



Drivers of spatial and temporal micro- and mesozooplankton dynamics in an estuary under strong anthropogenic influences (The Eastern Scheldt, Netherlands)

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ABSTRACT

The Eastern Scheldt estuary in the Netherlands has been anthropogenically changed by the construction of a storm surge barrier about 30 years ago, affecting abiotic conditions as well as phytoplankton and zooplankton (ZP). As ZP communities have not been sampled in the last decades, it is unclear to what extent they have changed during the last 30 years. We analyzed the micro- and mesozooplankton (microZP and mesozooplankton, mesozooplankton) composition and background parameters at eight stations in the Eastern Scheldt in spring, summer, and autumn 2018. Additionally, we conducted dilution experiments to estimate phytoplankton growth and microZP grazing rates. Seasonal variability of ZP was higher than spatial variability, but there were differences between stations. In agreement with data from the 1980s, we identified salinity and chlorophyll *a* as the most important factors in explaining the ZP community. Suspended particulate matter and water retention time were additionally identified for mesozooplankton, as well as dissolved inorganic nitrogen and phosphate concentration for microZP. We observed some changes in ZP composition with respect to the 1980s, most of which could be explained by the changes induced by the barrier construction. However, increased abundances of the cyclopoid copepod *Oithona* sp. were likely related to changes in phytoplankton composition due to large-scale bivalve cultivation in the estuary. Phytoplankton growth and microZP grazing rates were variable between seasons, which could be explained by changes in the phytoplankton community during the annual plankton succession. Overall, the observed alterations in the ZP community due to changed background parameters and intense bivalve filtration could affect the microbial loop as well as consumers of mesozooplankton and thus the functioning of the pelagic food web.

1. Introduction

Zooplankton plays a pivotal role in the pelagic food web as link between primary producers and higher trophic levels (Steinberg and Landry, 2017). Traditionally, mesozooplankton (mesozooplankton, mesozooplankton, 200 μm to 2 cm) and especially copepods were considered as the most important grazers of phytoplankton. However, the importance of microzooplankton (microZP, 20 to 200 μm) in pelagic food web dynamics has moved into focus in the last decades (Schmoker et al., 2013; Stoecker and Pierson, 2019). In estuaries, microZP can graze up to 60% of the daily primary production, thus exerting a strong top-down control on the phytoplankton community which makes them competitors for food for larger mesozooplankton (Calbet and Landry, 2004; Schmoker et al., 2013). In addition, the high lipid content of microZP and their ability to buffer

nutritional imbalances renders them high quality food items for mesozooplankton and newly hatched fish larvae (Malzahn et al., 2010; Stoecker and Pierson, 2019), especially when phytoplankton food quality is low or unpalatable taxa are present (Sommer et al., 2002; Fileman et al., 2007). In fact, microZP are often actively selected for by mesozooplankton, thereby bypassing the direct phytoplankton-copepod link (Saiz and Calbet, 2011; Landry and Décima, 2017). Deciphering the factors that control partitioning within and relative importance of different components of the pelagic food web remains challenging. Here, we take advantage of a detailed ZP dataset from an estuary that has undergone considerable hydrodynamic, trophic, and biological changes in the last decades.

Estuaries are productive ecosystems with high nutrient concentrations that can sustain substantial phytoplankton production resulting in high ZP abundances and grazing rates (Underwood and Kromkamp,

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1999; Calbet and Landry, 2004). Annual plankton succession includes a diatom-dominated spring bloom fuelled by high nutrient concentrations, followed by a nutrient-deplete summer phase, and an autumn bloom dominated by flagellates and cyanobacteria relying on recycled nutrients (Bakker et al., 1994; Underwood and Kromkamp, 1999). The importance of microZP in channelling carbon flows to higher trophic levels depends on nutrient concentrations and phytoplankton community composition (Sommer et al., 2002; Steinberg and Landry, 2017). During nutrient-rich spring blooms, the contribution of large diatom taxa to the phytoplankton community increases, which can be directly consumed by mesoZP (Stibor et al., 2004; Ray et al., 2016). The preference of mesoZP for grazing on diatoms reduces the importance of microZP in transferring carbon to higher trophic levels in these spring conditions (Stibor et al., 2004; Fileman et al., 2007). However, during nutrient-deplete conditions and autumn blooms, the phytoplankton community is dominated by pico- and nano-sized taxa below the copepods' preferred food size of $>5 \mu\text{m}$ (Sommer et al., 2002; Fileman et al., 2007), but within range of heterotrophic nanoflagellates and microZP (Steinberg and Landry, 2017). As the species-specific microZP food size spectrum ranges from $0.5 \mu\text{m}$ to more than their own size (Hansen and Calado, 1999; Haraguchi et al., 2018), they can proliferate in this situation despite of increased mesoZP grazing pressure. This can lead to fast nutrient recycling and can increase the relative importance of the microbial loop and the role of microZP in transferring carbon to higher trophic levels (Barber and Hiscock, 2006).

The Eastern Scheldt, located in the south-western Netherlands (Fig. 1), was transformed from an open estuary into a tidal bay. The connection to the North Sea has been partly closed by a storm surge barrier that was constructed from 1979 to 1986 in response to a major

storm surge in 1953 (Nienhuis and Smaal, 1994). The barrier is open most of the time to allow tidal exchange but reduces tidal volumes and associated tidal current velocities. Additionally, compartment dams severely restrict freshwater input to the system (Wetsteyn and Kromkamp, 1994). Overall, this led to approximately a doubling of residence time, with a gradient currently ranging from 52 to 112 days from west to east (Wetsteyn and Kromkamp, 1994; Jiang et al., 2019). Minor gradients in salinity, chlorophyll *a* (chl-*a*), and turbidity are present due to the North Sea influence in the western part and low amounts of freshwater inflow in the north-eastern part (Jiang et al., 2019). Furthermore, density of oysters, cockles, and mussels is particularly high in the central and eastern sectors, partly due to large-scale bivalve cultivation (Smaal et al., 2013; van den Ende et al., 2016; Jiang et al., 2019).

Changes in water quality and hydrodynamics caused by the barrier construction and the reduced freshwater input permanently changed plankton communities and their succession (Bakker et al., 1994; Nienhuis and Smaal, 1994). Mainly based on a combination of decreased nutrient input and increased ZP grazing pressure due to longer residence times, the phytoplankton composition shifted towards smaller taxa, and (meso)ZP biomass and abundance increased in the eastern part (Bakker et al., 1994; Bakker and van Rijswijk, 1994). ZP species composition appeared largely unaltered aside from a shift from the estuarine copepod *Acartia tonsa* to marine *A. clausi* (Bakker, 1994). In theory, shifting to smaller phytoplankton taxa and elongating the nutrient-deplete summer phase (Bakker et al., 1994) would favor increased mesoZP grazing on microZP due to the lack of appropriate phytoplankton food (Stibor et al., 2004). In the initial post-barrier times, this effect was indeed indicated by an overall lower microplankton biomass during the extended summer situation (Bakker and van Rijswijk, 1994).

