Size exclusion chromatography and stable carbon isotopes reveal the limitations of solid phase extraction with PPL to capture autochthonous DOM production

Gabriel Dulaquais, Pierre Fourrier, Jean François Maguer, Corentin Denis, Matthieu Waeles, Ricardo Riso

Size exclusion chromatography and stable carbon isotopes reveal the limitations of solid phase extraction with PPL to capture autochthonous DOM production.

Gabriel Dulaquais*, Pierre Fourrier, Jean François Maguer, Corentin Denis, Matthieu Waeles & Ricardo Riso

Laboratoire des Sciences de l'Environnement Marin CNRS UMR 6539, Institut Universitaire Européen de la Mer, Université de Bretagne Occidentale. Place Nicolas Copernic - 29280 Plouzané, France

*Corresponding author. Email: gabriel.dulaquais@univ-brest.fr.

Abstract

The study of the elemental and carbon isotopic composition of dissolved organic matter (DOM) is of great interest in deciphering the origin and transformations of organic matter in estuarine and coastal environments. Styrene-divinylbenzene copolymer (PPL) sorbent is currently the most commonly used material for the isolation of DOM from environmental samples. It is notably used for the development of molecular formula-based indices used to study DOM reactivity. However, any extraction procedure (including with PPL) will fractionate the DOM. If this fractionation is not well constrained it can lead to biased interpretations of the biogeochemical processes affecting DOM. In this work we investigate the fractionation effects of the PPL sorbent on the size class distribution of DOM and the carbon isotopic composition of the PPL retentate. The use of size exclusion chromatography, that does not require a pre-concentration step, allows a precise study of the fractionation of DOM (including aromaticity) by the PPL resin. Extractions performed on two types of humic substances dissolved in artificial seawater, using the PPL resin, showed high extraction yield (> 85%) and the stable isotopic carbon composition ($\delta^{13}C$) of these compounds was successfully recovered. These results indicate that salinity is not a parameter affecting extraction yield on PPL sorbent. For a hydrophilic compound (atropine) the extraction efficiency was low (33%) and $\delta^{13}C$ signature was underestimated. Size exclusion chromatography measurements, in samples collected along a salinity gradient, demonstrate that the PPL sorbent strongly fractionates DOM. Although the DOM size class distributions in freshwaters and marine waters were initially different, their retentates were marked by similar size class distributions. This work demonstrates that PPL resin captures DOM compounds with less nitrogenous content and it seems to have a lower affinity for aromatic compounds of marine origin than of terrigenous origin. The study of DOM distribution in a macro-tidal estuary demonstrated the limitations

of PPL resin extraction in capturing an internal N-rich DOM production event at the time of sampling. Futhermore, the isotopic composition of the PPL resin retentate appears to depend on the extraction efficiency of the more hydrophobic compounds which changed along the salinity gradient. This study recommends careful interpretations of data that only rely on PPL extractions, particularly for works tracking the origin of DOM in estuaries and comparing DOM composition across ocean biogeochemical domains.

1. Introduction

Marine dissolved organic matter (DOM) is a large reservoir of reduced carbon (662 Pg, Hansell et al., 2009) involved in key aquatic processes. The main constituent of DOM is carbon (~50%) that supports the metabolism of heterotrophic bacterioplankton production (Azam and Hodson, 1977 ; Ducklow, 1999). DOM also consists of various heteroatoms making DOM an alternative bioavailable reservoir of nitrogen and phosphorus for marine micro-organisms (Lønborg et al., 2009 ; Stepanauskas et al., 2002). Thereby, DOM plays an integral role in the biogeochemistry of aquatic systems as well as in the biological carbon pump.

Historically DOM has been studied through the prism of dissolved organic carbon (DOC) since the early 1980. With major analytical advances (high-temperature combustion method), intercomparison efforts (Hedges and Lee, 1993) and international oceanographic programs (e.g. JGOF, CLIVAR) the global distribution of oceanic DOC was assessed in the mid 2000 (Hansell and Carlson, 2002, Hansell et al., 2009). DOC mapping demonstrated that its concentration was not uniform along the water column but exhibited concentration gradients across biogeochemical domains and a distribution strongly influenced by overturning circulation and deep sea mineralization (Hansell et al., 2009). However, despite the growing community interest in DOM, its study was still limited by analytical issues. Low ambient concentrations (down to 30 µM-C), salts, and wide diversity in the molecular composition (Zark et al., 2017) have been a barrier for decades to a molecular characterization of marine DOM.

For DOM isolation, solid-phase extraction (SPE) using historical amberlite sorbents (e.g. DAX 8 and 4) was developed early to define hydrophobic and transphilic fractions of DOM. However, their implementation and evaporation steps were often time consuming and required large sample volumes. In the late 2000, Dittmar et al. (2008) published a rapid and

simple method to isolate DOM from any aquatic matrix, which was a new step for the scientific community. Among the 6 sorbents studied by Dittmar et al. (2008) the styrene divinyl benzene polymer (PPL) was the most promising with extraction efficiency of 43% for DOC for deep sea water and up to 65% for DOC in freshwater. This study permitted the emergence of the first analyses of oceanic DOM composition along the entire water column and recently in different biogeochemical domains (Broek et al., 2020 ; Hertkorn et al., 2013 ; Martinez-perez et al., 2017 ; Medeiros et al., 2015 ; Osterholz et aL., 2021 ; Seidel et al., 2015). PPL is also widely used for the isolation of soil organic matter (Patel et al., 2021) and for the development of molecular formula-based indices (notably the aromaticity index of DOM) used to study DOM reactivity (Zherebker et al., 2022).

SPE-DOC can be processed for stable isotope ($\delta^{13}C$), a useful tool to trace the sources of DOC. $\delta^{13}C$ of SPE-DOC helps to trace the estuarine mixing (Zhou et al., 2021), to distinguish the main source of DOC (C3 vs C4 photosynthetic pathway) in estuary (Marques et al., 2017) and to trace terrigenous inputs in coastal systems (Takasu et al., 2023) and in the ocean (Zigah et al., 2017). $\delta^{13}C$ of SPE has usually the same or lower $\delta^{13}C$ than bulk seawater (Broek et al., 2017; Zigah et al., 2017), suggesting that PPL resin selectively isolates compound groups and may have difficulty in capturing the DOM derived from the degradation of marine phytoplankton.

Extraction with PPL resin has also been applied to culture experiments with the aim of defining the molecular composition of DOM of phytoplankton or bacterial origin (Landa et al., 2013 ; Liu et al., 2020) or to study the lability of oceanic DOM (Shen and Benner 2018; 2020). These works have opened up a new field of research related to DOM ecology that focuses on the complex interactions between organic compounds and microbial cells (Dittmar et al., 2021).

Because PPL resin has become the most popular sorbent, understanding the representativeness of the DOM isolated using PPL resin is crucial. The few studies that address the selectivity of this sorbent have shown contrasting results. While DOM of terrigenous origin does not appear to be significantly fractionated during extraction (Raeke et al., 2016), the sample matrix composition has been shown to affect the extraction efficiency of a given compound (Johnson et al., 2017). Furthermore, incomplete elution of the PPL resin retentate can induce a bias in the determination of stable and radiocarbon isotopic composition (Lewis et al., 2020). It has also been shown that for seawater samples, the optical signature of the retentate from a PPL resin is substantially different from that of the corresponding bulk

samples (Wünch et al., 2018). Another key issue is the high selectivity (chemically based) of the PPL sorbent. Li et al (2017) analyzed retentates after SPE extractions using PPL resin for Suwannee River and North Sea water samples. Their results showed high compositional similarity in these two samples of different origin raising questions about the use of this sorbent to study DOM modifications. Considering the results of Li et al. 2017 and the growing number of studies reporting high similarities between DOM isolated across different oceanic basins and depths (Broek et al., 2020), it is important to investigate the selectivity/fractionation of DOM by the PPL sorbent during the extraction process using PPL sorbent.

The objectives of our study are to define the representativeness of DOM isolated using a PPL resin and to determine whether this type of extraction is adequate to capture internal processes in estuarine systems and to trace the origin of DOM inputs (allochthones vs autochthones). We first tested the accuracy of carbon stable isotope measurements of three different types of organic compounds after their isolation on a PPL resin and measured the stable carbon isotope composition in the PPL resin retentates. These experiments were done to determine if SPE using PPL can be used to trace the origin of DOM in estuarine and marine waters. Secondly, we studied the DOC size class distributions and DOM composition using size exclusion chromatography (SEC) of estuarine samples before and after their passage through the PPL resin in order to define the representativeness of the PPL retentate. SEC has the advantage of not requiring a pre-concentration step and allows access to the DOM composition without fractionation effects. This analytical tool has been successfully used for the analysis of freshwater (Marie et al., 2015), estuarine (Dulaquais et al., 2018) and marine waters (Fourrier et al., 2022) and allows in this study to determine precisely the fractionation of DOM by the PPL resin in terms of size class and composition (including aromaticity and nitrogen content).

