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Combined physical, chemical and biological factors shape *Alexandrium ostenfeldii* blooms in the Netherlands

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ABSTRACT

Harmful algal blooms (HABs) are globally expanding, compromising water quality worldwide. HAB dynamics are determined by a complex interplay of abiotic and biotic factors, and their emergence has often been linked to eutrophication, and more recently to climate change. The dinoflagellate *Alexandrium* is one of the most widespread HAB genera and its success is based on key functional traits like allelopathy, mixotrophy, cyst formation and nutrient retrieval migrations. Since 2012, dense *Alexandrium ostenfeldii* blooms (up to 4500 cells mL^{-1}) have recurred annually in a creek located in the southwest of the Netherlands, an area characterized by intense agriculture and aquaculture. We investigated how physical, chemical and biological factors influenced *A. ostenfeldii* bloom dynamics over three consecutive years (2013–2015). Overall, we found a decrease in the magnitude of the bloom over the years that could largely be linked to changing weather conditions during summer. More specifically, low salinities due to excessive rainfall and increased wind speed corresponded to a delayed *A. ostenfeldii* bloom with reduced population densities in 2015. Within each year, highest population densities generally corresponded to high temperatures, low DIN:DIP ratios and low grazer densities. Together, our results demonstrate an important role of nutrient availability, absence of grazing, and particularly of the physical environment on the magnitude and duration of *A. ostenfeldii* blooms. Our results suggest that predicted changes in the physical environment may enhance bloom development in future coastal waters and embayments.

1. Introduction

Global change is occurring at an unprecedented rate (Stocker et al., 2013), impacting ecosystems worldwide. In addition to climatic changes, anthropogenic activities have accelerated the rate and extent of eutrophication of many aquatic environments as well. These changes greatly affect phytoplankton, standing at the base of aquatic food webs. Over the past few decades, some phytoplankton species have become an increasing nuisance by forming harmful algal blooms (HABs; Anderson et al., 2002; Heisler et al., 2008). The global expansion of HABs has very often been attributed to eutrophication of coastal regions. Changes in nutrient loading, nutrient ratios and nutrient composition have a tremendous impact on phytoplankton communities living in rivers, estuaries and coastal zones (Anderson et al., 2002; Smith and Schindler, 2009). For instance, enhanced use of urea as a fertilizer and increases in the nitrogen and phosphorus to silicate ratios may promote proliferation of toxic dinoflagellates over diatoms (Glibert et al., 2001; Riegman, 1995). In addition, further changes in climate involving temperature shifts and subsequent weather changes, may lead to an expansion of the ecological niche of many HAB-forming species (Anderson et al., 2012b; Hallegraeff, 2010; Wells et al., 2015).

HABs are known for their adverse effects on ecosystems through their cascading impact on higher trophic levels (Anderson et al., 2002; Hallegraeff, 1993). For instance, HABs can produce toxic compounds that may accumulate in the food chain, leading to the death of fish, seabirds and marine mammals. Moreover, toxins accumulated in seafood may cause shellfish poisoning syndromes in humans (Wang, 2008). Proliferations of HABs can thus have far

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reaching ecological and economic consequences. The dinoflagellate *Alexandrium ostenfeldii* is a globally widespread toxic HAB-forming species (Balech, 1995; Fraga and Sanchez, 1985; Gribble et al., 2005; John et al., 2003; Levasseur et al., 1997; Mackenzie et al., 1996; Okolodkov and Dodge, 1996; Wang et al., 2006). Although it used to occur in low numbers in phytoplankton assemblages, in recent years dense blooms of this species have been reported (Borkman et al., 2012; Burson et al., 2014; Hakanen et al., 2012; Kremp et al., 2009; Tomas et al., 2012). *Alexandrium ostenfeldii* is also known to produce various toxins, including Paralytic Shellfish Poisoning (PSP) toxins and the cyclic imines gymnodimines and spirolides (Anderson et al., 1990; Cembella et al., 2000; Harju et al., 2016; Kremp et al., 2014). In addition to these toxins, *A. ostenfeldii* produces extracellular allelochemicals, which can lyse competing phytoplankton species and small protozoan grazers (Tatters et al., 2012; Tillmann et al., 2007; Tillmann and John, 2002; Van de Waal et al., 2015). Production of toxins and lytic compounds are key traits supporting *Alexandrium* proliferation (John et al., 2015; Wohlrab et al., 2016, 2010), particularly since dinoflagellates are typically poor competitors in terms of growth and nutrient uptake (Litchman et al., 2007; Smayda, 2002). Other important traits include heterotrophic feeding, cyst formation, and nutrient retrieval migrations (Smayda, 1997).

