Acta Oceanol. Sin., 2024, Vol. 43, No. 6, P. 86–95
https://doi.org/10.1007/s13131-024-2375-y
http://www.aosocean.com
E-mail: ocean2@hyxb.org.cn

Tracing nitrate sources in one of the world's largest eutrophicated bays (Hangzhou Bay): insights from nitrogen and oxygen isotopes

Zhi Yang[1], Jianfang Chen[1, 2*], Haiyan Jin[1], Hongliang Li[1], Zhongqiang Ji[1], Yangjie Li[1], Bin Wang[1], Zhenyi Cao[1], Qianna Chen[1]

[1] Key Laboratory of Marine Ecosystem Dynamics, Second Institute of Oceanography, Ministry of Natural Resources, Hangzhou 310012, China

[2] State Key Laboratory of Satellite Ocean Environment Dynamics, Second Institute of Oceanography, Ministry of Natural Resources, Hangzhou 310012, China

Received 27 February 2023; accepted 6 June 2023

Abstract

Eutrophication caused by inputs of excess nitrogen (N) has become a serious environmental problem in Hangzhou Bay (China), but the sources of this nitrogen are not well understood. In this study, the August 2019 distributions of salinity, nutrients [nitrate (NO_3^-), nitrite, ammonium, and phosphate], and the stable isotopic composition of NO_3^- ($\delta^{15}N$ and $\delta^{18}O$) were used to investigate sources of dissolved inorganic nitrogen (DIN) to Hangzhou Bay. Spatial distributions of nitrate, salinity, and nitrate $\delta^{18}O$ indicate that the Qiantang River, the Changjiang River, and nearshore coastal waters may all contribute nitrate to the bay. Based on the isotopic compositions of nitrate in these potential source waters and conservative mixing of nitrate in our study area, we suggest that the NO_3^- in Hangzhou Bay was likely derived mainly from soils, synthetic N fertilizer, and manure and sewage. End-member modeling indicates that in the upper half of the bay, the Qiantang River was a very important DIN source, possibly contributing more than 50% of DIN in the bay head area. In the lower half of the bay, DIN was sourced mainly from strongly intruding coastal water. DIN coming directly from the Changjiang River made a relatively small contribution to Hangzhou Bay DIN in August 2019.

Key words: nitrogen isotopes, oxygen isotopes, nitrogen cycle, nitrate sources, Hangzhou Bay

Citation: Yang Zhi, Chen Jianfang, Jin Haiyan, Li Hongliang, Ji Zhongqiang, Li Yangjie, Wang Bin, Cao Zhenyi, Chen Qianna. 2024. Tracing nitrate sources in one of the world's largest eutrophicated bays (Hangzhou Bay): insights from nitrogen and oxygen isotopes. Acta Oceanologica Sinica, 43(6): 86–95, doi: 10.1007/s13131-024-2375-y

1 Introduction

Nutrients support marine primary productivity, but excess nutrient loads can lead to eutrophication and harmful algal blooms and hypoxia (Anderson et al., 2002; Rabalais et al., 2002; Diaz and Rosenberg, 2008; Wang et al., 2016; Glibert, 2017). In recent decades, due to human activity, riverine inputs of nutrients (mainly nitrate, NO_3^-) to estuaries and coastal oceans have increased significantly (Galloway et al., 2004; Gruber and Galloway, 2008; Dai et al., 2011), and eutrophication in estuarine and coastal aquatic ecosystems has emerged as a global environmental problem. Thus, effective management practices are needed to reduce nutrient inputs to these valuable ecosystems. That goal requires that the sources of relevant nutrients are identified and quantified and that the biogeochemical processes controlling nutrient distributions are well understood.

Hangzhou Bay (Fig. 1) is the largest estuary of the East China Sea, covering an area of approximately 4 800 km^2 (length ~270 km and bay-mouth width ~100 km). This funnel-shaped bay is characterized by a large tidal range, strong tidal currents, high turbidity, and high nutrient concentrations (Che et al., 2003). The mean tidal height is 2.5 m at the bay mouth, increasing up-estuary to 4 m to 6 m at the bay head (He et al., 2016; Shi, 2011). The Qiantang River, which carries large amounts of terrestrial material, discharges directly into the upper bay. In autumn, the famous Qiantang tidal bore has a mean height of ~5 m and a maximum height of ~10 m.

In recent decades, due to increasing nitrogen (N) loads associated with agricultural, industrial, and domestic activities, the waters of Hangzhou Bay have become seriously eutrophic, with nitrate concentrations of up to ~150 μmol/L (Jia et al., 2014; Wu et al., 2019). One significant contributor to dissolved inorganic nitrogen (DIN) in Hangzhou Bay is NO_3^- from Qiantang River discharge (Dong et al., 1986; Gao et al., 1993; Wu et al., 2019). Several small rivers also connect to the bay, but their contribution to bay DIN is negligible because their runoff is much smaller than that of the Qiantang River (Gao et al., 1993). The Changjiang River (Fig. 1), where nitrate concentrations can be >100 μmol/L (Tang, 1990; Dai et al., 2011), contributes large amounts of NO_3^- to the area just south of the Changjiang River Estuary, and this NO_3^- has also been suggested to contribute significantly

Foundation item: The Zhejiang Provincial Natural Science Foundation of China under contract No. LZ22D060002; the Key R&D Program of Zhejiang under contract No. 2022C03044; the National Key Research and Development Program of China under contract No. 2021YFC3101702.

*Corresponding author, E-mail: jfchen@sio.org.cn

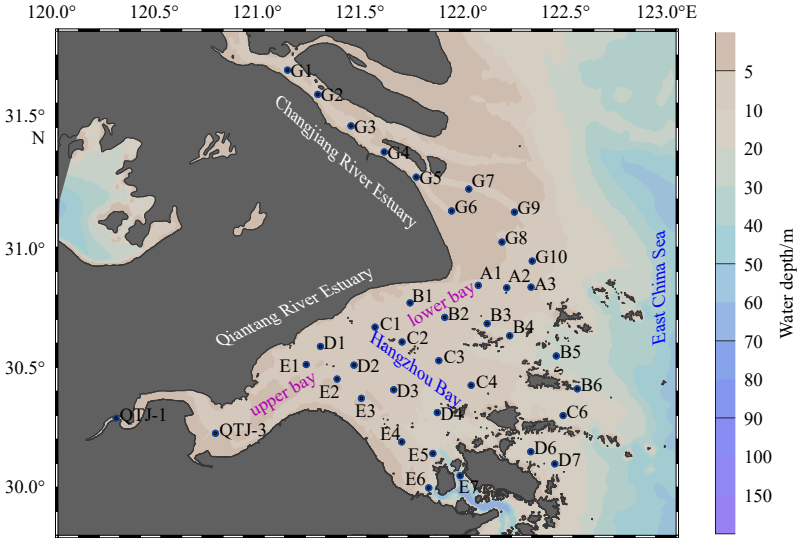

Fig. 1. Map of study area: Hangzhou Bay and adjoining waters. The basemap colors indicate water depth, and the labeled dots indicate August 2019 sampling sites.

to Hangzhou Bay DIN (Wu et al., 2019).

Despite this general knowledge of the likely sources of Hangzhou Bay DIN, the relative contributions of NO_3^- from the different sources remains unknown. Variation of $\delta^{15}N$ in nitrate ($\delta^{15}N_{NO_3^-}$) has been proven useful in identifying nitrogen sources and transformations in aquatic ecosystems (Kendall, 1998; Middelburg and Nieuwenhuize, 2001; Mayer et al., 2002; Ye et al., 2016; Yang et al., 2018; Chen et al., 2022), and many such studies have been conducted in the Changjiang River Estuary and adjacent area (Liu et al., 2009; Chen et al., 2013; Yan et al., 2017; Jiang et al., 2021), but to our knowledge such work has not been reported for Hangzhou Bay.

