



Harmful algal blooms as a sink for inorganic nutrients in a eutrophic estuary

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ABSTRACT: Phytoplankton-mediated nutrient fluxes typically provide only pulsed relief to adjacent coastal waters during the productive period, with nutrient export increasing in the absence of substantial phytoplankton biomass. On the warm temperate coastline of South Africa, the Sundays Estuary is characterised by highly regulated freshwater inflow patterns, nutrient-enriched conditions, and resident harmful algal blooms (HABs). Given these attributes, the study objective was to investigate the effect of these phytoplankton blooms on fluvial inorganic nutrient dynamics. To assess uptake, we analysed inorganic nutrient (phosphate, ammonium, NO_x) and phytoplankton concentrations in relation to salinity using data from 17 surveys. Property–salinity mixing diagrams and statistical analyses indicated a positive association between increasing phytoplankton biomass and decreasing NO_x flux ($p < 0.001$), and to a lesser degree phosphate flux ($p = 0.22$), along the gradient from low-salinity inner estuary to high salinity outer estuary. High biomass HAB accumulations of *Heterosigma akashiwo* ($>100 \mu\text{g chl } a \text{ l}^{-1}$) represent significant removal of available NO_x ($\sim 100\%$) and phosphate ($>75\%$) during warmer conditions ($>20^\circ\text{C}$). These events, together with continuous inorganic nutrient uptake during less severe bloom conditions, remove a substantial portion of annual NO_x and phosphate loads (36.5 and 36.4% flux, respectively). Although this buffers inorganic nutrient loading to adjacent coastal waters, it also represents an emerging legacy pollution issue in the form of a benthic accumulation of organic material in bottom waters subject to recurrent hypoxia. Future management efforts should adopt an ecosystem-based approach centred around simultaneous restoration of hydrological variability and dual nutrient reduction strategies (N and P).

KEY WORDS: Coastal filter · Eutrophication · *Heterosigma akashiwo* · Nutrient transformation · Phytoplankton

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1. INTRODUCTION

The transitional zone between terrestrial ecosystems and the open ocean has been referred to as the land–ocean aquatic continuum and is made up of an intricate series of systems that act as successive filters of nutrients, sediments and organic carbon (Billen et al. 1991). Positioned at the interface between the freshwater and marine environments, estuaries are considered a key component of this continuum. This

is primarily due to the natural dynamism of estuaries that can be largely attributed to its defining feature, the salinity gradient (Cloern et al. 2017). The mixing of land runoff and seawater inputs is responsible for establishing the hydrodynamic characteristics (e.g. density gradients, circulation patterns) that facilitate the unique species assemblages typically associated with these ecosystems (Potter et al. 2010). The ensuing dynamic interactions between abiotic components (geomorphology, hydrology, physico-chemistry)

and biotic composition enables estuaries to act as biogeochemical hotspots (e.g. uptake, burial and denitrification) that can significantly alter the flux, form and ratio of nutrients before entering coastal waters (Taljaard et al. 2009, Bouwman et al. 2013, Jickells et al. 2014, Xenopoulos et al. 2017). Thus, continued exploration of the mechanisms responsible for describing the rates and degree to which these transformations occur is imperative to a broader understanding of aquatic ecosystem functionality.

Detailed process-based assessments of rate measurements are required to fully describe the biogeochemical makeup of an estuary (Eyre 2000, Wild-Allen & Andrewartha 2016). Yet, these types of studies are often beyond the scope of routine monitoring programs due to the considerable logistical, financial and technical expertise requirements associated with such undertakings. The employment of mixing diagrams (i.e. property vs. salinity plots) provides an alternative approach to determining the biogeochemical processes responsible for nutrient transformations along the estuarine gradient, as demonstrated in previous studies (e.g. Boyle et al. 1974, Eyre 2000, Cloern et al. 2017). Also, property–salinity plots are beneficial in that they (1) facilitate inter-estuary comparisons due to the presence of a common axis (i.e. salinity gradient), and (2) are largely unaffected by tidal influence. Accordingly, these plots can be used to determine conservative (linear mixing line) and non-conservative (concave or convex mixing lines) behaviour of abiotic and biotic variables within an estuary. Conservative mixing indicates that physical processes (e.g. tidal exchange) are responsible for observed patterns, while curved mixing lines infer transformation of elements by biogeochemical processes (e.g. phytoplankton). This approach is particularly useful in estuaries where biological processes are driven by pelagic primary producer communities (phytoplankton) that act at a temporal scale in line with water residency periods, i.e. short turnover rates and rapid uptake.

Situated along the warm temperate south coast of South Africa, the agriculturally influenced Sundays Estuary catchment has been identified as a key source of dissolved inorganic nitrogen (DIN) loading (17%) to the adjacent coastal waters of Algoa Bay, introducing an estimated 1.5×10^5 kg DIN yr⁻¹ to the headwaters of the Sundays Estuary (Lemley et al. 2019a). These inputs have been explicitly linked to the occurrence of recurrent and high magnitude harmful algal blooms (HABs, e.g. *Heterosigma akashiwo*, *Heterocapsa rotundata*) in the Sundays Estuary (Lemley et al. 2017, 2018a,b), as well as being

implicated as a nutrient reservoir that potentially supports large-scale seasonal blooms of *Lingulodinium polyedra* in the adjacent nearshore environment (Lemley et al. 2019a). Specifically, the harmful nature of *H. akashiwo* blooms in the estuary has been evidenced by its role in (1) driving seasonal bottom water hypoxia (Lemley et al. 2017), (2) food-web restructuring (i.e. shaping the composition of co-occurring phytoplankton taxa; Lemley et al. 2018a, 2020), and (3) hindering the nursery function of the estuary (i.e. inducing mismatch of peak larval fish and prey densities; Smit et al. 2021). Repeated *in situ* measurements of physico-chemistry, inorganic nutrient concentrations and phytoplankton dynamics enable the detection of long-term patterns and rates of change, which, in turn, can provide insight regarding the underlying causes of observations. For example, studies in Northern (Glibert et al. 2008, Cloern 2019) and Southern (Larsson et al. 2017, Lemley et al. 2019b) Hemisphere estuaries have highlighted the value of such datasets in detecting ecosystem responses to environmental perturbations and management interventions.

In recent times, the Sundays Estuary has served as the focus of numerous research programs, and therefore abiotic and phytoplankton (including HABs) data are readily available. The temperate Sundays Estuary is unlike many other South African systems in that it exhibits consistent, albeit anthropogenically driven, freshwater inflow patterns (Taljaard et al. 2009, Lemley et al. 2017), thus, by and large, satisfying the steady-state assumptions required for effective application of mixing diagrams. Here, we have collated available spatio-temporal data from various monitoring surveys that encompass the length of the estuary (i.e. entire salinity gradient) with the objective of evaluating the efficacy of the phytoplankton community in the Sundays Estuary as an inorganic nutrient sink at the land–sea interface. Moreover, the study aimed to investigate the effect of recurrent estuarine phytoplankton blooms (e.g. HABs) on fluvial (allochthonous) inorganic nutrient dynamics. Given the increase in the frequency, magnitude, and variety of HAB incidences across the aquatic continuum globally (e.g. Glibert 2020), the Sundays Estuary case study provides a suitable platform from which to assess the consequences—negative or positive—that these phenomena can have both locally and in neighbouring coastal environments. Such information will aid the understanding of inorganic nutrient loading dynamics (e.g. timing, quantity), which is central to supporting effective management strategies.

2. MATERIALS AND METHODS

2.1. Study area

The predominantly open Sundays Estuary is located along the warm temperate south coast of South Africa, approximately 40 km northeast of Port Elizabeth. Exchange with the ocean is via a narrow, meandering channel (33° 43' 17.35" S, 25° 51' 11.34" E) that facilitates semidiurnal microtidal (≤ 1.5 m) exchanges with the Indian Ocean (Fig. 1). The shallow (< 2 m) and freshwater-dominated Sundays Estuary is approximately 24 km in length and exhibits a narrow, channel-like morphology with steep banks. Accordingly, the emergent macrophyte species *Phragmites australis* (common reed) is prevalent (~ 31.5 ha) throughout the oligo- to polyhaline reaches of the estuary, with salt marsh and submerged macrophytes largely absent due to the small intertidal zone (ca. 5–6 m in width) and turbid nature of the water column, respectively (Kotsedi et al. 2012, van Niekerk et al. 2019). The capacity of dam impoundments in the Sundays River catchment ($\sim 20\,730$ km²) is equivalent to present day mean annual runoff estimates (260×10^6 m³). Yet, freshwater inflows are augmented by an Orange-Fish-Sundays River inter-basin water transfer scheme that was designed to service the annual irrigation needs of an extensive citrus cultivation

industry (156.6×10^6 m³) in the lower catchment (Lemley et al. 2017) and to supplement (50–70 %) the urban demands of the Nelson Mandela Bay Municipality ($\sim 3 \times 10^5$ m³ d⁻¹) via the Nooitgedacht Water Treatment Works. The water is diverted from the Gariep Dam (30° 43' 48.86" S, 25° 46' 41.21" E) in the interior to the upper reaches of the Great Fish River catchment (31° 25' 25.11" S, 25° 38' 13.63" E), after which it is fed via a series of tunnels and canals to the Darlington Dam (33° 12' 19.06" S, 25° 8' 54.15" E) situated on the Sundays River. The constant inflow of nutrient-rich freshwater to the headwaters of the Sundays Estuary is the result of upstream activities (e.g. fertilizer application, irrigation return flows). Approximately 1.5×10^5 kg DIN (predominantly NO_x, i.e. nitrate + nitrite) and 3.6×10^3 kg dissolved inorganic phosphorus (DIP; phosphate) are introduced annually, with peak loading occurring in the autumn and summer months, respectively (Lemley et al. 2019a).

