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PRIMARY RESEARCH PAPER

Assessing phytoplankton composition and structure within micro-estuaries and micro-outlets: a community analysis approach

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Abstract Micro-estuaries and micro-outlets represent small coastal waterbodies that differ in their relative salinity and size, with the former being larger, more saline (mesohaline versus oligohaline), and exchanging with the sea more often than the latter. There are thousands of these waterbodies along the world's coastline, yet few of these very small systems have been identified and studied. We investigated systematic differences between micro-estuaries and micro-outlets in terms of phytoplankton community composition, including spatio-temporal variation in both community structure and biomass (chlorophyll-*a*). A multivariate analysis was used to assess differences in environmental variables, biomass and phytoplankton community composition across four seasons and the two waterbody types. A total of 260 (63 families) and 244 (74 families) phytoplankton taxa were identified within the micro-estuaries and micro-outlets, respectively. Nano- and picoplankton were the dominant groups in micro-estuaries, and pico- and microplankton in micro-outlets. Micro-estuaries were rich in phytoplankton taxa representative of marine, estuarine and freshwater conditions, with a

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successional sequence in dominance evident, from Chlorophyta during winter to Bacillariophyta in spring and Cyanophyta in summer. By contrast, micro-outlets were mostly dominated by freshwater taxa, with Chlorophyta remaining the dominant group across all four seasons. Higher phytoplankton biomass was recorded during the winter when increased nutrients were available following catchment flooding. Seasonal switching in phytoplankton was reflected not only in changing dominance patterns in both habitat types but also in complete replacement of some species in micro-outlets, despite Chlorophyta remaining dominant. Such temporal turnover, which is often accompanied by predictable seasonal changes in environmental conditions, can promote overall species richness by allowing more taxa to coexist in a single environment through temporal niche segregation.

Keywords Biomass · Chlorophyll-*a* · Micro-estuaries · Micro-outlets · Phytoplankton · Salinity

Introduction

Estuaries are regarded as one of the most productive of all aquatic ecosystem types, with the nutrient supply from freshwater inputs being crucial in sustaining the high primary production rates (Fisher et al., 1988; Vinayachandran & Mathew, 2003; Du et al., 2011; Dalu et al., 2018). In many parts of the world, the variability in estuary dynamics can involve switches between open (i.e. connected with the ocean) and closed (i.e. isolated from the ocean) mouth states, which, in turn, causes changes in the nutrient dynamics of these ecosystems (Roy et al., 2001). These hydrological shifts have been shown to cause large temporal and spatial variation in phytoplankton composition, biomass and production (Anandraj et al., 2008). Although coastal waters occupy only 0.5% of the total ocean volume, this zone can contribute up to 30% of overall marine primary production (Nixon et al., 1986; Longhurst et al., 1995; Pan et al., 2016).

Phytoplankton communities contribute significantly to this neritic production and to carbon fixation in estuaries and other coastal water bodies (Sand-Jensen & Borum, 1991; Ke et al., 2014; Lemley et al., 2016). Furthermore, phytoplankton abundance and species community composition reflect the environmental changes between and within riverine, estuarine and marine ecosystem types (Ke et al., 2012; Dalu & Froneman, 2016). Studies of phytoplankton composition are, therefore, useful in terms of providing background information for higher trophic level functioning and can also be used to inform the management of such systems (Fonge et al., 2013).

The dynamics of phytoplankton in South African estuaries have been widely investigated (Hilmer & Bate, 1991; Adams & Bate, 1999; Thomas et al., 2005; Anandraj et al., 2007; van der Molen & Perissinotto, 2011van Ginkel, 2012; Dalu et al., 2014, 2016) but no studies have been conducted on phytoplankton communities within the micro-estuaries or micro-outlets. Of the more than 100 coastal micro-estuaries and micro-outlets located on the African subcontinent, only a few have had very preliminary studies (Bate et al., 2017; Human et al., 2018). This contrasts with the vast amount of detailed information available from larger estuaries in the region (Allanson & Baird, 1999).

Micro-outlets are small freshwater-dominated systems with minimal water exchange with the sea and may even dry out during prolonged droughts, when stream flow ceases. In terms of salinity they are usually oligohaline (0.5–5.0 ppt) and often located above the spring high tide level (i.e. 'perched' systems; Fig. 1a, c). Micro-estuaries are slightly larger than micro-outlets, with water exchange with the sea occurring whenever the mouth is open. These systems are usually mesohaline (5–18 ppt) and less 'perched' than micro-outlets (Fig. 1b, d). Because of their limited estuarine features and unique physical and chemical characteristics, these systems and their biota provide a unique opportunity to explore when a coastal outlet becomes an estuary. This study aims to use the phytoplankton community composition and structure of micro-estuaries and micro-outlets to act as an environmental 'indicator' of whether these systems are estuarine or not. If both micro-estuaries and micro-outlets do possess true estuarine biota, then there is ecological significance in terms of coastal connectivity; i.e. micro-estuaries and/or micro-outlets could then fill 'estuarine gaps' along the coastline where larger estuaries are absent.

The primary aim of this investigation was to analyse whether there is any systematic difference between micro-estuaries and micro-outlets in phytoplankton community composition and to assess the

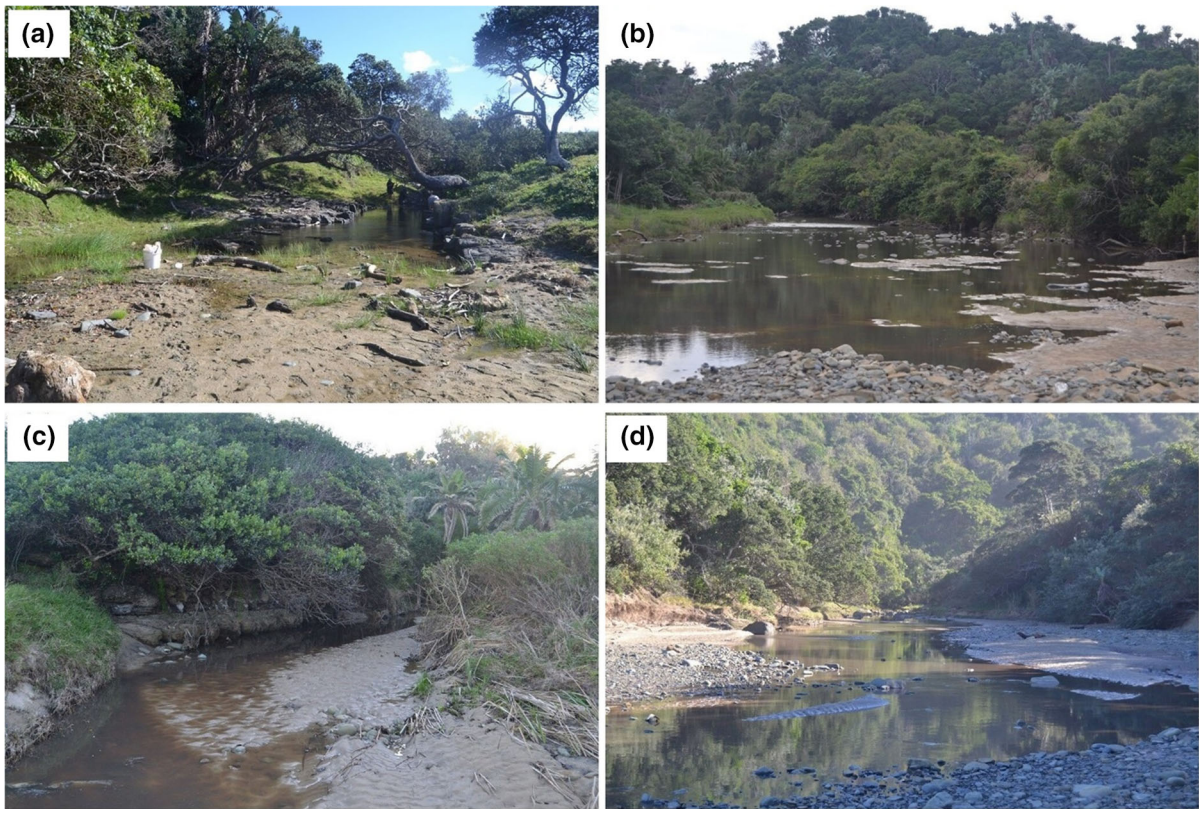

Fig. 1 Examples of sections from two micro-outlets, Black Rock (**a**) and Palm Tree (**c**), and two micro-estuaries, Mtendwe (**b**) and Mtwendwe (**d**), investigated during the current Eastern Cape micro-systems study

spatio-temporal variation in phytoplankton community structure and biomass over four seasons. We hypothesised that more freshwater taxa would occur in micro-outlets in comparison to micro-estuaries, primarily due to differences in marine connectivity, salinity ranges, system size and the relative influence of freshwater flows.

