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Harmful Algal Blooms Negatively Impact *Mugil cephalus* Abundance in a Temperate Eutrophic Estuary

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Abstract

The increased frequency and magnitude of harmful algal blooms (HABs) from anthropogenic nutrient enrichment is of great concern in estuarine systems worldwide. Despite estuaries being important fish nurseries, a paucity of knowledge exists on the impacts of HABs on estuarine fishes due to the sporadic and unpredictable nature of HABs. Thus, this study aimed to determine the frequency and magnitude of HABs and the subsequent impact on the abundance of a common estuarine-dependent Mugilidae species, *Mugil cephalus*, in the shallow low inflow and agriculturally altered warm temperate Sundays Estuary in South Africa. The sampling design adopted a monthly (monitoring over 2 years between 2018 and 2020) and twice weekly (for 7 weeks during known spring/summer HABs in 2018) day and night sampling approach. It was hypothesised that *Mugil cephalus* abundance would be negatively related to phytoplankton biomass since HABs would result in unfavourable daytime supersaturated dissolved oxygen surface water and night-time bottom-water hypoxia. This study found that the Sundays Estuary was dominated by the HAB species, *Heterosigma akashiwo*, which significantly altered the dissolved oxygen in the estuary resulting in frequent supersaturated surface waters and hypoxic bottom waters. *Mugil cephalus* abundances were negatively related to phytoplankton biomass, with instances of high abundance (CPUE > 10) only being found at sites characterised as not experiencing severe bloom conditions (< 60 Chl-*a* $\mu g.L^{-1}$). This avoidance of HABs suggests that if unsustainable levels of agricultural fertilisation continue, resulting in more severe and frequent HABs, a major decline in fish nursery quality in the estuary may occur.

Keywords Eutrophication · *Heterosigma akashiwo* · Hypoxia · Nursery habitat · Phytoplankton

Introduction

Eutrophication in estuaries is evidenced by high primary productivity resulting from nutrient enrichment, often manifesting as noxious algal blooms, oxygen depletion and benthic fauna mortality (Nixon 1995). Eutrophication has been reported from a variety of coastal marine ecosystems worldwide, and one of the major concerns is that the frequency and extent is increasing in coastal waters affected by anthropogenically altered riverine inflows (Nixon 1995; Cloern 2001; Heisler et al. 2008). The eutrophic condition of estuaries can be determined by assessing phytoplankton biomass, abundance and composition (Heisler et al. 2008; Coutinho et al. 2012; Lemley et al. 2016) because they play a vital role in the functioning of aquatic ecosystems, as they are largely responsible for directly or indirectly fuelling food webs through primary production (Nixon 1995).

Estuaries are particularly vulnerable to these pressures as they are transitional waters and need to endure the combined pressures from both marine and freshwater environments (Elliott and Whitfield 2011). These threats include the increase in waste-water production from domestic and industrial sources, as well as aquaculture and agricultural runoff, all of which leads to an increase in nutrient loading (phosphorus and nitrogen) and modifications to natural freshwater inflow patterns (Heisler et al. 2008). This,

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in turn, impacts phytoplankton biomass and community structure. The combination of nutrient enrichment, altered water residence times and subsequent temperature variations often results in enhanced algal growth rates and biomass (Eppley 1972; Heisler et al. 2008), which can lead to a change in water quality (Anderson and Taylor 2001; Cloern 2001; Coutinho et al. 2012).

Phytoplankton growth and biomass accumulation is generally limited by nutrients such as nitrogen and phosphorous. An increase in available limiting nutrients theoretically leads to increased phytoplankton growth, leading to an imbalance between algal production and consumption (Cloern 2001). This is followed by increased sedimentation of algal-derived organic matter, which stimulates microbial decomposition and oxygen consumption, resulting in the depletion of bottom-water oxygen (Anderson and Taylor 2001; Cloern 2001; Coutinho et al. 2012). Furthermore, because an increase in algal biomass often leads to a single species dominating the community, the likelihood of harmful algal bloom (HAB) formation is amplified (Heisler et al. 2008; Coutinho et al. 2012). Therefore, phytoplankton bloom phenomena have been used to assess the state of eutrophication in estuaries (Coutinho et al. 2012).

Recently, Lemley et al. (2017b) found that the phytoplankton composition of the eutrophied warm temperate Sundays Estuary, South Africa, is primarily driven by temperature, with multiple recurrent monospecific blooms of *Heterosigma akashiwo* exceeding 100 µg chl-*a* L^{-1} observed in the poly- to the mesohaline zone during the spring and summer period. These HABs coincided with the first account of seasonal bottom-water hypoxia (< 2 mg L^{-1}) in a South African estuary. (Lemley et al. 2017b). *Heterosigma akashiwo*, class Raphidophyceae, family Chattonellaceae, is a globally occurring microalgae species that has revealed itself as a nuisance species in temperate brackish waters (Hara and Chihara 1987; Martínez et al. 2010; Singh 2018). This species is now problematic in South African estuaries such as the Sundays Estuary (Hilmer and Bate 1990; Kotsedi et al. 2012; Lemley et al. 2017b; Adams et al. 2020). It is not toxic to humans but can be deleterious to aquatic fauna directly through a variety of pathways. Studies have reported that *H. akashiwo* can suppress the growth of co-occurring phytoplankton taxa (Yamasaki et al. 2009; Lemley et al. 2018b, 2020), as well as impact the feeding, growth and survival of invertebrates (Twiner et al. 2001; Wang et al. 2006; Yu et al. 2010; Almeda et al. 2011; Basti et al. 2016) and fishes (Black et al. 1991; Taylor and Haigh 1993; Rensel et al. 2010). However, the above deleterious impacts were concentration- and species-dependent with no clear mechanism of harm (Singh 2018; Bates et al. 2020; Anderson et al. 2021). Therefore, it is probable that different strains of *H. akashiwo* exist (Fredrickson et al. 2011; Gómez et al. 2022) with different potencies of toxicity (Higashi et al. 2017; Seoane et al. 2017) as defence mechanisms against predation or competition. Therefore, emphasising the need to study the ecological implications of HABs (such as *H. akashiwo*) in estuaries to prevent the possible loss of ecosystem services.

Estuaries are highly dynamic and productive ecosystems that serve as nursery areas for economically and ecologically important fish species (Beck et al. 2001; Potter et al. 2015). For a habitat to be considered a nursery, it must enhance the growth and survival of early-life stages of fishes (Gibson 1994; Beck et al. 2001; Dahlgren et al. 2006; Vasconcelos et al. 2011). This is highly dependent on various factors, which include food availability, predation, competition pressures and abiotic factors such as temperature, salinity, dissolved oxygen and turbidity (Clemmesen 1994; Beck et al. 2001; Able 2005; Strydom 2015). Acute, direct physiological impacts of HABs on estuarine fishes occur due to the production of various ichthyotoxins such as reactive oxygen radicals. However, most impacts are indirect through changes in growth and survival due to reductions in water quality (Morrell and Gobler 2020), food availability or quality (Smit et al. 2021) and behavioural changes such as the avoidance of once good food patches due to unfavourable water quality (Bornman et al. 2021). The recruitment of larval or juvenile fishes into estuaries normally coincides with periods of high productivity; however, in eutrophied warm temperate systems like the Sundays Estuary in South Africa, this coincides with HABs. Thus, potentially negatively impacting the fish nursery role of these important systems.

Research has largely focused on the impacts of HABs on farmed fishes due to the great economic losses incurred during these events (Anderson et al. 2021). However, the impact of HABs on the functioning of natural systems such as estuaries are largely understudied, despite the apparent increase in the occurrence of HABs globally (Heisler et al. 2008; Bates et al. 2020; Griffith and Gobler 2020). Furthermore, the few studies investigating HABs in estuaries have focussed on the nutrient loading and the phytoplankton community response and only inferred possible negative impacts on fishes that utilise the same habitat (Lemley et al. 2016, 2021a; Wallace and Gobler 2021). Fish kills are regularly attributed to HAB events in estuaries worldwide; however, these are seldomly sampled due to the unpredictability of HAB events. Thus, this study aimed to determine the frequency and magnitude of HABs in the eutrophied and low inflow warm temperate Sundays Estuary, which experiences recurrent HABs linked to seasonal changes and regulated river flow regimes. Additionally, the impact of these HABs on the abundance and distribution of a common estuarine-dependent Mugilidae has not been studied despite the critical role Mugilidae play in estuarine ecosystems. The ubiquitous flathead mullet, *Mugil cephalus*, was studied along the length of the estuary in relation to HAB areas using monthly (monitoring over 2 years between 2018 and 2020) and twice weekly (for 7 weeks during known spring/summer HABs in 2018) day and night sampling.