In this study, nitrogen and oxygen isotope ratios of NO_3^- were measured in water samples collected in August 2019 from Hangzhou Bay, the Qiantang River, the Changjiang River Estuary, and adjacent coastal waters. Concentrations of DIN [i.e., nitrate (NO_3^-), nitrite (NO_2^-), and ammonium (NH_4^+)] were also measured. These data were analyzed to investigate DIN transport from the Changjiang River to Hangzhou Bay and to quantify the relative contributions of DIN from different sources into Hangzhou Bay.

2 Material and methods

2.1 *Sampling sites*

In late August 2019, hydrographic data and seawater samples were collected at 39 stations during a research cruise (R/V *ZHEHAIKE 1 HAO*) in Hangzhou Bay, the Qiantang River, the Changjiang River Estuary, and adjacent coastal areas (Fig. 1). A rosette sampling system fitted with 6 Niskin bottles (10 L) and a CTD (conductivity–temperature–depth) sensor (SBE 917 plus, Sea-Bird Scientific) was used to measure *in situ* salinity and temperature and to bring seawater samples onboard. Generally, 1 to 3 depths were sampled at each station, depending on water depth. The analysis that follows focuses primarily on surface samples (collected approximately 2 m below the air/sea interface) and bottom samples (collected approximately 2 m above the sea bottom).

2.2 *Sample collection*

The seawater samples drawn from the Niskin bottles were pre-filtered through pre-combusted (at 450℃ for 4 h) GF/F membranes (Whatman, 0.7 μm) into 125 cm^3 HDPE bottles that had been previously rinsed with 10% HCl, distilled water, and the filtrate solution (three times). The samples were then stored at –20℃ for later analysis of nutrient concentrations and the nitrogen and oxygen isotope ratios of nitrate.

2.3 *Measurement of nutrient concentrations*

Concentrations of nitrate [NO_3^-] (where the square brackets denote concentration) and phosphate [PO_4^{3-}] were measured with an automated discrete chemical analyzer (Smartchem 600, AMS Alliance, Italy). Analytical precision for the NO_3^- analyses was better than 2% at concentrations >5 μmol/L. Analytical precision for the PO_4^{3-} analyses was better than 2% for concentrations >0.8 μmol/L. [NO_2^-] and [NH_4^+] were determined manually, using standard colorimetric methods (Hansen and Koroleff, 2009). Analytical precision for the NO_2^- analyses was better than 3% at concentrations >0.5 μmol/L. For the NH_4^+ analyses, analytical precision was approximately ±4% at 0.5 μmol/L and better than 3% at concentrations >1 μmol/L.

2.4 *Analysis of nitrogen and oxygen isotope ratios of* NO_3^-

The nitrogen and oxygen isotope ratios of NO_3^- were determined according to the method of Sigman et al. (2001) and Casciotti et al. (2002). First, NO_3^- was converted to gaseous nitrous oxide (N_2O) by cultured denitrifying bacteria (*Pseudomonas aureofaciens*, ATCC#13985). The product N_2O was then extracted from the sample vials, purified, and analyzed with an isotope ratio mass spectrometer (DELTA V Advantage, ThermoFisher Scientific). The international nitrate reference materials IAEA-NO-3 ($\delta^{15}N$ = 4.7‰; $\delta^{18}O$ = 25.6‰), USGS34 ($\delta^{15}N$ = –1.8‰; $\delta^{18}O$ = –27.9‰), and USGS35 ($\delta^{15}N$ = 2.7‰; $\delta^{18}O$ = 57.5‰) (Gonfiantini et al., 1995; Böhlke et al., 2003) were incorporated into every sample batch. This method is applicable to samples with [NO_3^-] ⩾ 1 μmol/L, yielding an average standard deviation of 0.2‰ for $\delta^{15}N$ and 0.5‰ for $\delta^{18}O$. For samples with [NO_2^-] ⩾ 0.5 μmol/L, the nitrite was removed with sulfamic acid according to the procedure of Granger and Sigman (2009). Isotope ratios are reported in delta (δ) notation in units of per mil (‰):

$$\delta^{15}\mathrm{N}(‰) = \left[\frac{\left(^{15}\mathrm{N}/^{14}\mathrm{N}\right)_{\mathrm{sample}}}{\left(^{15}\mathrm{N}/^{14}\mathrm{N}\right)_{\mathrm{reference}}} - 1\right] \times 1\,000,$$

$$\delta^{18}\mathrm{O}(‰) = \left[\frac{\left(^{18}\mathrm{O}/^{16}\mathrm{O}\right)_{\mathrm{sample}}}{\left(^{18}\mathrm{O}/^{16}\mathrm{O}\right)_{\mathrm{reference}}} - 1\right] \times 1\,000,$$

where $(^{15}\mathrm{N}/^{14}\mathrm{N})_{\mathrm{reference}}$ denotes N_2 in air and $(^{18}\mathrm{O}/^{16}\mathrm{O})_{\mathrm{reference}}$ denotes Vienna Standard Mean Ocean Water (VSMOW).

3 Results

In late August 2019, salinity across our study area (Fig. 1) ranged from 0.1 to 29.7 (Fig. 2a), with lowest values in the Qiantang River (station QTJ-1) and Changjiang River (stations G1–G7) and highest values in nearshore waters. Concentrations of dissolved oxygen (DO) ranged from 131 μmol/L to 232 μmol/L (Fig. 2b), with highest concentrations in upper Hangzhou Bay and lowest concentrations in nearshore coastal waters (the Qiantang River was not sampled for DO). Phosphate concentrations ranged from 0.8 μmol/L to 3.6 μmol/L (Fig. 2c), with most values being between 1 μmol/L and 2 μmol/L. Highest concentrations were found at the head of Hangzhou Bay (station QTJ-3), and lowest concentrations were found in nearshore waters.

Nitrate concentrations were highest (>120 μmol/L) in the Qiantang and Changjiang rivers (Fig. 3a) with lower concentrations (down to 22 μmol/L) in coastal waters. This pattern is opposite to that of salinity (Fig. 2a). At most stations, nitrite was <1 μmol/L (Fig. 3b) and ammonium was approximately 1 μmol/L (Fig. 3c). Consequently, DIN concentrations (Fig. 3d) were highest in the Qiantang River (158 μmol/L at station QTJ-1). Lowest DIN concentrations were found in coastal waters (down to 24 μmol/L in the near-bottom sample from station D7; Fig. 3d).

