Seasonal and spatial controls on N₂O concentrations and emissions in low-nitrogen estuaries: Evidence from three tropical systems

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Abstract

Estuarine N₂O emissions contribute to the atmospheric N₂O budget, but little is known about estuary N₂O fluxes under low dissolved inorganic nitrogen (DIN) conditions. We present high-resolution spatial surveys of N₂O concentrations and water-air fluxes in three low-DIN (NO₃⁻ < 30 μmol L⁻¹) tropical estuaries in Queensland, Australia (Johnstone River, Fitzroy River, Constant Creek) during consecutive wet and dry seasons. Constant Creek had the lowest concentrations of dissolved inorganic nitrogen (DIN; 0.01 to 5.4 μmol L⁻¹ of NO₃⁻ and 0.09 to 13.6 μmol L⁻¹ of NH₄⁺) and N₂O (93–132% saturation), and associated lowest N₂O emissions (– 1.4 to 8.4 μmol m⁻² d⁻¹) in both seasons. The other two estuaries exhibited higher DIN inputs and higher N₂O emissions. The Johnstone River Estuary had the highest N₂O concentrations (97–245% saturation) and emissions (– 0.03 to 25.7 μmol m⁻² d⁻¹), driven by groundwater inputs from upstream sources, with increased N₂O input in the wet season. In the Fitzroy River Estuary, N₂O concentrations (100–204% saturation) and emissions (0.03–19.5 μmol m⁻² d⁻¹) were associated with wastewater inputs, which had a larger effect during the dry season and were diluted during the wet season. Overall N₂O emissions from the three tropical estuaries were low compared to previous studies, and at times water-air N₂O fluxes were actually

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negative, indicating that N_2O consumption occurred. Low water column NO_3^- concentration (i.e. $< 5 \mu\text{mol L}^{-1}$) appears to promote negative water-air N_2O fluxes in estuary environments; considering the number of estuaries and mangrove creeks where DIN falls below this threshold, negative water-air N_2O fluxes are likely common.

Keywords: nitrous oxide, estuary, groundwater, wastewater

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1. Introduction

Humans have greatly altered the nitrogen cycle in estuarine and coastal waters through excess nitrogen loads from agriculture, wastewater and urban runoff (Howarth, 2008; Vitousek et al., 1997). Nitrogen over-enrichment drives the production of excess organic matter (eutrophication), which is one of the greatest threats to coastal ecosystems worldwide (Howarth and Marino, 2006; de Jonge et al., 2002). Nitrogen is biologically processed in estuaries via many pathways including assimilatory uptake and microbially-mediated redox-regulated processes (e.g. Salk et al., 2017; An and Gardner, 2002; Cook et al., 2004; Eyre et al., 2016). Some of these pathways produce nitrous oxide (N_2O), which is a potent greenhouse gas that contributes about 8% of the radiative ‘greenhouse gas’ effect (Bouwman et al., 1995; Stocker et al., 2013) and causes ozone depletion in the stratosphere (Ravishankara et al., 2009).

In coastal areas, the most common biological pathways of N_2O production are nitrification and denitrification. Denitrification is the reduction of NO_3^- to NO_2^- , N_2O , and N_2 (Firestone et al., 1980; Firestone and Davidson, 1989). Typically, heterotrophic microbes are responsible for denitrification, however autotrophic ammonia-oxidising bacteria can reduce NO_2^- to N_2O and N_2 via ‘nitrifier-denitrification.’ In contrast with denitrification, nitrification involves oxidation of NH_4^+ to N_2O , NO_2^- , and NO_3^- (Arp et al., 2002; Belser and Schmidt, 1978). Denitrification occurs in anoxic environments, such as anoxic layers of soil or sediment (Davidson and Swank, 1986; Firestone et al., 1979;

Thomas et al., 1994), while nitrification occurs in both sediment (Jenkins and Kemp, 1984; Usui et al., 2001) and the water column (Damashek et al., 2016; Iriarte et al., 1998; Somville, 1984), where O_2 is available.

High concentrations of nitrate (NO_3^-) in estuarine waters are linked to increased N_2O water-air emissions (Murray et al., 2015), although this may be attenuated by the estuarine flushing time (Wells et al., 2018). High NH_4^+ concentrations are also known to enhance N_2O production (De Wilde and De Bie, 2000). Most studies of N_2O in tidal aquatic environments have been undertaken in disturbed estuaries with high DIN concentrations (up to $500 \mu mol L^{-1}$) (Murray et al., 2015). Additionally, most N_2O water concentration sampling has been undertaken in temperate or higher-latitude estuaries, with only 11 water-air N_2O emission studies in estuaries located between $25^\circ S$ and $25^\circ N$ (Table 1). Waters low in dissolved inorganic nitrogen (DIN) can be a net sink for N_2O , particularly in small mangrove-lined creeks (Erler et al., 2015; Maher et al., 2016; Wells et al., 2018; Murray et al., 2018). N_2O undersaturation may be prevalent in mangrove creeks, but the extent and seasonal dynamics of N_2O undersaturation are not well understood due to the limited number of studies and lack of seasonal sampling.

There can be significant variability in water-air N_2O fluxes across larger estuaries due to N_2O exchange, groundwater input, and benthic/water-column microbial activity. Large spatial N_2O concentration gradients have been observed at ‘hot spots’ of N_2O influx (Mueller et al., 2016; Wong et al., 2013). However, most estuarine N_2O surveys employ discrete sampling at coarse resolution, which may not capture the full range of variation. Continuous sampling, employing real-time N_2O concentration monitoring, is emerging as a way of investigating spatial variability in estuaries at a very fine scale (Bange et al., 1998; Brase et al., 2016; Mueller et al., 2016; Wells et al., 2018), however there has only been one such sampling campaign in a tropical estuary (Mueller et al., 2016).

In the current study, we present real-time N_2O concentration measurements and interpret the drivers of N_2O production and loss in three Queensland es-

tuaries during the wet and dry seasons, focusing on catchments which are only
 55 slightly to moderately affected by anthropogenic disturbance. We hypothesise
 that in such environments, water-air N_2O fluxes should be positive and increase
 with DIN concentration, however N_2O may be undersaturated at very low DIN
 concentrations. The DIN- N_2O relationship may provide some indication of the
 biogeochemical conditions under which a tropical estuary can transition from a
 60 net sink to a net source of N_2O .

Table 1: Range of N_2O concentrations and water-air N_2O fluxes in tropical estuaries.

Study Location	N_2O saturation (%)	N_2O flux ($\mu\text{mol m}^{-2} \text{ day}^{-1}$)	Citation
Andaman Island, Indian Ocean	102 to 208	– 2.4 to 7.2	Barnes et al. (2006)
Jiulong River, China	112 to 4133	1.1 to 93.8	Chen et al. (2015)
Pearl River, China	101 to 3799	0.1 to 733	Lin et al. (2016)
Tianjin, China	100 to 1300	– 6.2 to 49.9	Liu et al. (2015)
Various estuaries in Australia	50 to 123	– 3.4 to 0.7	Maher et al. (2016)
Northwestern Borneo	102 to 1679	3.6 to 33	Mueller et al. (2016)
Queensland, Australia	86 to 154	– 2.2 to 9.4	Murray et al. (2018)
Adyar River, India	—	3.6 to 84.9	Rajkumar et al. (2008)
Various estuaries in India	72 to 5902	– 1.1 to 14.21	Rao and Sarma (2013)
Pichavaram Mangrove, India	112 to 157	10.1 to 31.2	Senthilkumar (2008)
Various estuaries in Australia	~ 90 to 400	– 0.6 to 11.6	Wells et al. (2018)

2. Study Locations

Surveys were conducted during March (wet season) and September (dry
 season) in northern Queensland, Australia, along the lengths of three estuaries:
 the Fitzroy River Estuary, the Johnstone River Estuary, and Constant Creek
 65 Estuary (Figure 1). These are wet and dry tropical estuaries where most of
 the rainfall occurs in summer, with a mean annual rainfall of 3152 mm in the
 Johnstone catchment, 1705 mm in the Constant Creek catchment, and 646
 mm in the Fitzroy catchment (Queensland Department of Environment and

Science). In the Johnstone and Fitzroy estuaries there is a strong seasonality in
70 freshwater supply, as can be seen in data from upstream river gauges (Figure
2). Under high flows, salt water can flush fully to the mouth and during the
dry season these systems can become hypersaline due to high evaporation and
low freshwater input (Eyre, 1995, 1994).

