Carbon and Hydrogen Isotope Signatures of Dissolved Methane in the Scheldt Estuary

Caroline Jacques1 · Thanos Gkritzalis2 · Jean-Louis Tison1 · Thomas Hartley1 · Carina van der Veen3 · Thomas Röckmann3 · Jack J. Middelburg4 · André Cattrijse2 · Matthias Egger5 · Frank Dehairs6 · Célia J. Sapart1,3

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Abstract
We collected water samples from the Scheldt estuary during December 2015 and November 2016 for methane (CH4) concentration and isotopic composition (δ13C and δD values) analyses, to investigate the origin of the excess dissolved CH4, which is a common feature in estuaries. The Scheldt estuary is a eutrophic, heterotrophic tidal estuary, located at the border between Belgium and the Netherlands. The gas chromatography and mass spectrometry analyses revealed (1) variable dissolved CH4 concentrations reaching up to 302.6 nM in surface waters of the Port of Antwerp, which fits within the higher range of values reported for European estuaries, and (2) the presence of surprisingly high isotopic signatures in the upper estuary. While microbial CH4 production dominates in the lower part of the estuary, we observe a clear trend towards isotopically heavier CH4 upstream where isotopic signatures as enriched as −25.2‰ for carbon and +101‰ for hydrogen were measured. We conclude that microbial oxidation of most of the CH4 pool could explain such enrichments, but that the origin of riverine CH4 enriched isotopic signatures remains to be explained. This study identifies peculiar features associated with CH4 cycling in the Scheldt estuary, paving the way for a more thorough biogeochemical quantification of various production/removal processes.

Keywords Dissolved methane · Stable isotopes · Scheldt estuary · Microbial production · Oxidation

Introduction
The distribution of dissolved CH4 in estuaries is governed mainly by riverine inputs, diffusion from the sediments, microbial production in micro-environments, leakage from oil and gas local industries, groundwater discharge, efflux to the atmosphere and microbial consumption (Scranton & McShane, 1991; Middelburg et al., 2002). Given the complexity of estuarine systems, the relative contribution of these processes to the dissolved CH4 pool is subject to wide spatial and temporal variations (Abril & Borges, 2004).

CH4 produced in anaerobic aquatic environments can be of thermogenic or microbial origin. The two main microbial production pathways are acetate fermentation (see, e.g. Whiticar, 1999):

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Caroline Jacques
caroline.jacques@ulb.ac.be
1 Laboratoire de Glaciologie, Université libre de Bruxelles, Avenue F.D. Roosevelt 50, 1050 Brussels, Belgium
2 Vlaams Instituut voor de Zee, Flanders Marine Institute, Wandelaarkaai 7, 8400 Ostend, Belgium
3 Institute for Marine and Atmospheric Research Utrecht, Utrecht University, Princetonplein 5, 3584 CC Utrecht, The Netherlands
4 Department of Earth Sciences, Utrecht University, Princetonlaan 8a, 3584 CB Utrecht, The Netherlands
5 The Ocean Cleanup Foundation, Batavierenstraat 15, 3014 JH Rotterdam, The Netherlands
6 Analytical, Environmental and Geochemistry Department, Vrije Universiteit Brussel, Pleinlaan 2, 1050 Brussels, Belgium
\[ \text{CH}_3\text{COOH} \rightarrow \text{CH}_4 + \text{CO}_2, \]

and CO₂ reduction:
\[ \text{CO}_2 + 4\text{H}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O} \]