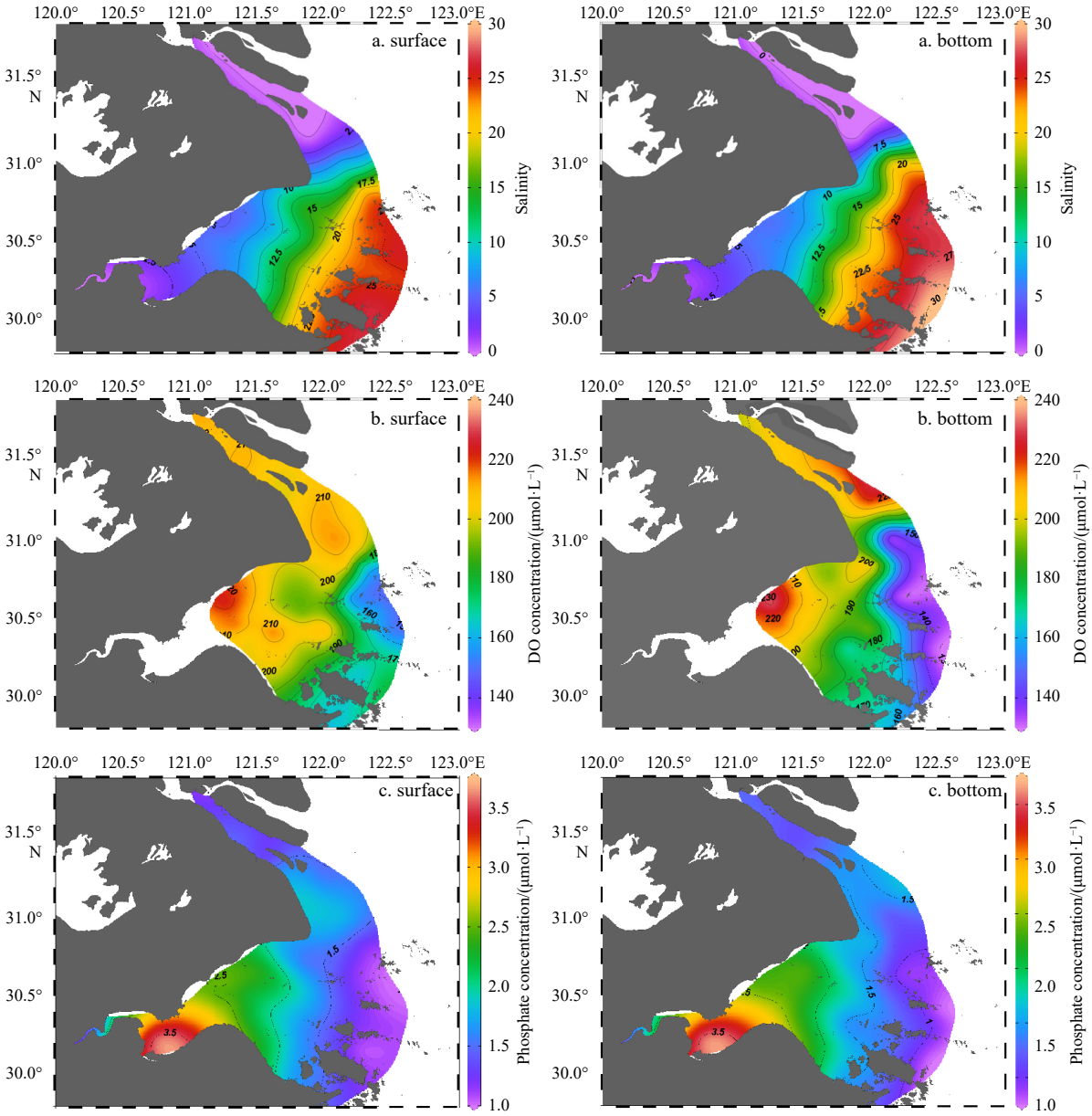

Fig. 2. Distributions of surface-water (left panels) and bottom-water (right panels) properties in the Qiantang River Estuary, Changjiang River Estuary, Hangzhou Bay, and adjacent nearshore waters in late August 2019: salinity (a), DO (b), and $[PO_4^{3-}]$ (c).

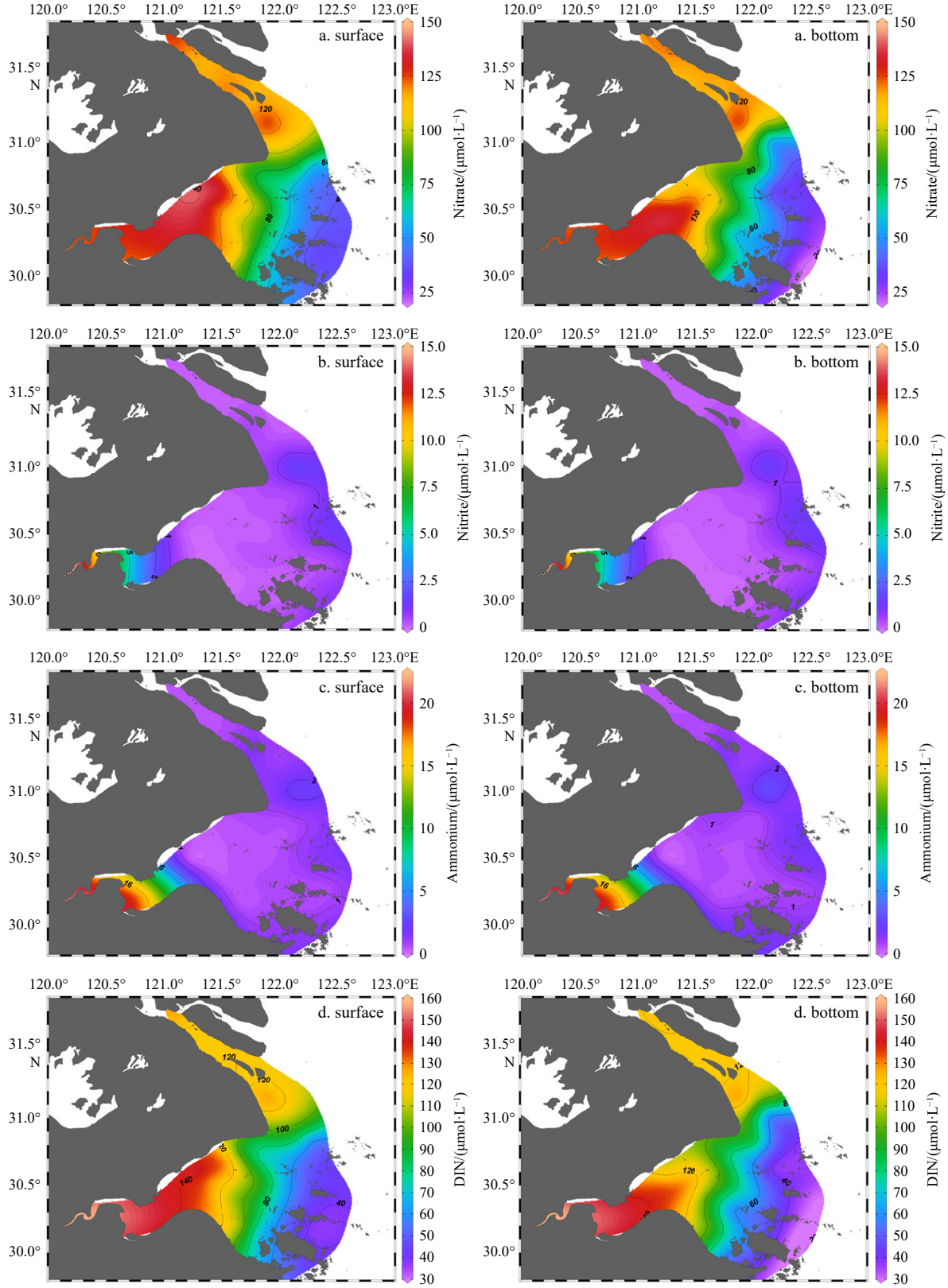

Fig. 3. Distributions of surface-water (left) and bottom-water (right) nutrients in the study area in late August 2019: nitrate (a), nitrite (b), ammonium (c), and DIN (d) concentrations.

Values of $\delta^{15}N_{NO_3^-}$ (Fig. 4a) ranged from 5.3‰ to 7.3‰ (averaging 6.8‰ ± 0.3‰), with generally small variations across the study area. The lowest value occurred in the Qiantang River. Values of $\delta^{18}O_{NO_3^-}$ (Fig. 4b) ranged from –0.4‰ to 3.5‰ (averaging 1.4‰ ± 0.9‰), with values increasing seaward from river to coastal waters. Low values of $\delta^{18}O_{NO_3^-}$ occurred in the Qiantang and Changjiang rivers, and high values occurred in coastal waters.

