Spatiotemporal variability of nitrous oxide in a large eutrophic estuarine system: The Pearl River Estuary, China

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ABSTRACT

The spatiotemporal variations of nitrous oxide (N2O) in the Pearl River Estuary, a large perturbed estuary, were investigated via six cruises covering both wet and dry seasons during 2007–2011. Significant spatial and temporal variabilities in N2O concentrations and N2O saturations were detected. Spatially, N2O was oversaturated in the entire estuary; ranging from 328 nmol L\(^{-1}\), or 38 times saturation in the O2-depleted Upper Estuary, down to 11–79 nmol L\(^{-1}\) in the Middle Estuary (163–905% saturation), and to ~7 nmol L\(^{-1}\) (slight supersaturation) in the Lower Estuary. Temporally, increased N2O up to 182 ± 82 nmol L\(^{-1}\) (1800 ± 750% saturation) was observed in the Upper Estuary during winter at low river discharge in comparison to 76 ± 19 nmol L\(^{-1}\) (1163 ± 287% saturation) in summer at high river discharge; whereas no significant seasonal difference was detected within the Middle and Lower Estuaries. The N2O fluxes decreased by 2 orders of magnitude from upstream to downstream (733 to lower than 5 μmol m\(^{-2}\) d\(^{-1}\)). Seasonally, the higher N2O fluxes integrated across the estuary were in spring and winter, and lower fluxes were exhibited in summer and autumn. The annual water–air N2O flux was estimated to be 37 ± 15 μmol m\(^{-2}\) d\(^{-1}\). This rendered a total emission of (1.67 ± 0.89) × 10\(^8\) g N\(_2\)O yr\(^{-1}\), which is equivalent to the revised total emission from 19 European inner estuaries (1.35 × 10\(^8\) g N\(_2\)O yr\(^{-1}\)). Moreover, this amount of N2O emission equals approximately 30% of reported CO2 emission from the Pearl River Estuary in terms of greenhouse warming potential. The N2O production was predominantly modulated by nitrification in the Upper Estuary while in the Middle and Lower Estuaries, estuarine mixing appeared to dominate the N2O behavior.

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1. Introduction

Nitrous oxide (N\(_2\)O), a trace gas with a 114 year lifespan in the atmosphere, has about 300 times greater greenhouse potential relative to CO2. It is increasing in concentration at a rate of ~0.25% annually due to increasing human activities (IPCC, 2007). Additionally, N2O is generated as a by-product from the first step of microbial nitrification, i.e., the oxidation of ammonium (NH\(_4\)\(^+\)) to nitrite (NO\(_2\)\(^-\)) (Dore and Karl, 1996; Yoshinari et al., 1997; Middelburg and Nieuwenhuize., 2000). N\(_2\)O is also known to be produced as an intermediate from denitrification, i.e., the reduction of nitrate (NO\(_3\)\(^-\)) to N\(_2\) (Naqvi et al., 2000; Walter et al., 2006; Yamagishi et al., 2007). Nitrification is principally autotrophic, whereas denitrification is heterotrophic. Both processes can occur either in the water column or sediments (Codispoti et al., 2001; Bange, 2008). In each case, the N\(_2\)O yield is believed to be highly dependent on the ambient dissolved O2 concentration in aquatic systems (Goreau et al., 1980; Codispoti et al., 2001; Naqvi et al., 2010). N2O production would be significantly enhanced under low O2 concentrations (Dai et al., 2008; Codispoti, 2010; Naqvi et al., 2010; Kim et al., 2013).

Marine waters are generally believed to be a major natural and anthropogenic source of atmospheric N\(_2\)O (Seitzinger et al., 2000). Coastal aquatic systems, including estuaries, are an important component of the marine N\(_2\)O cycle. However, N\(_2\)O emissions from estuaries demonstrate considerable uncertainty (Bange et al., 1996; Bange, 2006; Nevison et al., 2003; Barnes and Upstill-Goddard, 2011) due to major spatiotemporal variability and the limited data available. In addition, most early estimates of estuarine N2O emissions focused on relative small European estuaries (Bange et al., 1996; Bange, 2006; Barnes and Upstill-Goddard, 2011). Mounting evidence suggests that substantial differences occur in different estuarine systems, and the large Asian estuaries might hold an increasingly important role in budgeting the future global N\(_2\)O emission with increasing anthropogenic stress (Zhang et al., 2010; Rao and Sarma, 2013). Even within a single estuary, large spatiotemporal variations are present (Harley et al., 2015). This poses a big challenge to reliably constrain the estuarine N\(_2\)O effluxes at a global scale.