The determination of CH₄ stable isotope signatures (δ¹³C-CH₄ and δD-CH₄) has shown high potential to elucidate CH₄ sources and sinks in multiple studies (e.g. Schoell, 1988; Whiticar, 1999). Stable isotope signatures can provide information on the processes responsible for CH₄ production and uptake as they are associated with a characteristic isotopic fractionation. The acetate fermentation pathway produces CH₄ depleted in deuterium (δD from −400 to −285‰) compared to the CO₂ reduction pathway (δD from −250 to −170‰) (Witichar, 1999). Thermogenic CH₄ is typically enriched in ¹³C whereas microbial CH₄ is typically depleted. When sulphate-reducing bacteria outcompete methanogens for acetate in marine sediments, CO₂ reduction becomes the main microbial pathway (Witichar, 1999). More recently, aerobic pathways of CH₄ production from methylated compounds in oligotrophic oceanic waters have been reported (Karl et al., 2008; Damm et al., 2010). Sasakawa et al. (2008) reported δ¹³C values up to +5.9‰ in CH₄ emitted from sinking particles in subsurface seawater and attributed this strong enrichment to active microbial CH₄ oxidation occurring at anoxic/oxic boundaries present in these particles. Repeta et al. (2016) identified a new pathway, involving bacterial degradation of organic matter phosphonates, responsible for the production of CH₄ in aerobic surface waters. This process could resolve the marine CH₄ paradox, which refers to the ubiquitous CH₄ supersaturation in ocean surface waters, implying that methanogenesis occurs in the presence of oxygen. The δ¹³C signatures of the CH₄ produced by this pathway are in the range of enriched values commonly measured in the ocean surface, from −47 to −44‰ (Repeta et al., 2016).

The purpose of this study was to measure, using high precision techniques, both isotopic signatures of CH₄ in surface waters of the Scheldt estuary, which is a well-studied environment located at the border between Belgium and the Netherlands. We illustrate how the co-isotopic tool can be applied to an estuarine environment to investigate dissolved CH₄ dynamics.

**Study Location**

From its source in northern France, the Scheldt spreads over 355 km in Belgium and the Netherlands before flowing into the North Sea. The catchment basin is densely populated with approximately 13 million inhabitants (Baeyens et al., 1998) and is home to intense industrial and agricultural activities. The main city along the estuary is Antwerp whose port hosts several companies operating in the oil and chemical sector. A nuclear power plant composed of 4 nuclear reactors is located at Doel, downstream of the port area (Fig. 1a). For most of the twentieth century, wastewater originating mainly from Brussels was discharged in the river via the Rupel without further treatment, seriously deteriorating the water quality and leading to anoxia in the upper part of the estuary (Soetaert & Herman, 1995; Baeyens et al., 1998; Soetaert et al., 2006). Thanks to the implementation of an effective wastewater management plan in the 1980s, the water quality has gradually improved, with declining loads of total nitrogen and total phosphorus (Soetaert et al., 2006).

Given its hydrodynamical properties, the Scheldt estuary is conveniently divided into two zones (Baeyens et al., 1998). Taking the mouth of the estuary at Vlissingen as the 0 km reference point, the “lower estuary” extends up to 60 km upstream (at the Belgian-Dutch border) and is characterised by salinities typically comprised between 10 and 30. The “upper estuary” extends from 60 to 97 km (at the confluence of the Scheldt and the Rupel rivers), which includes the Port of Antwerp (Fig. 1a), and encompasses the zone of maximum turbidity, typically located at salinities between 2 and 10 (Baeyens et al., 1998). The location of the salinity transition between the upper and the lower estuary is not strict and varies with tides and river discharge. A dry period will result in an upstream shift (or salinity incursion), whereas a wet period will result in a downstream shift of this limit. Given the relatively low discharge of the Scheldt river (on average about 120 m³ s⁻¹), water exchange in the estuary is mostly governed by tides, leading to high residence times (months) and intense vertical mixing (Baeyens et al., 1998). Due to the large tidal range, the estuary is characterised by extensive intertidal areas, the greatest one being the brackish marsh of Saeftinghe, near the Belgian-Dutch border (green area in Fig. 1a).