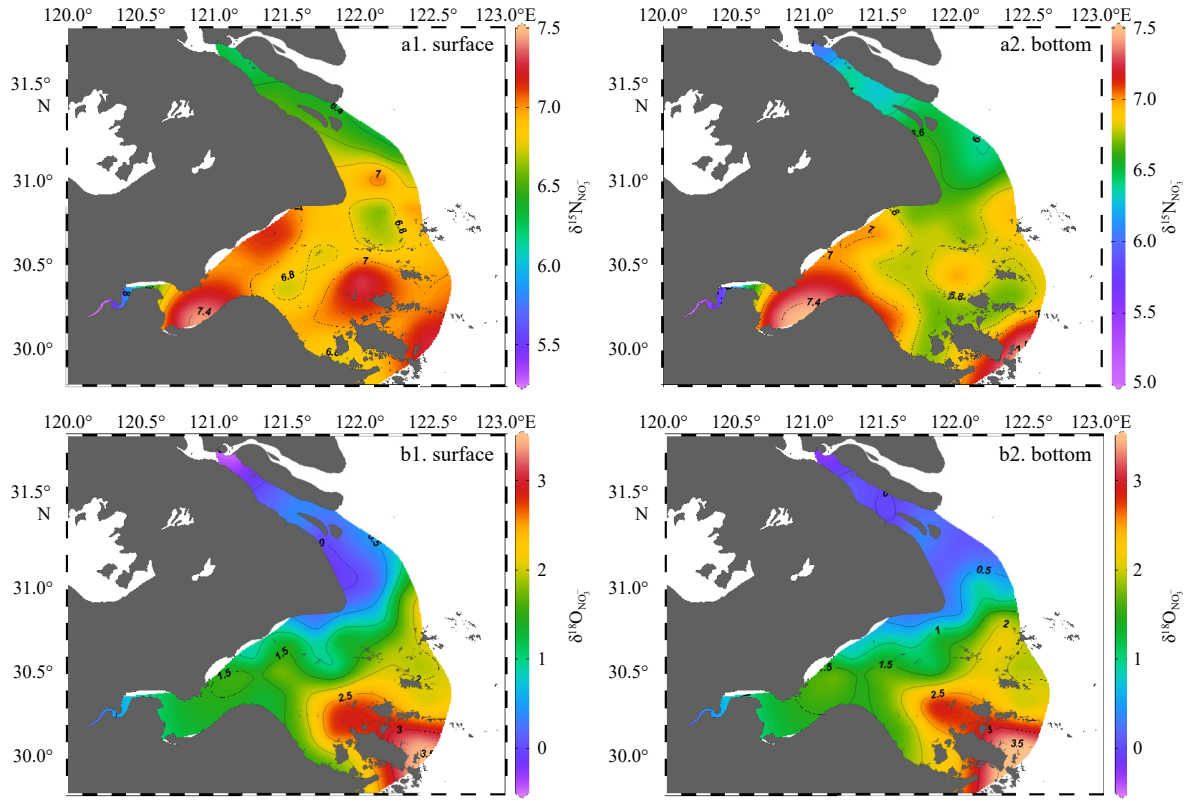

Fig. 4. Distributions of surface-water (left) and bottom-water (right) $\delta^{15}N$ and $\delta^{18}O$ of nitrate in late August 2019: $\delta^{15}N_{NO_3^-}$ (a) and $\delta^{18}O_{NO_3^-}$ (b).

4 Discussion

4.1 *Conservative mixing of nitrate and DIN from different sources to Hangzhou Bay*

Over most of our study area, NO_3^- accounted for more than 95% of DIN (Fig. 5a), while NO_2^- and NH_4^+ accounted for less than 1% and 4%, respectively (Figs 5b, c). High values of [DIN]/[PO_4^{3-}] (>30, nearly twice the Redfield ratio of 16; Fig. 5d) suggest that excessive input of DIN (mainly in the form of NO_3^-) to Hangzhou Bay has become a serious environmental problem.

The Qiantang River seems to have been one important source of NO_3^-, as indicated by the contrasting spatial patterns of [NO_3^-] and [DIN] (with values being highest in the river, decreasing seaward; Figs 3a and d) and salinity (lowest in the river, increasing seaward; Fig. 2a). Changjiang River water seems to intrude along the bay's north shore in both surface and bottom waters, evident as a plume of low-$\delta^{18}O_{NO_3^-}$ water (Fig. 4b). To the south, high-$\delta^{18}O_{NO_3^-}$ and high-salinity tongues of nearshore coastal water can be seen entering the mouth of the bay through the Zhoushan islands. The distributions of nitrate $\delta^{18}O$ and salinity (Fig. 4b) suggest that the Qiantang River, the Changjiang River, and nearshore coastal waters may all be contributing nitrate to Hangzhou Bay.

Previous studies have suggested that biological removal of nutrients in Hangzhou Bay is relatively insignificant (Dong et al., 1986; Gao et al., 1993) due to high turbidity and consequent light limitation of phytoplankton production (Li et al., 1993; Che et al., 2003). To assess whether the spatial distributions of nitrate and DIN might therefore be controlled mainly by water mixing, we examined relationships between nitrate and salinity and between DIN and salinity. We first divided the study area into three subregions (Fig. 6a) according to which inflowing water body tended to dominate inputs, as indicated by station location (Fig. 1) and values of salinity (Fig. 2a) and $\delta^{18}O_{NO_3^-}$ (Fig. 4b): (1) subregion CJA, deemed to be most strongly affected by Changjiang River inputs; (2) subregion QTJA, deemed to be most strongly affected by Qiantang River inputs; and (3) subregion CWA, where coastal-water inputs dominate. Stations B1 through B4 were included in the CJA subregion because the $\delta^{18}O_{NO_3^-}$ distribution (Fig. 4b) indicates a Changjiang River source for nitrate at these locations. Within subregion CWA, the average salinity at every station (surface water and bottom water) was >25.

Significant linear relationships were found between [NO_3^-] and salinity in the river-influenced QTJA and CJA areas (Fig. 6b) (R^2 = 0.93 for QTJA and 0.99 for CJA), consistent with observed overall patterns of $\delta^{18}O_{NO_3^-}$ and salinity (i.e., with lowest values in the rivers, increasing seaward to highest values in nearshore coastal waters; Figs 4b and 2a). Significant linear relationships were similarly found for DIN and salinity (Fig. 6c) (R^2 = 0.97 for QTJA and 0.99 for CJA). These results suggest that as river waters and nearshore waters meet in the vicinity of Hangzhou Bay, their DIN (and NO_3^-) are conservatively mixed, with little influence from nonconservative biological processes. These findings are consistent with earlier suggestions (mentioned above in this section) that the effects of phytoplankton assimilation on nitrate concentrations in Hangzhou Bay are insignificant due to high concentrations of suspended sediments.

The process of denitrification (i.e., the conversion of nitrate and nitrite to gaseous forms of nitrogen, thus removing DIN from seawater) in Hangzhou Bay is likely similarly insignificant. Denitrifying microbes use nitrite as an electron receptor only when oxygen is limited, and in the course of our cruise DO was always >130 μmol/L (Fig. 2b). The process of nitrification (i.e., conver-

