Estuarine, Coastal and Shelf Science 160 (2015) 10–21

Contents lists available at ScienceDirect

Estuarine, Coastal and Shelf Science

journal homepage: www.elsevier.com/locate/ecss

Environmental factors affecting methane distribution and bacterial methane oxidation in the German Bight (North Sea)

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ARTICLE INFO

Article history:
Received 14 July 2014
Accepted 22 March 2015
Available online 3 April 2015

Keywords:
methanotrophy
estuary
Elbe River

ABSTRACT

River estuaries are responsible for high rates of methane emissions to the atmosphere. The complexity and diversity of estuaries require detailed investigation of methane sources and sinks, as well as of their spatial and seasonal variations. The Elbe river estuary and the adjacent North Sea were chosen as the study site for this survey, which was conducted from October 2010 to June 2012. Using gas chromatography and radiotracer techniques, we measured methane concentrations and methane oxidation (MOX) rates along a 60 km long transect from Cuxhaven to Helgoland. Methane distribution was influenced by input from the methane-rich mouth of the Elbe and gradual dilution by methane-depleted sea water. Methane concentrations near the coast were on average 30 ± 13 nmol L^{-1}, while in the open sea, they were 14 ± 6 nmol L^{-1}. Interestingly, the highest methane concentrations were repeatedly detected near Cuxhaven, not in the Elbe River freshwater end-member as previously reported. Though, we did not find clear seasonality we observed temporal methane variations, which depended on temperature and presumably on water discharge from the Elbe River. The highest MOX rates generally coincided with the highest methane concentrations, and varied from 2.6 ± 2.7 near the coast to 0.417 ± 0.529 nmol L^{-1} d^{-1} in the open sea. Turnover times varied from 3 to >1000 days. MOX rates were strongly affected by methane concentration, temperature and salinity. We ruled out the supposition that MOX is not an important methane sink in most of the Elbe estuary and adjacent German Bight.

1. Introduction

Methane is the most abundant organic gas in the atmosphere. Being a potent greenhouse gas, it plays an important role in warming the Earth's atmosphere (Kirschke et al., 2013). Its contribution to global warming is attenuated by comparatively low concentrations and short lifetime in the atmosphere. Methane has the second-largest radiative forcing of the long-lived greenhouse gases, after CO_2 (Ramaswamy et al., 2001). The atmospheric concentration of methane has increased to a level unprecedented in at least the last 800,000 years (Stocker et al., 2013). Its average concentration nowadays is around 1.8 ppm (Kirschke et al., 2013). Changes in methane concentration, which are defined by the balance between sources and sinks of methane, have led to investigations on the microbial-driven methane cycle in various environments.

About 60% of the methane released to the atmosphere is from anthropogenic sources such as agriculture, waste treatment, biomass burning, and fossil fuels. The remaining 40% originates from natural sources, mainly wetlands (Kirschke et al., 2013). Among these sources, the world's oceans contribute <0.1–10% of the methane emissions (Scranton and McShane, 1991; Bates et al., 1996; Middelburg et al., 2002; Conrad, 2009). Digestive tracts of zooplankton (Bianchi et al., 1992; De Angelis and Lee, 1994), and

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http://dx.doi.org/10.1016/j.ecss.2015.03.028

organic particulate matter (Karl and Tilbrook, 1994) are the main methane sources in the open ocean. However, about 75% of global oceanic methane emission occurs in shelf areas and estuaries (Bange et al., 1994; Bange, 2006). The main sources of methane in these areas are sediments, adjacent rivers, tidal flats, and marshes (Bange et al., 1994; Bange, 2006; Abril et al., 2007; Grunwald et al., 2009; Bussmann, 2013). Sedimentary release of methane which follows methanogenesis (Koch et al., 2009; Bussmann, 2013) and abiotic methane production (Hovland et al., 1993) is supplemented with lateral methane transport. Water discharge from rivers, as well as tidal influence, are factors that greatly control methane distribution (Rehder et al., 1998; Grunwald et al., 2009). Thus, these areas represent diverse and complex hydro-dynamic systems. Besides, estuarine systems are subject to significant seasonal changes (Sansone et al., 1998; Middelburg et al., 2002; Abril and Borges, 2005), a factor which is not considered in many studies. Bacterial methane oxidation, along with degasing and dilution of methane-rich water, are also important factors controlling methane distribution in the water column (Grunwald et al., 2009). Up to 80–90% of the available methane can be metabolized and thereby disposed of by bacterial methane oxidation in the freshwater (Reeburgh et al., 1993; Guérin and Abril, 2007). In the marine environment, however, methane oxidation (MOX) rates are in general lower (Valentine et al., 2001; Mau et al., 2013). Though, knowledge on bacterial methane oxidation in the water column rapidly improves, MOX rates measurements in estuaries are still scarce. Additionally, an improved knowledge of the environmental factors controlling bacterial methane oxidation and the physiological properties of these organisms is crucial and this topic needs further investigation (Valentine, 2011; Mau et al., 2013).

The objectives of this study were to describe the spatial and seasonal variations of methane in the German Bight, near the Elbe estuary. The methane concentration, as well as several hydrochemical parameters from the bottom and surface waters, were measured over a period of two years along a 60 km long transect in the German Bight. Along with temperature, the concentrations of inorganic nutrients (ammonium, nitrite, nitrate, phosphate, and silicate) and suspended particulate matter (SPM) were measured. Salinity was an especially important parameter, as it is a direct indicator of the extent of water discharge of the Elbe River. The aim of this study was to bring the importance of bacterial methane oxidation as a significant methane sink into focus. Therefore, we made highly sensitive radiotracer measurements to estimate MOX rates in the water column (Valentine et al., 2001). Only on the basis of these comprehensive examinations we were able to determine the main environmental factors that control methane distribution and MOX rates.

2. Materials and methods

2.1. Study area

The North Sea (including its estuaries and fjords) has a surface area of about 750,000 km^2 and a volume of about 94,000 km^3 (Commission, 2000). The non-tidal circulation of the North Sea is dominated by a cyclonic residual current. Water from the Atlantic (Fair Isle Current, Shetland Flow) flows southward along the British coast and returns northward, together with influxes from the English Channel and various rivers along the coasts of the Netherlands, Germany, and Denmark (Rehder et al., 1998). The German Bight is the south-eastern bight of the North Sea, bounded by the Netherlands and Germany to the south, and Denmark and Germany to the east (the Jutland peninsula). To the north and west, it is bounded by the Dogger Bank. The depths in this area vary mainly from 20 to 40 m (Czitrom et al., 1988). The German Bight consists mainly of a mixture of the Central (Southern) North Sea water masses and continental coastal waters (Becker et al., 1992). The water column in the central (southern) North Sea can be stratified into two slightly different layers (Czitrom et al., 1988; Becker et al., 1992). Inshore water did not show any stratification in either summer or winter. Freshwater discharge from the Elbe and Weser rivers causes a large salinity contrast near the shore (Czitrom et al., 1988). Analysis of horizontal density gradients did not show a clear annual cycle either near the shore or offshore (Czitrom et al., 1988). Surface sediments affected by tidal or residual current, wave action, and heavy bottom trawling are very mobile (ICES, 1988; Becker et al., 1992).

The Elbe is one of the major rivers of central Europe, with a total catchment area of about 150,000 km^2. It runs from the Czech Republic through Germany, and reaches the German Bight in its north-eastern region, near Cuxhaven. The Elbe's mean annual discharge at the river mouth is 860 m^3 s^{-1}. The discharge regime is mainly controlled by rainfall and snowmelt, therefore it peaks in April/May (Simon, 2005).

