Spatial variability of nitrous oxide in the Minho and Lima estuaries (Portugal)

Célia Gonçalves, Maria José Brogueira

Instituto Português do Mar e da Atmosfera, IPMA Rua Alfredo Magalhães Ramalho, 6, 1495-006, Lisboa, Portugal.

(CG) (Corresponding author) E-mail: celia.pgoncalves@gmail.com, ORCID iD: http://orcid.org/0000-0001-5524-1256

(MJB) E-mail: mjbrogueira@gmail.com, ORCID iD: http://orcid.org/0000-0003-3692-4099

Summary: Nitrous oxide (N2O) is a potent long-lived greenhouse gas and estuaries represent potentially important sources of this biogas to the atmosphere. In this work, we analyse the first N2O data obtained in the Minho and Lima estuaries, and the processes and environmental factors that may regulate its production in these systems. In September 2006, N2O attained values of up to 20.0 nmol L–1 in the upper reaches of the Lima estuary and the river was, apparently, the main source of biogas to the system. In Minho N2O reached a maximum of 14.4 nmol L–1 and nitrification appeared to contribute to the enhancement of N2O. In the upper estuary, the relatively high concentrations of nitrification substrate NH4+, the positive correlations found between N2O level above atmospheric equilibrium (ΔN2O) and apparent oxygen utilization and NO2–, and the negative correlations between ΔN2O and NH4+ and pH can be interpreted as in situ N2O production through pelagic nitrification. Principal component analysis gave evidence of considerable differences between upper estuaries, particularly in terms of higher N2O in Lima and NH4+ in Minho. Surface waters of both estuaries were always N2O-supersaturated (101-227%) and estimated N2O emissions from Minho and Lima were 0.28 Mg N2O-N yr–1 and 0.96 Mg N2O-N yr–1, respectively, which represent a reduced fraction of N2O global emission from European estuaries.

Keywords: N2O; greenhouse gas; fluxes; emission; Portuguese estuaries.

INTRODUCTION

In the last few decades, the study of N2O has acquired greater importance due to its contribution to global climate change. N2O is an important long-lived greenhouse gas in terms of radiative forcing (0.17±0.03 W m–2) (Myhre et al. 2013) and represents the major anthropogenic contributor to stratospheric ozone destruc-
It has a long atmospheric lifetime of 131±10 years (Prather et al. 2012) and its global warming potential is 310 times greater than that of carbon dioxide, in a time horizon of 100 years. In 2011 atmospheric N2O levels (324.2±0.1 ppb) exceeded the pre-industrial levels (270±7 ppb) by about 20% (Myhre et al. 2013), largely due to increased agricultural activity and industry.

Estuaries have been considered significant N2O contributors to the atmosphere as a consequence of their high productivity and anthropogenic nitrogen loadings. N2O is mainly formed during the first step of nitrification, the aerobic oxidation of ammonium (NH4+) to nitrite (NO2−), mediated by ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA), and microbiological denitrification, the biological reduction of nitrate (NO3−) to N2O and, in turn, nitrogen gas (N2). Nitrification and denitrification often occur simultaneously in aquatic ecosystems and their relative contribution to total N2O production is difficult to disentangle. As nitrification is an aerobic process in well-oxygenated estuarine systems, the water column mostly contributes N2O through nitrification production (de Wilde and de Bie 2000, Barnes and Upstill-Goddard 2011). Denitrification is usually limited to zones under hypoxic conditions (DO <2 ml L−1), although some denitrification may occur even in relatively oxygenated waters (de Bie et al. 2002).

Both nitrification and denitrification are sensitive to the ongoing environmental changes and any natural or anthropogenic-induced shifts in the N availability have the potential to alter nitrogen cycling in coastal environments and affect N2O formation and release to the atmosphere (Bange et al. 2010). The extent of the denitrification is also strongly controlled by temperature, nitrate concentrations and the availability of organic carbon (e.g. Dong and Nedwell 2006). In addition to the supply of oxygen and ammonia, which are the main controls on nitrification, other environmental variables may affect this biological process: temperature (Dai et al. 2008), salinity (Bollmann and Laanbroek 2002) and pH (Strauss et al. 2002).