The Johnstone River is furthest north, at 17.5°S latitude, draining a catch-
75 ment of 2320 m². The mouth of the Johnstone River is just 5 km east of the
city of Innisfail, QLD, which sits at the confluence of two major tributaries:
the North Johnstone River and the South Johnstone River. In this catchment
there is a sewage treatment plant located along a mangrove-lined creek, which
empties into the Johnstone River about 2.5 km upstream of the mouth of the
80 estuary (Figure 1a). The major land uses are banana and sugarcane cultivation.
Constant Creek is located north of Mackay, QLD at about 21°S latitude, lined
by mangroves and draining sugarcane cultivation and grazing lands (Figure 1b).
The mouth of the Fitzroy River is located at about 23°S latitude, draining a
catchment of 142,570 km², and emptying into the ocean downstream of Rock-
85 hampton, QLD. In the Fitzroy catchment, grazing and production forestry are
the most common land uses. Several sewage treatment plants release effluent
into the estuary just downstream of Rockhampton, near the upstream end of
the estuary (Figure 1c).

3. Methods

90 3.1. Sample Collection and Analysis

Spatial surveys were performed on board a small vessel, starting at high tide
at the mouth of each estuary and then moving upstream to the head of the
estuary (Eyre, 2000). In the case of the Johnstone River estuary, the South
Johnstone tributary was surveyed for about 1 km before a return trip to the
95 fork in the river and a survey of the North Johnstone tributary. Water was
continuously sampled from just below the water line through a water pump and
hose, which were suspended from the side of the boat. This water was then fed

Figure 1: The three estuaries surveyed along the coast of Queensland, Australia: a) the Johnstone River Estuary, b) Constant Creek, and c) the Fitzroy River Estuary.

into a shower-head style equilibrator to allow any dissolved gases to come into equilibrium between the water and a head-space of air (Johnson, 1999).

100 The head-space was vented into a second equilibrator head-space, which was itself vented into the ambient air. This assured there would be no pressure build-up in the equilibrator, while minimising atmospheric contamination of the sample. The head-space in the primary equilibrator was connected by 4 mm Bev-A-line tubing into a Picarro cavity ring-down spectrometer (CRDS)
105 instrument (details described below). A DrieriteTM column was connected to this tubing, between the equilibrator and the analyser inlet, to reduce humidity in the incoming air stream and minimise infrared interference from H₂O. The analyser outlet was connected back to the equilibrator so that the CRDS and equilibrator formed a loop through which air continuously flowed. Initial
110 experimentation revealed that there was a small lag (~ 5 min) between water sampling and full equilibration of the water with the head-space.

Two different CRDS instruments were used to measure N₂O concentration. In the wet season (March), the Picarro G5101-I isotopic N₂O analyser (Picarro Inc., Santa Clara, CA. USA) was used (Erler et al., 2015). In the dry season
115 (September) technical problems prevented the use of this device, so a G2308 Picarro was used. Both of these detect N₂O by measuring the ‘ring-down’ time of infrared light, however there is a difference in precision; The G5101-I has a 1-minute 1- σ precision of ~ 0.3 ppbv while the G2308 is less precise (~ 8 ppbv). In both seasons a 350 ppbv N₂O standard gas mixture was analysed to
120 check for instrument drift before and after each deployment, and no significant instrument drift was recorded.

In addition to continuous N₂O measurements, discrete water samples were collected about every 2 ppt of salinity along the length of the survey. These samples were filtered (0.45 μ m, Whatman GF/F), stored in 10 mL polycarbonate
125 vials, and frozen for later DIN and $\delta^{15}\text{N}$ –DIN analysis. Salinity, temperature and luminescent dissolved oxygen (LDO) were measured every 5 minutes using a Hydrolab DS5X Sonde (AquaLab), which was suspended from the side of the vessel, just below the water line. This sonde was re-calibrated for salinity be-

fore and after each survey. ^{222}Rn , a groundwater tracer, was measured every
130 10 minutes by a radon-in-air analyser (RAD7, DurrIDGE) connected by a loop of
tubing to the shower-head exchanger (Santos and Eyre, 2011). ^{222}Rn data was
only collected in the dry season.

Wind speed, required for N_2O water-air flux calculations, was measured us-
ing an anemometer and augmented with 1-minute wind speed data procured
135 from three Bureau of Meteorology (BOM) stations — Rockhampton (station
number 039083), South Johnstone (032037), and MacKay (033045) (near Con-
stant Creek). Depth datapoints came from a Garmin GPS unit on-board the
boat. Discharge data ($\text{m}^2 \text{ s}^{-1}$ of stream flow) came from two stream gauges up-
stream of the Johnstone River (stream gauge numbers 112004A and 112101B),
140 and one stream gauge upstream of the Fitzroy River (stream gauge number
130005A) which were obtained from the Water Monitoring Information Portal
of the Queensland Department of Natural Resources and Mines.

Discrete water samples were analysed for nitrate (NO_3^-), dissolved organic
nitrogen (DON), and ammonium (NH_4^+) on a Lachat-flow injection device (see
145 Eyre and Pont (2003); Eyre et al. (2011) for methods, errors and detection
limits). Before $\delta^{15}\text{N}$ analysis, NO_3^- was converted to N_2O by denitrifying
bacteria following the method of Sigman et al. (2001). The resulting N_2O was
then stripped from the solute by helium sparging and then analysed in a Thermo
Fisher GasBench II which fed into to a Thermo Delta V Plus IRMS.

150 3.2. Calculations

The dissolved N_2O and saturation values were calculated based on the sol-
ubility equations of Weiss and Price (Weiss and Price, 1980), and apparent
oxygen utilisation (AOU) was derived from LDO using the solubility equations
of (Benson and Krause, 1984). Excess N_2O ($\Delta\text{N}_2\text{O}$) was calculated as the
155 difference between measured concentration of N_2O (in nmol L^{-1}) and the theo-
retical concentration at 100% saturation, which we refer to as the "background"
concentration:

Figure 2: Stream discharge ($\text{m}^3 \text{s}^{-1}$) measurements from three tributaries upstream of the Johnstone and Fitzroy Rivers, at the locations of (a) 'Tung Oil' upstream of the North Johnstone River, (b) 'Central Mill' upstream of the South Johnstone River, and (c) 'The Gap' upstream of the Fitzroy River.

$$\Delta N_2O = N_2O_{\text{measured}} - N_2O_{\text{background}}$$

There are many parameterisations available for estimating water-to-air fluxes. The commonly-cited options can produce very different results (Musenze et al., 2014; Rosentreter et al., 2017), so fluxes were calculated using 6 different parameterisations (equations in Appendix Table A1). The mean fluxes presented in this study represent the average of all 6 parameterisations, and the ranges represent the full range of all calculated values. Gas fluxes were calculated at 1-minute intervals, and any missing temperature, salinity, or wind data was linearly interpolated from the nearest measured values. The resulting water-air N_2O fluxes then were extrapolated over the area of the main channel of each estuary. To calculate the average area-weighted estuary water-to-air flux, it was first necessary to define a shape-file outlining the areal extent of each transect. This was done by manually digitising Landsat 8 satellite images for each estuary using QGIS software. After this step, the 1-minute-average water-air flux values (in $\mu\text{mol } N_2O \text{ m}^{-2} \text{ d}^{-1}$) were extrapolated over the water surface area at $\sim 3 \text{ m}$ resolution, using an inverse cost function – specifically, the GRASS GIS ‘v.surf.icw’ function (developed by Hamish Bowman) was used for this purpose. Finally, the area-weighted values were calculated as the average N_2O flux of all of the pixels in the resulting geoTIFF raster image.