The Pearl River (Zhujiang) Estuary is a large subtropical Asian estuary altered significantly by human-induced perturbation (Dai et al.,...
2006, 2008, 2014; He et al., 2014). This estuary is located in one of the most rapidly developing areas of the world during the past three decades. The estuarine environment was greatly affected by the rapid economic growth and anthropogenic stress from cities such as Guangzhou, Hong Kong, Macau, Shenzhen, and Zhuhai. Many environmental issues, such as ammonium contamination and hypoxia, have emerged (Zhai et al., 2005; Dai et al., 2006, 2008; Guo et al., 2009; He et al., 2014). This coupled high-nitrogen and low-oxygen system permits an opportunity to examine nitrogen transformation and the production of N\(_2\)O.

We conducted six cruises during 2007–2011 to constrain the water-air N\(_2\)O fluxes from the Pearl River Estuary that encompasses both spatial and temporal variations. An estimate of N\(_2\)O effluxes into the atmosphere was conducted based on the seasonal and zonal distributions of N\(_2\)O distribution. These fluxes and emissions were compared with other estuaries in Asia and Europe. Factors regulating N\(_2\)O production were discussed as well.

2. Material and methods

2.1. Study area

The Pearl River is the second largest river in China in terms of annual water discharge (3.26 \(\times\) 10\(^{11}\) m\(^3\) yr\(^{-1}\)). It spans for 2214 km, and drains an area of 452,000 km\(^2\) (Dai et al., 2014). The Pearl River has three main tributaries (Fig. 1): namely, the Xijiang (West River), Beijiang (North River), and Dongjiang (East River). Amongst them, the West River accounts for ~70% of the total freshwater discharge (China Bureau of Hydrology, Ministry of Water Resources, http://sqqx.hydroinfo.gov.cn/websq/). The water discharge rate shows significant seasonality, and ~80% of the discharge takes place in the wet season from April to September (Fig. 2). During winter, the monthly average river discharge is around 2000 m\(^3\) s\(^{-1}\). In contrast, the monthly average water flow rate during the summer can be 8 times higher; peaking at approximately 16,000 m\(^3\) s\(^{-1}\) in June.

For the convenience of N\(_2\)O flux estimation, we divided the survey region (with a total area of ~2789 km\(^2\)) into 3 zones in accordance to the N\(_2\)O level and the geometry of the estuary similar to Guo et al. (2009) (Fig. 1). These 3 zones are (1) Upper Estuary: Guangzhou section, the channel flowing through the city of Guangzhou to Humen Outlet, with a length of ~75 km and an area of ~107 km\(^2\); (2) Middle Estuary: Inner Lingdingyang, from Humen Outlet to Inner Lingding Island, with a length of ~40 km and an area of ~107 km\(^2\); (3) Lower Estuary: Outer Lingdingyang, from Inner Lingding Island to the Outer Estuary, with a length of ~50 km and an area of ~2100 km\(^2\) (Fig. 1, Table 1).

![Image](1566001730513104.png)
2.2.2. Analysis of \( N_2O \)

Samples for dissolved \( N_2O \) were immediately taken in 100 mL glass flasks, poisoned with 100 \( \mu L \) saturated HgCl\(_2\) solution and stoppered with a rubber septum without any headspace gas. The samples were stored in the dark at 4 °C prior to laboratory analysis. Sample analysis was performed within one month after collection.