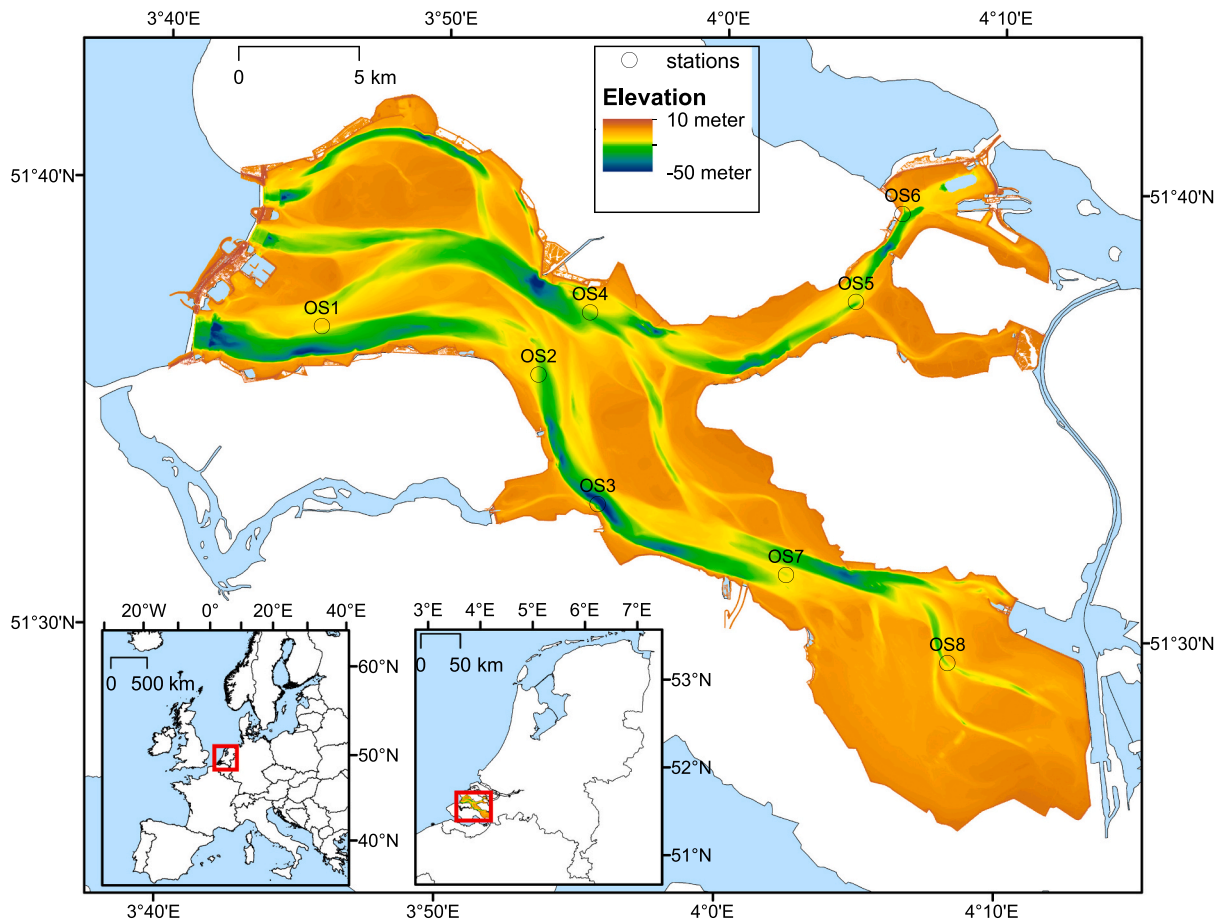


Fig. 1. Bathymetric map of the Eastern Scheldt with the storm surge barrier to the left and sampling stations OS1 to OS8 from 2018. The map inserts show the geographical location of the Eastern Scheldt within the Netherlands.

However, the Eastern Scheldt has also been subject to major biological changes. Since the 1980s, primary production decreased by about 50% while bivalve density substantially increased, with bivalve standing stocks now likely being higher than ecologically sustainable (Smaal et al., 2013; Jansen et al., 2019; Jiang et al., 2019). Bivalve filtration can significantly reduce phytoplankton concentrations, which could lead to ZP food limitation near bivalve beds or farms (Nielsen and Maar, 2007; Maar et al., 2008; Trottet et al., 2008). Furthermore, filtration activity can directly impact ZP as they are within the bivalves' size-dependent food size range of 3 μm to 4 mm (Dupuy et al., 2000; Lehane and Davenport, 2006; Mostajir et al., 2015). Generally, motile mesoZP like adult copepods are consumed less than larvae and microZP because they can better escape the filter feeding current (Kjørboe and Visser, 1999). If microZP becomes depleted, it can negatively impact higher trophic levels as observed in other estuaries experiencing high bivalve densities (Petersen et al., 2008; Cloern and Jassby, 2012). Nevertheless, bivalves also provide nutrients that may stimulate the microbial loop, thus indirectly promoting microZP by supplying adequate food particles despite of high grazing pressure (Mostajir et al., 2015).

The Eastern Scheldt presents a unique possibility to investigate controlling factors of micro- and mesoZP communities, and in particular the impact of enhanced bivalve filtration, in a spatially variable estuary with deep tidal channels, shoals, and extensive tidal flats. In this study, we collected micro- and mesoZP samples and a comprehensive set of background parameters at eight different stations in the Eastern Scheldt to identify the specific combination of abiotic and biotic factors shaping the ZP community. Sampling took place in spring, summer, and autumn 2018 to account for the seasonal plankton succession. Furthermore, we checked our data against information on ZP species composition, abundance, and parameters influencing the ZP community available from previous studies from 1978 to 1988 (see publications by Bakker, 1994; Bakker et al., 1994; Bakker and van Rijswijk, 1994, for details) to see if there were any major differences visible. In addition, we conducted dilution experiments during each sampling campaign to estimate instantaneous phytoplankton growth and microZP grazing rates and to further explore trophic interactions within the pelagic community.

2. Methods

2.1. Sampling area

The Eastern Scheldt is a semi-enclosed saline tidal bay with a total area of about 350 km² (Fig. 1). It has extensive intertidal areas, providing an important bird habitat, and is commercially important due to bivalve cultivation and recreational use. The Eastern Scheldt can be divided in four compartments; the western, central, eastern, and northern part, with residence times of about 52, 88, 112, and 106 days, respectively (Jiang et al., 2019). The phytoplankton community is strongly influenced by the nutrient regime, with nutrient-replete conditions only in winter and early spring, followed by a prolonged nutrient-deplete summer situation with high water transparencies, leading to low diatom abundances but high levels of small, often motile flagellates for most of the season (Bakker et al., 1994). MicroZP abundances show an increase from winter to summer, with a steep decrease in late summer connected to nutrient limitations and increased grazing pressure by mesoZP (Bakker and van Rijswijk, 1994). After low abundances during winter, mesoZP peaks in late spring, with copepods *Temora*, *Acartia*, and *Centropages* as the most abundant taxa, which can maintain high abundances during summer in the eastern part (Bakker and van Rijswijk, 1994). Meroplankton is dominated by barnacle, bivalve, and gastropod larvae, with overall higher abundances in the shallow eastern part (Bakker and van Rijswijk, 1994).

Dominant bivalves in the Eastern Scheldt are Blue mussels (*Mytilus edulis*), Pacific oysters (*Crassostrea gigas*), and cockles (*Cerastoderma edule*) (Jiang et al., 2019). *M. edulis* culture plots are mainly located in

the western and central part, *C. gigas* plots in the eastern part (Smaal et al., 2013). *C. edule* are not cultured but harvested from the natural stocks. Other bivalve species are present at varying, comparatively low abundances, contributing to the overall high filtration pressure (van den Ende et al., 2016).

Sampling of water and plankton took place at stations OS1 to OS8 in the Eastern Scheldt (Fig. 1) during three cruises with RV Navicula beginning of March (03–07 to 03–09, spring cruise), end of May (05–30 to 06–04, summer cruise), and September 2018 (09–17 to 09–19, autumn cruise). Stations were following transects from west to east and west to north, along the main tidal channels. Based on the information available on plankton succession from 1978 to 1988 (Bakker and van Rijswijk, 1994), these three cruises should have captured the spring bloom, summer depletion, and autumn bloom conditions. While assumptions on monthly or annual variability cannot be made from the data, the description of the three main conditions of the plankton succession during the productive period was possible.

2.2. CTD measurements and water column sampling

A CTD cast was performed at every station with a Seabird SBE911 Plus (March and September) or YSI 6600 V2 (May). CTDs were equipped with an SBE 43 or a ROX 6150 sensor for oxygen measurements, respectively. One surface water sample per station (± 1 m below surface) was taken with a 5 L Niskin bottle in March, while in May and September, triplicate Niskin samples were taken at each station. For pigment analysis, 0.9–1 L water were filtered over 25 mm GF 6 filters (Whatman) and stored at -80 °C. After extraction with 90% acetone (Wright et al., 1991), samples were analyzed by high performance liquid chromatography (HPLC) on a Shimadzu LC-04, combined with a fluorescence detector and a photodiode array absorption detector (Zapata et al., 2000). For the analysis of suspended particulate matter (SPM), 1 L was filtered over pre-combusted, weighted 47 mm GF/F filters. Filters were rinsed with ammonium carbonate to remove salt and stored at -20 °C. They were dried at 60 °C and analyzed on an elemental analyzer (Interscience).

DOC samples were filtered over pre-combusted GF/F filters and stored in 5 mL glass vials at -20 °C. They were analyzed for DOC concentration using a Formacs total organic carbon analyzer (Skalar). Furthermore, samples for dissolved inorganic nutrients – nitrite (NO_2^-), nitrate (NO_3^-), silicate (SiO_4), and phosphate (PO_4^{3-}) – were taken. Nutrient samples were filtered over a 0.45 μm PE filter, stored at -20 °C and measured with a QuAatro AutoAnalyzer (Seal Analytical, Inc.). However, ammonium (NH_4^+) measurements could not be used due to contamination of samples. During the cruise in March, DOC and nutrient samples could not be taken due to logistic constraints. Therefore, DOC and nutrient data for March were obtained from the Eastern Scheldt monitoring program conducted by the Dutch Ministry of Infrastructure and Water Monitoring (Rijkswaterstaat). These data had a lower spatial resolution, including only OS1, OS5, and OS8. They are shown in the result plots to indicate differences between seasons but were not included in the statistical analyses.

2.3. Zooplankton samples

MesoZP samples were taken with an oblique net haul to a depth of 10 m using a 150 μm -hand net (conical design, length 100 cm) equipped with a flowmeter (Hydro-Bios, Kiel). The content of the cod end was rinsed into a 200 mL plastic bottle using filtered seawater and fixed with 37% formaldehyde buffered with borax (final concentration 4%). Prior to visual inspection, samples were rinsed with tap water to remove the formaldehyde and adjusted to a volume of 100 mL. After mixing, a subsample was taken using a pipette with a large opening and counted using a stereomicroscope (Zeiss 475052–9901). At least 100 individuals of the two most common taxa were counted. Hence, the total counted volume varied between 5 and 20 mL depending on plankton abundance.