2.2. Estuarine sampling program

Seventeen surveys (Table 1) were conducted in the Sundays Estuary during the period from 2014–2019, with the exception of 2017 (i.e. no sampling occurred). Each of these selected surveys satisfies several criteria that meet the study objectives. Firstly, each

Table 1. Abiotic (salinity, temperature, river inflow rate, freshwater end member dissolved inorganic nitrogen [DIN]:dissolved inorganic phosphorus [DIP] ratio, and inorganic nutrient loads) and phytoplankton (chl *a*, dominant species and maximum cell density) data for each of the sampling surveys (where the dash indicates an absence of data regarding the dominant taxa). Seasonality is indicated by different colours, i.e. summer (red), spring (orange), autumn (yellow), and winter (blue). *H. akashiwo*: *Heterosigma akashiwo*; *H. rotundata*: *Heterocapsa rotundata*; *C. atomus*: *Cylotella atomus*

Category	Sampling date (d mo yr)	Salinity (min./max.)	Temp. (°C) (min./max.)	Chl <i>a</i> (µg l ⁻¹) (min./max.)	Flow rate (m ³ s ⁻¹)	DIN:DIP (Stn 6)	NO _x -N load (kg d ⁻¹)	PO ₄ -P load (kg d ⁻¹)	Dominant species	Max. density (cells ml ⁻¹)
Linear	24 Feb 15	1.9; 35.1	20.9; 23.4	2.1; 59.6	1.57	5.2	27.9	8.3	<i>H. akashiwo</i>	4731
	4 May 15	0.9; 34.5	17.2; 18.1	0.0; 10.7	4.61	13.6	389.3	27.4	–	–
	15 Aug 15	1.3; 34.2	13.3; 15.2	0.3; 42.3	2.33	19.7	271.0	13.8	<i>H. rotundata</i>	2179
	13 Jul 16	2.1; 33.3	13.5; 15.8	1.8; 16.0	1.13	21.5	163.0	7.7	<i>H. rotundata</i>	258
	15 Nov 19	3.7; 35.9	19.5; 23.4	4.7; 40.9	0.37	7.4	10.7	1.8	<i>C. atomus</i>	15 898
Press	29 Jan 14	1.1; 34.8	24.4; 27.0	12.4; 126.1	4.86	4.0	237.7	61.3	<i>H. akashiwo</i>	4182
	9 Aug 14	1.8; 31.6	16.4; 17.6	0.0; 49.4	1.72	22.8	270.8	10.6	–	–
	30 Jan 15	2.3; 33.3	20.8; 26.0	5.9; 29.0	0.75	4.5	38.7	7.7	<i>H. akashiwo</i>	186
	2 Jun 15	1.6; 34.1	15.8; 17.4	0.0; 65.7	1.43	13.2	207.4	16.8	<i>H. rotundata</i>	3083
	6 Jul 15	1.5; 34.7	13.7; 16.8	0.0; 548.8	1.55	18.5	229.3	10.3	<i>H. rotundata</i>	194 991
	17 Sep 15	1.3; 34.1	17.2; 18.5	0.0; 321.2	2.53	13.8	198.0	17.3	<i>H. rotundata</i>	51 615
	13 Oct 15	1.3; 34.6	18.8; 21.1	0.0; 71.6	1.88	15.1	132.8	10.3	<i>H. rotundata</i>	3097
	11 Apr 18	2.9; 34.6	18.2; 22.4	2.1; 100.2	1.41	12.5	107.2	8.6	<i>H. akashiwo</i>	5699
Pulse	31 Mar 15	1.3; 32.5	19.9; 23.1	1.8; 127.3	3.79	10.6	297.3	27.0	<i>H. akashiwo</i>	6930
	16 Nov 15	1.5; 34.8	18.2; 21.6	2.7; 549.6	1.14	15.1	88.7	5.9	<i>H. akashiwo</i>	29 392
	10 Dec 15	2.2; 33.4	22.6; 26.3	3.3; 158.1	0.82	6.2	39.8	6.7	<i>C. atomus</i>	5 350
	25 Oct 18	2.3; 28.2	19.2; 23.5	3.0; 142.1	0.59	17.6	45.7	2.6	<i>H. akashiwo</i>	7433

survey needed to encompass the length of the estuary (i.e. mouth to upper reaches) and, thus, encapsulate all salinity classes, i.e. oligo- (0.5–5), meso- (5–18), poly- (18–30), and mixoeuhaline (30–40). Next, the positions of the 6 sampling stations were consistent among all surveys (Fig. 1), except for January 2014, when Stn 5 was excluded. Finally, depth interval data for each of the selected abiotic (salinity, temperature, inorganic nutrients) and phytoplankton (biomass, cell abundance, dominant species information) parameters needed to have been recorded on the survey. Together, selected surveys represent all seasons: spring ($n = 5$), summer ($n = 4$), autumn ($n = 3$), winter ($n = 5$).

All variables were assessed at specified water depths, i.e. surface (0 m), middle (0.5 and/or 1 m), and bottom waters. Discrete salinity and water temperature ($^{\circ}\text{C}$) were recorded *in situ* using either a HANNA HI98194, YSI 650 MDS or a YSI 6600-V2 multiparameter meter. Water samples for inorganic nutrient analyses were filtered onboard through hydrophilic polyvinylidene difluoride (PVDF) 0.47 μm pore-size syringe filters and placed into acid-washed polyethylene bottles before being frozen. Orthophosphate (PO_4^{3-}), ammonium (NH_4^+) and total oxidised nitrogen (NO_x , i.e. $\text{NO}_3^- + \text{NO}_2^-$) concentrations were determined using standard spectrophotometric methods (Bate & Heelas 1975, Parsons et al. 1984). Chlorophyll *a* concentration (i.e. proxy for phytoplankton biomass) was measured by filtering replicate samples of a known volume (i.e. ranging from 100 to 500 ml) through 1.2 μm pore-size glass fibre filters (Whatman® GF/C or Munktell® MGC). Subsequently, filter papers were placed into aluminium foil and frozen prior to analysis. Once in the laboratory, filters were thawed and placed into glass vials with 10 ml of 95 % ethanol (Merck® 4111) for 24 h in a cold ($\sim 2^{\circ}\text{C}$), dark room to allow for chlorophyll *a* extraction. Thereafter extracts were refiltered through glass fibre 1.2 μm pore-sized filters to clear any suspended particles, and chlorophyll *a* concentrations were determined according to Nusch (1980). Absorbances before and after acidification with 1 N HCl were read using a Thermo Scientific™ GENESYS™ 10S UV-Vis spectrophotometer. Water samples collected for phytoplankton community analysis were preserved with 1 ml of 25 % glutaraldehyde solution (Sigma-Aldrich® Chemicals G5882). Next, a known volume of fixed samples (i.e. ranging from 5 to 50 ml) was placed into 26.5 mm diameter Utermöhl chambers and allowed to settle for 24 h before enumeration (cells ml^{-1} ; as per Snow et al. 2000), and identification was conducted using an inverted Leica DMIL phase contrast microscope ($\sim 630\times$ magnification).

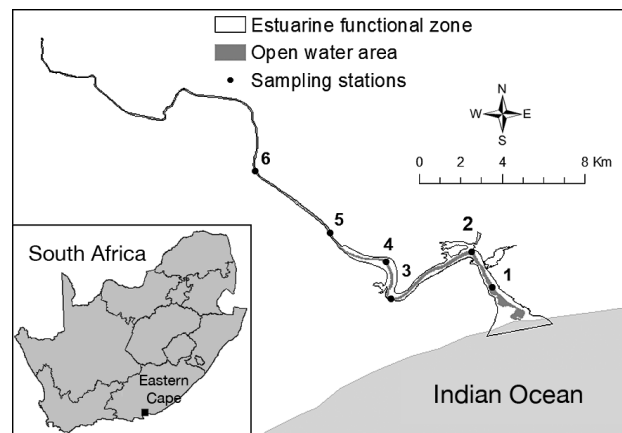


Fig. 1. The Sundays Estuary is located on the warm temperate south coast of South Africa. Sampling stations numbered 1 to 6 extend from the seawater dominated outer estuary to the freshwater dominated innermost estuary

2.3. Freshwater inflow and nutrient loading

Freshwater inflow data were obtained from the Department of Water and Sanitation (DWS), South Africa (www.dwa.gov.za/Hydrology/Verified/hymain.aspx). Water entering the Darlington Dam via the inter-basin scheme is transferred downstream to the Korhaans Weir ($33^{\circ}22'42.5''\text{S}$, $25^{\circ}21'17.3''\text{E}$), which is situated approximately midway between the dam and head of the estuary, where it then gets diverted into an irrigation canal that services the agricultural industry in the lower Sundays catchment. The freshwater inflow rate to the estuary was estimated (Table 1; $\text{m}^3 \text{s}^{-1}$) by summing the 7 d average (i.e. week prior to survey date) of river inflow (Stn N4H1) and 10% of the median monthly flow rates (i.e. steady-state inflow assumed) diverted into the irrigation canal (Stn N4H6). Both gauging stations are situated at the Korhaans Weir, with Stn N4H1 positioned just below the weir to capture overflow volumes and Stn N4H6 used as a proxy to estimate diffuse irrigation return flows. Inorganic nutrient loads were then calculated by multiplying this inflow rate by the nutrient concentration of the freshwater inflow (observed at Stn 6); nutrient concentrations ($\text{NO}_x\text{-N}$ and $\text{PO}_4\text{-P}$) were converted from μM to mg l^{-1} , and nutrient loading was expressed in kg d^{-1} (Table 1).