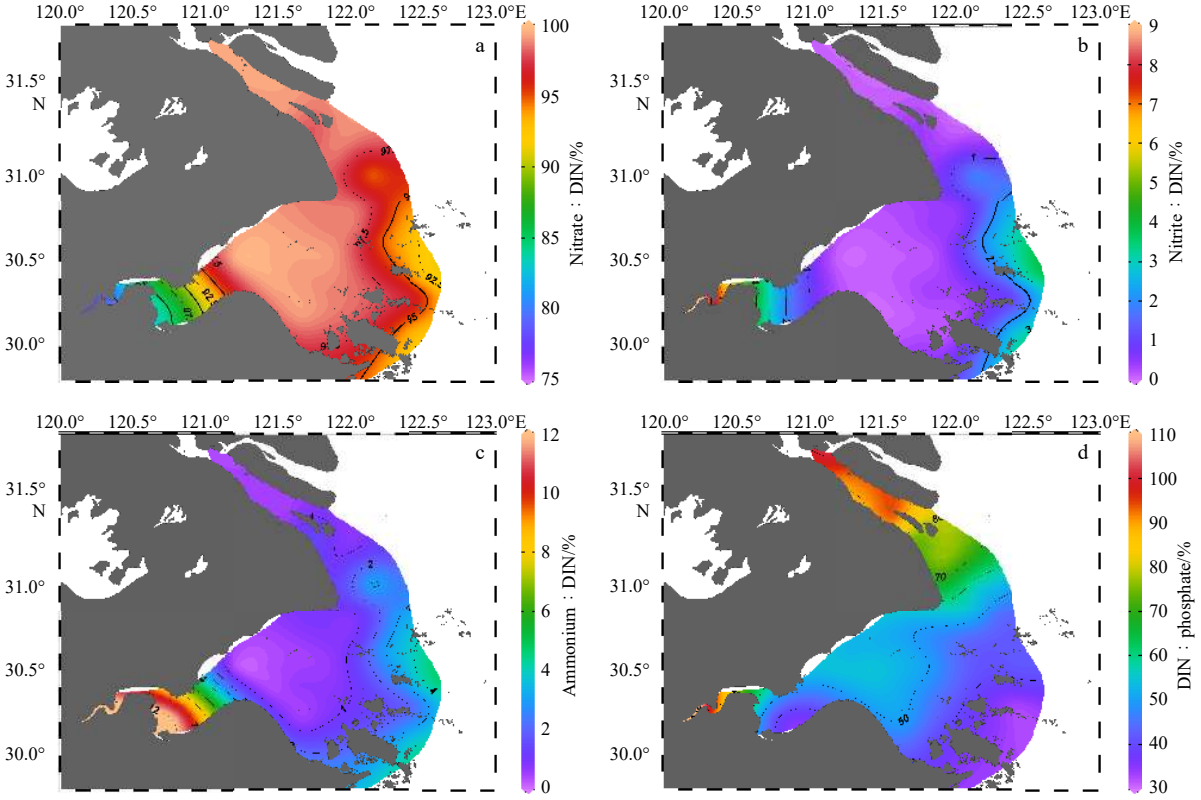

Fig. 5. Distributions of nitrogen and phosphate concentration ratios in late August 2019: nitrate/DIN (a), nitrite/DIN (b), ammonium/DIN (c), and DIN/phosphate (d) concentration ratios. Ratios at each station were computed using vertically averaged nutrient concentrations.

sion of ammonium to nitrate, which does not change DIN concentrations) does seem to have been occurring in the head of Hangzhou Bay, as evidenced by the unchanging concentration of nitrate from Qiantang River to the head of Hangzhou Bay concurrent with decreasing concentrations of ammonium and nitrite (Fig. 3). Still, nitrification seems not to have significantly affected the signal of conservative mixing of DIN from the various sources to Hangzhou Bay.

4.2 *Relative DIN contributions to Hangzhou Bay*

Conservative mixing models have been widely used to study nutrient dynamics in estuarine and coastal regions (Dähnke et al., 2008; Han et al., 2012; Wang et al., 2014). To estimate relative DIN contributions from different sources to Hangzhou Bay, we likewise used a three-endmember salinity-based conservative mixing model:

$$f_{CJ} + f_{QTJ} + f_{CW} = 1, \tag{1}$$

$$f_{CJ}S_{CJ} + f_{QTJ}S_{QTJ} + f_{CW}S_{CW} = S_{MIX}, \tag{2}$$

$$f_{CJ}\mathrm{DIN}_{CJ} + f_{QTJ}\mathrm{DIN}_{QTJ} + f_{CW}\mathrm{DIN}_{CW} = \mathrm{DIN}_{MIX}, \tag{3}$$

where the f terms represent the fractional contributions of the three endmember water masses denoted by the subscripts CJ (Changjiang River), QTJ (Qiantang River), and CW (coastal water). The terms S and DIN represent the parameters being mixed: salinity and dissolved inorganic nitrogen. The terms S_{MIX} and DIN_{MIX} represent the quantities in a mixture of the three endmembers.

The first step in the analysis was to assign S and DIN values to each contributing water mass (Table 1). For the Changjiang River, there was little variation in salinity and DIN concentration between stations G1 and G7 (Figs 1, 2a, and 3d), so we used the average of all values along that stretch. For the Qiantang River (water mass QTJ), we used the average values of salinity and DIN concentration measured at stations QTJ-1 and QTJ-3 (Figs 1, 2a, and 3d). For the nearshore coastal water (water mass CW), highest salinity was observed in the bottom water of station D7, along with the lowest concentration of DIN and the highest $\delta^{18}O$ of nitrate (Figs 1, 2a, 3d, and 4b). Thus, these values of salinity and DIN concentration were chosen as the CW endmember parameters.

By inserting endmember characteristics (Table 1) into the mixing model (Eqs 1, 2, and 3) and solving for f_{CJ}, f_{QTJ}, and f_{CW} at each station, we can estimate the fractional contributions of riverine and coastal water DIN across Hangzhou Bay in August 2019. The results indicate that the Qiantang River (Fig. 7a) was a significant source of DIN between the bay head and the central bay, possibly contributing more than 50% of DIN in the bay head area. The Changjiang River (Fig. 7b) notably contributed DIN to the northern bay mouth area, with its influence (i.e., percent contribution) decreasing southward. At the time of our cruise, discharge from the Changjiang River was relatively low and incursion of the nearshore water mass was relatively strong, as indicated by the distributions of salinity (Fig. 2a) and $\delta^{18}O_{NO_3^-}$ (Fig. 4b). Water exiting the Changjiang River was basically squeezed in by intruding coastal water to hug the northern shore of Hangzhou

