Estuarine zooplankton communities shift but are not depleted during recurrent harmful algal blooms of *Heterosigma akashiwo* in the Sundays Estuary, South Africa

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ABSTRACT: Anthropogenically derived eutrophication and harmful algal blooms (HABs) are both a direct and indirect threat to estuarine ecosystems globally. Micro- and mesozooplankton play a crucial intermediary role in the functioning of estuarine systems. Therefore, the impacts of HABs on zooplankton communities and estuarine productivity are of interest worldwide. This study aimed to determine the changes in the zooplankton community structure in the anthropogenically altered Sundays Estuary, South Africa, which is affected by predictable and recurrent HABs of the raphidophyte *Heterosigma akashiwo*. Monthly sampling was conducted over 24 mo between 2018 and 2021. This was coupled with twice weekly sampling of 2 complete HAB cycles comprising no bloom, accumulation, and hypereutrophic bloom phases (during the austral spring in 2018). Generalised linear models found that salinity (31.3%) and temperature (12.1%) were positively related to zooplankton in Sundays Estuary. There was no major zooplankton mortality resulting from hypoxia or HABs during the sampling interval adopted for this study. The ubiquitous calanoid copepod *Pseudodiaptomus hessei* occurred at similar abundances among the HAB phases and responded to similar triggers (temperature and salinity) as the HAB species. However, predatory or scavenging invertebrates such as the isopod *Cirolana fluviatilis* and the polychaete *Perinereis falsovariegata* were recorded in higher abundances, suggesting a shift in the community structure due to HABs. The possible negative effects of the *H. akashiwo* HABs on the zooplankton species are likely dependent on their concentration and duration, which may fluctuate spatially and temporally in estuaries based on the river inflow and temperature.

KEY WORDS: Eutrophication · Agricultural runoff · Hypoxia · Mesozooplankton · Nursery habitat · Trophic cascades

1. INTRODUCTION

Phytoplankton blooms occur during instances of rapid increases in primary productivity due to nutrient enrichment and are caused by the proliferation of monospecific phytoplankton species. Some of these species are known to be harmful to other aquatic organisms and are thus classified as harmful algal blooms (HABs) (Heisler et al. 2008, Pereira Coutinho et al. 2012). HABs have become more frequent and severe in coastal waters worldwide due to anthropogenic nutrient enrichment (Hallegraeff 1993, Wells et al. 2015, Adams et al. 2020), prompting large-scale restoration projects that have successfully reduced

their frequency (Oviatt et al. 2017). The negative impacts of HABs are complex, with the severity depending on the phytoplankton species and the frequency and scale of the blooms. They are often a co-stressor in eutrophic ecosystems such as estuaries, which accumulate other pollutants such as heavy metals (Hallegraeff 1993, Cloern 2001, Morrell & Gobler 2020).

Estuaries are highly dynamic and productive habitats that provide many ecosystem services, including serving as nursery, spawning, and feeding habitats to ecologically and economically important fish species (Beck et al. 2001, Able 2005, Potter et al. 2015, Sheaves et al. 2015). However, estuaries are particularly vulnerable to HABs, as they are transitional waters subject to combined threats such as eutrophication and habitat loss caused by unsustainable development (Adams et al. 2020). Despite this vulnerability, the ecological impacts of HABs on estuarine systems are relatively understudied (Bates et al. 2020, Griffith & Gobler 2020). Most estuarine *in situ* studies have focused on the bottom-up effects (from nutrients to primary producers) and only inferred a negative impact on species that are higher up the food chain, such as zooplankton (Almeda et al. 2011). Zooplankton communities are particularly important since micro- and mesozooplankton species are an important intermediary between the primary and higher trophic levels (Turner 2004, Steinberg & Landry 2017). Therefore, frequent and severe HABs in estuaries may be detrimental to the nursery value of these systems.

A growing body of laboratory studies has shown the deleterious effects of HABs on various grazer zooplankton species (Guisande et al. 2002, Yu et al. 2010, Almeda et al. 2011). However, there are few *in situ* data available on the responses of zooplankton communities to the effects of HABs in estuaries. Toxic or unpalatable HAB species may cause preferential grazing on non-harmful algal species, which reduces or eliminates grazing pressure, allowing the harmful species to outcompete other non-harmful microalgae and directly or indirectly promoting HAB formation (Mitra & Flynn 2006, Goleski et al. 2010). Alternatively, increases in primary productivity may be favoured by some herbivorous zooplankton species, which have adapted to graze successfully on toxic HAB species (Turner 2010, 2014). Additionally, scavenger or predatory zooplankton such as isopods may also become more abundant during or directly after HABs, during the low-oxygen decay phase (Breitburg et al. 2001). Therefore, HABs may alter the taxonomic composition and size structure of zoo-plankton assemblages, which may have detrimental consequences for ecosystem functioning.

Recently, severe HABs of *Heterosigma akashiwo*, directly linked with hypoxia, were recorded in the eutrophic Sundays Estuary on the warm-temperate south-east coast of South Africa (Lemley et al. 2017, 2018, 2020). *H. akashiwo* (class Raphidophyceae, family Chattonellaceae) occurs globally and is known for its status as a nuisance species (Anderson et al. 2021). It episodically forms HABs that result in fish mortality and have severe detrimental impacts on the aquaculture industry (Anderson et al. 2021). *H. akashiwo* is a euryhaline species that is predominantly found in high abundance in estuaries (Martínez et al. 2010), functioning as a mixotroph that can ingest bacteria and other particles to supplement its phototrophic physiology (Jeong 2011). This species is also highly mobile, moving up and down the water column with a mean vertical velocity of 60 to 80 μm s^{-1} (Kim et al. 2013) to maximise its growth (Hara & Chihara 1987, Lemley et al. 2018), and it can persist in a dormant cyst stage for long periods during unfavourable conditions (Shikata et al. 2007). *H. akashiwo* is not toxic to humans but can be deleterious to aquatic fauna through a variety of pathways, some of which include the production of hemolysins, reactive oxygen species (such as hydrogen peroxide; Diaz et al. 2018), excessive mucus comprising polyunsaturated fatty acids (Chang et al. 1990), and possibly brevetoxin-like compounds (Khan et al. 1997). However, the environmental conditions under which *H. akashiwo* produces these compounds and how these impact co-occurring species are still unclear (Bates et al. 2020). Studies have reported that *H. akashiwo* can suppress the growth of co-occurring phytoplankton taxa (Yamasaki et al. 2009, Lemley et al. 2018, 2020) and 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 (Horner 1998, Smit et al. 2021, Bornman et al. 2022a). However, the above deleterious impacts are often concentration- and species-dependent (Singh 2018, Bates et al. 2020, Anderson et al. 2021).

This study aimed to examine the interactions among the zooplankton community in the highly regulated and warm-temperate Sundays Estuary, which has recurrent HABs of *H. akashiwo*. The objectives were to (1) determine the current abundance and composition of zooplankton in the estuary; (2) assess the relationship between the zooplankton community and the 3 typical bloom phases; and (3) evaluate whether HABs cause species and guild shifts in the zooplankton community. It was hypothesised that

during peak HABs, which are often characterised by hypoxia, changes in the zooplankton community would be apparent due to differences in physiological tolerances, with shifting species-specific abundances during the bloom phases. Since eutrophication is a global threat to estuaries, an understanding of HABs and their impacts on zooplankton communities could provide an ecosystem-wide approach to nutrient input management and the conservation of estuarine functioning.

2. MATERIALS AND METHODS

2.1. Study site

The Sundays Estuary is situated in the warm-temperate biogeographic zone of South Africa (Fig. 1). Freshwater inflow from the river has been extensively altered due to an inter-basin transfer scheme and large upstream citrus agriculture resulting in no episodic flooding events and a regulated ebb flow of 0.47
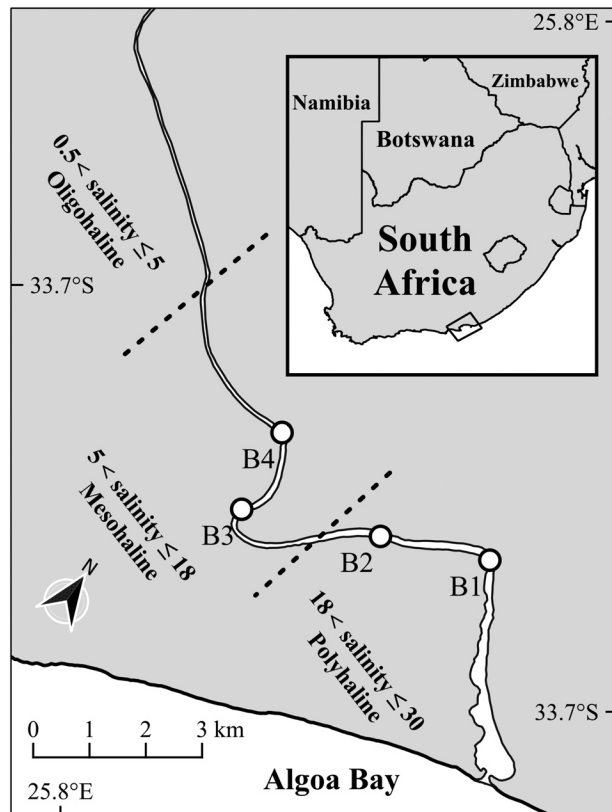