2.4. Property–salinity plots

When nutrient or phytoplankton concentration is plotted against salinity (a conservative water property), a linear relationship is obtained if that concen-

tration is also conservative (i.e. zero nutrient uptake, zero phytoplankton growth or decay). This conservative mixing line is entirely due to the mixing of freshwater with seawater and the associated dilution of higher seawater values. Alternately, upward curvature of the mixing line (i.e. convex) between freshwater and seawater end members indicates growth or production of a property in the estuary (not just mixing), while downward curvature (i.e. concave) indicates non-conservative decay or uptake processes. Here we use this approach to quantify fluvial inorganic nutrient uptake in the Sundays Estuary and identify 3 types of surveys based on NO_x -salinity plots: 'linear' relationships exhibited a straight conservative mixing line, 'press' relationships exhibited a concave mixing line (downward curvature), and 'pulse' relationships exhibited complete NO_x depletion ($\sim 0 \mu\text{M}$) within oligo- to mesohaline surface waters. This classification is based on the premise that nutrient enrichment of water that facilitates disturbances to the balance of an ecosystem (e.g. water quality, community composition) represents eutrophication (OSPAR 2003). In eutrophication literature (e.g. Tett et al. 2007), sustained 'press' and widespread episodic 'pulse' disturbances are considered undesirable due to the marked deterioration in the structure and resilience of the ecosystem (e.g. HABs).

NO_x and phosphate uptake rates can be estimated from these property-salinity plots for each of the 'press' ($n = 8$) and 'pulse' ($n = 4$) surveys. Conservative mixing lines (and equations) can be determined from nutrient (NO_x and phosphate) concentrations in the freshwater and seawater end members. Due to the low variability in seawater concentrations, we used median values, recorded for the period post-2013 at the adjacent coastal monitoring station (i.e. PELTER Reference Stn 3), of 35.0 for salinity and $5.00 \mu\text{M}$ (interquartile range: 2.82 to $9.59 \mu\text{M}$) and $0.77 \mu\text{M}$ (interquartile range: 0.50 to $1.34 \mu\text{M}$) for NO_x and phosphate concentrations, respectively (Lemley et al. 2019a). The freshwater concentrations were determined from our Stn 6 data taken on each survey. Once the conservative mixing line equations were obtained for each survey, the NO_x and phosphate uptake rate at each station along the estuary was calculated as a percentage of the NO_x and phosphate influx by determining the percentage difference between predicted and actual concentrations at a given salinity (assuming a uniform freshwater flux through the estuary, i.e. negligible evaporative loss due to small surface area). Although surrounding irrigated fields (MacKay & Schumann 1990) and the Alexandria dune field (Campbell & Bate 2005) are

known sources of groundwater, these inputs appear to be negligible given the monotonic longitudinal salinity gradients observed (see Fig. 4) and consistent with the observation that the dune field discharges directly to surf zone.

2.5. Statistical analyses

All plots and analyses were conducted in the R environment (R version 3.6.1, R Core Team 2019). For property-salinity plots, a locally weighted smoothing function ('loess', span = 0.66) or quantile regressions (i.e. 25th, 50th and 75th) were applied to aid visualization of trends for selected parameters along the salinity gradient. Using the 'vegan' package, principal components analysis (PCA) was applied to pooled spatial data collected for each sampling survey (salinity, water temperature, phosphate, NO_x , and phytoplankton biomass) to illustrate the factors likely responsible for shaping the 'linear', 'press' and 'pulse' states. The Shapiro-Wilk W -test was used to evaluate the normality of selected parameters (salinity, inorganic nutrients, phytoplankton biomass, water temperature, NO_x flux, and phosphate flux). The non-parametric (Shapiro-Wilk W -test: $p < 0.05$ in all cases) Kruskal-Wallis ANOVA with Dunn's multiple comparison post hoc test — using the Benjamini-Hochberg p -value adjustment method — was applied to assess differences between the 'linear', 'press' and 'pulse' classifications. Lastly, the associations between selected variables were evaluated using the non-parametric Spearman's rank correlation. All analyses were tested at an *a priori* significance level of $\alpha < 0.05$.

3. RESULTS

The entire salinity gradient from freshwater to seawater was captured during each of the 17 surveys (Table 1), with all salinity classes well represented by the 348 depth interval samples collected during the study period, i.e. oligohaline (36.5%), mesohaline (32.2%), polyhaline (19.5%), and mixoeuhaline (11.8%). Property-salinity plots of all data (Fig. 2) indicated that freshwater (fluvial) inputs are the primary source ($p < 0.001$) of NO_x ($r = -0.69$; Fig. 2A) and phosphate ($r = -0.55$; Fig. 2C) to the Sundays Estuary. The seasonal nature of NO_x inputs was highlighted ($r = -0.60$; $p < 0.001$) by elevated concentrations ($> 80 \mu\text{M}$; max. $130.1 \mu\text{M}$) coinciding with reduced water temperatures ($< 18^\circ\text{C}$), i.e. greatest influx in winter and autumn. Ammonium ($\text{NH}_4\text{-N}$) concentra-

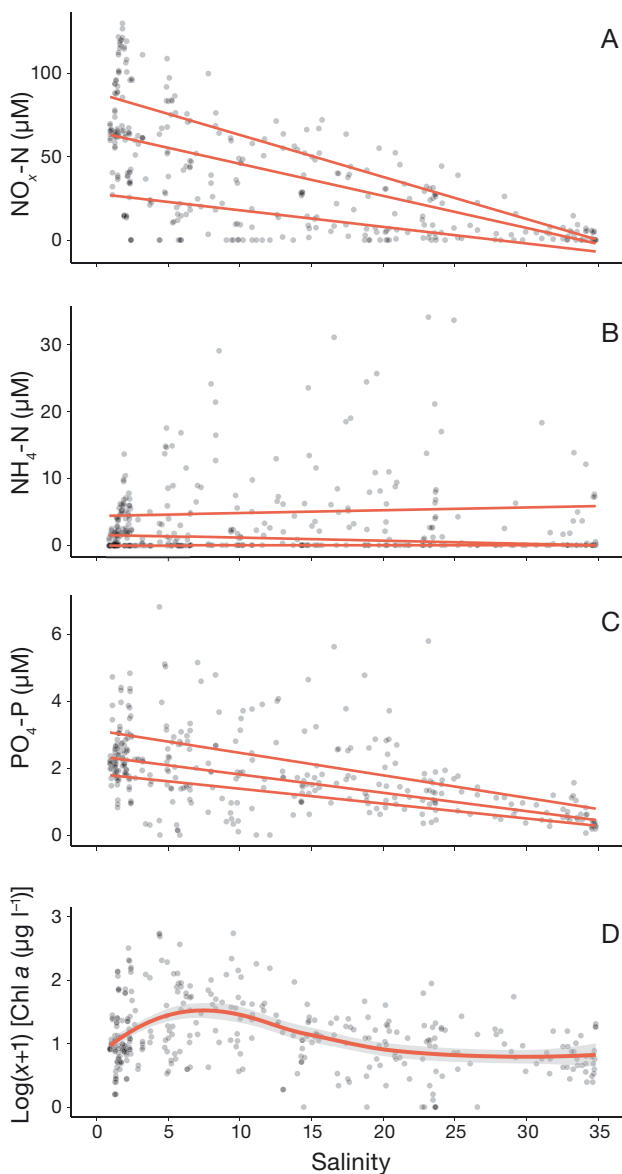


Fig. 2. Concentrations of (A) oxidised nitrogen, (B) ammonium, (C) phosphate and (D) phytoplankton biomass versus salinity for data from all 17 surveys in the Sundays Estuary. Points are plotted with a degree of transparency ($\alpha = 0.3$) to indicate observation frequency. Trendlines represent quantile regressions (25th, 50th and 75th; A–C) and locally weighted smoothing ('loess') fit with span 0.66 (shaded areas indicate the 95% confidence interval of the fitted trend; D)

tions (Fig. 2B) were typically low ($< 2 \mu\text{M}$) and showed no association with salinity ($p > 0.05$); however, ammonium peaks ($> 10 \mu\text{M}$) generally occurred in conjunction with elevated phosphate levels ($r = 0.19$; $p < 0.001$). This observation is indicative of bottom water remineralisation processes, a feature typical of the high retention middle reaches in the Sundays Estuary

(Lemley et al. 2017). The spatio-temporal distribution of phytoplankton biomass in the Sundays Estuary (Fig. 2D) was largely driven ($p < 0.001$) by reduced salinity conditions ($r = -0.18$) and elevated water temperatures ($r = 0.45$). Most notable is that phytoplankton biomass maxima ($> 80 \mu\text{g chl } a \text{ l}^{-1}$; $\log_{(x+1)} > 1.9$) were largely confined to mesohaline waters (5–18) and showed an inverse association with NO_x concentrations ($r = -0.16$; $p = 0.003$).