Estimates of N2O release from estuaries to the global inventory reveal wide uncertainties due to the large variability in N2O data (Bange et al. 2010, Barnes and Upstill-Goddard 2011). However, considerable efforts have been made in the last few decades to better understand the nitrogen cycle, the dynamics of N2O production and the quantification of the respective emission from European estuaries. More recently Murray et al. (2015) reviewed N2O global fluxes from estuarine environments and reported a variation of 0.17-0.95 Tg N2O-N yr−1.

Studies on the N2O dynamics and fluxes have been carried out in the Portuguese Tagus, Sado and Douro estuaries (e.g. Gonçalves et al. 2010, 2015, Teixeira et al. 2013). However, no data on N2O levels and fluxes are available for the Minho and Lima estuaries. In this work, we (1) report spatial variability of N2O concentration in these systems, (2) assess the contribution of different N2O sources, (3) evaluate the role of environmental properties on the increment of N2O fluxes, and (4) estimate N2O emission in a perspective of global N2O estuarine emissions.
Nyr–1, respectively, and effluents from domestic origin have great socio-economic importance from tourism, fishing and agriculture. However, increasing pressure has resulted from harbour activities (Viana do Castelo). However, nutrient loadings also originate from diffuse sources, largely agriculture (0.51 t N yr⁻¹), although the main N source to the estuary is the Lima River (1077 t N yr⁻¹) (Ferreira et al. 2005). This N load is, however, approximately 19 times lower than the load to the Minho estuary (Table 1).

**Sampling**

Water sampling was undertaken in September 2006 during ebb tide (at spring tide), at nine stations located along a main transect of both estuaries, from the upstream limit of tidal influence to the estuary mouth, covering a full range of salinity of 0-30 corresponding to a distance of 26.5 km in Minho and 15 km in Lima estuary (Fig. 1). Surface water (0.2 m depth) was collected using 2-L Niskin bottles (General Oceanics) for analysis of salinity (S), temperature (T), pH, dissolved inorganic nitrogen (nitrate NO₃⁻, nitrite NO₂⁻ and ammonium NH₄+), dissolved oxygen (DO) and nitrous oxide (N₂O). The hydrological characteristics of the Minho and Lima estuaries and the meteorological conditions observed during the sampling period are presented in Table 2.

**Analytical procedure**

Water temperature (T) was measured in situ with a Seabird SBE19/CTD probe with an accuracy of 0.01°C. Salinity (S) measurements were carried out using a temperature-controlled Guideline Salinometer (Portasal 8410A), and accuracy was 0.03 salinity. Equipment was calibrated with a certified IAPSO Standard Seawater reference.

Meteorological parameters (air temperature, pressure, and wind speed and direction) were determined using a portable meteorological station (Campbell Scientific CR510). Measurements represent the average of physical parameters taken using a sampling time of 5 seconds and a storage time of 1 minute. Wind speed 10-minute average was determined for each sampling
station and converted to wind speed values at 10 m height ($u_{10}$) using a logarithmic correction (Hartman and Hammond 1985).

The Minho and Lima River discharges were calculated as an average of the flow 10 days before sampling, at the hydrometric stations of Foz de Mouro and Ponte da Barca, respectively (SNIRH 2013).

Dissolved oxygen (DO, μmol L$^{-1}$) was measured using whole-bottle Winkler’s titration method (Amnot and Chaussepied 1983). A Methrom titrator was used to dispense small amounts of thiosulphate, and starch endpoint was detected visually. Precision of the method was in the range of 0.08% to 0.25%. DO saturation, expressed in percentage (%), was determined as the ratio of the oxygen concentration determined and the equilibrium values of DO calculated with the Weiss (1970) equation. Apparent oxygen utilization (AOU, μmol L$^{-1}$) was calculated as the difference between the saturation oxygen concentration and the dissolved oxygen concentration measured in the sample.

Water samples for determination of dissolved inorganic nitrogen were filtered through acetate cellulose filters (pore size 0.45 μm) and stored at −20°C until analysis. Analyses were carried out using a Traacs autoanalyser following colorimetric techniques outlined by the manufacturer. Estimated precision was ±0.8% for nitrate and nitrite (NO$_3^-$ and NO$_2^-$) and ±2.0% for ammonium (NH$_4^+$), at mid-scale concentrations. Accuracy of nutrient measurements was maintained by using CSK Standards (WAKO, Japan).

pH measurement was carried out immediately after collecting water samples using a Metrohm 704 pH-meter and a combined electrode (Metrohm), standardized against NBS buffers (6.865 and 9.180 pH). Precision of pH measurements was ±0.01.