2. Material and Methods

2.1 Sampling

11 samples were collected along the salinity gradient of the Aulne estuary-Bay of Brest estuarine system (Britanny, France) on May 31 2021. For salinities below 20 of practical salinity unit (PSU), sampling was operated from the R/V *Hésione* (INSU-CNRS-UBO) and was carried out 0.5 m below the surface with the arm fully covered by a plastic glove (92 cm, Polysem®) using acid cleaned high density polyethylene (HDPE) bottles. For salinities above 20, sampling was operated onboard of the R/V *Albert Lucas* (INSU-CNRS-UBO) and was done at 1 m depth using a Niskin bottle. All samples collected were filtered onboard the R/V *Albert Lucas* within two hours after sampling using precombusted 0.7-µm GF/F filters (Whatman®). The filtrates were then collected in acid-cleaned HDPE bottles, double bagged and stored in the dark at 4°C. It is worth noting that the cruise occurred during a low discharge period (9.1 $m^3\ s^{-1}$). This water flow was preceded by a month of low water regime. This choice of water regime was made in order to have a residence time of the waters in the estuary (~15 days) significantly longer than the timescale of sampling (10 hours) allowing a better visualization of the biogeochemical processes occurring in the estuary. Salinity (S) and pH (NBS scale) were measured *in-situ* using a multi-parameter probe (Hanna Instruments© 9829) calibrated on the day of sampling. The accuracy of the measurements is ± 0.1 $g\ kg^{-1}$ and ± 0.01 pH units respectively. Salinity and pH of the samples were remeasured in the lab at 25 °C. The marine end-member sample was collected in the Iroise Sea on October 22, 2018 during the FeLINE cruise (Riso et al., 2021) and stored at -20°C from collection until analysis. For all cleaning procedures and the preparation of all aqueous solutions we used ultrapure water (resistivity > 18.2 MΩ.cm, MilliQ Element, Millipore®) and HCl (Suprapur®, Merck).

2.2 Experimental and solid phase extraction procedures

The chart of the experimental procedure used in this work is shown in figure 1 and the details of the different steps are provided in the following sections.

For estuarine samples, solid phase extraction was operated once per sample. After acidification at pH 2 (Suprapur®, Merck), 0.5 L (S < 20) or 1 L of estuarine samples (S > 20) were passed through a 100 mg PPL cartridge (Agilent, Santa Clara, CA, USA) following the procedure described in Dittmar et al. (2008). The cartridges were first washed with 1 column volume of

methanol. Methanol (HPLC grade, Merck©) was rinsed from the cartridge with 5 volumes of ultrapure water acidified at pH 2 (HCl, Suprapur®, Merck) before the extraction started. Extractions were performed at a flow rate of 4 mL/min using a peristaltic pump (Watson Marlow © 205S) with TYGON® tubing. Before air-drying, the cartridges were rinsed with 5 volumes of ultrapure water acidified at pH 2 (HCl, Suprapur®, Merck) in order to remove salts. Then, after 5 min of air drying we started the elution of the cartridges. We used 1.5mL of HPLC grade methanol, which is within the range of the recommended volume (12 mL/g of resin) to avoid bias resulting from incomplete elution (Lewis et al., 2020). After elution, the methanol extracts were evaporated directly into smooth wall tin capsules (Elemental microanalysis ©) on a hot plate at 50°C. All of these steps were performed under a laminar flow bench (ISO-5). The tin capsules were then stored in precombusted glass vials in a desiccator until determination of elemental C and $\delta^{13}C$ (by Elemental Analyzer coupled to Isotope ratio mass spectrometer, EA-IRMS) within a week after extraction. It is worth noting that volatile organic compounds may be lost during the drying step inducing bias for elution recovery assessment.

For each sample, the DOC size class distribution was measured by size exclusion chromatography (SEC) before (bulk sample) and after (permeate, Non SPE-DOM) the sample passed through the PPL resin. The permeate is constituted of all the water samples that passed through the PPL resin during the extraction procedure. The retentate and eluate refer to the DOM retained by the PPL resin and recovered after a methanol elution. The extraction yield (EY%) for each class of compounds was determined according to equation 1. Since the retentate could not be injected in the SEC, the size class distribution of the retentate (SPE-DOC) was calculated by the difference between bulk and permeate DOC concentrations in each size class fraction. Elution recoveries (ER %) were estimated according to equation 2 by comparing the amount of C in the eluate (measured by an Elemental Analyze, EA , (Thermo Scientific EA Flash 2000) to the amount of DOC in the retentate (SPE-DOC multiplied by sample volume). We observed a contamination (with methanol) of the permeate during the extraction procedure for the sample at salinity 15.5. For this sample, the DOC recovery (50.7%) was estimated from the amount of C measured by an EA and considering 95% of ER. Recovery of the different fractions can not be assessed for this latter sample.

Equation 1 $$EY(\%) = \left\{1 - \frac{[DOC]_{permeate}}{[DOC]_{bulk}}\right\} * 100$$

Equation 2 $$ER(\%) = \frac{OC_{EA}}{\{([DOC]_{permeate} - [DOC]_{bulk})\} * V_{sample}} * 100$$

With ***EY*** the Extraction yield; ***ER*** the Elution recovery; ***[DOC]$_{eluate}$*** and ***[DOC]$_{bulk}$*** the dissolved organic carbon concentration measured by size exclusion chromatography before and after the sample passes through the PPL resin respectively. ***OC_{EA}*** *is* the amount of organic carbon measured by the elemental analyser in the tin capsule after elution and evaporation of the PPL resin; ***V_{sample}*** the volume of sample that passes through the PPL resin.

For the evaluation of stable carbon isotopic accuracy, solid phase extractions were performed for three certified referenced materials: Suwannee River Fulvic Acid (INTERNATIONAL HUMIC SUBSTANCES SOCIETY (IHSS), SRFA 1S101F), Leonardite Humic Acids (International humic substances society , LHA 1S104H) and Atropine (Elemental microanalysis , OAS 279202). These compounds were selected by considering their isotopic signature and elemental composition (Table 1). These materials were first dissolved in artificial seawater (final concentrations 2 mg / L) prepared as described in Fourrier et al., 2022 (see supplementatry informations, SI). Artificial seawater was then acidified to pH 2 (HCl) and 250 mL of these solutions were then passed through the PPL resin, eluted and evaporated as for the estuarine samples.