Once the total water-air N_2O flux ($\mu\text{mol d}^{-1}$) was calculated, it was compared with various inputs into the estuary, such as the total DIN load (the DIN concentration of freshwater multiplied by the total freshwater discharge into each estuary) and the load of NO_3^- and NH_4^+ . Additionally, the depth (m), salinity, and N_2O concentration (nmol L^{-1}) datasets were extrapolated using the same method as the water-air fluxes – the inverse-cost function – over the same area in order to calculate the total water volume of each estuary and the total volume of freshwater. The freshwater fraction was calculated using the QGIS ‘raster calculator’ function at each pixel of the ‘depth’ geoTIFF as:

$$\text{Freshwater fraction} = (S_{\text{mouth}} - S_{\text{measured}}) / S_{\text{mouth}}.$$

185 Where S_{mouth} is the salinity at the mouth of the estuary (the oceanic salinity) and S_{measured} is the salinity as measured (or extrapolated from measured values) at each point. The freshwater fraction values were multiplied by the depth and surface area at each pixel as:

$$\text{Freshwater m}^3 \text{ per pixel} = \text{Freshwater fraction} \times \text{pixel area} \times \text{depth}$$

The sum of all values in the resulting raster image, calculated using the
190 ‘raster statistics’ function in QGIS, was then the total amount (in m^3) of fresh-
water in the estuary. This information, along with the freshwater discharge
data, was required to calculate the freshwater flushing time using the fraction
of freshwater method (Kennish, 1986), where the total freshwater volume is di-
vided by the freshwater discharge at the upstream gauge. Instead of using the
195 discharge on the day previous to sampling, however, the discharge of preceding
days was summed until the total freshwater volume was reached (Eyre, 2000;
Kaul and Froelich, 1984). The exception was at Constant Creek, where freshwa-
ter residence time was not calculated due to the absence of upstream discharge
data.

200 The discharge data and total N_2O flux data were used to estimate the con-
tribution of ventilation of river N_2O to the total N_2O flux within each estuary
(modified from Abril et al., 2000; Borges and Abril, 2011). This value represents
the proportion of the positive water-air N_2O flux which would be needed to offset
all of the excess dissolved N_2O ($\Delta\text{N}_2\text{O}$) delivered by the river at the upstream
205 end of the estuary channel. It is possible for this proportion to be greater than
100% in estuaries where the water-air N_2O flux is too low to fully account for
all incoming dissolved N_2O – i.e. in estuaries where freshwater-derived N_2O is
exported to the ocean.

Independently, the theoretical ventilation time (the time required to equi-
 210 librate the entire water column with overlying air) was estimated as the ratio
 between the average depth and average gas transfer velocity (Bender et al., 2011;
 De Wilde and De Bie, 2000; Reuer et al., 2007). In addition, where N_2O con-
 centrations fell below the conservative salinity mixing line, the amount of N_2O
 removed from the water column (R_{N_2O}), was calculated as the sum of the devi-
 215 ations between observed N_2O concentrations and expected N_2O concentrations
 at each point.

For consistency, the area of each transect was defined as the maximum area
 that had sampling coverage for both seasons. In the Fitzroy, the survey extended
 from the mouth of the estuary to a point just downstream of the Rockhampton
 220 weir. In the Johnstone, the sampling vessel traveled up the South and North
 Johnstone for a few km. In Constant Creek the surveyed area extended upstream
 to a bridge just inland of the boat ramp.

4. Results

4.1. Johnstone River Estuary

225 Nitrous oxide concentrations in the Johnstone River Estuary ranged from
 132 to 245% saturation in the wet season and from 97 to 228% saturation
 in the dry season (Table 2; Figure 3e,f). Water-air N_2O fluxes were higher
 than in the Fitzroy River Estuary and Constant Creek, with a wide range of
 0 to $47.5 \mu\text{mol m}^{-2} \text{d}^{-1}$ over the salinity gradient in the wet season (Table 2;
 230 Figure 3g,h) and an average area-weighted water-air flux of $5.6 \mu\text{mol m}^{-2} \text{d}^{-1}$
 (Table 2). The range over the salinity gradient in the dry season was -0.3 to
 $28.8 \mu\text{mol m}^{-2} \text{d}^{-1}$ and the average area-weighted water-air flux was $3.6 \mu\text{mol}$
 $\text{m}^{-2} \text{d}^{-1}$. Nitrous oxide concentrations were generally highest at the freshwater
 end-member, particularly in the South Johnstone (Figure 4a,b), where N_2O
 235 saturations reached as high as 245% in the wet season and 225% in the dry
 season. In the North Johnstone, N_2O reached a maximum saturation of 204%
 in the wet season and 160% in the dry season.

The freshwater flushing time in the Johnstone River was 0.59 days (14 h) in the wet season, during which time the theoretical water-column ventilation time was about 2.02 days. The potential contribution of riverine N₂O evasion to N₂O emissions was about 450% (Figure 5) corresponding to a total riverine input of 70 mol of excess N₂O per day, as compared with the total water-air evasion of 15.4 mol d⁻¹. In the dry season, the freshwater residence time was 2.3 days, similar to the theoretical ventilation time of 2.26 days. The contribution of riverine N₂O was 124%; 9.8 mol d⁻¹ N₂O was emitted to the atmosphere, while the total excess freshwater N₂O contribution was 12.3 mol d⁻¹.

The Johnstone River was consistently the least saline of the three estuaries, with salinity range of < 1 to 34 in the dry season and < 1 to 12 in the wet season. In both seasons there was a negative relationship between salinity and N₂O. In both seasons, N₂O concentrations measured downstream of the confluence of the North and South Johnstone fell below the salinity–N₂O conservative mixing line, resulting in a total deficit of 6.3 mol in the wet season and 1.4 mol in the dry season (Figure 7). Over the same area, the total water-air N₂O flux was 8.9 mol d⁻¹ (wet season) and 4.2 mol d⁻¹ (dry season). Additionally, the freshwater residence times were lower below the confluence of the North and South Johnstone Rivers — 7 h for the wet season and 21 h for the dry season — and over the residence time the water-air N₂O flux could account for 41% and 260% of the missing N₂O in the wet and dry season, respectively. The ratio of the N₂O emissions (μmol d⁻¹) to total DIN load (μmol d⁻¹) was 0.02% in the wet season and 0.09% in the dry season.

In the dry season, NO₃⁻ concentrations were lower (mean value of 3.5 μmol L⁻¹) and NH₄⁺ values higher (mean of 1.3 μmol L⁻¹) (Figure 3b,d) than in the wet season (mean of 6.0 μmol L⁻¹ for NO₃⁻ and 0.1 μmol L⁻¹ for NH₄⁺) (Figure 3a,c). There was a strong positive correlation between NO₃⁻ and N₂O in dry season (r² = 0.77; p < 0.001) and a weak and non-significant relationship in the wet season (Figure 8a). Ammonium concentrations were low and showed no correlation with N₂O concentrations (Figure 8b). Nitrate levels decreased with increasing salinity in both seasons (Appendix Figure A1a), but NH₄⁺ showed no

clear correlation with salinity (Appendix Figure A1b). Dissolved oxygen levels
 270 varied between 90% and 100% saturation, with AOU values falling between 7
 and $36 \mu\text{mol L}^{-1}$ in the wet season and -30 and $16 \mu\text{mol L}^{-1}$ in the dry season
 (Appendix Figure A2a,b). Excess N_2O ($\Delta\text{N}_2\text{O}$) ranged from 2 to 10 nmol L^{-1}
 in the wet season and 0 to 4 nmol L^{-1} in the dry season.

The ^{222}Rn concentrations were negatively correlated with salinity and posi-
 275 tively correlated with both NO_3^- and N_2O concentration in the dry season
 (Figure 9e,g,h). Overall, wet season $\delta^{15}\text{N}-\text{NO}_3^-$ values ($+7.7$ to $+9.9\text{‰}$)
 were higher than dry season values ($+4.8$ to $+7.3\text{‰}$) (Figure 10a,b), with the
 lowest wet season value near the mouth of the estuary, and the highest value in
 the high- N_2O reaches of the north arm.

Table 2: N_2O concentrations (saturation %) and water-air N_2O fluxes, for the Johnstone River, the Fitzroy River, and Constant Creek, including area-weighted values.