Our experiments demonstrated no significant differences in dissolved \( N_2O \) concentrations from replicate samples (n = 12) analyzed after two months storage. The dissolved \( N_2O \) concentrations were measured at State Key Laboratory of Marine Environmental Science (MEL), Xiamen University. The analytical method was a modification of the method described by Chen et al. (2007). A purge and trap system (Tekmar Velocity XPT) coupled with a gas chromatograph was set up, with the analytical procedure as the following: the water sample of 5 mL volume was transferred into the glass purge vessel and subsequently purged with a 20 mL min\(^{-1}\) nitrogen gas flow of ultra-high purity (99.999%) for 10 min. The displaced gas was transferred to a purge trap (24 cm molecular sieve 5 A, mesh 80/100) at room temperature. During desorption (250 °C for 2 min) gases collected in the trap were transferred through a heated transfer line to the GC injector port. Gas chromatographic analyses were performed with a Hewlett-Packard Model 6890 equipped with a micro-electron capture detector (\( \mu ECD \)) operating at 300 °C. Chromatographic separation was achieved on a RT-Q PLOT wide-bore column (30 m × 0.53 mm i.D., df = 20 μm) (Restek). The column temperature was held at 50 °C. A 5% mixture gas of CH\(_4\)-Ar at a flow rate of 5 mL min\(^{-1}\), and 20 mL min\(^{-1}\) was used as carrier gas and make-up gas. Calibration of \( N_2O \) concentrations was calculated from the peak areas with standard gases of 1.0 and 5.0 ppmv \( N_2O/N_2 \) (Research Institute of China National Standard Materials). Certain volumes of standard gas were transferred into the glass purge vessel and subsequently analyzed by the same procedure used for water samples. When many water samples were analyzed standards were included every 5–10 samples. The precision of this method was estimated to be better than ±5%.

2.2.3. Ancillary measurements

Temperature and salinity were measured continuously using an YSI multi-parameter meter fitted in the under-way measurement system described in Zhai et al. (2005) and Dai et al. (2006). Discrete DO was measured on board using the Winkler titration method.

Nutrients samples were stored at −20 °C until analysis except for NH\(_4\)\(^+\), which was analyzed on board with the indophenol blue spectrophotometer method (Pai et al., 2001). NO\(_2\)-N and NO\(_3\)-N measurements were processed at Xiamen University, using classic colorimetric methods with a Technicon AA3 Auto-Analyzer (Blan-Lube). Nutrient sample analysis was performed within one month after collection. Nitrification rates were measured using an inhibitor technique. Both methods were described in Dai et al. (2008).

DO and nutrient data from April 2007 and August 2008 were cited from Guo et al. (2009), He et al. (2010), and He et al. (2014). The nitriﬁcation rates data in April 2007 and August 2008 were cited from He et al. (2014). The parameters observed in March 2010, August 2010, November 2010, and January 2011 were new observations.

2.3. Calculations

The excess \( N_2O (\Delta N_2O) \) was estimated as the diﬀerence between the calculated \( N_2O \) equilibrium concentration and the measured concentration of \( N_2O \) as the following equation;

\[
\Delta N_2O (\text{nmol L}^{-1}) = N_2O_{\text{observed}} - N_2O_{\text{equilibrium}}
\]

where \( N_2O_{\text{observed}} \) is the \( N_2O \) concentration measured in the water, and \( N_2O_{\text{equilibrium}} \) is the \( N_2O \) concentration at relative equilibrium.
with atmospheric concentration. The equilibrium values of \( \text{N}_2\text{O} \) were calculated with the equation given by Weiss and Price (1980). Atmospheric \( \text{N}_2\text{O} \) was not measured during these cruises. Global mean atmospheric \( \text{N}_2\text{O} \) mixing ratios of 320 ppb for 2007, 321 ppb for 2008, and 323 ppb for 2010 from the NOAA/ESRL halocarbons in situ program (http://www.esrl.noaa.gov/gmd) were used for the calculations in this study.

The \( \text{N}_2\text{O} \) flux through the air–sea interface was estimated based on Eq. (2):

\[
F = k \times \Delta \text{N}_2\text{O}
\]

where \( F \) (\( \mu \text{mol m}^{-2} \text{ d}^{-1} \)) is the flux across the air–sea interface, and \( k \) (cm h\(^{-1} \)) is the gas transfer velocity depending on wind and water temperatures.