Materials and methods

Study area

The study was conducted within the warm temperate biogeographic region of South Africa, an area that can receive precipitation at any time of the year, with slight peaks in rainfall often occurring during autumn and spring (Kopke, 1988). The coastline is greatly influenced by the Agulhas Current, with inshore coastal water temperatures varying between 14 and 20°C (Lutjeharms, 2006). Field work was conducted over four seasons: July/August 2015 (winter), November 2015 (spring), January 2016 (summer) and May 2016 (autumn) between Gonubie and Black Rock ($\sim$ 30 km coastal length; see Fig. 2).

Four micro-estuaries (channel width $\sim$ 10–20 m, channel length $\sim$ 100–300 m, catchment size $\sim$ 5–10 km^2), namely Kwesani, Cunge, Mtwendwe and Mtendwe; and four micro-outlets (channel width $\sim$ 2–5 m, channel length $\sim$ 30–60 m, catchment size $\sim$ 1 km^2), namely Stromatolite, Sandy Bottom, Palm Tree and Black Rock, were selected for this study. Samples were collected from three sites (lower, middle and upper) in the micro-estuaries and two sites (lower and upper) in the shorter micro-outlets (Fig. 2). All study ecosystems were open to the sea during winter and spring, and closed during summer and autumn, with the exception of Kwesani, which was closed throughout the study due to the presence of a boulder and pebble berm.

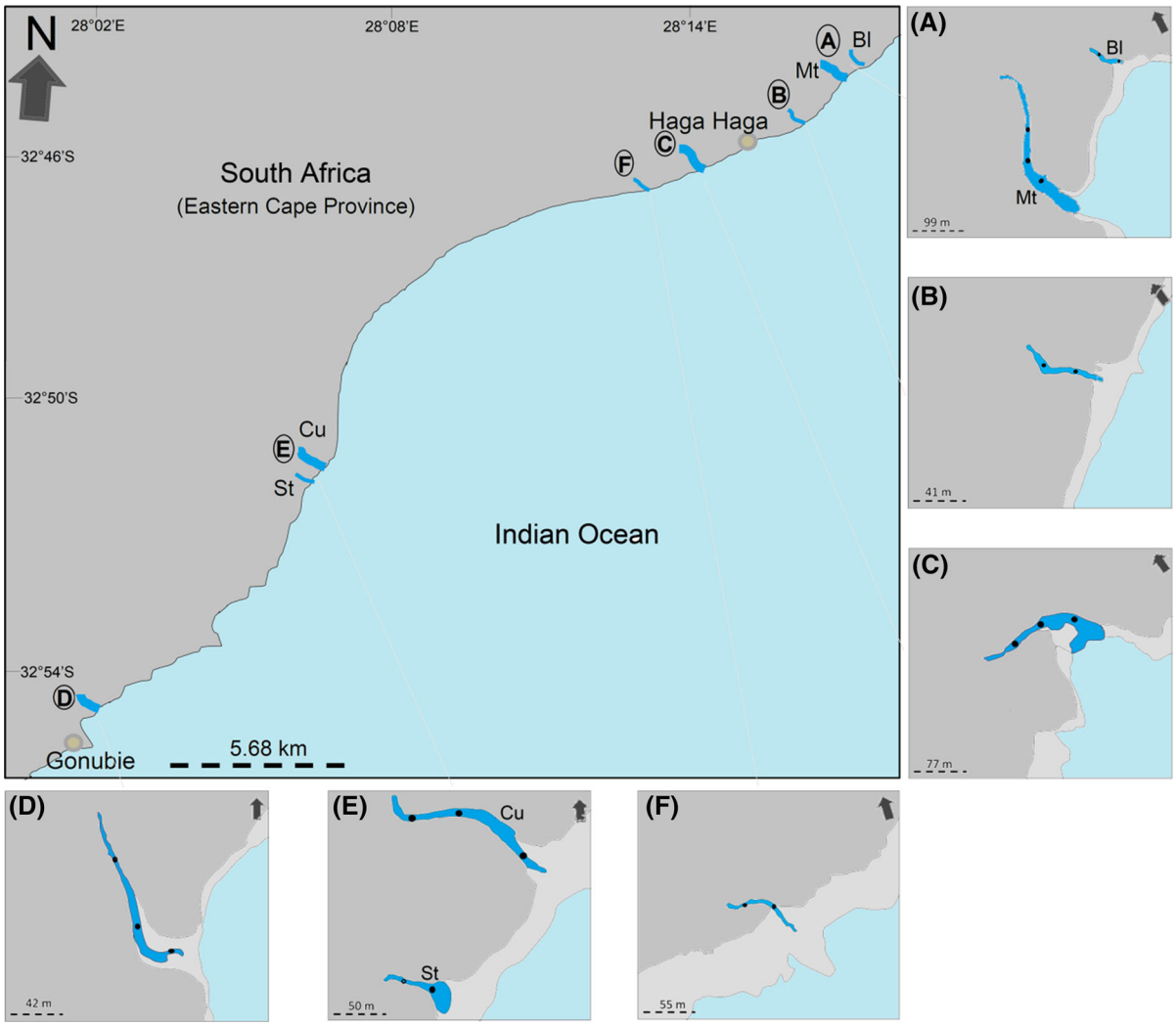

Fig. 2 Location of the four micro-estuaries: **A** (Mt) Mtendwe, **C** Mtwendwe, **D** Kwesani, **E** (Cu) Cunge, and four micro-outlets: **A** (Bl) Black Rock, **B** Palm Tree, **E** (St) Stromatolite, **F** Sandy Bottom. The solid black dots indicate study sites

Environmental variables

Dissolved oxygen, conductivity, pH, salinity and temperature were measured 5 cm below the water surface at each site using a YSI 6600-V2 Multi-probe meter (YSI Incorporated, Ohio). Where the water depth was > 50 cm, then additional readings were taken at the sediment–water interface. Water depth was measured using a graduated rod. Water samples (1 l) were collected from each site (n = 2) in each system and stored on ice for chlorophyll-*a* (chl-*a*) concentration (proxy for phytoplankton biomass) and total suspended solids (TSS) determinations in the laboratory. The water samples were collected at the subsurface level (0–30 cm) from the littoral zone and channel at each site (Dalu et al., 2014).

In the field, water samples for nutrient analysis in the laboratory were filtered through 0.45-µm syringe filters and stored on ice. The filtered water samples were then analysed for total oxidised nitrogen (TO_xN) using the reduced copper cadmium method (Bate & Heelas, 1975), and ammonium (NH_4^+) and soluble reactive phosphates (SRP) using standard spectrophotometric methods (Parsons et al., 1984). Total suspended solids were estimated after filtration of 250 ml of water (n = 2) for each site and system. Filtration was conducted in the laboratory on samples that had been stored on ice, using a Millipore (Swinnex

47 mm) filter holder and pre-weighed 0.7-µm Whatman GF/F filters. The filter membranes were then dried at 70°C for 48 h to determine total suspended solids.

Phytoplankton biomass

Chl-*a* concentration was used to determine the size-fractionated pelagic phytoplankton biomass. In the laboratory, water samples ($n = 2$ per site) were used for the determination of size-fractionated chl-*a* measurements by serially filtering aliquots (100–250 ml, vacuum < 5 cm Hg) through a 20-µm Nitex nylon mesh filter (microplankton > 20 µm), a 2-µm Millipore isopore membrane filter (nanoplankton 2–20 µm) and a 0.7-µm Whatman GF/F filter (picoplankton 0.7–2 µm; Sieburth et al., 1978). After filtration, the chl-*a* was extracted by placing filters in separate labelled vials containing 10 ml of 90% acetone for 24 h in the dark. A Turner Designs 10-AU fluorometer fitted with a narrow-band, non-acidification system was used to determine chl-*a* concentration through fluorescence measurements (Welschmeyer, 1994).

Phytoplankton communities

Three 10 l subsurface water samples (5–40 cm depth), one on either side of each site in each system (littoral zones) and one in the channel, were collected and then pooled together in a 40-l bucket and stirred. Thereafter, a 1 l subsample was taken as representative of the study site for the determination of phytoplankton composition and abundance and preserved in 5% non-acetic Lugol's iodine solution.

In the laboratory, the phytoplankton samples were left to settle for 48 h as per Utermöhl's technique. Final aliquots were ~ 10–100 ml of the samples after sedimentation depending on phytoplankton abundances in each sample. Species determination and enumeration were performed using an inverted Nikon TMS light microscope at ×400 magnification. In some cases, a phytoplankton subsample was digested using hot hydrochloric acid and potassium permanganate, a method used for detailed diatom identification (Taylor et al., 2005). Identification of diatom taxa was conducted at ×1000 magnification under oil immersion, with a minimum of 300–650 valves being counted for each sample using an Olympus CX compound microscope and the results were analysed qualitatively. All phytoplankton taxa were identified to the lowest possible level, i.e. genus or species using the keys of John et al. (2002) and Taylor et al. (2007).