We hypothesised that *M. cephalus* abundance would be negatively related to phytoplankton biomass since HABs would result in unfavourable daytime supersaturated dissolved oxygen surface water and night-time bottom-water hypoxia. The understanding of phytoplankton dynamics and HABs on estuarine fish populations has become important to fully elucidate due to the ever-increasing anthropogenic pressures placed on estuaries worldwide and to prevent the loss of ecosystem services that these communities provide.

Materials and Methods

Study Site

The predominantly open Sundays Estuary is located on the south-eastern coast of South Africa and is downstream to one of the world's largest citrus farming hubs (Van Niekerk et al. 2019). Nutrient-rich return flows into the estuary and a near-constant ebb flow of approximately 0.47 to 1.86 $m^3 s^{-1}$ (Lemley et al. 2017b) (due to a water transfer scheme) and lack of episodic flooding events (due to the Darlington Dam) (Lemley et al. 2017a) has resulted in the persistence of phytoplankton biomass in excess of 20 Chl-*a* $\mu g.L^{-1}$ (Lemley et al. 2018b). Recurrent monospecific blooms (> 1000 $cells.mL^{-1}$) of mainly *Heterosigma akashiwo* occurring in the spring/summer months, but also *Heterocapsa rotundata* in the winter months, have become the norm in the middle reaches of the Sundays Estuary (Kotsedi et al. 2012; Lemley et al. 2017b). Inorganic nutrient availability (nitrate and phosphate), mesohaline conditions and the presence of a well-defined halocline were found to be key bottom-up controls influencing the magnitude and duration of HABs (Lemley et al. 2018b). Algal decomposition and bacterial remineralisation of the above high biomass blooms and the relatively stable hydrodynamics of the middle reaches (MacKay and Schumann 1991) has resulted in periodic decreased water quality characterised by bottom-water hypoxia (< 2 $mg.L^{-1}$) in the mesohaline zone of the Sundays Estuary (Lemley et al. 2018a, b). Additionally, the estuary has no extensive saltmarsh or submerged macrophytes, with primary productivity mostly resulting from the phytoplankton community (Emmerson 1989; Lemley et al. 2017b; Van Niekerk et al. 2019). Thus, the low inflow Sundays Estuary has recurrent HABs that make for an ideal study site to investigate the impacts of HABs on the abundance of *Mugil cephalus*, which is a common fish species in South African estuaries (Fig. 1).

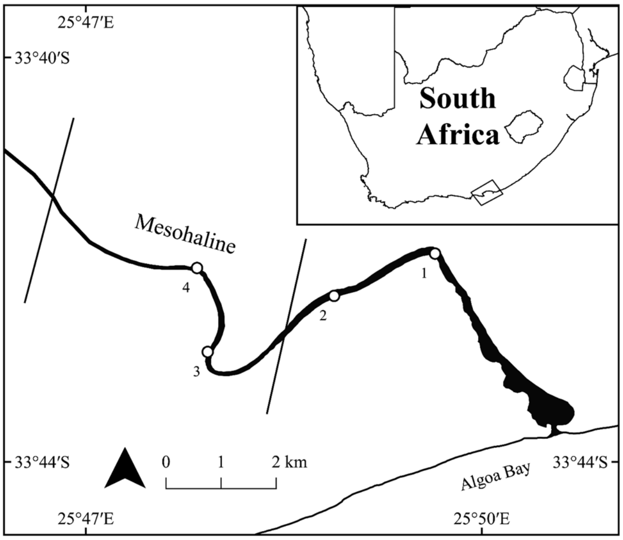


Fig. 1 Locations of the four fixed baseline sampling sites with moving intensive sites located within the mesohaline zone of the Sundays Estuary on the warm temperate coast of South Africa

Study Design

The current study is a continuation of recent microalgal studies done monthly (Lemley et al. 2017b), twice weekly (Lemley et al. 2018b) and bihourly (Lemley et al. 2018a) in the Sundays Estuary and used similar phytoplankton sampling techniques. The current study encompasses a baseline monitoring period of monthly sampling for 24 months between 2018 and 2020 and an intensive sampling period during which sampling took place twice weekly during the peak phytoplankton bloom period of October to November in 2018 (spring/summer in the Southern Hemisphere). Baseline sampling took place at four fixed sites, with two being in the mesohaline zone and two in the polyhaline zone of the estuary during a first-quarter moon phase (to limit tidal influences), while three intensive sampling sites were not fixed, and each site location was determined by surface water salinities corresponding to 18, 10 and 5 (upper, mid and lower mesohaline) (Fig. 1). Daytime supersaturated oxygen conditions and night-time bottom-water hypoxia are known to occur during HABs (Lemley et al. 2018a, b; Smit et al. 2021). Therefore, daytime and night-time sampling were done to determine differences in *M. cephalus* abundance and distributions in relation to phytoplankton bloom dynamics.

Field Sampling

Juvenile and adult *M. cephalus* abundance was sampled using a 50-m beach seine net (2 m deep and 12 mm mesh size). Netting took place at each site where the net was lowered off the bow of a boat in a semi-circle to the shoreline, taking care to minimise disturbances and limited to shallow (< 1.5 m deep) unobstructed, gently sloping muddy banks. The catch was gently lowered into a large bin filled with site-specific estuary water to lessen stress and aid in recovery. *Mugil cephalus* were field-identified using Whitfield (2019), counted and returned to the estuary as soon as possible. Phytoplankton in situ sampling used similar techniques described by Lemley et al. (2017a, b). Physico-chemical parameters, inorganic nutrients, chlorophyll-*a* and phytoplankton community samples were collected at the surface, 0.5 m, 1.0 m and near the bottom of the water column at each site.

Physico-chemical parameters were measured using a YSI ProDSS multiprobe and included temperature (°C), salinity, turbidity (NTU), pH and dissolved oxygen (mg. L^{-1}). Replicate water samples (of each depth) were collected with a weighted pop-bottle and gravity filtered through glass-fibre filters (Whatman© GF/C). Filtrates were stored in the dark and frozen until they were analysed (i.e., within a week after sampling) in the laboratory. Chlorophyll-*a* was subsequently extracted with 10 mL of 95% ethanol (Merck 4111) for 24 h in a cold (ca. 1–2 °C), darkroom. Spectrophotometric determinations of chlorophyll-*a* were performed according to Nusch (1980). Absorbances before and after acidification with 1 N HCl were read using a Thermo Scientific™ GENESYS™ 10S UV–Vis spectrophotometer at 665 nm. Water samples for inorganic nutrient analysis, at each depth, were filtered through hydrophilic polyvinylidene difluoride (PVDF) 0.47-µm pore-size syringe filters and subsequently frozen at −40 °C. The levels of nitrate, nitrite, phosphate, ammonium and silica were determined using a SEAL Autoanalyser 3 H.R. (SEAL Analytical, Inc.).

The phytoplankton community structure (at each depth and site) was determined by preserving 150 mL water samples with 1 mL of 25% glutaraldehyde solution (Sigma-Aldrich® Chemicals G5882). The Coulon and Alexander (1972) method was used to settle the samples overnight in 26.5-mm-diameter Utermöhl chambers. Two drops of Rose Bengal (Sigma Chemicals R3877) were added to a known volume (i.e., ranging from 10 to 50 ml) of preserved water samples and allowed to settle for 24 h before identification. After settling, a Zeiss IM 35 inverted microscope was used to count and identify the phytoplankton groups at a magnification of 630×, during which either a minimum of 200 frames or 200 cells were counted. The cells were classified according to phytoplankton classes, i.e., Bacillariophyceae, Chlorodendrophyceae, Cryptophyceae, Dinophyceae, Euglenophyceae, Raphidophyceae and *Mesodinium* cf. *rubrum*. The number of cells (per mL) for each group present was calculated using the equation described by Snow et al. (2000).

Flow Data

River discharge data were obtained from the Department of Water and Sanitation, South Africa (https://www.dws.gov.za/Hydrology/Verified/hymain.aspx). The freshwater inflow into the estuary was estimated by summing the flow volumes overtopping at the Korhaanspoort weir (Station N4H001), which is approximately 90 km upstream added with the possible return flow (ca. 10%) of water that was pumped from the weir via a canal (Station N4H006) for irrigation in the near catchment (Lemley et al. 2021a). A 24-h lag was added to river discharge data to account for the distance from the weir to the study sites.