\( k \) was calculated using the Borges et al. (2004) equation:

\[
k = \frac{0.31 \times (9.7 + 3.64 \cdot u_{10}) \times (\text{Sc}/600)^{-0.5}}{\text{Sc} = 600} \tag{3}
\]

where \( u_{10} \) is the wind speed at 10 m above the water surface. \( \text{Sc} \) is the Schmidt number calculated from temperature, which is the relationship between viscosity and the diffusion coefficient of \( \text{N}_2\text{O} \) in water that depends on the water temperature and salinity (Wanninkhof, 1992). In our study, \( S < 30 \) was treated as freshwater, while \( S > 30 \) was treated as seawater. The monthly average wind speeds obtained from Hong Kong Observatory (http://www.weather.gov.hk/cis/region_climat/CCH/CCH_mean_e.htm) from the meteorological station located at the estuary mouth were used for the calculation of the water–air \( \text{N}_2\text{O} \) fluxes.

As previously stated, the estuary was divided into 3 zones: Upper Estuary, Middle Estuary, and Lower Estuary. Each zone area was multiplied by the average \( \text{N}_2\text{O} \) fluxes in spring, summer, autumn and winter that was upscaled to give a seasonal emission value for each zone. They were subsequently summed to calculate the yearly emission for the whole estuary and sections of the estuary.

2.4. Statistical analysis

Significant differences of concentrations, saturations and fluxes in different zones and seasons were tested with t-tests. All statistical analyses were conducted in SPSS with a significance level of \( p < 0.05 \).

3. Results

3.1. Hydrochemistry

Freshwater discharge rates from the Pearl River system showed significant seasonal variations. Higher values were demonstrated in the

Fig. 3. Spatial distributions of salinity (a), temperature (b), DO (c), NH\(_4\)+ (d), NO\(_2\)− (e), and NO\(_3\)− (f) in the Pearl River Estuary based on six cruises (April 2007, August 2008, March 2010, August 2010, November 2010, and January 2011). The distance is positive for downstream and negative for upstream of the Humen Outlets. Black lines separate distinct reaches: Reach (1) Upper Estuary (Humen upstream); Reach (2) Middle Estuary (Inner Lingdingyang); Reach (3) Lower Estuary (Outer Lingdingyang and beyond). The DO and nutrient data for the Apr 2007 cruise were cited from Guo et al. (2009); He et al. (2010); He et al. (2014); and DO and nutrient data for the August 2008 cruise were cited from He et al. (2014).
summer (~13,700 m³ s⁻¹ in August 2008), and lower values during winter (~2200 m³ s⁻¹ in January 2011) (Fig. 2). The spatial distribution of salinity within the estuary was largely reflective of different freshwater discharge rates in different seasons. In the vicinity of Humen Outlet, the salinity was ~0–1 in summer (August 2008 and August 2010). In contrast, in winter (January 2011), estuarine mixing moved upstream and salinity of ~12 was exhibited at the Humen Outlet (Fig. 3a). Average salinity in the Middle and Lower Estuaries, demonstrated higher values in winter (~18 and ~29 respectively) than in summer (~5 and ~17, respectively) (Fig. 3a). In spring and autumn (April 2007, March 2010, and November 2010), the salinity distribution pattern was median to summer and winter (Fig. 3a). The surface water temperature ranged from 19.4–24.7 °C (in spring), 27.2–31.2 °C (in summer), 20.9–23.2 °C (in autumn), and 15.6–19.4 °C (in winter) (Fig. 3b).

Pronounced oxygen depletion was observed in the surface water in the Upper Estuary throughout the year (Fig. 3c), which has been reported previously (Dai et al., 2006, 2008; Guo et al., 2009; He et al., 2014). Seasonally, the most severe oxygen depletion of surface DO lower than 63 μmol L⁻¹ (2 mg L⁻¹) was detected in the entire upstream of the Humen Outlet during spring. The lowest concentration of 8–12 μmol L⁻¹ (~4% DO saturation) was observed in March 2010. In winter, estuarine mixing played a significant role in raising the oxygen content upstream of Humen, and the observed oxygen depletion area was relatively narrow compared to spring. Downstream, DO concentration increased gradually with the salinity gradient, reaching nearly saturated or supersaturated conditions in the Lower Estuary (Fig. 3c). In August 2010, there was a notable maximum oxygen concentration (~400 μmol L⁻¹) that was observed in the Lower Estuary (Fig. 3c) due to high net community production (Guo et al., 2009; He et al., 2014).

Similar distribution patterns and high levels of dissolved inorganic nitrogen (DIN) were detected in the Pearl River Estuary during our survey cruises (Fig. 3d–f). This observation was reported in previous studies (Dai et al., 2006, 2008; He et al., 2014). Low-nutrient seawater mixing accounted for the significant seaward-decreasing trend in all nitrogen species. In comparison to winter and spring, larger freshwater dilution and short water residence time may have accounted for the overall lower total DIN in summer.