Based on the curvature of survey-specific NO_x–salinity mixing plots (see Section 2 for details), the selected survey dates were classified as exhibiting either 'linear' (conservative mixing line, $n = 5$), 'press' (concave mixing line, $n = 8$) or 'pulse' (nutrient depletion, $n = 4$) disturbance states (Fig. 3). Contrastingly, the PO₄–salinity plots corresponding to these classifications exhibited conservative (linear) mixing lines for both 'linear' and 'press' conditions, with non-conservative (concave) mixing lines for phosphate only observed during 'pulse' events (as defined by NO_x curves). Convex mixing lines were observed for NH₄–salinity plots during 'press' surveys indicating bottom water remineralisation processes, while 'linear' and 'pulse' conditions elicited no discernible patterns and comparably lower concentrations (typically $< 10 \mu\text{M}$). During the 'pulse' state (Fig. 3), NO_x and phosphate concentrations declined rapidly at a salinity of approximately 5; however, unlike NO_x, phosphate levels were never fully depleted (median = $1.0 \mu\text{M}$).

When comparing the environmental parameters associated with each of the classifications, it was notable that salinity conditions were similar ($p > 0.05$), i.e. 'linear' (12.8 ± 11.4 , $n = 102$), 'press' (12.9 ± 11.1 , $n = 166$), and 'pulse' (12.0 ± 10.8 , $n = 80$). More specifically, longitudinal and vertical salinity gradients were similar across classifications (Fig. 4), with oligo-, meso- and polyhaline surface water conditions prevailing in the upper (Stns 5 and 6), middle (Stns 3 and 4) and lower (Stns 1 and 2) reaches, respectively. The freshwater end member site (Stn 6) was the only locality at which vertical salinity stratification was absent (i.e. well-mixed water column) during each sampling survey. This was key to ensuring the sampling survey selection criteria had been met, i.e. innermost station was at the landward end of the salinity intrusion.

Water temperature did exhibit differences between classifications, with warmer waters observed during 'pulse' ($22.5 \pm 2.1^\circ\text{C}$) compared with 'press' ($19.5 \pm 3.7^\circ\text{C}$) and 'linear' ($18.2 \pm 3.4^\circ\text{C}$) phases (Kruskal-Wallis $H = 61.5$; $p < 0.001$; $df = 2$). Also, warmer waters were observed during 'press' conditions more often

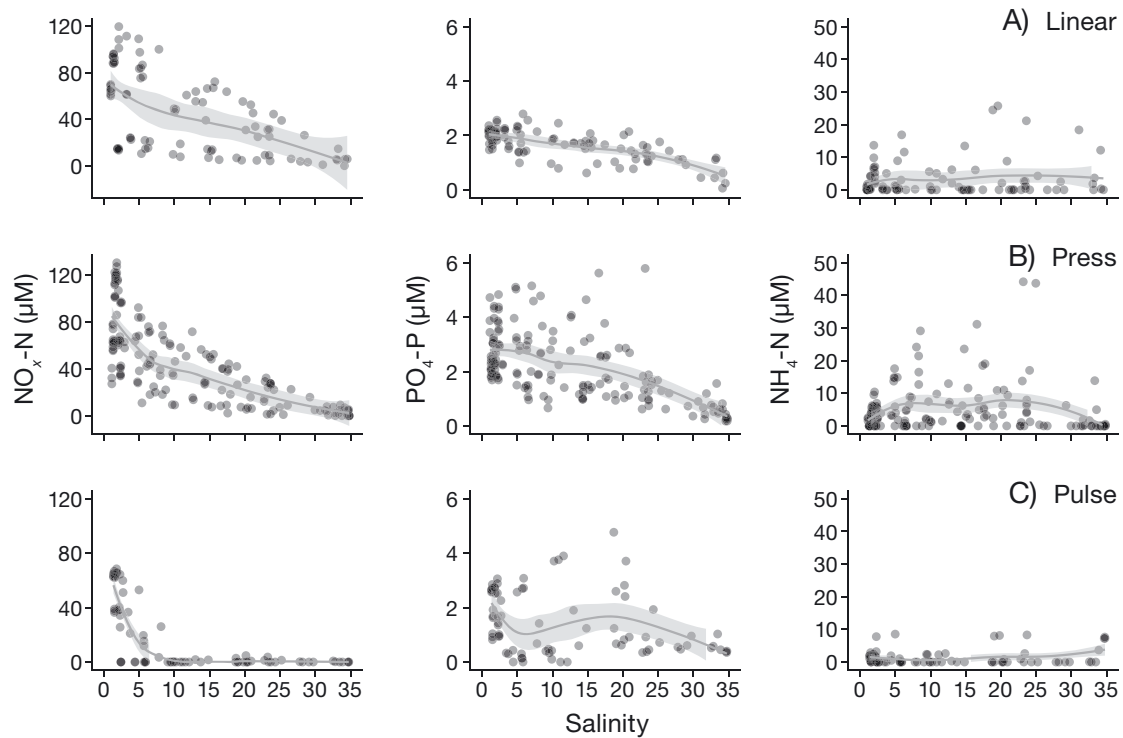


Fig. 3. Total oxidised nitrogen ($\text{NO}_x\text{-N}$), phosphate ($\text{PO}_4\text{-P}$) and ammonium ($\text{NH}_4\text{-N}$) distribution patterns along the salinity gradient representative of the (A) 'linear' ($n = 5$), (B) 'press' ($n = 8$) and (C) 'pulse' ($n = 4$) classification of surveys, based on $\text{NO}_x\text{-salinity}$ plots, in the Sundays Estuary. Points are plotted with a degree of transparency ($\alpha = 0.4$) to indicate observation frequency. Trendlines are locally weighted smoothing ('loess') fits with span 0.66, while the light grey shaded areas indicate the 95% confidence interval of the fitted trend

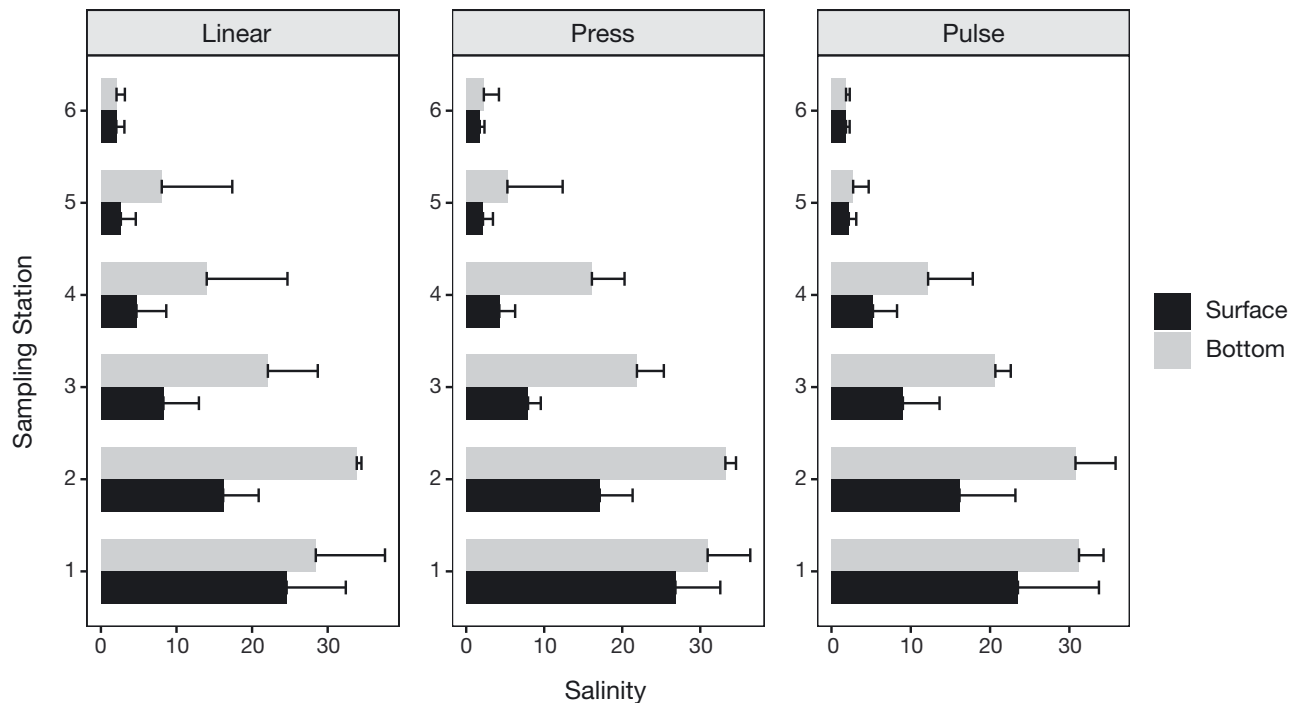


Fig. 4. Longitudinal and vertical differences in mean salinity (+SD) associated with each of the survey classifications