Individuals were analyzed to the lowest possible level (genus or species). Juvenile and adult stages were assessed as well (see Supplement Table S1 for detailed taxa list).

Fixation and analysis of mesoZP were handled the same way as in the 1980s data set (Bakker and van Rijswijk, 1994). However, Bakker and van Rijswijk (1994) used a submersible pump to take an integrated water sample of a smaller volume, which was filtered over a 63 μm mesh afterwards. As such a fine mesh size would not have been feasible for net tows due to clogging, a larger mesh was used in this study, even though it meant losing part of the very small-sized mesoZP. While it should be kept in mind that the results from the 1980s and from this study are thus not directly comparable, large deviations in abundances and taxonomic composition of copepods should become visible.

MicroZP samples were taken directly from the Niskin bottle, stored in 250 mL brown glass bottles, and fixed with Acidic Lugol's solution, final concentration 2%. Taxonomic composition of microZP was analyzed microscopically (Utermöhl, 1958) using sedimentation chambers (Hydro-Bios, Kiel). Either 50 or 100 mL of fixed sample was left to settle for 24 h and subsequently counted at 200 \times with an Olympus IMT-2 inverted microscope. The whole surface of the sedimentation chamber, or at least half of it in case of very high abundances, was counted to avoid a counting bias towards rare taxa. MicroZP were analyzed to lowest possible taxonomic level and otherwise grouped into morphotypes (see Supplement Table S1 for details of groups considered). Note that Lugol staining does not allow distinguishing between autotrophic and mixotrophic dinoflagellates. Given that essentially all dinoflagellates can be considered as mixotrophic (Sherr and Sherr, 2007), only genera known as primarily autotrophic were excluded from the microZP (e.g. *Triplos*).

2.4. Grazing experiments

To estimate phytoplankton growth rates and grazing rates of microZP on phytoplankton, dilution experiments were conducted during each of the three cruises (following Landry and Hassett (1982)). Water was collected from 1, 3, and 5 m depth using Niskin samplers that were gently emptied into a large carboy using a hose attached to the outflow to avoid damaging the microZP. The water was pre-screened with a 150 μm mesh to exclude mesoZP.

Sterile filtered water was obtained using a 0.45/0.2 μm filter cartridge (Sartobran P 300, Sartorius AG) and a peristaltic pump. Dilutions of 20, 40, 60, and 100% undiluted Eastern Scheldt water were set up in carboys to provide equal concentrations in the triplicate incubation bottles. Triplicate pigment filters were taken from each carboy for phytoplankton start concentrations. Incubation bottles were gently filled avoiding air bubbles. For the first experiment, 500 mL Schott glass bottles were used and thereafter 1 L polycarbonate bottles. To avoid sedimentation, bottles were attached to a slowly rotating plankton wheel placed in an acclimatized room providing ambient temperatures and light-dark rhythm. At the end of the 24 h-incubation, pigment filters were taken from each bottle and analyzed with HPLC as described in 2.2. For March, only chl-a values were available from the analysis.

Phytoplankton net growth rates k (d^{-1}) (incl. microZP grazing) were calculated using an exponential growth model $k = \ln(c_{\text{end}}) - \ln(c_{\text{start}})$, with c_{start} as the pigment concentration at the start of the experiment and c_{end} as the concentration after 24 h (Landry and Hassett, 1982). This was done for chl-a as proxy for the whole phytoplankton community and for pigments obtained from HPLC analysis with concentrations high enough to be measured in all dilutions. Phytoplankton groups derived from the different pigments following Jeffrey et al. (1997) are listed in Table 1.

The instantaneous phytoplankton growth rate μ (day^{-1}) (excl. grazing) was obtained from the y-axis intercept of a linear regression of k against the dilution factor α . The microZP grazing rate g (day^{-1}) was obtained from the negative slope of the regression (Landry and Hassett, 1982). Negative grazing rates were set to zero for the statistical

Table 1

Pigments from HPLC analysis and their corresponding algal groups. Only pigments with concentrations high enough to calculate instantaneous growth rates μ from dilution experiments are included.

Pigment	Algal group
Alloxanthin	Cryptophytes
b-Carotene	Chlorophytes, prasinophytes
Chl-a	Community
Chl-b	Chlorophytes, prasinophytes
Chl-c3	Haptophytes, diatoms
Chlorophyllid a	Degradation product of chl-a
Diadinoxanthin	Diatoms, some dinoflagellates
Fucoxanthin	Diatoms
Peridinin	Dinoflagellates
Phaeophytin a	Degradation product of chl-a
Zeaxanthin	Cyanobacteria

evaluation. Since nonlinear effects are often observed in dilution experiments due to, e.g., food saturation (Gallegos, 1989), regressions were also performed including a quadratic term to check if this provided a better fit to the data points as indicated by both visual inspection of the plots and comparison of the r^2 values. In addition, Akaike's Information Criterion (AIC) was compared for both models as it considers both the complexity of the model and the goodness of fit. If the model involving the quadratic term provided a better fit, μ was obtained from the y-axis intercept of the curve and g was calculated as established for two-point dilution series, using the equation $g = \mu - k_1$ with k_1 as the average k of the undiluted treatments (Morison and Menden-Deuer, 2017). Residuals of the regressions were checked for normality and heteroscedasticity.

2.5. Statistics

All statistical tests were performed with R (R Core Team, 2020) with the packages vegan (Oksanen et al., 2020), nlme (Pinheiro et al., 2020), and emmeans (Lenth, 2022). The threshold for a significant result was set to $\alpha = 0.05$.

We applied a non-metric multidimensional scaling technique (NMDS) to investigate changes in the ZP community composition over time. While NMDS does not assess effects of environmental gradients on ZP abundances, it can detect temporal and spatial patterns within the community. This ordination technique is rank-based and thus avoids potential problems arising from data sets containing groups with very different abundance magnitudes (Field et al., 1982; Dexter et al., 2018). Using the 'metaMDS' function from the vegan package, an NMDS ordination was performed using Bray-Curtis community dissimilarities calculated from square root transformed ZP abundances. To test if dissimilarities between treatments were significant, a permutational multivariate analysis of variance (PERMANOVA) was applied subsequently (Anderson, 2017).

To investigate the influence of abiotic variables on the ZP community, we performed a distance-based redundancy analysis (db-RDA) using function 'capscale' from the vegan package. Since this type of principle component analysis is based on distances, it has the advantage that ZP abundances do not have to be normally distributed (Legendre and Anderson, 1999). To account for the large differences in ZP abundance and frequent occurrence of zeros, square root transformed relative ZP abundances were used. Separate analyses were run for mesoZP and microZP data sets.

Prior to analysis, abiotic data were z-scored to account for their different scales. Variables used were temperature, salinity, O_2 , SPM, DOC, NO_x ($\text{NO}_2 + \text{NO}_3$), PO_4 , SiO_4 , chl-a, and retention time (RT). RT values were obtained from the study by Jiang et al. (2019). Due to a lack of some abiotic variables for March, this month was excluded from the db-RDA. Variables were checked for correlation by calculating Variance Inflation Factors (VIFs). The variable with the highest value was removed and the VIFs recalculated until the values of the remaining variables were ≤ 3 (Zuur et al., 2010). Remaining variables were added

to the model one by one in a forward model selection procedure while their significance was assessed with an ANOVA-like permutation test. The significant variables explaining most of the data set variations (based on their AIC values) were added to the model first, followed by the others until no further significant improvement of the model was observed (function ‘ordistep’).

Additionally, we performed a generalized least square analysis (GLS) to test for effects of month and station on microZP and mesoZP abundances since these data were non-normal distributed. The optimal variance-covariate structure was determined using the restricted maximum-likelihood (REML) estimation. Residuals of the models were tested for normality and homoscedasticity using Shapiro-Wilk tests and conditional plots, respectively. To fulfill the requirements for REML, abundance data were log-transformed. Subsequently, a post-hoc test for pairwise comparison of months and stations was performed (function ‘emmeans’).

3. Results

3.1. Abiotic data

A clear seasonal trend was observed for most abiotic parameters, with temperature, O₂, and SPM concentration differing noticeably between sampling months (Fig. 2). Spatial differences were much less pronounced for most parameters or inconsistent between seasons for SiO₄ and chl-a. However, the lowest values for salinity and SPM and highest values for DOC, NO₂, NO₃, and SiO₄ were usually measured at OS5 and OS6 in the northern branch. For PO₄, values were highest in the northern branch in May with 0.76 and 0.51 μmol L⁻¹, but not in September when OS7 and OS8 in the eastern part reached concentrations of ~1.51 μmol L⁻¹. Chl-a showed a different pattern, with the lowest values of 1.74 μg L⁻¹ for OS5 in March during the spring bloom compared to 3.96 to 7.15 μg L⁻¹ for the other stations. In May, chl-a values of 4.44 and 3.78 μg L⁻¹ at OS5 and OS6 were higher than at the other station that reached 2.72 to 3.16 μg L⁻¹, with exception of OS1 close to the storm-surge barrier where values of 4.76 μg L⁻¹ were

measured as well. This difference was especially pronounced in September during the autumn bloom when 6.30 and 8.33 μg L⁻¹ were measured at OS5 and OS6 in comparison to 2.99 to 5.52 μg L⁻¹ at the other stations. Furthermore, a gradient from west to east was visible for several parameters, but it was season dependent. While DOC, NO₂, and PO₄ increased towards the east in September, from 144 to 232, 0.31 to 0.47, and 0.66 to 1.59 μmol L⁻¹, respectively, this was not the case in May.