In all seasons, NH₄⁺ was the dominant species of DIN in the Upper Estuary, accounting for ~80% of DIN (Fig. 3d). NH₄⁺ concentrations were highest at the freshwater end-member, peaking at ~470 μmol L⁻¹ in April 2007. This region was directly impacted by regional wastewater discharge (Dai et al., 2008; He et al., 2014). Slightly downstream, NH₄⁺ concentrations rapidly declined in correlation with increasing NO₂⁻ and NO₃⁻ concentrations (Fig. 3e and f). This data supported the earlier contention that the upper Pearl River Estuary is a site of permanent and strong NH₄⁺ nitrification (Dai et al., 2006, 2008; He et al., 2014). With the chief drop in the Upper Estuary, NH₄⁺ concentrations gradually decreased to the detection limit in the seaward direction.

The NO₃⁻ distribution along the Pearl River Estuary showed a few peaks in the Middle or Upper Estuary (Fig. 3f). Occurrence of those peaks was related to local sewage inputs from major cities. In addition, their locations may have been affected by the tidal motion superimposed by the complex geometry of the Lingdingyang (Dai et al., 2006, 2008; He et al., 2014). The highest NO₃⁻ value in March 2010 was up to 230 μmol L⁻¹. During all cruises, NO₃⁻ concentrations gradually decreased in the Lower Estuary, due to dilution with seawater with lower NO₃⁻ contents (Fig. 3f).

3.2. Spatial and seasonal variations of N₂O concentration and its saturation

The N₂O distribution along the Pearl River Estuary displayed pronounced spatial variability, ranging from 6 to 329 nmol L⁻¹ that corresponded to saturations of 101–3800% (Figs. 4 & 5, Table 1). Hence, the Pearl River Estuary was a net source of atmospheric N₂O.

The general pattern was that N₂O was higher at the Upper Estuary, decreasing downstream during all seasons. Take March 2010 (Figs. 4 and 5c, Table 1) as an example. In the main north to south transect, the Upper Estuary (Zone 1) exhibited very high N₂O (55–329 nmol L⁻¹), which paralleled to saturations of 674–3800%. This level was similarly reported in April 2004 by Xu et al. (2005). N₂O decreased in the Middle Estuary to 11–49 nmol L⁻¹ (255–614% saturation). In the Lower Estuary, the N₂O concentration was reduced to about 6 nmol L⁻¹, which is in near-equilibrium with the atmosphere. In two transects crossing from west to east, slightly higher N₂O concentrations were observed in the west compared to the east. Values ranged from 13 to 17 nmol L⁻¹ in the Middle Estuary transect, and 8 to 10 nmol L⁻¹ in the Lower Estuary transect. These results are inversely proportional to salinity readings. Regarding the N₂O-salinity relationship, N₂O dropped rapidly against salinity from 329 nmol L⁻¹ in the Upper Estuary to 55 nmol L⁻¹ at ~12–13; progressively decreasing seaward with increasing salinity. Seaward of salinities with values of ~33, N₂O concentrations were close to atmospheric equilibrium (Table 1, Fig. 4b).

Additionally, the seasonal variation was also significant with generally higher N₂O concentrations during winter/spring than summer/autumn. Zonal average N₂O values in different seasons were summarized in Table 1 and further presented in Fig. 6.

In the Upper Estuary (Zone 1), average N₂O was higher in spring (152 ± 42 nmol L⁻¹ in April 2007, and 151 ± 104 nmol L⁻¹ in March 2010) and winter (182 ± 82 nmol L⁻¹ in January 2011), but much lower during summer (72 ± 56 nmol L⁻¹ in August 2008, and 76 ± 19 nmol L⁻¹ in August 2010). The average N₂O value in the Middle Estuary (Zone 2) and the Lower Estuary (Zone 3) ranged from 19 to 34 nmol L⁻¹ and 11 to 15 nmol L⁻¹, respectively (Table 1). The seasonal variation in the Middle and Lower Estuaries displayed no statistical significance compared to Upper Estuary. In the Upper Estuary, the intra-seasonal variation was small both in spring and summer.
3.3. Spatial and seasonal variations in N2O fluxes