Fig. 1. Locations of the 4 fixed baseline sampling sites with the moving intensive sites located within the mesohaline zone (5 < salinity ≤ 18) of the Sundays Estuary on the warm-temperate coast of South Africa

to 1.86 m^3 s^{-1}, causing a permanently open mouth state (Lemley et al. 2017). Additionally, the estuary is characterised by semi-diurnal tides (with a range of approximately 1 m), with distinct vertical salinity stratification profiles in the mesohaline surface waters and high residence times (Lemley et al. 2018). The above conditions have resulted in a near-permanent eutrophic state (>20 µg chl a l^{-1}), with multiple mono-specific HABs (>1000 cells ml^{-1}) of mainly *Heterosigma akashiwo* occurring in the mesohaline (5 < salinity ≤ 18) and polyhaline (18 < salinity ≤ 30) zones during the spring and summer that last for approximately 1 wk (Lemley et al. 2018). The predictable and recurrent HABs in this system make it an ideal *in situ* study site for investigating the impacts of HABs on estuarine ecology. Recent studies in this estuary have investigated the impacts of HABs on the phytoplankton community (Lemley et al. 2017, 2020), larval fishes (Smit et al. 2021), juvenile fish physiology (Bornman et al. 2022a), and small-scale movement patterns of young fishes (Bornman et al. 2021, 2022b). Despite extensive work on the zooplankton community in the Sundays Estuary, which showed that it is a typical South African warm-temperate estuary (Wooldridge & Melville-Smith 1979, Jerling & Wooldridge 1995b, Sutherland et al. 2013), the limited frequency of sampling (only seasonal) and the restricted scale (only one or 2 sites in the mesohaline zone) were inadequate to determine any changes in the zooplankton community during the periods of HABs.

2.2. Study design

Zooplankton samples were collected during a baseline monitoring period of monthly sampling for 24 mo between 2018 and 2020. This was accompanied by an intensive sampling period, with sampling taking place twice weekly during the peak phytoplankton bloom period in the austral spring (October to November) in 2018. Baseline sampling took place during the same moon phase each month (first quarter moon) to minimise any tidal effects while sampling at the 4 fixed sites within the known HAB zone, with 2 sites (baseline [B] Sites B1 and B2) being in the polyhaline zone of the estuary and 2 sites (Sites B3 and B4) being in the mesohaline zone. The intensive sampling was more frequent and thus required non-fixed sites (intensive [I] Sites I1–I3) to account for tidal fluctuations, with each of the sampling locations being determined by surface water salinities corresponding to 18 at Site I1, 10 at Site I2, and 5 at Site I3 (upper, middle, and lower mesohaline; Fig. 1). The

samples were collected at the near-surface and bottom of the water column during the night since HABs in the Sundays Estuary are known to display diel vertical migration, and a recent study found that the lowest dissolved oxygen concentrations occurred in the bottom waters during the night-time (Lemley et al. 2018).

2.3. Field sampling

Physicochemical variables (temperature, salinity, dissolved oxygen, turbidity, and pH) were determined at 0.5 m intervals from the surface throughout the water column using a ProDSS YSI multiparameter probe at each sampling site. Additionally, phytoplankton biomass was determined by measuring chlorophyll a (chl a; according to the methods of Lemley et al. 2017) in 2 replicate water samples that were collected at the surface, 0.5 m, 1.0 m, and the bottom of the water column, using a weighted pop bottle. The water samples were gravity-filtered through glass-fibre filters (Whatman GF/C), frozen, and stored in the dark until analysis (i.e. within 1 wk of sampling). Chl a was subsequently extracted with 10 ml of 95% ethanol (Merck 4111) for 24 h in a cold (ca. 1–2°C), dark room. The absorbances before and after acidification with 1 M HCl were determined using a Thermo Scientific™ GENESYS™ 10S UV–Vis spectrophotometer at 665 nm using methods similar to Nusch (1980). Bloom phases were classified using methods similar to Lemley et al. (2015) as follows: (1) accumulation ($20 \leq$ µg chl a l^{-1} ≤ 60); (2) hypereutrophic (>60 µg chl a l^{-1}); and (3) no bloom (<20 µg chl a l^{-1}). Two modified Working Party 2 plankton nets (570 mm mouth diameter; 0.2 mm mesh aperture) were fitted with calibrated General Oceanics flowmeters (model 2030R) and towed at a constant speed of 1 to 2 knots for 3 min after dark (minimising net avoidance). The 2 nets were simultaneously lowered and towed horizontally alongside a 5 m riverboat. One net was used to sample the near-surface waters (0–0.6 m) and the other was held down using a graduated pole to sample the near-bottom waters (1.4–2 m) at a mean volume of 189.76 ± 70.41 m^3 per net. An oblique course across the axis of the estuary was followed, enabling the samples to be taken near the margins and in the mid-channel (Wooldridge & Bailey 1982). The samples were immediately preserved in 10% buffered formalin after each tow.

Zooplankton species were identified and enumerated by following methods similar to Wooldridge & Melville-Smith (1979), whereby the samples were diluted by adding freshwater to a predetermined volume (up to 2 l on average), and 3 subsamples were drawn after agitation with a paddle. These samples were then placed on a tray and identified to the nearest taxon using a Leica M80 stereomicroscope. The original descriptions of the species (e.g. Connell & Grindley 1974, Brownell 1983, Wittmann 1992) were used for identification. The copepods were divided into classes, which were related to the degree of sexual maturity, following Jerling & Wooldridge (1989). These included mature males, ovigerous females, non-ovigerous females, and juveniles. Similarly, the Mysidacea classes were based on those described by Mauchline (1973): (1) juveniles, secondary sexual characteristics not developed; (2) immature males; (3) immature females; (4) females with developing young in the brood pouch; (5) females with rounded embryos; (6) females with empty marsupia and young released; (7) mature males. Species abundance was determined by the number of individuals of each taxon per cubic meter of water, following Wooldridge & Melville-Smith (1979):

$$\text{Zooplankton abundance} = \left(\frac{N}{s} \times d\right)/V \qquad (1)$$

where N is the total number of individuals, s is the subsample volume, d is the dilution volume, and V is the total volume filtered (m^{-3}) as measured by the General Oceanics flowmeters.

2.4. Statistical analysis

All data exploration and analyses were conducted using R statistical software version 4.1.0 (R Core Team 2021) with $\alpha = 0.05$. The Shapiro-Wilk test was used to assess the normality of the data, and Levene's test was used to test for the assumption of homogeneity of variance between the independent groups. However, despite applying several transformations to the data, neither of the above assumptions for performing parametric statistical analysis were met. Therefore, a Kruskal-Wallis test, with Bonferroni correction for the post hoc (Wilcox) tests, was used to explore the differences among the seasons, study sites, depths, and bloom phases of the zooplankton species abundance and environmental variables.

No time-lagged effects were observed when using the autocorrelation functions for species abundance over the sampled time scale, and thus all data were analysed without implementing any lag effects. The zooplankton abundance data were overdispersed, and therefore a negative binomial distribution was

used to model the response variable during the model-based approach. Since relationships between environmental variables (including the HAB) and zooplankton species abundance are often non-linear, both linear and non-linear models were tested. The multivariate generalised linear model (GLM) and generalised additive model (GAM) were fitted using the 'manyany' function of the 'mvabund' package (Wang et al. 2012). Akaike's information criterion (AIC) differed by less than 2 AIC units between the GLM and GAM. Therefore, the results of the simplest model (the GLM) are reported. Multicollinearity between the explanatory variables was tested using a variance inflation factor (VIF). VIF ≥ 3 was considered to be indicative of collinearity and any variables exceeding this threshold were removed from subsequent analyses. The model predictors included salinity, temperature, dissolved oxygen, pH, daily river inflow, and chl a, with spatial autocorrelation being considered by implementing a 'block' design with the depths nested within the study sites (Wang et al. 2012). The interactions between the explanatory variables and zooplankton abundance across the taxa were tested using likelihood ratio tests and resampled p-values (Monte Carlo resampling with 999 bootstrap iterations; Wang et al. 2012).

3. RESULTS

3.1. Environmental variability

Seasonal differences in the night-time water temperature were found, with the warmest temperatures occurring in the summer with a mean (min.–max.) of 23.9°C (18.7–26.6°C) and the coldest in winter with a mean of 15.7°C (14.0–18.4°C). Salinity was highest in the winter at 20.0 (7.8–36.1) and lowest during the spring intensive period at 13.5 (4.2–33.4). Turbidity was lower in winter at 8.7 NTU (3.1–36.4 NTU) than in spring at 13.7 NTU (2.5–44.5 NTU) and in summer at 14.2 NTU (2.9–73.9 NTU). Dissolved oxygen varied greatly, with bottom-water hypoxia (<2 mg l^{-1}) occurring in all but the winter months. The winter months had the most stable dissolved oxygen at 8.1 mg l^{-1} (4.7–11.8 mg l^{-1}) compared with that of the other seasons. Dissolved oxygen levels were lowest during the autumn at Site B4 (mid-mesohaline) and highest at Sites B1 (mid-polyhaline), B2 (lower polyhaline), and I2 (mid-mesohaline; Table 1). The baseline summer and intensive spring sampling periods had higher pH values than those of the other seasons. Additionally, the phytoplankton community was dominated by the

raphidophyte *Heterosigma akashiwo*, which is a known HAB species, and it was mainly responsible for the phytoplankton biomass maxima that were recorded during this study and a concurrent study (Bornman et al. 2022b). Phytoplankton biomass was the lowest in the winter at Site B1 (mid-polyhaline), with a mean of 4.2 µg chl a l^{-1} (0.0–10.1 µg chl a l^{-1}) and highest during the spring at Site I2 (mid-mesohaline), and Site I3 (lower mesohaline), with means of 56.5 µg chl a l^{-1} (5.3–254.6 µg chl a l^{-1}) and 57.0 µg chl a l^{-1} (1.2–328.6 µg chl a l^{-1}), respectively.

