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**Nitrification and
inorganic N in the
Pearl River Estuary**

Minhan Dai et al.

Nitrification and inorganic nitrogen distribution in a large perturbed river/estuarine system: the Pearl River Estuary, China

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Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Abstract

We investigated the spatial distribution and seasonal variation of dissolved inorganic nitrogen in a large perturbed estuary, the Pearl River Estuary, based on three cruises conducted in winter (January 2005), summer (August 2005) and spring (March 2006).

5 On-site incubation was also carried out for determining ammonium and nitrite oxidation rates (nitrification rates). We observed a year-round pattern of dramatic decrease in NH_4^+ , increase in NO_3^- but insignificant change in NO_2^- in the upper estuary at salinity $\sim 0\text{--}5$. However, species and concentrations of inorganic nitrogen at estuary significantly changed with season. In winter with low runoff the most upper reach of the
10 Pearl River Estuary showed relatively low rates of ammonia oxidation ($0\text{--}5.4 \mu\text{mol N L}^{-1} \text{d}^{-1}$) and nitrite oxidation ($0\text{--}5.2 \mu\text{mol N L}^{-1} \text{d}^{-1}$), accompanied by extremely high concentrations of ammonia (up to $>800 \mu\text{mol L}^{-1}$) and nitrate (up to $>300 \mu\text{mol L}^{-1}$). In summer, the upper estuary showed higher nitrification rates (ammonia oxidation rate $\sim 1.5\text{--}33.1 \mu\text{mol N L}^{-1} \text{d}^{-1}$, nitrite oxidation rate $\sim 0.6\text{--}32.0 \mu\text{mol N L}^{-1} \text{d}^{-1}$) with lower
15 concentrations of ammonia ($<350 \mu\text{mol L}^{-1}$) and nitrate ($<120 \mu\text{mol L}^{-1}$). The Most Probable Number test showed relatively lower nitrifier abundance in summer at most sampling stations, indicating a greater specific nitrification rate per cell in the warm season. Temperatures appeared to control nitrification rates to a large degree in different seasons. In addition to aerobic respiration, nitrification contributed significantly
20 to the consumption of dissolved oxygen (DO) and production of CO_2 at the upper estuary. Nitrification-induced DO consumption accounted for approximately up to one third of the total water column community DO consumption in the upper estuary during surveyed periods, boosting environmental stress on this large estuarine ecosystem.

1 Introduction

25 Rivers play a crucial role in the delivery of nutrients to the ocean, making coastal waters particularly prone to eutrophication, which may cause harmful algal blooms and/or hy-

BGD

5, 1545–1585, 2008

Nitrification and inorganic N in the Pearl River Estuary

Minhan Dai et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



**Nitrification and
inorganic N in the
Pearl River Estuary**

Minhan Dai et al.

[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[◀](#)[▶](#)[◀](#)[▶](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)

poxia, disrupting coastal ecosystems (e.g. Galloway et al., 2004; Howarth and Marino, 2006; Rabalais et al., 2002; Smith et al., 2006). Major research efforts have thus been devoted to the environmental consequence of the progressive increase in nutrient discharges associated with human activities, such as untreated domestic and industrial wastewaters, into estuarine and coastal environments (e.g. Gunnarsson et al., 2000; Howarth et al., 1996; National Research Council, 2000; Verity et al., 2006).

Among the various nutrients, nitrogen has received particular attention because of the magnitude of the associated environmental concerns and the complexity of nitrogen cycling after discharge into the aquatic environment (Galloway et al., 2004; Howarth and Marino, 2006). It is now recognized that oxidation of ammonium plays a pivotal role in generating a source of nitrate for denitrifying bacteria. The coupling of this obligately aerobic process (nitrification) with an anaerobic process (denitrification) leads to the loss of nitrogen to the atmosphere. Therefore, nitrification is crucial to the understanding of the nitrogen cycle in aquatic systems, particularly, in river/estuarine systems (Bianchi et al., 1997; Lomas and Lipschultz, 2006).

There have been many examples showing intensive nitrification in polluted rivers/estuaries that directly or indirectly (through organic nitrogen mineralization) receive a large amount of ammonium favorable to the development of nitrification (e.g. de Wilde and de Bie, 2000; Garnier et al., 2006; Magalhaes et al., 2005; Xu et al., 2005). Transformation of nitrogen species from ammonia and nitrite to nitrate in river/estuaries during transportation not only modulates their relative distributions but also enhances oxygen consumption (e.g. Bianchi et al., 1997; Brion et al., 2000; Lipschultz et al., 1986). However, the inter-relationship between environmental conditions and nitrification in aquatic environments remains unclear (Strauss and Lamberti, 2000) and only a few reports, which are mainly limited to the seasonal variation of nitrification rates, include any attempt to elucidate factors regulating estuarine/coastal nitrification (Berounsky and Nixon, 1990; de Bie et al., 2002; Huesemann et al., 2002; Xia et al., 2004).

The Pearl River (Zhujiang) Estuary is one of the most complex estuarine systems

**Nitrification and
inorganic N in the
Pearl River Estuary**

Minhan Dai et al.

[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[⏪](#)[⏩](#)[◀](#)[▶](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)

in the world. The Pearl River discharges to the South China Sea through three sub-estuaries, the Lingdingyang, Maodaomen and Huangmaohai sub-estuaries (Fig. 1a), via eight main outlets, or distributaries. This estuary is located in what has been one of the most rapidly developing areas of the world during the past two decades. The massive economic growth and urban development in this region has led to excessive release of wastes into the estuarine region (Tang, 1997). As a consequence, many environmental issues have emerged, such as ammonium contamination and hypoxia (Dai et al., 2006; Zhai et al., 2005), which at the same time offers an opportunity to examine nitrogen transformation in human-perturbed estuaries. Unfortunately, available data on the spatial and temporal variations of dissolved inorganic nitrogen are still limited in the open literature though research devoted to this region has grown recently in response to progressively worsening environmental conditions (Cai et al., 2004; Yin, 2002; Yin et al., 2001; Zhang et al., 1999). Moreover, most of the previous studies have focused on the Lingdingyang sub-estuary (one of the three sub-estuary complexes) leaving information on nutrient biogeochemistry in the other two sub-estuaries unavailable.

Based upon the distribution of inorganic nitrogen species and the stoichiometry of dissolved oxygen (DO) consumption and CO₂ production, we inferred that strong nitrification should occur in the water column in the upper reaches of the Pearl River Estuary (Dai et al., 2006). However, rates and controlling factors of nitrification have not been addressed. In this study, we conducted temporal and spatial investigations on dissolved inorganic nitrogen and nitrification rates; special attention being given to their interrelationship with environmental conditions.

2 Materials and methods

2.1 Study area

The Pearl River is the second largest river in China next to the Yangtze River (Changjiang) in terms of freshwater discharge. The Pearl River has three main tribu-

taries (Fig. 1a), namely the Xijiang (West River), Beijiang (North River) and Dongjiang (East River). The annual mean river discharge of the Pearl River is $3.26 \times 10^{11} \text{ m}^3 \text{ yr}^{-1}$, of which 80% occurs in the wet season from April to September (Zhao, 1990). The water discharge rate shows significant seasonality. During the dry winter, monthly average flow rate is around $2000 \text{ m}^3 \text{ s}^{-1}$, while, in the wet summer, monthly average water flow rate can be 8 times higher, reaching $16\,000 \text{ m}^3 \text{ s}^{-1}$ in July.

The Pearl River Delta is formed by two funnel-shaped bays, the Lingdingyang and the Huangmaohai sub-estuaries (Fig. 1a). The Lingdingyang sub-estuary, which is conventionally regarded as the Pearl River Estuary, is the largest sub-estuary with an area of 1180 km^2 and collects about 55% of the runoff and 46% of the suspended sediment load (see Table 1) of the entire Pearl River system (Zhao, 1990). The Lingdingyang is surrounded by a number of metropolises such as Guangzhou, Shenzhen, Macao and Hong Kong. The Huangmaohai sub-estuary has a surface area of 440 km^2 with an annual mean fresh water input of $4.05 \times 10^{10} \text{ m}^3 \text{ yr}^{-1}$, accounting for 13% of the total river discharge (Zhao, 1990). The Huangmaohai sub-estuary is also surrounded by rapidly developing cities such as Xinhui and Jiangmen (not shown in Fig. 1). Between the two bays is an arc like siltation zone with its apex at Modaomen. The Modaomen sub-estuary receives 28% of the total freshwater input. Eight outlets are distributed within this delta-estuary system. Four of the eight outlets, the Humen, Jiaomen, Hongqimen and Hengmen, drain water runoff from the East River, the North River and several branches of the West River into the Lingdingyang, which is a microtidal estuary with an average tidal range of 0.8–1.9 m increasing towards the Humen. The West River mainly discharges into the Modaomen sub-estuary via the Modaomen and Jitimen outlets. The Yamen and Hutiaomen deliver water runoff from two branches of the West River and a small local river (the Tanjiang River, not shown) to the Huangmaohai sub-estuary, which is a macrotidal estuary (Wu and Shen, 1999).

The water residence time in summer is shorter than in winter in all three sub-estuaries. The Lingdingyang sub-estuary has the shortest residence time (faster exchange) among the three sub-estuaries in both wet and dry seasons (Cheung et al.,

**Nitrification and
inorganic N in the
Pearl River Estuary**

Minhan Dai et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



2000). The estuarine water is usually stratified during the summer wet season and well mixed during the winter dry season. The salt wedge and turbidity maximum migrate seasonally due to changes in freshwater runoff (Mao et al., 2004). In the wet seasons, the fresh water affects the entire Lingdingyang resulting in a salinity of <math><20-25</math> at the surface; while in dry seasons, seawater may occupy the whole estuary with a salt wedge, even reaching Guangzhou (Tian, 1994).