The monthly average wind speeds, ranging from 4.7 to 5.6 m s\(^{-1}\), were used for the calculation of the N2O fluxes. Similar to the distribution of N2O concentration, the water–air N2O fluxes displayed considerable spatial variability by 2 orders of magnitude in the Pearl River Estuary. The N2O flux maximum of 733 μmol m\(^{-2}\) d\(^{-1}\) was observed at the Upper Estuary, while the N2O flux was reduced to values lower than 5 μmol m\(^{-2}\) d\(^{-1}\) towards the mouth of the estuary (Fig. 6c, Table 1).

On a seasonal time scale, substantially elevated N2O fluxes were exhibited during winter (330 ± 152 μmol m\(^{-2}\) d\(^{-1}\)) and spring (313 ± 150 μmol m\(^{-2}\) d\(^{-1}\)) in Upper Estuary than in summer (176 ± 106 μmol m\(^{-2}\) d\(^{-1}\)) (Fig. 6c). The intra-seasonal variation in the N2O fluxes was generally low both in summer and spring. The annual average N2O flux from entire Pearl River Estuary was estimated to be 37 ± 15 μmol m\(^{-2}\) d\(^{-1}\).

4. Discussions

4.1. Factors influencing N2O distribution

Factors contributing to the variations of N2O in an estuarine system include its production primarily via nitrification that is related to the substrate level (NH\(_4^+\) and NO\(_2^-\)) and its ambient environment; notably, DO and physical conditions such as river discharge, estuarine mixing and outgassing. Besides nitrification, denitrification may also produce or consume N2O. In this section, we discuss the main factors modulating the N2O distribution in the Pearl River Estuary. The Upper Estuary was characterized by strong nitrification fueled by high NH\(_4^+\) under oxygen conditions.
depleted conditions, while the Middle and Lower Estuaries were featured by estuarine mixing that played a major role in modulating the $\text{N}_2\text{O}$ variability.

4.1.1. Upper Estuary
As shown, the Upper Estuary featured very high concentrations of $\text{N}_2\text{O}$, $\text{NH}_4\text{+}$, DIN levels (Dai et al., 2006, 2008). Fig. 7 further demonstrated that excess $\text{N}_2\text{O}$ ($\Delta\text{N}_2\text{O}$) exhibited positive correlations with nitrogen loading ($\text{NH}_4\text{+}$ and DIN) in the Pearl River Estuary, indicating the significance of nitrification as observed in other eutrophic estuaries (Abril et al., 2000; de Wilde and de Bie, 2000; Garnier et al., 2006). High $\text{N}_2\text{O}$ production in the Upper Estuary would be attributed to high $\text{NH}_4\text{+}$ effluent (Fig. 7a). This validated that nitrification was the possible mechanism for $\text{N}_2\text{O}$ production. Barnes and Upstill-Goddard (2011) similarly reported high $\text{N}_2\text{O}$ production in the Tees and Tyne estuaries in UK, which was a nitrification product of $\text{NH}_4\text{+}$ derived from wastewater inputs.

As Fig. 8 showed, the nitrification rates were high in the surface waters of the upper Pearl River Estuary; with an ammonia oxidation rate of 8.8–22.8 $\mu\text{mol N L}^{-1} \text{ d}^{-1}$ in April 2007, and from below the detection limit to 27.0 $\mu\text{mol N L}^{-1} \text{ d}^{-1}$ during August 2008 (He et al., 2014).

Fig. 6. Zonal average of surface water $\text{N}_2\text{O}$ concentration (a), saturation (b), fluxes (c), and emissions (d) from the Pearl River Estuary.
These reports suggest a strong presence of nitrification in the Upper Estuary. The high abundance of ammonium oxidizing bacteria was also quantified in the prior study (Dai et al., 2008). The concentration of nitrifier bacteria within the water column of Pearl River Estuary was between 2 and 3500 cells mL$^{-1}$. The highest densities occurred at the Upper Estuary, and dramatically decreased with increasing salinity. The distribution pattern of nitrifier abundance was broadly consistent with nitrification rates (Dai et al., 2008). N$_2$O production was in agreement with the pattern of NH$_4^+$ oxidation rates. Both showed higher reading upstream, with values decreasing seaward (Fig. 8). This pattern implied that ammonium oxidation was an important N$_2$O source in the water column.