Three distinct bloom phases, based on the phytoplankton concentration, were apparent during intensive sampling, where 2 full bloom cycles were sampled during the austral spring (Fig. 2). Additionally, a very large *H. akashiwo* bloom occurred in the autumn at Site I1 (upper-mesohaline), with 905.8 µg chl a l^{-1} recorded in the bottom waters (Table S1 in the Supplement at www.int-res.com/articles/suppl/m723p019_supp.pdf). The night-time phytoplankton biomass was higher in the near-bottom waters than in the near-surface waters ($W = 72\,450$, $p < 0.001$; Fig. 2). Surface-water dissolved oxygen maxima and bottom-water dissolved oxygen minima occurred during periods of hypereutrophic blooms (Table S1). Moreover, no prolonged bottom-water hypoxia was observed during sampling (Fig. 3).

3.2. Spatial and temporal trends of zooplankton abundance and diversity

A total of 27 species from 12 families were identified during the study. The copepods *Pseudodiaptomus hessei* and *Paracartia longipatella* dominated the zooplankton community in terms of numerical abundance, representing 45.6 and 32.3% of the total catch, respectively. The Brachyura *Hymenosoma orbiculare* (10.9%) and the Mysidacea species *Mesopodopsis wooldridgei* (6.4%) and *Rhopalophthalmus terranatalis* (2.1%) were also found at high abundances. Among the zooplankton species, 22 accounted for less than 2.7% of the catch, which included species such as the copepod *Halicyclops* sp., the isopod *Cirolana fuviatillis*, and the euryhaline polychaete *Perinereis falsovariegata*.

Higher abundances of zooplankton occurred in the near-bottom waters with a mean (min.–max.) of 613.0 ind. m^{-3} (18.6–7356 ind. m^{-3}) compared with that of the near-surface waters at 525.0 ind. m^{-3} (9.7–7351.0 ind. m^{-3}; $W = 76\,176$, $p < 0.001$). Species diversity did not differ between the 2 depths ($W = 12\,006$, $p = 0.20$). Seasonal differences in abundance and

Table 1. Variation in the night-time physico-chemical variables and phytoplankton biomass during the baseline and intensive study periods in the Sundays Estuary. Mean (min.–max.) values and differing superscript letters that denote the nonparametric Kruskal-Wallis significance test values between sites and seasons are given, with p-values adjusted to account for multiple testing using the Bonferroni method

| Season	Baseline				Intensive		
	Site B1	Site B2	Site B3	Site B4	Site I1	Site I2	Site I3
Temperature (°C)							
Spring	20.0 (14.9–23.6)[d]	20 (14.7–23.9)[d]	20.2 (13.2–24.4)[cd]	20.0 (13.9–24.2)[cd]	22.2 (19.1–25.7)[b]	22.7 (19.8–26.0)[b]	22.6 (20.0–26.0)[b]
Summer	23.2 (18.7–26.6)[ab]	23.7 (21.9–25.9)[ab]	24.2 (22.2–26.1)[a]	24.3 (22.0–26.0)[a]			
Autumn	20.2 (16.5–24.7)[d]	20.4 (16.6–24.6)[cd]	20.5 (16.2–24.2)[cd]	20.4 (16.0–24.1)[cd]			
Winter	16.0 (14.6–18.0)[e]	16.0 (14.7–18.0)[e]	15.6 (14.3–18.2)[e]	15.3 (14.0–18.4)[e]			
Salinity							
Spring	24.9 (18.8–34.8)[a]	22.3 (16.2–31.6)[ab]	15.8 (11.1–22.3)[cd]	12.5 (5.3–18.8)[d]	20.6 (16.1–33.4)[e]	12.9 (9.0–26.6)[f]	7.1 (4.2–24.1)[g]
Summer	24.6 (16.5–35.6)[a]	21.0 (11.6–34.3)[ab]	14.0 (7.1–22.8)[cd]	11.2 (4.5–22.0)[de]			
Autumn	21.6 (10.4–34.9)[ab]	18.4 (8.4–33.6)[bc]	14.0 (4.8–25.9)[cd]	11.8 (3.4–23.7)[d]			
Winter	26.1 (16.9–36.1)[a]	22.5 (14.1–30.2)[ab]	17.2 (10.4–25.7)[bc]	14.0 (7.8–22.1)[cd]			
Turbidity (NTU)							
Spring	14.4 (4.4–44.4)[abc]	16.7 (5.9–41.1)[a]	12.9 (7.6–24.4)[ab]	11.0 (2.5–22.7)[abc]	12.5 (3.7–76.8)[e]	13.4 (1.2–62.3)[e]	12.2 (0.5–71.9)[e]
Summer	13.9 (5.2–36.7)[abc]	16.1 (6.0–49.1)[ab]	12.8 (2.9–29.7)[abc]	13.8 (3.0–73.9)[abc]			
Autumn	14.9 (5.1–48.2)[abc]	12.5 (4.9–27.7)[abc]	10.2 (4.6–22.6)[abc]	9.3 (4.9–16.9)[abc]			
Winter	10.2 (3.1–36.4)[c]	9.1 (3.9–22.3)[bc]	8.0 (3.9–23.7)[c]	7.6 (4.2–15.4)[c]			
pH							
Spring	8.2 (7.9–9.0)[a]	8.3 (7.8–9.0)[a]	8.3 (8.0–9.1)[a]	8.3 (8.0–9.2)[a]	8.8 (8.6–9.2)[e]	8.9 (8.3–9.2)[f]	8.9 (8.4–9.2)[f]
Summer	8.7 (7.8–11.4)[a]	8.8 (7.8–11.6)[ab]	8.9 (7.8–11.7)[ab]	8.9 (7.7–11.7)[ab]			
Autumn	8.4 (7.5–9.1)[a]	8.4 (7.7–9.1)[a]	8.3 (7.7–8.9)[a]	8.3 (7.8–8.8)[a]			
Winter	8.4 (7.8–9.6)[a]	8.4 (7.8–9.4)[a]	8.4 (7.8–9.3)[a]	8.4 (7.8–9.2)[a]			
Dissolved oxygen (mg l^{-1})							
Spring	7.3 (2.4–10.9)[abc]	6.6 (1.6–10.6)[abc]	7.2 (4.6–9.7)[abc]	7.2 (4.9–10.9)[abc]	7.4 (3.6–11.8)[e]	8.9 (2.7–14.8)[f]	7.6 (2.0–14.7)[e]
Summer	7.1 (5.6–8.8)[abc]	6.5 (1.4–9.5)[abc]	6.8 (1.9–9.8)[abc]	6.6 (0.9–11.1)[abc]			
Autumn	8.4 (2.8–24.1)[a]	7.2 (0.4–17.2)[abc]	6.0 (0.3–12.2)[bc]	5.5 (0.6–10.4)[c]			
Winter	8.4 (5.8–11.2)[a]	8.5 (4.7–11.8)[a]	8.1 (5.0–10.0)[ab]	7.6 (4.9–9.4)[abc]			
Phytoplankton biomass (µg chl a l^{-1})							
Spring	8.9 (0.0–21.3)[ef]	16.5 (2.4–136.2)[cdef]	15.0 (5.9–39.7)[bcde]	19.2 (4.7–59.2)[abcde]	24.5 (3.0–340.4)[h]	56.5 (5.3–254.6)[g]	57.0 (1.2–328.6)[k]
Summer	13.6 (3.6–24.9)[cde]	19.2 (7.1–54.5)[abcde]	38.9 (3.5–290.1)[abc]	37.0 (8.3–152.4)[ab]			
Autumn	43.6 (1.8–905.8)[def]	27.6 (1.8–223.0)[bcde]	34.9 (3.0–173.2)[abcd]	45.8 (1.2–256.0)[abcd]			
Winter	4.2 (0.0–10.1)[f]	13.0 (1.2–59.8)[def]	12.9 (1.8–29.6)[cdef]	20.6 (1.2–91.8)[cdef]			