Water samples for determination of dissolved N$_2$O were collected in triplicate in 20-mL glass headspace vials and poisoned with saturated aqueous mercury chloride (HgCl$_2$) to stop biological activity. The vials were stored upside down, in the dark, at 4°C in the refrigerator until analysis, performed within 10 days. Dissolved N$_2$O was determined by a headspace equilibration technique coupled with gas chromatographic separation (GC-3800, Varian). Briefly, 20 mL of sample was equilibrated with 5 mL of highly purified helium (purity =99.9999%) in a headspace CombiPAL autosampler. Gas chromatographic separation was carried out using a stainless steel column packed with 80/100 mesh Porapak. Oven and detector temperature was set at 50°C and 320°C, respectively, and high purity nitrogen (99.9999%) was used as the carrier gas (flow rate 30 mL min$^{-1}$). To remove water vapour and carbon dioxide, absorbent columns packed respectively with MgCl$_2$ and Carbosorb were located in the carrier gas line between the sample loop and the separation column. N$_2$O peak was detected with a $^{60}$Ni electron capture detector (ECD). Calibration of ECD response was performed using standard gas mixtures with 400, 780 and 1980 ppb N$_2$O in synthetic air (Air Liquide), and method precision was 2.6% (30 replicate measurements using samples containing 10 nmol L$^{-1}$ of N$_2$O). In situ concentration of N$_2$O (C, nmol L$^{-1}$) was calculated from the concentrations measured in the headspace according to the solubility equation of Weiss and Price (1980):

$$C = \beta (TS) x' P$$

where $x'$ is the measured N$_2$O dry mole fraction, P is the atmospheric pressure, and $\beta$ is the solubility coefficient, which is a function of the water temperature (T) and salinity (S). N$_2$O equilibrium concentrations were calculated assuming an atmospheric N$_2$O mixing ratio of 320.1 ppb (WMO 2006).

N$_2$O saturation, expressed in percentage (%), was determined as the ratio between the measured dissolved N$_2$O concentration and the equilibrium concentration. The N$_2$O water-air flux ($F_{N_2O}$) was estimated according to the following equation:

$$F_{N_2O} = k_{N_2O} \Delta N_2O$$

where $\Delta N_2O$ is the excess of N$_2O$, is the difference between the measured concentration and the equilibrium concentration with the atmosphere in the estuarine water at the local temperature and salinity; and $k_{N_2O}$ (cm h$^{-1}$) is the N$_2O$ transfer velocity, which is expressed as a function of the wind speed and the Schmidt number (Sc). Since no direct measurements of $k_{N_2O}$ were made in the Minho and Lima estuaries, both the k-wind relationships of Carini et al. (1996) (hereinafter referred to as C96) and Raymond and Cole (2001) (hereinafter referred to as RC01) were, respectively, used to compute k:

$$k_{C96} = 0.045 + 2.0277u_{10}$$

$$k_{RC01} = 1.91 e^{0.35u_{10}}$$

The gas transfer velocities and air-sea fluxes were estimated using in situ wind speeds normalized to 10 m height ($u_{10}$). The k coefficients were corrected for in situ temperature using the following relationship:

$$k_{N_2O} / k_{600} = (Sc_{N_2O}/600)^{0.5}$$

where $Sc_{N_2O}$ is the Schmidt number for N$_2O$ calculated according to the equation of Wanninkhof (1992):

$$Sc = 2301.1 – 151.1 T + 4.7364 T^2 + 0.057431 T^3$$

where T is the temperature (°C).

Statistical analysis

An unpaired t-test was used to identify statistical differences in levels of N$_2O$ and other environmental variables between and along estuaries. Pearson’s correlation analyses were performed to evaluate the existence of relationships between $\Delta N_2O$ and the variables NH$_4^+$, AOU, NO$_3^-$ and pH assumed to be connected with production pathways of this biogas. The multivariate techniques principal component analysis (PCA) and cluster analysis were applied to
environmental data in order to identify and compare inter-relationships between these variables in both estuaries. Data were log(x+1) transformed and handled using correlation-based PCA on the basis of standard Euclidean distance between samples to define their dissimilarity. PRIMER (version 6) was employed for the multivariate analysis.