Location	N_2O saturation (%)				N_2O flux ($\mu\text{mol m}^{-2} \text{ day}^{-1}$)			
	min	max	mean	Area-weighted mean	min	max	mean	Area-weighted mean
Johnstone River wet season	132	245	170	165	0	47.6	6.1	5.6
Johnstone River dry season	97	228	133	140	-0.3	28.8	2.8	3.6
Constant Creek wet season	93	104	99	100	-2.2	1.2	-0.1	0.02
Constant Creek dry season	97	132	106	116	-0.8	15.1	1.3	3.5
Fitzroy River wet season	100	139	113	107	-0.01	33.4	3.8	2.2
Fitzroy River dry season	103	204	128	112	-4.8	28.5	2.6	1.5

280 4.2. Constant Creek Estuary

N_2O concentrations were lower in Constant Creek than the Johnstone and Fitzroy river estuaries, ranging from 92.9% to 104.4% saturation in the wet season and 97.3% to 131.5% saturation in the dry season (Table 2; Figure 4c,d).

Figure 3: Box plots of wet and dry season NO_3^- , NH_4^+ concentrations, N_2O saturations, and water-air N_2O fluxes for the three estuaries.

Figure 4: The spatial variability of N_2O concentration (% saturation) in the Johnstone River Estuary, Constant Creek, and Fitzroy River Estuary in the wet and dry seasons.

In the dry season N_2O concentrations were highest at the seawater end-member
 285 and showed non-conservative behaviour with an uptake in the middle reaches of
 the estuary (Figure 4c). In the wet season water-air N_2O fluxes were negative
 over a large portion of the estuary (range of -2.2 to $1.2 \mu\text{mol m}^{-2} \text{d}^{-1}$) (Table 2),
 reflecting slightly undersaturated N_2O concentrations (93%-100%) in the middle
 of the estuary. The highest water-air fluxes (up to $15.1 \mu\text{mol m}^{-2} \text{d}^{-1}$) (Table
 290 2), occurred during the dry season, when N_2O saturations reached $\sim 130\%$ in
 the outer estuary (Figure 4d).

Theoretical water-column ventilation times were 2.1 days in the wet season
 and 1.2 days in the dry season, the difference caused by an increase in wind
 speed in the dry season. The total water-air flux was much lower in the wet
 295 season (1.4 mol d^{-1}) than in the dry season (8.9 mol d^{-1}). In both seasons,
 N_2O concentrations fell below the conservative mixing line, with an apparent
 total N_2O deficit of 1.4 mol in the wet season and 9.9 mol in the dry season.
 For water-air N_2O emissions to make up for this deficit, it would require ~ 28
 days in the wet season and only ~ 1 day in the dry season.

300 Constant Creek was the most saline of the three estuaries, with salinity
 ranging from 11 to 35 in the wet season and 30 to 35 in the dry season. NO_3^-
 concentrations were lower than in the Johnstone and Fitzroy river estuaries,
 ranging from 0.2 to $0.4 \mu\text{mol L}^{-1}$ in the wet season and from 0 to $5.4 \mu\text{mol}$
 L^{-1} in the dry season (Figure 3a,b). Similar to the Fitzroy and Johnstone river
 305 estuaries, NH_4^+ concentrations were extremely low in the wet season, ranging
 from 0.1 to $0.4 \mu\text{mol L}^{-1}$ (Figure 3c), but increased in the dry season, ranging
 from 0.4 to $13.6 \mu\text{mol L}^{-1}$ (Figure 3d). In the wet season the highest NO_3^-
 and NH_4^+ concentrations were at the seawater end-member. In contrast, in
 the dry season the highest NO_3^- and NH_4^+ concentrations were at the river
 310 end-member (Appendix Figure A1c,d).

Neither N_2O , NO_3^- nor NH_4^+ were correlated with ^{222}Rn (Figure 9i,j,k);
 ^{222}Rn increased at lower salinities (Figure 9l) while DIN and N_2O concentrations
 were highest in the outer estuary (Figure 4d). In the dry season dissolved
 oxygen was saturated ($\sim 100\%$), with AOU values between -8 and $13 \mu\text{mol}$

Figure 5: Residence time vs. the proportion of water-air N_2O flux explained by ventilation of river-derived (freshwater-derived) N_2O from upstream sources (%), for the Johnstone River Estuary and Fitzroy River Estuary

315 L^{-1} (Appendix Figure A2d). In the wet season dissolved oxygen was slightly undersaturated (85%), with AOU values between -19 and $34 \mu mol L^{-1}$. AOU and ΔN_2O show a negative correlation in the wet season (Appendix Figure A2c). Due to low NO_3^- concentrations, $\delta^{15}N-NO_3^-$ values were only measured at two locations during the dry season, when they were 2.8 and 4.2‰.

320 4.3. Fitzroy River Estuary

In the Fitzroy River Estuary, N_2O concentrations ranged from 100% to 139% saturation, in the wet season and 103% to 204% in the dry season (Table 2; Figure 3e,f). Despite higher N_2O concentrations in the dry season, water-air fluxes were higher in the wet season (area-weighted mean of $2.2 \mu mol m^{-2} d^{-1}$ in wet and $1.5 \mu mol m^{-2} d^{-1}$ in dry) due to higher k-values during the wet season (Appendix Table A2). In both seasons N_2O concentrations decreased from the upstream end to the ocean. However in the wet season there was a slight increase in the middle reaches of the estuary, near the mouth of small mangrove-lined tributary at about 30 km downstream of Rockhampton.

330 The freshwater flushing time was 5.7 days in the wet season, and the average ventilation time of the water-column was 0.9 days. With a freshwater excess N_2O load of 151 mol N_2O and a total water-air N_2O flux of 477 mol over the residence time, the ventilation of the freshwater (riverine) N_2O accounted for 32% of the N_2O emissions (Figure 5). In the dry season the flushing time was 72.6 days and the ventilation time was 2.2 days. The excess freshwater N_2O load was 67 mol, and over the duration of the residence time, the estimated total

water-air flux was 4150 mol, meaning that ventilation of river N_2O inputs can explain 1.6% of the water-air flux. In both seasons, N_2O concentrations showed non-conservative mixing, (Figure 6c); N_2O was as much as 3 nmol L^{-1} below the conservative mixing line despite N_2O saturation remaining above 100%. The
 340 estimated total N_2O deficit was 1.3 mol N_2O in the wet season and 150 mol in the dry season. Over the duration of the residence time, the water-air flux could account for 5100% and 2800% of the lost N_2O in the wet season and dry season, respectively. The N_2O water-air flux ($\mu\text{mol d}^{-1}$) accounted for 0.05% and 1.12%
 345 of the total DIN load ($\mu\text{mol d}^{-1}$) in the wet and dry seasons respectively.

Salinity was lower during the wet season, ranging from < 1 to 29 as compared to 24 to 35 in the dry season. In the dry season, NO_3^- and NH_4^+ concentrations were elevated (Figure 3b,d) compared to the Johnstone River Estuary and Constant Creek, showing a positive correlation with salinity (Appendix
 350 Figure A1e,f). The N_2O saturation was positively correlated with both NO_3^- and NH_4^+ concentrations in the dry season (Figure 8e,f). In contrast, in the wet season NO_3^- and NH_4^+ concentrations were lower (Figure 3a,c) and not correlated with N_2O concentrations (Figure 8e,f).

AOU values varied from 24 to $59 \mu\text{mol L}^{-1}$ in the wet season, increasing
 355 along the length of the freshwater plug until it reached a peak at the transition between freshwater (salinity < 0.5) and brackish water (Appendix Figure A2e). AOU increased again until about 30 km from Rockhampton, where both AOU and $\Delta\text{N}_2\text{O}$ reach a secondary, mid-estuary peak. In the dry season, AOU fell between 14 and $41 \mu\text{mol L}^{-1}$. The highest AOU values were observed at a
 360 salinity less than 25, where $\Delta\text{N}_2\text{O}$ was elevated in the upper estuary. At this location there was a positive correlation between AOU and salinity (Appendix Figure A2f). At a salinity of ~ 27 and a distance of ~ 11 km downstream of Rockhampton, the AOU- $\Delta\text{N}_2\text{O}$ relationship changes, but the values further downstream still show a positive correlation.

365 The ^{222}Rn concentrations showed a positive correlation with NO_3^- concentrations (Figure 9i) and a maximum at intermediate salinity values — (up to 3 pCi L^{-1} between salinities of 26 and 28) (Figure 9l). At the lowest salinity

values (< 25), where ^{222}Rn was at intermediate concentration, NO_3^- , N_2O and NH_4^+ were all relatively high, diverging from a simple linear correlation with ^{222}Rn . Nitrate $\delta^{15}\text{N}$ values were higher in the dry season (+ 8.7 to + 11.7‰),
 370 than in the wet season (+ 4.1 to + 7.0‰) (Figure 10) and the lowest wet-season $\delta^{15}\text{N}-\text{NO}_3^-$ values were found in the low-salinity upper reaches of the estuary.

