 3 5 7	May 1 3 5 7	June 1 3 5 7	July 1 3 5 7	August 1 3 5 7	September 1 3 5 7	October 1 3 5 7	November 1 3 5 7	December 1 3 5 7	SUM
Diatoms													
<i>Biddulphiales</i>	1	1	1	1 1	1 1 1								8
<i>Thalassiosiraceae</i> sp.			1						1				2
<i>asterionella</i> sp.			1 1										2
<i>Cerataulina</i>						1	1						2
<i>Chaetoceros</i>	1	1			1	1 1		1 1 1	1	1 1 1	1 1		14
<i>Detonula</i> sp.				1	1	1							3
<i>Ditylum</i> sp.			1	1 1	1	1 1	1 1	1 1		1			11
<i>Lauderia</i> sp.	1		1										2
<i>Leptocylindrus</i> sp.		1					1 1	1	1				5
<i>Licmophora</i> sp.			1			1							2
<i>Naviculaceae</i> sp.	1	1	1	1		1	1		1 1		1	1	10
<i>Nitzschia</i> sp.	1 1 1	1 1 1	1 1	1 1 1	1 1 1	1 1 1	1 1 1 1	1 1 1 1	1	1 1	1 1 1 1		32
<i>Odontella</i> sp.				1		1		1		1	1 1 1	1	8
<i>Coscinodiscus</i> sp.								1					1
<i>Actinocyclus</i> sp.										1			1
<i>Plagiogramma</i> sp.				1		1							2
<i>Pleurosigma</i> sp.		1 1	1 1		1 1	1 1	1 1	1			1		12
<i>Gyrosigma</i> sp.		1 1	1 1		1 1	1 1	1 1	1			1		12
<i>Pseudonitzschia</i> sp.						1 1 1		1		1		1	6
<i>Raphaneis</i> sp.			1		1								2
<i>Skeletonema</i> sp.		1 1 1	1 1 1 1	1 1 1	1 1	1 1 1 1	1 1	1 1 1 1	1	1	1		25
<i>Guinardia delicatula</i>		1			1 1	1			1 1		1		7
<i>Guinardia flaccida</i>				1									1
<i>Lauderia annulata</i>							1						1
<i>Dactylosolen fragilissima</i>						1							1
<i>Lithodismium undulatum</i>							1		1 1				3
<i>Paralia sulcata</i>	1 1 1 1	1 1 1	1	1 1		1 1 1	1	1	1 1	1	1 1 1	1 1	23
<i>Rhizosolenia imbricata</i>		1	1 1	1 1	1 1	1 1	1 1	1		1 1 1	1 1 1 1	1	20
<i>Rhizosolenia setigera</i>	1					1							2
<i>Thalasionema nitzschoides</i>				1	1	1	1	1	1		1 1	1	9
<i>Thalassiosira levanderi</i>		1											1
<i>Thalassiosira gravida</i>				1									1
<i>Thalassiosira rotula</i>				1									1
<i>Eucampia zodiacus</i>						1 1			1			1	4
<i>Meuniera membranacea</i>											1		1
<i>Brockmanniella brockmannii</i>												1	1
<i>Asterionellopsis glacialis</i>		1 1	1 1 1		1								6
<i>Bacillaria paxillifera</i>			1 1						1				3
XXX					1	1 1 1	1	1 1 1 1	1 1 1	1 1 1 1	1 1 1 1	1 1 1 1	20
SUM	6 2 5 2	1 7 7 7	10 7 5 5	8 7 6 2	8 6 4 1	11 13 5 9	8 7 6 2	9 7 4 5	9 6 2 1	7 6 3 1	11 8 5 3	5 6 1 1	5.56
Dinoflagellates													
<i>Protoperidinium</i> sp.		1				1	1	1	1	1			6
<i>Peridinium</i> sp.		1				1	1	1	1	1			6
<i>Scripsiella</i> sp.					1 1	1	1 1 1	1 1 1	1 1 1	1	1		14
<i>Gonyaulax</i> sp.				1	1 1	1 1	1	1	1 1				9
<i>Alexandrium</i> sp.		1					1	1					3
<i>Prorocentrum</i> sp.				1		1	1 1 1	1 1 1	1 1 1				11
<i>Gymnodiniaceae</i>							1	1 1	1 1				5
<i>Gyrodinium</i> sp.			1				1 1		1				4
<i>Akashiwo</i> sp.							1 1 1		1 1				5
<i>Torodinium</i> sp.	1							1	1				3
<i>Dinophysis</i> sp.					1 1		1		1				1
<i>Heterocapsa triquetra</i>					1							1	3
XXX				1			1	1 1	1				6
SUM	1 0 0 0	1 2 0 0	1 0 0 0	1 1 0 1	3 3 0 0	2 4 0 0	10 5 3 0	1 3 8 3	6 5 7 0	1 2 0 0	1 0 0 0	1 0 0 0	1.58
Other algal groups													
<i>Euglenaceae</i>	1	1		1 1	1 1						1 1		8
<i>Scenedesmus</i> sp.					1								1
<i>ulothrix</i>						1	1						2
<i>Dictyocha</i> sp.		1	1									1	3
<i>Pediastrum</i> sp.				1									1
<i>Ciliates</i>		1 1				1			1	1 1			6
SUM	1 0 0 0	1 2 1 0	1 0 0 0	1 1 1 0	1 1 1 0	1 0 0 2	0 0 0 0	0 0 0 0	0 0 1 0	0 1 0 1	1 1 0 0	0 1 0 0	0.44
TOTAL SUM	8 2 5 2	3 11 8 7	12 7 5 5	10 9 7 3	12 10 5 1	14 17 5 11	18 12 9 2	10 10 12 8	15 11 10 1	8 9 3 2	13 9 5 3	6 7 1 1	7.58