Figures were created in Golden Software Grapher program (version 9.6.1001).

RESULTS

N₂O levels and fluxes

Concentrations of N₂O and the studied environmental parameters plotted against salinity along both Minho and Lima estuaries are shown in Figure 2.

Distribution of N₂O exhibits a declining tendency towards the mouths of both estuaries. In general, concentrations were higher in Lima than in Minho (Fig. 2A). In Lima values ranged from 10.1 to 20.0 nmol L⁻¹ and were below the conservative mixing line. The maximum value was reached in the upper estuary, suggesting that the Lima River was the main source of N₂O to the estuarine system. From salinity 0 to 4 a sharp decrease was observed, suggesting a greater N₂O loss in the zone, presumably through water-air gas exchange. Downstream of salinity 4, N₂O decreased more slightly and the mixing with the N₂O-poorer seawater is well perceptible. In the Minho estuary, concentrations varied from 8.6 to 14.4 nmol L⁻¹ and by contrast with those observed in Lima were above the conservative mixing line, pointing to the existence of N₂O sources within the estuary. A major concentration increase from 10.5 to 14.4 nmol L⁻¹ was detected between 0 and 2.5 salinity, suggesting the existence of internal N₂O sources in this zone of the estuary, presumably from manufacturing industries located nearby. N₂O saturation values ranged from 101% to 166% along the Minho estuary and from 113% to 227% along the Lima estuary (see Table 4), indicating that both estuaries are potential N₂O sources to the atmosphere.

Surface waters of both estuaries were well oxygenated during our study period. Concentrations of DO were higher than 280 μmol L⁻¹ in the upper Lima estuary at salinity 0.2, decreasing to 240 μmol L⁻¹ at salinity 2.8 (Fig. 2C). Seawards of this salinity, an increasing tendency was observed and a concentration of 255 μmol L⁻¹ was also reached in the vicinity of the estuary mouth (salinity 30.2). In the Minho estuary, a decrease in DO was also detected in the upper estuary and the concentration dropped from 243 μmol L⁻¹ at 0.1 salinity to 199 μmol L⁻¹ at 2.3 salinity. Afterwards, a sharp increase in DO was measured along the estuary and a maximum value of 264 μmol L⁻¹ was reached at the estuary mouth (salinity 29.7). DO-enriched seawater probably accounted for the similar increasing trend seaward in both estuaries. Saturation values were higher than 70% in the Minho estuary and 90% in the Lima estuary.
Fig. 3. – Relationships between $\Delta N_2O$ and environmental parameters in the Minho estuary. Lower salinity sites (0-10 salinity) are represented by black circles and higher salinity sites (15-30 salinity) by open circles. $R^2$, correlation coefficient.

Fig. 4. – Relationships between $\Delta N_2O$ and environmental parameters in the Lima estuary.
In the Minho estuary pH increased from 7.3 in the more river-influenced zone to 8.1 at the estuary mouth (Fig. 2D). In the Lima estuary pH showed a larger range of values, increasing from 6.7 in the most river-influenced site to 8.0 at the estuary mouth.

NO$_3^-$ was the dominant species of inorganic nitrogen in both estuaries, reaching a similar maximum concentration in the river input (47.6 μmol L$^{-1}$ in Minho and 44.4 μmol L$^{-1}$ in Lima) (Fig. 2E). Values decreased seawards and in general followed the theoretical conservative mixing line. Along the Minho estuary both NO$_2^-$ and NH$_4^+$ exhibited an irregular behaviour, though the system seemed to function as an NO$_2^-$ source and an NH$_4^+$ sink (Fig. 2F, G). Between salinity 0 and ~5-7 the decline in NH$_4^+$ (~4.0 to 0.5 μM L$^{-1}$) was simultaneous with an increase in NO$_2^-$ (~0.6 to 1.0 μM L$^{-1}$) and N$_2$O (~10 to 13-14 nmol L$^{-1}$), suggesting the occurrence of nitrification.