**Methods**

Surface water was sampled at a total of 89 stations (numbered 1–49 and 100–139) starting from Ostend, at the Belgian coast, to Antwerp, following the rising tide, and back, on RV Simon Stevin, during fall 2015 and 2016 (8–9/12/2015 and 8–9/11/2016) (Fig. 2). The 2015 cruise reached the confluence between the Scheldt and the Rupel. During the 2016 cruise, the water column was also sampled at three depths (bottom, mid-depths and surface) at 9 stations (Fig. 1a and b). Water samples were collected using 60-mL borosilicate bottles, following established sampling protocols (Reeburgh, 2007; Borges et al., 2016): two bottles for concentration measurements and two bottles for isotope analyses. All samples were poisoned.
with HgCl₂ (1 μL mL⁻¹), closed with a butyl septum and an aluminium crimp seal and stored in the dark at ambient temperature until measurement. CH₄ concentrations were measured on a gas chromatograph (GC) equipped with a flame ionisation detector (FID), after headspace equilibration, as described in Borges et al. (2016). Raw data were corrected based on Upstill-Goddard et al. (1996) with Henry’s constants from Yamamoto et al. (1976). The measurement precision estimated from sample duplicates is ± 2.2 nmol L⁻¹.

Stable isotope analyses were conducted using a CH₄ preconcentration system coupled to a ThermoFinnigan DeltaPlus XL isotope ratio mass spectrometer (Brass & Röckmann, 2010; Sapart et al., 2011). In each sample, 20 mL of He was injected to create a headspace and bottles were then vigorously shaken for 1 min and left to equilibrate for an hour as described in Sapart et al. (2017). The headspace was retrieved by addition of 20 mL of MilliQ water to the bottles and injected into a 40-mL sample loop. All δ values are reported in ‰ vs VPDB for δ¹³C values and ‰ vs VSMOW for δD values. Precisions (1σ) for δ¹³C-CH₄ and δD-CH₄ measurements were ± 0.2‰ and ± 6.1‰, respectively. The standard deviation was calculated from reference air injections (Brass & Röckmann, 2010) for samples having similar concentrations.

Results

In December 2015, the dissolved CH₄ concentrations ranged between 15.9 and 94.7 nM in surface waters, with lowest values found in the upper estuary (Fig. 2a and Table S1). Saturation ratios were calculated with respect to CH₄ concentrations in equilibrium with an atmospheric mixing ratio of 1.9 ppm at in situ salinities and temperatures measured, following Wiesenburg and Guinasso (1979). Surface waters were supersaturated in CH₄ at all stations (Table S1), with supersaturation ratios ranging between 4.3 in the upper estuary and 28.6 in the lower estuary. The lower and upper parts of the estuary displayed distinct carbon isotopic signatures, from −65.6 to −51.4‰ and from −44.7 to −25.2‰, respectively (Fig. 2c). The signatures most enriched in heavy isotopes were measured near the confluence of the Scheldt river and the Rupel and in the Port of Antwerp, at salinities lower than 10. This spatial segregation was also apparent in the hydrogen isotopic signatures, with values ranging between −212 and +101‰ in the upper estuary (Fig. 2c).