Table 2. Spearman correlation coefficients obtained between the exopolysaccharides (TEP, S-EPS and B-EPS) and the biological, physical and chemical parameters (n=96 in the sub-surface layer, and n=48 close to the WSI), the pico/nano abundances (*Synechococcus*, Pico-eukaryotes and Cryptophyceae; n=72 in the sub-surface layer and n=48 close to the WSI) and the diatom and dinoflagellate abundances (n=48 in the sub-surface layer only). The coefficients were considered significant when the p-value was < 0.05, if not, it was noted “NS”).

Parameters	Sub-surface layer			Water sediment interface		
	TEP	S-EPS	B-EPS	TEP	S-EPS	B-EPS
Temperature	-0.69	0.46	0.24	-0.35	0.35	NS
Salinity	-0.22	NS	NS	-0.46	NS	NS
Irradiance	-0.60	0.52	0.62	-	-	-
Turbidity	0.81	-0.46	-0.35	0.67	NS	NS
SPM	0.50	-0.51	-0.56	0.77	-0.58	-0.56
DIN	0.36	-0.20	NS	0.54	NS	NS
P	NS	NS	-0.21	0.61	-0.32	-0.35
Si	0.42	-0.38	-0.19	0.54	NS	NS
F _v /F _M	NS	NS	NS	-0.53	NS	NS
Large Chl <i>a</i>	-0.36	0.29	0.23	0.33	NS	NS
Small Chl <i>a</i>	-0.50	0.29	0.25	NS	NS	NS
Total Chl <i>a</i>	-0.52	0.34	0.28	NS	NS	NS
TEP	-	-0.57	-0.40	-	-0.60	-0.54
S-EPS	-0.57	-	0.81	-0.60	-	0.93
B-EPS	-0.40	0.81	-	-0.54	0.93	-
<i>Synechococcus</i> Ab.	NS	NS	NS	NS	NS	NS
Pico-eukaryote Ab.	-0.42	NS	NS	NS	NS	NS
Cryptophyceae Ab.	NS	0.23	NS	-0.43	0.33	NS
Diatom Ab.	-0.31	0.28	0.28	-	-	-
Dinoflagellate Ab.	-0.36	0.28	NS	-	-	-