Although these traits are effective for supporting growth, the abiotic environment also plays a crucial role in HAB initiation and subsequent development. Dinoflagellates reside as resting cysts in the sediment and, besides using endogenous clocks observed in some species (Anderson and Keafer, 1987), require various environmental stimuli in order to germinate (Anderson et al., 2005). Specifically, temperature, oxygen concentration and light play a role in cyst germination (Anderson et al., 1987; Dale, 1983). Therefore, cyst resuspension induced by wind mixing may facilitate bloom initiation. Once emerged from the cystbank, however, dinoflagellates are very sensitive to turbulence (Berdalet et al., 2007; Berdalet and Estrada, 2005; Wyatt and Horwood, 1973), and their blooms are often associated with calm weather and water column stability (Berman and Shteinman, 1998; Margalef et al., 1979 Wyatt and Horwood, 1973). Other physical controls, such as temperature and salinity, as well as chemical controls, such as nutrient availability are also important in determining the development of HABs. Thus, a combination of environmental conditions will set the window of opportunity for HABs to develop (Anderson et al., 2012a).

Massive annual recurring *A. ostenfeldii* blooms only recently emerged in a Dutch brackish water creek (Burson et al., 2014), and were first observed in 2012. The inflow of water is derived from the agricultural hinterlands, subsequently supplying the creek ample nutrients. The outflow is regulated by a pumping station, which discharges into the Eastern Scheldt, an estuary with the main shellfish farming areas of the Netherlands (Van Der Heijden, 2007). Discharge of creek water with high *A. ostenfeldii* population densities thus forms a potential threat to the public health. Little, however, is known about the drivers underlying the proliferation of *A. ostenfeldii* in this creek. Therefore, we investigated how various physical, chemical and biological factors affected the timing and magnitude of *A. ostenfeldii* blooms in these brackish waters. To this end, we closely followed an *A. ostenfeldii* population for three consecutive years, together with temperature, wind speed, rainfall, salinity, nutrients, and zooplankton.

2. Material & methods

The brackish water creek Ouwerkerkse kreek (51°62′N, 3°99′E) is located in the Rhine-Muesse-Scheldt delta of the Netherlands (Fig. 1A; Burson et al., 2014; Van de Waal et al., 2015). It has a mean depth of 5 m with a maximum depth of 8 m, covering roughly 0.12 km^2.

Field data was collected for three consecutive years, starting in April 2013. At three locations in the creek (Fig. 1B), samples were taken for *Alexandrium ostenfeldii* population densities, toxin concentrations (2014–2015), and bacterial abundances (2015) once every week or every two weeks from spring until autumn. In the same period, additional monthly samples were taken at two locations for phytoplankton and zooplankton determination. Moreover, monthly samples were taken each year for salinity, inorganic nutrient concentrations and chlorophyll-a concentrations (Fig. 1B). Hourly meteorological data, i.e. temperature, wind speed and precipitation, was derived from the weather station in Vlissingen, approximately 20 km from our study site (with very similar meteorological conditions) and a moving average was calculated.