2.2. Water sampling

Samples were collected for 2 years from 2010 to 2012. Eleven one-day sampling cruises took place on the 7.10, 8.12 in 2010, 12.01, 2.03, 4.05, 6.07, 29.09 in 2011 and 11.01, 29.02, 28.03, 11.06 in 2012. All sampling procedures and some of the processing of the samples were done on board the research vessels 'Uthörn' and 'Ludwig Prandtl'. Seven sampling stations were distributed along the Helgoland–Cuxhaven transect (Fig. 1). The names of the stations were determined by their distance from the northernmost coastal point near Cuxhaven, so the most south-eastern station was denoted 'Sea kilometre (Sk) 1', and the most north-western, near Helgoland, 'Sk 59'. We determined stations Sk 1, Sk 14, and Sk 20 to be 'coastal stations', and stations Sk 27, Sk 32, Sk 49, and Sk 59 to be 'marine stations'. Water samples were collected with Niskin bottles from the water surface (1 m below the surface) and from the bottom (1 m above the bottom).

2.2.1. Oceanographic parameters

Water temperature was measured to monitor seasonality during the study period. Salinity was measured to account for the proportion of freshwater from the Elbe River in North Sea water. Oxygen concentration was measured to investigate its effect on MOX rate, and accordingly, on methane distribution. During the Prandtl cruises, temperature, salinity, and oxygen in the water column were measured immediately after sampling on board using a Universal Pocket Meter (Multi 340i) with precisions of 1% for salinity, 0.1 °C for temperature, and 0.5% for oxygen. Salinity was measured in μS cm^{-1}, and then converted according to the Practical Salinity Scale. For the Uthörn cruises, a sea-bird CTD sensor was mounted to the water sampling rosette. In July 2011, temperature measurements were not made due to technical problems. Thus, we obtained temperature data from the database of the River Basin Community Elbe (RBC Elbe; in German, 'Flussgebietsgemeinschaft (RBC) Elbe; http://www.fgg-elbe.de/elbe-datenportal.html). These data were collected near Cuxhaven two days before our cruise. Comparisons of RBC measurements with ours for other months did not reveal any significant difference (±1 °C). These data from July were excluded from the comparison of temperatures on the surface and on the bottom, but were used for the correlation analysis between temperature and other factors.

2.2.2. Suspended particulate matter (SPM)

Sampled water was filtered using pre-washed and pre-weighed GFC filters (Whatman TM), which were afterwards dried for 24

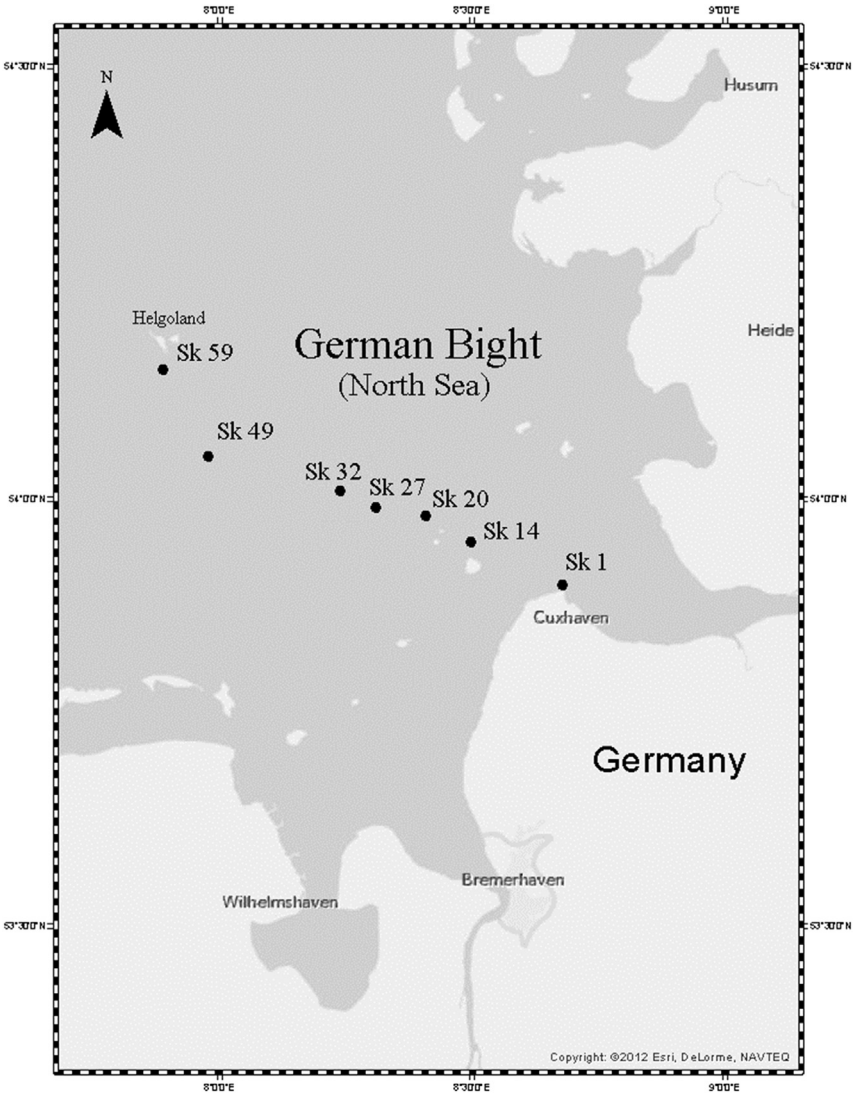

Fig. 1. Map of the German Bight with sampling stations along the Cuxhaven–Helgoland transect.

hours at 60 °C and weighed again. Water volumes varied from 100 to 250 ml, depending on turbidity. Filtration was done on board right after collecting water samples. The other procedures were done in the laboratory.

2.2.3. Inorganic nutrient analysis

Samples for inorganic nutrients (NO_2, NO_3, NH_4, PO_4) and silicate concentrations were taken after sampling for methane and MOX rates. During the Prandtl cruises, sampled water was filtered with GFC filters (Whatman TM), transferred into 50 ml Falcon tubes, and frozen until further analysis in the laboratory. Sampling during the Uthörn cruises and analysis of all the samples was performed by Karen Wiltshire's group (Wiltshire, 2011, 2012) following the technique described by Grasshoff et al. (1983). Nutrient analysis was performed only for surface waters.

2.3. Methane concentration

We measured methane concentrations along the transect in different months to assess seasonal and spatial variations. Right after sampling collected water was transferred, bubble-free, into 120 ml glass serum bottles. The bottles were rinsed with approximately two volumes of sample water, capped with black rubber stoppers, and sealed with an aluminium crimp.

To eliminate agents (such as soap) that inhibit methane oxidation, the glass bottles had been washed and soaked in 3% HCl for 12 hours, and then soaked in distilled water overnight. The stoppers had been autoclaved for 20 min at 120 °C three times, and then rinsed with distilled water.

To determine methane concentrations, samples from each depth and each station were collected in single (March–September 2011) or duplicate bottles (October 2010–January 2011, January–June 2012).

Immediately after filling the bottles, samples were poisoned with 0.3 ml of 25% H_2SO_4 (samples from marine stations) or 0.3 ml of 5N NaOH (samples from coastal stations) to prevent methane oxidation. Poisoning agents were chosen according to the results of preliminary testing of the efficiency of both chemicals in marine and brackish water (Bussmann et al., 2015). Methane concentrations were measured using a headspace technique, by adding 10 ml of N_2 according to McAuliffe (1971). Three 0.1 ml headspace aliquots from each sample were analysed with a gas chromatograph (GC 2014; Shimadzu) equipped with a flame ionization detector and a molecular sieve column (Hay Sep N, 80/100, Alltech). The

temperatures of the oven, the injector, and detector were 40, 120, and 160 °C, respectively. The carrier gas (N_2) flow was 20 ml min^{-1}. The gas flow of the FID was 40 ml min^{-1} for H_2 and 400 ml min^{-1} for synthetic air. Gas standards (Air Liquide) with concentrations of 10 and 100 ppm methane were used for calibration. The measurement error of the GC was less than 5%.

Equilibrium concentrations were calculated according the formula proposed by Wiesenburg and Guinasso Jr (1979). Data on the methane concentration in the atmosphere were obtained from the Mace Head Atmospheric Research Station, located on the west coast of Ireland (http://agage.eas.gatech.edu). Saturation rates were calculated as the ratio between observed methane concentrations in the water column and equilibrium concentrations with respect to the ambient methane concentrations in the atmosphere, multiplied with 100%.