In November 2016, on the contrary, the highest dissolved CH₄ concentrations in surface waters were measured in the upper estuary, with maximum values of 299.2 nM and
302.6 nM at stations 134 and 135, respectively (Fig. 2b). At the coastal stations and in the lower estuary, maximum concentrations reached 66.8 nM and 114.6 nM, respectively. Saturation ratios ranged from 5.3 to 94.2 (Table S1). As observed in December 2015, CH$_4$ was generally more depleted in heavy carbon isotopes in the lower estuary compared to the upper estuary where $\delta^{13}$C-CH$_4$ values up to $-30.3\permil$ were measured (Fig. 2d). However, at coastal stations, CH$_4$ was also relatively enriched in heavy carbon isotopes, with $\delta^{13}$C-CH$_4$ values between $-44.5$ and $-31.9\permil$. Hydrogen isotope signatures showed a similar pattern, with $\delta$D-CH$_4$ enriched in heavy isotopes up to $-66.3\permil$ at coastal stations and up to $+6.7\permil$ in the Port of Antwerp (Fig. 2f). In the water column, isotopic signatures amongst the most depleted in heavy isotopes were measured at stations “2” and “19”, whose depth profiles also show little change, possibly indicating good vertical mixing (lower estuary, Fig. 1b). Stations “39”, “68” (where dissolved CH$_4$ concentrations reach 398.8 nM at the bottom) and “71” show evidence of upward diffusion of CH$_4$ from the sediments, associated with an isotopic enrichment towards the surface. Stations “65”, “75” and “78” show interesting profiles, with little change in CH$_4$ concentrations and $\delta^{13}$C signatures, but a sudden hydrogen isotope shift towards the surface. Very enriched carbon (up to $-30.2\permil$) and hydrogen (up to $+64.4\permil$) isotopic signatures were measured at station “75” (upper estuary), along the depth profile. At station “80”, dissolved CH$_4$ concentration decreased from the surface towards the riverbed, concurrent with an enrichment in heavy isotopes at mid-depth. A noticeable feature during the second cruise (November 2016) is an increase in water temperature by up to 2 °C when passing the Doel nuclear power plant, near stations 125 and 136 (Figure S1).

**Discussion**

During both cruises, CH$_4$ concentrations significantly exceeded equilibrium solubility, indicating that surface waters of the Scheldt estuary acted as a source of CH$_4$ to the atmosphere, consistent with prior work in this and other European estuaries (Middelburg et al., 2002; Upstill-Goddard & Barnes, 2016). The non-conservative behaviour of dissolved CH$_4$ along the salinity gradient in December 2015 and November 2016 (Fig. 3a) has been observed in other estuaries (Sansone et al., 1999) and points towards localised sources and sinks such as riverine inputs, methanogenesis in the riverbed and intertidal sediments, microbial oxidation and efflux to the atmosphere. In December 2015, a local minimum in CH$_4$
concentration was observed at salinities between 2 and 6. In November 2016, we lack freshwater end-member samples, but CH₄ concentrations decreased again upstream of the maximum observed at salinities around 10. Riverine CH₄ likely undergoes intense oxidation in the estuarine turbidity maximum (ETM), typically located at these salinities, as highlighted in Abril et al. (2007). Unfortunately, we did not measure suspended particulate matter (SPM) concentrations that would support this hypothesis. In November 2016, CH₄ concentrations reached maximum values (up to 302.6 nM) at intermediate salinities. Such a local maximum (also observed at salinities between 5 and 10 in Middelburg et al. (2002) during May, June, October and December) is present, but to a lower extent (67.5 nM), in our December 2015 data set and might be attributed to CH₄ formation from localised peaty sediments in the Port of Antwerp (Baeyens et al., 1998). Vertical profiles of CH₄ at stations “68” and “71” show highest concentrations towards the sediment, progressively decreasing towards the surface, in agreement with this hypothesis (Fig. 1b). Dissolved CH₄ concentrations are subject to large temporal variability, given the influence of physical environmental factors such as tidal mixing and river discharge, as illustrated by the differences in surface concentrations between both years (Fig. 3a). For example, falling tides influence the concentration of CH₄ by exerting a lower hydrostatic pressure on sediments, favouring the release of CH₄ bubbles accumulated in pore waters due to the low solubility of the gas (Chanton et al., 1989; Middelburg et al., 1996). At low tide, the upward migration of gas bubbles in the shallow water column is fast, reducing the gas exposure to microbial oxidation, i.e. it bypasses the microbial CH₄ consumption zone. Conversely, bubble production and release are suppressed at high tide, which also increases CH₄ exposure to microbial oxidation and further reduces the amount of CH₄ emitted to the atmosphere. River discharge and subsequent mixing with seawater will also influence dissolved CH₄ concentrations in the estuary (de Angelis & Lilley, 1987). According to the Belgian Royal Meteorological Institute (RMI), precipitation was abnormally low in September 2016, leading to a low river discharge in fall 2016 (Maris & Meire, 2017), likely explaining the salinity intrusion in the upper estuary in November 2016 (Figure S2), and the shift of the local CH₄ maximum to salinities between 10 and 15 (Fig. 3a).