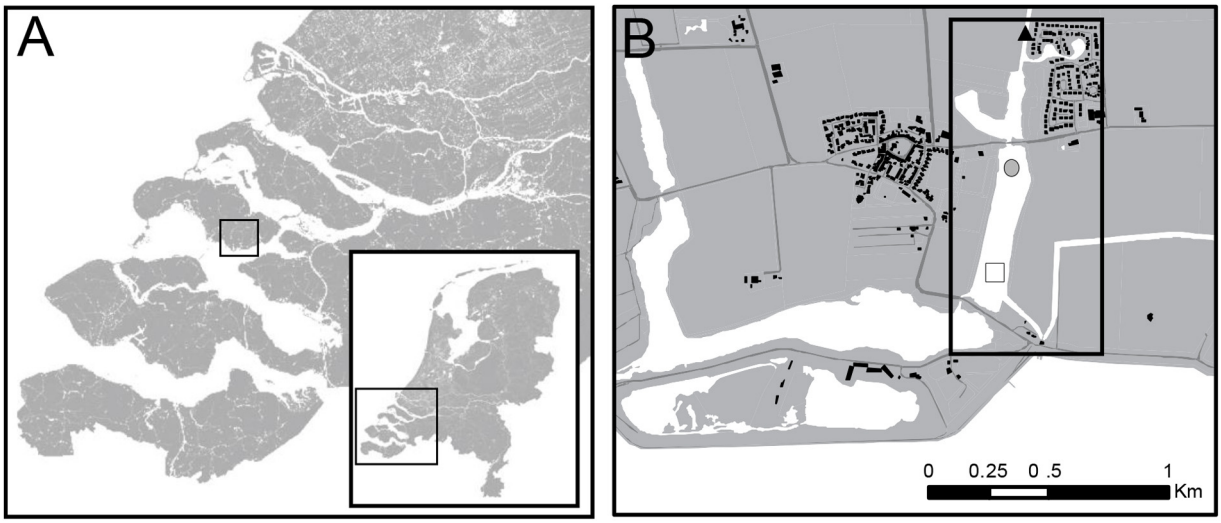

Fig. 1. Location of A) the Ouwerkerkse kreek in the Netherlands, and B) sampling points in the Ouwerkerkse kreek, where the triangle (northern part) and square (southern part) represent the sample locations for all measurements and the circle (middle part) the extra sample location for *A. ostenfeldii* abundances and toxins.

2.1. Alexandrium ostenfeldii *abundances*

An integrated water sample was taken of the upper 1 m of the water column at all sample locations (Fig. 1B), from which a 50 mL subsample was fixed with Lugol's iodine solution (Lugol) to a final concentration of 1% and stored in the dark at 4 °C until analysis. *A. ostenfeldii* cells were counted in an Utermöhl chamber on an inverted microscope (DMI 4000B; Leica Microsystems CMS GmbH, Mannheim, Germany). *A. ostenfeldii* population densities were determined by counting at least 200 cells or 100 fields of view (200× magnification). Earlier morphological and molecular analyses showed that 20 isolated clones from this population belonged to Group 1 of *A. ostenfeldii* (Kremp et al., 2014; Van de Waal et al., 2015). The start and end of a bloom in each year was set when *A. ostenfeldii* population densities reached 10% of its maximum.

2.2. Chlorophyll-a

Chlorophyll-a extractions were performed with 80% ethanol according to Nusch (1980). Samples were subsequently analyzed spectrophotometrically at 665 and 750 nm (UV-VIS Spectrophotometer UV1650-PC, Shimadzu Europe, Duisburg, Germany), with an additional measurement at both wavelengths after addition of 0.4 M HCl to a final concentration of 4 mM for phaeopigment correction. Calculations were performed after Nusch and Palme (1975), using a chlorophyll-a extinction coefficient of 8.16 L g^{-1} mm^{-1}, and a ratio between chlorophyll-a and phaeopigment extinction coefficients of 1.7.