A: Baseline
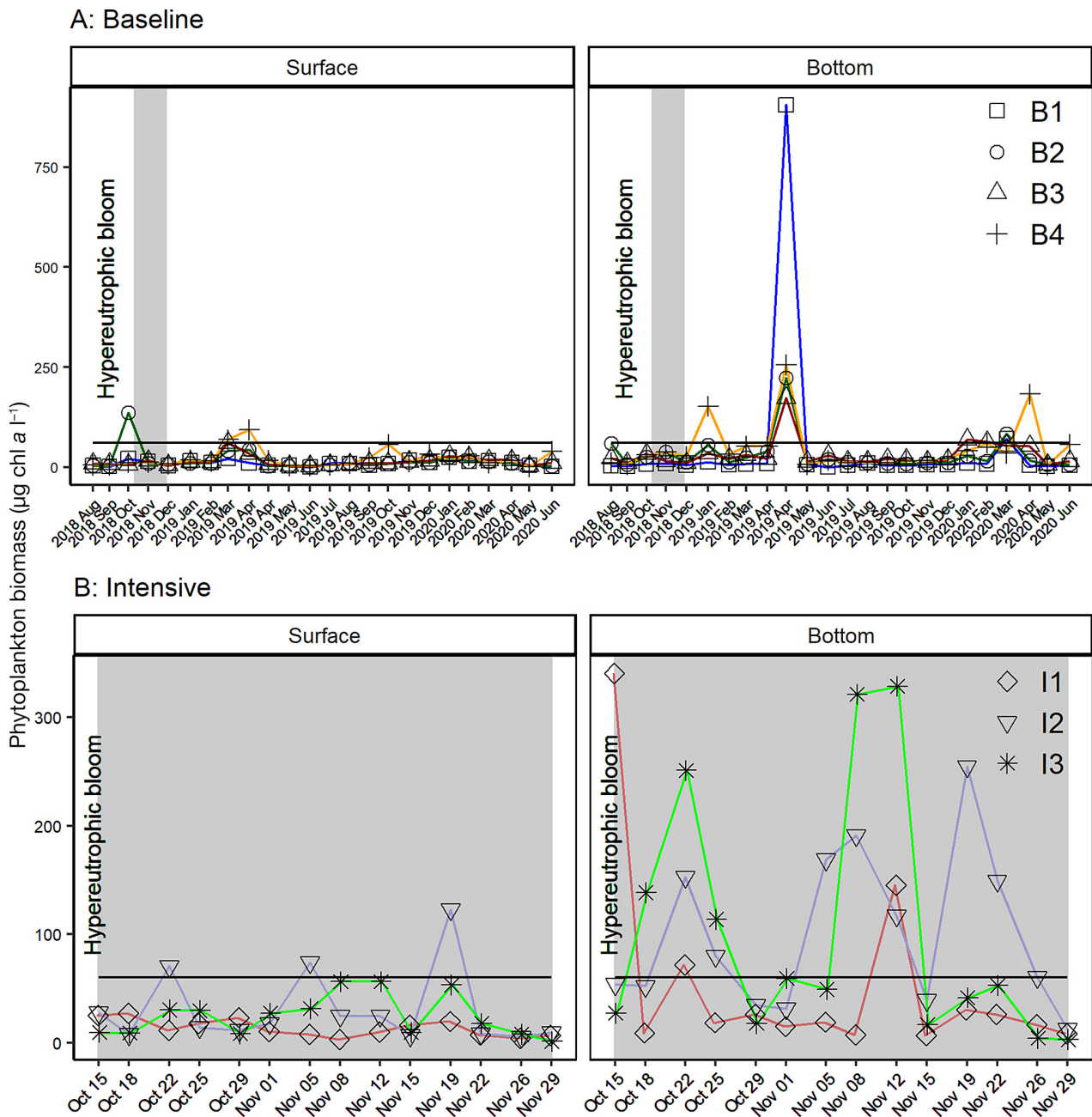
B: Intensive

Fig. 2. Frequency and intensity of the night-time hypereutrophic blooms (>60 μg chl a l^{-1}) in the near-surface (<0.5 m) and near-bottom (>1.5 m) waters at each site (points) in the Sundays Estuary during the (A) baseline (white background) and (B) intensive (grey background) study period in 2018–2020

diversity were recorded (Fig. 4). Abundance was highest in the spring during the baseline study period, with a mean (min.–max.) of 1133.9 ind. m^{-3} (37.1–7356.1 ind. m^{-3}), and it was higher (Kruskal-Wallis $\chi^2 = 68.0$, df = 4, p < 0.001) than those that occurred in the summer, at 570.0 ind. m^{-3} (15.3–3848.7 ind. m^{-3}), autumn at 314.1 ind. m^{-3} (9.7–1239.8 ind. m^{-3}), and winter at 154.8 ind. m^{-3} (11.7–932.0 ind. m^{-3}). Species diversity was significantly lower in the autumn

during the baseline period, with a Shannon diversity index of 0.6 (0.0–1.6) compared with that of the other seasons (Kruskal-Wallis $\chi^2 = 38.3$, df = 4, p < 0.001; Fig. 4).

Copepods were most abundant in the zooplankton samples and were found at their highest abundance in the spring. The abundance of $P.$ $hessei$ peaked in the spring during the baseline study at 470.0 ind. m^{-3} (12.3–681.0 ind. m^{-3}) compared with that of the other

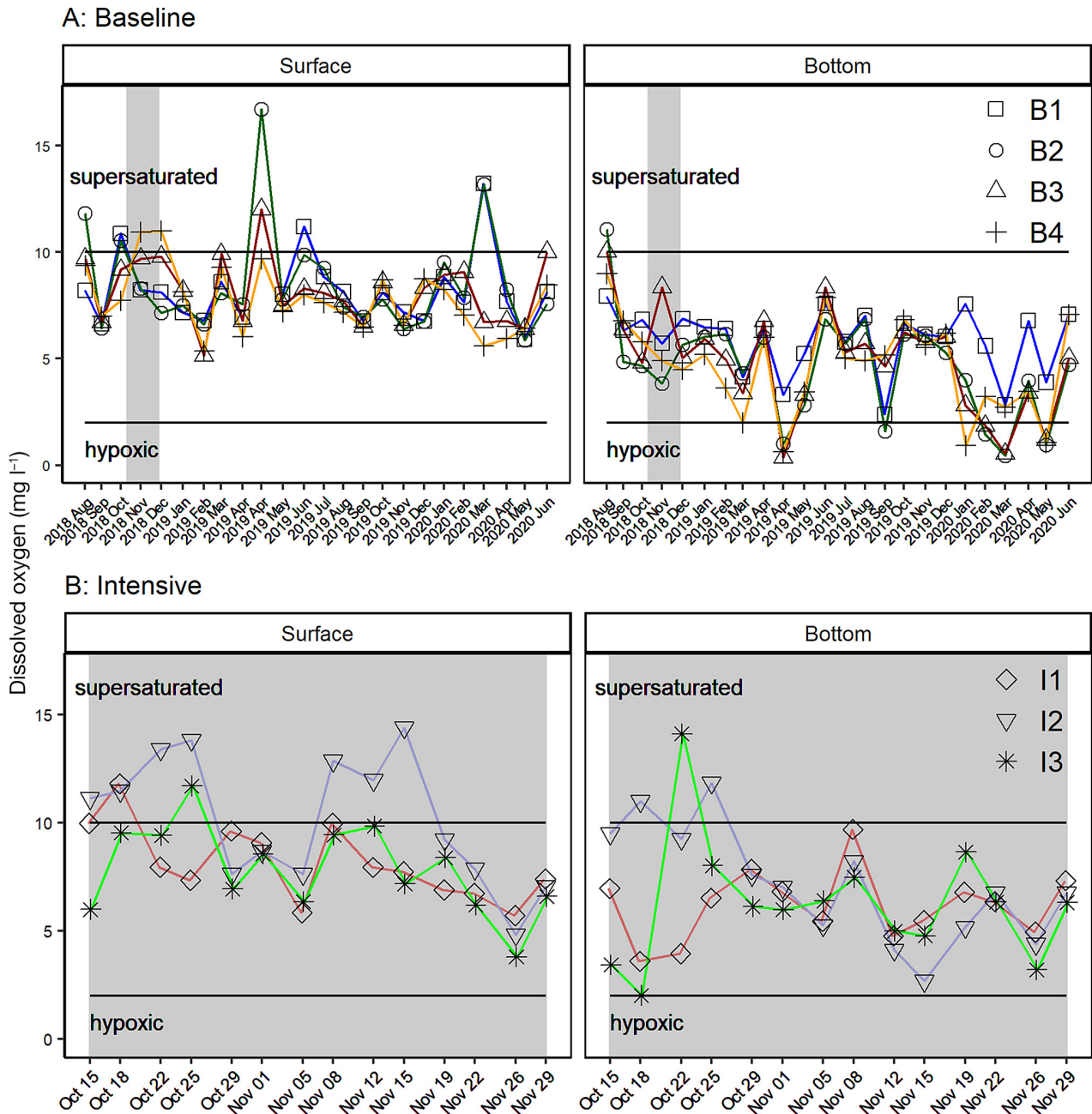Fig. 3. Night-time near-surface (<0.5 m) and near-bottom (>1.5 m) water hypoxia (<2 mg l^{-1}) and supersaturation (>10 mg l^{-1}) at each study site (points) during the (A) baseline (white background) and (B) intensive (grey background) study period in the Sundays Estuary (2018–2020)

seasons (Kruskal-Wallis χ^2= 57.5, df = 4, p < 0.001). This was mostly due to the increased abundance of ovigerous females and juveniles during the spring season (Table S2). Similarly, lower *P. longipatella* abundances were found during the autumn than in the other seasons. *Halicyclops* sp. and *Acartiella natalensis* were found at low abundances during this study at 0.1 ind. m^{-3} (0.0–1.9 ind. m^{-3}) and 3.8 ind. m^{-3} (0.0–284.0 ind. m^{-3}), respectively. *A. natalensis*