3.2. ZP abundance and taxonomic composition

Overall, we observed low mesoZP abundances in March with 7 to 13 ind. L⁻¹, except for OS2 in the western part with 91 ind. L⁻¹ (Fig. 3). This peak was caused by cirripedia larvae (mainly young *Balanus* nauplii), calanoid copepods (mainly *Temora longicornis*), and copepod nauplii of different taxa. Likewise, cirripedia larvae reached high percentages of ~38% at the other stations. Cyclopoid copepods (*Oithona* sp.) were almost exclusively found at OS2.

Highest abundances of mesoZP were found in May, reaching 143 to 466 ind. L⁻¹. The exception was OS8 in the eastern part with 956 ind. L⁻¹ due to high abundances of polychaete and gastropod larvae that were not found elsewhere, representing 23% and 19% of the community, respectively. Once more, calanoid copepods contributed ~33% to the community, but this time it was mainly *Acartia* spp. Cirripedia larvae were still important contributors at OS5, OS6, and OS8 (~35%), while bivalve larvae only reached high abundances at OS6 and OS8 (~18%). Comparable to March, highest abundances of cyclopoid *Oithona* sp. (23%) were recorded at OS2.

In September, we observed overall low mesoZP abundances. At OS2, 41 ind. L⁻¹ were reached, followed by OS7 with 35 ind. L⁻¹, and 6 to 16 ind. L⁻¹ at the other stations. However, unlike in March, OS2 was dominated by echinoderm larvae, mainly brittle stars (32%), and the appendicularian *Oikopleura* cf. *dioica* (21%). This community composition was observed both at OS1 and OS2 in the western part, even though abundances differed. Except for OS1 and OS2, cirripedia larvae were still present at high percentages, as a mix of older *Balanus* sp. larval stages

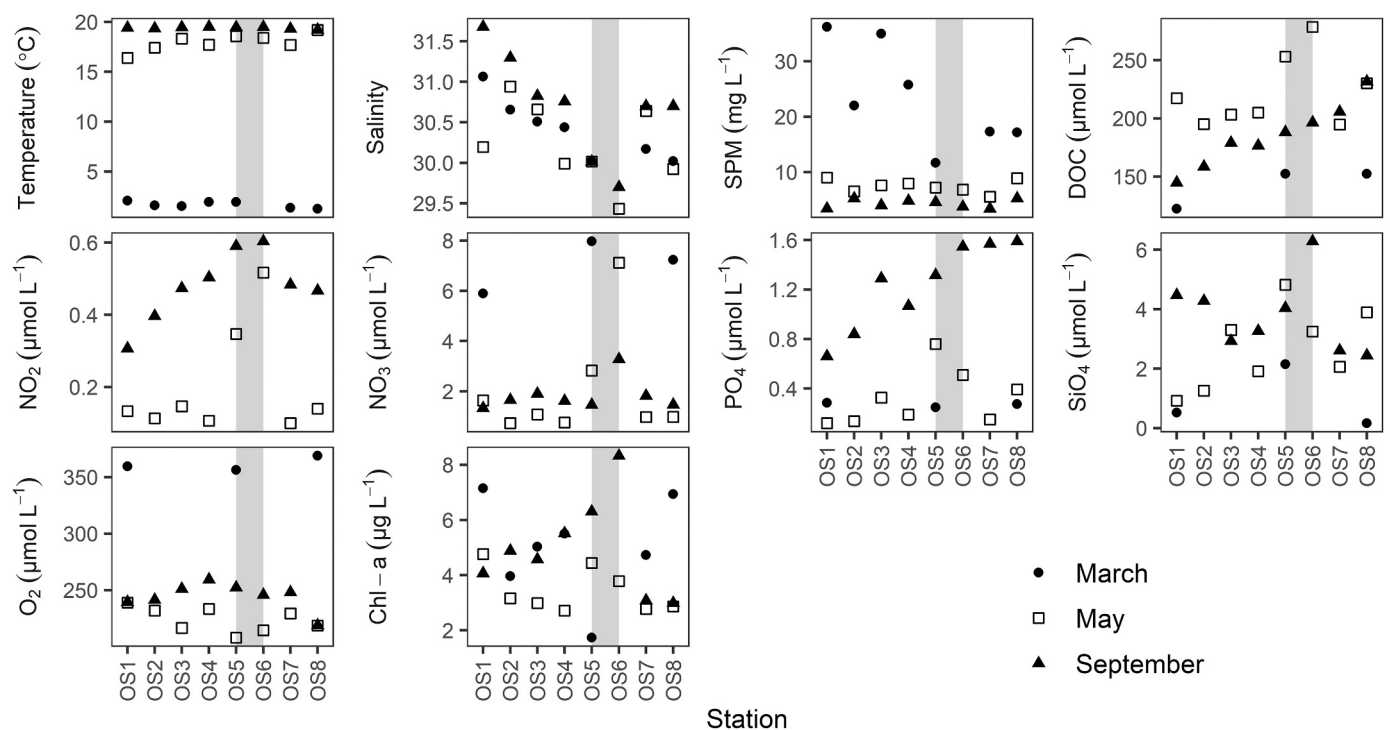


Fig. 2. Abiotic parameters from surface water samples in March (black circles), May (white squares), and September (black triangles). Stations are sorted west to east, with grey shading indicating stations OS5 and OS6 in the northern branch of the Eastern Scheldt. OS6 was not sampled in March.

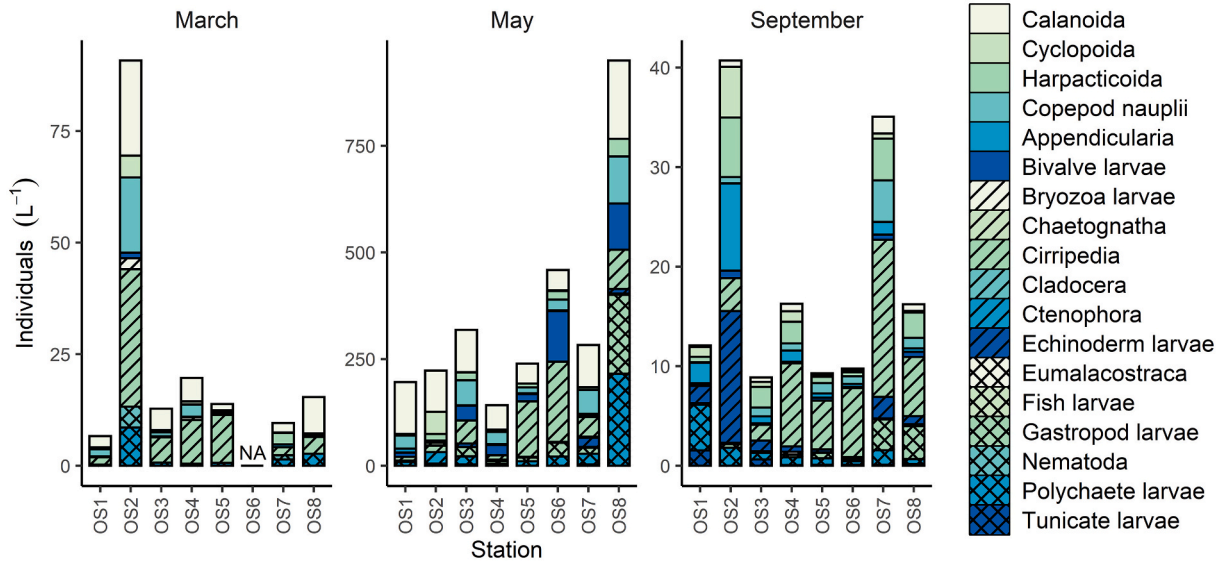


Fig. 3. Taxonomic composition of the mesozooplankton community (individuals L⁻¹). Note the different scale of the y-axis between plots. OS6 was not sampled in March.

(~46%). Furthermore, the copepod community was no longer dominated by calanoids but by pelagic harpacticoids (~13%, mainly *Euterpina acutifrons*) and especially at OS2 by cyclopoid *Oithona* sp. (12%). Two *Pseudodiaptomus marinus* copepodites were found at OS3, a taxon not recorded in the Eastern Scheldt before.

In March, microZP abundances were comparatively low at the western stations OS1, OS2, OS4, and OS5 with 2.19×10^3 to 6.46×10^3 ind. L⁻¹, while we already found 1.41×10^4 to 2.17×10^4 ind. L⁻¹ at stations OS3, OS7, and OS8 (Fig. 4). The ciliate community consisted mainly of small and medium-sized *Strombidium* spp. (oligotrichs). At station OS5, OS7, and OS8 small-sized *Balanion comatum* (prorodontids) contributed up to 31% to the community. Mixotrophic and heterotrophic dinoflagellates were almost exclusively represented by small-sized thecate taxa (<30 μm) at all stations.