2.3. Zooplankton

For zooplankton determination, a 5 L integrated water sample was filtered over a 100 μm mesh and fixed with 96% ethanol. The sample was settled in a large sedimentation chamber with a cuvette and analyzed with an inverted microscope (DMI 4000B; Leica Microsystems CMS GmbH, Mannheim, Germany). Zooplankton was counted and identified up to genus level. Zooplankton dimensions were assessed using image analysis, and organism volume was subsequently calculated from these dimensions assuming a most similar geometric shape (typically a sphere). Biovolume was subsequently calculated by multiplying the organism volume with its counts.

2.4. Salinity

Salinity was calculated using water temperature and conductivity from surface water (<1 m) according to the Standard Methods for the Examination of Water and Wastewater (Clesceri et al., 1999). For further details on the salinity gradient we refer to Martens et al. (2016).

2.5. Nutrients

Field samples of 15 mL for inorganic nutrients were taken (Fig. 1B) and stored at −20 °C until analysis. Dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphorus (DIP) were subsequently measured using continuous flow analysis coupled with spectrophotometric detection (San++ Automated Wet Chemistry Analyzer, Skalar Analytical B.V., Breda, the Netherlands) according to the ISO 13395:1996 and the ISO 15681-2:2003 protocol, respectively.

2.6. Toxin measurements

Subsamples for toxin analyses were taken from the integrated water sample by filtration of 20–60 mL over glass fiber filters (GF/F, Whatman, Maidstone, UK), which were stored at −20 °C until further analysis.

2.6.1. Analysis of PSP toxins

PSP toxins were determined by ion pair liquid chromatography coupled to post-column derivatization and fluorescence detection, as described in Krock et al. (2007) and Van de Waal et al. (2015).

2.6.2. Analysis of cyclic imine toxins by triple quadrupole mass spectrometry

The cyclic imine toxin measurements were performed on an Agilent 1100 LC liquid chromatograph (Waldbronn, Germany) coupled to a 4000 Q Trap triple-quadrupole mass spectrometer (AB-Sciex, Darmstadt, Germany) with a Turbo V ion source. Toxins were quantified by external calibration curves of SPX-1 and GYM A with standard solutions ranging from 10 to 1000 pg μL^{-1}, each. Other SPX and GYM for which no standards are available were calibrated against the SPX-1 and GYM A calibration curve, respectively, and expressed as SPX-1 or GYM A equivalents. For further details, please also see Van de Waal et al. (2015).

2.7. Statistical analysis

In order to assess the correlation between *A. ostenfeldii* abundances and different environmental variables, we used Spearman's rho (ρ), as a linear relationship could not be assumed. The relationships between the significant environmental variables were also determined with Spearman's rho (ρ) to test for collinearity among variables.

3. Results

3.1. Bloom development

An *Alexandrium ostenfeldii* bloom was observed in all three years. The magnitude of the bloom varied greatly between the years (Fig. 2). In 2013, the highest densities were observed, with values up to 4500 cells mL^{-1}. Population densities in 2014 were somewhat lower, reaching 3200 cells mL^{-1}. In 2015, population densities were substantially lower than previous years, only reaching 800 cells mL^{-1}. The duration of the bloom also varied greatly between the years, with the longest bloom period in 2014,

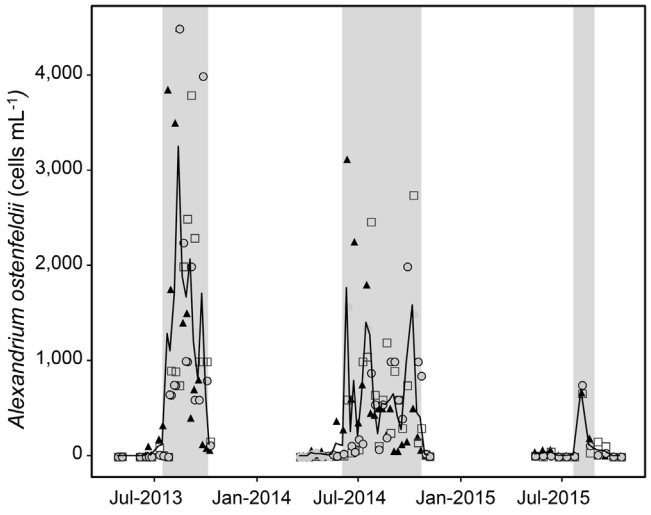

Fig. 2. Seasonal dynamics of *A. ostenfeldii* population densities, where symbols (triangle the northern part, square the southern part and circle the middle part of the creek) represent the population densities at the three sample locations and the black line indicates the average.