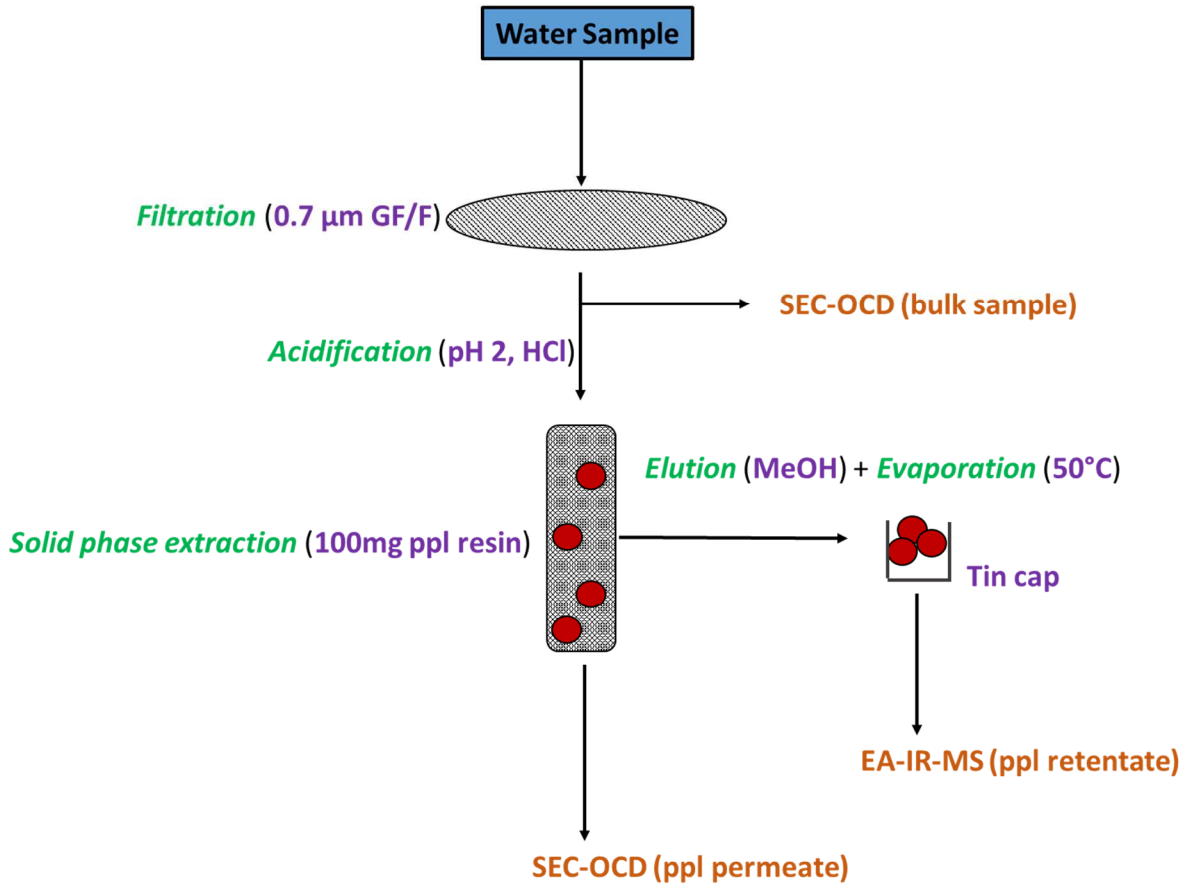


Figure 1: Chart of the experimental procedure followed in this study

2.3 DOM size class distribution

Measurements of the organic compound class distribution were performed by SEC coupled with C, N and UV detectors (DOC-Labor®, Karlsruhe, Germany) as previously described by Huber et al. (2011) for freshwater, and adapted for estuarine and marine waters by Dulaquais et al. (2018b). All the chemicals used for SEC analyses were those described in Fourrier et al. (2022). Repeated analysis of Deep Sea Reference samples (DSR, Hansell lab, Miami, USA $[DOC]_{DSR}$ = 43.2 ± 1.7 µmol L^{-1}; n = 5; consensus value of lot #10-18: 43 – 45 µmol L^{-1}) ensures an accurate determination of DOC. The determination of C:N ratios and of the percentage of aromatic carbon ($\%C_{arom}$) were conducted similarly as described in Riso et al. (2021) and Fourrier et al. (2022) (see SI file for additional information regarding the procedures). The device, equipped with two chromatographic columns (250 mm × 20 mm, TSK HW-50S, 3000 theoretical plates, Toso, Japan), permits the separation of DOM into six fractions of organic compounds with an optimal resolution. These fractions were described in order of retention as biopolymers (BP, high molecular weight compounds (HMW) > 10 kDa), humic substances (HS, 0.5 – 10 kDa), building blocks (BB, 0.3 – 0.5 kDa), low molecular weight acids (LMW acids, < 0.3kDa, charged), low molecular weight neutrals (LMW neutrals < 0.3 kDa) and the non-chromatographable DOC (retained by the column at a pH of 7) called hydrophobic DOC (HOC). The respective compositions of these operationally defined fractions are described in details in Huber et al. (2011) and Dulaquais et al. (2018b). We provide additional information considering the recent work of Fourrier et al. (2022). The BB fraction, initially thought as degradation by-products of HS, may actually correspond to degradation by-products of BP; LMW monoprotonic acids and LMW neutral compounds correspond to small-degradated humic substances and small hydrophilic compounds, respectively.

2.4 Stable carbon isotopic measurements

Stable carbon isotope ratios and C content analyses were performed using an Elemental Analyzer (Thermo Scientific EA Flash 2000) coupled to an Isotope Ratio Mass Spectrometer (IRMS Thermo Scientific Delta Plus). Isotopic ratios were expressed in conventional δ notation and were reported in parts per thousands (‰) as :

$$\delta^{13}C = [(({}^{13}C/{}^{12}C)_{Sample} / ({}^{13}C/{}^{12}C)_{VPDB}) - 1] * 1000$$

where $(^{13}C/^{12}C)_{VPDB}$ = 0.0112372, the ratio of ^{13}C to ^{12}C in the international reference Vienna-Pee Dee Belemnite (V-PDB) standard. Replicate analysis, using atropine as working standard (calibrated to the VPDB) indicated an analytical precision of ± 0.1‰ and ±0.6% for $\delta^{13}C$ and for C content, respectively.

The determination of bulk $\delta^{13}C$ composition (before solubilization) of the SRFA (- 27.6 ‰ ± 0.1 n = 3) and LHA (- 23.7 ‰ ± 0.1 n = 3) reference material were in keeping with the values (- 27.6 ‰ and - 23.8 ‰ respectively) provided by IHSS for these compounds (https://humic-substances.org/elemental-compositions-and-stable-isotopic-ratios-of-ihss-samples/), ensuring accurate of $\delta^{13}C$ measurements for natural organic matter.

2.5 Statistical analysis

T-tests were operated to determine if the PPL extraction procedure significantly affects the initial $\delta^{13}C$ signature of the 3 organic compounds tested in this study (SRFA, LHA and atropine). If *p-value* < 0.05 then the dataset are considered significantly different. The possibility of an effect of the PPL extraction procedure onto DOM size fractionation was tested using a two factor Wilcoxon test (two groups, permate and retentate) for each of the six size class fractions.

3. Results and discussion

3.1 Accuracy of $\delta^{13}C$ measurement after extraction

Table 1 shows the $\delta^{13}C$ results (blank corrected) for the three referenced materials after their solubilization in artificial seawater and extraction using the PPL sorbent. We found that the PPL resin (≥ 88%) efficiently extracted SRFA and Leornadite Humic Acids. Their $\delta^{13}C$ isotopic signatures were in agreement with those certified by the IHSS (table 1; *p-value* > 0.05). In contrast, atropine was not extracted efficiently (33%) and we measured a $\delta^{13}C$ isotopic signature (-19.9 ± 0.3; *p-value* < 0.05) significantly lower than the certified value (-18.1 ± 0.1). On the one hand, our results demonstrate that humic substances of both freshwater and terrigenous origin can be efficiently extracted from seawater matrix using a PPL sorbent. This process does not induce isotopic fractionation of stable carbon isotopes. On the other hand, our results suggest that hydrophilic molecules with low C/N ratio are not efficiently retained by the PPL sorbent. This low extraction yield seems to induce an important bias for the $\delta^{13}C$ isotopic signature. Here we suggest that the $\delta^{13}C$measurement of a sample after solid phase

extraction using the PPL sorbent is strongly influenced by the type of compounds in solution but not sensitive to the salinity of the sample.

Table 1 : *Certified elemental and stable carbon isotopic composition ($\delta^{13}C$) of the three certified referenced materials used in this study. Recovery and $\delta^{13}C$ isotopic signature measured after their dissolution in artificial seawater and extraction using a PPL sorbent.*

	SRFA	LHA	Atropine
Elemental O/C	0.60	0.37	0.18
Elemental H/C	0.99	0.7	1.37
Elemental C/N	85.0	60.5	17.0
$\delta^{13}C$ certified	-27.6	-23.8	-18.1
$\delta^{13}C$ measured in solid (n = 3)	-27.6 ± 0.1	-23.7± 0.1	-18.1 ± 0.1
Number of independent extraction	10	3	3
% recovery in artificial seawater	88	92	33
$\delta^{13}C$ measured after solubilization and PPL extraction	-27.8 ± 0.2	-23.9 ± 0.1	-19.9 ± 0.3

3.2 Retention yields for the samples collected in the salinity gradient

The extraction yield (EY) of the PPL resin was determined for DOC and for the different size classes operationally defined by SEC analysis. The elution recoveries were all > 95% ensuring no elution bias for EA-IRMS analysis of the retentate. Along the salinity gradient, DOC EY (Table 2) decreased from 52% in the Aulne freshwater (S = 0) to 35% in the marine sample (S = 35.15). A similar decrease in EY from fresh to marine waters was initially observed by Dittmar et al. (2008).

The different operational fractions exhibited varying EYs along the salinity gradient. On the one hand, the EY% of the HS fraction decreased from ~60% to 45%. Those of the biopolymers and LMW neutrals also decreased from freshwater to marine waters, from ~40% to ~13% and ~30 % to ~1%, respectively. These results demonstrate the weak ability of the PPL sorbent to capture these different hydrophilic compounds in marine samples. On the other hand, the EY of the most hydrophobic organic compounds was enhanced for higher salinities (EY% range 90-100% for S>30) compared to the low salinity samples (EY% range 35-65%). Because riverine and soil humic substances were accurately recovered in artificial seawater (S = 35, see section 3.1, table 1), salinity can not be considered as the controlling parameter for DOM adsorption onto the PPL sorbent. Thereby, the changes of EY we observed along the salinity gradient

indicate changes in the DOM molecular composition. This selectivity of the PPL sorbent implies that analyses of PPL retentate will only be representative of a fraction of DOM.