The photosynthetic available radiation (PAR) in the range 400–700 nm was measured on-site, 5 cm below the water surface and immediately above the sediment, using a LI-COR light meter fitted with a LI-193SA spherical quantum sensor (LI-COR, Nebraska). PAR values were used to calculate the diffusive light attenuation coefficient (K_d), using the following equation:

$$K_d = -\ln(I_{z2} \div I_{z1})/(z_2 - z_1),$$

where I_{z2} is irradiance (mmol m^{-2} s^{-1}) at depth z_2 (m) and I_{z1} is irradiance at depth z_1 (Kühl et al., 1997; Perissinotto et al., 2010).

Data analysis

Two-way analysis of variance (ANOVA) was used to assess the differences in environmental variables, chl-*a* concentration and phytoplankton species richness across the study seasons (winter, spring, summer, autumn) and waterbody type (micro-estuaries, micro-outlets) using SPSS 16.0 for Windows software (SPSS Inc., 2007). Prior to multivariate analysis, all phytoplankton composition and abundance data were $\log_{10}(x + 1)$-transformed to reduce heteroscedasticity. Distance-based Permutational Analysis of Variance (PERMANOVA; Anderson, 2001) was conducted using PRIMER v6 add-on package two-way PERMANOVA+ (Anderson et al., 2008) to determine whether the phytoplankton community and full suite of normalised environmental variables differed among study seasons and waterbody types. Each term in the analysis was tested using 9999 permutations of the correct relevant permutable units (Anderson & ter Braak, 2003), with significant terms investigated using a posteriori pair-wise comparison with the PERMANOVA *t*-statistic (Anderson et al., 2008).

The similarity percentages routine (SIMPER) was used to determine the phytoplankton taxa contributing to any differences among waterbody types. SIMPER analysis breaks down the contribution of each taxon to the observed similarity and/or dissimilarity between samples and allows the identification of taxa that are most important in creating the observed pattern of similarity and/or dissimilarity. To evaluate seasonal changes in phytoplankton community structure per

system and among-site separation within each system, *n*-MDS analysis was carried out (Kruskal & Wish, 1978) using PRIMER in autopilot mode to allow the programme to choose the best solution at each dimensionality. The Bray–Curtis dissimilarity was used as a measure of distance.

Results

Environmental variables

All systems were saline throughout the study period (Fig. 3), with higher salinity values recorded in micro-estuaries due to a more regular marine connection (Fig. 3; Table 1). That is, micro-outlets were mostly oligohaline (0.5–5.0 ppt) and micro-estuaries mostly mesohaline (5–18 ppt) during the study period, with the exception of the spring sampling event when all systems were oligohaline due to high freshwater inputs. This shows that different hydrological processes operate within each waterbody type, which is reflected in their physical and chemical properties.

Significant differences (2-way ANOVA) between waterbody types were recorded for temperature ($F_{(1,77)} = 8.724$, $P = 0.004$), salinity ($F_{(1,77)} = 13.836$, $P < 0.001$), DO ($F_{(1,77)} = 6.516$, $P = 0.013$) and NH_4 ($F_{(1,77)} = 8.724$, $P = 0.004$;

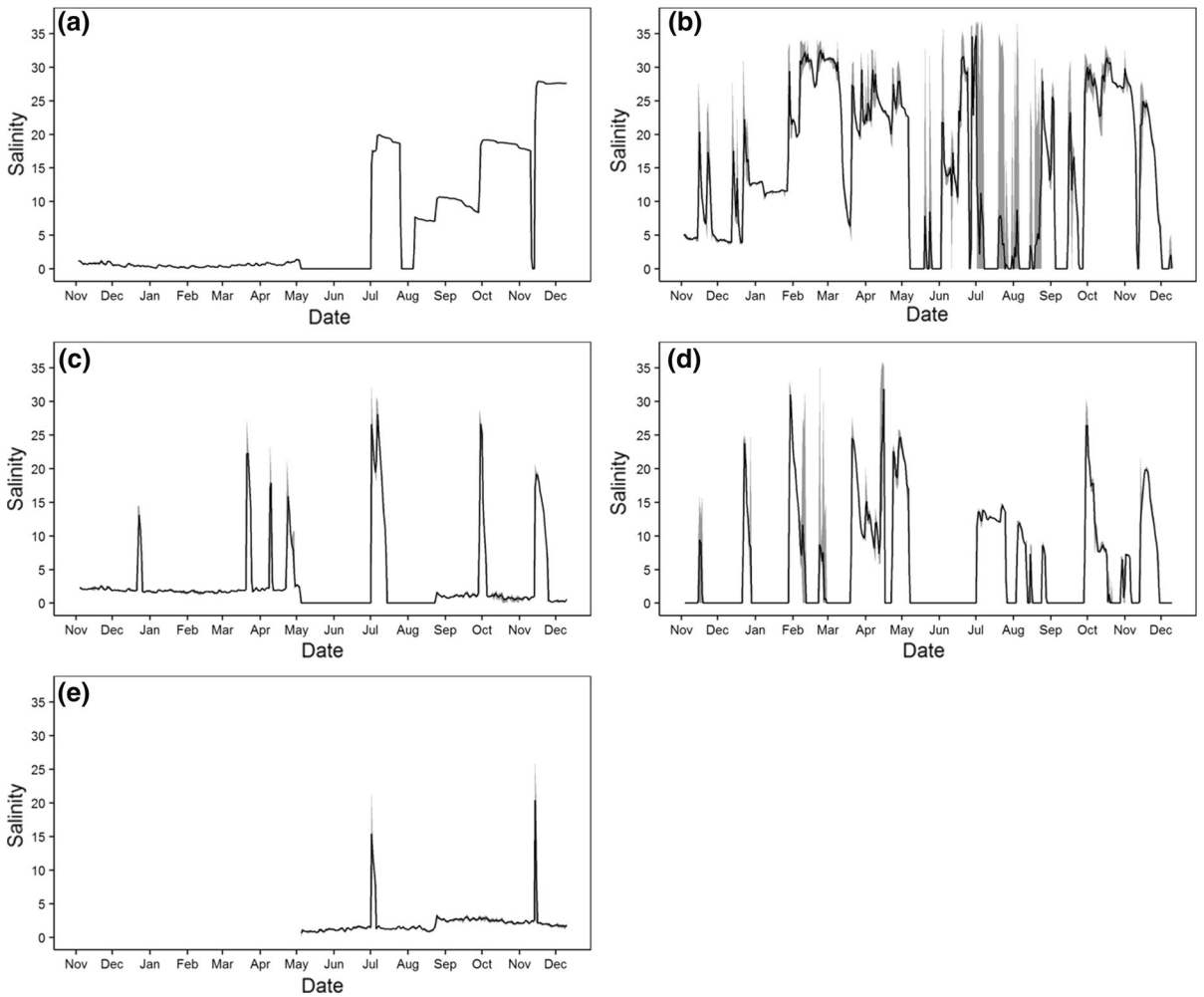

Fig. 3 Daily mean (black solid line; $n = 24$) and range (shaded grey ribbon; minimum–maximum) of salinity values recorded in three selected micro-outlets, namely **a** Black Rock, **c** Palm Tree and **e** Sandy Bottom, and two selected micro-estuaries, namely **b** Mtendwe and **d** Mtwendwe, between November 2015 to December 2016

Table 1 Seasonal environmental variables recorded in the micro-estuaries and micro-outlets during 2015 and 2016