2.4. Methane oxidation (MOX) rate

In addition to the chemical parameters, we also measured the microbial methane oxidation, as a possible important methane sink. Samples for MOX rates were collected from each depth and each station in triplicate bottles. According to Bussmann et al. (2015) the coefficient of variation in this case is about 23 ± 11% at low activities (<10 nmol $L^{-1}d^{-1}$) and 7 ± 5% at higher activities (>10 nmol $L^{-1}d^{-1}$). MOX rates were measured following radiotracer techniques using tritiated methane (American Radiolabeled Chemicals, 20 Ci $mmol^{-1}$) according to a method modified from Valentine et al. (2001). A diluted tracer (0.1 ml) was added to the samples (2 kBq ml^{-1}). Samples were vigorously shaken and incubated for 24 h in the dark at near *in situ* temperatures. After incubation, methane oxidation was stopped by adding 0.3 ml of 25% H_2SO_4 (for marine station samples) or 5N NaOH (for coastal station samples). Controls were stopped before the addition of the tracer.

Under oxic conditions methane is oxidized according the following reaction:

$$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O \quad (1)$$

The consumption of tritiated methane (C^*H_4) leads to the production of tritiated water ($^*H_2O$) and to the decrease of radioactive tracer in the gas phase:

$$C^*H_4 + 2O_2 \rightarrow CO_2 + 2^*H_2O \quad (2)$$

Then, the total radioactivity ($^3H{-}CH_4$ and $^3H{-}H_2O$) added to the sample, and the fraction of the labelled gas oxidized or produced water ($^3H{-}H_2O$) have to be measured. To determine the total radioactivity of the sample, the sample bottle is opened, and 2 ml of the subsample is mixed with a 5 ml scintillation cocktail. To determine the radioactivity of the tritiated water, the rest of the sample was sparged with air to expel all methane. Bottles were then half emptied, a long needle was inserted to the bottom of the bottle, and the sample was purged with air for 30 min. Two ml water aliquots after sparging were mixed with 5 ml of the scintillation cocktail (Ultima Gold LLT, Perkin Elmer) and analysed on a liquid scintillation counter (Tri-Carb® 2910 TR, Perkin Elmer) using decays per minute (dpm) values. The MOX rate in nmol L^{-1} d^{-1} was calculated by taking the ratio of the produced radioactive water to the amount of added tracer ($r = {}^*H_2O/C^*H_4$, in dpm) and multiplying it with the ambient methane concentration ($[CH_4]$ in nmol L^{-1}) and correcting for the incubation time (t in d) (Valentine et al., 2001)

$$MOX = r \times [CH_4]/t \quad (3)$$

The turnover time (tt in days) is the time it would take to oxidize all the methane at a given MOX rate. It was calculated as the inverse of the ratio (r), corrected for the incubation time (t). Thus, this parameter is independent of the ambient methane concentration, and gives a good measure of the methanotrophic potential (Heintz et al., 2012).

However, there might be some $^*H_2O$ which was not the result of methane oxidation but was a contamination of the tracer. The $^*H_2O$ from the killed controls reveals this value. In marine waters, about 0.06% of the injected tracer was found to be 'abiotic water'. In freshwater, the percentage increased to about 0.62%. The value of $^*H_2O$ were corrected for this 'abiotic' water.

2.5. Statistical analysis

For comparison of surface and bottom values and for comparison of values at different stations (spatial variation), we used a paired *t*-test in the case of a normal distribution, and a non-parametric Wilcoxon signed- rank test when a normality test (Shapiro–Wilk) failed. Marine and coastal stations were analysed separately. When no significant difference between data sets was found, the respective data were pooled. To investigate the dependence of methane concentration, turnover time, and MOX rate on hydrographical and chemical factors such as temperature, salinity, methane, phosphate, oxygen, and SPM, we performed simple linear regression analyses. If the linear correlation was not significant, we performed a Spearman rank order correlation analysis, which shows if variables are related monotonically, i.e. an increase of one variable causes an increase/decrease of the other variable. The Spearman correlation coefficient (r_s) is defined as the Pearson correlation coefficient between the ranked variables. For a sample of size *n*, the *n* raw scores X_i, Y_i are converted to ranks x_i, y_i, and r_s is computed as:

$$r_s = \frac{\sum_i (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_i (x_i - \bar{x})^2 \sum_i (y_i - \bar{y})^2}}. \quad (4)$$

Calculations were performed using SigmaPlot for Windows Version 11.0 software.

3. Results

3.1. Oceanographic parameters

No significant difference between surface and bottom temperature was observed (paired *t*-test, $n = 39$, $p = 0.917$), so the data were pooled. The lowest temperatures were measured in January 2011, with an average of 2.6 ± 1.2 °C. The highest temperature (19.0 ± 1.4 °C) was observed in September 2011.

Surface water generally had a lower salinity than bottom water (paired *t*-test, $n = 49$, $p < 0.001$). The stratification of the water column was most pronounced from June to September. Due to the input of freshwater from the Elbe River, salinity increased with distance from the shore. This is well illustrated by two independent sampling campaigns in the summer (July 2011) and winter (February 2012) (Fig. 2). For all sampling dates, at marine station Sk 59, the average salinity was 32.2 ± 1.0, while coastal station Sk 1 had an average salinity of 16.3 ± 5.7. The lowest salinity at this station (7.8) was detected in September 2011, and the highest salinity at this station (24.7) was detected in June 2012.

3.2. Suspended particulate matter, oxygen and inorganic nutrients

Values for SPM ranged from 8 to 167 mg L^{-1}, but neither seasonal nor spatial (along the transect) differences could be detected.

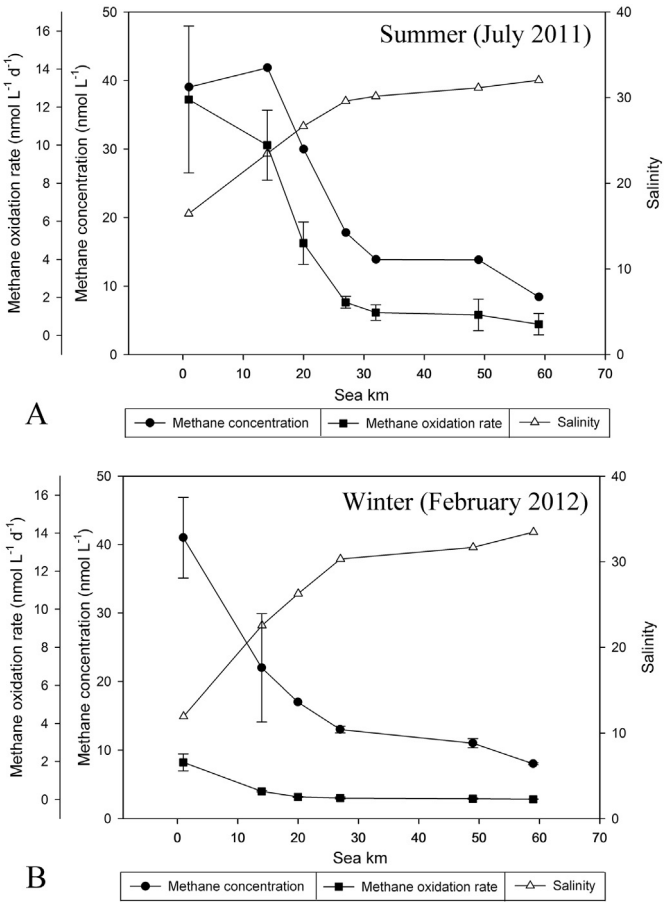

Fig. 2. Distribution of methane concentration, MOX rate, and salinity along the transect in A) summer (July 2011) and B) winter (February 2012). Shown is the average of surface and bottom water.