Data Analysis

One-way analysis of variance (ANOVA) was used to test for differences in environmental variables recorded at each site and depth. Linear mixed models (LMM) and generalised least square (GLS) approaches were used to analyse the drivers of phytoplankton biomass. All possible predictors were tested for multicollinearity using variance-inflation factor (VIF) tests and only included in the model if VIF ≤ 3. Predictors included the physico-chemical variables (temperature, salinity, dissolved oxygen, pH) and inorganic nutrients (SRP, DSi, NO_x, NH_4^+), altogether with the dominant phytoplankton species densities. All likely predictor variables were first centred (mean of zero) and scaled (standard deviation of one) using the 'scale' function in *R*. A 'top-down' model selection process was followed as recommended by Zuur et al. (2009), with the most parsimonious model determined using the 'dredge' function from the 'MuMIn' package with Akaike information criterion (AIC) used as goodness of fit.

The drivers of phytoplankton species densities were assessed using generalised linear models (GLM) using the R statistical software with the 'manyglm' function of the 'mvabund' package (Wang et al. 2012). This model-based approach uses resampling-based hypothesis testing to make taxon-specific inferences about which environmental variables are associated with multivariate abundances. The effects of explanatory variables retained in both models were assessed using the multivariate 'anova' function (p.uni = 'adjusted', nBoot = 10,000:Wang et al. 2012). To account for temporal autocorrelation, diel state nested in study site was included as repeated measures. This allowed for the analysis of data from all depths. The model selection process followed that described by Zuur et al. (2009). Zero-inflated negative binomial GLMMs, of the 'glmmTMB' package, were used to investigate the relationship among environmental variables, phytoplankton densities and the abundance and distribution of *M. l cephalus*. To control for spatial pseudoreplication effects, the 'study site' was considered random and diel state nested in study site were considered fixed. All final model assumptions that were checked included: (1) linearity using variance-inflation factor tests (VIF ≤ 3), (2) normality using Shapiro–Wilk's tests, and (3) homogeneity of variances using Levene's tests.

Results

Freshwater Inflow

Daily average river inflow ranged from 0.01 to 7.62 $m^3\ s^{-1}$ with a mean (SD) of 1.45 (1.25) $m^3.s^{-1}$ (Fig. 2). Two peaks occurred, one at the start of September 2018 and one in March 2020 (Fig. 2). October, November and December had the lowest inflow to the estuary in both 2018 and 2019 (Fig. 2). This study occurred during an extended drought period in the region. Note that only 5 days of flow values were available during the Intensive sampling period.

Physico-Chemical Variability

Dissolved oxygen (DO) differed between sites ($F_{6,1104}$ = 18.8, $P < 0.001$) and depths ($F_{3,1104}$ = 124.6, $P < 0.001$) (Table 1). DO varied greatly between depths with frequent instances of hypoxia (< 2 $mg.L^{-1}$) occurring in the bottom waters (water depth > 1 m) (Fig. 3). Mean DO of baseline sites were similar ($F_{3,768}$ = 3.2, $P = 0.07$), while intensive site 1 (upper mesohaline with surface salinity of 18) mean DO was less than the other two intensive sites ($F_{2,336}$ = 8.3, $P = 0.004$). Seasonal variation in pH were evident with more alkaline conditions observed in the warmer summer and spring months with mean and standard deviation (SD) of 8.8 (0.6) and 8.6 (0.5) compared to winter and fall of 8.5 (0.7) and 8.4 (0.4) respectively ($F_{3,1104}$ = 21.3, $P = 0.03$) (Table 1). Turbidity differed between sites ($F_{6,1104}$ = 5.1, $P < 0.001$) and depths ($F_{3,1104}$ = 321.9, $P < 0.001$) (Table 1). Bottom mean (SD) turbidity at baseline site 4 (mid mesohaline) of 19.8 (12.6) NTU was less than baseline site 2 (lower polyhaline) of 27.3 (10.2) NTU and baseline site 1 (mid polyhaline) of 30.1 (12.7) NTU (Table 1). Persistent haloclines were evident during the study as bottom waters were more saline than surface waters (Table 1). Baseline sites 1 and 2 (mid and lower polyhaline) salinities were similar but were significantly higher than baseline sites 3 and 4 (upper and mid mesohaline) (Table 1). Intensive site 1 had significantly higher salinities than intensive site 3 (lower mesohaline with surface salinity of 5) across all depths (Table 1). The temperature was similar between sites and depths for both study periods (Table 1).

Nutrients

Inorganic nutrients showed seasonal trends, with mean (SD) phosphate concentrations (SRP) of 2.5 (1.3) μmol.L^{-1} and mean total oxidised nitrogen (NO_x) of 25.4 (13.2) μmol.L^{-1}, which peaked in summer and winter, respectively. Consequently, the dissolved inorganic nitrogen to dissolved inorganic phosphate ratio (DIN:DIP) peaked in the winter months with a mean of 24.6 (9.0). Dissolved silica (DSi) and ammonium (NH_4^+) peaked in December 2018 with a mean of 251.0 (230.3) μmol.L^{-1} and 24.2 (28.0) μmol.L^{-1}, respectively. Nutrient concentrations were also highest at sites further up the estuary, suggesting nutrient uptake by the phytoplankton community further down towards the mouth (Fig. 4).

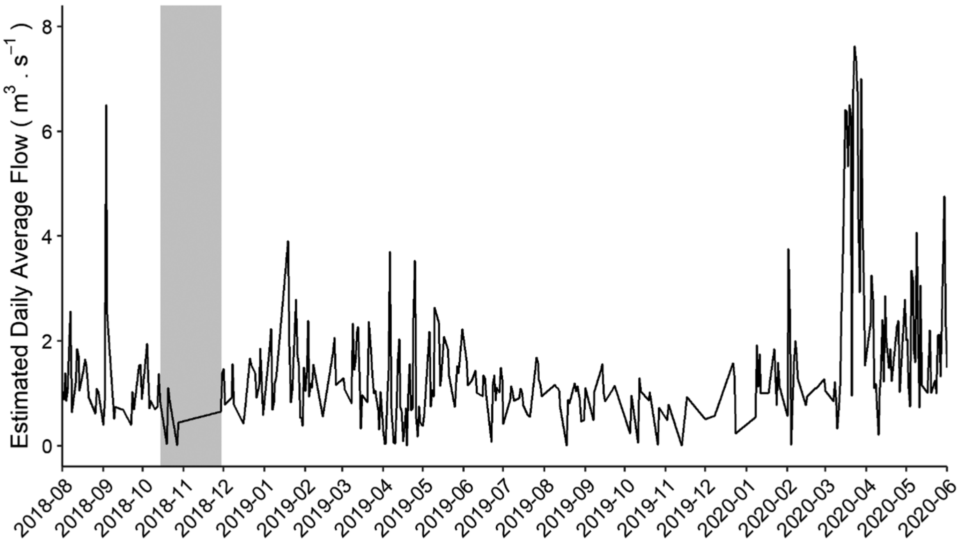


Fig. 2 Estimated daily average river inflow into the Sundays Estuary over the study period (2018–2020). Shaded area indicates the intensive study period

Table 1 Physico-chemical measures during the baseline and intensive study period. Mean and (SD) values reported for the four sampled depths