MicroZP abundances peaked in May with 3.26×10^4 to 4.59×10^4 ind. L⁻¹. Only OS1, OS5, and OS8 abundances stayed at 1.59×10^4 to 2.00×10^3 ind. L⁻¹. The ciliate community was dominated by *B. comatum* (~22%) and to a lesser degree by *Strombidium* spp. of

different size classes (~19%). In addition, a higher percentage of *Mesodinium rubrum* (a cyclotrichid) was found, especially at OS6 in the northern branch (36%). Furthermore, strobilidiids (choreotrichids) increased in abundance. Much like in March, OS5 and OS6 in the northern branch had a somewhat different community composition, with the lowest numbers of choreotrichids compared to the other stations. For dinoflagellates, an increase in heterotrophic athecate gymnodiniales was observed at all stations (~16%). Thecate *Protoperidinium* spp. (peridinales) of different size classes also increased in abundance (~8%).

In September, microZP abundances peaked at OS5 and OS6 in the northern branch, reaching 4.73×10^4 and 3.44×10^4 ind. L⁻¹, respectively. Aside from oligotrichid ciliates (33 and 18%, mainly *Strombidium* spp.), peridinales (19 and 37%), and gymnodiniales (17 and 14%) were the main contributors to this peak. Prorocentrales were present at lower percentages (3 and 8%). While the taxonomic composition was similar at the other stations, overall abundances were lower (1.35×10^4 to 1.66×10^4 ind. L⁻¹). Only OS2 was similar to OS5 and OS6 with abundances

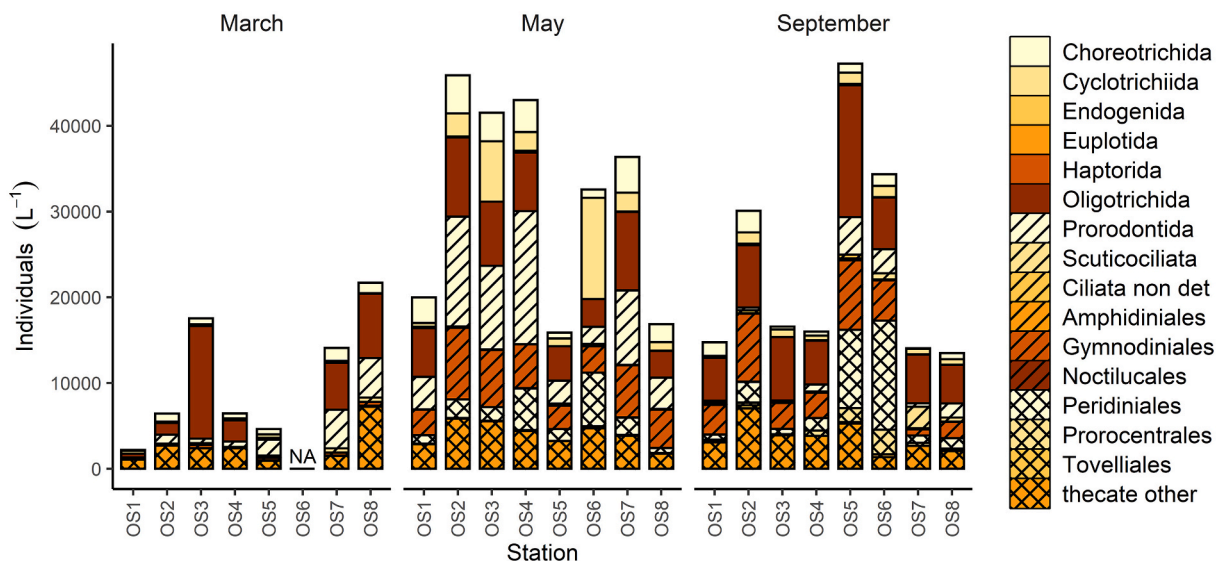


Fig. 4. Taxonomic composition of the microzooplankton community in individuals L⁻¹. OS6 was not sampled in March.

of 3.01×10^4 ind. L⁻¹.

As indicated by the GLS and subsequent post-hoc analysis testing for effects of month and station on ZP abundances, (log) mesoZP abundance in May was different from March and September ($p < 0.001$, Table 2). In addition, mesoZP abundances at stations OS2 and OS8 were different from the others ($p = 0.001$ and $p = 0.049$). For (log) microZP abundance, March differed significantly from May and September, but there were no differences between stations.

3.3. ZP community composition and variation

The temporal and spatial community variation of total ZP is presented by the NMDS results in Fig. 5. With a stress value of 0.127, the results can be considered as reliable for the highly variable ecological data used. As indicated by the PERMANOVA, dissimilarities between months were significant ($p = 0.001$), but not between stations. Hence, temporal differences between communities were evidently more pronounced than spatial differences.

The permutation test identified SPM, salinity, chl-a, and residence time (in order of their importance) as the most important abiotic parameters shaping the mesoZP community composition in May and September, as visualized in the db-RDA plot (Fig. 6A and B). The first two factors explained 36 and 21% of the community variation, while the 3rd and 4th together added 6%. It must be noted though that SPM and chl-a were on opposite sides of one axis, with salinity and residence time forming a 2nd axis, indicating that those parameter pairs were not independent of each other. Clustering of the mesoZP taxa around the center indicated that many mesoZP taxa were not strongly affected by one of the parameters. However, as indicated by their position along the gradient arrows, calanoid copepods *T. longicornis* (Tem) and *Centropages hamatus* (Cen) were positively associated with SPM. Furthermore, cyclopoid *Oithona* sp. (Oit), echinoderm larvae (Ech), and appendicularians (App) were found to be associated with high salinity and chl-a values. Cirripedia larvae (Cir) were strongly associated with chl-a and retention time (Fig. 6A). While there was a clear seasonal pattern visible, also spatial differences between stations became obvious. In September, there was a cluster of eastern and northern stations visible in the mesoZP plot, the ones most distant from the North Sea and with the highest residence times (OS5 to OS8). Those were the stations with the highest abundances.

For microZP, the influential factors were identified as PO₄, salinity, NO_x (NO₂ + NO₃), and chl-a (Fig. 6C and D). The first two factors explained 34 and 15% of the community variation, with the 3rd and 4th adding 8 and 6%, respectively. Unlike for mesoZP, residence time and SPM were not identified as significantly influential for microZP. The majority of microZP was not strongly associated with one of the parameters as visualized by the clustering around the centre of the plot. Especially connected to NO_x concentrations was cyclotrichid *M. rubrum* (MeS), a mixotrophic ciliate. This finding is related to stations OS5 and OS6 in the northern branch, where the highest abundances of this

Table 2

Results from the generalized least square analysis (GLS) testing the effects of month and station on (log₁₀) mesozooplankton and (log₁₀) microzooplankton abundances. Stations and months with significantly different abundances are indicated by bold numbers. SE: standard error, Sept: September.

	Mesozooplankton				Microzooplankton			
	Coefficient	SE	t-value	p-value	Coefficient	SE	t-value	p-value
OS2	0.676	0.227	2.979	0.011	0.380	0.212	1.795	0.096
OS3	0.210	0.227	0.924	0.372	0.424	0.212	2.002	0.067
OS4	0.254	0.227	1.121	0.283	0.280	0.212	1.324	0.208
OS5	0.121	0.227	0.534	0.602	0.315	0.212	1.487	0.161
OS6	0.320	0.257	1.244	0.235	0.442	0.240	1.843	0.088
OS7	0.355	0.227	1.566	0.141	0.348	0.212	1.647	0.124
OS8	0.493	0.227	2.174	0.049	0.294	0.212	1.390	0.188
March–May	1.289	0.146	8.810	<0.001	0.508	0.136	3.727	0.003
March–Sept	-0.028	0.146	-0.190	0.852	0.364	0.136	2.670	0.019
May–Sept	-1.316	0.139	-9.470	<0.001	-0.144	0.130	-1.112	0.286

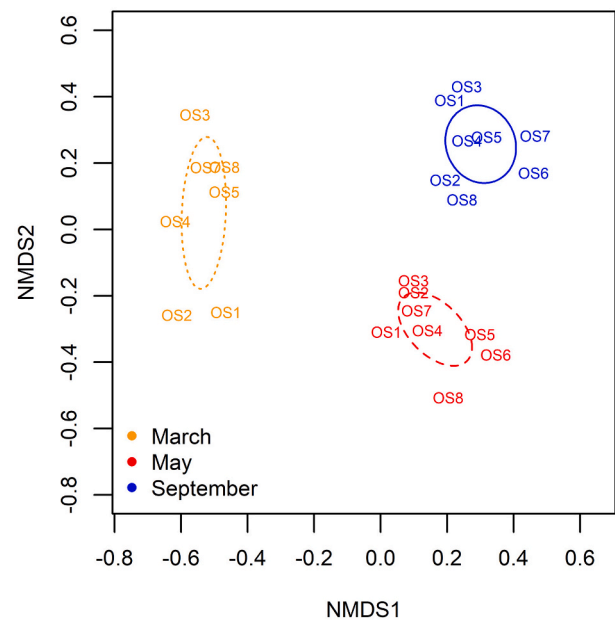


Fig. 5. NMDS plot of micro- and mesozooplankton community data (stress value 0.127). Colours represent sampling months and ellipses standard deviations (March: orange, dotted ellipse; May: red, dashed; September: blue, solid). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

species were recorded in May.