Table 2 : *Dissolved organic carbon concentrations (µM-C) measured in estuarine samples before and after (permeate) the solid phase extraction for the 6 operationally defined fractions. Associated extraction yields (EY %) for each fraction and total load of carbon in the PPL cartridge are provided. ND means not determined due to a methanol contamination during the extraction procedure.*

Salinity	Sampling location	Sample Volume (L)	Biopolymers (BP)			Humic substances (HS)			Biopolymers byproducts (BB)			Low molecular weight acids (LMW acids)			Low molecular weight neutrals (LMW neutrals)			Hydrophobic carbon (HOC)			DOC			Carbon in cartridge
			[DOC] initial	[DOC] permeate	EY (%)	[DOC] initial	[DOC] permeate	EY (%)	[DOC] initial	[DOC] permeate	EY (%)	[DOC] initial	[DOC] permeate	EY (%)	[DOC] initial	[DOC] permeate	EY (%)	[DOC] initial	[DOC] permeate	EY (%)	[DOC] initial	[DOC] permeate	EY (%)	µmol C
0.17	**48°12'57.8"N 4°04'30.0"W**	440	15.7	9.3	40.8	117.6	47.2	59.9	23.9	12.2	49.0	1.0	0.3	70.0	22.5	18.9	26.0	36.9	17.1	53.7	230.0	105.0	54.3	55.0
5.5	**48°13'16.3"N 4°06'59.1"W**	494	11.8	9.2	22.0	119.3	48.7	59.2	17.2	9.1	47.1	0.8	0.6	25.0	30.0	21.1	29.9	32.2	11.6	64.0	211.3	100.3	52.5	54.8
10	**48°14'43.3"N 4°07'11.9"W**	462	12.2	7.3	40.2	114.8	44.6	61.1	14.1	7.0	50.4	0.8	0.5	37.5	32.1	23.5	32.9	23.9	12.8	46.4	197.9	95.8	51.6	47.2
15.5	**48°14'47.6"N 4°09'48.3"W**	470	8.2	nd	nd	101.9	nd	nd	14.0	nd	nd	4.5	nd	nd	29.5	nd	nd	19.3	nd	nd	177.4	nd	nd	44.5*
20.8	**48°15'15.8"N 4°13'43.8"W**	475	8.0	7.1	11.3	95.6	48.3	49.5	12.3	6.0	51.2	5.9	2.6	55.9	24.8	22.6	16.7	19.9	4.9	75.4	166.5	91.5	45.0	35.6
25.5	**48°16'06.8"N 4°15'43.7"W**	950	8.8	7.3	17.0	91.9	39.1	57.5	6.8	6.5	4.4	4.9	2.8	42.9	26.3	23.2	0.4	10.2	0.0	100.0	149.0	79.0	47.0	66.5
27.7	**48°16'41.5"N 4°16'56.0"W**	950	9.6	6.7	30.2	79.6	32.1	59.7	9.6	7.9	17.7	4.3	2.6	39.5	24.7	22.9	7.6	8.3	1.2	85.5	136.0	73.2	46.2	59.6
30.4	**48°17'09.5"N 4°15'30.2"W**	950	9.6	6.9	28.1	64.9	31.1	52.1	10.7	7.3	31.8	4.4	2.6	40.9	25.3	24.0	5.2	5.2	0.0	100.0	120.1	71.9	40.1	45.8
32.2	**48°18'30.5"N 4°24'32.5"W**	950	10.4	7.2	30.8	46.5	23.4	49.7	9.0	7.1	21.1	3.1	2.4	22.6	23.4	23.2	1.2	4.7	0.0	100.0	97.2	63.2	35.0	32.3
32.5	**48°20'57.5"N 4°32'39.1"W**	950	10.6	8.6	18.8	43.5	21.8	49.8	8.8	6.7	23.9	2.9	2.3	20.7	23.4	23.1	1.4	7.0	0.6	91.4	96.3	63.1	34.5	31.6
35.15	**48°13'15.4"N 4°44'04.0"W**	250	8.0	7.0	12.9	39.1	21.3	45.5	8.0	5.9	26.3	2.5	2.5	0.0	24.9	24.4	0.0	6.5	0.0	100.0	89.8	61.9	34.7	7.0

284 * Calculated using EA-IRMS carbon measurement and an elution recovery of 95% (ER = 95%). See text for details

285

3.3 DOC distribution and partitioning along the salinity gradient

The distributions of DOC and the six operationally defined size fractions along salinity gradient are shown in figure 2a. DOC ranged from 234 µM-C in the freshwater sample (Aulne River) to 96.3 µM-C at S=32.5 (Bay of Brest). The North Atlantic surface water used in this work is a sample collected from the Iroise Sea in 2018 (Riso et al., 2021) with a DOC concentration of 89.8 µM-C. At the time of sampling, DOC showed a nearly conservative distribution along the salinity gradient (DOC = 235µM-C -3.9*Salinity ; n = 11; R^2 = 0.96) with a slight positive anomaly at S > 20 (Figure S1). DOM partitioning was similar to those previously reported in the same estuary (Dulaquais et al., 2018) with (1) the predominance of HS across the system (from 60% of DOC at S < 10 to 45% at S > 32); (2) a decrease in HOC contribution of HOC from 15 % in freshwater to 4 % in the most marine waters (36 µM at S = 0.17 to 4 µM at S> 32.5); (3) an increase in the contribution of LMW neutrals with salinity (from 10% in freshwater to 25% in marine waters), and (4) a low and near constant concentration of biopolymers (HWM DOC, 10.5 ± 2.3 µM, n = 11). With the exception of HOC, the mixing diagrams show non-conservative behavior for all fractions studied (Figure S1), indicating that internal process were affecting the distribution of DOM during the sampling period behind the quasi-conservative distribution of DOC along the salinity gradient.

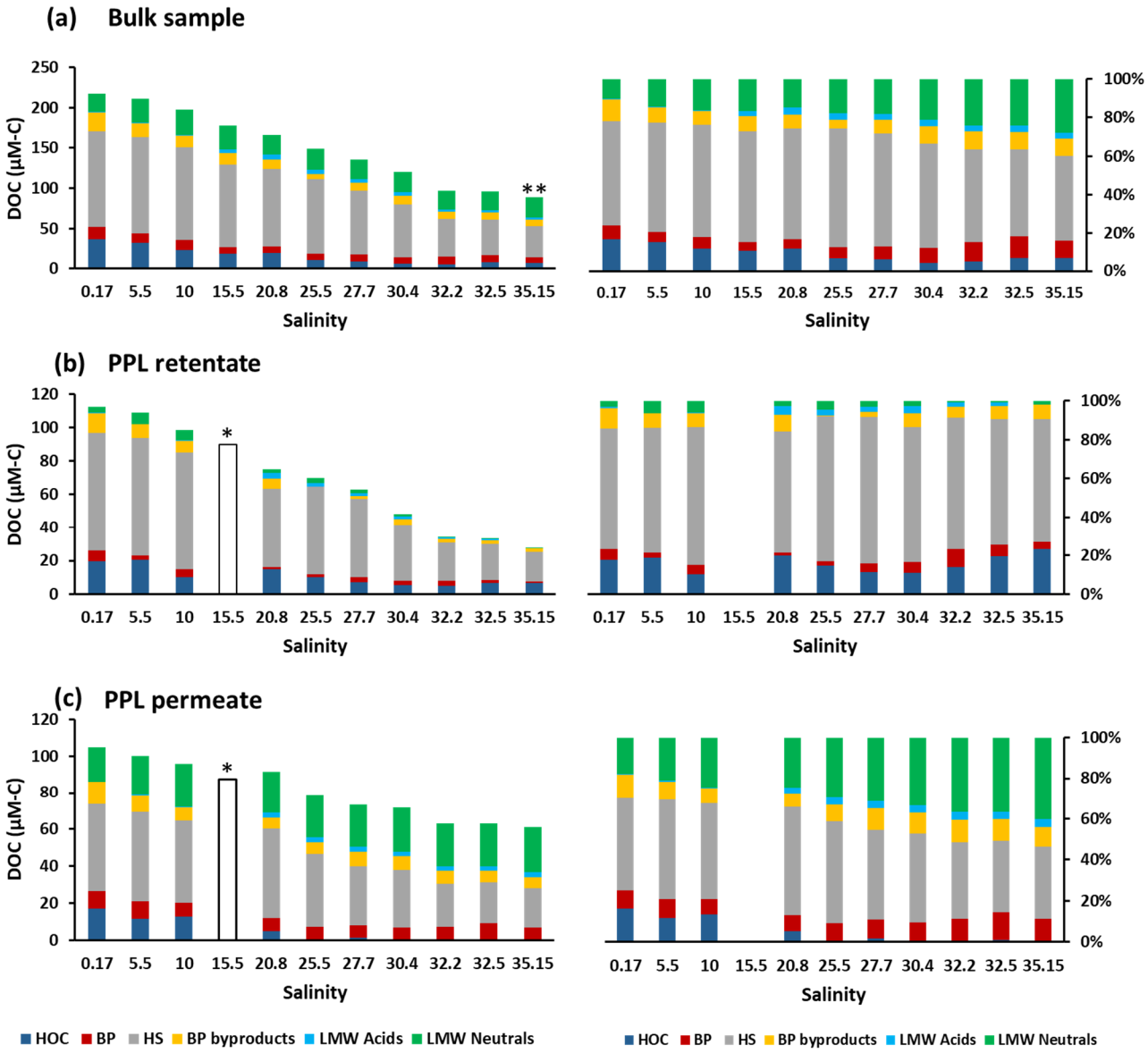


Figure 2: Dissolved organic carbon (DOC in µM-C) concentrations (left panels) and relative contribution (right panels) in the six fractions operationally defined by size exclusion chromatography for (a) filtered sample, (b) the PPL-retentate (SPE-DOM), (c) the PPL-permeate (Non SPE-DOM). It is worth noting that a contamination with methanol prevents the analysis of the S=15.5 sample by SEC after the extraction procedure (PPL permeate and retentate). ** Sample collected in 2018 and kept frozen until analysis.