5. Discussion

5.1. Johnstone River Estuary

375 The highest concentrations of N_2O (both wet and dry seasons) over the three estuarine systems were found in the Johnstone River Estuary. In contrast to the two other systems, N_2O concentrations in the Johnstone River Estuary were higher during the wet season rather than the dry season. Below we discuss these differences in terms of the potential sources of N_2O , the observed seasonal
 380 pattern in N_2O concentration, and the distribution of N_2O within the Johnstone River Estuary.

Compared with the other two estuaries, the Johnstone River Estuary is subject to much higher freshwater input from its tributaries (Figure 2a,b,c). As such, the estimated N_2O loads are high enough, and the residence time is low
 385 enough, that some of the N_2O arriving from upstream sources is exported to the ocean. In both the wet and dry season, the proportion of N_2O emissions explained by ventilation of riverine N_2O inputs is greater than 100% (Figure 5). In other words the potential N_2O ventilation is higher than the observed N_2O water-air flux, and any in situ production of N_2O is overwhelmed by the river
 390 load.

Discharge-driven increases in estuarine N_2O concentration are relatively common. For example, across 28 estuaries in India, high wet-season N_2O concentrations resulted from mobilisation of N_2O from upstream sources (Rao and Sarma, 2013). Similar to the Johnstone River, the Saribas and Lupar Rivers in
 395 Borneo also exhibited greater N_2O variability and higher maximum N_2O concentrations in the wet season, with a clear source of N_2O near the midpoint of

Figure 6: N_2O concentrations plotted as a function of salinity in wet and dry seasons in the Johnstone River Estuary (a), Constant Creek (b) and Fitzroy River Estuary (c).

the spatial transect (Mueller et al., 2016). In the dry season however, Mueller et al. (2016) found that N_2O concentrations were less variable and most of the N_2O came from upstream, freshwater inputs.

400 One of the main differences between the Johnstone River and the two rivers in Borneo is the relationship between N_2O and NO_3^- . Mueller et al. (2016) found no correlation between DIN and N_2O in either season, and noted that despite the relatively low DIN concentrations of the waterways ($10\text{--}37\ \mu\text{mol L}^{-1}$) N_2O was present in very high concentrations in some places (up to 1679% 405 saturation). In contrast, in the Johnstone, N_2O concentrations showed a correlation with NO_3^- in both seasons (Figure 8a,b), indicating that N_2O production was enhanced by the higher NO_3^- concentrations, or that N_2O and NO_3^- were entering the system together.

The salinity– NO_3^- mixing plots indicate that most of the NO_3^- in the Johnstone River was from freshwater sources from the North and South Johnstone 410 tributaries (Appendix Figure A1a); NO_3^- was not generated in large quantities below the confluence of the river. It is difficult to tell if any N_2O was produced in the estuary. In both seasons, the N_2O load from freshwater input is high enough to more than account for all of the emitted N_2O (Figure 5) and in-situ 415 production is not needed to explain the observed patterns. In some estuaries and coastal waters, a positive correlation between N_2O and apparent oxygen utilization has been interpreted as a sign of in-situ nitrification (Nevison et al., 2003; Goncalves and Brogueira, 2017), however this correlation was not observed in the Johnstone (Figure A2a,b), and neither did we observe a negative correlation 420 between N_2O and NH_4^+ (Figure 8b), as would be expected if nitrification were consuming large quantities of NH_4^+ .

Another possible pathway of N_2O production or consumption is denitrification. Sediments and groundwater can be major sites of denitrification and N_2O production (Marzadri et al., 2017), and the higher $\delta^{15}\text{N}\text{--}\text{NO}_3^-$ values observed 425 in the wet season could indicate a greater influence of denitrification during the high- N_2O season (Figure 10). Denitrification increases the $\delta^{15}\text{N}\text{--}\text{NO}_3^-$ in the remaining NO_3^- pool (Granger et al., 2008; Middelburg and Nieuwenhuize,

2001), therefore the higher $\delta^{15}\text{N}-\text{NO}_3^-$ could result from an increased inflow of DIN from sediment pore-water or groundwater, where denitrification is likely to occur. However, similar to nitrification, there was little evidence that denitrifi-

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cation occurred in-situ; dissolved N_2O could have come from upstream sources. A denitrification pathway for N_2O production would be consistent with the strong correlation between ^{222}Rn and both NO_3^- and N_2O in the dry season (Figure 9a,c), which suggest that at least some NO_3^- and N_2O came from groundwater inputs. The CO_2 and CH_4 concentrations were also correlated with ^{222}Rn suggesting a similar groundwater source (Rosentreter et al., 2018). Although we were unable to obtain ^{222}Rn concentrations for the wet season, it seems likely that groundwater input would increase with higher precipitation. This would drive pore-water NO_3^- and N_2O into the upper river systems. Overall, we propose that the higher concentration of N_2O in the Johnstone River Estuary relative to the Constant Creek and the Fitzroy River Estuaries, and the increase in N_2O concentration in the wet season relative to the dry season, is a consequence of higher rainfall and increased groundwater flow.

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5.1.1. Spatial Variability

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The Johnstone Estuary itself has two main sections (see Figure 1): the North and South tributaries. The South Johnstone tributary was the strongest source of N_2O -rich freshwater in both the wet and dry seasons, accounting for 51–55% of the N_2O load despite only contributing 32–34% of the freshwater volume (according to the upstream gauges). This high N_2O concentration water mixed within the main body of the estuary with water from the North Johnstone tributary.

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Below the confluence of the North and South Johnstone, N_2O concentrations fall below the conservative mixing line in both the wet and dry seasons (Figure 6a). If N_2O concentrations were near or below 100% saturation, this mid-estuary minimum of N_2O might indicate N_2O reduction to N_2 , as has been observed in some low-DIN tidal waterways (Daniel et al., 2013; Rao and Sarma, 2013; Erler et al., 2015; Maher et al., 2016; Murray et al., 2018). However, where N_2O is

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supersaturated, as in the Johnstone River Estuary, it doesn't seem likely that N_2O consumption could explain the loss of N_2O .

460 The N_2O –salinity relationship in the Johnstone River Estuary is similar to the eutrophic Pearl River in China (Lin et al., 2016), where N_2O was non-conservative despite dissolved N_2O concentrations consistently above 100% saturation (101–3799%). Lin et al. (2016) estimated N_2O removal rates which nearly balanced out water-air N_2O fluxes, suggesting that all of the apparent
465 N_2O loss could be explained by evasion.

In the Johnstone River, in contrast, there was a net export of freshwater-derived N_2O (55 mol d^{-1}) in the wet season, despite the fact that evasion could only explain $\sim 40\%$ of the N_2O lost below the confluence of the North and South Johnstone. In the dry season, this N_2O loss was dwarfed by the water-air
470 evasion (280%), a fact that might indicate in-situ N_2O production, yet the net export of freshwater-derived N_2O was lower (2.4 mol d^{-1}). The residence time was very low below the confluence of the North and South Johnstone in both seasons, and a small change in either residence time or water volume could have a large effect on the calculations.

475 5.2. Constant Creek Estuary

In contrast to the Johnstone River, the low DIN and N_2O concentrations in Constant Creek suggest relatively low anthropogenic input of nitrogen from agriculture or other sources. Similar to the Johnstone River Estuary, N_2O concentrations in Constant Creek fall below the conservative mixing line (Figure
480 6c). In this case, however, N_2O concentrations in the middle reaches of the estuary are close to or slightly below 100% saturation. This suggests that the N_2O deficit results from consumption of N_2O by denitrifiers.

In the wet season there was some evidence for in-situ consumption. While the overall area-weighted flux was positive, negative water-air fluxes (as low as
485 $-2.2 \mu\text{mol m}^{-2} \text{ d}^{-1}$) were observed in the middle section of the transect (Table 2). The undersaturation of N_2O has been observed previously in other relatively pristine creeks in Australia (Erler et al., 2015; Maher et al., 2016; Murray et al.,

Figure 7: N_2O mixing plot for the Johnstone River. The map shows the difference, in $\text{nmol N}_2\text{O}$ between the measured N_2O concentration and the value expected from saltwater/freshwater mixing alone, with 'hot spots' indicated in red.