3.4. Growth and grazing rates

Taking chl-a as a proxy for the whole phytoplankton community, instantaneous phytoplankton growth rates μ ranged from -0.32 day⁻¹ in May and 0.02 day⁻¹ in September to 0.32 day⁻¹ during the spring bloom in March (Fig. 7). Likewise, the chl-a specific microZP grazing rate g was zero in May, 0.14 day⁻¹ in September, and 0.22 day⁻¹ in March.

The other pigments measured in both September and March were chl-b, Fucoxanthin, and Alloxanthin. For chl-b (chlorophytes/prasinophytes), μ was negative in both months while g was zero in May and 0.34 day⁻¹ in September. The situation differed for Fucoxanthin (diatoms) where μ was negative and g zero in May but $\mu = 0.40$ day⁻¹ and $g = 0.24$ day⁻¹ in September. For Alloxanthin (cryptophytes), μ was 0.13 day⁻¹ in May and as low as 0.07 day⁻¹ in September while g stayed almost the same with 0.27 and 0.30 day⁻¹, respectively. Overall, cryptophytes were the only group for which a positive μ was calculated in May.

In September, there were some additional pigments measured,

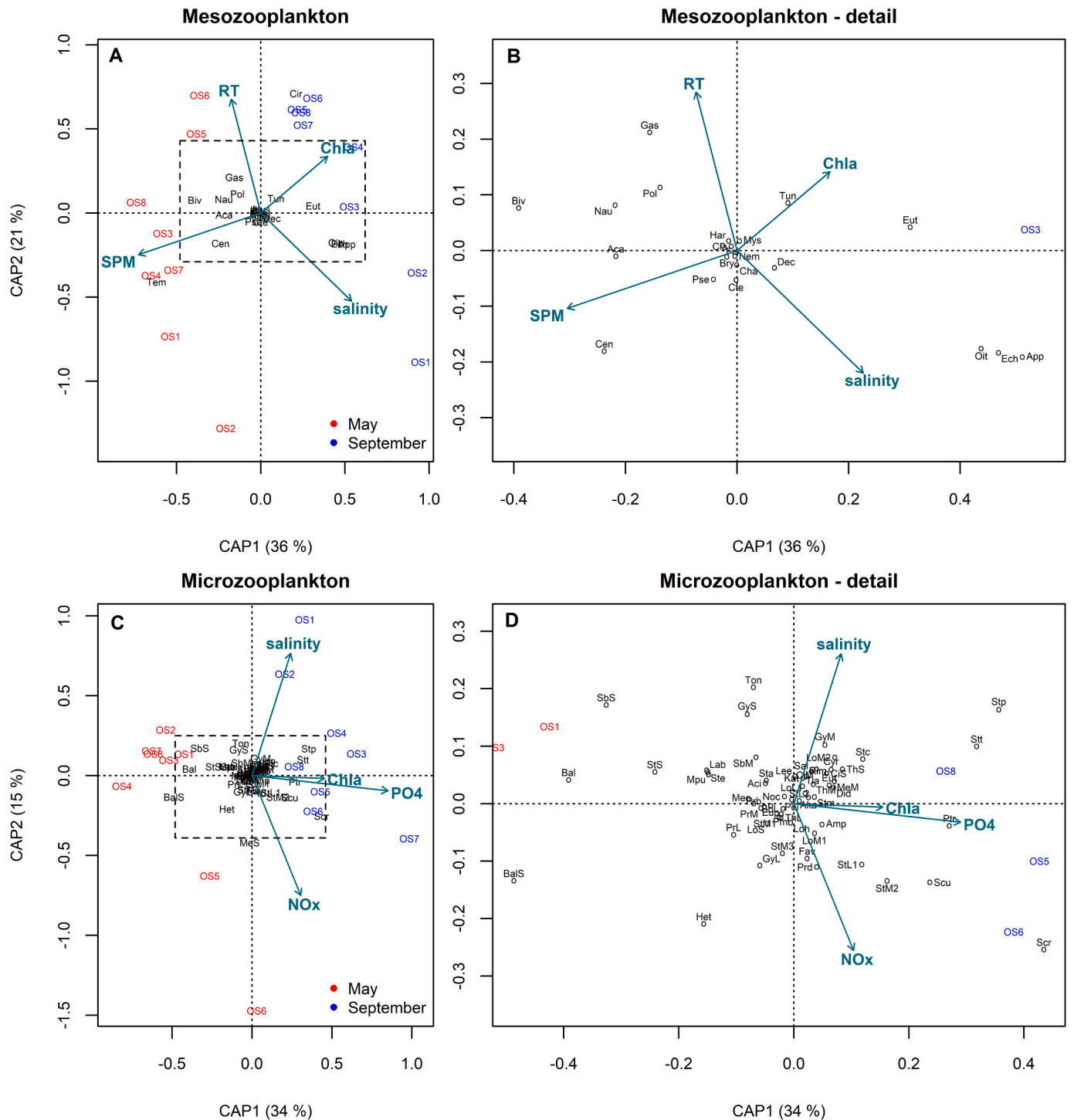


Fig. 6. Results of the db-RDA for (A) mesozooplankton, with the box indicating the zoom-in shown in (B) for a clearer view of the overlapping taxa in the centre. (C) Results for microzooplankton abundances; and (D) zoom-in of the central part. Colours of stations OS1 to OS8 indicate sampling months (May: red, September: blue). Arrows denote factors significantly associated with the zooplankton communities. Chla: chlorophyll a, NOx: sum of NO₂ and NO₃, RT: residence time, SPM: suspended particulate matter. See Table S1 for taxa abbreviations. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

mirroring the composition of an autumn bloom with high contributions of cyanobacteria and dinoflagellates. For Zeaxanthin (cyanobacteria), chl.-c3 (bacteria), and Peridinin (dinoflagellates), μ was between 0.42 and 0.55 day⁻¹, with g ranging from 0.02 to 0.39 day⁻¹. For Diadinoxanthin (diatoms/some dinoflagellates) and b-Carotene (chlorophytes/prasinophytes), we observed the opposite with $\mu = 0.30$ and 0.48 day⁻¹ and values for g slightly higher than μ with 0.43 and 0.51

day⁻¹, respectively. For Chlorophyllid a (degradation product of chl-a), μ was 0.73 day⁻¹ and g 1.56 day⁻¹ in May. In September, μ was 1.27 day⁻¹, while g was lower with 0.24 day⁻¹. Phaeophytin a, another degradation product of chl-a, was present at high levels in September only, with $\mu = 1.19$ day⁻¹ and g being marginally higher than μ with 1.38 day⁻¹.

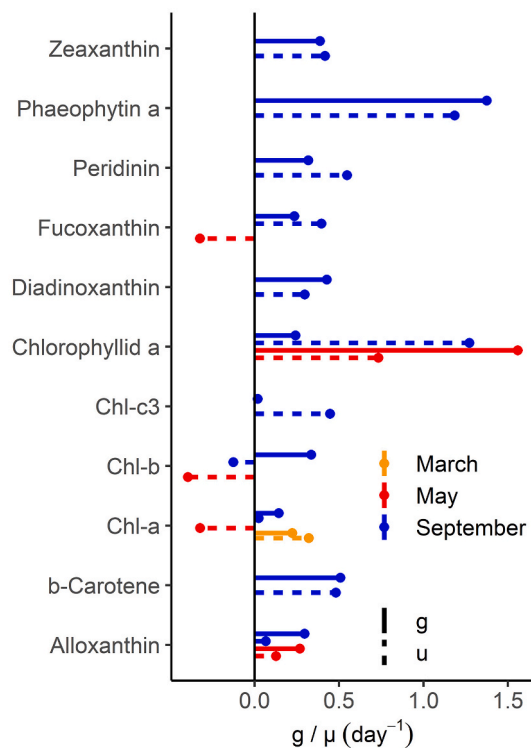


Fig. 7. Instantaneous phytoplankton growth rates μ (day^{-1} , dashed lines) and microzooplankton grazing rate g (day^{-1} , solid lines) calculated for the different pigments measured. Colours indicate grazing experiments conducted in March (orange), May (red), and September (blue). In May, g was zero for chl-a, chl-b, and Fucoxanthin. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

4.1. Phytoplankton growth and microzooplankton grazing

In March, results from the dilution experiments were indicative for typical spring bloom conditions, in which phytoplankton growth (i.e. μ) outweighs microZP grazing (i.e. g) such that ZP grazing is not able to reduce phytoplankton biomass (as discussed by, e.g., Strom et al., 2001; Sherr and Sherr, 2009). This was supported by the comparatively high chl-a concentration ($\sim 5 \mu\text{g L}^{-1}$) and low microZP abundance found in the field samples in March. It has been hypothesized that favourable abiotic factors for phytoplankton, combined with a low grazing pressure, create a “loophole” that enables phytoplankton blooms in early spring in the first place (Irigoiien et al., 2005).