To obtain a more comprehensive view of the Pearl River Estuary, this study not only focused on the Lingdingyang sub-estuary but also the Huangmaohai sub-estuary and off-estuary zones. To ease the discussion, we divided the Lingdingyang sub-estuary into three parts based on the relative distance to the Humen Outlet, namely, Guangzhou upstream (-100 to -30 km), Guangzhou downstream (-30 to 0 km) and Humen downstream (>0 km) (see Fig. 4). Similarly, the Yamen is used as a reference point for the transect in the Huangmaohai sub-estuary.

2.2 Sampling

Water samples were collected during three cruises undertaken in January 2005, August 2005 and March 2006, representing the cold and dry season with low water flow, the warm and flood season with high water flow and the transitional season with medium flow, respectively. During our sampling cruises in January 2005 and March 2006, the monthly average discharge was slightly higher than the long-term average, while in August 2005 the monthly average discharge was 54% lower than the long-term average discharge.

Samples were taken along a main transect as shown in Fig. 1 where stations are dotted (Fig. 1b-d). In January 2005 and August 2005, we expanded our sampling to the Huangmaohai sub-estuary. Discrete sampling was guided by the salinity gradient within the estuarine mixing zone and by distance when no salinity changes occurred at the upstream Humen in the wet season. At selected stations, we took incubation samples to determine nitrification rates. These stations are numbered in Fig. 1b. It should be noted that Stations 1-2 and Station 4 are located in the main channel of

Nitrification and inorganic N in the Pearl River Estuary

Minhan Dai et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



the upstream Humen, and Stations 3 and 5 are located at the branches of the lower East River. Station 6 is located in the downstream Humen of the upper Lingdingyang sub-estuary and Station 7 is located upstream of the Yamen Outlet (Fig. 1).

Water column samples for nutrients, bacterial abundance, nitrifying bacteria and pH were collected using 2.5 L Go-Flo bottles. Surface samples for nitrifying bacteria were taken with a clean pumping system equipped with a FloJet[®] pump and Teflon lined tubing. Surface samples for nutrients were collected using the same system with the addition of on-line cartridge filters (pore size $\sim 1 \mu\text{m}$).

2.3 Incubation experiments

To determine nitrification rates, on-deck incubation was carried out in 4 L narrow neck amber glass bottles using an inhibitor technique, which has been widely used to measure nitrification rates in coastal marine environments (Bianchi et al., 1997; de Bie et al., 2002; Feray et al., 1999). Water samples were homogenized in a 20-L pre-cleaned polyethylene container, and then dispensed into the incubation bottles. One aliquot was incubated with the addition of allythiourea (ATU, at 100 mg L^{-1}), which inhibits the oxidation of ammonia (NH_3) to nitrite (NO_2^-). Another aliquot was incubated by adding NaClO_3 (10 mg L^{-1}) to inhibit oxidation of NO_2^- to nitrate (NO_3^-). A third aliquot acted as a control, without adding any inhibitors. The concentrations of both inhibitors were optimized based on a set of pre-experiments both in the laboratory and on site (data not shown). The experiments were carried out on deck with running through water to maintain the in situ water temperature. Sub-samples were taken out at 4–8 h intervals for analyzing nutrients. Nitrification rates were estimated from the evolution of nutrient concentrations in incubation bottles during the exponential phase of increase or decrease in NO_2^- (Bianchi et al., 1992). Bulk oxygen consumption rates were determined as previously described in Dai et al. (2006).

BGD

5, 1545–1585, 2008

Nitrification and inorganic N in the Pearl River Estuary

Minhan Dai et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



2.4 Analyses

2.4.1 Nutrients

Samples were stored at -20°C until analysis except for NH_4^+ , which was analyzed on deck with the indophenol blue spectrophotometric method (Pai et al., 2001). NO_2^- and NO_3^- measurements were run in our land-based laboratory at Xiamen University according to classic colorimetric methods with a Technicon AA3 Auto-Analyzer (Bran-Lube). The detection limits for NO_2^- , NO_3^- and NH_4^+ were 0.02, 0.07 and $0.16 \mu\text{mol L}^{-1}$, respectively.

2.4.2 Abundance of nitrifying bacteria

The abundance of nitrifying bacteria (nitrifiers) was determined using the most probable number (MPN)-Griess technique (Abril et al., 2000; Man, 1975; Pauer and Auer, 2000). Prior to incubation, all the tubes, the culture media, and the NaCl solution (0.9%, for dilution) were sterilized in an autoclave at 120°C for 20 min. The media that we used were identical to those used by Chen and Zheng (1985), which dissolve 2.0 g of $(\text{NH}_4)_2\text{SO}_4$, 1 g of K_2HPO_4 , 0.5 g of MgSO_4 , 2 g of NaCl, 0.4 g of FeSO_4 and 5 g of CaCO_3 in 1 L of distilled water. The pH of the media solution was adjusted to 7.2. Samples were diluted to a different degree (from 10^{-1} to 10^{-7}) using sterilized NaCl solution. One mL of inoculum with a different degree of dilution was then added to a series of four tubes containing 9 mL of the culture media. These tubes were then incubated for 45–60 days at $28 \pm 2^{\circ}\text{C}$ in the dark. Finally, Griess reagent and diphenylamine were applied to examine whether nitrite or nitrate was formed. The abundance of nitrifiers was estimated based on classic MPN statistics (Man, 1975).

This MPN-Griess technique has been widely used and it is capable of diagnosing the existence of nitrifiers and estimating bacterial densities (e.g. Diab et al., 1993; Koops and Pommerening-Roser, 2001; Pauer and Auer, 2000). It should be noted that the MPN-Griess method has been criticized for its potential underestimation of bacterial

BGD

5, 1545–1585, 2008

Nitrification and inorganic N in the Pearl River Estuary

Minhan Dai et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



populations (Belser, 1979). However, when compared with the immunofluorescence technique or MPN-PCR method, it is considered to lead to an insignificant underestimation of cell counts (Fera y et al., 1999; Malhautier et al., 1998). The MPN-Griess technique is also considered to be advantageous for enumerating the autotrophic nitrifying community because many defects may exist in other methods (Fera y et al., 1999; Konuma et al., 2001). We have conducted a series of laboratory experiments using model nitrifiers and the results revealed a good consistency of cell abundances in duplicate samples. Standard deviations between replicates were 30%, 38% and 21% for cell ranges of $<50 \text{ cell mL}^{-1}$, $>100 \text{ cell mL}^{-1}$, and $>1000 \text{ cell mL}^{-1}$, respectively. Replicates were also taken for field samples in March 2006. Results showed that the standard deviation for field samples was better than 40%.

2.4.3 Temperature, salinity, DO and pH

Temperature and salinity were measured continuously using a YSI 6600 multi-parameter meter fitted in the underway measurement system described in Zhai et al. (2005) and Dai et al. (2006). Discrete DO was measured on board using the Winkler method. pH was measured on board within 4 h of sampling with a Corning[®] 350 pH/ions analyzer and Orion Ross combination pH electrode calibrated against 3 NIST traceable buffers.

3 Results

3.1 Spatial-temporal distributions of inorganic nutrients and DO

Table 2 summarizes chemical data collected in this study and reported in the literature at (or near) the freshwater endmembers (the Humen and Yamen outlets) of the Pearl River. All the data listed in Table 2 (except that in January 2005 at the upstream Yamen) were measured under a salinity <5 . Wide concentration ranges at the freshwater

BGD

5, 1545–1585, 2008

Nitrification and inorganic N in the Pearl River Estuary

Minhan Dai et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



endmembers were noticeable for all nutrients and DO in the study area (Figs. 3 and 4). The highly variable endmember concentrations were apparently influenced by the seasonal variation of river discharge, which had profound effects on the supply of organics and nutrients as well as the water mixing of the estuary.

5 The maximum NO_3^- concentrations observed in this study were $190 \mu\text{mol L}^{-1}$ in January 2005, $260 \mu\text{mol L}^{-1}$ in March 2006 and $110 \mu\text{mol L}^{-1}$ in August 2005. PO_4^{3-} was overall at a level of $1 \mu\text{mol L}^{-1}$ with the maximum concentration ($5.6 \mu\text{mol L}^{-1}$) observed in August 2005 at the upstream Humen. These very high NO_3^- and considerably low PO_4^{3-} concentrations are characteristic of the Pearl River as compared to other world major rivers (Cai et al., 2004; Yin et al., 2001; Zhang et al., 1999). Observed N/P ratios (total dissolved inorganic nitrogen to phosphate) in the upper reaches of the Pearl River Estuary ranged from 470–510 in January 2005 to 300–470 in March 2006, and 70–230 in August 2005 showing a significant seasonal variability with higher N/P ratios in the dry winter. This N/P ratio declined seaward (not shown), reaching ~80–100 in the vicinity of the Humen in both winter and summer, similar to what was previously documented (Cai et al., 2004; Dai et al., 2006; Zhang et al., 1999). Silicate ranged between 50 and $150 \mu\text{mol L}^{-1}$, which is comparable with historical data (Cai et al., 2004; Dai et al., 2006; Lin et al., 1985; Zhang et al., 1999).