Variable	Winter		Spring		Summer		Autumn	
	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
Micro-estuaries								
Temperature (°C)	11.2–15.8	13.2 ± 1.2	16.7–19.9	18.7 ± 1.1	24.1–37.4	27.9 ± 3.9	15.1–20.3	17.2 ± 1.4
Conductivity (ppt)	0.5–29.7	11.3 ± 12.2	0.6–5.8	2.3 ± 2.1	1.8–36.6	14.2 ± 12.7	4.6–37.6	27.9 ± 14.2
Salinity (ppt)	0.2–18.4	6.8 ± 7.4	0.3–3.2	1.2 ± 1.1	0.9–24.0	9.0 ± 9.2	2.5–23.9	17.5 ± 9.2
DO (mg l^{-1})	6.8–10.1	8.8 ± 1.3	6.9–9.6	8.5 ± 0.9	3.9–11.2	7.0 ± 2.6	3.1–8.6	6.1 ± 1.5
pH	8.0–8.7	8.4 ± 0.2	8.4–8.8	8.6 ± 0.1	8.0–9.8	8.8 ± 0.5	7.4–8.6	8.1 ± 0.4
Water depth (m)	0.14–0.82	0.29 ± 0.22	0.10–0.36	0.20 ± 0.09	0.01–0.30	0.16 ± 0.08	0.01–0.26	0.12 ± 0.06
Turbidity (NTU)	4.7–147.1	74.7 ± 52.3	8.5–661.0	167.9 ± 225.3	0.0–35.3	11.6 ± 11.7	0.0–18.2	2.6 ± 5.1
TSS (mg l^{-1})	19.0–152.6	62.6 ± 39.6	16.0–81.6	32.9 ± 22.8	10.8–102.8	45.1 ± 33.1	15.2–106.6	71.1 ± 32.7
NH_4 (μM)	0.0–7.3	2.8 ± 0.7	0.01–5.6	1.4 ± 0.5	3.9–10.9	7.1 ± 0.4	4.2–21.9	11.0 ± 0.5
TO_xN (μM)	0.0–7.9	3.3 ± 1.0	0.2–5.1	1.8 ± 0.5	0.1–2.3	0.6 ± 0.2	0.01–7.6	2.3 ± 0.4
SRP (μM)	0.0–6.1	2.2 ± 0.5	0.02–0.8	0.4 ± 0.1	0.5–4.5	1.1 ± 0.4	0.04–3.5	0.9 ± 0.1
Micro-outlets								
Temperature (°C)	12.3–16.4	13.8 ± 1.4	16.1–20.0	18.5 ± 1.5	13.0–26.8	22.7 ± 4.5	14.3–18.4	16.0 ± 1.7
Conductivity (ppt)	0.8–30.3	7.8 ± 17.3	0.6–1.0	0.8 ± 0.1	1.1–43.3	13.3 ± 30.9	1.0–19.2	4.2 ± 6.1
Salinity (ppt)	0.4–33.0	4.9 ± 11.4	0.3–0.5	0.4 ± 0.1	0.6–1.7	1.0 ± 0.4	0.5–11.6	2.4 ± 3.7
DO (mg l^{-1})	8.2–10.5	9.7 ± 0.7	8.2–9.9	9.3 ± 0.6	3.3–12.4	7.7 ± 3.1	4.5–13.0	7.9 ± 2.8
pH	7.8–8.8	8.3 ± 0.3	8.2–9.3	8.7 ± 0.4	8.2–9.3	8.7 ± 0.4	7.6–8.8	8.3 ± 0.4
Water depth (m)	0.13–0.63	0.30 ± 0.17	0.12–0.29	0.21 ± 0.06	0.07–0.34	0.19 ± 0.09	0.05–0.26	0.15 ± 0.08
Turbidity (NTU)	38.5–118.2	70.6 ± 23.2	24.5–99.5	52.9 ± 26.4	0.2–8.6	3.0 ± 3.0	0.0–6.4	1.2 ± 2.3
TSS (mg l^{-1})	15.4–149.0	55.8 ± 52.2	11.0–42.7	26.8 ± 9.7	8.8–36.2	18.4 ± 9.8	5.6–196.8	44.9 ± 65.5
NH_4 (μM)	0.4–2.5	0.7 ± 0.6	0.2–12.9	2.1 ± 2.0	5.8–63.7	14.7 ± 13.2	8.6–47.8	15.6 ± 8.1
TO_xN (μM)	0.7–8.2	4.6 ± 0.6	0.3–4.3	2.1 ± 0.3	0.2–0.6	0.4 ± 0.03	0.1–2.1	0.8 ± 0.2
SRP (μM)	0.02–3.8	1.6 ± 0.7	0.2–0.9	0.4 ± 0.1	0.5–3.0	1.4 ± 0.1	0.02–3.9	1.1 ± 0.8

DO dissolved oxygen, *NH_4* ammonium, TO_xN total oxidised nitrogen, nitrate + nitrite, *SD* standard deviation, *SRP* soluble reactive phosphorus, *TSS* total suspended solids

Table 2). Both waterbody types were alkaline (mean pH 8.0–9.0). The mean water temperature ranged from 13.2°C (winter) to 27.9°C (summer) for micro-estuaries and a smaller range was recorded for micro-outlets, namely 13.8°C (winter) to 22.7°C (summer). The dissolved oxygen (DO) concentration was generally higher in winter for micro-outlets (range 8.2–10.5 mg l^{-1}) when compared to micro-estuaries (6.1–10.1 mg l^{-1}; Table 1).

Turbidity was higher in spring (micro-estuary mean 167.9 NTU) and winter (micro-outlet mean 70.6 NTU) and lower in autumn for both ecosystem types (< 3.0 NTU). Mean total suspended solids, TO_xN and SRP were generally higher for the micro-estuaries, with NH_4 being higher for micro-outlets (Table 1). There were significant seasonal differences (ANOVA, $P < 0.05$) in all environmental variables for both system types. Overall, environmental conditions

Table 2 Two-way analysis of variance (ANOVA) based on environmental variables and size-fractionated chl-*a* concentration to identify differences among the two main waterbody types, viz. micro-outlets versus micro-estuaries

Variable	Season		Type		Season × Type	
	$F_{(3,75)}$	P	$F_{(1,77)}$	P	$F_{(3,75)}$	P
Environmental						
Temperature	**75.279**	**< 0.001**	**8.724**	**0.004**	**5.379**	**0.002**
Conductivity	**4.147**	**0.009**	3.899	0.052	**3.188**	**0.029**
Salinity	**5.497**	**0.002**	**13.836**	**< 0.001**	**4.466**	**0.006**
DO	**6.402**	**0.001**	**6.516**	**0.013**	0.29	0.832
pH	**9.868**	**< 0.001**	0.009	0.927	1.037	0.381
Water depth	**5.912**	**0.001**	0.931	0.338	0.087	0.967
Turbidity	**5.952**	**0.001**	2.243	0.139	1.674	0.181
TSS	**3.44**	**0.021**	2.444	0.122	0.661	0.579
SRP	**7.747**	**< 0.001**	0.509	0.478	1.759	0.163
TOxN	**24.774**	**< 0.001**	0.858	0.358	1.665	0.182
NH_4	**25.812**	**< 0.001**	**6.049**	**0.016**	1.703	0.174
K_d	0.869	0.461	0.991	0.323	0.732	0.536
PAR top	**3.925**	**0.012**	0.311	0.579	1.273	0.29
PAR bottom	**7.272**	**< 0.001**	0.035	0.852	1.729	0.169
Chlorophyll-*a*						
Picoplankton	**4.47**	**0.006**	0.096	0.757	1.416	0.245
Nanoplankton	**5.069**	**0.003**	0.819	0.368	1.223	0.308
Microplankton	**4.529**	**0.006**	**8.298**	**0.005**	**4.596**	**0.005**
Community metrics						
Taxa richness	**25.843**	**< 0.001**	2.477	0.12	1.732	0.168

Bold values indicate significant differences ($P < 0.05$)

differed between seasons (PERMANOVA Pseudo-$F_{(2,76)} = 8.327$, $P < 0.001$) and waterbody type (Pseudo-$F_{(1,77)} = 3.478$, $P = 0.001$), but there was also a significant interactive effect between season and waterbody type (Pseudo-$F_{(3,75)} = 1.787$, $P = 0.023$).

Light attenuation coefficients (K_d) in the micro-estuaries peaked during spring (93.4 m^{-1}) before decreasing to a low of 5.2 m^{-1} in summer (Fig. 4b). The K_d coefficient decreased from winter (10.2 m^{-1}) to summer (3.1 m^{-1}). Coefficients for both micro-estuaries and micro-outlets increased gradually from a summer low to an autumn high (Fig. 4b), but no significant differences ($P > 0.05$) across seasons and waterbody types were recorded (Table 2). For the micro-estuaries, surface irradiance (PAR) varied between 366.6 and 560.2 µmol m^{-2} s^{-1}, while bottom irradiance (PAR) levels ranged between 78.6 and 281.4 µmol m^{-2} s^{-1}. Peak irradiance for both surface and bottom values was recorded in summer (Fig. 4c, d). In the micro-outlets, surface irradiance ranged between 68.5 (summer) and 534.4 µmol m^{-2} s^{-1} (spring), while the bottom irradiance levels were 26.7 (winter) to 461.1 µmol m^{-2} s^{-1} (summer; Fig. 4c, d). Strong seasonal differences were recorded for top and bottom PAR values in micro-outlets (Table 2).