Stable isotope analysis can represent a powerful tool to determine the origin of CH₄ in aquatic environments (e.g. Schoell, 1988; Whiticar, 1999), because of the characteristic isotopic fractionation associated with CH₄ production and consumption pathways. The pattern of δ¹³C and δD along the salinity gradient was similar for both years (Fig. 3b and c). We observe a gradual shift from signatures enriched in heavy isotopes at low salinities (the upper estuary) to depleted

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**Fig. 3** Evolution of (a) dissolved CH₄ concentrations (nM), (b) δ¹³C-CH₄ (‰ vs VPDB) and (c) δD-CH₄ (‰ vs VSMOW) with salinity in surface waters of the Scheldt estuary in December 2015 (red circles) and November 2016 (blue triangles). The grey dashed line represents the range of equilibrium solubilities (2.8–3.8 nM) calculated for the temperatures and salinities measured during both cruises and with an atmospheric mixing ratio of 1.9 ppm.
signatures at higher salinities (lower estuary), followed by an enrichment at the mouth of the estuary and the coastal area.

We also report our isotopic data on a dual isotope plot (Fig. 4). They show a linear trend and fit in between and above the three domains defined by Whiticar (1999) for the main CH4 production pathways: acetate fermentation, CO2 reduction and thermogenic degradation. Isotopic values that are so enriched in both heavy isotopes (up to −25.2‰ for δ13C and +101‰ for δD) are quite unusual. The gradual depletion of isotopic signatures from the upper estuary to the mouth could indicate an increasing contribution of microbial CH4 production, likely originating from the mineralisation of locally produced organic matter (Middelburg et al., 1996). The lower estuary indeed harbours extensive intertidal flats and marshes, known to release CH4 (Middelburg et al., 1996, 2002). The reasons behind the unusually enriched isotopic signatures measured in the upper estuary however remain unclear.

The linear array of δ13C and δD in Fig. 4 could also possibly reflect an increasing contribution of closed system microbial oxidation modifying a single source signal. This would occur in case methanotrophs are active, since these preferentially consume lighter CH4, leaving the remaining pool more enriched in both heavy isotopes. An extreme case of that process could lead to the unusually high isotopic values observed in the upper estuary. However, in the case of a simple closed system, the isotopic enrichment due to oxidation should be accompanied by CH4 consumption and therefore a decrease in CH4 concentration, which is clearly not observed for the whole Scheldt estuary data set of Fig. 4. This would confirm that local CH4 sources and sinks play a key role in this complex system, evolving far from a single source-closed system.

Sansone et al. (1999) observed a wide range of isotopic signatures in five American estuaries, with δ13C values as enriched as −36.2‰ in freshwater end-members, and values as depleted as −60.4‰ at the mouth. Unfortunately, δD was not measured in their study. To our best knowledge, our work is the first to measure both CH4 isotopic signatures in an estuary, at high spatial resolution, and to report such enriched values. Few studies report extremely positive values for δD-CH4 from gas seeps (Etiope et al., 2011; Daskalopoulou et al., 2018; Milkov & Etiope, 2018), but this has never been observed in estuarine environments. In order to investigate whether microbial oxidation alone could lead to isotopic signatures as enriched as the ones we measured in the upper estuary, we plotted all (surface and profiles) our δ13C and δD data as a function of the logarithm of their CH4 concentration in Fig. 5a and b, respectively. We grouped the stations by zone and by year and calculated linear regressions (Table 1).