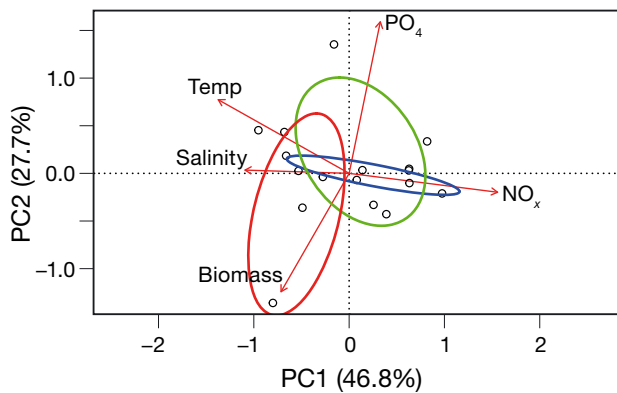


Fig. 5. Principal components analysis (PCA) ordination plot of sampling intervals based on *in situ* environmental and phytoplankton biomass data. The first 2 components (PC1 and PC2) together explain 74.5 % of overall variance. Sampling dates clustered using ellipses according to classifications: 'linear' (blue; high NO_x , low temperature and low biomass), 'press' (green; moderate biomass), and 'pulse' (red; high biomass and low nutrients)

than during the 'linear' conservative mixing periods ($p = 0.02$). Phytoplankton biomass ranged from 0 to $549.6 \mu\text{g chl } a \text{ l}^{-1}$ and mirrored the trends observed for water temperature across the classifications. Concentrations associated with 'pulse' ($69.9 \pm 110.0 \mu\text{g chl } a \text{ l}^{-1}$) and 'press' ($26.7 \pm 58.7 \mu\text{g chl } a \text{ l}^{-1}$) were greater ($H = 46.6$; $p < 0.001$) than those recorded during 'linear' ($9.1 \pm 9.4 \mu\text{g chl } a \text{ l}^{-1}$) surveys. The role that phytoplankton biomass plays in structuring the fluvial inorganic nutrient dynamics (N and P) in the Sundays Estuary is highlighted by its clear association with 'pulse' disturbance conditions (Fig. 5). Furthermore, conservative mixing periods ('linear') are generally associated with high NO_x concentrations and cooler water temperatures. These observations are evidenced by the markedly lower NO_x ($H = 51.7$) and ammonium ($H = 17.6$) concentrations during 'pulse' (NO_x : $18.1 \pm 25.4 \mu\text{M}$; NH_4 : $1.4 \pm 2.4 \mu\text{M}$) conditions when compared with 'linear' (NO_x : $42.9 \pm 34.2 \mu\text{M}$; NH_4 : $3.4 \pm 5.6 \mu\text{M}$) and 'press' (NO_x : $43.9 \pm 35.1 \mu\text{M}$; NH_4 : $4.8 \pm 7.3 \mu\text{M}$) states – all $p < 0.001$. Similarly, phosphate availability was lower ($H = 23.6$) during 'pulse' ($1.4 \pm 1.2 \mu\text{M}$) compared to 'press' ($2.2 \pm 1.3 \mu\text{M}$; $p < 0.001$) conditions, while concentrations characteristic of 'linear' surveys ($1.6 \pm 0.6 \mu\text{M}$) were similar ($p > 0.05$).

The influx of fluvial NO_x and phosphate loads were calculated for each survey using freshwater end member nutrient concentrations (Stn 6) and river inflow rates. Freshwater inflow ranged from 0.37 to $4.86 \text{ m}^3 \text{ s}^{-1}$, with inflow rates less than $2 \text{ m}^3 \text{ s}^{-1}$ for most surveys (Table 1). Inorganic nutrient loading to the Sundays Estuary was greatest during 'press' con-

ditions ($177.7 \pm 77.9 \text{ kg NO}_x \text{ d}^{-1}$ and $17.9 \pm 17.9 \text{ kg PO}_4 \text{ d}^{-1}$), while loading was considerably lower during 'pulse' conditions ($<100 \text{ kg NO}_x \text{ d}^{-1}$ and $<10 \text{ kg PO}_4 \text{ d}^{-1}$). Another distinction was the prevalence of the HAB-forming species, with *Heterosigma akashiwo* (max. $29\,392 \text{ cells ml}^{-1}$) during 'pulse' conditions when nutrients were more depleted and *Heterocapsa rotundata* (max. $194\,991 \text{ cells ml}^{-1}$) during 'press' states when nutrients were less depleted (Table 1). The diatom *Cylotella atomus* was also abundant during this study, but blooms were more stochastic and fluvial in nature (Lemley et al. 2018b) and had little influence on inorganic nutrient uptake.

The seaward flux of nutrients can be estimated at each station from nutrient–salinity data, as outlined in Section 2. The decrease in seaward flux between stations then provides an estimate of the uptake of NO_x (Fig. 6) and phosphate (Fig. 7) along the length of the estuary for 'press' and 'pulse' conditions. Uptake is negligible for 'linear' states (i.e. conservative mixing). There is a positive association between phytoplankton biomass (chlorophyll *a* concentration) and NO_x uptake ($r = 0.27$; $p < 0.001$, $n = 101$) but only a weak relationship between phosphate uptake and phytoplankton biomass ($r = 0.12$; $p = 0.22$). However, uptake of NO_x and phosphate were positively correlated ($r = 0.38$; $p < 0.001$), particularly during 'pulse' events, an observation supported by previous studies (e.g. Lemley et al. 2018a,b). As such, NO_x ($99.9 \pm 0.1\%$; range: 99.5 – 100%) and phosphate ($80.4 \pm 5.6\%$; range: 69.2 – 95.9%) uptake occurred concomitantly with peak surface water phytoplankton biomass readings during 'pulse' conditions. Nutrient uptake rates were proportionally lower during 'press' surveys, i.e. NO_x ($41.7 \pm 4.7\%$; range: 29.4 – 70.4%) and phosphate ($31.1 \pm 8.1\%$; range: 0 – 73.7%). In contrast, during 'linear' surveys inorganic nutrient uptake was negligible (conservative mixing), and the majority of fluvial nutrients (NO_x and phosphate) introduced to the Sundays Estuary during these periods entered the nearshore coastal waters of Algoa Bay, although diluted in the estuary by seawater of lower nutrient concentration.

4. DISCUSSION

Estuaries are important nutrient filters. In systems where primary production is largely constrained to the water column, phytoplankton can act as effective biogeochemical hotspots for nutrient uptake given suitable growing conditions (e.g. adequate water residency, nutrients, and light) (Painting et al. 2007,

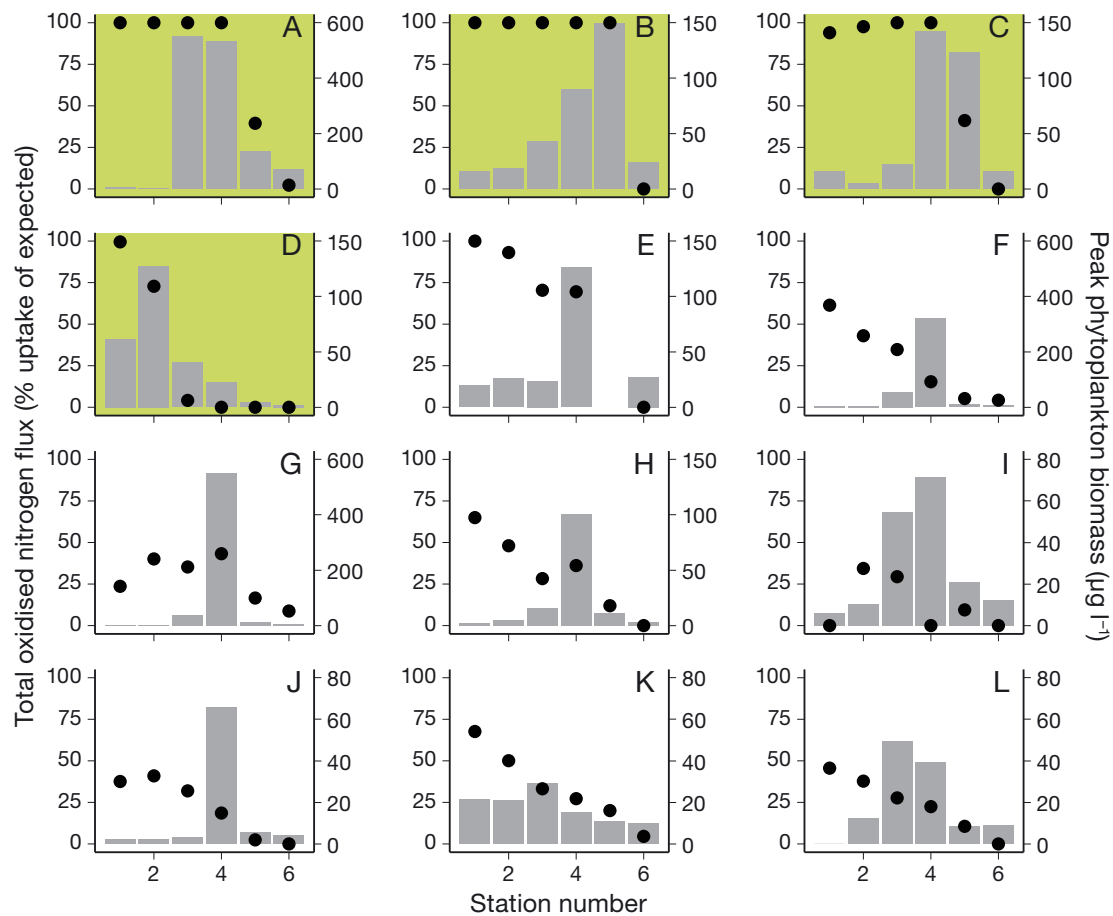


Fig. 6. Calculated NO_x uptake as a percentage of total fluvial flux into estuary (primary y-axis; black dots) and maximum near surface phytoplankton biomass (secondary y-axis; grey bars; data from 0 to 1 m depth). Phytoplankton biomass are from chlorophyll *a* concentrations observed at that station and uptake rates are from observed reduction in nutrient concentration between that station and the adjacent upstream station. Results are shown for surveys categorised as 'pulse' ($n = 4$; highlighted in green) and 'press' ($n = 8$), namely: (A) Nov 15, (B) Dec 15, (C) Oct 18, (D) Mar 15, (E) Jan 14, (F) Sep 15, (G) Jul 15, (H) Apr 18, (I) Oct 15, (J) Jun 15, (K) Jan 15, (L) Aug 14; see Table 1 for details