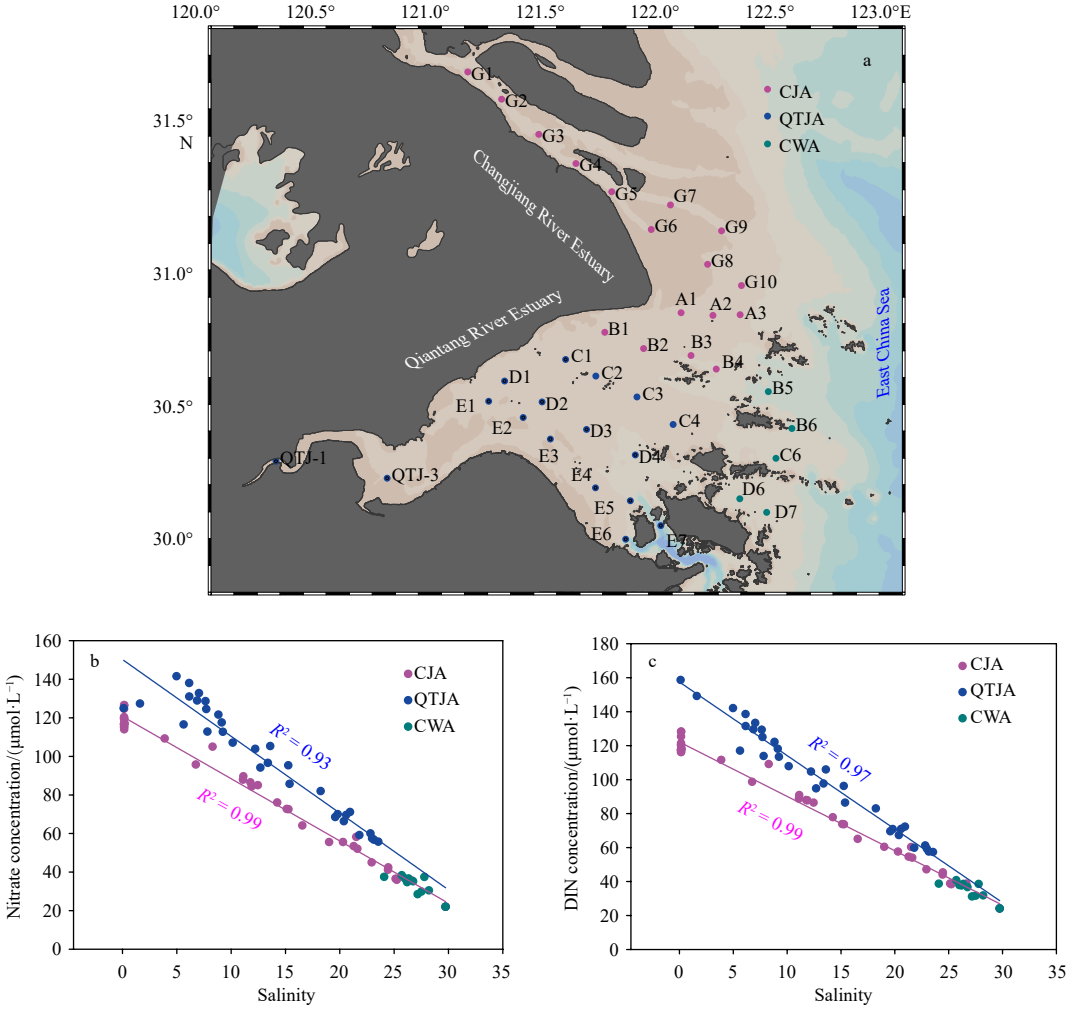

Fig. 6. Study area subregions and mixing properties in each. a. Map of study area, showing the three subregions defined according to predominating water mass inputs: QTJA, influenced primarily by Qiantang River inputs (blue dots); CJA, influenced primarily by Changjiang River inputs (pink dots); and CWA, influenced primarily by coastal water inputs (dark cyan dots). b. Nitrate concentrations as a function of salinity. c. DIN concentrations as a function of salinity.

Table 1. Endmember parameters used in the mixing model of Eqs 1–3. For an explanation of each endmember salinity and DIN, see Section 4.2

Endmember	Salinity	DIN/(μmol·L^{-1})
CJ (Changjiang River)	0.16 ± 0.01	120.2 ± 4.1
QTJ (Qiantang River)	0.86 ± 1.06	153.9 ± 6.7
CW (coastal water)	29.74	24.1

Bay. Coastal waters (Fig. 7c) contributed most of the DIN in the lower bay, from the bay mouth to the central bay.

4.3 *Primary sources of nitrate to Hangzhou Bay*

As discussed in Section 4.1, the Qiantang River, the Changjiang River, and nearshore coastal waters all contributed nitrate to Hangzhou Bay, and nitrate generally behaved conservatively in our study area. Thus, the classical dual isotope approach (Kendall et al., 2007; Xue et al., 2009; Yang et al., 2018) could be used to identify nitrate sources.

In the Changjiang River, concentrations of NO_3^- ranged from 114.0 μmol/L to 124.7 μmol/L [averaging (119.0 ± 4.1) μmol/L, stations G1 to G7] in August 2019, close to previously published values (Dai et al., 2011; Jiang et al., 2021). Multiple sources of NO_3^- have been suggested for this river, such as chemical fertilizer, urban sewage effluent, degradation of terrestrial organic nitrogen, and atmospheric deposition (Li et al., 2010; Dai et al., 2011). Among these possibilities, soil N and synthetic N fertilizer (mostly in the form of ammonium salt or urea) have been suggested to be the most important contributors to Changjiang River nitrate, based on isotopic signatures (Li et al., 2010; Jiang et al., 2021). Soil nitrogen is thought to be a primary source of nitrogen in various aqueous environments (Deutsch et al., 2006; Kendall et al., 2007; Chen et al., 2022). Most of the N in soils is bound in organic forms, with dissolved inorganic nitrogen (mainly NO_3^-) constituting ~1% of the total N and $\delta^{15}N$ signatures, possibly reflecting the effects of various anthropogenic activities (Kendall and Aravena, 2000). Soil NO_3^- produced from fertilizer generally has a lower $\delta^{15}N$ value (4.7‰ ± 5.4‰) than does animal waste ($\delta^{15}N$ = 14.0‰ ± 8.8‰) (Kendall et al., 2007). N fertilizer applications have greatly increased in China since the 1980s and are well correlated with the increase of [NO_3^-] in the Changjiang River (Jiang et al., 2021). In our Changjiang River samples, $\delta^{15}N_{NO_3^-}$ ranged from 6.1‰ to 6.7‰ and $\delta^{18}O_{NO_3^-}$ ranged from –0.4‰ to 0.4‰, likely reflecting a mixed source of nitrate, mainly from soil N and synthetic N fertilizer to the river (Fig. 8).

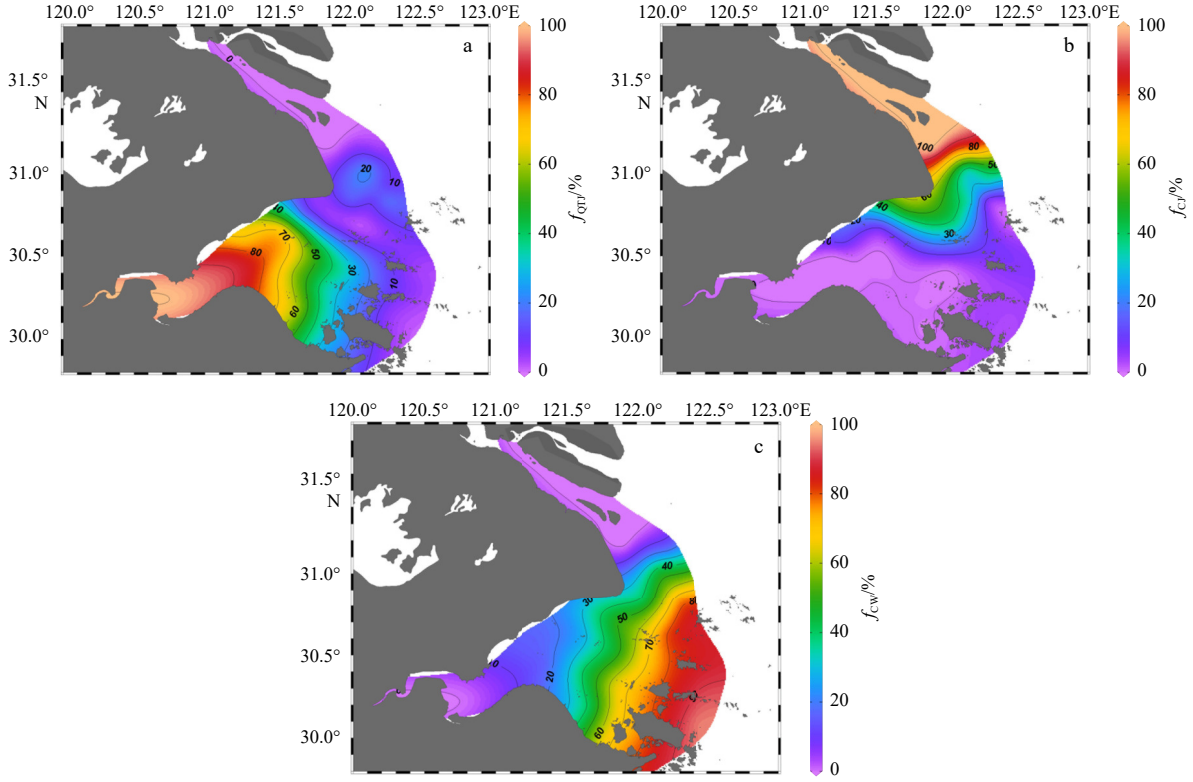

Fig. 7. Percent contributions of DIN to Hangzhou Bay from the Qiantang River (a), the Changjiang River (b), and nearshore coastal waters (c).