Bottom water was always more turbid than corresponding surface water (paired *t*-test, $n = 49$, $p < 0.001$, data not shown).

Oxygen concentrations did not show any spatial differentiation along the transect, nor any seasonal trend. The lowest concentration of oxygen (6.1 mg L^{-1}) was detected in October 2010 at station Sk 59, while the highest concentration (11.4 mg L^{-1}) was detected in January 2012 near station Sk 14. Values near the surface (average value of 8.7 mg L^{-1}) were generally slightly higher than near the bottom (average value of 8.5 mg L^{-1}) (paired *t*-test, $n = 42$, $p = 0.021$).

Overall concentrations of NO_2, NO_3, NH_4, and PO_4 decreased towards the sea and were nearly depleted at a distance of 30 km from the coast. Fig. 3 shows representative results from two individual sampling campaigns. Phosphate concentrations ranged from 0.3 μmol L^{-1} (May 2011) to 1.8 μmol L^{-1} (December 2010) at station Sk 59, and from 1.2 μmol L^{-1} (May 2011) to 3.3 μmol L^{-1} (January 2012) at station Sk 1. Nitrite concentrations ranged from 0.1 μmol L^{-1} (October 2010) to 1.2 μmol L^{-1} (March 2011) at station Sk 59, and from 0.7 μmol L^{-1} (May 2011) to 1.5 μmol L^{-1} (January 2012) at station Sk 1. Nitrate concentrations ranged from 0.1 μmol L^{-1} (October 2010) to 25.0 μmol L^{-1} (May 2011) at station Sk 59, and from 81.6 μmol L^{-1} (May 2011) to 219.0 μmol L^{-1} (February 2012) at station Sk 1. Ammonium concentrations ranged from 0.1 μmol L^{-1} (May 2011) to 3.8 μmol L^{-1} (July 2011) at station Sk 59, and from 0.5 μmol L^{-1} (May 2011) to 14.6 μmol L^{-1} (February 2012) at station Sk 1. In the summer months, e.g. July 2011, the ammonium concentration varied significantly over the entire transect. From Sk 1 to Sk 49, the concentration decreased, but suddenly rapidly increased again, until it reached a maximum at Sk 59, which is similar to Sk 1. Silicate concentrations ranged from 1.6 to 15.1 μmol L^{-1} at station Sk 59, and from 21.8 to 155.7 μmol L^{-1} at station Sk 1 in May 2011 and February 2012, respectively.

3.3. Methane concentrations

The results of all the measurements are summarized in Fig. 4 and are published in the data library PANGAEA (Bussmann et al., 2014a). No significant difference between surface and bottom values was observed either for the marine (paired *t*-test, $n = 28$, $p = 0.412$), or coastal stations (paired *t*-test, $n = 20$, $p = 0.522$), so the data were pooled. The dissolved equilibrium concentrations varied from 2.9 to 3.1 nmol L^{-1}. Methane concentrations in the water column for both marine and coastal stations were supersaturated. At the coastal stations saturation rate varied from 340 to 1880%, at the marine stations it varied from 170 to 1110%. At the coastal stations, methane concentrations were, in general, higher than at the marine stations, and decreased offshore from station Sk 1 to station Sk 20. The only exception was detected in September 2011, when values were significantly higher than during the rest of the season. These data were the only ones that did not follow the trend, and stood apart from the rest of the observations, so we omitted it from further processing. The three coastal stations had an average methane concentration of 30 ± 13 nmol L^{-1}. The average methane concentration at station Sk 1 was 40.9 ± 9.4 nmol L^{-1}, ranging from 24.0 nmol L^{-1} in May 2011 to 58.3 nmol L^{-1} in March 2012. The average methane concentration at station Sk 20 was 19.8 ± 8.9 nmol L^{-1}, ranging from 10.0 nmol L^{-1} in May 2011 to 36.0 nmol L^{-1} in June 2012. For the marine stations, no spatial variation of methane concentrations was observed, so the data were pooled. The average methane concentration for the marine stations was 14.0 ± 4.8 nmol L^{-1} ($n = 56$), with a minimum of 5 nmol L^{-1} in March 2011 and a maximum of 32 nmol L^{-1} in December 2010.

To highlight the strong effect of the riverine water and the seasonal trends, two individual sampling campaigns (summer/winter) are shown in Fig. 2. At the coastal stations, methane concentrations were higher in summer (July 2011) than in winter (February 2012). At the marine stations, methane concentrations were approximately the same. Methane concentration decreased offshore, clearly coinciding with the salinity increase in both summer and winter. Regression analysis showed that about 57% of methane variability could be explained by salinity (for all data, $n = 93$, Fig. 5). On four occasions, we were able to sample all stations on one day. For these dates (May 2011, July 2011, January 2012, and February 2012), the correlation was much stronger and the salinity explained 70–98% of the methane variability. When the regression line (Fig. 5) was extrapolated to a salinity of zero, we obtained a prospective methane concentration of 72 nmol L^{-1} for Elbe waters. The corresponding methane concentrations from the single cruise analysis ranged from 56 to 80 nmol L^{-1}.

No linear correlation between methane concentration and temperature was observed, however we detected a temperature dependence after rank transformation of the data. At the coastal stations, the highest methane concentrations were found during periods of highest temperatures ($r_s = 0.49$, $n = 36$). Since two independent parameters correlated with methane concentration at the coastal stations, we applied multiple linear regressions between methane concentration, salinity, and temperature, and obtained a model with an even higher correlation coefficient ($r^2 = 0.74$, $n = 30$) (Fig. 6).

No linear correlation, but a weak positive dependence of methane concentration on oxygen, was found along the entire

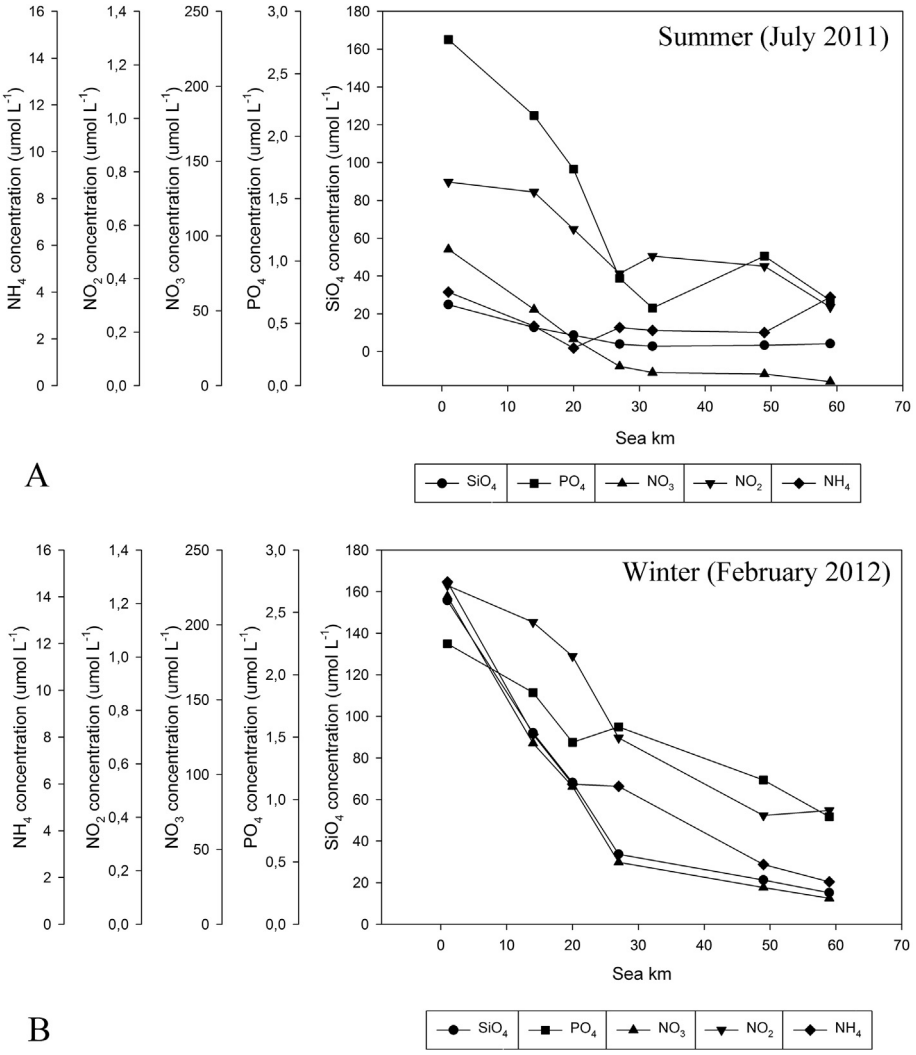

Fig. 3. Distribution of inorganic nutrients in A) summer (July 2011) and B) winter (February 2012). Shown are only data from surface water.