Phytoplankton communities

A total of 341 phytoplankton taxa belonging to 12 phyla and 77 families were identified from 130 different genera. Bacillariophyta was the most abundant division (161 species), followed by the Chlorophyta (62 taxa), Euglenophyta (50 taxa) and Cyanobacteria (30 taxa). A total of 260 (63 families) and 244 (74 families) taxa were identified within the micro-estuaries and micro-outlets, respectively. Unique phytoplankton taxa for the micro-estuaries and micro-outlets numbered 77 and 108 taxa, respectively (Table S1). The most abundant species in micro-estuaries were *Euglena* sp. 1, *Microcystis flos-aquae* (Wittrock) Kirchner, *Chlorella minutissima* Fott & Nováková, *Monoraphidium contortum* (Thuret) Komárková-Legnerová, *Trachelomonas volvocina*

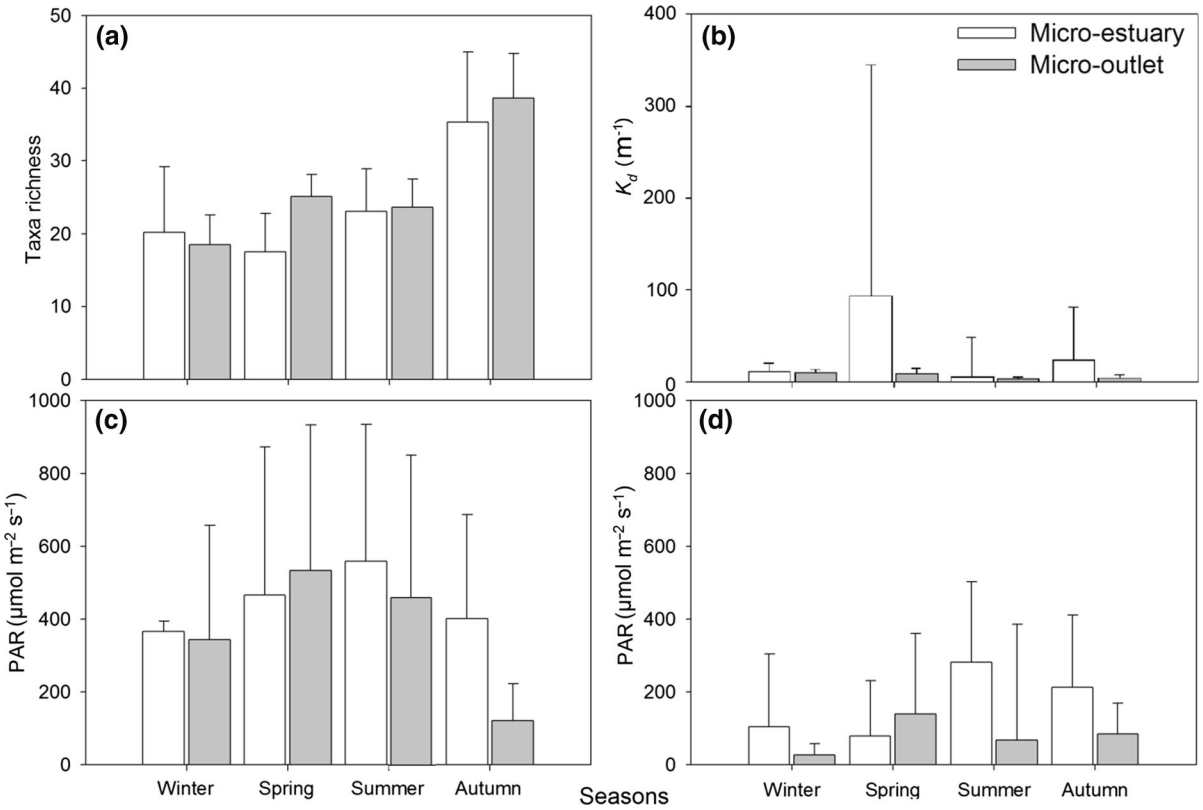

Fig. 4 Variation in **a** phytoplankton taxa richness, **b** light attenuation coefficient (K_d), **c** photosynthetic available radiation (PAR) top and **d** PAR bottom among the micro-estuaries and micro-outlets. Error bars represent standard deviations

var. *subglobosa* Lemmermann, *Scenedesmus communis* E.Hegewald and *Amphora coffeaeformis* (C.Agardh) Kützing, while *Euglena* sp. 1, *Chroococcus dispersus* (Keissler) Lemmermann, *Chlamydomonas crassa* H.R.Christen, *Euglena ehrenbergii* G.A.Klebs, *Peridinium* sp., *Trachelomonas* sp. 1, *Merismopedia glauca* (Ehrenberg) Kützing and *Anabaena* sp. 1 were most abundant in micro-outlets. Taxa richness increased from winter to autumn (Fig. 4a), with a strong seasonal variation (ANOVA, $P < 0.05$) but no significant difference between waterbody types (Table 2).

The *n*-MDS ordination based on phytoplankton taxa (all seasons pooled) was discriminated among waterbody types (stress values of 0.20 indicated a useful two-dimensional representation of the groups; Fig. 5). The strong overlap observed between micro-estuaries and micro-outlets, especially during winter and some autumn and summer sites, could be attributed to marine water intrusion, causing the freshwater-dominated micro-outlet systems to attain short-term salinity levels similar to with those measured in the micro-estuaries (Fig. 5). The sites were separated on a salinity gradient along the first axis, with *Staurosirella pinnata* (Ehrenberg) D.M.Williams & Round, *Oscillatoria* sp., *Cyclotella meneghiniana* Kützing and *Diploneis elliptica* (Kützing) Cleve associated with less saline sites (i.e. mostly freshwater-dominated micro-outlets), whereas *Chlorella minutissima* Fott & Nováková, *M. flos-aquae, Euglena oblonga* F.Schmitz and *Achnanthidium minutissimum* (Kützing) Czarnecki were associated with more saline sites (i.e. mostly micro-estuaries).

The similarity percentage (SIMPER) analysis revealed an overall average dissimilarity between micro-estuaries and micro-outlets of 94% (Table 3). Approximately 74 phytoplankton taxa caused about 60% of the dissimilarity observed, with 18 taxa each contributing > 1% of the dissimilarity between micro-estuaries and micro-outlets (Table 3). Based on PERMANOVA analyses, phytoplankton communities varied across seasons (Pseudo-$F_{(1,77)}$ = 3.190,

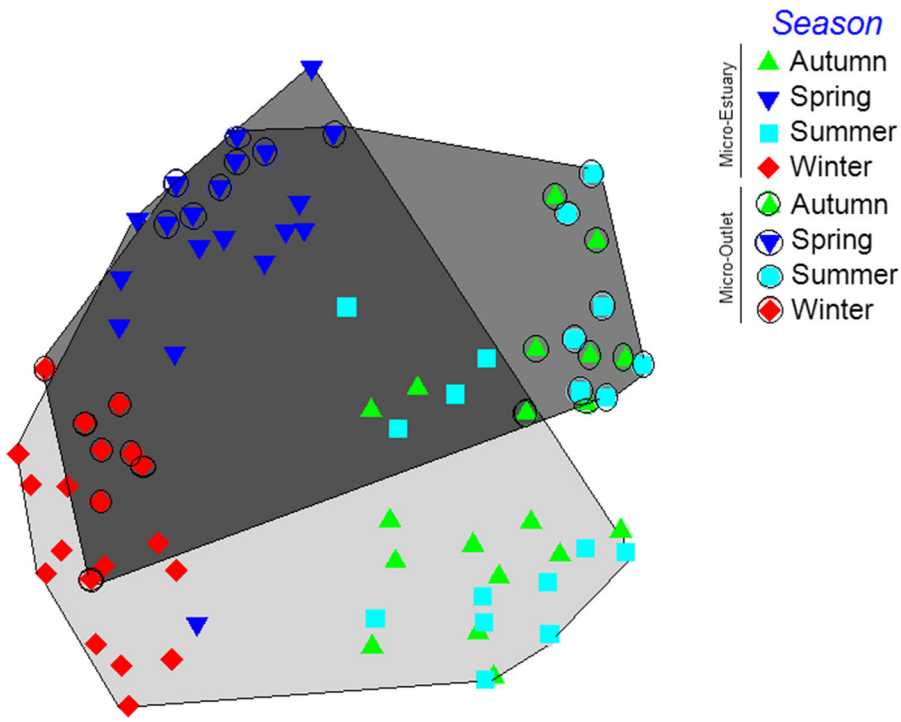

Fig. 5 *n*-MDS ordination highlighting variation of phytoplankton communities across seasons and waterbody types. Polygons indicate the two waterbodies: light grey—micro-estuaries and dark grey—micro-outlets

Table 3 SIMPER results (> 1% dissimilarity contribution) for dissimilarity (93.87%) in phytoplankton taxa between waterbody types, i.e. between micro-estuaries and micro-outlets