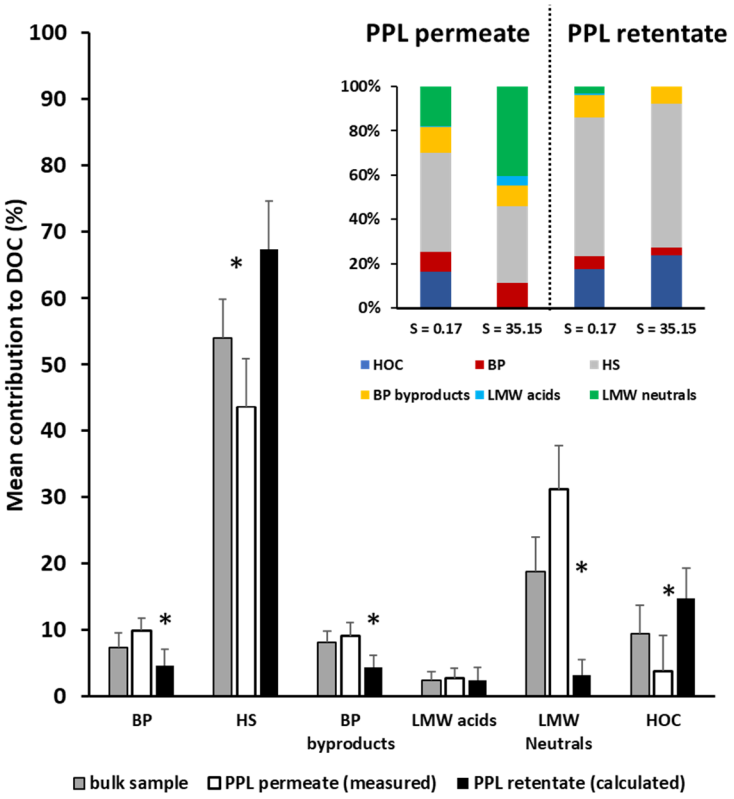
3.4 Selectivity of the PPL sorbent

In order to study the representativeness of a PPL resin retentate we studied the size class partitioning of DOC along the salinity between the PPL retentate (referred to as SPE-DOC hereafter) and its permeate (referred to as non-SPE-DOC hereafter) .

The size class fractionations shown in Figure 2 clearly demonstrate a high selectivity of the PPL sorbent for some fractions. The size class composition of the SPE-DOC samples was completely different from that of the corresponding bulk samples (Figure 2) with an over-representation

of HOC and humic fractions in the SPE-DOC, at the expense of biopolymers and LMW neutrals fractions. To further identify which size fractions are selectively retained by the PPL sorbent, we determined the discrete (Figure S1) and average (with associated standard deviation) contribution of each size fraction to the SPE-DOC pool and to the unextractable DOC (using PPL) along the salinity gradient (Figure 3). Among the six fractions studied, five were retained with a selectivity that induced a significant difference (Wilcoxon test) between the retentate and the permeate (Figure 2, 3 and S1). Furthermore the high selectivity of the PPL sorbent induced a similar distribution of SPE-DOC size classes between freshwater and the marine waters, whereas before the extraction procedure, the samples had different compositions (Figure 2 & 3).

The previous results demonstrate that during the extraction procedure, the size fractionation of DOC changes. Thereby the DOC retained by the PPL resin is not representative of the bulk sample. Such fractionation may lead to biased interpretations in the absence of additional data (e.g. SEC measurements). Our results also showed that the selectivity of the resin was however not total for a given fraction, suggesting that in a fraction operationally defined by the SEC, the PPL resin is able to retain specific compounds according to their chemical properties. To investigate this hypothesis, we compared the elemental C/N ratios and aromaticity of compounds retained by the PPL sorbent to those that escape the extraction procedure.



336

337 **Figure 3:** Mean DOC distribution in the bulk sample (grey bars), PPL permeate (non-SPE-DOC, white
338 bars) and in the PPL-retentate (SPE-DOC, dark bars) for the 11 samples collected along the Aulne-Iroise
339 Sea land-sea continuum. * indicate a significant difference between white and dark bars based on a
340 two-factor Wilcoxon test (two groups, permate and retentate). Insert represents the DOM
341 fractionation of PPL permeate and of PPL retentae for the freshwater (S = 0.17) and marine (S = 35.15)
342 endmembers.

343 The C/N ratios measured in the PPL permeate were consistently lower than those measured
344 in the samples before the extraction procedure indicating that the PPL resin has a low affinity
345 for N-containing molecules. Since high nitrogen content is accepted as a proxy of DOM lability
346 (Fourrier et al., 2022) and C/N ratio increases with DOM ageing (Walker et al., 2016); the ability
347 of the PPL resin to capture labile and freshly produced DOM seems limited. This result is of
348 particular importance because the decrease of C/N after PPL processing was observed in both
349 humic substances (Figure 4a) and biopolymer (data not shown) fractions, which have different
350 production pathways and labilities. Biopolymers (> 10kDa) can be considered as young and
351 labile DOM derived from phytoplankton degradation (cell lysis). Humic substances are
352 compounds that result from more intense processing (condensation, bacterial degradation),
353 they can be considered as a more refractory fraction of DOM. Our results also show that the
354 aromaticity of the biopolymers and humic substances fractions was also affected by the
355 extraction procedure. We observed a much higher aromaticity of DOM in the PPL permeate

at salinities higher than 20 compared to the untreated samples (Figure 4b). This result indicates that the PPL resin has a lower affinity for marine aromatic DOM than for riverine/terrigenous aromatic compounds. Considering the low affinity of PPL for chemical nitrogen groups (Figure 4a) our observation can be related to a higher proportion of aromatic moities containing heteroatoms (e.g. O, N) in the marine DOM compared to the freshwater DOM. Overall, such high selectivity for hydrophobic and aliphatic compounds at the expense of aromatic and N-rich compounds strongly limits the use of the PPL resin to monitor the production or to study the composition of labile DOM in marine waters.

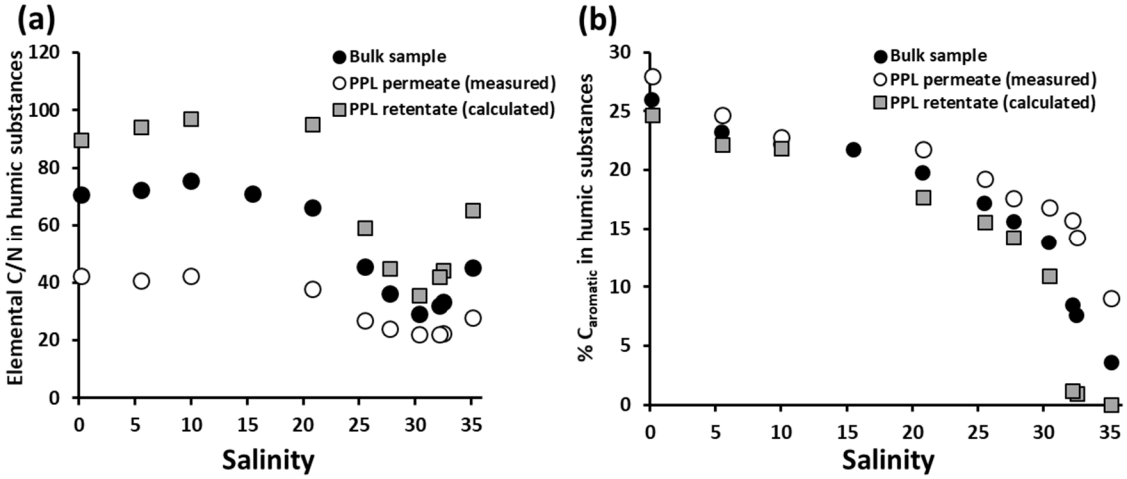


Figure 4 : Elemental C/N ratios **(a)** and percentage of aromatic carbon **(b)** measured in the bulk samples (dark dots), in the permeate of a PPL resin (white dots) and calculated for the retentate of a PPL resin (grey squares) for the humic substances fraction along the land-sea continuum of the Aulne-Iroise Sea.