Study	Site	Depth	N	Dissolved oxygen (mg.L^{-1})		Turbidity (NTU)		pH		Salinity		Temperature (°C)	
				mean (SD)	min–max	mean (SD)	min–max	mean (SD)	min–max	mean (SD)	min–max	mean (SD)	min–max
Baseline	1	Surface	48	8.3 (2.9)	5.6–24.1	8.1(4.5)	3.1–33.5	8.4 (0.8)	7.1–11.7	18.8 (5.3)	8.1–35.2	19.6 (3.6)	13.4–26.5
		0.5	48	8.1 (2.6)	5.6–20.4	8.0 (2.3)	4.0–12.5	8.5 (0.8)	7.6–11.8	19.2 (5.1)	9.2–35.8	19.7 (3.5)	13.5–26.6
		1	48	7.1 (1.9)	3.0–14.5	9.4 (3.1)	4.4–19.4	8.4 (0.8)	7.7–11.8	22.4 (3.4)	14.6–36.0	19.9 (3.2)	13.7–25.3
		Bottom	48	5.5 (1.8)	0.8–7.9	30.1 (12.7)	6.3–57.3	8.4 (0.8)	7.7–11.7	32.7 (2.9)	18.4–37.3	19.1 (2.5)	15.1–24.2
	2	Surface	48	8.1 (2.2)	4.5–16.7	8.4 (4.1)	3.8–26.8	8.5 (0.8)	7.7–11.7	16.1 (4.4)	6.7–24.0	19.8 (3.6)	13.6–25.8
		0.5	48	9.3 (8.6)	4.4–17.2	9.1 (3.1)	4.9–19.0	8.5 (0.8)	7.8–11.7	16.8 (4.3)	8.6–26.2	20.0 (3.5)	13.8–25.9
		1	48	6.4 (1.8)	2.4–11.2	12.7 (5.7)	6.4–40.8	8.4 (0.8)	7.0–11.7	21.0 (3.3)	10.7–27.9	20.1 (3.2)	14.4–25.0
		Bottom	48	4.5 (2.1)	0.4–11.1	27.3 (10.2)	10.3–56.8	8.3 (0.8)	7.7–11.7	27.7 (3.4)	20.0–34.3	19.8 (2.7)	15.3–24.5
	3	Surface	48	8.3 (1.8)	5.1–12.1	7.3 (2.8)	2.7–14.5	8.5 (0.8)	7.5–11.6	11.2 (3.4)	4.8–17.3	20.0 (3.9)	13.2–25.9
		0.5	48	7.9 (1.8)	4.9–12.2	8.8(2.9)	3.6–16.1	8.5 (0.8)	7.6–11.7	13.3 (9.8)	4.8–23.4	20.0 (3.9)	13.5–26.1
		1	48	6.6 (2.1)	2.3–12.0	11.5 (3.6)	5.8–21.1	8.4 (0.8)	7.6–11.7	15.8 (4.2)	5.3–25.2	20.3 (3.6)	14.1–25.7
		Bottom	48	4.5 (2.5)	0.3–10.0	20.7 (11.4)	8.9–67.1	8.3 (0.8)	7.3–11.7	21.0 (3.0)	15.7–26.4	20.4 (3.4)	14.3–25.1
	4	Surface	48	8.2 (1.9)	5.4–13.7	7.3 (2.9)	3.0–14.1	8.6 (0.9)	7.4–11.7	8.8 (3.2)	3.4–15.3	20.0 (4.0)	13.5–26.3
		0.5	48	8.2 (2.0)	5.2–14.2	7.9 (3.2)	2.9–15.1	8.6(0.8)	7.6–11.6	9.2 (3.2)	3.6–15.7	20.1 (4.1)	13.4–26.3
		1	48	7.8 (6.8)	1.3–13.2	9.9 (3.2)	3.9–16.1	8.5 (0.8)	7.6–11.6	12.5 (4.7)	3.8–23.9	20.3 (3.9)	13.9–25.9
		Bottom	48	4.1 (2.2)	0.3–9.0	19.8 (12.6)	2.5–73.9	8.3 (0.8)	7.6–11.6	19.1 (3.8)	9.2–26.0	20.5 (3.5)	13.9–25.5
Intensive	1	Surface	28	8.2 (1.5)	5.7–11.8	6.8 (2.1)	2.8–12.7	8.8 (0.2)	8.1–9.2	18.1 (1.1)	16.1–20.9	22.6 (2.1)	19.4–26.2
		0.5	28	7.8 (1.5)	5.3–10.5	8.2 (2.5)	3.4–16.6	8.8 (0.1)	8.5–9.1	19.5 (1.7)	17.1–24.4	22.3 (1.9)	19.5–25.8
		1	28	7.1 (1.3)	4.5–9.7	11.1 (4.5)	5.3–24.5	8.8 (0.1)	8.3–9.0	22.3 (3.4)	17.7–30.0	21.8 (1.7)	18.8–25.3
		Bottom	28	5.8 (1.4)	3.1–9.7	33.3 (27.7)	9.3–126.3	8.7 (0.2)	7.7–8.9	26.6 (4.8)	18.9–34.9	21.0 (1.8)	17.0–24.1
	2	Surface	28	11.0 (3.6)	4.8–20.6	7.2 (3.8)	1.2–22.2	9.0 (0.2)	8.7–9.2	10.3 (0.7)	9.0–11.7	23.0 (2.1)	19.8–27.3
		0.5	28	10.5 (3.9)	4.4–21.7	8.4 (2.8)	2.7–14.3	9.0 (0.2)	8.7–9.2	11.8 (2.3)	9.1–21.0	23.0 (2.0)	20.1–26.5
		1	28	8.3 (3.1)	2.7–14.8	13.0 (10.4)	3.8–62.3	8.9 (0.1)	8.6–9.2	15.3 (4.6)	10.4–28.2	22.4 (1.8)	19.6–25.5
		Bottom	28	6.9 (2.6)	2.7–12.7	21.2 (11.6)	7.1–62.3	8.8 (0.2)	8.3–9.0	17.5 (5.6)	10.7–30.1	22.3 (1.9)	19.3–25.3
	3	Surface	28	9.3 (4.4)	3.8–20.7	7.4 (3.4)	0.5–13.6	9.0 (0.1)	8.8–9.2	5.4 (0.7)	4.2–6.8	22.8 (2.0)	20.0–26.3
		0.5	28	9.7 (4.8)	3.4–20.9	8.2 (3.0)	4.2–12.9	9.0 (0.1)	8.7–9.2	5.7 (0.9)	4.4–8.0	22.9 (1.9)	20.3–26.5
		1	28	9.2 (4.5)	3.2–19.4	16.2 (23.3)	4.9–132	8.9 (0.2)	8.7–9.2	7.1 (2.1)	4.4–13.9	23.0 (1.7)	20.5–26.0
		Bottom	28	7.1 (3.5)	0.9–16.2	21.2 (15.1)	5.3–71.9	8.8 (0.2)	8.4–9.1	10.0 (5.5)	4.4–24.1	22.7 (1.7)	20.5–26.0