Table 1
Spearman's rank correlation coefficients (ρ) describing relationships between *A. ostenfeldii* abundances, temperature and salinity (** $P < 0.001$, * $P < 0.05$).

	A. ostenfeldii	Temperature	Salinity
Temperature	0.74**		
Salinity	0.42*	0.40*	
Wind speed	−0.51**	−0.56**	−0.38*
Precipitation	0.16	0.13	−0.41*
DIN	−0.11	−0.57**	−0.54*
DIP	0.64*	0.85**	0.47*
Copepods	−0.38	−0.11	0.17
Rotifers	−0.17	0.39	0.35
Week	0.43**	0.41**	0.07

where it lasted a total of 139 days from the 17th of July until the 4th of October, followed by 2013, with 79 days from the 4th of June until the 21st of October. In 2015, the bloom lasted only 35 days from the 23rd of June until the 27th of August.

3.2. Physical environment

A. ostenfeldii abundances corresponded strongly to changes in temperature ($\rho = 0.74$, $P < 0.001$; Table 1). During bloom periods temperatures were generally above 15 °C, corresponding to summer conditions (Fig. 3A). Moreover, some collinear relationships between temperature and other environmental factors were also found, namely wind speed ($\rho = -0.56$, $P < 0.001$), salinity ($\rho = 0.40$, $P < 0.05$), and DIP concentrations ($\rho = 0.85$, $P < 0.001$; Table 1).

We observed a negative correlation between *A. ostenfeldii* abundances and wind speed ($\rho = 0.51$, $P < 0.001$; Table 1). Average wind speed was generally lower during the blooms of 2013 and 2014 compared to the rest of the year (around 5 m s^{-1} against 7 m s^{-1}), with increases in the middle and at the end of the 2014 bloom period (Fig. 3B). In 2015, however, wind speed remained relatively high during the bloom period with only a brief decline towards the end of the bloom.

Precipitation fluctuated strongly throughout the year, including the bloom periods. Distinct peaks in precipitation could be recognized at the end of the bloom in 2013, in the middle of the bloom in 2014 and during the bloom period of 2015 (Fig. 3C). *A. ostenfeldii* abundances did not show a correlation with precipitation (Table 1). Precipitation did, however, show a negative correlation with salinity ($\rho = 0.41$, $P < 0.05$; Table 1).

Over the course of a year, large fluctuations were observed in salinity (Fig. 3D), with high values up to 23 in summer and low values down to 4.9 in winter. During the bloom periods of 2013 and 2014 salinities were largely above 15, while in 2015 salinities remained below 15 with extremely low values down to 3.7 at the end of the bloom. *A. ostenfeldii* abundances generally increased with salinity ($\rho = 0.42$, $P < 0.05$; Table 1).

3.3. Chemical environment

Dissolved inorganic nutrients showed a clear annual cycle (Fig. 4A, B). Nitrogen concentrations were high in winter (~150 μmol L^{-1}), decline in spring (down to ~6 μmol L^{-1}) and subsequently increased again in autumn (Fig. 4A). This pattern was reversed for phosphorus, with concentrations becoming high in summer (up to ~20 μmol L^{-1}) and low in winter (down to ~1 μmol L^{-1}; Fig. 4B). Consequently, DIN:DIP ratios during the *A. ostenfeldii* blooms were low, with an average around 0.5 (Fig. 4C). Towards the end of each bloom, DIN:DIP ratios increased again. *A. ostenfeldii* abundances showed a positive correlation with DIP ($\rho = 0.63$, $P < 0.05$), while no correlation with DIN and DIN:DIP ratios was observed (Table 1).