2018; Wells et al., 2018), a salt marsh (Daniel et al., 2013), and some monsoonal estuaries in India (Rao and Sarma, 2013). However, N₂O undersaturation is not
 490 observed in all pristine estuaries. Barnes et al. (2006) documented N₂O saturations between 100% and 208% in a pristine mangrove creek where NO₃⁻ values were 0–5 μmol L⁻¹ and NH₄⁺ values were 0–25 μmol L⁻¹, similar to values found at Constant Creek (0–5.4 μmol L⁻¹ NO₃⁻ and 0–13.6 μmol L⁻¹ NH₄⁺). The creek on Andaman Island had relatively low salinity (0–28) compared with
 495 Constant Creek (10–35); the shared characteristics of undersaturated estuaries seem to be low DIN and high salinity (Maher et al., 2016; Rao and Sarma, 2013). Saline conditions can inhibit water-column nitrification (Rysgaard et al., 1999), and this might help to prevent in-situ N₂O production.

In the dry season, it was not clear that N₂O was consumed in the estuary,
 500 due to the fact that that N₂O concentrations were generally elevated above 100% (Figure 3f). Constant Creek was the only estuary where the highest N₂O concentrations were found at the downstream end of the transect, and we propose that the extra N₂O was most likely produced on the extensive shallow shoals which are found in the outer estuary (Figure 4d). Shallow benthic sediments are
 505 known to be a strong source of N₂O in many estuaries (e.g. Usui et al., 2001; Ferrón et al., 2007). Due to the downstream N₂O source, the salinity–N₂O data-points to deviate below the conservative mixing line. Considering that N₂O concentrations remain at or above 100% over the transect, we cannot say that the non-conservative behaviour of N₂O is due to in-situ consumption. Fur-
 510 thermore, the water-air N₂O flux would require only 1.1 days to balance out the negative deficit, which is not an unreasonably long time.

In the dry season, ²²²Rn concentrations were highest at low N₂O concentra-
 tions, indicating that groundwater was not a strong source of N₂O (Figure 9g). Water-column nitrification is also unlikely to produce N₂O in this environment.
 515 As mentioned previously, a positive linear correlation between AOU and ΔN₂O is considered a sign of nitrification activity because nitrification produces N₂O and consumes O₂. However in Constant Creek there is a clear negative correlation between excess N₂O and AOU in the wet season (Appendix Figure A2),

suggesting that nitrification was not a strong source of N_2O , but that denitrifiers
 520 reduced N_2O to N_2 , especially under low-DIN conditions.

5.3. Fitzroy River Estuary

In the Fitzroy River Estuary, there was a clear source of N_2O at the upstream
 end of the survey (Figure 4e,f), with higher concentrations during the dry sea-
 son (Figure 4f). Similar to Constant Creek, but in contrast to the Johnstone
 525 River Estuary, the seasonal differences in N_2O concentrations reflect a funda-
 mental shift in the source and/or processing of DIN, and not just a change in
 freshwater discharge. While total freshwater discharge was higher in the wet
 season, this extra freshwater did not have enough N_2O to increase N_2O con-
 centration higher than in the dry season (Figure 6c), and freshwater may even
 530 dilute N_2O concentrations. In the dry season the weak relationship between
 N_2O and ^{222}Rn shows that pore-water/groundwater was not the only source of
 N_2O in the Fitzroy River Estuary (Figure 9k). Near Rockhampton, there was
 a source of low-salinity water with relatively low ^{222}Rn concentrations but high
 N_2O , NO_3^- and NH_4^+ concentrations (Figure 9i,j,k,l).

535 Looking at the strong correlation between N_2O saturation and DIN (Fig-
 ure 8e,f) and the relationship between ^{222}Rn and DIN, it seems likely that the
 wastewater treatment plants located near the head of the Fitzroy River Es-
 tuary (Figure 1) are the ultimate source of a large portion of the NO_3^- , N_2O
 and NH_4^+ present in the waterway. In the case of N_2O , the treatment plants
 540 may be a direct or indirect source: N_2O may be discharged directly as a re-
 sult of wastewater processing, or alternately the organic matter and DIN from
 the wastewater treatment plants may spur N_2O production downstream of the
 wastewater outlet. The low-salinity, low- ^{222}Rn wastewater had a greater effect
 on NH_4^+ and N_2O concentrations than on NO_3^- concentrations (Figure 9i,j,k).
 545 For a given NO_3^- concentration in the dry season N_2O concentrations were
 much lower in the Fitzroy than in the Johnstone where NO_3^- and N_2O were
 sourced from groundwater (Figure 3b,f). This suggests that although most of
 the N_2O and some of the NO_3^- were sourced from the wastewater treatment

Figure 8: NO_3^- and NH_4^+ vs N_2O concentrations for the Johnstone River Estuary, Constant Creek, and the Fitzroy River Estuary.

Figure 9: ^{222}Rn vs. NO_3^- , NH_4^+ , and N_2O (nmol L^{-1}) concentrations, and salinity vs. ^{222}Rn concentration, for the Johnstone River Estuary, Constant Creek, and the Fitzroy River Estuary

plant the N_2O was not directly linked to the NO_3^- . Nitrification of wastewater-
 550 derived NH_4^+ , occurring at or just downstream of the wastewater outlet could
 also have elevated N_2O and NO_3^- .

Wastewater effluent is associated with increased NH_4^+ concentrations and
 N_2O production in other tropical and subtropical estuaries. For example, in
 the Adyar River in southeast India high NH_4^+ concentrations resulted from
 555 wastewater and use of diammonium phosphate fertiliser led to elevated N_2O
 driven by water-column nitrification (Rajkumar et al., 2008). Many of the estu-
 aries on the east and west coast of India, where N_2O production is attributed to
 water-column nitrification, receive wastewater effluent as well and show a strong
 correlation between NH_4^+ and N_2O concentration (Rao and Sarma, 2013). Sim-
 560 ilarly, strong DIN– N_2O correlations were observed in the wastewater-affected
 Pearl River in China (Lin et al., 2016).

Upstream freshwater N_2O inputs could not account for the entire water-air
 N_2O flux in either season, which indicates that there is a secondary source of
 N_2O downstream of Rockhampton, either from tributary/groundwater inputs
 565 or in-situ production. In the wet season, some of this extra N_2O entered the es-
 tuary about 30 km downstream from Rockhampton, where there was an obvious
 increase in N_2O concentration over a region of constant salinity. Considering
 that this occurs near the mouth of a creek where we observed high N_2O concen-
 trations, it seems likely that some of this extra N_2O was allochthonous. However
 570 the N_2O increase was also associated with an increase in AOU, indicating a pos-
 sible role of nitrification. AOU declines again downstream of the mid-estuary
 N_2O peak, indicating that nitrification was likely responsible for very little N_2O
 production in the low- N_2O lower reaches of the estuary.

In the dry season, the total contribution of freshwater-derived N_2O is not
 575 well known because freshwater N_2O concentrations were not directly measured
 (minimum salinity in the estuary is 22). Additionally, estimating ‘freshwater’
 N_2O concentration is difficult where wastewater inputs contribute to N_2O pro-
 duction, as wastewater could have an extremely high N_2O concentration. While
 excess N_2O ($\Delta\text{N}_2\text{O}$) was assumed to be similar to the highest observed values

580 at the upstream end of the estuary (8.0 nmol L^{-1}), if we assume the average freshwater $\Delta\text{N}_2\text{O}$ was close to wet season values ($\sim 2.8 \text{ nmol L}^{-1}$), the riverine contribution to N_2O production would be 0.4%. At the high value of 20 nmol L^{-1} the contribution would be 4%, and to reach 100%, the average freshwater $\Delta\text{N}_2\text{O}$ would have to be $\sim 500 \text{ nmol L}^{-1}$.