Species	Av. Dis.	Contrib.%	Cum.%
Euglena sp. 1	2.90	3.09	3.09
Trachelomonas sp. 1	1.92	2.05	5.13
Peridinium sp.	1.56	1.66	6.79
Chroococcus dispersus (Keissler) Lemmermann	1.52	1.61	8.40
Navicula erifuga Lange-Bertalot	1.44	1.53	9.93
Achnanthidium crassum (Hustedt) Potapova & Ponader	1.24	1.32	11.25
Euglena sp. 2	1.20	1.28	12.53
Achnanthidium minutissimum (Kützing) Czarnecki	1.19	1.27	13.8
Amphora coffeaeformis (Agardh) Kützing	1.15	1.22	15.03
Staurosirella pinnata (Ehrenberg) D.M.Williams & Round	1.13	1.20	16.23
Anabaena sp. 1	1.07	1.14	17.37
Trachelomonas volvocina (Ehrenberg) Ehrenberg	1.04	1.11	18.48
Trentepohlia sp.	1.03	1.09	19.57
Microcystis flos-aquae (Wittrock) Kirchner	1.03	1.09	20.67
Merismopedia glauca (Ehrenberg) Kützing	0.99	1.05	21.72
Unidentified filamentous algae sp. 2	0.98	1.04	22.76
Chaetomorpha crassa (Agardh) Kützing	0.96	1.02	23.78
Trachelomonas sp. 2	0.94	1.00	24.78

Av. Dis. average dissimilarity, *Contrib.%* percentage contribution, *Cum.%* percentage cumulative contribution

$P < 0.001$) and waterbody types (Pseudo-$F_{(3,75)} = 3.682$, $P < 0.001$). Using PERMANOVA pair-wise comparisons, strong similarities in the phytoplankton communities within each system were recorded for summer versus autumn ($t = 0.609$, $P = 0.9963$), but with significant differences ($P < 0.01$) being documented across all other seasons.

Phytoplankton biomass

The chl-*a* concentration, as a proxy of phytoplankton biomass, showed significant variation among seasons (PERMANOVA, Pseudo-$F_{(3,75)} = 13.811$, $P < 0.001$) and waterbody types (PERMANOVA, Pseudo-$F_{(1,77)} = 7.673$, $P < 0.001$; Figs. 5 and S1). Generally, the highest chl-*a* concentrations were recorded during winter and the lowest during summer. Pico- (0.7–2 μm) and nanoplankton (2–20 μm) were the dominant phytoplankton size fractions in micro-estuaries, with the latter being more prevalent. Micro- (> 20 μm) and nanoplankton (2–20 μm) were the dominant phytoplankton size fractions in micro-outlets (Fig. 6). Two-way ANOVAs showed significant differences ($P < 0.01$) among respective chl-*a* concentrations (i.e. phytoplankton size fractions among seasons), whereas microplankton (> 20 μm) was significantly different among the different waterbody types ($F = 8.298$, $P = 0.005$; Fig. 6, Table 2).

Discussion

Phytoplankton community structure was found to be significantly different across the different waterbody types, thereby supporting the hypothesis that the phytoplankton community could potentially be used to identify the differences between micro-estuaries and micro-outlets. The communities were highly seasonal across the two waterbody environments and exhibited strong oscillations in structure (see Table S2 for dominant taxa), with turnover from one unique phytoplankton community type to another occurring across seasons. Hence, it seems that seasonality played a key role in promoting spatio-temporal diversity of phytoplankton communities within micro-outlets and micro-estuaries.

We also found that collectively the phytoplankton across all systems (both micro-estuaries and micro-outlets) was highly diverse, with the total number of genera ($n = 130$) for both types of systems (see Table S1) being higher than that recorded within much larger and permanently open Eastern Cape estuaries such as the Kowie (Dalu et al., 2014) and Sundays (Janse van Vuuren & Taylor, 2015). This could be attributed to wind-mixing which plays an important role in phytoplankton community composition due to turbulence causing bottom water, loaded with nutrients and some benthic diatoms, to be upwelled to the water surface (Demers et al., 1987; Abbate et al., 2017), as well as high freshwater inflows bringing freshwater taxa into these systems (see Snow & Adams, 2007; Lemley et al., 2016). Such processes highlighted above could have resulted in the continuous physical mixing of freshwater, estuarine and marine phytoplankton species, thus creating an 'apparent' high diversity. The number of phytoplankton taxa was, however, very similar or slightly lower for each individual micro-estuary and/or micro-outlet when compared to individual larger permanently open estuaries within the region (e.g. Dalu et al., 2014; Janse van Vuuren & Taylor, 2015).

The higher fish and zooplankton biomass recorded in the micro-estuaries compared to micro-outlets (Magoro et al., *unpublished data*) could partially explain the differences observed in phytoplankton community structure. The phytoplankton biomass decreased from winter, through spring and summer, to autumn in both system types, possibly due to increased grazing by invertebrates and heterotrophic activity during spring and summer. A similar pattern to that described above has been recorded by Tucker et al. (1984), Livingston et al. (2002) and Domingues et al. (2011). It is noteworthy that the phytoplankton biomass in the micro-systems investigated in our study was generally lower than that recorded in Eastern Cape river systems (see Dalu et al., 2014 for a freshwater—estuarine chl-*a* concentration comparison). In addition, the slightly higher pH levels observed during spring and summer for both micro-estuaries and micro-outlets might also have had a profound negative effect on phytoplankton productivity (see Hinga, 2002).

Significant differences in phytoplankton biomass occurred across the two waterbody types, with the microplankton size fraction being significantly different between micro-estuaries and micro-outlets. Phytoplankton biomass (chl-*a* concentration) recorded during this study in micro-estuaries and micro-outlets was relatively similar to that observed in smaller temporarily open/closed ecosystems (Nozais et al., 2001; Perissinotto et al., 2002; Kruger & Strydom, 2011; Dalu et al., 2014). The micro-estuaries and micro-outlets generally followed a similar trend to what has been observed in the Kowie (Dalu et al., 2014) and St Lucia (Perissinotto et al., 2010) estuaries, where nano- and picoplankton were the dominant