Jickells et al. 2014, Kadiri et al. 2014). So, while development of high biomass HABs is a characteristic symptom of eutrophication, with the increased frequency and magnitude of such events closely linked to anthropogenic nutrient enrichment (Glibert 2017, 2020), these HABs can also ameliorate nutrient impacts elsewhere in the system. HABs are commonplace in the permanently eutrophic Sundays Estuary (Hilmer & Bate 1991, Kotsedi et al. 2012, Lemley et al. 2017, 2018b), where dense blooms often exceed $500 \mu\text{g chl } a \text{ l}^{-1}$, typically dominated by *Heterosigma akashiwo* (Raphidophyceae) in spring/summer and *Heterocapsa rotundata* (Dinophyceae) in winter. Despite the known detrimental impacts that these HABs have on the estuarine environments in which they occur (e.g. hypoxia, reduced species diversity, smothering of benthic organisms), such large phytoplankton accumulations can shield adjacent estuarine and

coastal environments from land-derived pressures. This is evident from our study in which near depletion of available NO_x ($\sim 100\%$ influx is taken up in estuary) and phosphate ($>75\%$ influx taken up in estuary) were observed in association with high biomass HABs. Similar observations have been made in the temperate non-turbid Southampton Water estuarine system (UK), where phytoplankton were reported to be capable of removing 100% of riverine N loads (particularly ammonium) during the productive period (Torres-Valdés & Purdie 2006), but typically these nutrient removal events are brief and terminated when phytoplankton is flushed from the estuary.

Hydrodynamics determine estuarine circulation patterns, stratification and flushing rates (Eyre 2000, Kadiri et al. 2014, Wild-Allen & Andrewartha 2016). These factors may modulate eutrophic responses and, in turn, control the susceptibility of an ecosys-

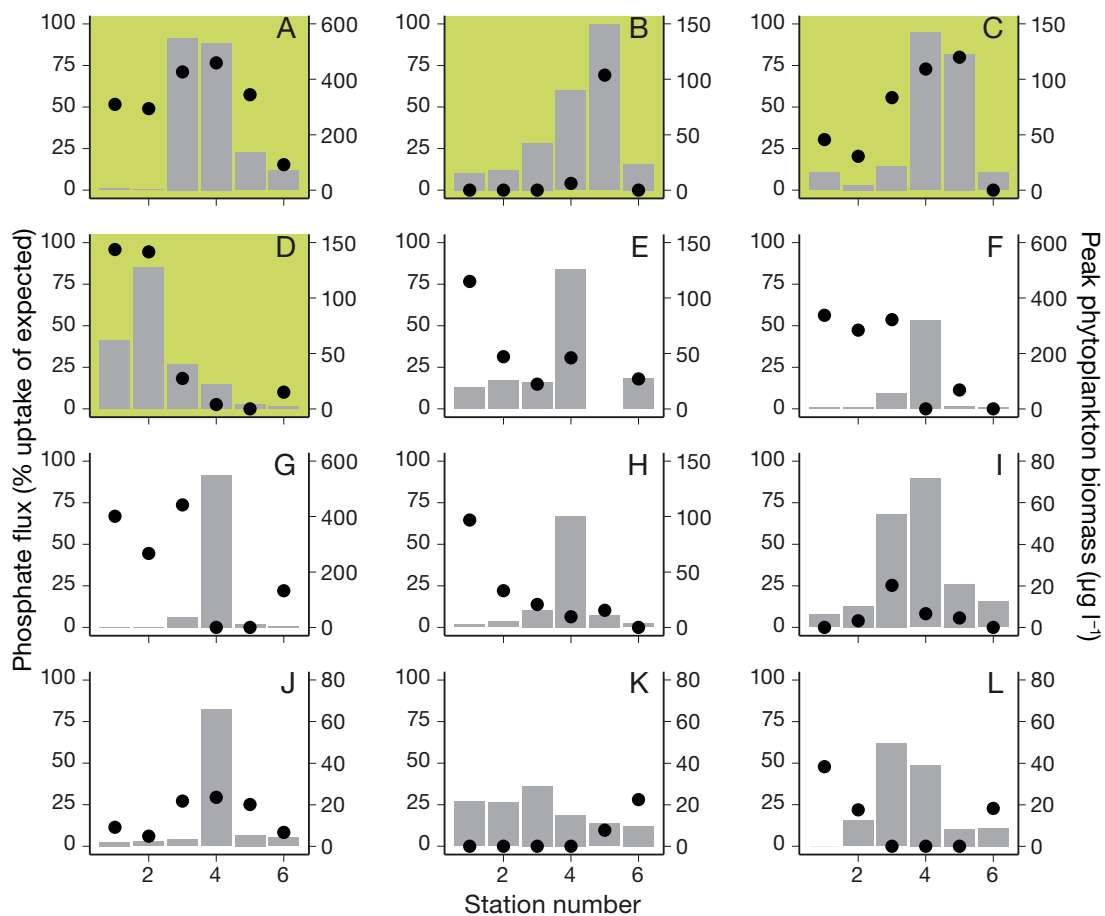


Fig. 7. Calculated phosphate uptake as a percentage of total fluvial flux into estuary. Details as in Fig. 6

tem to nutrient enrichment pressures. This was evident in 2 UK estuaries where, despite both being nutrient enriched, phytoplankton productivity was hindered by a combination of consistent rapid flushing and tidally induced vertical mixing conditions (Kadiri et al. 2014). However, the unutilized nutrients are then simply exported to the coastal ocean, and while concentrations may be lower owing to dilution, the entire mass of nutrients is still discharged to the ocean. In the predominantly open Sundays Estuary, the highly regulated freshwater inflow has severely reduced the likelihood of flushing events, thus culminating in environmental conditions that support effective inorganic nutrient uptake by phytoplankton (Scharler & Baird 2005, Lemley et al. 2017, 2018a,b). Ideal growing conditions for resident HAB species are provided by the interaction between micro-tidal estuary–ocean exchange and persistent nutrient-rich freshwater inflow that results in an environment characterised by prolonged water residence times (>40 d) and distinct vertical salinity profiles in the meso- to polyhaline middle reaches (see Fig. 4).

Therefore, unlike other systems where pulsed nutrient uptake of estuarine phytoplankton blooms provides only temporary relief to adjacent coastal waters (Eyre 2000, Torres-Valdés & Purdie 2006, McGlathery et al. 2007, Jickells et al. 2014), phytoplankton blooms are retained in the Sundays Estuary, and HABs are seen to persistently remove inorganic nutrients from inflowing waters (~70 % of surveys are classified as 'press' or 'pulse'). The persistence of the *H. akashiwo* and *H. rotundata* blooms requires that even at high concentrations loss through tidal exchange with the ocean does not exceed production in the estuary. This is largely due to the reverse diel vertical migrations of these phytoplankton, accessing near surface nutrients and light during daylight hours and sinking to depth overnight (Lemley et al. 2018a). Beyond trophic benefits, vertical migratory behaviour interacts with estuarine circulation that has a landward mean at depth and a seaward mean near surface. So, every night these phytoplankton are moved landward again—but they cannot move beyond the salt wedge so instead they accumulate

in the mesohaline region (the landward end of the salt wedge, i.e. the estuarine turbidity maximum). Through sinking across streamlines, this accumulation can reach very high concentrations. The fact that these phytoplankton are not simply flushed/mixed seaward is evident in the convex curve of phytoplankton–salinity plots (Fig. 2D), which indicates an apparent loss of phytoplankton seaward of the chlorophyll maxima in mesohaline waters (5–18). This ‘loss’ of phytoplankton can be partly attributed to flagellated HAB taxa (e.g. *H. akashiwo*, *H. rotundata*) dropping out of the seaward flowing surface layer and being transported back landward to the phytoplankton maximum in the inner estuary.