585 Unlike the wet season, the mid-estuary tributaries don't seem to have a huge effect on N_2O concentration. Above the salinity of ~ 27 transition at $\sim 11 \text{ km}$ from Rockhampton, some N_2O production could result from nitrification; AOU concentrations were slightly higher at salinities below 25 (up to 1.3 mg L^{-1}) where they exhibited a positive relationship with $\Delta\text{N}_2\text{O}$ (Appendix Figure 590 A2f). However, given that low- O_2 waters were likely quickly mixed with more oxygenated waters further downstream, it is possible that the AOU- $\Delta\text{N}_2\text{O}$ pattern is merely the result of mixing and N_2O evasion, which could cause both AOU and $\Delta\text{N}_2\text{O}$ to decline.

Considering the estimated freshwater N_2O inputs and water-air fluxes, it 595 seems that the Fitzroy was the only estuary where residence times were long enough for significant in-situ N_2O production to occur in both seasons. This raises the possibility that denitrification at the sediment-water interface could also contribute N_2O . Previous research has identified a relationship between residence time and denitrification in temperate estuaries (Eyre et al., 2016; Nixon 600 et al., 1996), which would predict that over a residence time of 72 days (as in the dry season) about 25% of NO_3^- inputs should be consumed by denitrifiers. For residence times of less than 1 month, as in the wet season and in the Johnstone River, the predicted consumption of NO_3^- by denitrifiers would be much lower than 25%. In theory, at higher temperatures these values could increase 605 (Barnes and Owens, 1999), however we should note that NO_3^- concentrations do not seem to show non-conservative behaviour indicative of significant NO_3^- consumption along the length of the estuary (Appendix Figure A1a).

In contrast to the Johnstone River, the $\delta^{15}\text{N}-\text{NO}_3^-$ data in the Fitzroy seem to indicate more intense denitrification of NO_3^- during the dry season, 610 rather than the wet season, which could reflect processing during wastewater

Figure 10: NO_3^- concentration vs $\delta^{15}\text{N}$ values of nitrate ($\delta^{15}\text{N}-\text{NO}_3^-$) during the wet and dry seasons in the Johnstone River Estuary, the Fitzroy River Estuary, and Constant Creek.

treatment and subsequent NO_3^- consumption by denitrifiers in the sediment downstream of the wastewater inlet (Figure 10c,d). Without the wastewater source and long residence time, we might expect that the Fitzroy dry season $\delta^{15}\text{N}-\text{NO}_3^-$ values would be lower than the wet season values, perhaps similar
615 to the dry season values observed at Constant Creek. However the Fitzroy is similar to the other two estuaries in that the seasonal increase in NO_3^- input is associated with increased $\delta^{15}\text{N}-\text{NO}_3^-$ values and higher N_2O concentrations.

5.4. Nitrous oxide load and residence time

Residence time is an important control on the amount of nitrogen and phosphorus processing that occurs in estuaries, with greater processing as residence
620 time increases (Balls, 1994; Nixon et al., 1996; McKee et al., 2000; Eyre et al., 2016). Here, we show that residence time is also an important control on the freshwater N_2O load that is lost as an estuarine water-air N_2O flux (i.e. res-

idence time vs. the % riverine ventilation; Figure 5). This is similar to the
625 findings that residence time affects the amount of freshwater DIC that is lost as
a water-air CO_2 flux, (i.e. residence time vs. the % riverine ventilation) (Borges
and Abril, 2011).

In the Johnstone River, N_2O was delivered along with freshwater (surface
water and/or groundwater), causing a clear negative relationship between N_2O
630 concentration and salinity (Figure 6a) and a wet-season dominance of N_2O
(Figure 4a,b). This is typical of many temperate (Barnes and Owens, 1999;
Robinson et al., 1998; Zhang et al., 2010) and some tropical estuaries (Rao and
Sarma, 2013); in these cases, the dominant control on N_2O concentration is
delivery of freshwater rather than in-situ N_2O production. In the Johnstone
635 River, the dominance of freshwater-derived N_2O can be seen in Figure 5, where
the amount of freshwater-derived N_2O exceeds the total water-air N_2O flux over
the surface area of the transect, i.e. the proportion of water-air fluxes that can
be explained by riverine N_2O inputs is greater than 100%.

When residence time increases, however, freshwater inputs play a lesser role
640 compared with in-situ processes. For example, when N_2O concentrations are
higher in the dry season (Chen et al., 2015; Rajkumar et al., 2008; Senthilkumar,
2008), often N_2O production is attributed to stagnation of DIN-rich water in
river or estuary water bodies, leading to water- column nitrification. In the wet
season, the in-situ nitrification source of N_2O is suppressed because of the lower
645 residence time and higher flushing rate (Chen et al., 2015). A similar pattern
is evident in the Fitzroy River, and can be seen in the relationship between
residence time and % riverine N_2O ventilation (Figure 5). In the wet season
the larger freshwater input dilutes the wastewater effluent and decreases NO_3^- ,
 NH_4^+ and N_2O concentrations in the estuary. This leads to a lower residence
650 time and a higher proportion of freshwater-derived N_2O , even though overall
 N_2O concentrations are lower (Figure 4c,d). In the dry season, freshwater input
is low and the residence time is high (~ 72 days), strengthening the relative
contribution of in-situ production to the water-air N_2O flux.

Figure 11: (a) Average annual NO_3^- concentration ($\mu\text{mol L}^{-1}$) vs N_2O water-air flux ($\mu\text{mol m}^{-2} \text{day}^{-1}$); modified from (Murray et al., 2015). (b) Data from the Johnstone River Estuary, Constant Creek, and the Fitzroy River Estuary, plotted against data presented in (Murray et al., 2015) along with results from several mangrove creeks (Barnes et al., 2006; Maher et al., 2016; Murray et al., 2018) and southeast Queensland estuaries (Wells et al., 2018).

Table 3: NO_3^- and NH_4^+ concentrations in tropical marine systems.

Citation	Environment	Country	NH_4^+ ($\mu\text{mol L}^{-1}$)	NO_3^- ($\mu\text{mol L}^{-1}$)
Agawin et al. (1996)	seagrass	Phillippines	1.6 to 1.9	0.4 to 0.8
Carruthers et al. (2005)	seagrass	Panama	0.20 to 0.26	0.20 to 0.27
Erfteimeijer and Herman (1994)	seagrass	Indonesia	2.2 to 3.2	0.9 to 1.4
Heminga et al. (1994)	seagrass	Kenya	0.4 to 0.8	0.3 to 0.45
Uku and Björk (2005)	seagrass		1.6 to 1.9	0.4 to 0.8
Boto and Wellington (1988)	mangrove	Australia	0.1 to 1.6	< 0.1 to 5.8
Davis et al. (2001a)	mangrove	Florida	0.1 to 5.2	0.1 to 5.8
Davis et al. (2001b)	mangrove	Florida	0.1 to 6.3	0.2 to 5.8
Ohowa et al. (1997)	mangrove	Kenya	0.3 to 3	0.2 to 8
Rivera-Monroy et al. (1995)	mangrove	Mexico	1.1 to 51.7	0.2 to 4.9
Rivera et al. (2007)	mangrove	Florida	< 0.1 to 1.4	1.8 to 11.8
Eyre and Balls (1999)	estuary main channel	Australia	< 0.1 to 4	< 0.1 to 7
Eyre (1994)	estuary main channel	Australia	—	2.3 to 31.2

5.5. Role of water column DIN concentrations and N_2O emissions

655 The low Fitzroy River Estuary and Johnstone River Estuary N_2O concentrations were typical of some tropical or subtropical estuaries or creeks at lower salinity which have a low freshwater source of DIN (Barnes et al., 2006; Maher et al., 2016; Senthilkumar, 2008; Wells et al., 2018) (Table 1). For example, most estuaries surveyed in India exhibited N_2O saturations between 100% and 660 300% (Rao and Sarma, 2013), with the exception of a few which had large anthropogenic N inputs. Similar values were observed in the more saline, downstream reaches of more eutrophic estuaries (Lin et al., 2016; Mueller et al., 2016; Rao and Sarma, 2013), where a strong upstream N_2O source is diluted by mixing with seawater. More anthropogenically disturbed tropical estuaries, with a 665 large population living in the catchment and significant wastewater or agricultural DIN inputs, can reach much higher maximum saturations, typically at the upstream, freshwater end of the estuary (Chen et al., 2015; Lin et al., 2016; Liu et al., 2015; Rao and Sarma, 2013).