3.5 Isotopic composition of SPE-DOC along the estuary

Along the Aulne estuary, $\delta^{13}C$ values measured inPPL eluates ranged from -28.6 ± 0.1 ‰ in the Aulne river freshwater to -24.2‰ ± 0.1 at S = 35.15. The depletion of $\delta^{13}C$ in the riverine end-member is consistent with the terrigenous origin of DOM in the river (Opsahl et al., 1999). Since $\delta^{13}C$ was not determined in the marine sample we must assume an isotopic signature of -22.7 ‰ +- 0.1 in North Atlantic waters identical to the one reported in the work of Medeiros et al. (2016) for North Atlantic SPE-DOC (using PPL). The increasing $\delta^{13}C$ isotopic signature we observed along the estuary (Figure 5) is consistent with the increasing contribution of marine waters. From these two end-members (e.g. Aulne River and North Atlantic), a mixing line can be generated using a mass balance of the freshwater fraction (*f*) at each point according to Equation 3.

Equation 3 $\delta^{13}C_{SPE\text{-}DOCtheo} = \{(\delta^{13}C_{Aulne} * [SPE\text{-}DOC]_{Aulne} * f) + (\delta^{13}C_{Atlantic} * [SPE\text{-}DOC]_{Atlantic} * (1-f))\} / \{([SPE\text{-}DOC]_{Aulne} * f) + ([SPE\text{-}DOC]_{Atlantic} * (1-f))\}$

With $\delta^{13}C$ SPE-DOC_{theo} the theoretical stable isotopic carbon signature of the PPL retentate; $[SPE\text{-}DOC]_{Aulne}$ and $[SPE\text{-}DOC]_{Atlantic}$ the DOC concentrations of the PPL retentate in the Aulne river and in the Iroise Sea samples respectively; f the fraction of freshwater, f = 1 if S = 0.17 ; f = 0 if S = 35.15

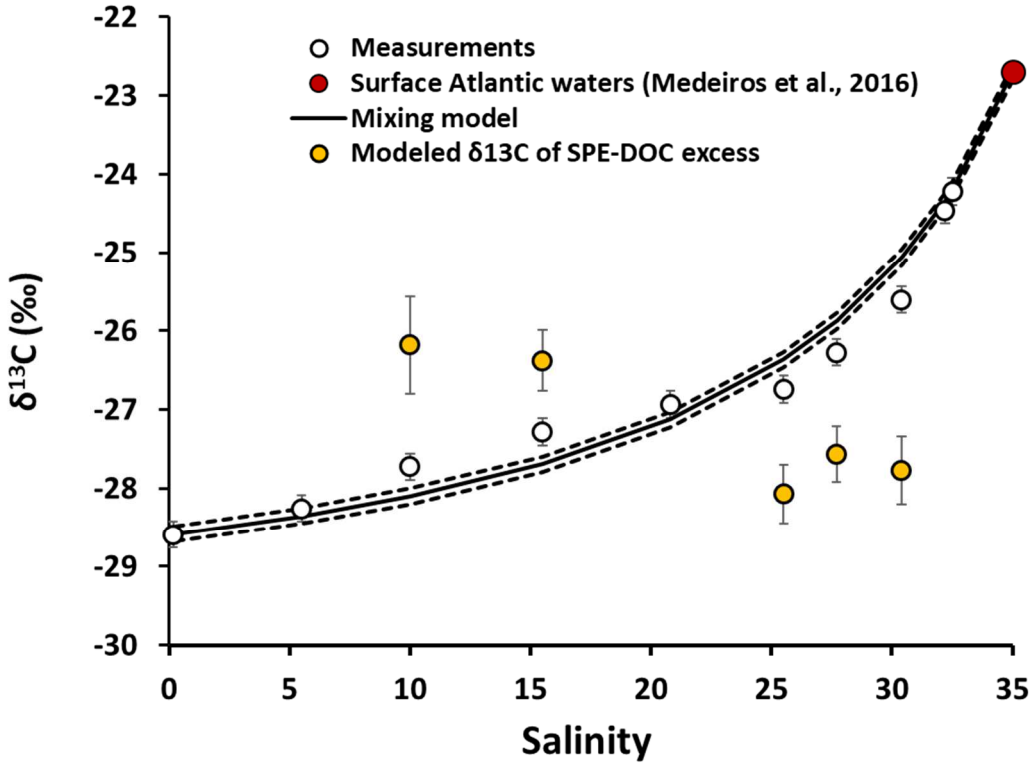
Along the estuary, values of $\delta^{13}C$ (Figure 5, white dots) were close to the mixing line. Closer examination, however, indicates some deviations from this line, with heavier $\delta^{13}C$ values in the upstream part of the estuary (S < 21) and lighter $\delta^{13}C$ when S > 21. Given the uncertainties of both mixing line and samples, five data points drift significantly from the mixing line. Based on the SPE-DOC excess estimate (Equations 4 & 5), we used a mass balance (Equation 6) to determine the $\delta^{13}C$ signature of the apparent SPE-DOC excess ($\delta^{13}C$ SPE-DOC_{excess}, Figure 5, yellow dots). The calculated $\delta^{13}C$ SPE-DOC_{excess} was -26.3 ± 0.2 for the two points in the salinity range 10-16 and was-27.8 ± 0.2 for the three points in the 25 - 31 salinity range.

Equation 4 $[SPE\text{-}DOC]_{excess} = [SPE\text{-}DOC]_{measured} - [SPE\text{-}DOC]_{theo}$

Equation 5 $[SPE\text{-}DOC]_{theo} = \{([SPE\text{-}DOC]_{Aulne} - [SPE\text{-}DOC]_{Atlantic}) / (S_{Aulne} - S_{Atlantic})\} * S_{measured}$

Equation 6 $\delta^{13}C\ SPE\text{-}DOC_{excess} = \{(\delta^{13}C_{SPE\text{-}DOC\ measured} * [SPE\text{-}DOC]_{obs}) - (\delta^{13}C_{SPE\text{-}DOC\ theo} * [SPE\text{-}DOC]_{theo})\} / [SPE\text{-}DOC]_{excess}$

Interpretation based strictly on $\delta^{13}C$ data, may lead to a false conclusion and/or biased interpretations. The $\delta^{13}C$ SPE-DOC_{excess} values in the salinity range 25-31 indicate a lighter organic $\delta^{13}C$ source that could be erroneously associated with terrigenous DOM inputs. Our study of the distribution of DOM size class by SEC clearly indicates an internal input of humic substances in this part of the estuary (Figure S1). At these salinities, the humic substances have aromaticities and C/N ratios much lower than the values measured for the Aulne end-member (Figure 4). Based on these properties, we relate this input of humic substances to an internal autochthonous production that may result from bacterial degradation of phytoplankton cells rather than to an external terrigenous input. If correct, these autochthonous humic substances should have a high $\delta^{13}C$ (phytoplankton origin) and increase the $\delta^{13}C$ signature of the DOM. Thereby there is a discrepancy between the SPE-DOC $\delta^{13}C$ values that suggest a terrigenous input and the SEC measurements that indicate autochthonous input.



409

410 **Figure 5:** Stable isotopic organic carbon composition ($\delta^{13}C$) measured in the PPL resin eluate (white
411 dots) along the land-sea continuum of the the Aulne-Iroise land-sea continuum. Solid and dashed lines
412 predict the conservative mixing and associated uncertainties calculated using the $\delta^{13}C$ value measured
413 in the Aulne river (this study) and the $\delta^{13}C$ of the surface Atlantic ocean (red dot) determined by
414 Medeiros et al. (2016). The $\delta^{13}C$ associated with excess SPE-DOC (yellow dots) were calculated by mass
415 balance between $\delta^{13}C$ measurements and the excess SPE-DOC. See text for details of calculations.

416 Two distinct processes can explain this discrepancy. First, autochthonous DOM production in
417 the salinity range 25-31 resulted in an input of N-rich humic substances. Considering their
418 aquatic origin, these humic substances produced in the estuary are probably enriched in ^{13}C.
419 These N-rich humic substances are weakly retained by the PPL resin (Figure 4a) and thus not
420 available for the $\delta^{13}C$ measurement. Second, the extraction efficiency of HOC compounds
421 increasing along the salinity gradient (Figure 6) may have affected the $\delta^{13}C$ values. Indeed, the
422 PPL retained HOC very efficiently (> 95%) at salinities above 25, the extraction yield was much
423 lower (35-65%) at salinities below 20. These HOC are primarily river-borne (Figure 6) are likely
424 characterized by a light $\delta^{13}C$ consistent with their terrigenous origin. Therefore, any changes
425 in the recovery of these compounds by the PPL sorbent will also affect the SPE-DOC $\delta^{13}C$
426 measurements.