For the upstream Humen, extremely high NH_4^+ concentration ($>800 \mu\text{mol L}^{-1}$) was observed in January 2005, but it was lower ($\sim 560 \mu\text{mol L}^{-1}$) in March 2006 and dropped to $\sim 340 \mu\text{mol L}^{-1}$ in August 2005. For comparison, NH_4^+ at the Yamen was considerably lower, ranging from a few $\mu\text{mol L}^{-1}$ in August 2005 to $<50 \mu\text{mol L}^{-1}$ in January 2005, revealing a similar seasonal pattern. The NH_4^+ decreasing tendency from dry to wet seasons was likely to be due to freshwater dilution. It must be pointed out that these high levels of NH_4^+ were observed in the upstream Humen, in the Guangzhou section (see Fig. 3) of the Pearl River, which has been directly impacted by regional waste discharge. This waste discharge has been increased due to regional development that began ~20 years ago. This is manifested in Fig. 2 which clearly shows the

Nitrification and inorganic N in the Pearl River Estuary

Minhan Dai et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



increase in discharges of total wastewater, chemical oxygen demand, and ammoniacal nitrogen, which began to be intensified in the new century.

The spatial distributions of NH_4^+ , NO_2^- and NO_3^- and DO during different cruises are demonstrated, respectively, in Fig. 3b–e for the Lingdingyang sub-estuary and in Fig. 3g–j for the Huangmaohai sub-estuary. Concentrations against salinity along the two transects are presented, respectively, in Fig. 4a–d and Fig. 4e–h. In the dry season (January 2005) with low freshwater input, surface salinity could be as high as 18 in the vicinity of the Humen Outlet (similar to February 2004 reported in Dai et al., 2006). By contrast, surface salinity was <1 at the same station even at high tide in August 2005 and ~ 2 in March 2006 (Fig. 3a). A similar salinity pattern was observed in the vicinity of the Yamen Outlet (Fig. 3f), which was ~ 0 and ~ 13 in August 2005 and January 2005, respectively. During low flow, seawater apparently intruded landward and this seawater intrusion supplied DO-rich seawater to the upstream area (Fig. 3b and g).

Sampling in all cruises covered a full range of salinity from 0 to >30 . Over such a wide range, sampling from the upstream Guangzhou to the downstream Humen (left panels in Fig. 3), covered total DIN ranges of $90\text{--}1000 \mu\text{mol L}^{-1}$, $40\text{--}700 \mu\text{mol L}^{-1}$ and $0\text{--}380 \mu\text{mol L}^{-1}$ in January 2005, March 2006 and August 2005, respectively. Short water residence time and larger freshwater dilution may have accounted for this overall lower total DIN in August. Yet, a few peaks of NH_4^+ , NO_2^- and NO_3^- appeared at the upper reach above the Humen. Occurrence of those peaks was related to branch or local sewage inputs from major cities, and meanwhile, their locations may have been affected by the tidal motion superimposed by the complex geometry of the Lingdingyang sub-estuary (Fig. 3). Low-nutrient and DO-rich seawater mixing accounted for the significant seaward-increasing trend in DO and seaward-decreasing trend in all nitrogen species. High levels of inorganic nitrogen and similar distribution patterns have been observed in the hypoxic area of the polluted Scheldt and the Seine estuaries (Abril and Frankignoulle, 2001; de Wilde and de Bie, 2000; Garnier et al., 2001).

In all seasons, NH_4^+ was the dominant species of inorganic nitrogen in the upper reach of the Lingdingyang sub-estuary. In both Fig. 3 and Fig. 4, we see that NH_4^+

BGD

5, 1545–1585, 2008

Nitrification and inorganic N in the Pearl River Estuary

Minhan Dai et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



**Nitrification and
inorganic N in the
Pearl River Estuary**

Minhan Dai et al.

dropped rapidly against salinity following the typical removal curve. This removal was associated with an increase in NO_3^- concentration. The relative abundance of nitrate became important seaward, reaching a concentration equivalent with that of NH_4^+ at a salinity ~ 10 (Fig. 4b–d). The maximum NO_2^- concentration was $65 \mu\text{mol L}^{-1}$ at Station 1 in January 2005 and it decreased seaward as salinity increased. In March 2006 and August 2005, the highest NO_2^- concentrations appeared at the sites where branches of the East River merge to the main channel (Fig. 3e). In March 2006, NO_2^- concentration was lowest, with a maximum of $30 \mu\text{mol L}^{-1}$, although the concentrations of NH_4^+ and NO_3^- were not the lowest among the seasons surveyed.

Surface DO was $>6 \text{ mg L}^{-1}$ in the vicinity of the Humen Outlet (Station 6) and decreased upstream in January 2005. At Station 2, DO was $<1 \text{ mg L}^{-1}$, similar to what was reported for February 2004 (Dai et al., 2006). In August 2005, surface DO ranged from $\sim 4.5 \text{ mg L}^{-1}$ at Station 6 to near zero at Station 2. Among the three cruises, the low DO area was largest in March 2006 (Fig. 3b). The most oxygen depleted zone (always $<3 \text{ mg L}^{-1}$) was between Station 4 and Station 2, where branches of the East River merge into the main channel of the Pearl River. DO concentration remained at a level of $1\text{--}2 \text{ mg L}^{-1}$ at the upstream Humen in March 2006. DO concentration between Station 1 and Station 2 was slightly higher than at the area between Station 2 and Station 4 in the summer cruise, implying the input of oxygenated freshwater. It is interesting to note that in March 2006, DO concentrations in areas around Station 6 were the lowest among the three seasons. In all three seasons, DO concentration along the salinity gradient of the estuary ranged from hypoxic conditions at low salinity to nearly saturated in the Lingdingyang. Saturation was overall lower in summer due mainly to the higher water temperature. It should also be noted that DO at salinity ~ 15 in August 2005 was very high (Fig. 4a), likely due to a regional algae bloom, where partial pressure of CO_2 ($p\text{CO}_2$) was as low as $180 \mu\text{atm}$ (Dai, unpublished data) as a result of enhanced photosynthesis. During all seasons, estuarine water upstream of the Humen was well mixed but stratified downstream as seen from the salinity and temperature vertical distributions as well as from the vertical profiles of both DO and

[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[◀](#)[▶](#)[◀](#)[▶](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)

nutrients (data not shown).

In the Huangmaohai sub-estuary (right panels in Fig. 3), DO concentrations mostly remained $> \sim 6 \text{ mg L}^{-1}$ in the upstream Yamen (Figs. 3g, 4e); yet, lower DO concentrations were found seaward around the sand bars (see Fig. 1b, c), which might be caused by local circulation and re-suspension. Similar to what was observed in the Lingdingyang sub-estuary, all inorganic nitrogen concentrations decreased with increasing salinity in the Huangmaohai sub-estuary, yet concentrations were much lower and the distribution pattern was much simpler due to less sewage input. In the Huangmaohai sub-estuary, both NO_3^- and NH_4^+ followed theoretical conservative mixing (Fig. 4f–g). Compared to the winter cruise, NH_4^+ concentrations in August 2005 were much lower ($< 5 \mu\text{mol L}^{-1}$) due to runoff dilution; however, NO_3^- concentrations were slightly higher. This inverse trend is attributable to the enhanced nitrification process and runoff dilution in summer. Contrasting to the upstream Humen, nitrate was the dominant species of inorganic nitrogen in the upper reaches of the Huangmaohai sub-estuary (Fig. 3h–j). This pattern should be due to the higher oxygen content in the water column.

3.2 Nitrifying activity and nitrifiers

Figure 5 shows an example of the evolution of NO_2^- , NH_4^+ and NO_3^- during the incubation experiment for the determination of nitrification rates. This example sample was taken from the surface at Station 2 in August 2005. With the addition of ATU, and hence an inhibition of NH_4^+ oxidation, this sample showed a linear decrease in NO_2^- with an increase in NO_3^- while NH_4^+ stayed constant (Fig. 5a). This suggests the production of NO_3^- from NO_2^- . In contrast, there was a linear NH_4^+ decrease and NO_2^- increase with no change of NO_3^- in the sample with NaClO_3 added, indicating that NO_2^- oxidation was efficiently blocked by NaClO_3 as expected (Fig. 5b). On the other hand, decreases in both NO_2^- and NH_4^+ and an increase in NO_3^- appeared due to the oxidation sequence from NH_4^+ to NO_2^- and then to NO_3^- in the control sample (Fig. 5c). The

Nitrification and inorganic N in the Pearl River Estuary

Minhan Dai et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



changing rate of NH_4^+ in the control was similar to the sample with NaClO_3 added while the rate of NO_2^- change in the control apparently represented the difference resulting from the two reactions. It should be noted that NO_2^- in the control showed nearly no change at the beginning of the incubation due to the fact that the changing rate of NO_2^- in the NaClO_3 added sample was almost equal to that in the ATU added sample. After this initial stage, NO_2^- in the control decreased when most of the NH_4^+ was consumed. Throughout the incubation experiment, all three species of inorganic nitrogen presented a constant sum (data not shown) indicating that a mass balance was reached during the transformation among nitrogen species, which strongly supported that our incubation experiments and analytical measurements were in order. The NH_4^+ and NO_2^- oxidation rates can therefore be estimated from the linear regression of nitrite in NaClO_3 or ATU added samples, respectively. The incubation experiment suggested a decline of the nitrification rate after 60 h, and so the last data point was excluded from the rate calculation.