In the Qiantang River and at its mouth (Stations QTJ-1 and QTJ-3), concentrations of NO_3^- were slightly higher than in the Changjiang River, ranging from 125.0 μmol/L to 127.4 μmol/L [averaging (126.2 ± 1.7) μmol/L]. Values of $\delta^{15}N_{NO_3^-}$ (5.3‰ to 7.3‰) and $\delta^{18}O_{NO_3^-}$ (0.4‰ to 1.3‰) suggest that soil N was an important source of nitrate in the Qiantang River (Fig. 8). Synthetic N fertilizer could also be an important contributor, as [NO_3^-] in the Qiantang River Estuary has greatly increased since the 1980s (from <70 μmol/L in the 1980s to >120 μmol/L in 2019; Dong et al., 1986, and Fig. 3a), concurrent with greatly increasing fertilizer applications in China (Jiang et al., 2021). Significantly high concentrations of NH_4^+ were also observed in the Qiantang River (18.0 μmol/L to 20.0 μmol/L) in combination with high concentrations of phosphate and nitrite (Figs 2c and 3a), which suggests a local input of NO_3^- derived from manure and sewage. A local manure and sewage input is also suggested by the slight increase in $\delta^{15}N_{NO_3^-}$ between upriver station QTJ-1 and river-mouth station QTJ-3, as nitrate sourced from manure and sewage is generally characterized by relatively high $\delta^{15}N$ (Fig. 8).

Intrusions of coastal water into Hangzhou Bay and the Changjiang River Estuary are indicated by the high-salinity tongues in those areas during our study period (Fig. 2). This coastal water was possibly formed in the summer (the flood season), when a low-salinity, high-nitrate river plume could have dispersed offshore to mix with high-salinity, oligotrophic surface waters of the East China Sea shelf and also the nearshore Kuroshio Branch Currents (Chang and Isobe, 2003; Zhou et al., 2009). According to previous studies, this shelf water is almost depleted of NO_3^- and the branch-current waters generally contain [NO_3^-] of ~13.0 μmol/L (Wang et al., 2016; Yan et al., 2017). Thus, the Changjiang River ([NO_3^-], ~119 μmol/L) still seems to be a key source of nitrate in the coastal water.

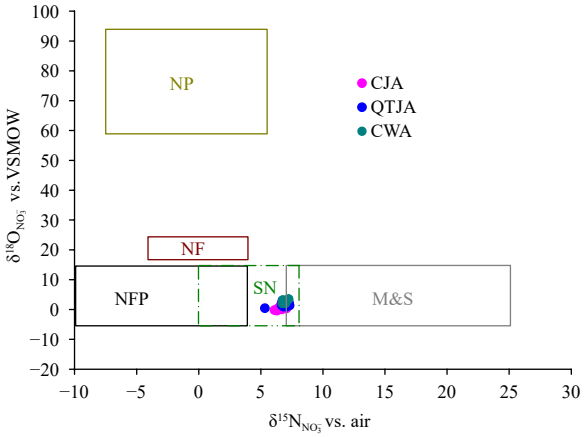

Fig. 8. Values of $\delta^{15}N$ and $\delta^{18}O$ in various nitrate source reservoirs and in seawater samples in the study area. The boxes indicate ranges of typical isotopic compositions for five potential sources of nitrate: NP : NO_3^- in precipitation; NF : NO_3^- in fertilizer; NFP : NH_4^+ (or urea) in fertilizer and precipitation; SN : NO_3^- derived from soil N; and M&S : NO_3^- derived from manure and sewage (Kendall et al., 2007; Xue et al., 2009; Yang et al., 2018). The colored dots show values of $\delta^{15}N$ and $\delta^{18}O$ in seawater samples in each of the three subregions defined in Fig. 6.

In conclusion, based on the relative DIN (mainly NO_3^-) contributions from different water sources to Hangzhou Bay (Fig. 7) and the overall ranges of $\delta^{15}N_{NO_3^-}$ (5.3‰ to 7.3‰) and $\delta^{18}O_{NO_3^-}$ (−0.4‰ to 3.5‰) in our study area, we suggest that the NO_3^- in Hangzhou Bay in late August 2019 was likely derived mainly from soils, synthetic N fertilizer, and manure and sewage.

5 Conclusions

Over most of Hangzhou Bay in late August 2019, DIN ranged from 54 μmol/L to 149 μmol/L, with nitrate accounting for more than 95% of DIN in most areas. The [DIN]:[PO_4^{3-}] ratio ranged from 40 to 56, suggesting that excessive input of DIN to Hangzhou Bay has become a serious environmental problem. The spatial distributions of nutrients (nitrate, nitrite, ammonium, and phosphate) and the signatures of the stable isotopes of nitrate ($\delta^{15}N_{NO_3^-}$ and $\delta^{18}O_{NO_3^-}$) together suggest that the DIN in Hangzhou Bay originated mainly from soils, synthetic N fertilizer, and manure and sewage. In the upper bay, the Qiantang River was a significant DIN source, possibly accounting for more than 50% of DIN in the bay head area. In the lower bay, coastal water inputs contributed most of the DIN. DIN coming directly from the Changjiang River made a relatively small contribution to the Hangzhou Bay DIN at that time.

Acknowledgements

We thank the crew members of R/V *ZHEHAIKE 1 HAO* for their assistance with sample collection. We are also grateful to the anonymous reviewers for their constructive comments on this manuscript. We thank Tonya Clayton for her professional editorial English assistance.

References

Anderson D M, Glibert P M, Burkholder J M. 2002. Harmful algal blooms and eutrophication: nutrient sources, composition, and consequences. Estuaries, 25(4): 704–726, doi: 10.1007/BF02804901

Böhlke J K, Mroczkowski S J, Coplen T B. 2003. Oxygen isotopes in nitrate: New reference materials for ^{18}O: ^{17}O: ^{16}O measurements and observations on nitrate-water equilibration. Rapid Communications in Mass Spectrometry, 17(16): 1835–1846, doi: 10.1002/rcm.1123

Casciotti K L, Sigman D M, Hastings M G, et al. 2002. Measurement of the oxygen isotopic composition of nitrate in seawater and freshwater using the denitrifier method. Analytical Chemistry, 74(19): 4905–4912, doi: 10.1021/ac020113w

Chang P H, Isobe A. 2003. A numerical study on the Changjiang diluted water in the Yellow and East China seas. Journal of Geophysical Research: Oceans, 108(C9): 3299

Che Yue, He Qing, Lin Weiqing. 2003. The distributions of particulate heavy metals and its indication to the transfer of sediments in the Changjiang Estuary and Hangzhou Bay, China. Marine Pollution Bulletin, 46(1): 123–131, doi: 10.1016/S0025-326X(02)00355-7

Chen Fajin, Chen Jianfang, Jia Guodong, et al. 2013. Nitrate $\delta^{15}N$ and $\delta^{18}O$ evidence for active biological transformation in the Changjiang Estuary and the adjacent East China Sea. Acta Oceanologica Sinica, 32(4): 11–17, doi: 10.1007/s13131-013-0294-4

Chen Fajin, Deng Ziyun, Lao Qibin, et al. 2022. Nitrogen cycling across a salinity gradient from the Pearl River Estuary to offshore: insight from nitrate dual isotopes. Journal of Geophysical Research: Biogeosciences, 127(5): e2022JG006862, doi: 10.1029/2022JG006862

Dähnke K, Bahlmann E, Emeis K. 2008. A nitrate sink in estuaries? An assessment by means of stable nitrate isotopes in the Elbe Estuary. Limnology and Oceanography, 53(4): 1504–1511, doi: 10.4319/lo.2008.53.4.1504

Dai Zhijun, Du Jinzhou, Zhang Xiaoling, et al. 2011. Variation of riverine material loads and environmental consequences on the Changjiang (Yangtze) Estuary in recent decades (1955– 2008). Environmental Science & Technology, 45(1): 223–227

Deutsch B, Mewes M, Liskow I, et al. 2006. Quantification of diffuse nitrate inputs into a small river system using stable isotopes of oxygen and nitrogen in nitrate. Organic Geochemistry, 37(10): 1333–1342, doi: 10.1016/j.orggeochem.2006.04.012

Diaz R J, Rosenberg R. 2008. Spreading dead zones and consequences for marine ecosystems. Science, 321(5891): 926–929, doi: 10.1126/science.1156401

Dong Henglin, Jiang Guochang, Wang Yuheng. 1986. Distribution of reactive silicate, nitrate and phosphate in the water near the mouth of the Hangzhou Bay. Donghai Marine Science (in Chinese), 4(4): 95–104