Results from the experiment in May represented a common post-bloom scenario, with low or non-detectable pigment concentrations (chl-a $\sim 3 \mu\text{g L}^{-1}$) and high concentrations of degradation products. Phytoplankton growth rates calculated from the dilution experiments were negative in most cases. This effect can be attributed to viral lysis and cell senescence during bloom degradation (Suffrian et al., 2008; Brussaard et al., 2013). An exception was the positive growth rate of cryptophytes. MicroZP abundances observed in the Scheldt in May were more than twice the values of March, which further supports the strong top-down control (i.e. $\mu < g$) of microZP on phytoplankton, especially cryptophytes, as indicated by the dilution experiment. This is in line with the observed high abundances of ciliate *Mesodinium rubrum* in the Scheldt, a functional autotroph relying on cryptophyte prey to obtain phototrophy and thus usually blooming during cryptophyte blooms (Stoecker et al., 2009).

Results from the September experiment were typical for an autumn bloom, with high pigment concentrations indicative for diatoms,

cyanobacteria, and dinoflagellates. Measured growth rates outweighed grazing rates (i.e., $\mu > g$) for these groups, an effect that can often be observed in autumn (Strom et al., 2001; Rollwagen-Bollens et al., 2011). Bacterial growth, based on chl-c3, was also found to be positive in the experiment, given the comparatively high growth ($\mu = 0.45 \text{ day}^{-1}$) and negligible grazing on this marker. This may be due to low *Balanion comatum* abundances in the Scheldt at this time point, one of the few taxa that can feed on bacterial cells (Johansson, 2004). In general, microZP abundance and grazing impact in September were lower than in May and comparable to March.

The overall pattern of primary production and grazing observed in these experiments is typical for temperate areas (Strom et al., 2001; Schmoker et al., 2013; Stoecker et al., 2017), and is similar to results from a series of dilution experiments conducted in the San Francisco Bay estuary (Rollwagen-Bollens et al., 2011). Interestingly, based on specific pigments, we found growth rates (μ) of most phytoplankton groups in the Eastern Scheldt to be high in September. In contrast, chl-a based growth rates reflecting the entire phytoplankton community were comparatively low (0.02 d^{-1}). This shows the value of considering the full pigment spectrum when estimating phytoplankton growth rates and the respective microZP grazing rates. In September, microZP were mainly grazing on chlorophytes/prasinophytes, diatoms, dinoflagellates, and cyanobacteria as indicated by the markers.

4.2. Factors controlling ZP community composition

Estuaries are typically characterized by large environmental gradients that give rise to distinct ZP communities in the upper and lower reaches (Soetaert and van Rijswijk, 1993; Le Coz et al., 2017). While minor gradients in salinity, nutrients, SPM, and chl-a were observed from west to east in the Eastern Scheldt in this study, they have been much reduced in comparison to pre-barrier times when they were comparable to typical open estuaries (Wetsteyn and Kromkamp, 1994). The absence of such pronounced gradients could explain why major differences in the current ZP community in the Eastern Scheldt were related to season rather than space.

For both microZP and mesoZP, salinity and chl-a were identified as important factors in structuring the community in this study. In addition, SPM and retention time were structuring factors for mesoZP, and PO_4 and NO_x for microZP. We expected a stronger influence of temperature based on the results from other estuaries (Soetaert and van Rijswijk, 1993; Tackx et al., 2004; Cloern and Jassby, 2012; Marques et al., 2018). The lack of effect could be related to the low temporal resolution of our data set since the db-RDA was limited to May and September. Furthermore, an explanatory power of 63% indicated that there might have been additional factors not measured during our sampling campaigns.

Current SPM concentrations in the Eastern Scheldt were higher in spring than in summer or autumn, but we did not detect any spatial pattern. The taxa positively associated with SPM were the two coastal calanoid copepods *Temora longicornis* and *Centropages hamatus*. SPM concentrations also had a positive effect on calanoid copepod abundances in the Western Scheldt (Le Coz et al., 2017), an estuary located south of the Eastern Scheldt, which has not been closed off from the North Sea since it is the route to the port of Antwerp. Yet, the average SPM concentrations of $\sim 10 \text{ mg L}^{-1}$ that we measured in the Eastern Scheldt represent a strong decrease compared to pre-barrier times, a trend already observed by Wetsteyn and Kromkamp (1994) in the 1980s. The decrease could be explained by a combination of reduced riverine inflow, reduced tidal influence, and high clearing by bivalve filtration (Wetsteyn and Kromkamp, 1994; Smaal et al., 2013). Contrastingly, increased light availability due lower SPM concentrations could also enhance algal growth and thus food availability for ZP, as it has been the case in other estuaries (Petersen et al., 2008; Cloern and Jassby, 2012).

A sharp salinity gradient is common in estuaries, often resulting in lower mesoZP abundances in the high-salinity downstream sections (Le

Coz et al., 2017). Despite of reduced freshwater inflow in the Eastern Scheldt and small spatial salinity differences that were measured between stations in this study (29.4 to 31.7), salinity was still identified as an important factor explaining ZP community variation. While it cannot be ruled out that there might have been another factor related to salinity causing the effect, such as the distance to the North Sea, the limited freshwater inflow in the northern branch (OS5 and OS6) and the North Sea water inflow from the west (OS1 and OS2) were apparently sufficient to influence the ZP community in the Eastern Scheldt. The taxa associated with higher salinities were *Oithona* sp., appendicularians, and echinoderm larvae, groups that are also common in the open North Sea (Greve et al., 2004). For microZP, results were not conclusive.

Chl-a concentration significantly influenced the ZP community in this study, albeit with a lower explanatory power. Even though most copepods are omnivores and microZP are often mixotrophs, the majority of ZP taxa currently present in the Eastern Scheldt belongs to groups that are known to rely on phytoplankton as main food source at some life stage (Sommer et al., 2002; Sherr and Sherr, 2007; Stoecker et al., 2009). The observed connection of high chl-a concentrations and thus food availability with ZP abundances in general is in accordance with results from other estuaries (Cloern and Jassby, 2012; Le Coz et al., 2017). However, chl-a concentrations in the Eastern Scheldt used to be higher in pre-barrier times; they decreased from 40–70 $\mu\text{g chl-a L}^{-1}$ (Bakker and van Rijswijk, 1987) to present 4–10 $\mu\text{g chl-a L}^{-1}$. Our results are thus in line with the long-term analysis by Smaal et al. (2013) indicating a decreasing trend in primary production in the Eastern Scheldt.

The mesoZP community observed in this study was also strongly connected with residence time, especially cirripedia larvae. To a lesser degree, gastropod larvae, polychaete larvae, and copepod nauplii were found to correlate positively with longer residence times. Given their small size and minute swimming abilities, these larvae could profit from a comparatively stable environment with respect to residence time as it reduces the chance of being displaced. The barrier construction increased residence time in the Eastern Scheldt considerably: from 5 to 52 days in the western, 25 to 88 in the central, 35 to 106 in the northern, and 50 to 112 in the eastern part (Weststeyn and Kromkamp, 1994; Jiang et al., 2019). Positive effects of a longer residence time on ZP abundances were also observed in the close-by Western Scheldt (Appeltans et al., 2003; Le Coz et al., 2017).

For microZP, PO_4 and NO_x were identified as important factors. This could be explained by the frequent occurrence of mixotrophy in microZP, enabling them to switch their nutritional mode from heterotrophy to autotrophy if food items are scarce but sufficient nutrients, including PO_4 and NO_x , and light are present. Even functional autotrophy can occur, as, e.g., in the cyclotrichid *M. rubrum* (Stoecker et al., 2009; Mitra et al., 2016), the species associated strongest with NO_x . *M. rubrum* is often present in high abundances when nutrients are plentiful (Stoecker et al., 2017), which was indeed observed at OS6 in the northern branch in May. Autotrophy presents an advantage under food-limited conditions, while heterotrophy usually allows faster growth (Dolan and Perez, 2000), which could explain why chl-a concentrations were also important for total microZP abundance. However, we used Lugol's solution as fixative for microZP samples which made it impossible to identify mixotrophs as their chloroplasts became invisible. Nevertheless, many microZP taxa are known to be mixotrophs, such as oligotrichid strombidiids and *M. rubrum* (Stoecker et al., 2009; Mitra et al., 2016) which dominated the ciliate community at different times. Overall, PO_4 , NO_x , and chl-a were highest in the northern branch in September, when the highest microZP abundances were recorded. In March and May, however, other factors must have been important in controlling microZP since abundances were higher in the central and eastern parts.