transect ($r_s = 0.37$, $n = 80$). We also found a strong correlation between concentrations of methane and inorganic nutrients, except NO_2 (r_s varied from 0.43 to 0.66 depending on the inorganic nutrient). However, we assumed that this was mainly due to the input of nutrient-rich waters from the mouth of the Elbe, as salinity and inorganic nutrient concentrations were also strongly correlated (r^2 for different mineral nutrients varied from 0.46 to 0.87).

No linear correlation between SPM and methane concentrations was found. Moderate negative dependence was indicated after rank transformation of the marine data. Thus, we can state that at the marine stations, the highest methane concentrations were found at the lowest SPM values ($r_s = -0.45$, $n = 54$).

3.4. Methane oxidation rate

The results of all the measurements are summarized in Fig. 7 and are published in the data library PANGAEA (Bussmann et al., 2014a). No significant differences between surface and bottom values were observed either for the coastal stations (paired *t*-test, $n = 18$, $p = 0.154$), or for the marine stations (paired *t*-test, $n = 28$, $p = 0.290$), so the data were pooled. At the coastal stations, MOX rates were in general higher than at the marine stations, and decreased offshore from station Sk 1 to station Sk 20. The coastal stations had an average MOX rate of 2.6 ± 2.7 nmol $L^{-1}d^{-1}$. The average MOX rate at station Sk 1 (nearest the coast) was 5.26 ± 4.72 nmol $L^{-1}d^{-1}$, ranging from 0.78 nmol $L^{-1}d^{-1}$ in June 2012 to 16.5 nmol $L^{-1}d^{-1}$ in July 2011. The average MOX rate at station Sk 20 was 0.97 ± 1.85 nmol $L^{-1}d^{-1}$, ranging from 0.04 nmol $L^{-1}d^{-1}$ in May 2011 to 5.81 nmol $L^{-1}d^{-1}$ in July 2011. The average MOX rate for the marine stations was 0.417 ± 0.529 nmol $L^{-1}d^{-1}$, with a minimum of 0.009 nmol $L^{-1}d^{-1}$ in February 2012 and a maximum of 1.96 nmol $L^{-1}d^{-1}$ in July 2011.

For the turnover time no significant differences between surface and bottom values were observed either for coastal stations (paired *t*-test, $n = 18$, $p = 0.182$), or marine stations (paired *t*-test, $n = 28$, $p = 0.136$). Thus, the data were pooled. The coastal stations had an average turnover time of 64 ± 75 days. The average turnover time at station Sk 1 was 16 ± 15 days, with a minimum of 3 days in July 2011 and a maximum of 56 days in June 2012. The average turnover time at station Sk 20 was 120 ± 92 days, with a minimum of 5 days in July 2011 and a maximum of 293 days in October 2010. The average turnover time for the marine stations was 196 days, with a minimum of 9 days in July 2011 and a maximum of 1049 days in February 2012. To highlight the strong effect of the riverine water and the seasonal trends, two individual sampling campaigns (summer/winter) are shown in Fig. 2. MOX rates along the transect

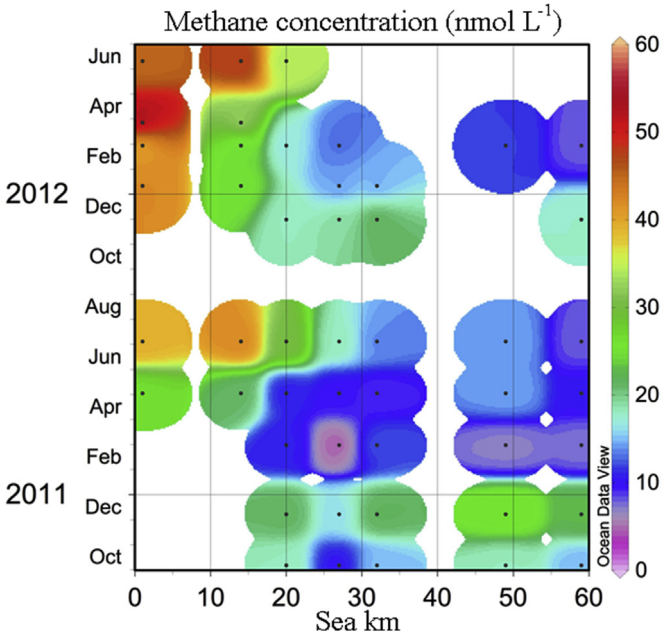

Fig. 4. Methane concentration along the transect during the whole period of investigation (surface and bottom).

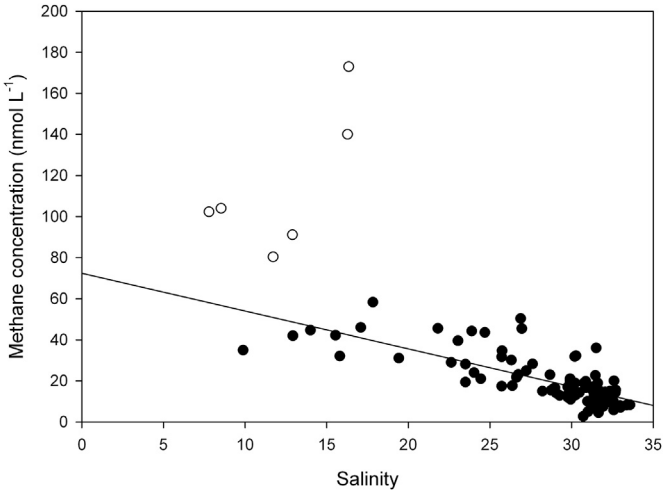

Fig. 5. Linear regression between salinity and methane concentration. Empty circles indicate outliers, which were excluded from regression analysis (measurements from September 2011).

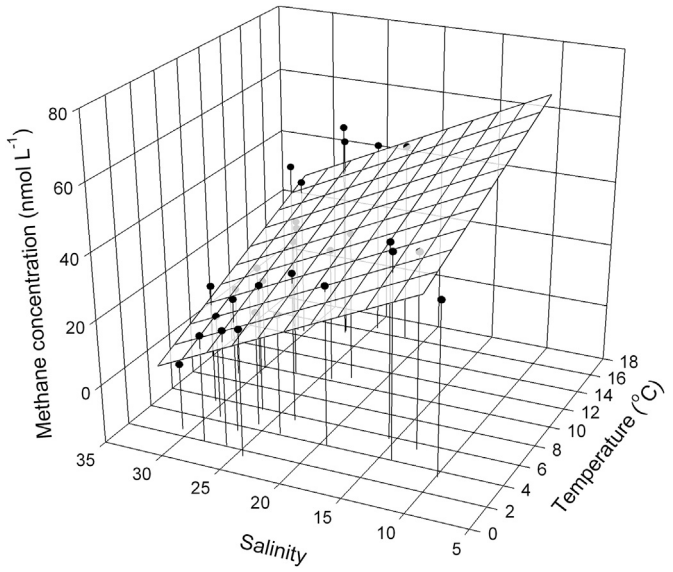

Fig. 6. Methane distribution at the coastal stations according to salinity and temperature. Multiple linear regression: CH_4 = 55.798 + (1.697*Temperature) − (1.709*Salinity); r^2 = 0.737.