Like N₂O saturations, calculated N₂O water-air fluxes across the three estu-
 aries were at the low end of the range typical for estuarine waters. This reflects
 670 relatively low DIN concentrations across the three estuaries (below 30 $\mu\text{mol L}^{-1}$
 for both NO₃⁻ and NH₄⁺). Over a large range of NO₃⁻ concentrations (i.e. 0
 to 500 $\mu\text{mol L}^{-1}$ of NO₃⁻) water-column NO₃⁻ concentrations appear to be a
 driver of N₂O water-air fluxes (Figure 11a) (Murray et al., 2015). Including
 675 more recent work in mangrove creeks (Daniel et al., 2013; Erler et al., 2015;
 Maher et al., 2016; Murray et al., 2018) and other low concentration estuaries
 (Wells et al., 2018), it appears that negative water-air fluxes occur where water
 column NO₃⁻ concentration falls below $\sim 5 \mu\text{mol L}^{-1}$ (Figure 11b), despite the
 weaker relationship over the smaller NO₃⁻ range. NO₃⁻ concentrations below
 680 $5 \mu\text{mol L}^{-1}$ are commonly found in many undisturbed and slightly disturbed
 tropical estuaries and mangrove creeks (Table 3), suggesting N₂O uptake may
 be widespread.

The ratio of N₂O emissions to total DIN load was quite low (0.02 – 0.09%),
 except for the Fitzroy dry season (1.12%). For comparison, N₂O emissions
 685 accounted for 0.26% of DIN load in the Changjiang River in China (Zhang
 et al., 2010), about 0.29% of TN in the Colne River (Robinson et al., 1998),
 and 0.3% of DIN is assumed to be converted to N₂O in estuaries in some global
 models of coastal N dynamics (Kroeze and Seitzinger, 1998). However, the ratio
 between DIN load and N₂O emissions, referred to as the emissions factor, can
 690 vary significantly and lower values are common. In a review of emissions factors
 in rivers, Hu et al. (2016) found a range of values from 0.003% to 7.13%, with a
 median of 0.15%. They found that the emissions factor increases as freshwater
 discharge declines, which might explain why the value was so high in the Fitzroy
 River during the dry season (1.12%), when discharge was low (Figure 2c) and
 695 residence time was high (Figure 5).

6. Conclusions

In the Johnstone River, N_2O came from a freshwater source upstream of the sampled estuary, with the highest contribution of N_2O coming from the South Johnstone River. The N_2O concentration of freshwater was similar in both the wet and the dry season, but the amount of freshwater delivered to the estuary in the wet season was much greater. In both seasons, the freshwater residence time was low enough that DIN and dissolved N_2O moved through the estuary with little evidence of in-situ production or consumption. Apart from the N_2O emitted through water-air exchange, dissolved N_2O was largely exported to the ocean.

The Fitzroy River was affected by wastewater input from the sewage treatment plants near Rockhampton. This was most obvious in the dry season, when freshwater input was low, and led to a stronger production and water-air flux of N_2O . The residence time was long, compared with the Johnstone River; Excess N_2O from the riverine source was completely lost to the atmosphere and additional N_2O from in-situ processes and downstream tributaries was added along the length of the transect.

Constant Creek was the site of N_2O consumption in the wet season, due to the unusually low concentrations of DIN in the water-column. In the dry season there was a slight N_2O production in some offshore shoals, but the N_2O concentration and fluxes in the creek itself remained very low. Comparing these results with previous studies, it seems that a NO_3^- concentration of less than $5 \mu\text{mol L}^{-1}$ is conducive to a negative water-air N_2O flux.

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1065 Appendix

Table A1: The parameterization equations used to calculate gas transfer k-values (in cm s^{-1}) from U_{10} (wind speed adjusted to 10 m altitude, in m s^{-1})

Citation	Equation
Clark et al. (1995)	$k = (2 + 0.24 * U_{10}^2) * (Sc/600)^{-0.5}$
Wanninkhof (2014)	$k = 0.251 * U_{10}^2 * (Sc/600)^{-0.5}$
Liss and Merlivat (1986)	$\begin{cases} 0.17 * U_{10} * (Sc/600)^{-2/3} & \text{If } U_{10} \leq 3.6 \\ (2.85 * U_{10} - 9.65) * (Sc/600)^{-0.5} & \text{If } 3.6 > U_{10} > 13 \\ (0.59 * U_{10} - 49.3) * (Sc/600)^{-0.5} & \text{If } U_{10} > 13 \end{cases}$
Ro and Hunt (2006)	$k = 5.9 * U_{10}^{1.81} * (Sc/600)^{-0.5}$
Nightingale et al. (2000)	$k = (0.222 * U_{10}^2 + 0.333) * U_{10} * (Sc/600)^{-0.5}$
Raymond and Cole (2001)	$k = 1.91 * e^{0.35 * U_{10}} * (Sc/600)^{-0.5}$

Table A2: Calculated k-values using 6 different parameterisations. The mean of all k-values is in parentheses.

Location/season	k value (cm s^{-1})					
	C 95	W 14	L&M 86	R&H 06	N 2000	R&C 01
Johnstone River wet season	2.2–12.6 (5.8)	0–11.3 (4)	0–1.2 (0.7)	0–7.3 (2.8)	0–11.9 (4.6)	2.1–18.8 (7.7)
Johnstone River dry season	2.2–12.2 (5.4)	0–10.8 (3.4)	0–1.2 (0.6)	0–7.1 (2.4)	0–11.4 (4)	2.1–17.8 (7.1)
Constant Creek wet season	4.7–12.6 (8.5)	2.8–11.4 (6.9)	– 0.8–9 (4.6)	2.1–7.4 (4.6)	3.5–11.9 (7.6)	6.2–18.8 (11.6)
Constant Creek dry season	8–18.5 (13.6)	6.5–17.9 (12.5)	4.3–14 (9.9)	4.4–11.1 (8)	7.2–18 (12.9)	10.9–33.2 (21.3)
Fitzroy River wet season	4.8–34.6 (16.9)	2.8–35.3 (16)	– 0.9–24.7 (12.2)	2.1–20.7 (10)	3.6–34 (16.2)	6.3–91.4 (29.8)
Fitzroy River dry season	2.5–18.6 (7.7)	0.5–18.3 (6.3)	– 5.6–14 (3.9)	0.4–11.2 (4.2)	0.9–18.2 (6.9)	3.1–36 (11)

Figure A1: Salinity vs NO_3^- and NH_4^+ during the wet and dry season in the Johnstone River Estuary, Constant Creek, and the Fitzroy River Estuary.

Figure A2: AOU vs ΔN_2O during the wet and dry season in the Johnstone River Estuary, Constant Creek, and the Fitzroy River Estuary.

Table A3: Results of calculations for water-air N_2O flux, using 6 different parameterisations. The mean flux is in parentheses.

Location/season	k value (cm s^{-1})					
	C 95	W 14	L&M 86	R&H 06	N 2000	R&C 01
Johnstone River wet season	1.4–31.9 (8.2)	0–28.8 (5.8)	0–3.12 (0.96)	0–18.5 (4.1)	0–30 (6.7)	1.4–47.5 (11)
Johnstone River dry season	– 0.2–20.9 (3.6)	0–17.3 (2.6)	0–2.4 (0.5)	0–11.5 (1.9)	– 0.24–19.0 (3.1)	– 0.24–28.8 (5.0)
Constant Creek wet season	– 1.4–1.0 (– 0.2)	– 1.2–0.7 (0.1)	– 1.0–0.7 (– 0.1)	– 0.7–0.5 (0.1)	– 1.2–0.7 (– 0.2)	– 2.2–1.2 (– 0.2)
Constant Creek dry season	– 0.5–8.4 (1.4)	– 0.5–8.2 (1.2)	– 0.5–6.5 (1.0)	– 0.2–5.0 (0.7)	– 0.48– 8.16 (1.2)	– 0.7–15.1 (2.2)
Fitzroy River wet season	0–14.9 (3.8)	0–14.9 (3.6)	0–11.0 (2.9)	0–8.9 (2.2)	0–14.4 (3.6)	0–33.4 (6.7)
Fitzroy River dry season	0.5–19.5 (3.1)	0.2–17.3 (2.4)	– 4.8–13.7 (1.2)	0.2–11.3 (1.7)	0.2–18.2 (2.6)	0.5–28.6 (4.3)