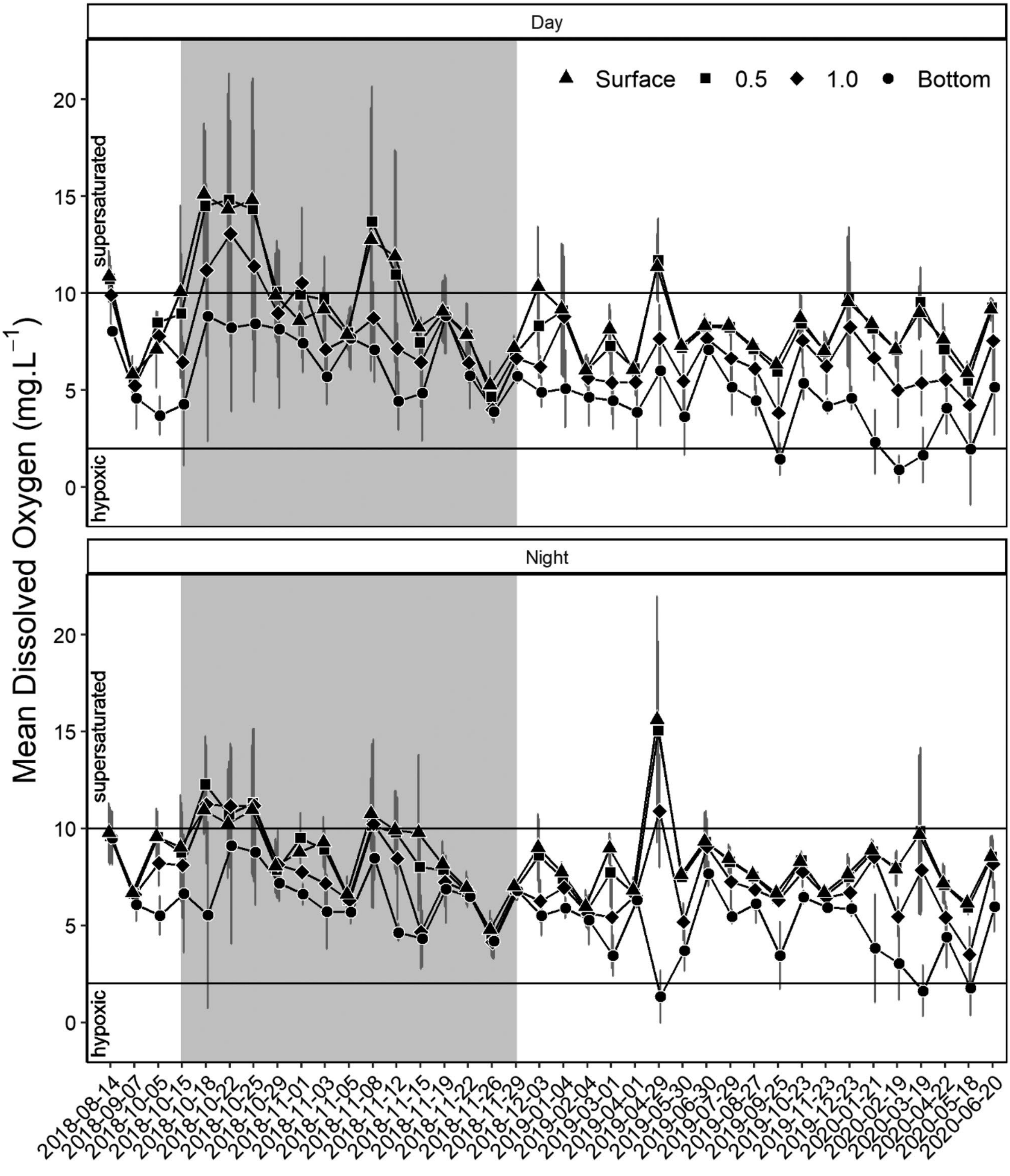


Fig. 3 Frequency of supersaturated (> 10 mg.L^{-1}) surface water and hypoxic (< 2 mg.L^{-1}) bottom water conditions in the Sundays Estuary over the study period (2018–2020). Mean dissolved oxygen over the four sampling depths with standard deviation bars shown. Shaded area is from the intensive sampling period

Phytoplankton Community

The raphidophyte, *Heterosigma akashiwo*, dominated the phytoplankton community during all but the winter months (Fig. 5). This HAB species was abundant (> 1000 cells.mL^{-1}) in the mesohaline zone (baseline sites 3 and 4 and intensive sites 2 and 3) for most of the study period. A peak density of ca. 70 000 cells.mL^{-1} occurred during an extensive bloom at the end of April 2019 at 0.5 m depth of site 1 during the baseline study (Fig. 6). This bloom event coincided with decreased salinities due to riverine inflow. Dinophyceae, Bacillariophyceae and *Mesodinium* cf. *rubrum* also occurred in high densities over the study period, with *M.* cf. *rubrum* co-occurring, whilst Dinophyceae and Bacillariophyceae densities mismatched with Raphidophyceae densities. The multivariate deviance of dominant phytoplankton taxa densities was best explained by season (32.9%) and sampling date (24.4%) (Table 2). Individual species univariate models which were nested within the multivariate model

Fig. 5 Total cell counts for dominant phytoplankton classes for day ▸ (grey) and night (white) as well as the total cell counts (black) for all the sampling depths during the entire sampling period from 2018 to 2020 in the Sundays Estuary

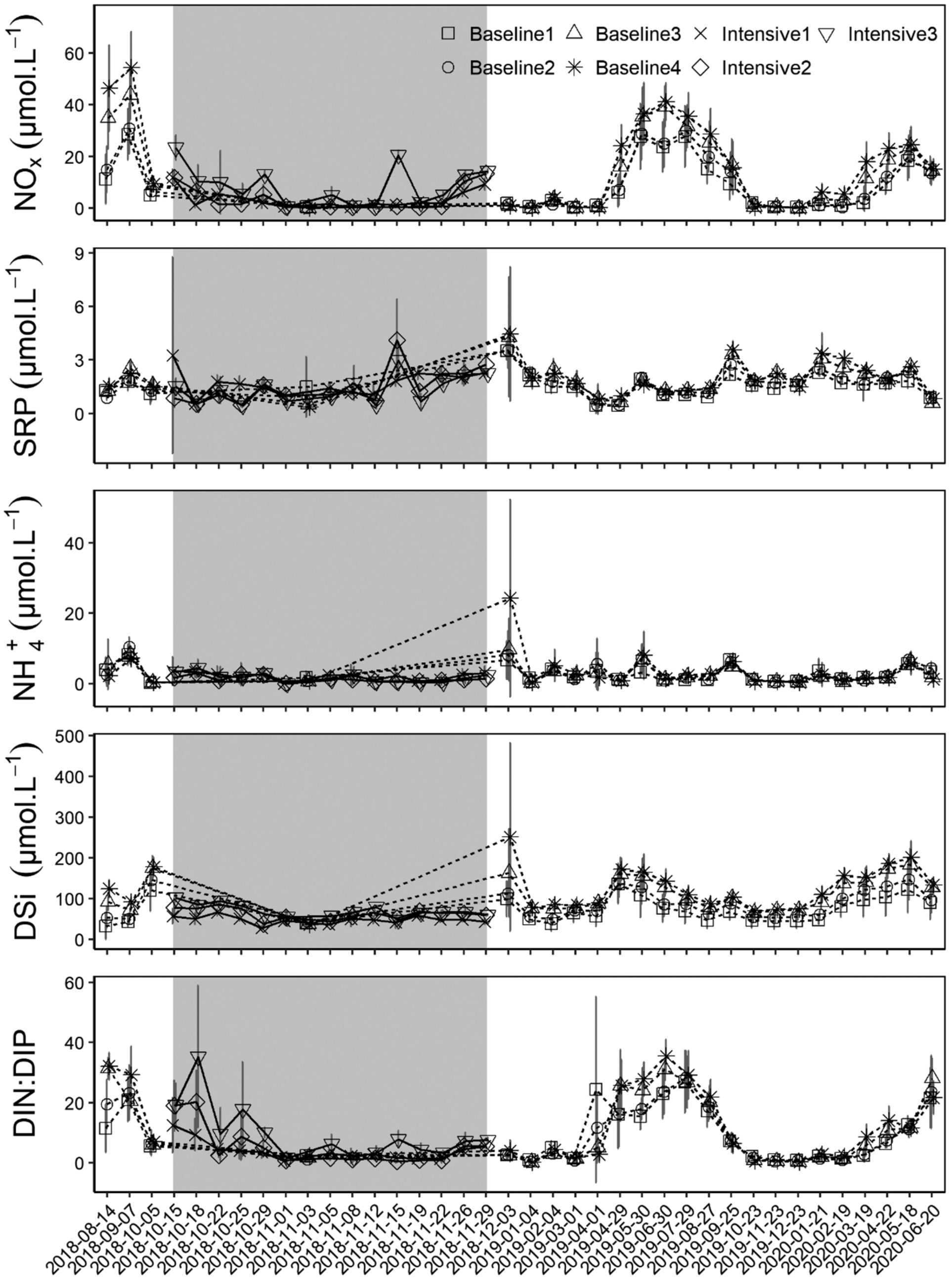
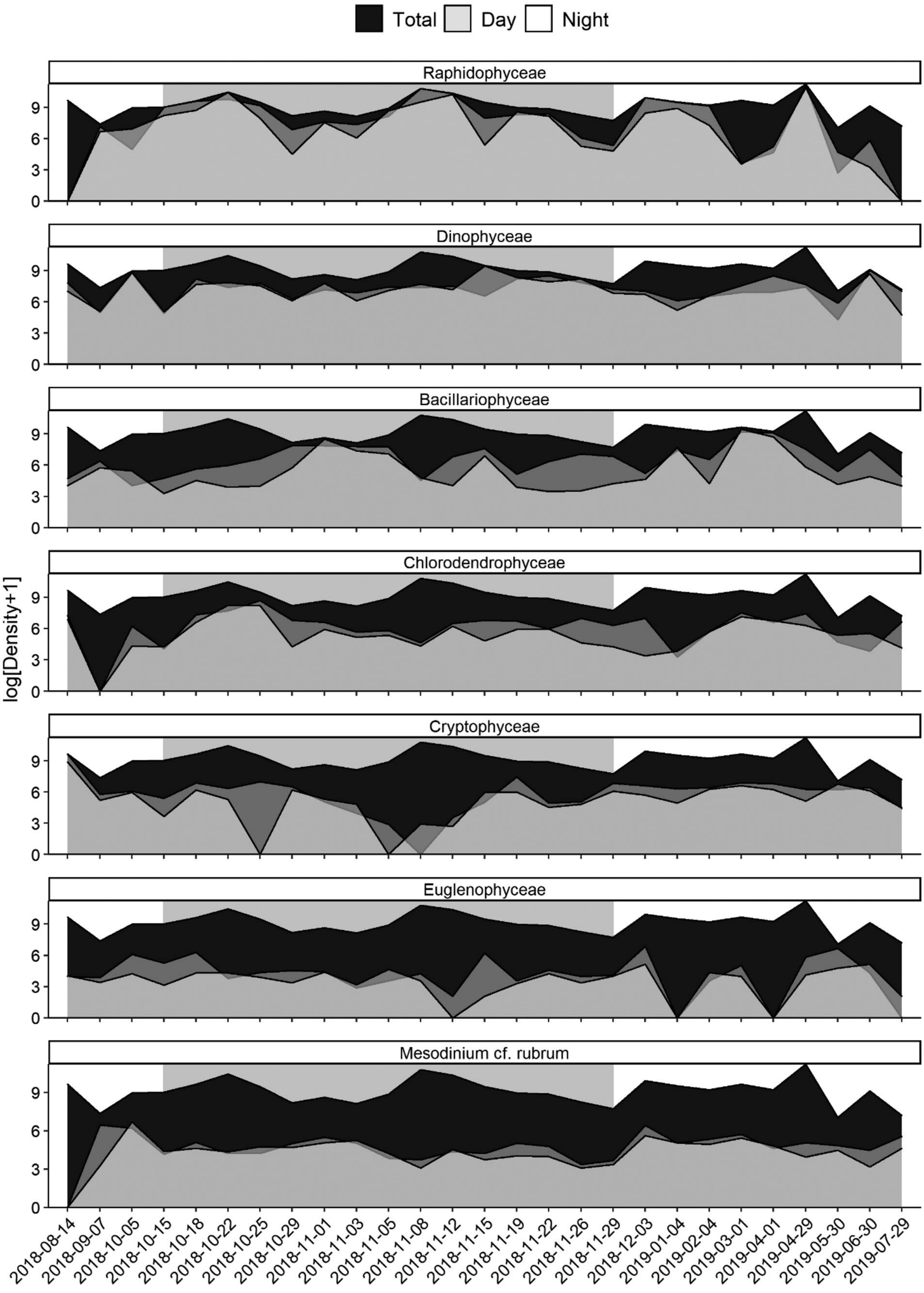