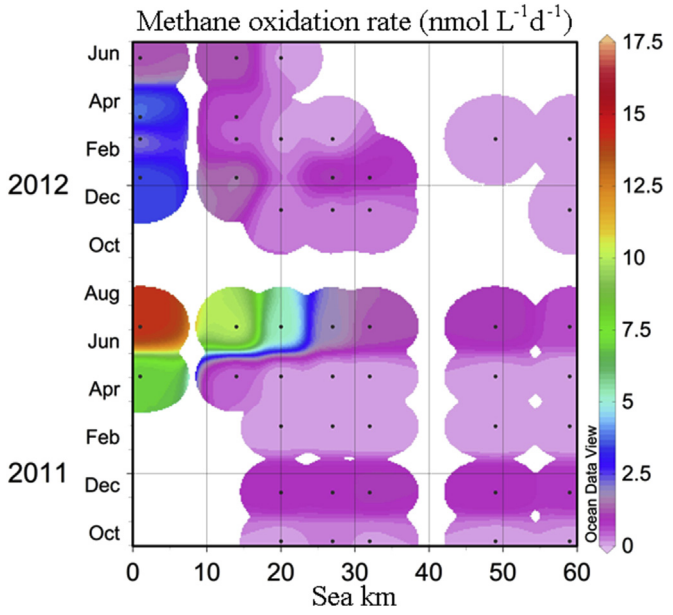

Fig. 7. MOX rates along the transect during the whole period of investigation (surface and bottom).

generally followed a trend similar to the methane concentrations and salinity. However, MOX rates were significantly lower in winter (February 2012) than in summer (July 2011), especially at the coastal stations.

Because of great variability in the turnover times and MOX rates, linear regression analysis did not reveal clear results. Therefore, we rank transformed the data and performed Spearman rank order correlation tests (Table 1). The tests showed that the variability of the turnover time was influenced by methane concentration ($r_s = -0.60$, $n = 94$) in a negative way, i.e. highest turnover times (the slowest rates) were found at lowest methane concentrations. Salinity influenced the variability of the turnover time in a positive way ($r_s = 0.56$, $n = 91$), especially in the coastal area ($r_s = 0.64$, $n = 37$), i.e. highest turnover times were found at highest salinities. Turnover time was moderately correlated in a negative way with temperature ($r_s = -0.46$, $n = 86$), i.e. highest turnover times were found at lowest temperatures. Thus, we found that turnover time was correlated to a greater or lesser extent with three factors: methane concentration, salinity, and temperature.

Because of the dilution of the methane-rich freshwater, we mostly found low methane concentrations at high salinities. To exclude this interaction, and to check for the 'real' influence of salinity on MOX rate and turnover time, we grouped the methane concentrations, according to their frequency distribution, to low methane concentrations (<15 nmol L^{-1}, $n = 37$), medium methane concentrations (15–30 nmol L^{-1}, $n = 41$), and high methane concentrations (>30 nmol L^{-1}, $n = 21$, Table 1). Only when methane

Table 1
Spearman rank correlation of the turnover time and MOX rates with hydrographic parameters.

Dependent parameter	Independent parameter	Spearman rank order correlation	Comments
Turnover time	Methane concentration	−0.60	
	Salinity	+0.56	Due to co-correlation of salinity with methane, methane data were grouped, then only with methane >30 nmol L^{-1}, salinity influences turnover time with $r_s = 0.39$
	Temperature	−0.46	
MOX rate	Methane Concentration	n. d.	
	Salinity	−0.66	Due to co-correlation of salinity with methane, methane data were grouped, than only with methane >30 nmol L^{-1}, salinity influences MOX with r_s = - 0.34
	Temperature	+0.43	

n.d. – not determined.

concentrations were high (>30 nmol L^{-1}), MOX rate and the turnover time (rank transformed data) were correlated with salinity ($r_s = 0.39$ and 0.34, respectively). At lower methane concentrations, these parameters were independent of salinity. Thus at 'high' methane concentrations, salinity had a negative effect on the turnover time and MOX rate. At lower methane concentrations, the most important factor for the turnover time was simply the methane concentration.

The same tendency was observed for MOX rates (Table 1), which were negatively correlated with salinity ($r_s = -0.66$, $n = 92$), but positively correlated with methane concentration ($r_s = 0.78$, $n = 94$) and temperature ($r_s = 0.43$, $n = 87$), i.e. highest MOX rates were detected at lowest salinities and highest methane concentrations and temperatures. However, the correlation between MOX rate and methane concentration should be viewed with caution, because MOX rate calculations are dependent on methane concentrations.

MOX rates were correlated with phosphate concentrations ($r_s = 0.54$, $n = 42$), but phosphate was strongly correlated with salinity. Therefore, to circumvent the co-correlation of phosphate and salinity, we grouped the salinity data into meso-, poly-, and euhaline (5–18, 18–30, and >30; (Caspers, 1959). For each of these salinity groups, no influence of phosphate on turnover time or MOX rate was observed (rank transformed data). No influence of the other inorganic nutrients, SPM, or oxygen on MOX rates or turnover times could be detected by statistical analyses.

4. Discussion

Methane distribution is defined as the balance between methane sources and sinks. Estuaries are one of the main sources of methane in the North Sea. They, in turn, are supplied with methane by riverine input, tidal flats, and marshes (Rehder et al., 1998; Middelburg et al., 2002; Abril and Borges, 2005; Grunwald et al., 2009). The main methane sinks are represented by the dilution of methane-rich waters with methane-depleted waters, outgassing, and bacterial methane oxidation (Scranton and McShane, 1991).

Methane concentrations were monitored over two years along a transect from the Elbe estuary towards the German Bight. The highest methane concentrations were detected near the coast (the first coastal station, Sk 1). Methane concentrations for the next 20 km offshore, decreased until they reached a plateau, and did not significantly vary any further (20–60 km). Therefore, we can conclude that in terms of methane distribution, the direct impact zone of the Elbe riverine waters is in the range of 20 km from the river mouth in Cuxhaven. The coastal stations (1–20 km from the coast) had an average methane concentration of 30 ± 13 nmol L^{-1}, comparable with the measurements of Rehder et al. (1998), which were in the range of 10–40 nmol L^{-1}. Methane concentrations at the marine stations were on average 14 ± 6 nmol L^{-1}. These concentrations are slightly higher than those reported by Rehder et al. (1998), but remarkably lower than values reported by Grunwald et al. (2009), which were around 200 nmol L^{-1} near Helgoland. In general, methane concentrations in the Elbe estuary and in the German Bight are comparable with those measured in other estuaries (de Angelis and Scranton, 1993; Abril and Iversen, 2002; Middelburg et al., 2002; Silvennoinen et al., 2008; Zhang et al., 2008; Grunwald et al., 2009) and open ocean locations (Kelley, 2003; Mau et al., 2013) (Table 2).

For all stations and at all times the water was supersaturated with methane (170–1880 %), thus the German Bight acts as a methane source to the atmosphere both at the coastal and at the open sea part.

As was done in previous studies, we extrapolated methane concentration at zero salinity using a linear correlation model. In March and June 2012, we had the opportunity to also sample water from the Elbe River itself. These data are published in the PANGAEA data base (Bussmann et al., 2014b). Thus, we were able to examine the actual riverine input more closely (Fig. 8). At salinities of 10–15, methane concentrations were 65 ± 5 nmol L^{-1} (Bussmann et al., 2014b), which is very close to our interpolated value of 72 nmol L^{-1}. The corresponding stations EC-719 and EC-724 are close to the port of Cuxhaven. In contrast, the real riverine methane concentrations with salinities <5 (river stations EC-679, 699 and 709) were much lower (26 ± 8 nmol L^{-1}).

This shows that the Elbe is not the main methane source in the estuary, and that the mixing model can only be applied at salinities 10–15. Thus, application of extrapolating approaches as proposed by Rehder et al. (1998) is not possible.