was only found in the spring and summer months during the baseline study. The larval stages of the common brachyuran *H. orbiculare* were only found at higher abundances during the spring months of the intensive study (Kruskal-Wallis χ^2 = 83.5, df = 4, p < 0.001). The abundances of the Mysidacea *M. wooldridgei* were higher in the spring during the baseline sampling at 81.2 ind. m^{-3} (0.0–721.0 ind. m^{-3}) compared with that during the other seasons,
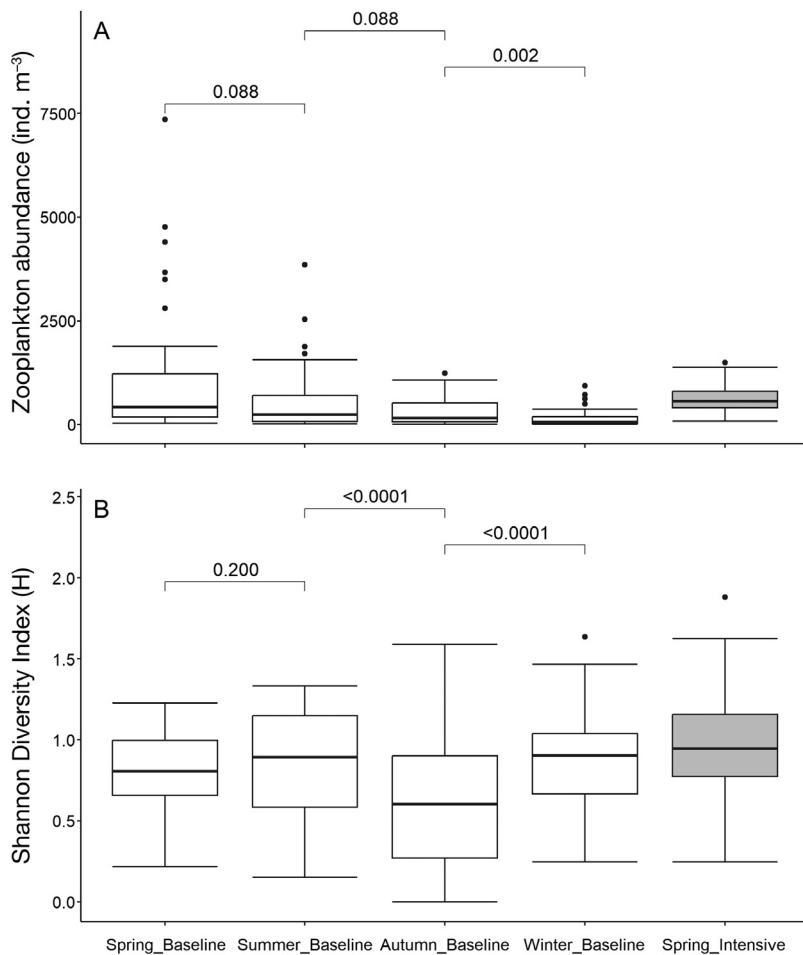
Fig. 4. Seasonal variation in the night-time zooplankton species abundance and diversity during the study period in the Sundays Estuary. Brackets indicate Bonferroni-corrected Wilcox significance tests between the seasons during the baseline (white) and intensive sampling (grey) periods. Boxplot parameters: mean and interquartile range; whiskers: minimum and maximum values; points: outliers

to the estuary mouth, in the polyhaline zone (Kruskal-Wallis $\chi^2 = 120.1$, df = 6, p < 0.001). When *A. natalensis* was present in the spring and summer months, it was only found in the lower polyhaline to mid-mesohaline zone (10 < salinity ≤ 18) of the estuary, while *Halicyclops* sp. occurred at the highest abundance at the sites that were furthest from the mouth; specifically, Site I3 of the spring intensive study at 22.6 ind. m^{-3} (0.0–284.0 ind. m^{-3}; Table 2). Similarly, *H. orbiculare* larvae occurred at the sites that were furthest from the mouth during the spring, with higher abundances occurring at Site I2 at 173.0 ind. m^{-3} (0.0–907.0 ind. m^{-3}) and Site I3 at 412.0 ind. m^{-3} (19.0–1094.0 ind. m^{-3}) than at Site I1 at 3.6 ind. m^{-3} (0.0–27.0 ind. m^{-3}; Kruskal-Wallis $\chi^2 = 180.2$, df = 6, p < 0.001). The Mysidacea species *M. wooldridgei* were in the highest abundances at sites closer to the mouth during the baseline (Kruskal-Wallis $\chi^2 = 41.2$, df = 3, p < 0.001) and intensive study periods (Kruskal-Wallis $\chi^2 = 44.8$, df = 2, p < 0.001). However, *R. terranatalis* occurred at higher abundances at Sites B2 (15.7 ind. m^{-3}; 0.1–115.0 ind. m^{-3}) and B3 (8.7 ind. m^{-3}; 0.0–79.8 ind. m^{-3}) than at Sites B1 (4.0 ind. m^{-3}; 0.0–25.5 ind. m^{-3}) and B4 (3.59 ind. m^{-3}; 0.0–48.0 ind. m^{-3}; Kruskal-Wallis $\chi^2 = 88.3$, df = 6, p < 0.001). Both Mysidacea species occurred at higher abundances in near-bottom waters than in near-surface waters (Table 2).

GLMs indicated that zooplankton species abundance was positively explained by salinity (31.3%) and temperature (12.1%; or differences in the sites and seasons), and to a lesser extent, by phytoplankton biomass (2.3%; Table 3). Abundances of *P. hessei* and *P. longipatella*, the 2 dominant copepod species during this study, were positively related to temperature and salinity (Table 3). Abundance of the brachyuran *H. orbiculare* was negatively related to salinity (26.4%) and pH (3.9%), while temperature (7.3%) and phytoplankton biomass (2.4%) were positively related to its abundance (Table 3). The abundance of the polychaete *P. falsovariegata* was also positively related to salinity and phytoplankton biomass and explained 31.7 and 13.0% of the deviance, respectively (Table 3). The abundance of the Mysidacea species *M. wooldridgei* and

with the lowest being in the winter at 12.4 ind. m^{-3} (0.1–90.8; Kruskal-Wallis $\chi^2 = 26.4$, df = 4, p < 0.001). The larger-sized mysid *R. terranatalis* also occurred at higher abundances during spring and summer (Kruskal-Wallis $\chi^2 = 15.7$, df = 4, p = 0.004). The above result was mainly due to increased numbers of immatures and females, either with eggs or developing young in their brood pouches (Table S2).

The dominant species *P. hessei* occurred at higher abundances at sites that were closer to the mouth of the estuary in the polyhaline zone (18 < salinity ≤ 30) than in the mesohaline (5 < salinity ≤ 18) sites (Kruskal-Wallis $\chi^2 = 70.0$, df = 6, p < 0.001; Table 2). *P. hessei* occurred at higher abundances in the near-bottom waters than in the near-surface waters ($W = 38226$, p < 0.001; Table 2). Similarly, *P. longipatella* abundances were higher at the sites that were closer

Table 2. Spatial variation in the dominant zooplankton abundance mean (min.–max.) in the near-surface and near-bottom water during the baseline and intensive study in the Sundays Estuary from 2018 to 2022

Species	Baseline				Intensive		
	Site B1	Site B2	Site B3	Site B4	Site I1	Site I2	Site I3
Surface							
Pseudodiaptomus hessei	448.2 (25.0–2265.0)	247.3 (5.4–870.2)	76.5 (3.1–396.7)	57.4 (4.3–404.0)	165.2 (40.0–358.0)	287.3 (7.0–681.0)	255.1 (20.0–632.0)
Paracartia longipatella	688.5 (0.0–5332.6)	339.2 (0.0–3951.0)	9.2 (0.0–84.4)	0.6 (0.0–3.7)	97.1 (1.0–563.0)	9.4 (0.0–113.0)	0.1 (0.0–1.0)
Acartiella natalensis	0.0 (0.0–0.0)	0.1 (0.0–1.2)	0.1 (0.0–1.9)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)
Halicyclops sp.	0.8 (0.0–7.6)	0.5 (0.0–2.0)	2.2 (0.0–26.4)	3.0 (0.0–18.6)	0.4 (0.0–2.0)	2.8 (0.0–20.0)	14.6 (0.0–91.0)
Hymenosoma orbiculare larvae	0.0 (0.0–0.4)	0.3 (0.0–2.2)	2.9 (0.0–21.6)	11.8 (0.0–101.6)	4.9 (0.0–27.0)	172.1 (0.0–772.0)	353.5 (19.0–626.0)
Mesopodopsis wooldridgei	78.5 (0.0–708.0)	50.3 (0.3–225.8)	19.2 (0.0–258.0)	8.3 (0.0–122.0)	101.1 (0.0–192.0)	24.8 (0.0–126.0)	0.7 (0.0–5.0)
Rhopalophthalmus terranatalis	3.1 (0.0–25.5)	7.6 (0.1–39.7)	4.7 (0.0–30.2)	1.4 (0.0–13.4)	34.8 (0.0–81.0)	25.5 (0.0–77.0)	1.1 (0.0–14.0)
Bottom							
P. hessei	521.4 (34.7–2549.0)	358.2 (15.0–1090.0)	171.6 (4.8–1762.5)	155.1 (3.9–521.0)	303.1 (113.0–576.0)	315.0 (77.0–660.0)	301.6 (56.0–589.0)
P. longipatella	633.1 (0.5–4644.5)	257.3 (0.0–2157.9)	18.5 (0.0–149.8)	0.8 (0.0–7.0)	95.2 (0.0–351.0)	76.1 (0.0–597.0)	4.9 (0.0–55.0)
A. natalensis	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.6)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)
Halicyclops sp.	0.2 (0.0–2.5)	0.3 (0.0–2.3)	1.1 (0.0–9.5)	6.3 (0.0–79.3)	0.5 (0.0–3.0)	2.1 (0.0–8.0)	30.6 (0.0–284.0)
H. orbiculare larvae	0.0 (0.0–0.0)	0.2 (0.0–1.6)	3.1 (0.0–27.8)	10.3 (0.0–92.6)	2.3 (0.0–22.0)	173.0 (0.0–907.0)	470.2 (125.0–1094.0)
M. wooldridgei	59.3 (1.2–720.7)	37.6 (0.1–165.9)	19.2 (0.0–115.0)	8.2 (0.0–67.8)	80.9 (15.0–216.0)	26.5 (0.0–168.0)	2.1 (0.0–8.0)
R. terranatalis	4.9 (0.1–22.9)	23.7 (0.4–114.9)	12.6 (0.0–79.8)	5.8 (0.0–48.0)	32.4 (0.0–59.0)	26.6 (0.0–76.0)	6.9 (0.0–92.0)

R. terranatalis was also positively related to temperature and salinity (Table 3).

3.3. Zooplankton trends during the HAB phases

Zooplankton abundances differed between the 3 bloom phases during the intensive study period, in which 2 HAB cycles were sampled during spring in 2018. The lowest zooplankton abundance of 504.6 ind. m^{-3} (84.0–1384.0 ind. m^{-3}) occurred when HABs were absent, while the accumulation (733.7; 296.0–1298.0 ind. m^{-3}) and hypereutrophic bloom (675.8; 293.0–1497.0 ind. m^{-3}) phases were similar (Kruskal-Wallis $\chi^2 = 12.6$, df = 2, p > 0.05; Fig. 5).