Fig. 4 Spatial and temporal trends in inorganic nutrients over the study period in the Sundays Estuary (2018–2020). Mean with standard deviation bars given. Shaded area is from the intensive sampling period


Total
Day
Night
Raphidophyceae
Dinophyceae
Bacillariophyceae
Chlorodendrophyceae
Cryptophyceae
Euglenophyceae
Mesodinium cf. rubrum
log[Density+1]
0
3
6
9
2018-08-14
2018-09-07
2018-10-05
2018-10-15
2018-10-18
2018-10-22
2018-10-25
2018-10-29
2018-11-01
2018-11-03
2018-11-05
2018-11-08
2018-11-12
2018-11-15
2018-11-19
2018-11-22
2018-11-26
2018-11-29
2018-12-03
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2019-06-30
2019-07-29

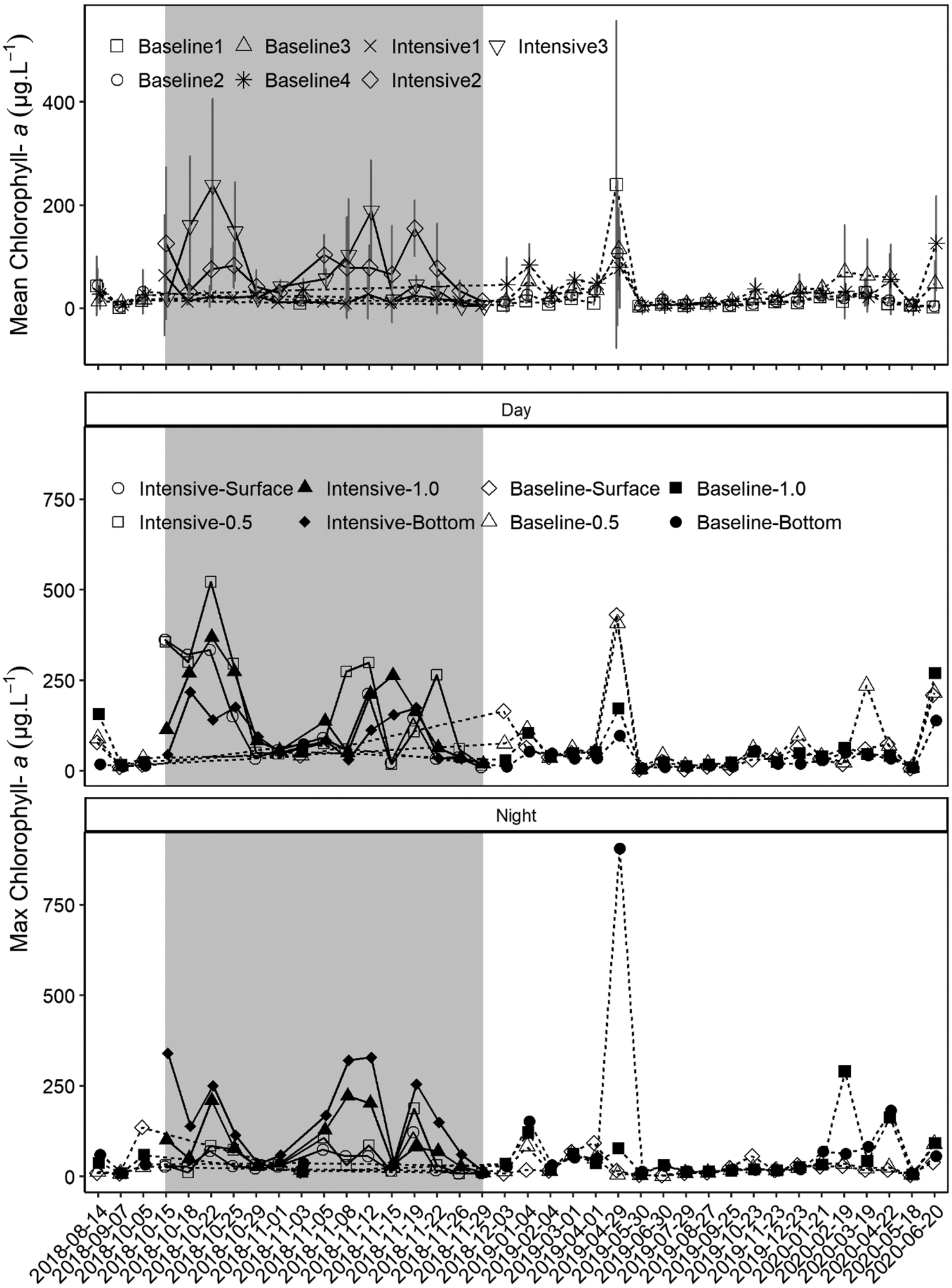

Fig. 6 Phytoplankton biomass over study period. Mean biomass at study sites (top), max biomass over depths at daytime (middle) and night-time (bottom). Shaded area is data from the intensive study period and error bars are standard deviation

demonstrated that salinity was a significant predictor with a negative relationship across taxa apart from *M.* cf. *rubrum*. Dissolved silica (DSi) was a significant positive predictor of *M.* cf. *rubrum* and Raphidophyceae densities (8.1 and 13.0% D, respectively) (Table 3). Dissolved oxygen had a significant positive relationship with Raphidophyceae and Cryptophyceae densities, while a negative relationship was observed with Dinophyceae and *M.* cf. *rubrum* densities (Table 3). Bacillariophyceae, Cryptophyceae and *M.* cf. *rubrum* showed a marked diel difference in densities, with significantly lower densities observed during the night-time (Table 3).