Middelburg et al. (2002) measured a significantly higher methane concentration of 111 nmol L^{-1} at zero salinity in 1997. However, as the water quality in the Elbe has improved over the last 20 years (Amann et al., 2012), the methane concentration may have decreased. Besides, Middelburg et al. (2002) made measurements during only one sampling campaign in April, and as we have shown, methane concentrations are subject to significant temporal variations (this will be discussed later).

Explanations for the methane increase near Cuxhaven are: a) increased methane production in the underlying sediment, b) lateral input. Additionally, we would expect decreased methane oxidation in the estuary in comparison with the freshwater end-member (discussed later). Methane is mostly produced in anoxic sediment zones, from where it can diffuse into the overlying water column. Our comparison of bottom and surface waters gave no indication of strong methane production in the sediments. The sediment in this area is supposed to be very coarse due to the strong currents, and even though methanogenesis also occurs in oxygenated, organic-rich sediments just several cm under the sediments surface (Deborde et al., 2010), we did not observe a strong direct sedimentary input. This is in agreement with the

Table 2
Methane concentrations and MOX rates, measured in different estuaries and in the open ocean.

Location	Methane concentrations (nmol L^{-1})	MOX rates (nmol $L^{-1}d^{-1}$)	Reference
Estuaries			
Elbe (German Bight)	3–60	0.01–17	This study
Elbe	4–111	n.m.	(Middelburg et al., 2002)
North Sea around Helgoland	200	n.m.	(Grunwald et al., 2009)
Southern Bight (North Sea)	3–22	<<1.3	(Scranton and McShane, 1991)
Ems	91–51	n.m.	(Middelburg et al., 2002)
Thames	5–273	n.m.	(Middelburg et al., 2002)
Rhine	4.1–1437	n.m.	(Middelburg et al., 2002)
Scheldt	20–485	n.m.	(Middelburg et al., 2002)
Loire	16–671	n.m.	(Middelburg et al., 2002)
Gironde	4–559	n.m.	(Middelburg et al., 2002)
Douro	15–128	n.m.	(Middelburg et al., 2002)
Sado	37–40	n.m.	(Middelburg et al., 2002)
Hudson river estuary	48–858	0.1–68	(de Angelis and Scranton, 1993)
Randers Fjord, Denmark	28–420	<0.2–15.2	(Abril and Iversen, 2002)
Bay of Temmesjoki River (Baltic Sea)	62–588	n.m.	(Silvennoinen et al., 2008)
Estuary of the Yangtze River	3–89	n.m.	(Zhang et al., 2008)
Open ocean			
Gulf of Mexico	10–343	0–57	(Kelley, 2003)
Cape Lookout Bight	18–246	0	(Kelley, 2003)
Arctic fjord Storfjorden	5–80	0–3	(Mau et al., 2013)

n.m. – not measured.

observation made by Scranton and McShane (1991), who stated that sandy sediments of the North Sea are not a significant methane source.

Tidal flats in the North Sea are known to be active sites of mineralization of organic material, which eventually leads to methane accumulation. Due to advective flow in the tidal areas, which is of special importance in permeable sandy sediments, pore waters enriched in re-mineralized nutrients and methane are actively released from sediments into the overlying water column (Beck and Brumsack, 2012; Segarra et al., 2013). As Cuxhaven is surrounded by tidal flats, we assume a strong lateral input of methane from these tidal flats.

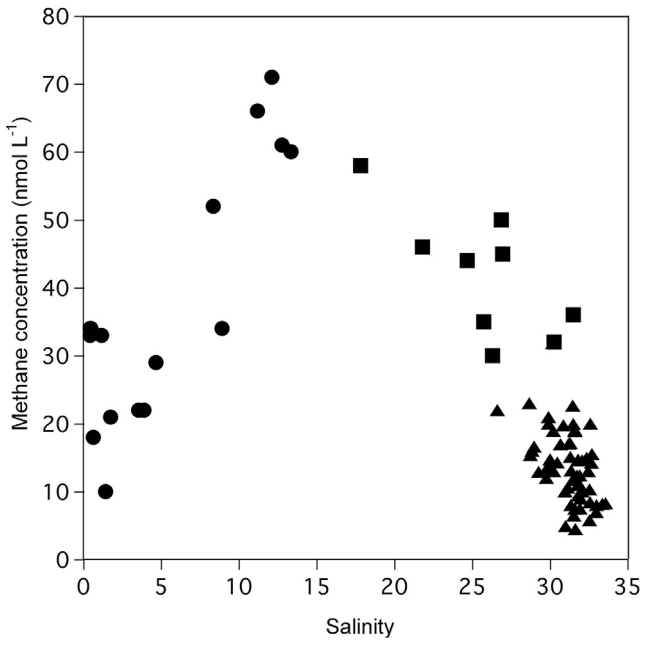

Fig. 8. Methane concentrations with corresponding salinities. Circles indicate river stations (Bussmann et al., 2014b), and squares indicate coastal stations from this study, both for March and June 2012. The triangles represent the values from all marine stations from this study.

Other potential methane sources are inputs from other rivers draining into the estuary, and sewage discharge. The Oste, a small river which flows into the North Sea near Cuxhaven, has low water velocities and therefore could have much higher methane concentrations. However, no data were available for this river. A wastewater treatment plant in Cuxhaven, as well as industrial activity, could lead to additional input of organics which might trigger *in situ* methane production both in sediments and in the water column. Input of inorganic nutrients was also detectable in the hydrochemical data; the phosphate and nitrate concentrations were especially high at the nearby station. We can thus conclude that the Elbe River gets enriched with methane at its mouth due to additional methane sources, whereby methanogenesis might also play an important role. Furthermore, water in the estuary gets diluted with methane-depleted water from the North Sea. The last section of the transect represents North Sea water almost exclusively, and methane concentrations there do not vary significantly.

We expected a seasonality for our methane concentration data, with higher methane concentrations in summer and low ones in winter. However, we could not find any clear seasonal pattern, which may be also due to the too large sampling intervals. But as seasonality is also reflected in changing temperatures, we could find a correlation with water temperature. The effect of temperature on methane production has been shown for many aquatic systems (Pulliam, 1993; Bange et al., 1994; Duc et al., 2010; Lofton et al., 2014), including river and estuary systems (Fedorov et al., 2003). Enhanced methane production in the warmer months is known for various river and estuary systems, such as the Don River, with the Taganrog Bay in the Sea of Azov, Russia (Fedorov et al., 2003), and the Baltic Sea (Bange et al., 1994). Clear seasonality with increased methane in the summer and autumn was also shown for the Rhine estuary (Middelburg et al., 2002). However, in the Scheldt and Gironde estuaries, seasonality was not pronounced (Middelburg et al., 2002), nor in Humber estuary, where methane concentrations were lowest in the summer (Upstill-Goddard et al., 2000). In our investigation, moderate correlation of methane concentration with temperature was shown, but only for the coastal stations ($r_s = 0.49$). Absence of the correlation at the marine stations can be explained by insignificant methane production and rather stable temperature regimes in the open sea and sediments.

Further on, we have to consider that a seasonality of methane concentrations could also originate from variation in the Elbe water discharge. As discussed before, methane distribution along the transect was correlated with salinity. Finally, a multiple linear regression combining salinity and temperature as parameters affecting the methane concentration explained 74% of the methane distribution. Water discharge measured at Neu Darchau from September 2010 to June 2012 ranged from 300 to 3500 m^3 s^{-1} (River Basin Community Elbe, http://www.fgg-elbe.de). Indeed, during the entire period of our sampling campaign at station Sk 1, the salinity varied widely from 8 to 25. However, we did not manage to find a strong correlation between water discharge and methane variations. This can be partly explained by the remoteness between Neu Darchau and station Sk1. Additionally, tides are likely to also be a significant factor affecting short-term methane distribution, as shown in previous studies (Grunwald et al., 2009). However, in our investigation, we did not have enough information to correlate methane concentration with the tidal surge.