Figure 3 displays relationships between $\Delta$N$_2$O and AOU, NH$_4^+$, NO$_2^-$, NO$_3^-$ and pH in the Minho estuary. In the mentioned salinity zone (0 and ~5-7) a significant positive correlation was found between $\Delta$N$_2$O and AOU ($R^2=0.75$). This indicates the occurrence of nitrification as a source of N$_2$O and the respective slope provides an estimate of the biological N$_2$O yield per mole O$_2$ consumed (Yoshinari 1976). Further, the simultaneous (negative) correlations between $\Delta$N$_2$O and the primary substrate for nitrification, NH$_4^+$ ($R^2=0.40$), and pH ($R^2=0.80$), and the positive correlation between $\Delta$N$_2$O and the byproduct of nitrification, NO$_2^-$ ($R^2=0.41$), are consistent with the predominance of nitrification as a mechanism of N$_2$O production in the upper part of the Minho estuary. No correlation was found with NO$_3^-$ whose high concentrations were mostly riverine derived.

In the Lima estuary no relationships were found, suggesting the occurrence of nitrification (Fig. 4).

The application of PCA to the studied environmental variables in the Minho and Lima estuaries allowed us to identify two main composite variables, PC1 and PC2 (eigenvalues >1.0), which explain 82% of the variance (Table 3) and represent a good description of the environmental structure across the estuarine sampled sites.

PC1 explained 53% of variance and had the highest positive loading for NO$_3^-$, T and N$_2$O and a negative loading for S and pH (Fig. 5). This component represents the separation of major river-influenced stations from major marine-influenced ones in both estuaries. PC2 explained 27% of the variance and correlated positively with DO and negatively with NO$_2^-$ and NH$_4^+$. This component appears to represent relevant parameters to N dynamics, particularly in Minho estuary. In fact, projection of stations along PC2 reveals a clear separation of sites from the upper Lima estuary (L1-L5) (Fig. 5), mostly associated with higher values of N$_2$O, and sites from the upper Minho (M1-M5) more associated with higher NO$_2^-$ and NH$_4^+$, apparently from river origin, as the N load from the Minho River is considerable at this point (Table 1). It was also observed that the stations from both middle/lower estuaries did not differ in terms of studied environmental variables.

N$_2$O water-air fluxes are shown in Figure 6. Positive values prevailed at all stations but decreased, in general, between the upper and lower zone of the estuaries. This tendency was more pronounced in the Lima estuary, where higher N$_2$O fluxes in the river-influenced area were about twice (~12.0 μmol m$^{-2}$ d$^{-1}$; C96) (Fig. 6B) those observed in the upper part of the Minho estuary (~6.0 μmol m$^{-2}$ d$^{-1}$; C96) (Fig. 6A).
higher fluxes in the Lima estuary were mainly associated with the higher levels of N₂O observed in the upper estuary area (St.1 to St.3; Fig. 2), indicating that the low salinity zone (0-5) is an important source of N₂O to the atmosphere.

Using the two different parameterizations (C96, RC01) to calculate the gas transfer coefficients, the averaged N₂O water-air fluxes from Minho estuary to the atmosphere ranged between 4.0±3.3 μmol m⁻² d⁻¹ (RC01) and 4.1±2.8 μmol m⁻² d⁻¹ (C96), corresponding to a mean N₂O concentration of 11.3±1.3 nmol L⁻¹ (132±22% saturation) and a mean wind speed of 3.9±0.1 m s⁻¹. Slightly higher N₂O water-air fluxes were found in the Lima estuary, with averaged values ranging between 4.7±1.9 μmol m⁻² d⁻¹ (RC01) and 5.0±2.0 μmol m⁻² d⁻¹ (C96), corresponding to a higher mean N₂O concentration of 13.7±1.6 nmol L⁻¹ (153±26% saturation) and a lower mean wind speed level (2.4±0.1 m s⁻¹).

N₂O fluxes from the Minho and Lima estuaries were regressed versus the first two PCs’ ordination of station scores to test their ability to predict the fluxes. We found out that only PC1 showed to be correlated with N₂O flux, with a strong positive correlation (R²=0.61) (Fig. 7). N₂O flux also increased along a gradient of increasing NO₃⁻, T and N₂O. These results suggest that future global changes in these parameters will result in an increase of N₂O flux in these estuarine systems.