It is known that low dissolved O$_2$ favors nitrification, and subsequently N$_2$O production (Kim et al., 2013). The upper Pearl River Estuary was severely depleted in O$_2$ (Dai et al., 2006, 2008; He et al., 2014). The gradient of oxygen depletion ($\Delta O_2 = [O_2]_{eq} - [O_2]$) along the estuary impacted the occurrence and intensity of nitrification, which additionally impacted the N$_2$O production (Codispoti, 2010; Kim et al., 2013). The relationships between $\Delta$N$_2$O and $\Delta$DO showed positive correlation during all seasons, with high N$_2$O emerging at low DO concentrations (Fig. 9). This pattern was similar with previous studies reported in other estuaries (McElroy et al., 1978; De Wilde and De Bie, 2000) and ocean margins (Cohen and Gordon, 1979; Patra et al., 1999; Walter et al., 2006; Löscher et al., 2012).

The N$_2$O concentration in the Upper Estuary showed remarkable seasonal variations. Significantly higher fresh water discharge in summer ($13,700$ m$^3$ s$^{-1}$) than in winter ($2200$ m$^3$ s$^{-1}$) would significantly dilute both the N$_2$O and its substrate such as DIN. In addition, the water residence time of Upper Estuary in summer (~3 days) was lower than that in winter (~5 days). Longer residence time benefits N$_2$O accumulation. In fact, the water column N$_2$O production rate of 30 nmol N L$^{-1}$ d$^{-1}$ can be derived by assuming an ammonium oxidation rate of 20 $\mu$mol N L$^{-1}$ d$^{-1}$ and the yield of the N$_2$O during nitrification of 1.5‰ (Elkins et al., 1978; De Wilde and De Bie, 2000). Obviously, the N$_2$O concentration can be built up to observed levels within a few days without outgassing. The role of denitrification in our study remains unclear although our calculation supports that nitrification was a primary source for water column N$_2$O.

4.1.2. Middle and Lower Estuaries

In the Middle and Lower Estuaries, N$_2$O rapidly dropped with increasing salinity (Fig. 4b); indicating that low N$_2$O seawater was diluting the high N$_2$O estuarine water. Thus the estuarine mixing process might play an important role in the modulation of N$_2$O distribution in the Pearl River Estuary. Here, we applied two end-members mixing model between freshwater and seawater to derive the conservative N$_2$O. This was subsequently compared with the field observations to derive the net alteration of N$_2$O during the estuarine mixing. Variations of seawater end-members in different seasons were small: 7 nmol L$^{-1}$ for all cruises. However, the variation of the freshwater end-member at the Humen Outlet, where the estuarine mixing initiated for the middle and lower estuarine mixing, was large in different cruises. Different freshwater end-members were used, with higher values in during winter and spring (70–140 nmol L$^{-1}$) in contrast to summer (~55–75 nmol L$^{-1}$).

The N$_2$O difference between the two end-member mixing and observation, denoted here as $\Delta$N$_2$O (the model prediction minus field observation), would suggest biogeochemically mediated and/or the outgassing portion of N$_2$O. The result was shown in Fig. 10a, the...
RN2O decreased with salinity in all cruises in the Middle and Lower Estuaries. Increased values were observed during winter and spring (0–70 nmol L−1) than in summer (0–50 nmol L−1). This indicated that more N2O in winter and spring was removed than in summer at the same salinity level. If we assume the water residence time was 2000; Guo et al., 2009), and the average depth of the Lingdingyang Estuaries. Increased values were observed during winter and spring (0–70 nmol L−1) than in summer (0–50 nmol L−1). This indicated that more N2O in winter and spring was removed than in summer at the same salinity level. If we assume the water residence time was 3 days in summer and 5 days in other seasons (Wong and Cheung, the same salinity level. If we assume the water residence time was 2000; Guo et al., 2009), and the average depth of the Lingdingyang

Consequently, in the Upper Estuary, N2O production was enhanced under high-DIN loading and high-oxygen depletion condition. Strong nitrification appeared to largely contribute to the high N2O production. In the Lower and Middle Estuaries, mixing with lower N2O seawater along with water–air N2O exchanges was responsible for the variability of N2O concentrations.