The abundances of *P. hessei* were similar between bloom phases (Fig. 6); however, juveniles occurred at a higher abundance during the hypereutrophic bloom (>60 µg chl *a* l^{-1}) at 79.9 ind. m^{-3} (0.0–286.6 ind. m^{-3}) compared with instances of no blooms at 45.6 ind. m^{-3} (0.0–382.0 ind. m^{-3}; Kruskal-Wallis $\chi^2 = 9.1$, df = 2, p = 0.002; Fig. 7). *P. longipatella* and *M. wooldridgei* were found at lower abundances in the hypereutrophic bloom phase than in the periods when the blooms were absent (Fig. 6). Additionally, higher *H. orbiculare* abundances occurred during the hypereutrophic bloom phase (Fig. 6), and *R. terranatalis* occurred at a similar abundance among the bloom phases (Fig. 6). The opportunist isopod *C. fluviatilis*, the amphipod *Monocorophium acherusicum* (a burrowing benthic suspension feeder), and the euryhaline polychaete *P. falsovariegata* were all found at higher abundances in the accumulation and hypereutrophic bloom phase than in the no-bloom phase (Fig. 6).

4. DISCUSSION

The Sundays Estuary is an important ecological and economic system that serves as both a nursery and feeding habitat for numerous commercial and recreational fish species. Some of these species are predatory fishes that are already facing population declines due to overfishing (Cockcroft & Dudley 2020) and rely on the abundant primary consumer zooplankton populations in estuaries as a direct or indirect food source during their juvenile stage (Whitfield 2019). Therefore, any major changes to the zooplankton community due to changes in water quality could have far-reaching consequences for the entire estuarine food web. Despite the importance of the Sundays Estuary as a nursery, water quality has been degraded by agricultural activities, threatening its ecological integrity.

The presence of recurrent HABs of *Heterosigma akashiwo* has led to frequent super-saturated surface and hypoxic bottom-water conditions. These conditions have been found to affect the estuarine zooplankton community in previous studies worldwide, showing a complete collapse during HABs in Florida Bay (Goleski et al. 2010), Raritan Bay (Rothenberger et al. 2014), and in the Mediterranean (Papantoniou et al. 2020). However, results from this study show that the abundance of zooplankton remained high even during extensive hypereutrophic blooms (>60 µg chl *a* l^{-1}) of *H. akashiwo*.

The present study showed that salinity and temperature were the primary drivers of the zooplankton community in the warm-temperate Sundays Estuary, and no die-off of mesozooplankton occurred because of hypoxia or HAB presence. Specifically, the dominant species *Pseudodiaptomus hessei*, a common calanoid planktivorous copepod that is found in warm-temperate South African estuaries, exhibited high abundance during the spring season, with abundances that were similar to those previously found in the Sundays Estuary (Wooldridge & Bailey 1982, Jerling & Wooldridge 1995b, Sutherland et al. 2013). Furthermore, the sampling frequency of the present investigation allowed for a comparison of zooplankton abundance during 3 bloom phases, and no significant difference in the abundance of *P. hessei* was observed during the accumulation, hypereutrophic, and no-bloom phases.

The findings from this study contrast the recent results by Papantoniou et al. (2020), who reported a significant reduction in mesozooplankton due to co-occurring anoxic conditions and HABs in Papas Lagoon (Mediterranean). Papantoniou et al. (2020)

found that *Acartia clausi* and *Paracartia latisetosa* (<1 mm) completely disappeared from the water column during HABs of a Raphidophyceae that reached a maximum biomass of ca. 38 µg chl *a* l^{-1}. The abundance of the above copepod species was negatively correlated with dissolved oxygen (Papantoniou et al. 2020). During the present study, the similarly sized (<1 mm) and common warm-temperate South African copepod, *P. longipatella*, was also found at higher abundance in non-bloom conditions than in hypereutrophic conditions. However, unlike in the non-tidal Papas Lagoon, the abundance of *P. longipatella* was correlated with salinity and temperature and not dissolved oxygen and chl *a*. Another common warm-temperate South African copepod, *A. natalensis*, was relatively scarce in this study, which is consistent with prior findings (Wooldridge & Melville-Smith 1979) but differed from previous research in that its presence was limited to spring and summer months. Therefore, the distribution of mesozooplankton in the Sundays Estuary was mostly determined by species' physicochemical preferences or predator–prey interactions rather than the influence of HABs at the current concentration levels.

The model results suggested that the observed zooplankton abundance trends were primarily related to salinity and temperature (41% explained) rather than dissolved oxygen fluctuations (<1% explained) or HAB concentration. A positive relationship was also observed between the abundance of *P. hessei* and both salinity and temperature. Notably, temperature emerged as the most significant factor in explaining the seasonal and temporal patterns of this species. These findings are in contrast with previous research, which classified *P. hessei* as a 'pioneer' species that typically thrives in low-salinity conditions to exploit post-flood habitats (Wooldridge & Melville-Smith 1979). This discrepancy was also noted by Strydom et al. (2014), who reported that the highest abundance of larval fishes and copepods occurred in the euhaline zone (i.e. salinity > 30) in the Sundays Estuary. Conversely, in comparable estuaries, peak abundances were observed in the highly productive mesohaline zone (i.e. 5 < salinity ≤ 18). Strydom et al. (2014) attributed this observation to the potential avoidance of HABs by late-stage larval fishes. However, in the present study, *P. hessei* was consistently found at higher abundance near the estuary mouth, and these abundances did not fluctuate in response to HABs, even during hypereutrophic bloom phases with extreme fluctuations in dissolved oxygen.

Notably, this study found no correlation between the abundance of *P. hessei* and *H. akashiwo*. There-

Table 3. Multivariate generalised linear model output of the dominant zooplankton in the Sundays Estuary. The deviance explained (D) by each predictor variable, directional coefficients (C) with the standard error (SE) of each predictor variable, and significance level of the likelihood-ratio-test statistic (p) are shown as *p < 0.05, **p < 0.01, and ***p < 0.001. – : not applicable

	df	Overall D (%)	p	*Pseudodiaptomus hessei* D (%)	C (SE)	p	*Paracartia longipatella* D (%)	C (SE)	p	*Hymenosoma orbiculare* larvae D (%)	C (SE)	p
Phytoplankton biomass (µg chl a l⁻¹)	1	2.3	***	0.5	0.0(0.1)	0.486	1.2	−0.2(0.1)	0.311	2.4	0.1(0.1)	*
Dissolved oxygen (mg l⁻¹)	1	0.9	0.056	0.5	0.0(0.1)	0.756	0.3	1.0(0.2)	0.756	1.2	0.3(0.1)	0.227
Salinity	1	31.3	**	9.4	0.1(0.1)	**	58.7	3.3(0.3)	**	26.4	−0.2(0.3)	**
Temperature (°C)	1	12.1	**	22.8	0.4(0.1)	**	4.4	0.2(0.2)	**	7.3	0.6(0.2)	**
pH	1	2.3	*	1.7	−0.2(0.1)	0.276	2.5	−0.3(0.1)	*	3.9	−0.4(0.1)	0*
Daily river inflow (m³ s⁻¹)	1	5.3	0.200	1.3	−0.1(0.1)	0.253	6.6	−0.3(0.2)	0.248	3.2	0.1(0.1)	0.253
Year	2	8.1	0.217	4.5	–	0.273	2.1	–	0.273	9.3	–	0.273
Season	3	6.9	***	29.7	–	***	3.5	–	0.100	4.3	–	**
Study site	6	28.6	***	27.5	–	***	14.1	–	***	41.8	–	***
Depth	1	2.2	***	2	–	0.216	6.5	–	**	0.4	–	0.55

fore, similar to other calanoid copepods, *P. hessei* may be more sensitive to salinity and temperature than dissolved oxygen, which ranged from supersaturated (>10 mg l⁻¹) to hypoxic (<2 mg l⁻¹) during the present study. However, the possibility that *P. hessei* is able to graze on HABs, such as *H. akashiwo* or other intermediate species, cannot be ruled out (Turner 2014), as there is limited knowledge about the grazing preferences of South African estuarine mesozooplankton. Nonetheless, it should be noted that mortalities may occur in *P. hessei* populations during prolonged hypoxic or anoxic conditions as has been observed in other calanoid copepod species that occur in estuarine systems with longer retention times (Grodzins et al. 2016, Papantoniou et al. 2020).