3.4. Biological environment

Chlorophyll-a concentrations generally showed two distinct peaks, with a phytoplankton spring bloom in all three years, and another bloom during the summer or autumn (Fig. 5). The spring blooms were dominated by green algae and were followed by diatoms and subsequently dinoflagellates (data not shown). Cyanobacteria were not prominently present in 2014, but reached high population densities in 2013 and 2015 during spring and autumn.

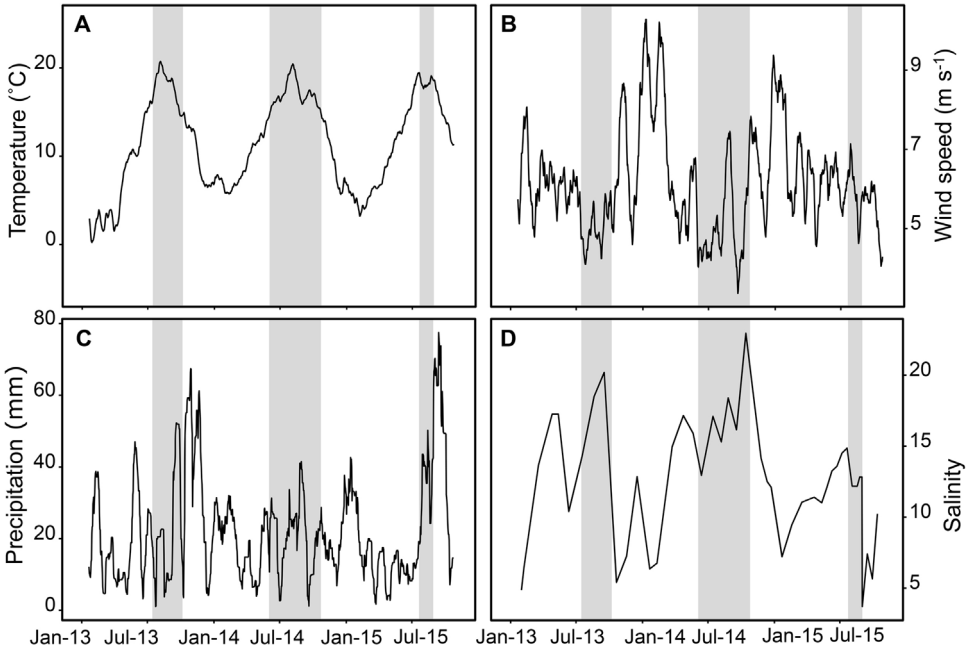

Fig. 3. Seasonal dynamics of A) temperature, B) wind speed, C) precipitation, and D) salinity, where the black lines represent the moving average. The light grey areas in the background of each graph indicate the occurrence of an *A. ostenfeldii* bloom event.