4.2. N2O emission and comparison with European and other Asian estuaries

Wide spatiotemporal integrated monitoring provided us to obtain reliable N2O emission from the entire Pearl River Estuary. The annual water–air N2O emission was estimated to be (3.8 ± 2.0) × 107 mol yr−1. Converting to equivalent greenhouse effect, this emission (1 × 1010 mol CO2) accounts for approximate ~30% of CO2 emission (3 × 1010 mol CO2) in the Pearl River Estuary (Guo et al., 2009).

The uncertainties of N2O emission estimation in the Pearl River Estuary arise from various sources: scaling errors from the estuary, uncertainties of the transfer velocity versus wind speeds, and bias in mean N2O values due to the spatial and temporal variances. The errors from area of estuary estimation were believed to be within 5% (Guo et al., 2009; Dai et al., 2014). The transfer velocity versus wind speeds (varied between 4.7 and 5.6 m s−1) carried an error of up to 25%. The uncertainty in estuarine N2O saturation could be 20% (1σ of the estuarine mean) including the uncertainty in N2O analysis. Using the individual errors above, the maximum uncertainty in our N2O emission estimate for the Pearl River Estuary was up to ±54%.

The previous reports for the European and Asian estuaries, N2O saturation varied over a wide range of 84% to 650% at various temporal and spatial scales (Table 2). The observed N2O concentrations in

![Fig. 10. RN2O vs. salinity (a) and N2O removal rates vs. water–air N2O fluxes (b) in the Pearl River Estuary (Middle- and Lower Estuary). RN2O (the model prediction minus field observation) represent the removal portion of N2O during the estuarine mixing.](image)
the annual water-air N$_2$O emission of the Pearl River Estuary was estimated to be (1.67 ± 0.89) × 10$^9$ g N$_2$O yr$^{-1}$ within an area of 2789 km$^2$. For comparison, this total emission from one anthropogenically impacted estuary was equivalent to the total emission from 19 European inner estuaries ($1.35 × 10^9$ g N$_2$O yr$^{-1}$), covering an area of ~1840 km$^2$ (Barnes and Upstill-Goddard, 2011). The previous estimates of N$_2$O emission in estuarine systems were mostly based on relative small European estuaries (Bange et al., 1996; Bange, 2006; Barnes and Upstill-Goddard, 2011). Clearly, with increasing anthropogenic stress, the Asian estuaries might hold an increasingly important role in budgeting the future global N$_2$O emission. Wide temporal and spatial variations of N$_2$O emission in Pearl River Estuary suggested that intensive monitoring is required; particularly for un-explored regions to obtain accurate contribution of emissions to atmospheric N$_2$O.

5. Conclusions

Dissolved N$_2$O concentrations and water-air N$_2$O fluxes in the Pearl River Estuary showed wide spatiotemporal variation. A wide range of N$_2$O saturation levels varied from ~100% to 3800%, with substantially higher N$_2$O and N$_2$O fluxes observed during spring and winter in Upper Estuaries than in summer. Annual water-air N$_2$O emission from the Pearl River Estuary was estimated to be 3.8 × 10$^9$ mol, equivalent to ~30% of CO$_2$ emission in terms of greenhouse effect. The weighted average annual emission of N$_2$O from the Pearl River Estuary amounts to (1.67 ± 0.89) × 10$^9$ g N$_2$O yr$^{-1}$, which was equivalent to the revised emission estimate from 19 European inner estuaries ($1.35 × 10^9$ g N$_2$O yr$^{-1}$).

Variations of N$_2$O in the Pearl River Estuary were influenced by multiple factors in the Upper Estuary. N$_2$O production was enhanced under high-DIN loading and high-oxygen depletion conditions. Strong nitrification largely contributed to the high N$_2$O production. In the Lower and Middle Estuaries, mixing with lower N$_2$O seawater along with water-air N$_2$O exchanges was responsible for the variability of N$_2$O concentrations. Further research on direct N$_2$O production rate measurements in the water column and sediments (especially in the upper estuarine zone) from nitrification and denitrification are required to concretely define the controlling mechanism of N$_2$O in the Pearl River Estuary. High spatial and temporal observations are mandatory to further reduce the uncertainty of N$_2$O emission estimation.

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