Phytoplankton Biomass

Two definite blooms were sampled during the Intensive sampling period (Fig. 6). The upper two intensive sites (2 and 3) had higher phytoplankton biomass than the site closer to the mouth area (Fig. 6). Nine of the 24 monthly baseline sampling events had sites that were in a high biomass bloom state (> 60 Chl-*a* µg.L^{-1}). Extensive blooms were found in April 2019, with blooms reaching a maximum of 800 Chl-*a* µg.L^{-1} during the night in the bottom waters at site 1. Diel vertical migration (DVM) of phytoplankton was evident with

Table 2 Multivariate generalised linear model (GLM) output of dominant phytoplankton densities. The deviance explained (*D*) by each predictor variable, directional coefficients (*C*) with standard error (SE) of each predictor variable and the significance level of the likelihood-ratio-test statistic (*P*) are shown

		Overall		Bacillariophyceae			Cryptophyceae			Dinophyceae			*Mesodinium* cf. *rubrum*			Raphidophyceae		
	df	*D* (%)	*P*	*D* (%)	C(SE)	*P*	*D* (%)	C(SE)	*P*	*D* (%)	C(SE)	*P*	*D* (%)	C(SE)	*P*	*D* (%)	*C* (SE)	*P*
Salinity	1	4.4	< **0.001**	0.1	−0.5 (0.2)	0.651	2.5	−0.3(0.2)	**0.026**	6.5	−0.1 (0.1)	< **0.001**	7.3	0.3 (0.1)	< **0.001**	8.9	−0.1 (0.2)	< **0.001**
Temperature (°C)	1	4.6	< **0.001**	6.2	0.3 (0.2)	< **0.001**	12.7	0.2(0.2)	< **0.001**	0.7	0.2 (0.1)	0.404	0.6	0.2 (0.1)	0.512	0.6	−0.3 (0.2)	0.512
DO (mg.L^{-1})	1	5.4	< **0.001**	0.1	−0.1 (0.1)	0.301	14.1	0.1(0.1)	< **0.001**	2.1	−0.1 (0.1)	0.228	1.8	−0.1 (0.1)	0.069	9.8	0.4 (0.1)	**0.027**
SRP (µmol.L^{-1})	1	4.9	< **0.001**	13.9	−0.2 (0.1)	< **0.001**	0.1	−0.1(0.1)	0.975	0.1	0.1 (0.1)	0.975	0.7	−0.1 (0.1)	0.969	3.7	−0.3 (0.1)	< **0.001**
DSi (µmol.L^{-1})	1	4.2	< **0.001**	0.1	−1.4 (0.2)	0.381	0.7	−0.2(0.1)	0.343	0.8	0.5 (0.1)	0.381	8.1	0.4 (0.1)	**0.013**	13.0	1.0 (0.1)	< **0.001**
NH^+_4 (µmol.L^{-1})	1	1.5	< **0.001**	0.1	−0.3 (0.1)	0.187	1.4	0.1(0.1)	0.116	8.6	−0.6 (0.1)	1.000	0.1	−0.1 (0.1)	0.721	0	−0.6 (0.2)	0.912
Diel	1	1.6	< **0.001**	1.5	-	1.000	2.9	-	**0.042**	0.1	-	< **0.001**	2.9	-	**0.042**	0.2	-	0.496
Date	1	24.4	< **0.001**	4.4	-	< **0.001**	24.8	-	< **0.001**	41.6	-	< **0.001**	55.7	-	< **0.001**	14.6	-	< **0.001**
Season	3	32.9	< **0.001**	67.4	-	1.000	31.3	-	< **0.001**	20.9	-	< **0.001**	3.2	-	< **0.001**	17.8	-	1.000
Study	1	3.9	**0.013**	0.7	-	0.345	0.1	-	0.349	4.1	-	1.000	5.6	-	0.440	10.7	-	**0.012**
Site: depth	28	12.1	< **0.001**	5.7	-	0.168	9.5	-	0.157	14.6	-	< **0.001**	14.0	-	0.507	20.7	-	< **0.001**

Chl-*a* peaking at the 0.5-m depth during the daytime, whilst night-time Chl-*a* peaked in the bottom waters (> 1 m).

The predictors that best explained phytoplankton biomass were diel state, depth nested within diel state, temperature, salinity, dissolved oxygen and the densities of Raphidophyceae and Dinophyceae. Temperature and dissolved oxygen had a significant positive relationship, whilst salinity had a negative relationship. Phytoplankton biomass tended to be greater during the day but also during the night in the bottom waters (> 1 m) (Table 3). The phytoplankton biomass of the Sundays Estuary was primarily comprised of the raphidophyte *Heterosigma akashiwo* and the dinoflagellate *Heterocapsa rotundata*, which are both known as harmful algal bloom species (HABs) (Table 3). Phytoplankton biomass significantly correlated with changes in dissolved oxygen (Table 3), with supersaturated surface- and hypoxic bottom-water conditions coinciding with high biomass (> 60 Chl-*a* µg.L^{-1}) bloom conditions (Table 1).

Mugil cephalus Abundance

No diel nor sampling site differences in *M. cephalus* abundances were found. However, seasonal changes in abundances were evident ($H_{3,276} = 11.7$, $P = 0.008$) with highest mean (min–max) abundance during fall 2.4 (0–33) and lowest in the spring season of 1.0 (0–32) (Fig. 7). This was probably due to the increased incidence of bloom conditions and not due to the increased sampling frequency (intensive study) (Table 4). Instances of high abundance (CPUE > 10) of *M. cephalus* were only found at sites that did not have high biomass bloom conditions (< 60 Chl-*a* µg.L^{-1}) (Fig. 7). The zero-inflated negative binomial model (ZINB) found that *M. cephalus* abundances were significantly negative related to phytoplankton biomass (test statistic = −2.05) and the spring season (−2.67) (Table 4). Despite phytoplankton biomass being explained by dissolved oxygen (among other predictors), dissolved oxygen minima were not a significant ($P = 0.07$) predictor of *M. cephalus* abundances (Table 4).

Discussion

The phytoplankton community in the Sundays Estuary was dominated by the HAB species, *Heterosigma akashiwo*. This species significantly altered the dissolved oxygen in the estuary resulting in frequent supersaturated surface waters and hypoxic bottom waters during blooms exceeding 1000 cells.mL^{-1}, confirming findings by (Lemley et al. 2017b, 2018a, b) in the same estuary and other South African eutrophic low inflow estuaries like the Hartenbos Estuary (Lemley et al. 2021b). Lemley et al. (2018a) also found that this species was dominant in the Sundays Estuary during warmer spring/summer months in the meso- to polyhaline zone and was limited by low temperatures and availability of oxidised nitrogen (NO_3,

Table 3 Generalised least square (GLS) model output of phytoplankton biomass

	Phytoplankton biomass			
Predictors	Estimates	CI	Statistic	*P*
(Intercept)	0.37	0.00–0.73	1.98	**0.047**
Temperature (°C)	0.13	0.11–0.14	21.02	**< 0.001**
Salinity	-0.03	–0.03 to –0.02	–8.36	**< 0.001**
Dissolved oxygen (mg.L^{-1})	0.03	0.01–0.04	3.55	**< 0.001**
Dissolved silica (μmol.L^{-1})	-0.00	–0.00 to 0.00	–1.89	0.060
Raphidophyceae (cells.mL^{-1})	0.01	0.01– 0.01	17.68	**< 0.001**
Dinophyceae (cells.mL^{-1})	0.01	0.01–0.02	10.38	**< 0.001**
DoN [night]	-0.37	–0.50 to –0.23	–5.39	**< 0.001**
Depth [1]	0.14	0.01–0.28	2.14	**0.033**
Depth [bottom]	0.15	0.01 – 0.29	2.03	**0.043**
Depth [surface]	-0.20	–0.33 to –0.07	–3.08	**0.002**
DoN [night] × depth [1]	0.10	–0.09 to 0.29	1.03	0.302
DoN [night] × depth[bottom]	0.43	0.24–0.62	4.45	**< 0.001**
DoN [night] × depth[surface]	0.20	0.01–0.39	2.08	**0.037**
Observations	1104			
R^2	0.517			
AIC	2249.260			

i.e. nitrate) and phosphate (PO_4-P) (Lemley et al. 2018a). The findings of this study found that the densities of *H. akashiwo* were negatively related to phosphate and positively related to dissolved silica. Inorganic nutrient concentrations were also highest at sites further up the estuary, which suggests that they are derived from the catchment and that the decline towards the mouth is mainly due to the uptake by the phytoplankton community and partly due to the dilution by seawater. These findings support previous studies (Lemley et al. 2017a, b, 2018a, 2021a) and support the persistent eutrophic conditions found to be characteristic of this estuary.