Galloway J N, Dentener F J, Capone D G, et al. 2004. Nitrogen cycles: past, present, and future. Biogeochemistry, 70(2): 153–226, doi: 10.1007/s10533-004-0370-0

Gao Shengquan, Yu Guohui, Wang Yuhen. 1993. Distributional features and fluxes of dissolved nitrogen, phosphorus and silicon in the Hangzhou Bay. Marine Chemistry, 43(1–4): 65–81, doi: 10.1016/0304-4203(93)90216-B

Glibert P M. 2017. Eutrophication, harmful algae and biodiversity—Challenging paradigms in a world of complex nutrient changes. Marine Pollution Bulletin, 124(2): 591–606, doi: 10.1016/j.marpolbul.2017.04.027

Gonfiantini R, Stichler W, Rozanski K. 1995. Standards and intercomparison materials distributed by the International Atomic Energy Agency for stable isotope measurements. In: Reference and Intercomparison Materials for Stable Isotopes of Light Elements, Proceedings of a Consultants Meeting. Vienna: IAEA, 13–29

Granger J, Sigman D M. 2009. Removal of nitrite with sulfamic acid for nitrate N and O isotope analysis with the denitrifier method. Rapid Communications in Mass Spectrometry, 23(23): 3753–3762, doi: 10.1002/rcm.4307

Gruber N, Galloway J N. 2008. An Earth-system perspective of the global nitrogen cycle. Nature, 451(7176): 293–296, doi: 10.1038/nature06592

Han Aiqin, Dai Minhan, Kao S J, et al. 2012. Nutrient dynamics and biological consumption in a large continental shelf system under the influence of both a river plume and coastal upwelling. Limnology and Oceanography, 57(2): 486–502, doi: 10.4319/lo.2012.57.2.0486

Hansen H P, Koroleff F. 2009. Determination of nutrients. In: Grasshoff K, Kremling K, Ehrhardt M, eds. Methods of Seawater Analysis. 3rd ed. Weinheim, New York, Chichester, Brisbane, Singapore, Toronto: Wiley-VCH, 159–228

He Zhiguo, Huangfu Kailong, Yuan Yeping, et al. 2016. Variations of temperature, salinity and current in the southern tidal passage of the Hangzhou Bay, China. Acta Oceanologica Sinica, 35(3): 30–37, doi: 10.1007/s13131-016-0819-8

Jia Haibo, Shao Junbo, Cao Liuyan. 2014. Analysis of the changes and development trend of ecological environment in Hangzhou Bay. Environmental Pollution and Control (in Chinese), 36(3): 14–19, 25

Jiang Shan, Jin Jie, Zhang Guosen, et al. 2021. Nitrate in the Changjiang diluted water: an isotopic evaluation on sources and reaction pathways. Journal of Oceanology and Limnology, 39(3): 830–845, doi: 10.1007/s00343-020-0149-8

Kendall C. 1998. Tracing nitrogen sources and cycling in catchments. In: Kendall C, McDonnel J J, eds. Isotope Tracers in Catchment Hydrology. Amsterdam: Elsevier Press, 519–576

Kendall C, Aravena R. 2000. Nitrate isotopes in groundwater systems. In: Cook P G, Herczec A L, eds. Environmental Tracers in Subsurface Hydrology. Boston, MA: Springer US, 261–297

Kendall C, Elliott E M, Wankel S D. 2007. Tracing anthropogenic inputs of nitrogen to ecosystems. In: Michener R, Lajtha K, eds. Stable Isotopes in Ecology and Environmental Science. 2nd ed. Oxford: Blackwell Publishing Ltd., 375–449

Li Yan, Li Jing, Su Jilan, et al. 1993. Study on fluctuations of plume front and turbidity maximum in the Hangzhou Bay by remote sensing data. Acta Oceanologica Sinica, 12(1): 51–62

Li Siliang, Liu Congqiang, Li Jun, et al. 2010. Assessment of the sources of nitrate in the Changjiang River, China using a nitrogen and oxygen isotopic approach. Environmental Science & Technology, 44(5): 1573–1578

Liu Xiujuan, Yu Zhiming, Song Xiuxian, et al. 2009. The nitrogen isotopic composition of dissolved nitrate in the Yangtze River

(Changjiang) Estuary, China. Estuarine, Coastal and Shelf Science, 85(4): 641–650

Mayer B, Boyer E W, Goodale C, et al. 2002. Sources of nitrate in rivers draining sixteen watersheds in the northeastern U. S.: Isotopic constraints. Biogeochemistry, 57(1): 171–197, doi: 10.1023/A:1015744002496

Middelburg J J, Nieuwenhuize J. 2001. Nitrogen isotope tracing of dissolved inorganic nitrogen behaviour in tidal estuaries. Estuarine, Coastal and Shelf Science, 53(3): 385–391

Rabalais N N, Turner R E, Wiseman W J Jr. 2002. Gulf of Mexico hypoxia, A. K. A. "The dead zone". Annual Review of Ecology and Systematics, 33(1): 235–263, doi: 10.1146/annurev.ecolsys.33.010802.150513

Shi J Z. 2011. Tide-induced fine sediment resuspension in the well-mixed Hangzhou Bay, East China Sea, China. Earth and Environmental Science Transactions of the Royal Society of Edinburgh, 102(1): 25–34, doi: 10.1017/S1755691011010061

Sigman D M, Casciotti K L, Andreani M, et al. 2001. A bacterial method for the nitrogen isotopic analysis of nitrate in seawater and freshwater. Analytical Chemistry, 73(17): 4145–4153, doi: 10.1021/ac010088e

Tang R. 1990. Biogeochemical behavior of nitrogen and phosphate in the Changjiang Estuary and its adjacent waters. In: Yu G, Martin J M, Zhou J, et al, eds. Biogeochemical Study of the Changjiang Estuary. Beijing: China Ocean Press, 322–334

Wang Kui, Chen Jianfang, Jin Haiyan, et al. 2014. Summer nutrient dynamics and biological carbon uptake rate in the Changjiang River plume inferred using a three end-member mixing model. Continental Shelf Research, 91: 192–200, doi: 10.1016/j.csr.2014.09.013

Wang Hongjie, Dai Minhai, Liu Jinwen, et al. 2016. Eutrophication-driven hypoxia in the East China Sea off the Changjiang Estuary. Environmental Science & Technology, 50(5): 2255–2263

Wu Bin, Jin Haiyan, Gao Shengquan, et al. 2019. Nutrient budgets and recent decadal variations in a highly eutrophic estuary: Hangzhou Bay, China. Journal of Coastal Research, 36(1): 63–71, doi: 10.2112/JCOASTRES-D-18-00071.1

Xue Dongmei, Botte J, De Baets B, et al. 2009. Present limitations and future prospects of stable isotope methods for nitrate source identification in surface- and groundwater. Water Research, 43(5): 1159–1170, doi: 10.1016/j.watres.2008.12.048

Yan Xiuli, Xu Minnina, Wan Xianhui, et al. 2017. Dual isotope measurements reveal zoning of nitrate processing in the summer Changjiang (Yangtze) River plume. Geophysical Research Letters, 44(24): 12289–12297

Yang Zhi, Chen Jianfang, Li Hongliang, et al. 2018. Sources of nitrate in Xiangshan Bay (China), as identified using nitrogen and oxygen isotopes. Estuarine, Coastal and Shelf Science, 207: 109–118

Ye Feng, Jia Guodong, Xie Luhua, et al. 2016. Isotope constraints on seasonal dynamics of dissolved and particulate N in the Pearl River Estuary, South China. Journal of Geophysical Research: Oceans, 121(12): 8689–8705

Zhou Feng, Xuan Jiliang, Ni Xiaobo, et al. 2009. A preliminary study of variations of the Changjiang Diluted Water between August of 1999 and 2006. Acta Oceanologica Sinica, 28(6): 1–11