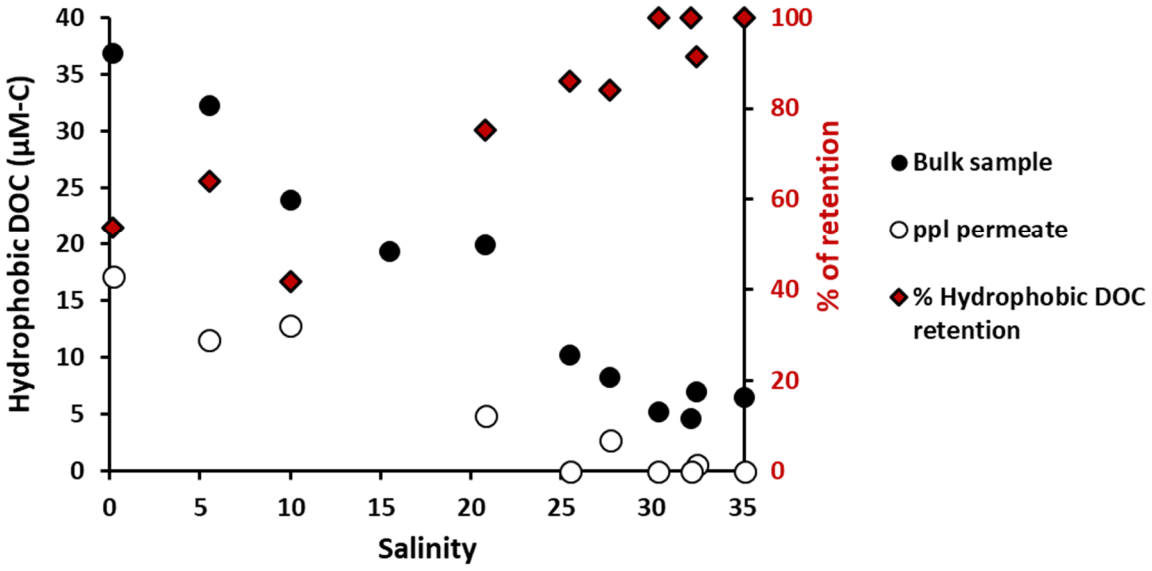


Figure 6: Hydrophobic DOC concentrations (left axis) in the bulk samples (dark dots) and in the PPL-permeates (white dots) measured along the Aulne-Iroise Sea land-sea continuum. The associated percentages of hydrophobic DOC extraction yields (red diamonds, right axis) increase from fresh to marine waters.

3.6 Implication for oceanic studies

Extraction with PPL as sorbent is commonly used to isolate DOM in order to trace molecular changes in DOM through biogeochemical processes (Osterholz et al., 2021). Here, we observed that extraction with a PPL sorbent alters the size class distribution of the initial DOM and that this resin is not optimal for capturing N-rich or aromatic marine DOM (Figure 4). In addition, we observed a high selectivity of the PPL resin for specific DOM fractions. This selectivity, already reported in other studies (Li et al., 2017; Wünch et al., 2018), generates a quasi-constant composition of the DOM isolated by the PPL resin whether in freshwater or marine waters (Figure 2 and 4). The PPL sorbent is often reffered as having a high affinity for refractory DOM compounds, which would make this sorbent effictive for studying this DOM fraction. It is clear that an PPL extraction of DOM with PPL in the deep ocean will trap a substantial part of the refractory DOC (up to 60%), however any analysis at the molecular level of the retentate from a PPL resin cannot be assigned to the intrinsect signature of refractory DOC. This is supported in particular by the work of Broek et al. 2020. These authors observed significant compositional differences between bulk DOM and PPL DOM retentates in term of C/N ratio, $\delta^{13}C$ and $\Delta^{14}C$ in the deep Pacific waters. At these Pacific depths, DOM there can be

considered fully refractory (Fourrier et al., 2022), so the existence of compositional differences between retentate from a PPL resin and untreated sample demonstrates that the PPL resin captures only a fraction of the refractory DOM. Furthermore in a controlled laboratory experiment conducted by Wienhausen et al., 2017, the PPL resin retained a portion of the freshly produced (labile) DOM, again supporting that this sorbent fractionates the DOM not in terms of lability but in terms of chemical properties.

These important results provide evidence that PPL resin fractionates DOM through chemical properties and that DOM refractability can not only be defined as the chemical properties of compounds retained by a PPL resin.

Due to the high selectivity of the PPL resin, we suggest that both labile and refractory DOM, can be isolated using a PPL resin. Consequently, a DOM isolated using the PPL resin will have the same chemical properties and molecular composition (in broad terms) in any aquatic system. As a result, the refractory nature of DOM can not be assigned solely in terms of chemical composition. This hypothesis is supported by systematic fractionation of DOM that we observed in PPL retentated from marine, estuarine and freshwater (Figure 2) while the main origin of DOM is still discernable using carbon isotopes (Figure 5). This is also supported by the accumulation of recent studies showing extraordinary homogeneity in DOM composition from surface to the deep ocean , between oceanic basins and in different environments (Broek et al., 2020 ; Li et al., 2017 ; Osterholz et al., 2021). Analysis of PPL retentates by any powerful technique (e.g. Fourier Transform Ion Cyclotron Resonance Mass Spectrometry*;* Nuclear Magnetic Resonance*)* provides useful information to study this defined fraction. However, one should not lose sight of the fact that the PPL retentate is not representative the refractory DOM alone but fractionate DOM in both in the refractory and non-refractory pool.

4. Conclusions

We studied the fractionation of DOM by solid-phase extraction with a PPL sorbent along an estuarine system. Our study confirms that the composition of the material is the main parameter controlling the extraction efficiency of DOM by the PPL resin. PPL sorbent has a high affinity for aliphatic hydrophobic compounds and retains poorly high molecular weight compounds as well as those enriched in nitrogen. Salinity does not seem to affect the

extraction significantly. In this work we have demonstrated that at low extraction yields (< 33%) of a compound, the measurement of $\delta^{13}C$ in the retentate of a PPL resin is in error. For yields > 88% the $\delta^{13}C$ measurement is valid.

The study of the size class fractionation of estuarine samples before and after their treatment on a PPL resin allowed us to observe that (i) the composition of a PPL resin retentate was not representative of the original sample, (ii) the distribution of DOC by size class was constant in the different retentates without taking into account the origin of the sample (freshwater and marine water). We observed a high selectivity of the PPL resin based on chemical properties (C/N ratio, aromaticity). This selectivity was observed for both refractory (humiques substances) and semi-labile (biopolymers) organic matter.

The carbon isotope study showed that the extraction efficiency of the compounds strongly influenced the $\delta^{13}C$ data obtained from the retentate analysis of a PPL resin. In this work, a high retention of the most hydrophobic compounds and a low extraction of humic substances enriched in nitrogen and produced in the estuary induced an underestimation of the $\delta^{13}C$ at the highest salinities (S > 20).

This work shows that without control of the extraction process, biased interpretations can be made based on the analysis of a PPL resin rententate alone. We call for a cautious interpretation of the DOM data from PPL extraction because this sorbent strongly fractionates the DOM pool on chemical and molecular properties and not on the lability.

Acknowledgement

We thank Jerôme Lepioufle, professeur agrégé d'anglais, for his help in proofreading and editing. We thank F. Quéré, E. Armice and the crew of the R/V Albert Lucas for help during the cruise and for collecting samples along the Aulne-Iroise Sea Land Sea continuum. This work is a contribution to the FeLINE project (Fer Ligands In the aulNe Estuary, Ifremer, Politique de site DS, 2019–2020) and was funded by Ifremer, grant number R204-12-MS-02.

References

Azam, F., & Hodson, R. E. (1977). Size distribution and activity of marine microheterotrophs 1. *Limnology and Oceanography*, *22*(3), 492-501.

Broek, T. A., Walker, B. D., Guilderson, T. P., Vaughn, J. S., Mason, H. E., & McCarthy, M. D. (2020). Low molecular weight dissolved organic carbon: Aging, compositional changes, and selective utilization during global ocean circulation. *Global Biogeochemical Cycles*, *34*(6), e2020GB006547.

Dittmar, T., Koch, B., Hertkorn, N., & Kattner, G. (2008). A simple and efficient method for the solid-phase extraction of dissolved organic matter (SPE-DOM) from seawater. *Limnology and Oceanography: Methods*, *6*(6), 230-235.

Dittmar, T., Lennartz, S. T., Buck-Wiese, H., Hansell, D. A., Santinelli, C., Vanni, C., ... & Hehemann, J. H. (2021). Enigmatic persistence of dissolved organic matter in the ocean. *Nature Reviews Earth & Environment*, *2*(8), 570-583.

Ducklow, H. W. (1999). The bacterial component of the oceanic euphotic zone. *FEMS Microbiology Ecology*, *30*(1), 1-10.

Dulaquais, G., Breitenstein, J., Waeles, M., Marsac, R., & Riso, R. (2018). Measuring dissolved organic matter in estuarine and marine waters: size-exclusion chromatography with various detection methods. *Environmental Chemistry*, *15*(7), 436-449.