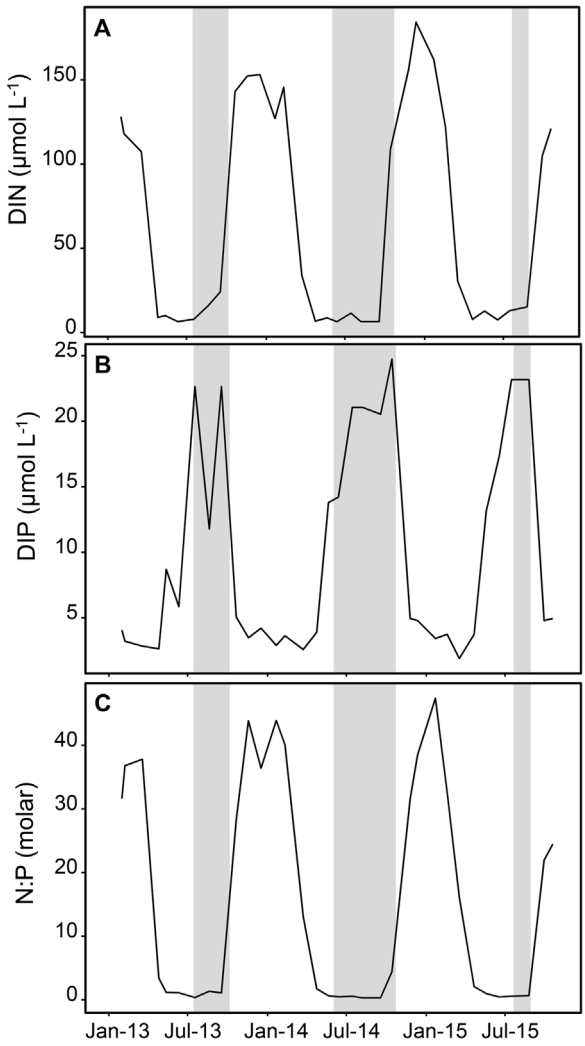

Fig. 4. Seasonal dynamics in A) DIN, B) DIP and C) DIN:DIP ratios. The light grey areas in the background of each graph indicate the occurrence of an *A. ostenfeldii* bloom event.

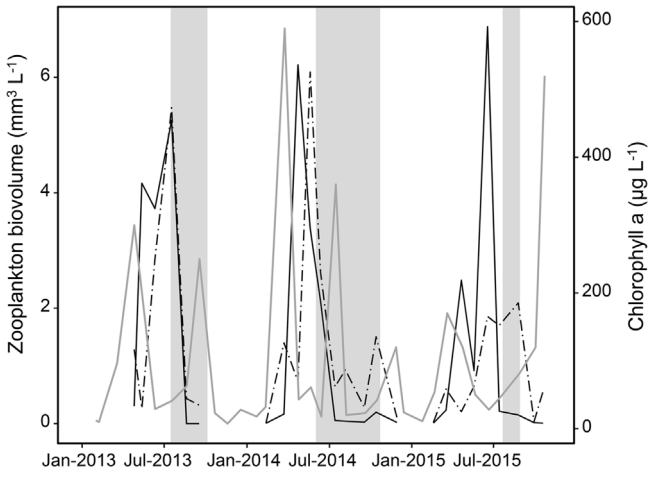

Fig. 5. Seasonal dynamics in the abundances of copepods (black line) and rotifers (black dotted line), and in the concentrations of chlorophyll-a (grey line). The light grey areas in the background of each graph indicate the occurrence of an *A. ostenfeldii* bloom event.

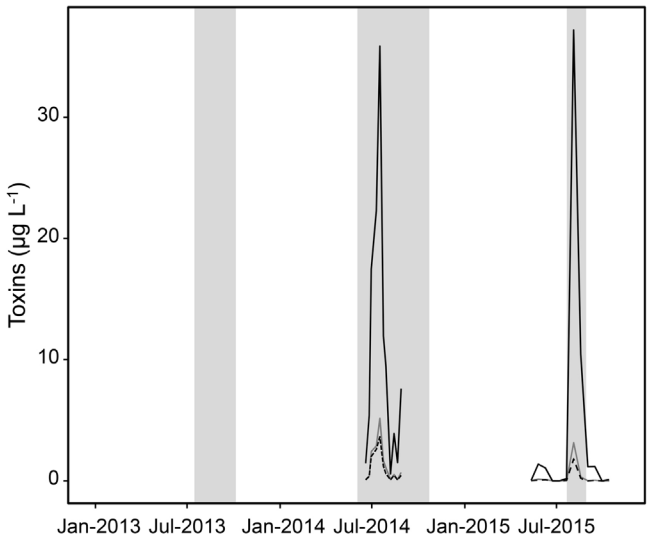

Fig. 6. Seasonal dynamics in PSP toxins (black line), spirolides (grey line) and gymnodimines (grey dotted line) during the *A. ostenfeldii* blooms of 2014 and 2015. The light grey areas in the background of each graph indicate the occurrence of an *A. ostenfeldii* bloom event.