4.3. Changes in ZP species composition

The Eastern Scheldt ZP community has been intensively sampled

before, during, and shortly after the construction of the storm surge barrier (Bakker, 1994). Some copepod taxa, i.e., *Anomalocera patersoni*, *Labidocera wollastoni*, and *Calanus* sp. that used to be present at low abundances in the 1980s were not found during our sampling campaign, even though the limited temporal resolution of our data must be kept in mind. These copepods are typical coastal and oceanic species, thus frequently found in the open North Sea, while only few individuals get mixed into estuaries (Fransz et al., 1991; Beaugrand, 2004). The obstructed exchange with the North Sea due to the storm surge barrier likely explains their disappearance. Another copepod that was not encountered anymore was the estuarine *Eurytemora affinis*. This species proliferates at low salinities (Appeltans et al., 2003; Tackx et al., 2004), and the reduced freshwater input is probably the reason for its disappearance. In addition, the reduced chl-a concentrations measured in this study may have negatively affected *E. affinis*, as other studies showed reduced abundances in estuaries where nutrient load and chl-a decreased while bivalve grazing intensified (Soetaert and van Rijswijk, 1993; Cloern and Jassby, 2012).

We also found two specimens of the neozoa *Pseudodiaptomus marinus* at OS3. This calanoid copepod originates from Asia and was most likely brought to Europe by ballast water, the reason why higher abundances are usually found in the vicinity of harbours (Sabia et al., 2015; Deschutter et al., 2018). In the North Sea, *P. marinus* was first recorded in 2010 at the French coast (Brylinski et al., 2012) and in 2011 in the Western Scheldt and the German Bight (Tackx et al., 2011; Jha et al., 2013). While observations of ovigerous females suggest successful reproduction (Brylinski et al., 2012), it remains largely unclear if *P. marinus* has established stable populations outside harbours (Sabia et al., 2015; Deschutter et al., 2018). We found *P. marinus* copepodites in this study, which might indicate reproduction in the Eastern Scheldt. However, it has to be kept in mind that the central part of the Eastern Scheldt has Vlakte canal as a connection to the Western Scheldt, where *P. marinus* has been recorded occasionally since 2011 (Tackx et al., 2011), so the individuals we encountered might have been carried in.

An important change we observed in the mesoZP community was the increase in *Oithona* sp. abundance, especially at OS2. This small cyclopoid copepod is an euryhaline taxon that forms stable populations in the North Sea and can become the dominant copepod during post-bloom conditions when food is comparatively scarce and predation by fish larvae and other planktivores is high (Sabatini and Kjørboe, 1994; Turner, 2004). *Oithona* may also replace larger copepod taxa in estuarine areas where eutrophication caused a shift in the phytoplankton community towards small flagellates (Turner, 2004). In the past, this copepod had been recorded in the Eastern Scheldt occasionally, but it contributed up to 23% to the total copepod abundance in our data set. As described by Bakker (1994), the irregular historical observations were attributed to advective transport from the North Sea into the estuary. It is unlikely that *Oithona* was overlooked during the 1980s sampling campaigns as a higher temporal resolution and a smaller mesh size were used (Bakker, 1994). The *Oithona* establishment we observed could indicate a shift in the functioning of the Eastern Scheldt pelagic food web. This shift is likely based on the combination of decreased primary production and phytoplankton cell size with increased salinity in post-barrier times, which were already considered as unfavourable for larger, estuarine copepod taxa by previous studies (Bakker et al., 1994; Nienhuis and Smaal, 1994; Smaal et al., 2013).

4.4. Functioning of the pelagic food web

Small cyclopoids like *Oithona* are usually eurytherm, euryhaline, omnivorous, and have a lower respiration and feeding rate than most calanoids, which enables them to live in a wider range of habitats (Paffenhöfer, 1993; Turner, 2004). They are able to feed efficiently on small-sized phytoplankton and flagellates, which is an advantage during autumn and winter when the phytoplankton community is often composed of cells too small to be fed upon by the majority of copepods

(Sabatini and Kjørboe, 1994; Sommer et al., 2002). In addition, microZP make up an important part of their diet, and they graze on copepod nauplii of, e.g., *Acartia* (Paffenhöfer, 1993; Nakamura and Turner, 1997), a common taxon in the Eastern Scheldt. A sudden increase in *Oithona* was also observed in the San Francisco Bay estuary after an increase in salinity and a bivalve invasion led to a change in the food web structure (Cloern and Jassby, 2012). The decrease in common ZP taxa with a concomitant increase in cyclopoids was attributed to the arrival of an invasive bivalve, *Corbula amurensis*, that outcompeted native species and remarkably reduced chl-a and SPM (Cloern and Jassby, 2012).

In the Eastern Scheldt, changes in the ZP community were less dramatic. While the increased bivalve filtration activity due to the *Crasostrea gigas* invasion and intense Blue mussel cultivation affected phytoplankton composition and size classes, mesoZP abundances did not markedly decrease in the first years after the dam was built (Bakker and van Rijswijk, 1994), and our newer data set confirms these findings. Additionally, many of the observed changes in ZP community composition in comparison to older data could be related to changes in salinity as outlined above. The more subtle changes could be related to the presence of bivalves prior to the barrier construction and reduction of freshwater inflow. Furthermore, the reduction of primary production in the Eastern Scheldt of about 50% and the increase in bivalve density were gradual changes observed over a course of 15 years (Smaal et al., 2013), while in the reduction of chl-a and increase in bivalve density in San Francisco Bay estuary happened within about two years (Cloern and Jassby, 2012). Another difference are the food size spectra of the two invasive bivalves. *C. amurensis* in the San Francisco Bay estuary feeds not only on mesoZP, microZP, and phytoplankton, but also on small-sized phytoplankton and bacteria, thus consuming all parts of the planktonic food web and disrupting the carbon flow to higher trophic levels (Werner and Hollibaugh, 1993; Greene et al., 2011). By reducing microZP abundances, the main food source for mesoZP during times of flagellate-dominated phytoplankton assemblages is removed (Stibor et al., 2004). In contrast, *C. gigas* in the Eastern Scheldt does not efficiently retain cells smaller than 5 µm (Dupuy et al., 2000; Mostajir et al., 2015). Hence, small-sized phytoplankton and bacteria remain available as food sources for microZP, which can be consumed by mesoZP when phytoplankton availability is low. This might explain why mesoZP in the Eastern Scheldt are still reaching abundances comparable to those prior to the arrival of *C. gigas* despite of chl-a concentrations <10 µg L⁻¹ during the bloom nowadays, which can be considered as a threshold value for ZP food limitation in estuaries (Müller-Solger et al., 2002).

Given the shallow water depths of estuaries, benthic-pelagic coupling and bivalve grazing generally play a more significant role than in coastal areas (Underwood and Kromkamp, 1999). The introduction of *C. gigas* in the Eastern Scheldt indeed increased filtration pressure and resulted in a higher water transparency (Bougrier et al., 1995; van den Ende et al., 2016). Simultaneously, a shift in phytoplankton composition towards smaller taxa occurred after the construction of the barrier (Bakker and van Rijswijk, 1994). This combination of factors has been shown to cause a decrease in ZP in other estuaries (Petersen et al., 2008; Cloern and Jassby, 2012). Yet, mesoZP abundances did not notably decrease in the Eastern Scheldt until 1988, but rather increased in the eastern part shortly after the construction (Bakker and van Rijswijk, 1994), which is in line with our recent observations. The combination of higher residence time, reduced tidal action, and higher water transparency likely enabled mesoZP to better detect and escape the filter feeders' current (Kjørboe et al., 1999). Overall, the oyster culture plots in the eastern part did not seem to directly negatively affect mesoZP abundances, even though the high oyster clearance rate of 2.08 L h⁻¹ (Bougrier et al., 1995) should remove large parts of the phytoplankton and microZP community thus impacting them indirectly.

4.5. Conclusions

In summary, we observed minor spatial gradients in abiotic factors in the Eastern Scheldt. While there were some differences in ZP abundances between stations, especially between the most distant ones, seasonal variability between the three investigated time points representing typical spring bloom, post-bloom, and autumn bloom scenarios was evidently higher. Phytoplankton growth and microZP grazing rates were variable but in line with what could be expected for the time points chosen, with changed phytoplankton communities explaining differences between the bloom phases. The ZP community was strongly associated with salinity and chl-a, even though differences between stations were not overly pronounced due to the levelling of gradients based on the storm surge barrier construction. In addition, SPM and retention time were identified as relevant factors for mesoZP, especially for larvae, and PO₄ and NO_x concentration for microZP, likely connected to occurrence of mixotrophic taxa. In comparison to information on ZP abundances and taxonomic composition from the 1980s, we observed changes in the taxonomic composition. While some could be explained by the reduced connection to the open North Sea, increased *Oithona* abundances seem to be more likely related to a change in phytoplankton species and size composition. Even though a higher temporal resolution would be necessary to confirm a consistent increase in *Oithona* abundances outside of the time points sampled in this study, the fact that this copepod had not been noticed so far emphasizes the importance of including microZP and mesoZP in regular monitoring programmes. Any change in the taxonomic composition of mesoZP will also affect grazing pressure on lower levels. This might in turn impact the functioning of the pelagic food web and the role of the microbial loop therein, especially in combination with the intense bivalve grazing in the Eastern Scheldt.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The research data is available at ZENODO (<https://doi.org/10.5281/zenodo.7595501>).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.seares.2023.102357>.

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