Competition and predation greatly influence the abundance of mesozooplankton such as copepods, which are a key prey source for many estuarine species including mysids and larval and juvenile fishes (Strydom et al. 2014). The 2 most commonly encountered mysid species in warm-temperate South African estuaries were also present in high abundance during the present study (Wooldridge & Bailey 1982). *Mesopodopsis wooldridgei* occurred at high abundance in the mid- to upper polyhaline zones that were closest to the estuary mouth during night sampling and were found at the near bottom of the water column. *Rhopalophthalmus terranatalis*, although present at lower abundances than *M. wooldridgei*, were observed more frequently in the upper polyhaline to the mid-mesohaline zone. The differential spatial distribution of the 2 species could be attributed to predation by larger *R. terranatalis* on juvenile *M. wooldridgei* and larger copepods, as evidenced by previous studies (Wooldridge & Webb 1988, Jerling & Wooldridge 1995a,b). Therefore, competition and pre-

dation may be more important in driving species distributions and abundance in the Sundays Estuary than HABs.

The abundance of *M. wooldridgei* was found to be greater during periods of HAB absence, while *R. terranatalis* abundance remained similar between bloom phases. The observed trends in species distribution were likely due to differences in salinity and temperature and predator–prey interactions as opposed to dissolved oxygen or HAB sensitivity. While little is known about the water quality tolerances of the species in question, other estuarine mysids, such as *Tenagomysis novae-zealandiae*, which are commonly found in New Zealand estuaries, are tolerant to low oxygen concentrations (<4 mg l⁻¹), with mortalities occurring only at dissolved oxygen levels less than 0.5 mg l⁻¹ (Larkin et al. 2007). The ability of the 2 mysids to tolerate low dissolved oxygen levels may have significant implications for their competitive and predatory interactions with other species, particularly larval fishes, during HABs (Smit et al. 2021).

The larvae of the crown crab *Hymenosoma orbiculare* are common in South African estuaries and can develop in a wide range of salinities; however, late-stage larvae were absent, which suggests that they have a marine stage in their life history (Papadopoulos et al. 2002, 2006). Adult hymenosomatids can occur in high abundances in estuaries and are common in the gut contents of fishes, playing a significant role in estuarine food webs (Hill & Forbes 1979). During the present study, *H. orbiculare* larvae occurred at high abundance during the accumulation and hypereutrophic bloom phases and were weakly positively related to phytoplankton biomass. Very little is known about the feeding habits of *H. orbiculare* larvae; however, it may be that this species can suc-

Table 3 (continued)

| Mesopodopsis wooldridgei			Rhopalophthalmus terranatalis			Cirolana fluviatilis			Monocorophium acherusicum			Perinereis falsovariegata		
D (%)	C (SE)	p	D (%)	C (SE)	p	D (%)	C (SE)	p	D (%)	C (SE)	p	D (%)	C (SE)	p
1.5	−0.1(0.1)	0.322	0.8	0.0(0.1)	0.486	4.0	0.1(0.2)	0.136	0.8	0.1(0.1)	0.353	13.0	0.6(0.2)	**
0.7	0.4(0.1)	0.756	0.1	0.0(0.1)	0.874	2.5	−0.4(0.2)	0.351	1.7	−0.4(0.2)	0.222	0.1	−0.4(0.3)	0.874
39.2	1.0(0.2)	**	3.5	0.4(0.2)	*	14.7	0.1(0.3)	**	42.8	−0.7(0.3)	**	31.7	0.3(0.5)	**
5.6	0.4(0.1)	**	24.8	0.6(0.1)	**	28.8	0.9(0.2)	**	12.5	1.0(0.2)	**	2.9	0.8(0.4)	0.078
0.5	−0.4(0.1)	0.447	5.0	0.0(0.1)	*	1.1	−0.7(0.4)	0.447	0.9	0.1(0.1)	0.402	1.9	0.4(0.3)	0.402
6.4	0.1(0.1)	0.253	5.4	0.1(0.1)	0.253	5.2	−0.2(0.2)	0.253	6.4	−0.4(0.1)	0.248	11.7	−0.2(0.2)	0.251
18.2	−	0.273	20.7	−	0.273	10.4	−	0.273	2.7	−	0.273	4.2	−	0.273
1.8	−	0.351	5.0	−	0.281	4.5	−	0.334	2.0	−	0.334	7.2	−	0.211
22.6	−	**	32.4	−	***	28.7	−	***	28.8	−	***	26.5	−	***
3.6	−	0.053	2.2	−	0.269	0.1	−	0.773	1.5	−	0.216	0.6	−	0.649
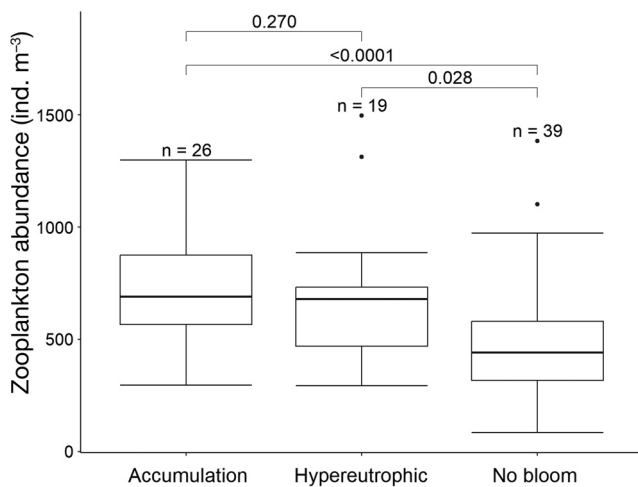
Fig. 5. Night-time zooplankton abundance over the 3 harmful algal bloom phases — accumulation ($20 \leq$ µg chl a l$^{-1} \leq 60$), hypereutrophic (>60 µg chl a l^{-1}) and no bloom (< 20 µg chl a l^{-1}) — in the Sundays Estuary during the intensive study period. Brackets indicate Bonferroni-corrected Wilcox significance tests between the bloom phases; boxplot parameters as in Fig. 4

cessfully feed on *H. akashiwo,* such as seen in some polychaete larvae on the west coast of Vancouver Island (Almeda et al. 2011). Alternatively, their increased abundance during these events could be due to decreased predation. However, gut-content analysis of common larval and juvenile fishes has suggested that they avoid feeding on *H. orbiculare* larvae, despite occurring at high abundances, and prefer to feed on the copepods *P. hessei* and *P. longipatella* (Strydom et al. 2014). The protruding spines of *H. orbiculare* may be a successful deterrent and act as a defence mechanism against predation. Therefore, predation pressure is less likely to have

been related to the measured changes in abundance of *H. orbiculare* larvae between bloom phases.

Predatory or opportunist species, such as the isopod *Cirolana fluviatilis* and polychaete *Perinereis falsovariegata*, were found in higher abundances during the accumulation and hypereutrophic bloom phases, with abundance of *P. falsovariegata* being positively related to phytoplankton biomass. *C. fluviatilis* are voracious scavengers and have caused major fish losses in cage aquaculture (Newman et al. 2007, Sanil et al. 2009). Low oxygen concentrations can greatly increase predation on fish larvae by invertebrate predators compared with that of vertebrate predators (Breitburg et al. 1994, 1997) since fish larvae are less tolerant to fluctuating dissolved oxygen concentrations. This predation is likely to be lower for copepods, which may be more tolerant to low oxygen conditions and thus are more of a challenge for predators as they use the hypoxic bottom waters as refugia during these conditions (Breitburg et al. 1997, Craig 2012, Keister & Tuttle 2013). Hypereutrophic blooms have been found to cause a decline in the larval grow-out in the Sundays Estuary (Smit et al. 2021). Therefore, the more abundant predator or scavenger zooplankton species during hypereutrophic bloom conditions, as was seen in the present study, may be preying on fish larvae that are more sensitive to low oxygen conditions than copepods (Breitburg et al. 1994, 1997). Therefore, low dissolved oxygen and HABs have the potential to drive taxonomic and size shifts in zooplankton communities, which can cause major alterations in the spatial ecology and, ultimately, the food web of eutrophic estuaries (Turner 2014, Papantoniou et al. 2020).