The quasi-steady state of resident phytoplankton blooms in the Sundays Estuary demonstrated a preference for warmer conditions. As such, instances of widespread pulse disturbances, characterised by high biomass HABs of *H. akashiwo* ($>100 \mu\text{g chl } a \text{ l}^{-1}$) and fluvial inorganic nutrient depletion, were largely confined to conditions in which water temperatures exceeded 20°C . During these sampling surveys, approximately 100 and 80 % of available NO_x and phosphate, respectively, were taken up from surface waters by phytoplankton. Additionally, a previous study in the estuary estimated that the duration of spring/summer *H. akashiwo* blooms are at least 1 wk and occur up to twice a month, during which NO_x and phosphate levels remain largely depleted (Lemley et al. 2018b). Based on these assumptions and previously reported spring/summer nutrient loads (Lemley et al. 2019a), an estimated $2.6 \times 10^4 \text{ kg N}$ (16.8 % of annual) and $7.9 \times 10^2 \text{ kg P}$ (21.8 % of annual) is prevented from entering the adjacent coastal waters of Algoa Bay annually by HABs during widespread pulse disturbances. Despite this, however, high biomass phytoplankton blooms occur year-round in the Sundays Estuary owing to its permanently eutrophic condition and species-specific preferences (e.g. *H. rotundata* more abundant in winter). During this study, these persistent bloom conditions induced drawdown of fluvial inorganic nutrients (particularly NO_x) along the length of the estuary and constituted extensive press disturbances that are undesirable due to the marked deterioration they cause in ecosystem structure (Tett et al. 2007). Using the frequency of occurrence (47 %) and approximate NO_x (42 %) and phosphate (31 %) flux estimates typical of press disturbance conditions recorded in this study, annual nutrient uptake by phytoplankton was estimated to be $3.0 \times 10^4 \text{ kg N}$ (19.7 % of annual) and $5.3 \times 10^2 \text{ kg P}$ (14.6 % of annual). Overall, phytoplankton activity during press and pulse disturbances acts as important sinks of the

major fluvial inorganic nutrient forms, removing a substantial proportion of annual NO_x (36.5 %) and phosphate (36.4 %) loads introduced to the Sundays Estuary before entering downstream coastal waters. Thus, phytoplankton-dominated systems such as these are comparable to benthically driven estuaries where submerged macrophytes (e.g. seagrasses) and salt marshes are responsible for significant annual nutrient fluxes (McGlathery et al. 2007, Eyre 2016, Aoki et al. 2020).

Upon decay, these HABs contribute a significant source of oxidizable organic matter to the Sundays Estuary (Lemley et al. 2017, 2018a,b). During the decomposition phase, these populations settle below the halocline in the more saline, less oxygenated ($<5 \text{ mg l}^{-1}$) bottom waters. Preliminary findings (G. Matcher unpubl. data) indicate that distinct bacterial assemblages characterise the bloom and decay phases of HABs in the Sundays Estuary, with aerobic members of the family *Rhodobacteraceae* illustrating a close association with high biomass accumulations of *H. akashiwo* (i.e. dissolved $\text{O}_2 >11 \text{ mg l}^{-1}$). Bacteria belonging to this lineage are known to be fuelled by the assimilation and remineralisation of phytoplankton-derived organic matter (e.g. Buchan et al. 2014). The role of bacteria as a nutrient-mediating feature is suggested by the increased concentrations of ammonium (e.g. N conservation through dissimilatory nitrate reduction to ammonium) and phosphate observed in polyhaline bottom waters in this study (Fig. 5), particularly in conjunction with ‘press’ conditions that encompass various stages of phytoplankton blooms (‘boom–bust’ cycle). Thus, there may be a net seaward efflux of these inorganic nutrient forms, together with any organic forms resulting from algal lysis. However, the absence of flushing events due to upstream flow management has led to these bottom water remineralisation ‘hotspots’ being confined to the highly stratified middle reaches of the estuary (MacKay & Schumann 1990). The increased retention in these remineralised nutrient reservoirs may allow for uptake by HAB species that supports continued growth or secondary blooms in the absence of sufficient fluvial inorganic nutrient sources (Lemley et al. 2018b).

Nutrient stoichiometry is considered an important factor shaping phytoplankton community dynamics (Glibert 2017 and references therein), an observation supported by the strong positive association ($p < 0.001$) observed between increasing phytoplankton biomass and NO_x fluxes in this study. Furthermore, despite phosphate fluxes being less responsive ($p = 0.22$) to phytoplankton fluctuations, co-limitation of

nutrients occurred during widespread pulse disturbances in warmer conditions. This is evidenced by the near depletion of both NO_x and phosphate concentrations during high biomass HABs. Therefore, even during periods of low fluvial NO_x availability in surface waters, phytoplankton blooms are able to persist due to the physiological strategies adopted by the dominant HAB-forming taxa that reside in the estuary. For example, both *H. akashiwo* and *H. rotundata* exhibit asynchronous diel vertical migratory behaviour that enables enhanced utilisation of bottom water nutrient stocks (inorganic and organic forms) and access to bacterial prey populations (phagotrophy) during unfavourable nutrient conditions (Seong et al. 2006, Lemley et al. 2018a). Additionally, *H. akashiwo* populations are capable of rapid nutrient uptake, osmotrophy (e.g. urea, glutamic acid), and luxury P uptake (Zhang et al. 2006, Herndon & Cochlan 2007, Verity 2010), all of which aid the (1) suppression of co-occurring taxa and (2) circumvention of nutrient limited conditions in the Sundays Estuary.

The timing of these spring/summer HABs of *H. akashiwo* in the Sundays Estuary is notable given that this is also the period in which nearshore regions within Algoa Bay are most susceptible to phytoplankton accumulations (e.g. *Lingulodinium polyedra*, *Noctiluca scintillans*) (Lemley et al. 2019a). These estuarine HABs buffer the adjacent coastal waters from considerable inorganic nutrient pulses through continuous uptake of NO_x and phosphate (~36% removed), thus mitigating any role the anthropogenic nutrients introduced via the Sundays Estuary may have in sustaining high biomass blooms in Algoa Bay. The manipulation of nutrient loads and ratios entering aquatic ecosystems from surrounding catchment inflows is viewed as a means of inhibiting eutrophication symptoms (e.g. algal biomass). Historically, management efforts typically focused on P reductions to achieve this, yet such interventions can culminate in exacerbated eutrophic symptoms (e.g. structural community shifts, increased biomass) in downstream estuaries and coastal waters that are often N-limited (Paerl et al. 2004). This was highlighted in a study on Irish estuaries where P-reduction interventions had culminated in a weakening of the estuarine N filter, subsequently resulting in marked increases in downstream movement of N to adjacent coastal waters (O'Boyle et al. 2015). Subsequently, dual nutrient reduction strategies are key to effective management of eutrophication, including HABs (Conley et al. 2009). The value of a dual approach was highlighted in an Australian estuary where significant reductions in N loading from chronic point sources lowered the prob-

ability and magnitude of HABs in the most susceptible warmer months (Larsson et al. 2017). Future management efforts in the Sundays Estuary catchment should be centred around simultaneously restoring an element of natural hydrological variability and reducing nutrient loads, with the objective of minimising the detrimental consequences of eutrophic symptoms downstream. Although phytoplankton blooms were identified as an effective sink for fluvial inorganic nutrients, thus limiting the flux of the most bioavailable form of anthropogenic nutrient inputs (i.e. NO_x and phosphate) to adjacent coastal waters, it would be misguided to allow continued degradation of the significant ecosystem services provided by estuarine ecosystems (e.g. fish nursery area, recreational value). This requirement for improved management is further necessitated by the potential for seaward export of remineralised and organic nutrient forms released as a by-product during the decay of these persistent phytoplankton blooms. Therefore, a catchment-to-coast approach is critical to the long-term sustainability of ecosystem health and functionality along the land–ocean aquatic continuum.

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LITERATURE CITED

- ✦ Aoki LR, McGlathery KJ, Oreska MPJ (2020) Seagrass restoration reestablishes the coastal nitrogen filter through enhanced burial. *Limnol Oceanogr* 65:1–12
- ✦ Bate GC, Heelas BV (1975) Studies on the nitrate nutrition of two indigenous Rhodesian grasses. *J Appl Ecol* 12:941–952
- Billen G, Lancelot C, Meybeck M (1991) N, P, and Si retention along the aquatic continuum from land to ocean. In: Mantoura RFC, Martin JM, Wollast R (eds) *Ocean margin processes in global change*. John Wiley & Sons, New York, NY, p 19–44
- ✦ Bouwman AF, Bierkens MFP, Griffioen J, Hefting MM, Middelburg JJ, Middelkoop H, Slomp CP (2013) Nutrient dynamics, transfer and retention along the aquatic continuum from land to ocean: towards integration of ecological and biogeochemical models. *Biogeosciences* 10:1–23
- ✦ Boyle E, Collier R, Dengler AT, Edmond JM, Ng AC, Stallard RF (1974) On the chemical mass-balance in estuaries. *Geochim Cosmochim Acta* 38:1719–1728