The best fit (R2 = 0.78 for δ13C and 0.83 for δD), obtained for upper estuary stations in November 2016 (dark blue triangles in Fig. 5b), indicates that the low river discharge encountered that year, created particular conditions that could be approximated by a closed system, where dissolved CH4 is consumed by a single sink. According to the Rayleigh fractionation model, the slope of this relationship can be used to derive isotopic fractionation factors (ε) of 12.8‰ for carbon and hydrogen, respectively. Conversely, we attribute the weak correlations obtained in December 2015 and in the lower estuary in November 2016, to the fact that a single process (e.g. microbial oxidation) does not dominate the CH4 dynamics but rather a combination of processes. This demonstrates the limitation of applying the Rayleigh approach to our data set, since the basic assumption of single source-closed system conditions is not satisfied. Nevertheless, this interpretation qualitatively illustrates how concentration and isotope signatures are expected to co-evolve due to oxidation, and allows investigating whether this oxidation could be responsible for such unexpected observed isotopic signatures, at least for part of the data set. Moreover, the deduced ε values fall within the ranges that are expected from previous investigations in the literature. To our knowledge, only one study reports fractionation factors associated with CH4 oxidation in estuaries, with εC values ranging from 4.2 to 12‰ (Sansone et al., 1999). The range of εC and εD values attributed to

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**Fig. 4** Dissolved CH4 concentrations (nM) in surface waters of the Scheldt estuary in December 2015 (a) and November 2016 (b), reported on a dual isotope plot together with the typical isotopic composition of oceanic CH4 sources, adapted from Whiticar (1999) and delimited by locations (see Fig. 1). Note the different concentration scales.
anaerobic oxidation of CH$_4$ in coastal sediments is typically of 8–12‰ and 100–180‰, respectively (e.g. Alperin & Reeburgh, 1988; Martens et al., 1999). The isotopic fractionation induced by aerobic oxidation measured in laboratory incubations ranges from 13 to 26‰ for carbon and from 97 to 350‰ for hydrogen (Coleman et al., 1981).

Based on our best fit estimates of $\varepsilon_C$ and $\varepsilon_D$ for the upper estuary stations in November 2016, theoretical oxidation lines incubations ranges from 13 to 26‰ for carbon and from 97 to 350‰ for hydrogen (Coleman et al., 1981).

Based on our best fit estimates of $\varepsilon_C$ and $\varepsilon_D$ for the upper estuary stations in November 2016, theoretical oxidation lines

**Table 1** Correlation coefficients of the linear regressions performed on the relationship between the $\delta$ signatures and the logarithm of their concentrations, in the upper and lower part of the estuary, in December 2015 and November 2016

| Year            | Zone                              | $R^2$ $\delta^{13}C$ vs ln (CH$_4$) | $R^2$ $\delta D$ vs ln (CH$_4$) |
|-----------------|-----------------------------------|-------------------------------------|----------------------------------|
| December 2015   | Lower estuary                     | 0.20                                | 0.51                             |
|                 | Upper estuary                     | 0.04                                | 0.01                             |
| November 2016   | Lower estuary with coastal stations | 0.36                                | 0.30                             |
|                 | Lower estuary without coastal stations | 0.06                                | 0.33                             |
|                 | Upper estuary                     | 0.78                                | 0.83                             |
were drawn taking the highest CH$_4$ concentration in each group, as the initial value (Fig. 5a and b). This extrapolation to the lower estuary and to the December 2015 data set allows investigating the influence of microbial oxidation on potential CH$_4$ sources, surmising fractionation factors were constant in time and space. In November 2016, the oxidation line reproduces quite well the pattern observed for upper estuary stations, implying that in theory, aerobic oxidation has the potential to lead to $\delta^{13}$C and $\delta$D values as enriched as $-30.3\%e$ and $+64.4\%e$, respectively. These enriched signatures coincide with very low remaining fractions of dissolved CH$_4$ (<0.2). This implies that either the observed signatures are inherited from upstream riverine oxidation processes or that 80% of the CH$_4$ produced by the mineralisation of organic matter in the upper estuary would need to be oxidised despite the low exposure time induced by the shallow water column at that location. This is in agreement with the findings of de Angelis and Scranton (1993) and Abril and Iversen (2002) who showed that oxidation rates are particularly high in the upper part of an estuary, at low salinities. This can be attributed to a salinity effect on methanotrophic activity (de Angelis & Scranton, 1993; Osudar et al., 2015; Osudar et al., 2017) and to the relative high biomass of nitrifying and methanotrophic bacteria in estuarine turbidity zones in the upper estuary (Owens, 1986; Abril et al., 2000; Abril et al., 2007). In any case, the origin of riverine CH$_4$ highly enriched in heavy isotopes (our first option) is still conjectural and cannot be resolved with our current data set. Reasonable assumptions include methanogenesis from highly oxidised organic matter, aerobic oxidation of CH$_4$ produced upstream or groundwater discharge, which can carry dissolved CH$_4$ quite enriched in heavy isotopes (Vigneron et al., 2017). Additional sampling upstream is required to investigate these hypotheses.