Laboratory studies have reported that *H. akashiwo* can directly or indirectly impact the feeding, growth,
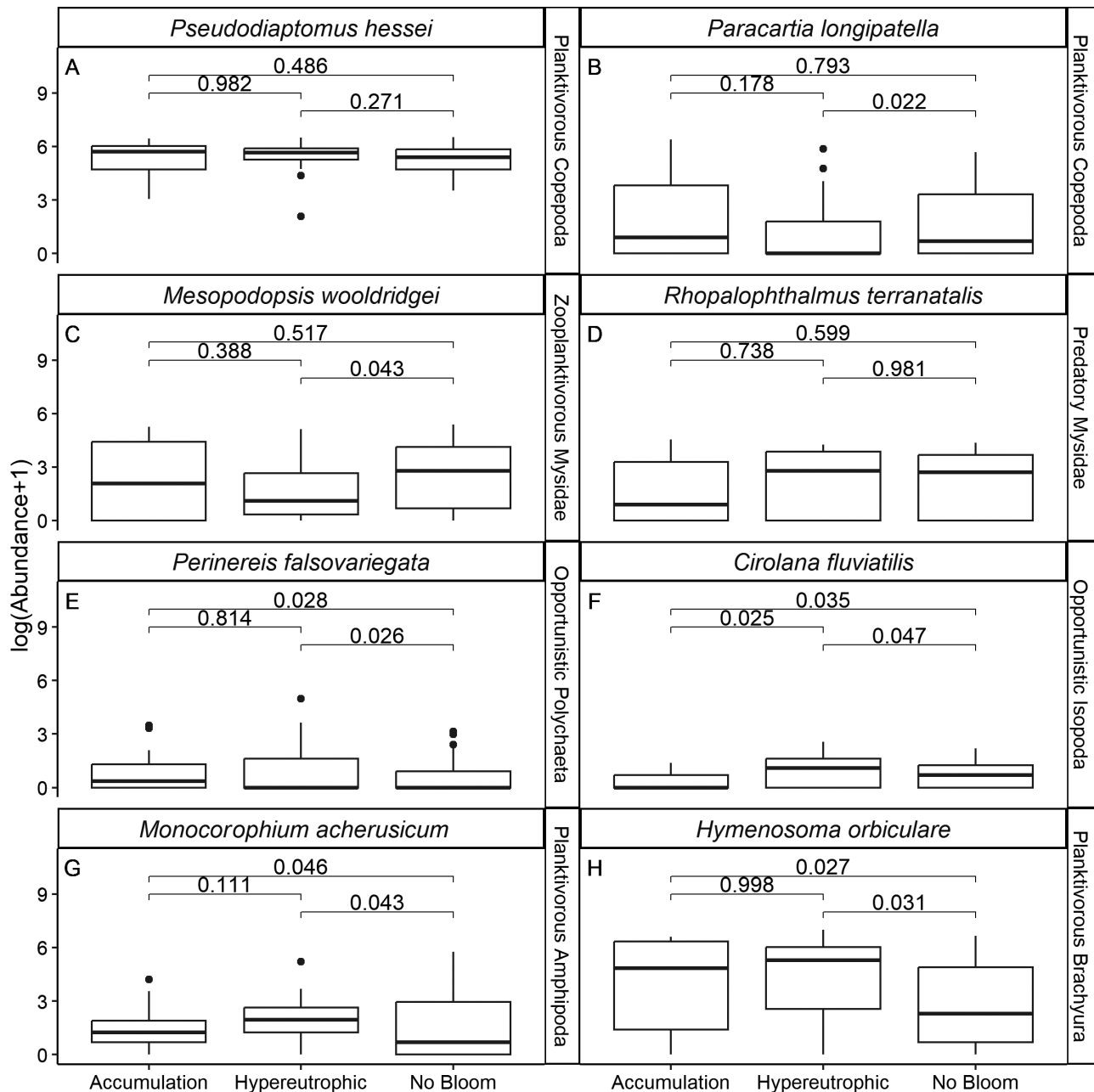
Fig. 6. Variation in the dominant zooplankton species during the intensive study period between the 3 bloom phases: accumulation (20 ≤ µg chl *a* l⁻¹ ≤ 60), hypereutrophic (>60 µg chl *a* l⁻¹) and no bloom (<20 µg chl *a* l⁻¹). Brackets indicate Bonferroni-corrected Wilcox significance tests between the bloom phases; boxplot parameters as in Fig. 4

and survival of invertebrates through a variety of pathways (Wang et al. 2006, Yu et al. 2010, Almeda et al. 2011). However, the effects of *H. akashiwo* may vary depending on the species, developmental stage, cell concentration, and exposure time (Yu et al. 2010, Almeda et al. 2011). The possible negative effects of HABs and extreme fluctuations in dissolved oxygen are also relatively short-lived, with extremes often

limited to a small area of the estuary over a short temporal scale (Lemley et al. 2018). Therefore, on larger spatial and temporal scales, the abundance of higher trophic levels resulting from high productivity due to nutrient enrichment is often greater than losses due to oxygen depletion (Breitburg et al. 1997, Breitburg 2002). However, the impacts may vary between eutrophic estuaries since systems that experi-
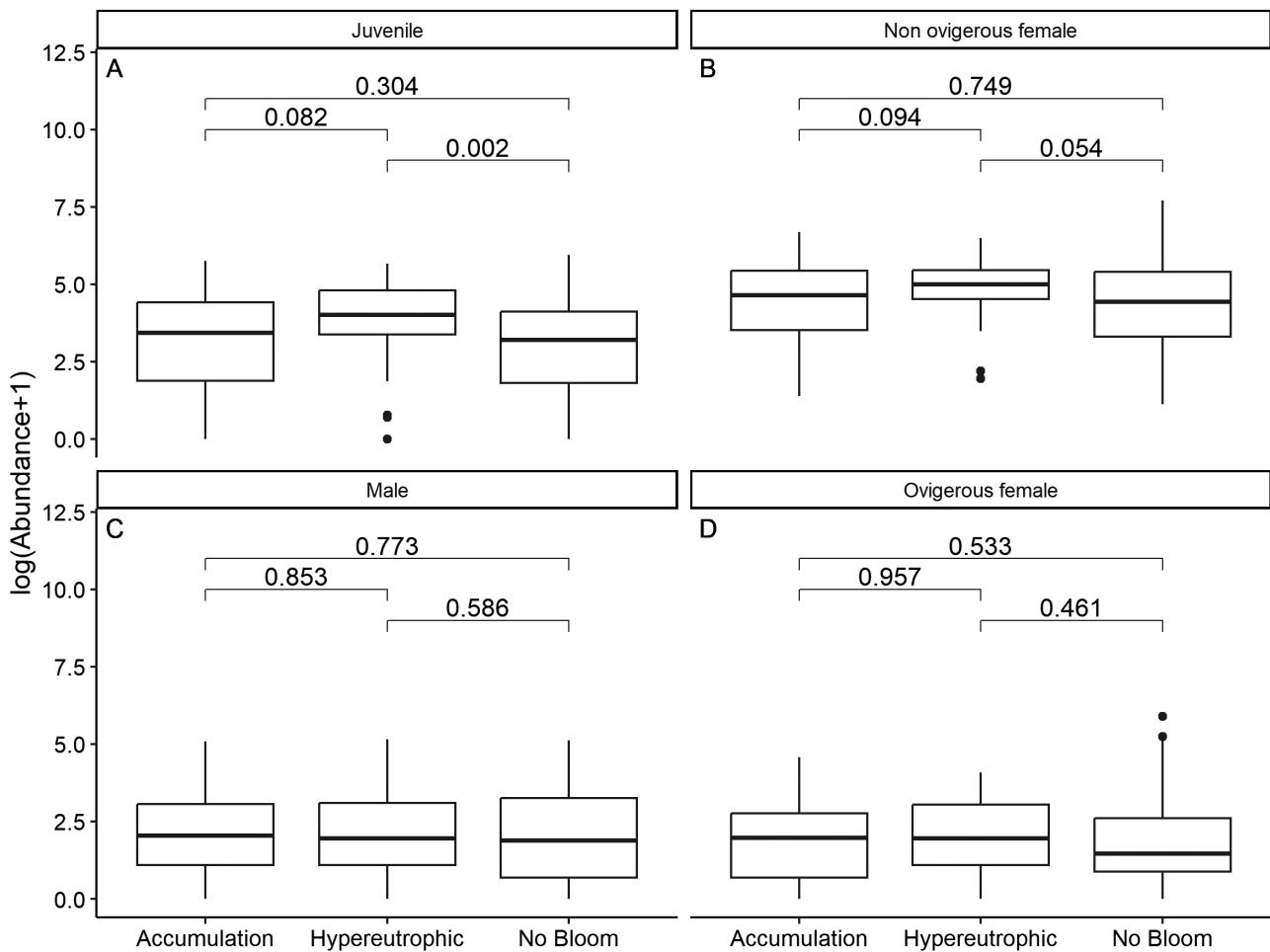
Fig. 7. Abundances of *Pseudodiaptomus hessei* sexual maturity classes during the intensive study period between the 3 bloom phases: accumulation ($20 \leq$ µg chl *a* l$^{-1} \leq 60$), hypereutrophic (>60 µg chl *a* l^{-1}) and no bloom (<20 µg chl *a* l^{-1}). Brackets indicate Bonferroni-corrected Wilcox significance tests between the bloom phases; boxplot parameters as in Fig. 4

ence less mixing and have longer retention times and a strong halocline may have extended periods of bottom water hypoxia or anoxia, which would cause increased mortalities during HAB events (Papantoniou et al. 2020).

In conclusion, our study highlights the importance of understanding the ecological drivers of zooplankton communities in estuarine ecosystems, particularly in the context of environmental stressors such as HABs. While previous international research has shown that estuarine zooplankton communities are severely impacted by HABs and hypoxic conditions, findings from this study suggest that some species may be more resilient to these stressors than previously thought. However, very little research has been conducted on the impacts of HABs on zooplankton communities, and the global understanding of these impacts is an emerging field of study. Moreover, it is still unclear

whether the HABs of *H. akashiwo* that occur in South African estuaries are a different strain than those that occur in other parts of the world. Consequently, future studies should investigate the genetics and toxicity of *H. akashiwo* in South African estuaries and include a global comparison of the range of this species. This is particularly important since recent mass fish mortality events have been reported in 2 other South African estuaries which were related to *H. akashiwo* HABs (Adams et al. 2020, Lemley et al. 2021). Furthermore, since the intensity of *H. akashiwo* blooms is related to increased temperatures and eutrophication, future work should include possible species and abundance shifts in zooplankton communities resulting from HABs to prevent a possible loss in the nursery value of estuaries subject to unsustainable development trends and changes in rainfall patterns due to global climate change.

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