Table 3 lists all the nitrification rates from the incubation experiments and concurrent inorganic nitrogen concentrations during sampling. The NH_4^+ oxidation rate ranged from 0 to $5.4 \mu\text{mol N L}^{-1} \text{d}^{-1}$ in January 2005; 0.1 to $14.8 \mu\text{mol N L}^{-1} \text{d}^{-1}$ in March 2006; and 1.5 to $33.1 \mu\text{mol N L}^{-1} \text{d}^{-1}$ in August 2005. Similarly, the NO_2^- oxidation rate ranged from 0 to $5.2 \mu\text{mol N L}^{-1} \text{d}^{-1}$ in January 2005; 0.2 to $5.2 \mu\text{mol N L}^{-1} \text{d}^{-1}$ in March 2006; and 0.6 to $32.0 \mu\text{mol N L}^{-1} \text{d}^{-1}$ in August 2005. The maximum nitrification rate appeared at Station 2. NH_4^+ and NO_2^- oxidation rates varied concomitantly showing similar spatial distributional patterns in all seasons. It is noticeable that the NH_4^+ oxidation rate was higher than the NO_2^- oxidation rate at most stations regardless of season. Such difference may cause NO_2^- accumulations. The nitrification activity at the end of the south branch of the East River (Station 5) was high, and the NH_4^+ oxidation rate reached $5.9 \mu\text{mol N L}^{-1} \text{d}^{-1}$ in March 2006 and $33.1 \mu\text{mol N L}^{-1} \text{d}^{-1}$ in August 2005. At Station 2, NH_4^+ and NO_2^- oxidation rates showed insignificant differences between bottom and surface samples. Similarly, hydro-chemical parameters showed insignificant

Nitrification and inorganic N in the Pearl River Estuary

Minhan Dai et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



differences in depth profiles (data not shown), suggesting that nitrification took place throughout the water column.

At Station 2, the highest NH_4^+ oxidation rate occurred in August 2005, $\sim 2\text{--}3$ times higher than that in March 2006, and $\sim 5\text{--}6$ times higher than that in January 2005. The nitrite oxidation rate did not show such a significant sequence. At Station 6 in the upper Lingdingyang, the nitrification rate peaked in March 2006 rather than in August 2005, when the nitrification rate reached $12.5 \mu\text{mol N L}^{-1} \text{d}^{-1}$ for NH_4^+ oxidation and $5.0 \mu\text{mol N L}^{-1} \text{h}^{-1}$ for NO_2^- oxidation. In comparison, nitrification activities at Station 7 in the Yamen upstream of the Huangmaohai sub-estuary showed a very low rate in August 2005 (Table 3). Figure 6c presents the sectional nitrification rates, classifying the study area into the upstream Guangzhou, downstream Guangzhou, and downstream Humen for the Lingdingyang sub-estuary; and the result from the upstream Yamen is also shown. The mean nitrification rates declined gradually seaward from the upstream Guangzhou, the region most impacted by regional wastewater discharges.

Compared to other estuaries in the world with low NH_4^+ concentrations, the nitrification rate in the Pearl River Estuary is high. For example, in the Rhone River plume, a nitrification rate of $0.2\text{--}2.2 \mu\text{mol N L}^{-1} \text{d}^{-1}$ at $\text{NH}_4^+ \sim 1\text{--}10 \mu\text{mol L}^{-1}$ is reported (Bianchi et al., 1997). Similarly in the Tamar Estuary with $\text{NH}_4^+ \sim 5 \mu\text{mol L}^{-1}$, a nitrification rate of up to $3 \mu\text{mol L}^{-1} \text{d}^{-1}$ is reported (Owens, 1986). In Narragansett Bay, this level is $0.9\text{--}11.0 \mu\text{mol N L}^{-1} \text{d}^{-1}$ at $\text{NH}_4^+ \sim 45 \mu\text{mol L}^{-1}$ (Berounsky and Nixon, 1990). However, nitrification rates in the Pearl River Estuary are lower than estuaries with high NH_4^+ concentrations, such as in the Scheldt Estuary, where the nitrification rate is reported to be $\sim 45\text{--}80 \mu\text{mol N L}^{-1} \text{d}^{-1}$ at a NH_4^+ concentration level of $150\text{--}500 \mu\text{mol L}^{-1}$ (de Bie et al., 2002; Somville, 1984). Elsewhere in the turbid water and fluid mud of the Gironde Estuary, the potential nitrification rate is very high, reaching $240\text{--}336 \mu\text{mol N L}^{-1} \text{d}^{-1}$, although the NH_4^+ concentration in the fluid mud is $<15 \mu\text{mol L}^{-1}$ (Abril et al., 2000). This may suggest that nitrification reactions are also modulated by other environmental factors in addition to NH_4^+ being the primary substrate. This will be further addressed in the Discussion section.

BGD

5, 1545–1585, 2008

Nitrification and inorganic N in the Pearl River Estuary

Minhan Dai et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



**Nitrification and
inorganic N in the
Pearl River Estuary**

Minhan Dai et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

The abundance of nitrifying bacteria in the water column of the Pearl River Estuary ranged between 2 and 2500 cells mL⁻¹, 3–3000 cells mL⁻¹ and 2–1150 cells mL⁻¹, respectively, in January 2005, March 2006 and August 2005 (Fig. 6). The density of nitrifying bacteria was overall high in spring (March 2006) and low in summer (August 2005), while the level in winter was intermediate. The highest nitrifier densities occurred at the upstream Guangzhou with low DO (Fig. 6). Nitrifier populations in most observed stations showed a dramatic decrease with increasing salinity in the Lingdingyang sub-estuary in all seasons. In summer and spring, the whole water column had an approximately similar nitrifier density (Table 3). The distribution pattern in nitrifier abundance was broadly consistent with that of the nitrification rates based on section category (Fig. 6).

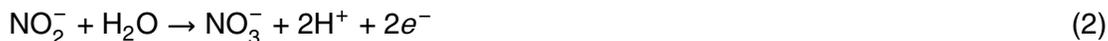
Compared to other estuaries in the world, the maximum nitrifier density observed in the water column of the Pearl River Estuary was 3×10^3 cell mL⁻¹, which is close to the level in the fluid mud of the Gironde Estuary (4×10^3 cell mL⁻¹) (Abril et al., 2000), but this density value was much lower than those in the Seine Estuary (Cébron and Garnier, 2005), where the nitrifier density was 3×10^7 cell mL⁻¹.

The maximum total prokaryote density was 2.9×10^6 cell mL⁻¹ and 2.63×10^6 cell mL⁻¹ in March 2006 and August 2005, respectively, in the pelagic waters of the Pearl River Estuary (Zhang and Jiao, unpublished data), the highest of which was also located at the upstream Humen. Bacterial density gradually decreased downstream due to mixing with the low-nutrient seawater, and the density dropped to 3.6×10^5 cell mL⁻¹ and 7.7×10^5 cell mL⁻¹ at the sea end in March 2006 and August 2005, respectively. The distribution of total prokaryotes against salinity was similar to the nitrifiers, although the ratio of nitrifiers to total prokaryotes was low.

4 Discussion

4.1 Interrelationship between nitrification and environmental conditions

Nitrification is typically composed of two reactions: ammonia oxidation (Eq. 1) and nitrite oxidation (Eq. 2).



Nitrifiers are obligate for nitrification reactions and are commonly observed in terrestrial and aquatic environments (Holt et al., 1995). Their growth rates are controlled by substrate (ammonia-N) concentrations, temperature, pH, light, DO, microbial abundance and community composition. In wastewater treatment systems and the soil environment, there has been extensive research on the media conditions affecting nitrifier growth and nitrification rates (e.g. Bhaskar and Charyulu, 2005; Kemmitt et al., 2006; Kesik et al., 2006; Lyssenko and Wheaton, 2006). In real aquatic systems, however, such research has been limited (Chenier et al., 2006; Dollhopf et al., 2005) due to the magnitude and the complexity of natural systems like the Pearl River Estuary. Below, we attempt to elucidate the interplay between nitrification and environmental conditions.