DISCUSSION

The present study reveals that the Minho and Lima estuaries, particularly the upper reaches, behave differently regarding N₂O levels, sources and fluxes. N₂O distribution exhibits a pronounced spatial variability in both estuaries but in the Lima estuary concentrations are higher than in Minho. The Lima River was the main N₂O contributor to the Lima estuary, whereas the occurrence of nitrification seems to represent an additional N₂O source within the Minho estuary. As NH₄⁺ is a primary substrate for nitrification, low NH₄⁺ concentration may limit nitrification. It has been suggested that the AOA and AOB niches are defined by ammonium concentrations (Martens-Habbena et al. 2009), with AOA dominating in ammonia-limited acid, whereas AOB have a tolerance of high ammonia concentrations.

Though no information on benthic AOA and AOB communities along the Minho and Lima estuaries is available, NH₄⁺ concentration in the upper Minho estuary (maximum 4.4 μmol L⁻¹) seems more suitable for the occurrence of nitrification than in the Lima estuary (maximum 1.8 μmol L⁻¹). Nitrification reactions typically happen within a DO range of 15.6-78.0 μmol L⁻¹, and the Minho and Lima surface waters were well...
above these concentrations, leading to nitrification conditions. However, only in the upper Minho do correlations found between RN2O and AOU, NH4+, NO2− and RN2O suggest that nitrification may have been acting as an NH4+ sink and a source of RN2O. The calculated biological RN2O yield (0.060 nmol per μmol O2 consumed) falls within the range observed in marine systems and in particular in the Atlantic off the Iberian coast (Nevison et al. 2003). The effect of salinity on nitrification is well documented and in many estuarine systems nitrification rates are highest at lower and intermediate salinities (Bianchi et al. 1999, Teixeira et al. 2013). Our results from the upper Minho are in accordance with these findings, as the potential nitrification occurred at low salinity (between ~2 and ~10). The community composition of nitrifying microbes is very dependent on salinity, but a combination of other environmental factors may shape AOB diversity along an estuary (Mosier et al. 2008).

pH may regulate nitrification, and Wild et al. (1971) found an ideal pH range for nitrification between 7.5 and 8.5. As nitrifiers are known to decrease pH, the sharp negative correlation found between pH and RN2O in the pH range 7.2-7.4 in the upper Minho estuary (Fig. 3E) may be a direct result of nitrification. RN2O saturation values ranging from 101% to 166% in the Minho estuary and 113% to 227% in the Lima estuary indicate that both estuaries behave as a potential RN2O source to the atmosphere. Positive RN2O water-air fluxes prevailed in all sampling stations, decreasing in general from upper to lower estuaries. However, this tendency was more pronounced in the Lima estuary, where higher RN2O fluxes in the river-influenced area were about twice (~12.0 μmol m−2 d−1; C96) those observed in the upper part of the Minho estuary (~6.0 μmol m−2 d−1; C96). It is likely that a greater turbulence of Minho upper estuary waters leads to a more rapid degassing of RN2O to the atmosphere in this part of the system.

Estimated RN2O fluxes were similar to those reported from the IPMA Oceanography Laboratory for their assistance in sampling, technical and analytical procedures. The authors also want to thank the Instituto Hidrográfico for their assistance during sampling. The research was supported by the PoPesca MARE project (22-05-01-FDR-001) and by the FCT-Portuguese Foundation of Science and Technology (POCI 2010 and FSE) through grant SFRH/BD/28569/2006.

ACKNOWLEDGEMENTS

Acknowledgements are due to colleagues from the IPMA Oceanography Laboratory for their assistance in sampling, technical and analytical procedures. The authors also want to thank the Instituto Hidrográfico for their assistance during sampling. The research was supported by the PoPesca MARE project (22-05-01-FDR-001) and by the FCT-Portuguese Foundation of Science and Technology (POCI 2010 and FSE) through grant SFRH/BD/28569/2006.

REFERENCES

Barnes J., Upstill-Goddard R.C. 2011. N2O seasonal distributions and air-sea exchange in UK estuaries: Implications for the trop-