The abundance of the important mugilid *M. cephalus* was negatively correlated to phytoplankton biomass with no catches exceeding 10 individuals recorded at sites that were

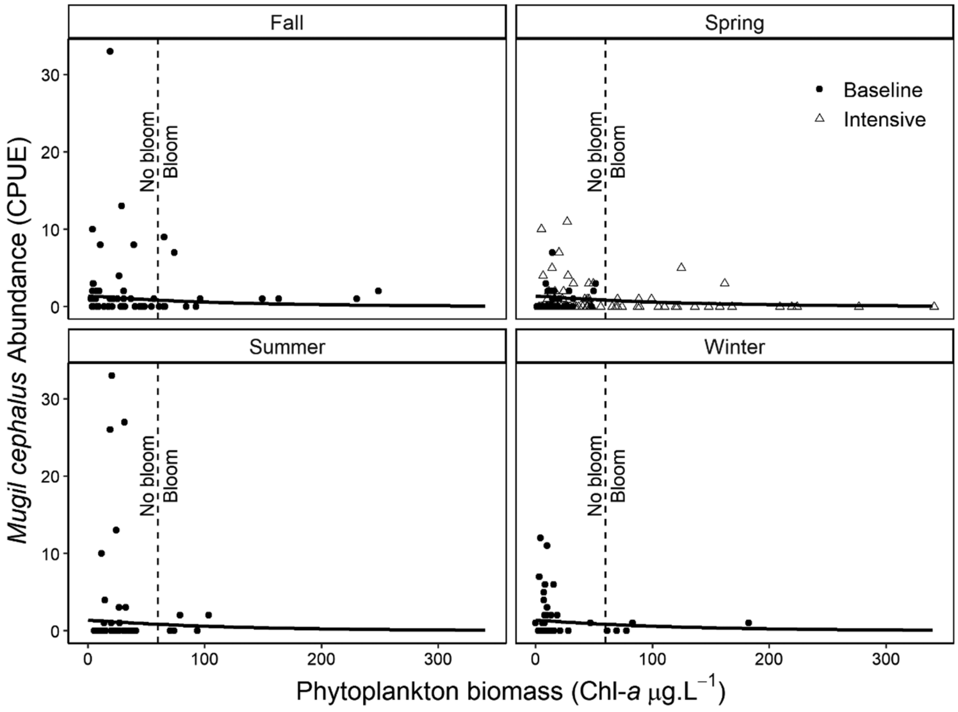

Fig. 7 The relationship of *Mugil cephalus* abundances and phytoplankton biomass (chlorophyll-*a*) sampled using a seine net between 2018 and 2020 in the Sundays Estuary. Zero-inflated negative binomial model is the fitted line

in a high biomass bloom state (> 60 Chl-*a* μg.L^{-1}). Recently, an acoustic telemetry study found that *M. cephalus* actively avoided HABs in the Sundays Estuary and spent more time free roaming areas adjacent to HAB maxima (Bornman et al. 2021). Therefore, HABs have a constricting effect on otherwise favourable habitats for *M. cephalus* which may limit feeding opportunities to *M. cephalus* and other piscivorous predators such as fishes (*Lichia amia*, *Argyrosomus japonicus*) or birds (*Haliaeetus vocifer*) which are reliant on this important fodder fish species (Whitfield et al. 2012; Whitfield 2019).

Furthermore, this study found that phytoplankton biomass and the spring season were negatively related to *M. cephalus* abundances. Juveniles of *M. cephalus* recruit into estuaries on the south-eastern coast of South Africa in the winter and spring months (Bok 1979) or specifically into the Sundays Estuary in August/September (Beckley 1984), just before peak HABs were observed in the current study. Therefore, HABs coinciding with the recruitment of juvenile *M. cephalus* (15–40 mm fork length) may impact growth and survival through poorer feeding opportunities or increased physiological stress (Smit et al. 2021; Bornman et al. 2022). Water quality during the current study, specifically dissolved oxygen, was related to HAB dynamics with supersaturated surface water and bottom water hypoxia occurring during peaks of phytoplankton biomass. Despite this, dissolved oxygen was not a significant predictor of *M. cephalus* abundance, which may be due to the adaptability of the species. The species is known to have a wide physiological tolerance to dissolved oxygen and even display jumping behaviour during times of low dissolved oxygen (Hoese 1985; Whitfield et al. 2012). A recent in situ cage study found prominent histological gill alterations of Mugilidae kept within HABs in the Sundays Estuary suggesting that HABs cause great physiological stress (Bornman et al. 2022).

Comparing the abundances of *M. cephalus* to other similar estuaries that do not experience HABs are difficult due to the myriad of gear types used in historical studies quantifying fish abundances in estuaries. Comparing fish abundances before HABs became prominent in the Sundays Estuary is also problematic since nutrient enrichment due to agricultural runoff was also first noted in the early 1970s (Forbes and Allanson 1970); thus, no baseline data is available. However, a recent

Table 4 Zero-inflated negative binomial model (ZINB) of *Mugil cephalus* abundances

	Mugil cephalus abundance			
Predictors	Incidence rate ratios	CI	Statistic	*P*
Count model				
(Intercept)	3.01	0.87–10.35	1.75	0.081
Temperature (°C)	1.43	0.88–2.33	1.45	0.147
Dissolved oxygen minima (mg.L^{-1})	1.38	0.97–1.95	1.80	0.072
Phytoplankton biomass (Chl-*a* μg.L^{-1})	0.73	0.54–0.99	–2.05	**0.040**
Season [spring]	0.30	0.12–0.72	–2.67	**0.008**
Season [summer]	0.86	0.28–2.68	–0.25	0.800
Season [winter]	0.81	0.24–2.77	–0.34	0.734
Baseline × site × diel (day)	1.12	0.78–1.60	0.60	0.546
Intensive × site × diel (day)	0.87	0.49–1.55	–0.46	0.647
Baseline × site × diel (night)	1.00	0.62–1.61	–0.02	0.987
Intensive × site × diel (night)	0.89	0.41–1.89	–0.31	0.755
(Intercept)	0.38			
Zero-inflated model				
(Intercept)	0.84	0.10–6.85	–0.17	0.868
Site × diel (day)	0.52	0.07–3.67	–0.66	0.509
Site × diel (night)	0.71	0.13–3.79	–0.40	0.688
Random effects				
σ^2	1.92			
$\tau_{00\mathrm{Site}}$	0.03			
ICC	0.01			
N_{Site}	7			
Observations	276			
Marginal R^2/conditional R^2	0.204 / 0.215			
AIC	811.853			

study by Nel (2018) found that the abundances of *M. cephalus* were similar between neighbouring permanently open estuaries. The above study used the same net and sampled at six sites, which encompassed the mesohaline zone, along each of the estuaries in the spring/summer months (October to December) during 2014 and 2015. Therefore, the Sundays Estuary still supports a high abundance of *M. cephalus*. Thus, the increases in primary productivity due to eutrophication may outweigh the negative impacts of HABs at larger temporal and spatial scales. This is particularly true for mobile juvenile fishes (such as *M. cephalus*), which may move away from HAB zones if not favoured. Despite the above, this study and others found that *M. cephalus* avoid high biomass HABs (> 60 Chl-*a* μg.L^{-1}) which cause physiological stress, periodically reducing favourable habitats to areas adjacent to HAB maxima (Bornman et al. 2021, 2022; Smit et al. 2021).

The possible direct and indirect deleterious impacts of HABs on estuaries are dependent on a variety of factors, including the specific phytoplankton species that are in a bloom state, the concentration of the HAB, and the species that are exposed to the HAB. However, this study provides valuable insight on the impacts of HABs in the Sundays Estuary. The high biomass blooms of *H. akashiwo* and subsequent dissolved oxygen fluctuations as seen in this study and other eutrophied low inflow South African Estuaries (Lemley et al. 2021b) are rarely encountered elsewhere in the world, with hypoxia normally attributed to other HAB species (Anderson et al. 2001). There is also no global consensus on the direct impacts of *H. akashiwo* on fishes, and therefore it is likely that the avoidance of fishes to this HAB species are related to complex indirect impacts such as food availability (Black et al. 1991; Rensel et al. 2010). Therefore, impacts of HABs such as *H akashiwo* are likely to vary among estuarine ecosystems globally.

Anthropogenic eutrophication and HABs are set to become more frequent and severe due to climate change and lead to further unsustainable development. Therefore, more research that focuses on the impacts of HABs on estuarine fishes is needed to limit the potential loss of estuarine ecosystem services. This is particularly important in low inflow estuaries which are less resilient to anthropogenic changes and are thus more susceptible to the formation of HABs since they have long flushing times. Therefore, management or mitigation strategies with a catchment-wide approach that focuses on reducing anthropogenic nutrient enrichment in estuaries are recommended to prevent further harm caused by HABs.

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