4.1.1 Nitrification vs. temperature

Much literature has documented that the nitrification rate in both marine and freshwater systems increases exponentially with temperature. For example, the nitrification rate in Narragansett Bay ranges from near zero in winter to $\sim 1 \mu\text{mol N L}^{-1} \text{d}^{-1}$ in summer, with an apparent $Q_{10} \sim 6.8$. (Q_{10} represents the increase in the rate of a process at each 10°C increase in temperature (See Berounsky and Nixon, 1990)). It is reported that the mean Q_{10} for the potential nitrification rate is ~ 2.5 within the temperature range

BGD

5, 1545–1585, 2008

Nitrification and inorganic N in the Pearl River Estuary

Minhan Dai et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



2–22°C (Hansen et al., 1981). In this study, the nitrification rate was consistently higher in summer, ~2–6 times higher than in other seasons except at Station 6. Especially at Station 2 in the Guangzhou section of the Pearl River, the ammonia oxidation rate in August 2005 was ~2 times higher than that in March 2006, and ~3 times higher than that in January 2005. At the same time, the surface water temperature in August 2005 was 15°C higher than in January 2005. Good correlation existed between the mean nitrification rate and temperature, exemplified in Fig. 7a, upstream and downstream of Guangzhou, suggesting a strong control of temperature on the nitrification rate. Moreover, regressions of the logarithm of the NH_4^+ oxidation rate vs. temperature also showed a significant positive relationship ($R^2=1.0$), and the calculated apparent Q_{10} value was 1.60 at Station 2, which is close to the value of ~2.0, based on the conservative estimate by the EPA (EPA, 1993). This value also is within the typical range of 1–3.5 for nitrification processes by cultured marine and freshwater nitrifiers (Jones et al., 2005). It is noteworthy that in coastal marine systems, other nitrogen species transformations such as denitrification, nitrogen fixation, phytoplankton growth, and regeneration of ammonium, are also sensitive to temperature with a Q_{10} ~1.2 to 9.4 (Berounsky and Nixon, 1990)

4.1.2 Nitrification vs. nitrogen substrate

Excess nitrogen in the form of ammonia may drive nitrification. As mentioned above, a dramatic decrease in NH_4^+ , an increase in NO_3^- and the invariant NO_2^- were observed at salinity 0 to 5 in the Pearl River Estuary in January 2005. A similar trend was observed in August 2005, when NO_3^- dropped to ~110 $\mu\text{mol L}^{-1}$ and remained at this level from Station 4 to Station 6 despite the fact that NH_4^+ and NO_2^- concentrations were low. High nitrifying activity dominated this section of the Pearl River Estuary between Station 2 and Station 6, which may explain the high NO_3^- concentration at this location. At the end of the south branch of the East River, we also observed high nitrifying activity, coinciding with the decrease of NH_4^+ and increase of NO_3^- downstream. This nitrifying

Nitrification and inorganic N in the Pearl River Estuary

Minhan Dai et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



**Nitrification and
inorganic N in the
Pearl River Estuary**

Minhan Dai et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



activity and nutrient distribution pattern, together with the nitrogen transformation inferred, strongly supported the contribution of nitrification processes in this location. A very similar case is documented in the Delaware River (Lipschultz et al., 1986). However, being a primary substrate for nitrification, low NH_4^+ concentration may also limit the nitrification activity. For example, the nitrification activity at Station 7 in the Yamen upstream of the Huangmaohai sub-estuary showed a very low rate in August 2005, which could be explained by the low level of NH_4^+ ($<5 \mu\text{mol L}^{-1}$) despite the relatively high nitrifier abundance.

It should be noted that we did not find a linear and simple correlation between the nitrifying activity and inorganic nitrogen in the Pearl River Estuary. In the winter (January 2005), the most upper reaches of the Pearl River Estuary showed relatively low NH_4^+ oxidation ($5.4 \mu\text{mol N L}^{-1} \text{d}^{-1}$) and NO_2^- oxidation rates ($5.2 \mu\text{mol N L}^{-1} \text{d}^{-1}$). However, this region was characterized by very high NH_4^+ ($>800 \mu\text{mol L}^{-1}$) and NO_3^- ($>300 \mu\text{mol L}^{-1}$) concentrations. In summer, both surface and bottom waters of the upper estuary showed very high nitrification rates (NH_4^+ oxidation rate $\sim 31.5 \mu\text{mol N L}^{-1} \text{d}^{-1}$, NO_2^- oxidation rate $\sim 29.5 \mu\text{mol N L}^{-1} \text{d}^{-1}$) accompanied by relatively low NH_4^+ and NO_2^- concentrations as compared to winter. Significant positive correlations between the NH_4^+ and NO_2^- oxidation rates ($R^2=0.70$, data not shown), and between NO_2^- oxidation rates and NO_2^- concentration (Fig. 7b) were observed. This phenomenon elucidates the substrate effect on the nitrification rate. On the other hand, no significant correlation between NH_4^+ and NO_2^- oxidation rates was found, which is similar to the case in the Rhone River plume and the Indian sector of the Southern Ocean (Bianchi et al., 1994, 1997).

4.1.3 Nitrification vs. nitrifier activity

Nitrifier abundance is reflective of nitrification potential. As shown in Fig. 6c–d, the distribution of the nitrification rate was overall consistent with that of nitrifier abundance, except at downstream Humen in March 2006, when a low nitrifier density was asso-

**Nitrification and
inorganic N in the
Pearl River Estuary**

Minhan Dai et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

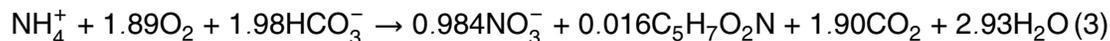


ciated with a higher nitrifying activity. In addition, nitrifier density at Station 2 were relatively low but with the highest nitrifying activity when compared with other stations in August 2005 (Table 3). The low nitrifier densities and high ammonium oxidation rate revealed a much greater specific nitrification rate per cell. There are other reports on similar cases. In Lake St. George, less than 1–2 cells mL⁻¹ were found, however, it would need a nitrifier population of 1.6×10²–1.0×10⁴ cells mL⁻¹ according to the in situ nitrifying activity of 13 μg N L⁻¹ d⁻¹ (Knowles and Lean, 1987).

It should be pointed out that most nitrifiers are attached to particles (Stehr et al., 1995). Particle aggregates provide microsites in which oxygen concentrations are usually lower than in the surrounding waters, which favors bacterial growth. The accumulation of particles in estuaries was shown to enhance the growth of the nitrifying population and nitrifying activity in the Gironde, Seine and Yellow River/Estuary (Abril et al., 2000; Brion et al., 2000; Xia et al., 2004). In August 2005, Station 2 showed a high nitrification rate despite a relatively low nitrifier density, which may be due to the high turbidity (total suspended solids (TSS) up to be 49 mg L⁻¹). In March 2006, nitrifier densities and inorganic nitrogen was low at Station 6 located at downstream Humen as compared to other stations, however it showed moderate nitrifying activity, which may be explained again by high TSS concentration (Table 3). Finally, it must be pointed out that our MNP method for determining nitrifier abundance does not distinguish between the two groups of nitrifiers.

4.2 Nitrification and community oxygen consumption

Coupling the two oxidation processes (Eqs. 1 and 2) and assimilation reactions, the overall reaction describing the complete nitrification process should be (see Dai et al., 2006, after US EPA, 1993):



The stoichiometric coefficients imply that 1 mole NH₄⁺ removal through nitrification required a 1.98 mole of TALK consumption and produced 1.90 mole of CO₂. Implied from

the above reaction is that the carbonate system was greatly related to the nitrification process, which consumes oxygen and produces CO_2 and hence acid. Below we further elucidate the importance of nitrification in community oxygen consumption in our system.

Oxygen is essential during both the ammonia and nitrite oxidation processes (Deri, 1991). DO, as the growth-limiting substrate, values for the half-saturation coefficient are reported as $0.15\text{--}2.0\text{ mg L}^{-1}$ in biological waste treatment systems (EPA, 1993). Nitrification reactions typically happen within a DO range of $0.5\text{--}2.5\text{ mg L}^{-1}$ although they can still proceed at a DO level as low as 0.05 mg L^{-1} (Abeliovich, 1987). Under certain conditions, nitrification can impose significant oxygen demands leading to localized oxygen minima in the upper reaches of estuaries (Balls et al., 1996).

Here in the Pearl River Estuary, we found complex relationships between DO and nitrification rates, but an overall reverse trend was observed in all seasons and most of the higher ammonia oxidation rates were associated with a DO range of $0.5\text{--}2.5\text{ mg L}^{-1}$ (Fig. 7c), consistent with the previous studies mentioned above. The decrease in DO concentration also coincided with an increase in NO_2^- in most cases, which may suggest that DO is one of the most important factors interplaying with nitrification in the Pearl River Estuary. At station 6, DO concentration was lowest in March 2006, while the nitrification rate was the highest among the three surveyed seasons (Fig. 3 and Table 3). This suggests that nitrification may represent an important mechanism contributing to O_2 consumption.

In fact, nitrification-induced DO consumption has been reported previously. Nitrification accounts for around 25% of the total biological oxygen demand in the West Schelde Estuarine maximum turbidity zone and for $>20\%$ in the water column (Pakulski et al., 1995; Soetaert and Herman, 1995). Capers (1981) reports that intense nitrification is correlated with a depletion of the oxygen concentration in a large zone of the freshwater section in the Elbe Estuary. In the Mississippi River plume, DO consumption associated with potential nitrification amounts to $20\sim 34\%$ of the community DO consumption at the mid-salinity zone (Pakulski et al., 1995). At Lake St. George,

**Nitrification and
inorganic N in the
Pearl River Estuary**

Minhan Dai et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



nitrification even results in 71% of average oxygen consumption under winter ice, and promotes oxygen depletion and possibly fish kills in winter (Knowles and Lean, 1987).