Figure 1. Map of the Seine estuary (Longitude: 0.2327, latitude: 49.4326 (WGS84) - Normandy, France) showing the study area. Poses is the upper limit of tidal propagation. The sampling transect from site 1 to site 8 followed the salinity gradient from the euhaline zone (Site 1- L: 0.1116, l: 49.4335) to the oligohaline zone (site 8 - L: 0.5149, l: 49.4841). The sites were sampled monthly throughout 2015.

Figure 2. Variations in the parameters in the sub-surface layer (1 m under the surface) of the Seine estuary from January to December, 2015. With total, small cell and large cell chla concentrations ($\mu\text{gchla.L}^{-1}$); S-EPS, B-EPS (mgG.L^{-1}) and TEP concentrations (mgXGeq.L^{-1}); *Synechococcus*, *Pico-eukaryotes*, *Cryptophyceae*, diatom and dinoflagellate abundance (cells.L^{-1}). The dinoflagellate:diatom abundance ratio is also given. Values were previously smoothed using the Loess non-parametric regression method.

Figure 3. Variations in the parameters close to the water/sediment interface (1 m above the sediment) in the Seine estuary from January to December, 2015. With total, small cell and large cell *chla* concentrations ($\mu\text{gchla.L}^{-1}$); S-EPS, B-EPS (mgG.L^{-1}) and TEP concentrations (mgXGeq.L^{-1}); and *Synechococcus*, *Pico-eukaryotes*, and *Cryptophyceae* abundances (cells.L^{-1}). Values were previously smoothed using the Loess non-parametric regression method.

Figure 4. Cytometric signatures of the different populations observed in 2015 along the Seine estuary. A 1 µm self-fluorescent latex marble was added to each sample as a size and fluorescence reference. The graphs show the FL3 median (phycoerythrin), FL4 median (chlorophyll) and SSC median (size). With the two *Synechococcus* populations (A), the two pico-eukaryote populations that were not significantly differentiated (B) and the six *Cryptophyceae* populations (C).

Figure 5. Representation of Principal Component Analysis (PCA) using the abiotic parameters as qualitative variables (Irradiance (J cm^{-2}), flow ($\text{m}^3 \text{s}^{-1}$), temperature ($^{\circ}\text{C}$), salinity (PSU), SPM (g L^{-1}) and nutrients ($\mu\text{mol L}^{-1}$): DIN, P and Si) **and the biological parameters as quantitative variables** (total chl *a* (T.Chla) and chl *a* of small and large cells (S.Chla and L.Chla) in $\mu\text{g L}^{-1}$, Transparent exopolymeric particles (TEP) in mgXGeq L^{-1} , Soluble and Bound exopolymeric substances (S.EPS and B.EPS) concentrations in mgEPS L^{-1} and the abundance of phytoplankton community (*Synechococcus* (Syn), *Cryptophyceae* (crypto), Pico-eukaryotes (Picoeuk), diatoms (Diat.) and dinoflagellates (Dino.) in cells L^{-1})). The sub-surface water was represent in the left panel and the water sediment interface in the right panel.

Figure 6. Primary production as a function of the species richness of the Seine estuary. All the spatial (sites 1, 3, 5 & 7) and temporal (from January to December) data were used in this plot. Species richness was calculated as a function of the microscopic observations made in this study (table 1) and expressed in $\log_{10}+1$. The primary production data ($\text{gC.m}^{-2}.\text{d}^{-1}$) are detailed in Morelle et al. (submitted). The dynamic fit was performed on SigmaPlot 12.5 after 200 iterations of fits and the final equation was $\text{PP} = e^{2.5 \times \text{SR}}$ ($p < 0.001$; $R^2=0.35$).