- ✦ Buchan A, LeClerc GR, Gulvik CA, González JM (2014) Master recyclers: features and functions of bacteria associated with phytoplankton blooms. *Nat Rev Microbiol* 12: 686–698
- Campbell EE, Bate GC (2005) Tide-induced pulsing of nutrient discharge from an unconfined aquifer into an *Anaulus australis*-dominated surf-zone. *Water SA* 24:365–370
- ✦ Cloern JE (2019) Patterns, pace, and processes of water-quality variability in a long-studied estuary. *Limnol Oceanogr* 64:S192–S208
- ✦ Cloern JE, Jassby AD, Schraga TS, Nejad E, Martin C (2017) Ecosystem variability along the estuarine salinity gradient: examples from long-term study of San Francisco Bay. *Limnol Oceanogr* 62:S272–S291
- ✦ Conley DJ, Paerl HW, Howarth RW, Boesch DF and others (2009) Controlling eutrophication: nitrogen and phosphorus. *Science* 323:1014–1015
- ✦ Eyre BD (2000) Regional evaluation of nutrient transformation and phytoplankton growth in nine river-dominated sub-tropical east Australian estuaries. *Mar Ecol Prog Ser* 205:61–83
- Eyre BD, Maher DT, Sanders C (2016) The contribution of denitrification and burial to the nitrogen budgets of three geomorphically distinct Australian estuaries: importance of seagrass habitats. *Limnol Oceanogr* 61:1144–1156
- ✦ Glibert PM (2017) Eutrophication, harmful algae and biodiversity—challenging paradigms in a world of complex nutrient changes. *Mar Pollut Bull* 124:591–606
- ✦ Glibert PM (2020) Harmful algae at the complex nexus of eutrophication and climate change. *Harmful Algae* 91: 101583
- ✦ Glibert PM, Kelly V, Alexander J, Codispoti LA, Boicourt WC, Trice TM, Michael B (2008) *In situ* nutrient monitoring: a tool for capturing nutrient variability and the antecedent conditions that support algal blooms. *Harmful Algae* 8:175–181
- ✦ Herndon J, Cochlan WP (2007) Nitrogen utilization by the raphidophyte *Heterosigma akashiwo*: growth and uptake kinetics in laboratory cultures. *Harmful Algae* 6: 260–270
- ✦ Hilmer T, Bate GC (1991) Vertical migration of a flagellate-dominated bloom in a shallow South African estuary. *Bot Mar* 34:113–121
- ✦ Jickells TD, Andrews JE, Parkes DJ, Suratman S, Aziz AA, Hee YY (2014) Nutrient transport through estuaries: the importance of estuarine geography. *Estuar Coast Shelf Sci* 150:215–229
- ✦ Kadiri M, Bockelmann-Evans B, Rauen WB (2014) Assessing the susceptibility of two UK estuaries to nutrient enrichment. *Cont Shelf Res* 88:151–160
- ✦ Kotsedi D, Adams JB, Snow GC (2012) The response of microalgal biomass and community composition to environmental factors in the Sundays Estuary. *Water SA* 38: 177–190
- ✦ Larsson ME, Ajani PA, Rubio AM, Guise K and others (2017) Long-term perspective on the relationship between phytoplankton and nutrient concentrations in a south-eastern Australian estuary. *Mar Pollut Bull* 114:227–238
- ✦ Lemley DA, Adams JB, Taljaard S (2017) Comparative assessment of two agriculturally-influenced estuaries: similar pressure, different response. *Mar Pollut Bull* 117: 136–147
- ✦ Lemley DA, Adams JB, Rishworth GM (2018a) Unwinding a tangled web: a fine-scale approach towards understanding the drivers of harmful algal bloom species in a eutrophic South African estuary. *Estuaries Coasts* 41: 1356–1369
- ✦ Lemley DA, Adams JB, Strydom NA (2018b) Triggers of phytoplankton bloom dynamics in permanently eutrophic waters of a South African estuary. *Afr J Aquat Sci* 43:229–240
- ✦ Lemley DA, Adams JB, Bornman TG, Campbell EE, Deyzel SHP (2019a) Land-derived inorganic nutrient loading to coastal waters and potential implications for nearshore plankton dynamics. *Cont Shelf Res* 174:1–11
- ✦ Lemley DA, Adams JB, Rishworth GM, Bouland C (2019b) Phytoplankton responses to adaptive management interventions in eutrophic urban estuaries. *Sci Total Environ* 693:133601
- ✦ Lemley DA, Adams JB, Rishworth GM, Purdie DA (2020) Harmful algal blooms of *Heterosigma akashiwo* and environmental features regulate *Mesodinium cf. rubrum* abundance in eutrophic conditions. *Harmful Algae* 100: 101943
- ✦ MacKay HM, Schumann EH (1990) Mixing and circulation in the Sundays River Estuary, South Africa. *Estuar Coast Shelf Sci* 31:203–216
- ✦ McGlathery KJ, Sundbäck K, Anderson IC (2007) Eutrophication in shallow coastal bays and lagoons: the role of plants in the coastal filter. *Mar Ecol Prog Ser* 348:1–18
- Nusch EA (1980) Comparison of different methods for chlorophyll and phaeopigment determination. *Arch Hydrobiol Beih Ergeb Limnol* 14:14–36
- ✦ O'Boyle S, Wilkes R, McDermott G, Ni Longphuirt S, Murray C (2015) Factors affecting the accumulation of phytoplankton biomass in Irish estuaries and nearshore coastal waters: a conceptual model. *Estuar Coast Shelf Sci* 155: 75–88
- OSPAR (2003) Strategies of the OSPAR commission for the protection of the marine environment of the north-east Atlantic (reference number: 2003-21). In: OSPAR Convention for the Protection of the Marine Environment of the Northeast Atlantic: Ministerial Meeting of the OSPAR Commission, Bremen, 25 June 2003, Vol Annex 31 (Ref. B-4.2)
- ✦ Paerl HW, Valdes LM, Joyner AR, Piehler MF (2004) Solving problems resulting from solutions: evolution of a dual nutrient management strategy for the eutrophying Neuse River Estuary, North Carolina. *Environ Sci Technol* 38:3068–3073
- ✦ Painting SJ, Devlin MJ, Malcolm SJ, Parker ER and others (2007) Assessing the impact of nutrient enrichment in estuaries: susceptibility to eutrophication. *Mar Pollut Bull* 55:74–90
- Parsons TR, Maita Y, Lalli CM (1984) A manual of chemical and biological methods for seawater analysis. Pergamon Press, New York, NY
- ✦ Potter IC, Chuwen BM, Hoeksema SD, Elliott M (2010) The concept of an estuary: a definition that incorporates systems which can become closed to the ocean and hypersaline. *Estuar Coast Shelf Sci* 87:497–500
- R Core Team (2019) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. www.r-project.org
- Scharler UM, Baird D (2005) The filtering capacity of selected Eastern Cape estuaries, South Africa. *Water SA* 31:483–490
- ✦ Seong KA, Jeong HJ, Kim S, Kim GH, Kang JH (2006) Bacterivory by co-occurring red-tide algae, heterotrophic nanoflagellates, and ciliates. *Mar Ecol Prog Ser* 322:85–97

- ✦ Smit T, Lemley DA, Adams JB, Strydom NA (2021) Preliminary insights on the fine-scale responses in larval *Gilchristella aestuaria* (Family Clupeidae) and dominant zooplankton to estuarine harmful algal blooms. *Estuar Coast Shelf Sci* 249:107072
- Snow GC, Bate GC, Adams JB (2000) The effects of a single freshwater release into the Kromme Estuary. 2. Microalgal response. *Water SA* 26:301–310
- ✦ Taljaard S, van Niekerk L, Joubert W (2009) Extension of a qualitative model on nutrient cycling and transformation to include microtidal estuaries on wave-dominated coasts: southern hemisphere perspective. *Estuar Coast Shelf Sci* 85:407–421
- ✦ Tett P, Gowen R, Mills D, Fernandes T and others (2007) Defining and detecting undesirable disturbance in the context of marine eutrophication. *Mar Pollut Bull* 55: 282–297
- ✦ Torres-Valdés S, Purdie DA (2006) Nitrogen removal by phytoplankton uptake through a temperate non-turbid estuary. *Estuar Coast Shelf Sci* 70:473–486
- van Niekerk L, Adams JB, Lamberth SJ, MacKay CF and others (eds) (2019) South African National Biodiversity Assessment 2018: Technical Report, Vol 3. Estuarine realm. CSIR report number CSIR/SPLA/EM/EXP/2019/0062/A. Report number SANBI/NAT/NBA2018/2019/Vol3/A. South African National Biodiversity Institute, Pretoria
- ✦ Verity PG (2010) Expansion of potentially harmful algal taxa in a Georgia Estuary (USA). *Harmful Algae* 9:144–152
- ✦ Wild-Allen K, Andrewartha J (2016) Connectivity between estuaries influences nutrient transport, cycling and water quality. *Mar Chem* 185:12–26
- ✦ Xenopoulos MA, Downing JA, Kumar MD, Menden-Deuer S, Voss M (2017) Headwaters to oceans: ecological and biogeochemical contrasts across the aquatic continuum. *Limnol Oceanogr* 62:S3–S14
- ✦ Zhang Y, Fu F, Whereat E, Coyne KJ, Hutchins DA (2006) Bottom-up controls on a mixed-species HAB assemblage: a comparison of sympatric *Chattonella subsalsa* and *Heterosigma akashiwo* (Raphidophyceae) isolates from the Delaware Inland Bays, USA. *Harmful Algae* 5: 310–320

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