Fourrier, P., Dulaquais, G., Guigue, C., Giamarchi, P., Sarthou, G., Whitby, H., & Riso, R. (2022). Characterization of the vertical size distribution, composition and chemical properties of dissolved organic matter in the (ultra) oligotrophic Pacific Ocean through a multi-detection approach. *Marine Chemistry*, *240*, 104068.

Hansell, D. A., Carlson, C. A., & Suzuki, Y. (2002). Dissolved organic carbon export with North Pacific Intermediate Water formation. *Global Biogeochemical Cycles*, *16*(1), 7-1.

Hansell, D. A., Carlson, C. A., Repeta, D. J., & Schlitzer, R. (2009). Dissolved organic matter in the ocean: A controversy stimulates new insights. *Oceanography*, *22*(4), 202-211.

Hedges, J.I., and C. Lee. 1993. Measurement of dissolved organic carbon and nitrogen in natural waters. Marine Chemistry 41(1–3):1–290.

Hertkorn, N., Harir, M., Koch, B. P., Michalke, B., & Schmitt-KoPPLin, P. (2013). High-field NMR spectroscopy and FTICR mass spectrometry: powerful discovery tools for the molecular level characterization of marine dissolved organic matter. *Biogeosciences*, *10*(3), 1583-1624.

Huber, S. A., Balz, A., Abert, M., & Pronk, W. (2011). Characterisation of aquatic humic and non-humic matter with size-exclusion chromatography–organic carbon detection–organic nitrogen detection (LC-OCD-OND). *Water research*, *45*(2), 879-885.

Johnson, W. M., Kido Soule, M. C., & Kujawinski, E. B. (2017). Extraction efficiency and quantification of dissolved metabolites in targeted marine metabolomics. *Limnology and Oceanography: Methods*, *15*(4), 417-428.

Landa, M., Cottrell, M. T., Kirchman, D. L., Kaiser, K., Medeiros, P. M., Tremblay, L., ... & Obernosterer, I. (2014). Phylogenetic and structural response of heterotrophic bacteria to dissolved organic matter of different chemical composition in a continuous culture study. *Environmental microbiology*, *16*(6), 1668-1681.

Lewis, C. B., Walker, B. D., & Druffel, E. R. (2020). Isotopic and optical heterogeneity of solid phase extracted marine dissolved organic carbon. *Marine Chemistry*, *219*, 103752.

Li, Y., Harir, M., Uhl, J., Kanawati, B., Lucio, M., Smirnov, K. S., ... & Hertkorn, N. (2017). How representative are dissolved organic matter (DOM) extracts? A comprehensive study of sorbent selectivity for DOM isolation. *Water Research*, *116*, 316-323.

Liu, S., Baetge, N., Comstock, J., Opalk, K., Parsons, R., Halewood, E., ... & Carlson, C. A. (2020). Stable isotope probing identifies bacterioplankton lineages capable of utilizing dissolved organic matter across a range of bioavailability. *Frontiers in microbiology*, 2364.

Lønborg, C., Davidson, K., Álvarez–Salgado, X. A., & Miller, A. E. (2009). Bioavailability and bacterial degradation rates of dissolved organic matter in a temperate coastal area during an annual cycle. *Marine Chemistry*, *113*(3-4), 219-226.

Marie, L., Pernet-Coudrier, B., Waeles, M., Gabon, M., & Riso, R. (2015). Dynamics and sources of reduced sulfur, humic substances and dissolved organic carbon in a temperate river system affected by agricultural practices. *Science of the Total Environment*, *537*, 23-32.

Marques, J. S., Dittmar, T., Niggemann, J., Almeida, M. G., Gomez-Saez, G. V., & Rezende, C. E. (2017). Dissolved black carbon in the headwaters-to-ocean continuum of Paraíba do Sul River, Brazil. *Frontiers in Earth Science*, *5*, 11.

Martínez-Pérez, A. M., Osterholz, H., Nieto-Cid, M., Álvarez, M., Dittmar, T., & Álvarez-Salgado, X. A. (2017). Molecular composition of dissolved organic matter in the Mediterranean Sea. *Limnology and Oceanography*, *62*(6), 2699-2712.

Medeiros, P. M., Seidel, M., Powers, L. C., Dittmar, T., Hansell, D. A., & Miller, W. L. (2015). Dissolved organic matter composition and photochemical transformations in the northern North Pacific Ocean. *Geophysical Research Letters*, *42*(3), 863-870.

Medeiros, P. M., Seidel, M., Niggemann, J., Spencer, R. G., Hernes, P. J., Yager, P. L., ... & Hansell, D. A. (2016). A novel molecular approach for tracing terrigenous dissolved organic matter into the deep ocean. *Global Biogeochemical Cycles*, *30*(5), 689-699.

Opsahl, S., Benner, R., & Amon, R. M. (1999). Major flux of terrigenous dissolved organic matter through the Arctic Ocean. *Limnology and Oceanography*, *44*(8), 2017-2023.

Osterholz, H., Kilgour, D. P., Storey, D. S., Lavik, G., Ferdelman, T. G., Niggemann, J., & Dittmar, T. (2021). Accumulation of DOC in the South Pacific subtropical gyre from a molecular perspective. *Marine Chemistry*, *231*, 103955.

Patel, K. F., Fansler, S. J., Campbell, T. P., Bond-Lamberty, B., Smith, A. P., RoyChowdhury, T., ... & Bailey, V. L. (2021). Soil texture and environmental conditions influence the biogeochemical responses of soils to drought and flooding. *Communications Earth & Environment*, *2*(1), 1-9.

Raeke, J., Lechtenfeld, O. J., Wagner, M., Herzsprung, P., & Reemtsma, T. (2016). Selectivity of solid phase extraction of freshwater dissolved organic matter and its effect on ultrahigh resolution mass spectra. *Environmental Science: Processes & Impacts*, *18*(7), 918-927.

Riso, R., Mastin, M., Aschehoug, A., Davy, R., Devesa, J., Laës-Huon, A., ... & Dulaquais, G. (2021). Distribution, speciation and composition of humic substances in a macro-tidal temperate estuary. *Estuarine, Coastal and Shelf Science*, *255*, 107360.

Seidel, M., Yager, P. L., Ward, N. D., Carpenter, E. J., Gomes, H. R., Krusche, A. V., ... & Medeiros, P. M. (2015). Molecular-level changes of dissolved organic matter along the Amazon River-to-ocean continuum. *Marine Chemistry*, *177*, 218-231.

Shen, Y., & Benner, R. (2018). Mixing it up in the ocean carbon cycle and the removal of refractory dissolved organic carbon. *Scientific reports*, *8*(1), 1-9.

Shen, Y., & Benner, R. (2020). Molecular properties are a primary control on the microbial utilization of dissolved organic matter in the ocean. *Limnology and Oceanography*, *65*(5), 1061-1071.

Stepanauskas, R., JØrgensen, N. O., Eigaard, O. R., Žvikas, A., Tranvik, L. J., & Leonardson, L. (2002). Summer inputs of riverine nutrients to the Baltic Sea: bioavailability and eutrophication relevance. *Ecological monographs*, *72*(4), 579-597.

Takasu, H., Okamura, T., Komorita, T., Shiragaki, T., & Uchino, K. (2023). Contribution of riverine dissolved organic carbon to organic carbon decomposition in the Ariake Sea, Japan, a coastal area suffering from summer hypoxia. *Aquatic Sciences*, *85*(1), 1-10.

Walker, B. D., Beaupré, S. R., Guilderson, T. P., McCarthy, M. D., & Druffel, E. R. (2016). Pacific carbon cycling constrained by organic matter size, age and composition relationships. *Nature Geoscience*, *9*(12), 888-891

Wienhausen, G., Noriega-Ortega, B. E., Niggemann, J., Dittmar, T., & Simon, M. (2017). The exometabolome of two model strains of the Roseobacter group: a marketplace of microbial metabolites. *Frontiers in microbiology*, *8*.

Wünsch, U. J., Geuer, J. K., Lechtenfeld, O. J., Koch, B. P., Murphy, K. R., & Stedmon, C. A. (2018). Quantifying the impact of solid-phase extraction on chromophoric dissolved organic matter composition. *Marine Chemistry*, *207*, 33-41.

Zark, M., Christoffers, J., & Dittmar, T. (2017). Molecular properties of deep-sea dissolved organic matter are predictable by the central limit theorem: Evidence from tandem FT-ICR-MS. *Marine Chemistry*, *191*, 9-15.

Zherebker, A., Rukhovich, G. D., Sarycheva, A., Lechtenfeld, O. J., & Nikolaev, E. N. (2022). Aromaticity Index with Improved Estimation of Carboxyl Group Contribution for Biogeochemical Studies. *Environmental Science & Technology*, *56*(4), 2729-2737.

Zhou, Y., He, D., He, C., Li, P., Fan, D., Wang, A., ... & Sun, Y. (2021). Spatial changes in molecular composition of dissolved organic matter in the Yangtze River Estuary: Implications for the seaward transport of estuarine DOM. *Science of The Total Environment*, *759*, 143531.