It must be pointed out that the DO level and its consumption involves many complex processes, such as aerobic respiration, water residence times, and air-sea oxygen exchanges (Dai et al., 2006; Griffith et al., 1990; Hopkinson et al., 1989; Zhai et al., 2005). The contribution of nitrification to DO consumption is typically confined to a narrow range of an estuarine zone, such as in the upper reaches. In the present study, if we assumed that nitrification consumed 1.4 mol and 0.5 mol O₂ when oxidizing 1 mol of NH₄⁺ and 1 mol of NO₂⁻, DO consumption caused by nitrification was 0.3 μmol O₂ L⁻¹ h⁻¹ in January 2005, 0.7 μmol O₂ L⁻¹ h⁻¹ in March 2006 and 2.5 μmol O₂ L⁻¹ h⁻¹ in August 2005 at Station 2. At Station 1, this value was 0.4 μmol O₂ L⁻¹ h⁻¹ in January 2005. At the same time, the total oxygen consumption rate was 2.7 μmol O₂ L⁻¹ h⁻¹ at Station 2 in March 2006 and 7.5 μmol O₂ L⁻¹ h⁻¹ in August 2005. Therefore, the ratio of O₂ consumption due to nitrification to community O₂ consumption was ~33%, ~26% for summer and spring, respectively, at Station 2. Although this ratio cannot represent the entire Pearl River Estuary and the respiration process involved a complex suit of variables, what can be certain is that community O₂ consumption by nitrification was significant in the upper Pearl River Estuary. Heterotrophic bacteria are generally considered to be the principle consumers of O₂ in marine ecosystems. As a comparison, Jensen et al. (1990) reported that organisms other than heterotrophic bacteria were responsible for >50% of community O₂ consumption in a highly eutrophic region of the shallow Danish Estuary. So we assert that heterotrophic bacteria and other organisms (zooplankton, protozoa etc.) consumed most O₂ in spring or winter, while nitrifiers were the key consumers of DO in summer in the upper Pearl River Estuary.

As shown by the formation of hydrogen ions (acid) in Eqs. (1) and (2), reductions in pH and alkalinity are expected to inhibit the nitrification reaction. An ideal pH range for nitrification is between 7.5 and 8.5 (Engel and Alexander, 1958; Wild et al., 1971) although nitrifying bacteria can adapt to environments outside this range. Alkalinity is also consumed during the nitrification process and thus sufficient alkalinity must be

**Nitrification and
inorganic N in the
Pearl River Estuary**Minhan Dai et al.

[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[◀](#)[▶](#)[◀](#)[▶](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)

**Nitrification and
inorganic N in the
Pearl River Estuary**

Minhan Dai et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

present to buffer the acids produced during nitrification. A number of studies have suggested that NH_4^+ oxidation might be inhibited at low pH. Huesemann et al. (2002) found that relative to pH 8, the nitrification rate dramatically drops with pH, by ~50% at pH 7 and by >90% at pH 6.5 when incubating seawater samples taken from either the euphotic or aphotic zone. Earlier, Srna and Baggaley (1975) report a ~50% reduction in marine ammonium oxidation rates when pH drops from 7.8 to 7.3.

During our March 2006 cruise, pH ranged between 6.94 and 7.25. The ammonia oxidation rate showed a significant positive correlation with pH (Fig. 7d). This trend is similar to the result observed by Huesemann (2002). However, the correlation between pH and nitrification did not appear to exist in other cruises, which again emphasized the complexity of the interplay between these two variables. pH is regulated by many physicochemical factors, such as NH_4^+ , TALK, DIC concentration, acid wastewater, biological production and respiration, and the fact that the Pearl River Estuary is indeed a complex system with large temporal or spatial variations of physicochemical factors.

Nitrification apparently interplayed with other carbonate parameters according to our observation in all cruises. We observed that high nitrifying activities accompany high $p\text{CO}_2$, TALK and DIC in the upper estuary of the Pearl River in winter (Dai et al., 2006; Guo et al., 2008). Dai et al. (2006) speculate that nitrification together with aerobic respiration contribute to the consumption of DO and may have significant impact on the distribution pattern of the carbonate system in the upper Pearl River Estuary. In summer (August 2005), our incubation experiment showed very high nitrification rates. Meanwhile, we found significant DIC change and TALK decrease during the incubation due likely to the CO_2 outgassing and nitrification for the surface or bottom water of the upper estuary (data not shown). If DIC change in the incubation was completely due to the CO_2 outgassing, then the rate of DIC decrease would be equal to the rate of CO_2 outgassing. The $\Delta[\text{CO}_2]/-\Delta[\text{NH}_4^+]$ quotient at both the surface and bottom of Sta 2 in Aug. 2005 ranged between 1.78 and 2.33, which was close to the theoretical nitrification quotient of 1.90 shown in Eq. (3).

Taken together, $p\text{CO}_2$ mirrored DO for all incubation stations in all seasons (Fig. 7e) though the correlation coefficient for both was not very high ($R^2=0.47$). This phenomenon has been indicated previously (Dai et al., 2006; Zhai et al., 2005). Nitrification rates varied concomitantly with $p\text{CO}_2$, particularly in summer, revealing its important role in consuming DO and producing free CO_2 in the upper Pearl River Estuary.

5 Concluding remarks

Extremely high concentrations of NH_4^+ and depleted DO in the water sound an alarm concerning the ecosystem changes due to heavy pollution in the Pearl River Estuary. Freshwater runoff, sewage input and in-estuary nitrification processes strongly affected the concentration and relative distribution of various inorganic nitrogen forms on a spatial scale. A strong nitrification process was observed in the upper Pearl River Estuary and its intensity changed over time with the highest nitrification rate occurring in summer, when temperature was high and O_2 solubility was low. Evidence from the present investigations indicated that nitrification was an important mechanism contributing to O_2 consumption in the upper Pearl River Estuary, which counted for about 20–30% of the community O_2 consumption in the oxygen depleted zone. Temperature appeared to control the nitrification rate to a large degree; additionally, the nitrification rate may also have been influenced by oxygen, pH, nitrifier density and TSS.

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BGD

5, 1545–1585, 2008

Nitrification and inorganic N in the Pearl River Estuary

Minhan Dai et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



References

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BGD

5, 1545–1585, 2008

Nitrification and inorganic N in the Pearl River Estuary

Minhan Dai et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



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BGD

5, 1545–1585, 2008

Nitrification and inorganic N in the Pearl River Estuary

Minhan Dai et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



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BGD

5, 1545–1585, 2008

Nitrification and inorganic N in the Pearl River Estuary

Minhan Dai et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



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BGD

5, 1545–1585, 2008

Nitrification and inorganic N in the Pearl River Estuary

Minhan Dai et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



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BGD

5, 1545–1585, 2008

Nitrification and inorganic N in the Pearl River Estuary

Minhan Dai et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



**Nitrification and
inorganic N in the
Pearl River Estuary**

Minhan Dai et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



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BGD

5, 1545–1585, 2008

**Nitrification and
inorganic N in the
Pearl River Estuary**

Minhan Dai et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Nitrification and inorganic N in the Pearl River Estuary

Minhan Dai et al.

Table 1. Basic hydrograph of major sub-estuaries of the Pearl River.

Sub-estuary	Outlet	Area ^a (km ²)	Volume ^a (10 ⁶ m ³)	Water ^b discharge (%)	Sediment ^c load (%)	Tidal ^d exchange (%)
Lingdingyang	Humen (HUM)	1180	8068	55	46	~60
	Jiaomen (JOM)					
	Hongqimen (HQM)					
Modaomen	Hengmen (HEM)	350	1750	28	33	
	Modaomen (MDM) Jitimen (JTM)					
Huangmaohai	Hutiaomen (HTM)	440	1760	13	11	~28
	Yamen (YAM)					

^aWong and Cheung (2000)

^bCheung et al. (2000)

^cPRWRC/PRRCC (1991)

^dCheng (2001)

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Nitrification and inorganic N in the Pearl River Estuary

Minhan Dai et al.

Table 2. Summary of nutrient ($\mu\text{mol L}^{-1}$) and DO (mg L^{-1}) data in the literature and in this study at the low-salinity zone of the two sub-estuaries of the Pearl River: Lindingyang and Huangmaohai. DIN denotes the sum of NO_2^- , NH_4^+ and NO_3^- .

Survey time	Location	Sal.	NO_2^-	NH_4^+	NO_3^-	DIN	PO_4^{3-}	SiO_2	DO	Ref.
Jan 1984	Humen vicinity	~0.0	–	–	–	91.0	0.5	–	–	Lin et al. (1985)
Feb 2004	Upstream Humen	2.2	36.2	455.9	272.0	764.0	0.5	85.6	0.4–2.2	Dai et al. (2006)
Jan 2005	Upstream Humen	0.7–0.9	46.7–67.8	615.3–832.1	107.1–190.1	852.1–1007.0	1.8–2.0	31.3–84.7	0.3–2.6	This study
Jan 2002	Jiaomen vicinity	4.1	11.1	2.6	143.5	157.0	1.3	–	–	Wang et al. (2003)
Jan 2005	Upstream Yamen	6.6	18.0	42.3	58.4	118.7	0.8	48.7	7.7	This study
Mar 2006	Upstream Humen	0.1–0.9	12.5–33.0	194.0–559.2	91.8–266.2	326.0–698.2	1.0–1.8	96.7–157.3	0.9–2.7	This study
Mar 1997	Jiaomen vicinity	1.1	–	–	–	90.0	0.5	–	4.4	Yang and Zhang (1999)
Aug 1996	Humen vicinity	~	–	up to 22.0	up to 76.0	–	up to 1.0	up to 180.0	–	Zhang et al. (1999a)
Jul 2000	Humen vicinity	<1.0	1.6–7.8	4.8–12.0	70.0–100.0	75.0–110.0	0.2–1.2	126.0–141.0	3.5–7.2	Cai et al. (2004)
Aug 2005	Upstream Humen	0.0–0.8	7.5–55.8	29.4–341.9	16.9–111.5	151.0–381.8	1.0–5.6	61.6–165.4	0.2–0.4	This study
Jul 1996	Jiaomen vicinity	0.7	–	–	–	746.0	1.7	–	4.1	Yang and Zhang (1999)
Aug 2005	Upstream Yamen	~0.1	1.9–8.9	1.8–4.9	75.3–119.3	80.0–124.4	0.5–1.6	52.3–128.3	3.6–7.3	This study

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Nitrification and inorganic N in the Pearl River Estuary

Minhan Dai et al.