MOX rates, as well as turnover times, were calculated to assess the role of methane oxidation as a methane sink, and to define its role in methane distribution. The highest MOX rates were found at the coastal stations (2.6 ± 2.7 nmol $L^{-1}d^{-1}$), and the lowest MOX rates were found at the marine stations (0.4 ± 0.5 nmol $L^{-1}d^{-1}$). Despite the importance of methane oxidation processes in the water column, very few measurements have been made in estuaries. MOX rates in the Hudson estuary (in the salt intrusion area) varied from 0.1 to 68 nmol $L^{-1}d^{-1}$ (de Angelis and Scranton, 1993). In the Randers fjord MOX rates reached 15 nmol $L^{-1}d^{-1}$ (Abril and Iversen, 2002). Summarized data on aerobic methane oxidation in ocean waters from different locations show that MOX rates are generally in the range of 0.001–10 nmol $L^{-1}d^{-1}$ (Kelley, 2003; Mau et al., 2013). MOX rates measured at different estuaries and in the open ocean are summarized in Table 2. Thus, our measurements along the entire transect are in the same range of MOX rates in other aquatic environments.

For the southeast coast of the North Sea a flushing time of 11 days and for the central North Sea of 40 days is given by Ilyina et al. (2006). Our average turnover times for the coastal stations and marine stations were 64 days and 196 days, respectively. Thus, the water masses are faster exported than MOX could consume the methane. Scranton and McShane (1991), who studied methane distribution in the Southern Bight (North Sea) also came to a conclusion that bacterial methane oxidation was not a significant methane sink. However, a more detailed modelling would be necessary to adequately address the question if methane oxidation is a significant methane sink in the Elbe estuary and adjacent German Bight.

Microbial activities are often controlled by substrate concentration, following the enzyme kinetics as described by Michaelis Menten. In our case this means, that with increasing methane concentration the MOX rate also increases, until (enzyme) saturation is reached and no further increase of MOX can be observed. To describe this phenomenon and make it comparable between different studies, the parameter V_{max} and K_m are used to describe the maximal velocity and the half saturation concentration (Lehninger, 1985). Unfortunately, to our knowledge there are no kinetic studies on marine methanotrophs, which usually endure at low methane concentrations. Recent studies from arctic lakes reveal K_m from 4 to 10 μM (Lofton et al., 2014) and the review from Hanson and Hanson (1996) gives a K_m of 1 μM. In a study on the kinetics of cultured methanotrophic strains under low methane concentration (10–100 ppm), it was shown that these strains, which have a K_m within the above described range, are able to grow under these limiting conditions (Knief and Dunfield, 2005). In our study, natural methane concentrations ranged from 3 to 58 nmol L^{-1}, which are concentrations well below half saturation. Thus we assume that the methanotrophic population of the North Sea is strongly limited by methane concentration and an increase in methane concentration would lead to increased activity. With our data we did not calculate such a relation between methane concentration and MOX rate due to methodological restraints (the MOX rate is calculated by multiplying the ratio of consumed tracer with the methane concentration). But even when setting these restraints apart only a weak correlation was observed.

The effect of salt as a chemical agent inhibiting methane oxidation in freshwater has been shown before (de Angelis and Scranton, 1993). The changing salinity likely causes osmotic stress for freshwater methanotrophic bacteria (Hanson and Hanson, 1996). At the same time, most studies report that microbial methane oxidation is already significantly reduced at salinities <10 (Abril and Borges, 2005), whereas in our investigation, high MOX rates (17 nmol L^{-1} d^{-1}) were detected even at a salinity of 17. Our results show that salinity, as such, only has a (negative) effect when methane concentrations are >30 nmol L^{-1}. Thus, we conclude that, at the coastal stations, the negative effect of osmotic stress is counteracted by the positive effect of high methane concentrations in the estuary. An explanation might be that microorganisms which are frequently exposed to changing salinity (like the Elbe estuary, due to the significant influence of tides) are adapted to changing salinities. However, it remains unclear as to what extent freshwater or marine methanotrophic bacteria participate in methane oxidation.

Our results show that temperature also has a moderate effect on methane oxidation. Thus, the lowest turnover times and highest MOX rates were detected during the highest temperatures. This is in contrast with observations made by Lofton et al. (2014), who states that temperature can influence methane oxidation only in substrate-saturated environment.

Another factor which can stimulate methane oxidation is turbidity or SPM. It was mentioned before that SPM can serve as a vehicle for methanogens, and the same principle can be applied to methane-oxidizing microorganisms (Middelburg et al., 2002; Abril et al., 2007). *In vitro* experiments showed that removal of particles smaller than 11 μm can decrease the MOX rate by 50% (de Angelis and Scranton, 1993). In our study, we did not detect any correlation of turnover times/MOX rates with SPM. The easiest explanations would be that stronger factors (such as methane concentration and temperature) simply suppressed the influence of SPM.

Oxygen is another important factor for methane oxidation. Summarized data on oxygen half-saturation constants for aerobic methane oxidation (the concentration at which the growth rate of bacteria reaches the half-maximum) show a wide range, from 0.14 to 58 μM (Guérin and Abril, 2007). Oxygen concentrations measured along the transect in our study were within this range, thus oxygen was never a limiting factor, and no correlation with methane oxidation was detected.

Methanotrophs are regarded as slow growing bacteria, and hence tend to lose out on nutrients to faster growing heterotrophs. Besides, some mineral nutrients, like ammonium, are known to inhibit methane oxidation (Alam and Jia, 2012) while others, like phosphate, increase MOX rates (Boiesen et al., 1993). Also, the availability of nitrogen can become a limiting factor for the growth of methanotrophs in nitrogen-limited environments (Bodelier et al., 2000). In our study, large quantities of inorganic nutrients, were brought to the German Bight from the mouth of the Elbe, thus providing all the inorganic nutrients needed for intensive methane oxidation. However, we did not detect any correlation between MOX rates and any mineral nutrients at the marine stations. Due to low MOX rates at the marine stations, the supply of inorganic

nutrients was still sufficient, and thus did not influence the methanotrophy.

5. Conclusion

We observed a wide variation of methane concentration in the water column along a 60 km transect from Cuxhaven to Helgoland. Highest concentrations were detected near the coast, where the water is enriched with methane by river water and lateral sources. Over the next 20 km, in the direction of Helgoland, we observed decrease in methane concentration due to dilution with methane-depleted sea water from the German Bight. The last 40 km of the transect represents seawater almost exclusively, with a consistently low methane concentration. We also discovered that most of the methane in the Elbe estuary does not come from the Elbe River itself, as was thought before, but from an area near Cuxhaven. Thus, the conservative mixing model, which describes methane distribution as a simple dilution of methane-rich freshwater from the river with marine water, is only applicable at salinities >10. Possible methane sources near Cuxhaven are input from small rivers, and methane rich tidal flats.

Though we did not find any clear seasonal pattern, sampling through the year also enabled us to discover that the methane distribution in the Elbe estuary was subject to significant temporal changes. We assume that these changes were controlled by the interaction of Elbe water discharge and methane concentrations in the mouth of the Elbe, which were higher in the warmer seasons. More information on the tidal surge, as well as the current regime would be useful for a better interpretation of the methane variations. However in the present study we could show that salinity explained about 57% of the methane variability.

Methane oxidation measurements were made in this area for the first time during at least the last 10 years. We discovered that methane oxidation was likely not a significant methane sink in most of the Elbe estuary. However, more data on water residence time is needed to make definite conclusions. The main factors affecting methanotrophic activity were methane concentration, salinity, and temperature. However, further kinetic studies would be useful to gain more insight into the influence of methane concentrations on MOX.

Acknowledgements

Many thanks are given to the scientific parties and crews of the research vessels Prandtl and Uthörn. We thank K.W. Klings for excellent technical assistance and the group of Prof. Wiltshire (K. Carstens) for providing hydrographic and chemical data. The work of A.M. was funded by the DAAD, by the Grant Agency of the Czech Republic (Grant no. 13-00243S) and a grant of the Faculty of Science, University of South Bohemia (GAJU 04-145/2013/P).

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