Except for the upper estuary in November 2016, the potential oxidation lines drawn in Fig. 5a and b for the other groups only explain part of the data distribution, implying that other processes are at stake. In this very dynamic estuarine system, mixing between dissolved CH$_4$ molecules produced at different locations and at different stages of oxidation should also be considered. With this in mind, we drew a few exemplative dilution curves between extreme end-members to illustrate the impact of this process on the data distribution and how it would blur the simplistic trend attributed to aerobic oxidation (dashed lines, Fig. 5a and b). In the specific case of upper estuary stations in November 2016, the dilution curve reproduces well the dispersion of the observed distribution (dark blue triangles in Fig. 5a and b). This study identifies three potential dissolved CH$_4$ pools with distinct isotopic signatures (Fig. 5c): riverine CH$_4$ with surprisingly enriched $\delta$ values (red ellipse), highly concentrated CH$_4$ with depleted $\delta$ values produced locally in the sediments of the Port of Antwerp (orange ellipse), and CH$_4$ with depleted $\delta$ values from newly produced organic matter in the lower estuary (yellow ellipse). The concurrent action of aerobic oxidation and mixing therefore supports the wide range of isotopic signatures and concentrations measured in this study. Figure 5d summarises these ranges in each zone and highlights the spatial prevalence of each CH$_4$ pool in the lower and upper parts of the estuary.

Finally, it is worth noting the special case of the coastal stations. These are located in an area where high dissolved CH$_4$ concentrations were reported due to the presence of gassy sediments (Borges et al., 2016). Based on the enriched signatures we measured in surface waters of this area, the origin of the CH$_4$ could be either thermogenic or microbial with a longer exposure to oxidation due to the increase in water column depth. The first option is supported by the position of some of the coastal samples in Fig. 4b. The latter option cannot be confirmed by the limited coastal data set in Fig. 5a and b. Further sampling above the sediments and in the water column for concentration and isotopic analyses will help identify the origin of this CH$_4$ enriched in heavy isotopes.

**Conclusion**

Our data confirm literature reports of very high and variable dissolved CH$_4$ concentrations in the Scheldt estuary. We have complemented these measurements with a unique data set of both CH$_4$ stable isotopes ($\delta^{13}$C and $\delta$D). We show that CH$_4$ dissolved in the upper estuary is unusually enriched in $^{13}$C and D and results either from an intense aerobic oxidation in the upper estuary, an unknown upstream source or both. This enriched CH$_4$ is further exposed to aerobic oxidation along the estuary and undergoes mixing with depleted CH$_4$ produced by methanogenesis in the sediments, before entering the North Sea. Complex biogeochemical models should be used at a later stage to refine the analysis and more strictly quantify the processes identified in this study. To further investigate the estuarine CH$_4$ cycling, we recommend additional water sampling upstream to characterise the contribution of riverine input to the enriched signatures measured at salinities close to 0. We further recommend that future studies also include atmospheric and flux measurements in order to better understand the role of the estuary in terms of CH$_4$ emissions. Our observed CH$_4$ isotope anomalies in the Scheldt estuary highlight that important unknowns remain to be resolved for the estuarine CH$_4$ cycle.

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