Table 3. Ammonia oxidation rate (V_a), nitrite oxidation rate (V_n), and other parameters at all incubation stations.

Location	Station	Dates	Nitrifying activity ($\mu\text{mol L}^{-1} \text{d}^{-1}$)		NO_2^-	NH_4^+	NO_3^-	Nitrifier (cell mL^{-1})	TSS (NTU)	
			V_a	V_n						$(\mu\text{mol L}^{-1})$
Upstream Guangzhou	Station 1	Jan 2005	3.9	4.6	67.8	832.1	107.1	950	–	
		Aug 2005	12.5	1.5	23.1	341.9	16.7	20	37.0	
		Mar 2006	2.9	2.2	25.8	341.9	266.2	3500	16.8	
	Station 2 (surface)	Jan 2005	5.4	5.2	17.4	188.3	185.3	2500	–	
		Aug 2005	31.5	29.5	65.0	111.5	60.4	20	49.0	
		Mar 2006	10.1	5.2	19.9	377.8	131.5	3500	20.4	
		Station 2 (bottom)	Aug 2005	28.2	32.0	65.8	100.1	62.0	20	–
			Mar 2006	14.8	3.0	18.8	367.1	133.2	3000	–
			Aug 2005	1.5	0.6	13.4	62.7	62.7	165	35.4
Downstream Guangzhou	Station 3	Mar 2006	0.1	0.2	6.8	133.7	102.1	200	25.8	
		Jan 2005	0.0	0.0	20.6	100.5	95.0	12	–	
	Station 4	Mar 2006	0.3	0.4	16.0	236.9	99.4	35	–	
		Aug 2005	4.1	1.0	9.8	58.6	42.7	2	23.9	
		Aug 2005	33.1	12.1	7.3	61.0	97.2	1150	35.1	
	Station 5	Mar 2006	5.9	3.3	15.2	261.8	95.3	1650	21.3	
		Aug 2005	3.9	1.1	11.2	50.2	74.3	10	16.2	
	Downstream Humen	Station 6	Mar 2006	12.5	5.0	31.7	132.2	121.5	200	51.2
			Aug. 2005	0.0	1.6	4.0	1.2	119.4	165	42.8
Yamen	Station 7	Aug. 2005	0.0	1.6	4.0	1.2	119.4	165	42.8	

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Nitrification and inorganic N in the Pearl River Estuary

Minhan Dai et al.

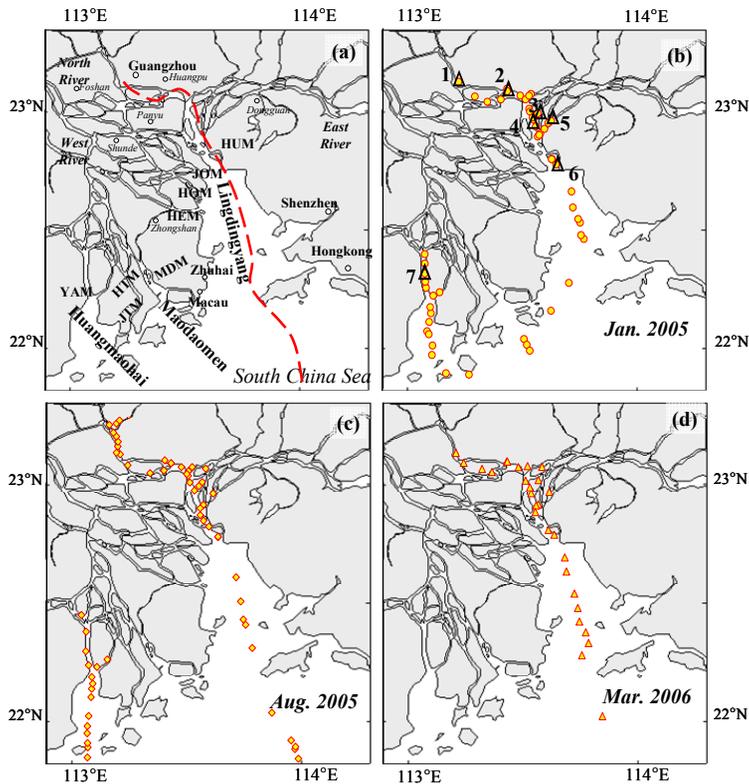


Fig. 1. Map of the Pearl River Estuary (a) and sampling sites (b, c, d) in different cruises. HUM, JOM, HQM and HEM designate Humen, Jiaomen, Hongqimen, and Hengmen, respectively, which are the eastern four outlets of the Pearl River Estuary. YAM and MDM designate, respectively, the Yamen and Madaomen outlets. The three sub-estuaries, Lindingyang, Madaomen and Huangmaohai, are also indicated. The red dashed line in panel (a) shows the main cruise track of the cruises. Nutrient sampling sites for January 2005, August 2005 and March 2006 cruises are in yellow symbols in panels (b), (c) and (d), respectively. Open triangles in panel (b) mark those stations for incubation.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Nitrification and inorganic N in the Pearl River Estuary

Minhan Dai et al.

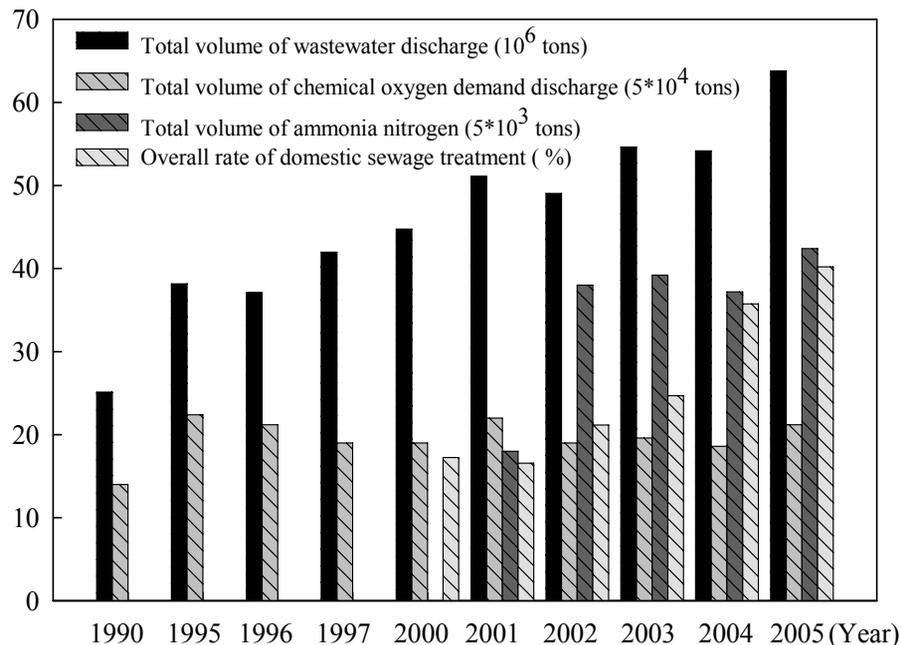


Fig. 2. Quantity of wastewater discharge from Guangdong Province, China between 1990 and 2005. Data between 1990 and 1997 are from Ho and Hui (2001), while the rest of the data are based on Environmental Status Bulletins of Guangdong Province, China (<http://www.gdepb.gov.cn>).

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Nitrification and inorganic N in the Pearl River Estuary

Minhan Dai et al.

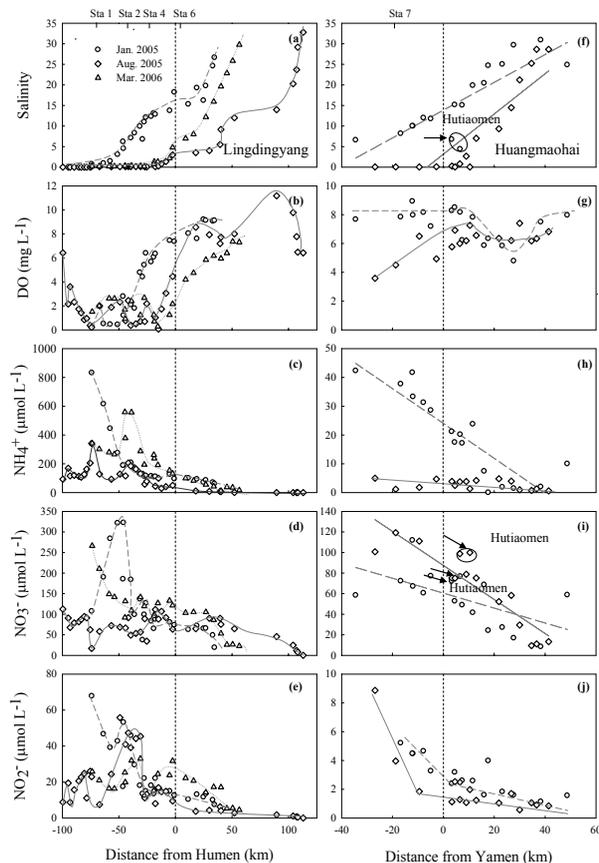


Fig. 3. Salinity, DO and inorganic nitrogen vs. distance from Humen or Yamen along the sampling transects in this study. The broken vertical lines represent the location of the Humen and Yamen outlets. Positive numbers denote downstream and negative values are upstream of the outlets. The left panels are for the Lingdingyang sub-estuary and the right ones are for the Huangmaohai sub-estuary.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Nitrification and inorganic N in the Pearl River Estuary

Minhan Dai et al.