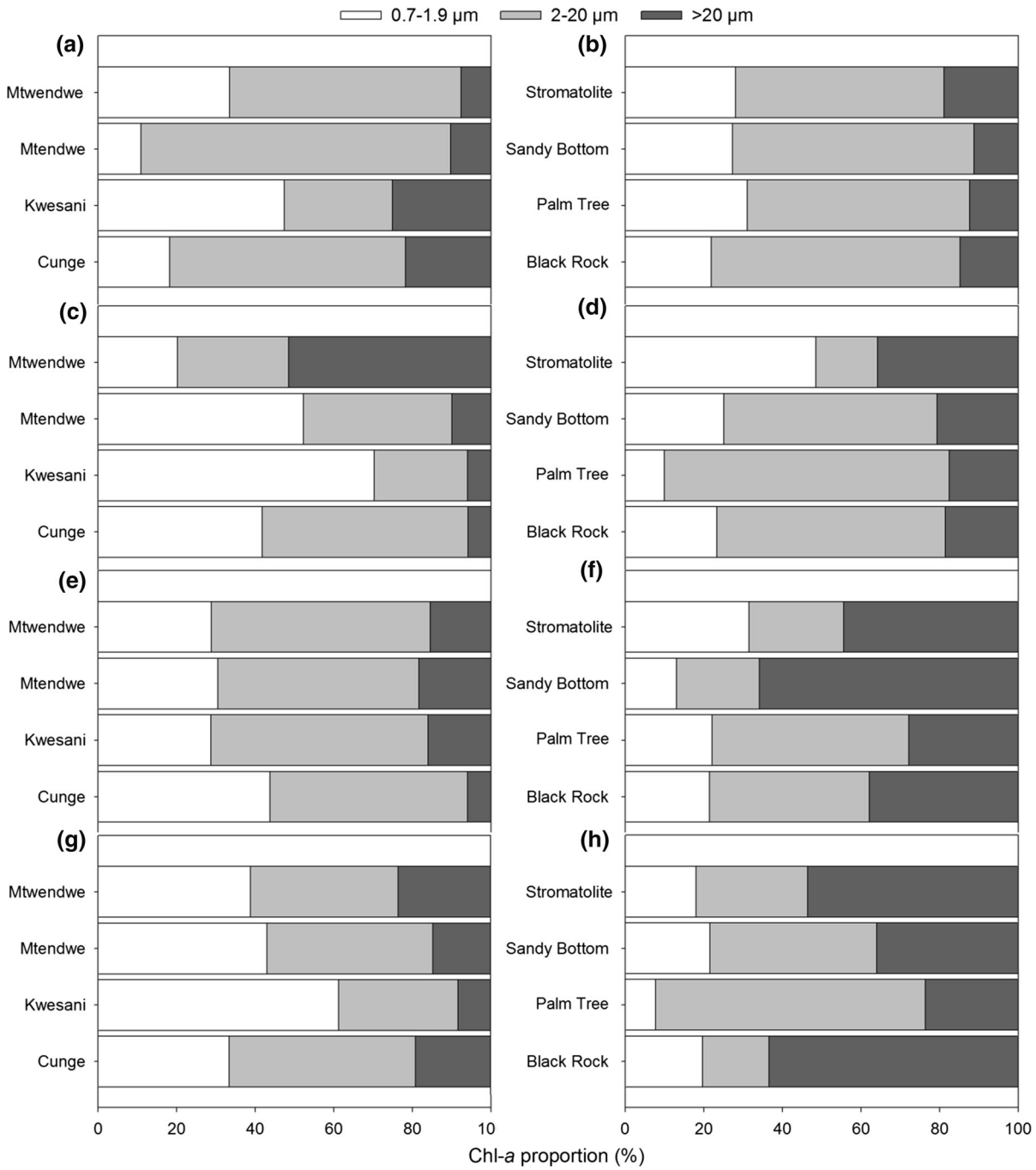

Fig. 6 Spatial and temporal variation in phytoplankton size fractions among study sites along the warm temperate region of South Africa during the study seasons: **a, b** winter, **c, d** spring, **e, f**, and **g, h** autumn; micro-estuaries—Cunge, Kwesani, Mtendwe, Mtwendwe; micro-outlets—Black Rock, Palm Tree, Sandy Bottom, Stromatolite

groups. However, for the micro-outlets, there was a switch in dominance from nano- and picoplankton (winter, spring) to pico- and microplankton (summer, autumn). This switch was also highlighted by changes in phytoplankton community composition, as some species were lost and replaced by new ones. That is,

there were different species occupying the same site during different seasons in micro-estuaries, thus increasing the total species richness for these systems, a common phenomenon in predictably seasonal environmental regimes, such as in these micro-systems (Tonkin et al., 2017).

When nutrient supply inputs are sudden, as is expected for the micro-estuaries influenced by intermittent or pulsed flow events (i.e. freshwater or marine inputs), phytoplankton are expected to be characterised by alternating succession dynamics, a phenomenon well documented for temperate inland water bodies (Roelke & Spatharis, 2015). In our study, the increased frequency and duration of inflows (see Human et al. (2018) for more detailed information) was concurrent with the emergence of filamentous algal mats. The potential for flow inputs to be a significant predictor of algal abundance has also been shown by Hensley & Cohen (2017). When nutrient supply is suddenly increased due to pulsed river flow or tidal influence, phytoplankton communities will show alternating successional patterns driven by nutrient availability and competition for available resources (Roelke & Spatharis, 2015). Therefore, the Euglenophyta (i.e. *Euglena* spp. and *Trachelomonas* spp.) were observed across all seasons, with peak dominance during winter after the floods.

During open-mouth conditions in winter and spring (with exception of Kwesani), the phytoplankton communities comprised a high number of brackish water species that were completely different to those observed under closed mouth conditions when more freshwater taxa were dominant (see Table S2). It was further noted that seasonal switching in phytoplankton communities was reflected not only in dominance patterns in both habitat types, but, more clearly in the complete replacement of some species in micro-outlets. Such temporal turnover, which is often accompanied by predictably seasonal changes in environmental conditions, can promote overall biodiversity by allowing more species to coexist in a single environment through temporal niche segregation (Tonkin et al., 2017). Indeed, we found this in our study, with greater overall diversity in micro-outlets (Table S1).

In micro-estuaries, which we presume to be similar to other estuary types where nutrients are usually non-limiting, the observed phytoplankton taxa succession was restricted to the Margalef (1958) first-stage succession model (i.e. small cells with high growth rates), which involves typical species for frequently destabilised environments (Levasseur et al., 1984). Our results demonstrate that the water column destabilisation frequency, due to intermittent and regular tidal action in micro-outlets and micro-estuaries, respectively (see Fig. 3), along with freshwater inflow events, controls the growth rates of phytoplankton cells through nutrient additions, as highlighted by Snow & Adams (2007) and Snow & Bate (2009). Light intensity determines the occurrence of non-motile forms such as diatoms due to photosynthetic requirements, and temperature sets the conditions for optimal metabolic activity. These drivers, including salinity, will determine phytoplankton composition and succession, e.g. flagellate numbers and diatom species succession (Levasseur et al., 1984). This finding was especially true for the micro-estuaries, where a phytoplankton succession was more evident, with the dominance of Chlorophyta in winter changing to Bacillariophyta dominance in spring and Cyanobacteria in summer (Table S2). However, in the micro-outlets, while the dominance of different individual taxa changed, Chlorophyta remained the dominant class during all seasons.

In conclusion, the distinct seasonality in both micro-outlets and micro-estuaries allowed a considerable number of phytoplankton species to coexist through temporal turnover in assemblages between the different seasons. Surprisingly, despite the ephemeral nature of micro-outlets over both short and longer time scales, these systems are highly diverse and rich in phytoplankton species that are representative of marine, estuarine and freshwater sources. The clear differences in community structure across the different waterbody types lend support to the hypothesis that phytoplankton communities could be used to identify differences between micro-estuaries and micro-outlets. These results highlight the importance of valuing and protecting these coastal ecosystems, which harbour considerable biodiversity and may play an important role in maintaining connectivity among larger coastal waterbodies globally.

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References

Abbate, M. C. L., J. C. Molinero, V. A. Guinder, G. M. Perillo, R. H. Freije, U. Sommer, C. V. Spetter & J. E. Marcovecchio, 2017. Time-varying environmental control of phytoplankton in a changing estuarine system. Science of the Total Environment 609: 1390–1400.

Adams, J. B. & G. C. Bate, 1999. Primary producers—Estuarine microalgae. In Allanson, B. & D. Baird (eds), Estuaries of South Africa. Cambridge University Press, Cambridge.

Allanson, B. R. & D. Baird (eds), 1999. Estuaries of South Africa. Cambridge University Press, Cambridge.

Anandraj, A., R. Perissinotto & C. Nozais, 2007. A comparative study of microalgal production in a marine versus a river-dominated temporarily open/closed estuary, South Africa. Estuarine, Coastal and Shelf Science 73: 768–780.

Anandraj, A., R. Perissinotto, C. Nozais & D. Stretch, 2008. The recovery of microalgal production and biomass in a South African temporarily open/closed estuary, following mouth breaching. Estuarine, Coastal and Shelf Science 79: 599–606.

Anderson, M. J., 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecology 26: 32–46.

Anderson, M. J. & C. J. F. ter Braak, 2003. Permutation tests for multi-factorial analysis of variance. Journal of Statistical Computation and Simulation 73: 85–113.

Anderson, M. J., R. N. Gorley & K. R. Clarke, 2008. PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods. PRIMER-E, Plymouth.

Bate, G. C. & B. V. Heelas, 1975. Studies on the nitrate nutrition of two indigenous Rhodesian grasses. Journal of Applied Ecology 12: 941–952.

Bate, G. C., M. Nunes, B. Escott, A. Mnikathi & J. Craigie, 2017. Micro-estuary—a new estuary type recognised for South African conditions. Transactions of the Royal Society of South Africa 72: 85–92.

Dalu, T. & P. W. Froneman, 2016. Diatom based water quality monitoring in Africa: challenges and future prospects. Water SA 42: 551–559.

Dalu, T., P. W. Froneman & N. B. Richoux, 2014. Phytoplankton community diversity along a river–estuary continuum. Transactions of the Royal Society of South Africa 69: 107–116.

Dalu, T., N. B. Richoux & P. W. Froneman, 2016. Distribution of benthic diatom communities in a permanently open temperate estuary, in relation to physico-chemical variables. South African Journal of Botany 107: 31–38.

Dalu, T., J. B. Adams, J. C. Taylor, G. C. Bate, M. Nunes, P. W. Froneman & R. J. Wasserman, 2018. An overview of the ecology and status of estuarine microphytobenthos research in South Africa. African Journal of Marine Science 40: 1–12.

Demers, S., J. C. Therriault, E. Bourget & A. Bah, 1987. Resuspension in the shallow sublittoral zone of a macrotidal estuarine environment: wind influence. Limnology and Oceanography 32: 327–339.

Domingues, R. B., A. B. Barbosa, U. Sommer & H. M. Galvão, 2011. Ammonium, nitrate and phytoplankton interactions in a freshwater tidal estuarine zone: potential effects of cultural eutrophication. Aquatic Sciences 73: 331–343.

Du, Y., L. Yang & S. P. Xie, 2011. Tropical Indian Ocean influence on northwest Pacific tropical cyclones in summer following strong El Niño. Journal of Climate 24: 315–322.

Fisher, T. R., L. W. Harding Jr., D. W. Stanley & L. G. Ward, 1988. Phytoplankton, nutrients, and turbidity in the Chesapeake, Delaware, and Hudson estuaries. Estuarine, Coastal and Shelf Science 27: 61–93.

Fonge, A. B., B. G. Chuyong, A. S. Tening, A. C. Fobid & N. F. Numbisi, 2013. Seasonal occurrence, distribution and diversity of phytoplankton in the Douala Estuary. Cameroon, African Journal of Aquatic Science 38: 123–133.

Hensley, R. T. & M. J. Cohen, 2017. Flow reversals as a driver of ecosystem transition in Florida's springs. Freshwater Science 36: 14–25.

Hilmer, T. & G. C. Bate, 1991. Vertical migration of a flagellate-dominated bloom in a shallow South African estuary. Botanica Marina 34: 113–121.

Hinga, K. R., 2002. Effects of pH on coastal marine phytoplankton. Marine Ecology Progress Series 238: 281–300.

Human, L. R. D., M. Magoro, T. Dalu, R. Perissinotto, A. K. Whitfield, J. B. Adam, S. H. P. Deyzel & G. M. Rishworth, 2018. Natural enrichment in pristine micro-estuaries and micro-outlets. Science of the Total Environment 624: 945–954.

Janse van Vuuren, S. & J. C. Taylor, 2015. Changes in the algal composition and water quality of the Sundays River, Karoo, South Africa, from source to estuary. African Journal of Aquatic Science 40: 339–357.

John, D., B. Whitton & A. Brook (eds), 2002. The freshwater algal flora of the British Isles: an identification guide to freshwater and terrestrial algae. Cambridge University Press, Cambridge.

Ke, Z. X., Y. H. Tan, L. M. Huang, J. L. Zhang & S. M. Lian, 2012. Relationship between phytoplankton composition and environmental factors in the surface waters of southern South China Sea in early summer of 2009. Acta Oceanologica Sinica 31: 109–119.

Ke, Z., Y. Tan, Y. Ma, L. Huang & S. Wang, 2014. Effects of surface current patterns on spatial variations of phytoplankton community and environmental factors in Sunda shelf. Continental Shelf Research 82: 119–127.

Kopke, D., 1988. The climate of the Eastern Cape. In Bruton, M. N. & F. W. Gess (eds), Towards an Environmental Plan for the Eastern Cape. Rhodes University, Grahamstown.

Kruger, M. & N. A. Strydom, 2011. Plankton dynamics associated with the convergence zone of a shear front in the permanently open Kowie Estuary, South Africa. African Zoology 46: 47–59.

Kruskal, J. B. & M. Wish, 1978. Multidimensional Scaling. Sage University Paper series on Quantitative Applications in the Social Sciences. Sage Publishers, Beverly Hills and London.

Kühl, M., C. Lassen & N. P. Revsbech, 1997. A simple light meter for measurements of PAR (400–700 nm) with fiber-optic microprobes: application for P vs E0 (PAR) measurements in a microbial mat. Aquatic Microbial Ecology 13: 197–207.

Lemley, D. A., J. B. Adams & G. C. Bate, 2016. A review of microalgae as indicators in South African estuaries. South African Journal of Botany 107: 12–20.

Levasseur, M., J.-C. Therriault & C. Legendre, 1984. Hierarchical control of phytoplankton succession by physical factors. Marine Ecology Progress Series 19: 211–222.

Livingston, R. J., A. K. Prasad, X. Niu & S. E. McGlynn, 2002. Effects of ammonia in pulp mill effluents on estuarine phytoplankton assemblages: field descriptive and experimental results. Aquatic Botany 74: 343–367.

Longhurst, A., S. Sathyendranath, T. Platt & C. Caverhill, 1995. An estimate of global primary production in the ocean from satellite radiometer data. Journal of Plankton Research 17: 1245–1271.

Lutjeharms, J. R. E., 2006. The Agulhas Current. Springer-Verlag, Heidelberg.

Margalef, R., 1958. Temporal succession and spatial heterogeneity in phytoplankton. In Buzzati-Traverso, A. A. (ed), Perspective in Marine Biology. University of California Press, Berkeley and Los Angeles.

Nixon, S. W., C. Oviatt, J. Frithsen & B. Sullivan, 1986. Nutrients and the productivity of estuarine and coastal marine ecosystems. Journal of the Limnological Society of South Africa 12: 43–71.

Nozais, C., R. Perissinotto & S. Mundree, 2001. Annual cycle of microalgal biomass in a South African temporarily-open estuary: nutrient versus light limitation. Marine Ecology Progress Series 223: 39–48.

Pan, C.-W., Y.-L. Chuang, L.-S. Chou, M.-H. Chen & H.-J. Lin, 2016. Factors governing phytoplankton biomass and production in tropical estuaries of western Taiwan. Continental Shelf Research 118: 88–99.

Parsons, T. R., Y. Maita & C. M. Lalli, 1984. A Manual of Chemical and Biological Methods for Seawater Analysis. Pergamon Press, New York.

Perissinotto, R. & C. Nozais, 2002. Spatio-temporal dynamics of phytoplankton and microphytobenthos in a South African temporarily-open estuary. Estuarine, Coastal and Shelf Science 55: 47–58.

Perissinotto, R., D. Pillay & G. Bate, 2010. Microalgal biomass in the St. Lucia Estuary during the 2004–2007 drought period. Marine Ecology Progress Series 405: 147–161.

Roelke, D. L. & S. Spatharis, 2015. Phytoplankton succession in recurrently fluctuating environments. PLoS One 10: e0121392.

Roy, P. S., R. J. Williams, A. R. Jones, I. Yassini, P. J. Gibbs, B. Coates, R. J. West, P. R. Scanes, J. P. Hudson & S. Nichol, 2001. Structure and function of south-east Australian estuaries. Estuarine, Coastal and Shelf Science 53: 351–384.

Sand-Jensen, K. & J. Borum, 1991. Interactions among phytoplankton, periphyton, and macrophytes in temperate freshwaters and estuaries. Aquatic Botany 41: 137–175.

Sieburth, J. M., V. Smetacek & J. Lenz, 1978. Pelagic ecosystem structure: heterotrophic compartments of the plankton and their relationship to plankton size fractions. Limnology and Oceanography 23: 1256–1263.

Snow, G. C. & J. B. Adams, 2007. Relating microalgal spatial patterns to flow, mouth and nutrient status in the temporarily open/closed Mngazi estuary, South Africa. Marine and Freshwater Research 58: 1032–1043.

Snow, G. C. & G. C. Bate, 2009. Response of the microalgae to changes in freshwater inflow in the Berg Estuary, southern Africa. Transactions of the Royal Society of South Africa 64: 189–203.

SPSS Inc., 2007. SPSS Release 16.0.0 for Windows. Polar Engineering and Consulting. SPSS Inc., Chicago.

Taylor, J. C., W. R. Harding & C. G. M. Archibald, 2005. A Methods Manual for the Collection, Preparation and Analysis of Diatom Samples. Version 1.0. WRC Report No. TT 281/07. Water Research Commission, Pretoria.

Taylor, J. C., W. R. Harding & C. G. M. Archibald, 2007. An illustrated guide to some common diatom species from South Africa. WRC Report TT 282/07. Water Research Commission, Pretoria.

Thomas, C. M., R. Perissinotto & I. Kibirige, 2005. Phytoplankton biomass and size structure in two South African eutrophic, temporarily open/closed estuaries. Estuarine, Coastal and Shelf Science 65: 223–238.

Tonkin, J. D., M. T. Bogan, N. Bonada, B. Rios-Touma & D. A. Lytle, 2017. Seasonality and predictability shape temporal species diversity. Ecology 98: 1201–1216.

Tucker, C. S., S. W. Lloyd & R. L. Busch, 1984. Relationships between phytoplankton periodicity and the concentrations of total and unionized ammonia in channel catfish ponds. Hydrobiologia 111: 75–79.

van der Molen, J. S. & R. Perissinotto, 2011. Microalgal productivity in an estuarine lake during a drought cycle: The St. Lucia Estuary, South Africa. Estuarine, Coastal and Shelf Science 92: 1–9.

van Ginkel, C. E., 2012. Algae, phytoplankton and eutrophication research and management in South Africa: past, present and future. African Journal of Aquatic Science 37: 17–25.

Vinayachandran, P. N. & S. Mathew, 2003. Phytoplankton bloom in the Bay of Bengal during the northeast monsoon and its intensification by cyclones. Geophysical Research Letters 30: 1572.

Welschmeyer, N. A., 1994. Fluorometric analysis of chlorophyll *a* in the presence of chlorophyll *b* and pheopigments. Limnology and Oceanography 39: 1985–1992.