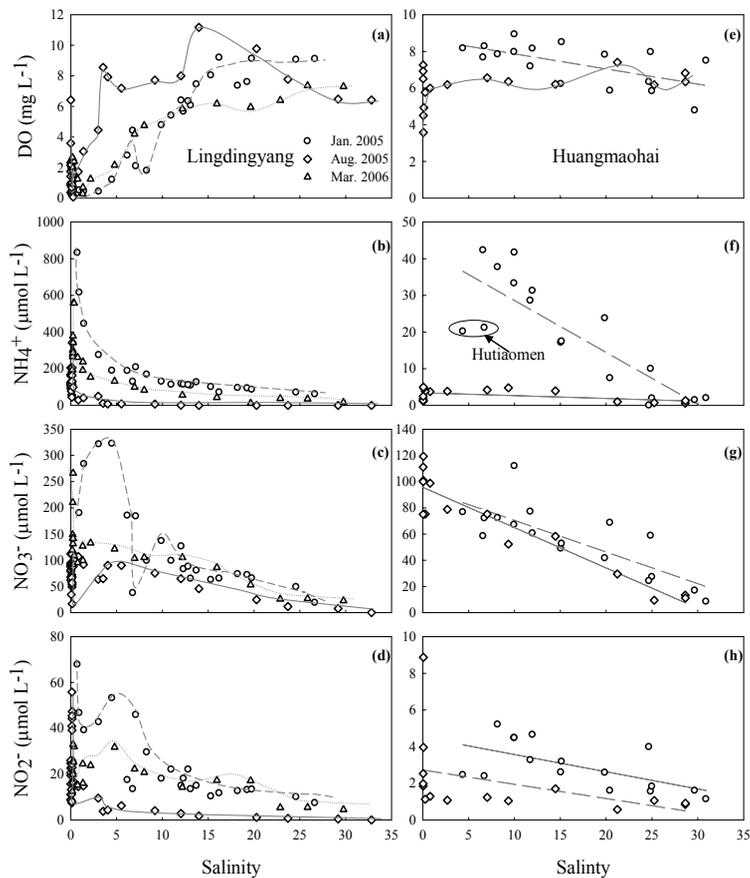


Fig. 4. Dissolved oxygen and dissolved inorganic nitrogen vs. salinity along the Humen and Yamen transects.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Nitrification and inorganic N in the Pearl River Estuary

Minhan Dai et al.

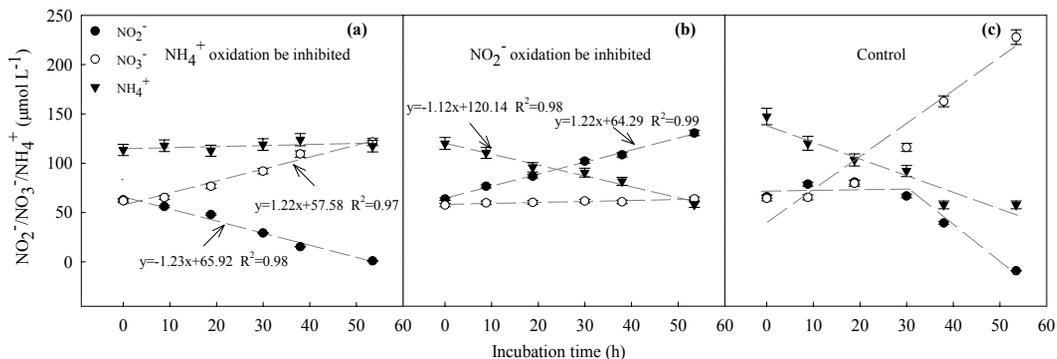


Fig. 5. Evolution trend of ammonium (NH_4^+), nitrite (NO_2^-), and nitrate (NO_3^-) concentrations in the course of nitrification incubations for samples taken from Station 2 in August 2005. **(a)** oxidation of ammonium inhibited; **(b)** oxidation of nitrite inhibited; **(c)** control sample without any inhibitors.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



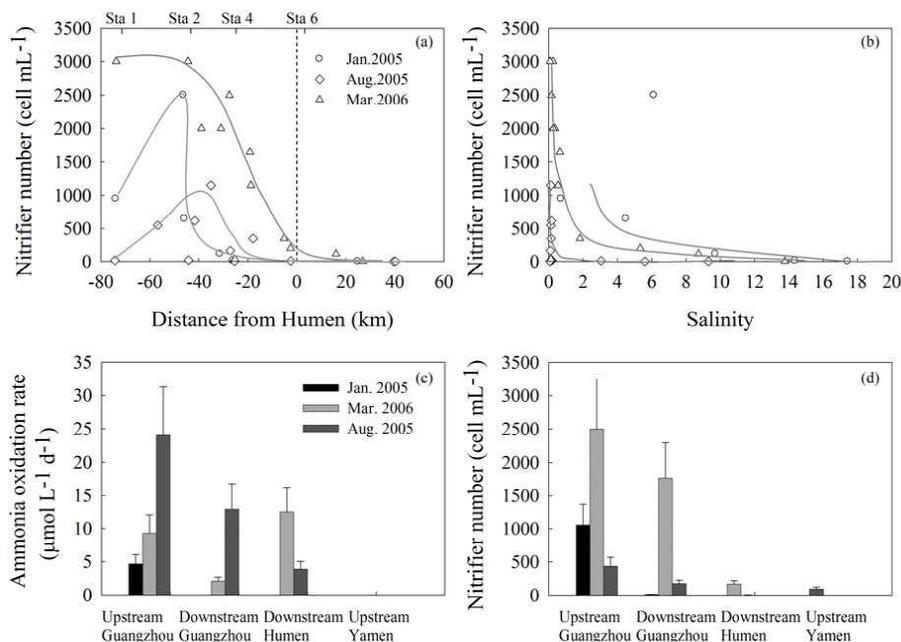


Fig. 6. Spatial distributions of nitrifier and ammonia oxidation rates in the Pearl River Estuary. **(a)** Nitrifier density vs. distance from the Humen and; **(b)** Nitrifier density vs. salinity along the Humen transects; **(c)** Sectional distribution of ammonia oxidation rate, and **(d)** Sectional distribution of nitrifier density. Upstream of Guangzhou is defined as a distance of -100 to -30 km from the Humen; downstream of Guangzhou is defined as a distance of 0 to -30 km from the Humen; downstream of the Humen is defined as a distance >0 km; upstream of the Yamen is defined as a distance <0 km from the Yamen.

Title Page

Abstract Introduction

Conclusions References

Tables Figures

◀ ▶

◀ ▶

Back Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



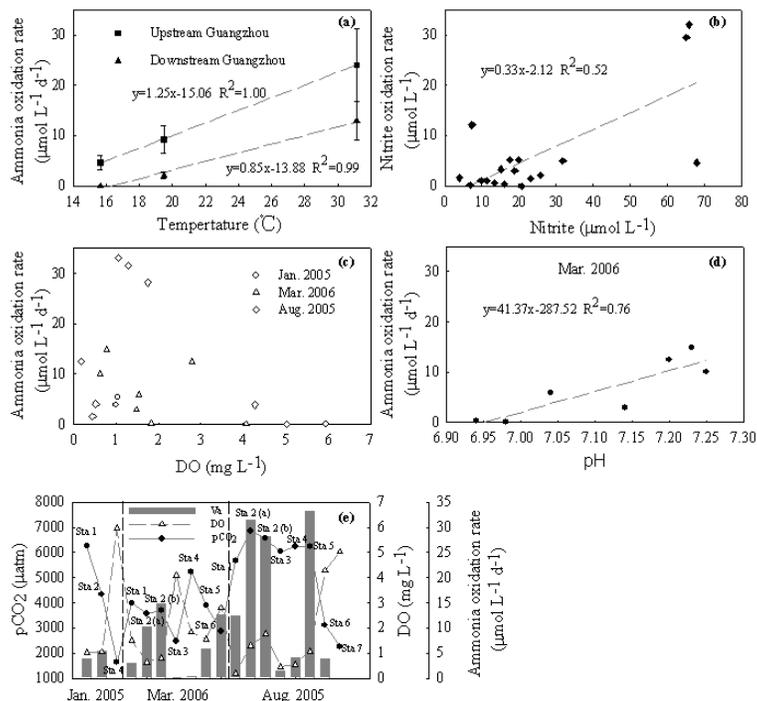


Fig. 7. Interrelationship between ammonia oxidation rates and environmental variables. **(a)** average ammonia oxidation rates at upstream and downstream of Guangzhou vs. temperature during all cruises; **(b)** nitrite oxidation rates vs. nitrite concentration for all cruises; **(c)** ammonia oxidation rates vs. DO concentration for different cruises; **(d)** ammonia oxidation rates vs. pH for spring (March 2006) cruise; and **(e)** ammonium oxidation rates vs. $p\text{CO}_2$ and DO for all nitrification incubation stations for all the three cruises. $p\text{CO}_2$ data of January 2005 and August 2005 are cited from Dai et al. (unpublished data). $p\text{CO}_2$ data in March 2006 are derived from DIC and TALK, salinity, in situ temperature, silicate and phosphate concentration with the CO_2SYS program (Lewis and Wallace, 1998).

Title Page

Abstract Introduction

Conclusions References

Tables Figures

◀ ▶

◀ ▶

Back Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