Each year, copepods were most abundant prior to the *A. ostenfeldii* blooms, reaching total biovolumes of 6.88 $mm^3 L^{-1}$ (Fig. 5). Copepod densities started to decline rapidly at the onset of each bloom and remained low throughout the bloom period. Other grazers, particularly rotifers, were also abundant in the creek. Rotifers reached biovolumes of 6.22 $mm^3 L^{-1}$ prior to each bloom, and showed subsequent steep declines during the 2013 and 2014 blooms (Fig. 5). In 2015, rotifer densities remained generally low, with average biovolumes around 1.9 $mm^3 L^{-1}$ during the bloom period. A correlation between *A. ostenfeldii* abundances and grazer densities was not found.

3.5. Toxins

PSP toxins, gymnodimines and spirolides were measured during the blooms of 2014 and 2015. In both years, the amount of cell-bound toxins corresponded to *A. ostenfeldii* population densities, being highest at the peak of the blooms reaching average values of 37.2 and 35.9 $\mu g L^{-1}$ for PSP toxins, 3.0 and 1.8 $\mu g L^{-1}$ for gymnodimines and 3.2 and 5.1 $\mu g L^{-1}$ for spirolides, in 2014 and 2015, respectively (Fig. 6). In 2014, toxins were only measured from June 19th until August 28th.

No major changes in toxin composition were observed between and within the years (data not shown). For PSP toxins, C1/C2 analogues comprised most of the toxin profile (80%), and relative contributions of saxitoxin and GTX2/3 were comparable with ~10% each. The cyclic imine toxin profile consisted for gymnodimines of gymnodimine A, and for spirolides of 13-desmethyl spirolide C.

4. Discussion

Since the detection of high abundances of the dinoflagellate *Alexandrium ostenfeldii* in 2012 (Burson et al., 2014), annual blooms recurred in the Ouwerkerkse Kreek. Yet, the dynamics of the blooms varied strongly between the monitored years (2013–2015). Our results indicate that the combination of physical, chemical, and biological factors determines the occurrence of *A. ostenfeldii* blooms, while physical factors play a crucial role in the magnitude and duration of a bloom.

Nutrient dynamics were largely comparable between the three years, with low DIN concentrations around 10 $\mu mol L^{-1}$ and high

DIP concentrations around 20 μmol L^{-1} during each bloom (Fig. 4A, B). DIN concentrations were low as a result of an earlier phytoplankton spring bloom consisting primarily of green algae, while DIP concentrations were presumably high due to anoxic conditions near the bottom of the creek leading to the release of phosphate from the sediment (Mortimer 1941; Fig. S1; Appendix A). Such anoxic conditions, in turn, may be the result of increased surface temperatures ($\rho = 0.85$, $P < 0.001$; Table 1). *Alexandrium ostenfeldii* abundances showed a significant correlation with DIP concentrations ($\rho = 0.66$, $P < 0.05$; Table 1), although this might be due the collinearity between DIP and temperature, corresponding to summer conditions. *Alexandrium* species have previously been shown to proliferate independently of inorganic nutrient concentrations, also when nitrogen concentrations were low (Collos et al., 2007; Hakanen et al., 2012; Laanaia et al., 2013; Vila et al., 2005). Despite these low nitrogen concentrations, and the generally poor competitive ability of dinoflagellates in terms of growth and nutrient uptake (Litchman et al., 2007; Smayda, 1997; Tang, 1996), dense *A. ostenfeldii* blooms did occur each year. Several *Alexandrium* species were shown to utilize organic substrates (Anderson et al., 2012a; Collos et al., 2007, 2004; Jacobson and Anderson, 1996). Such heterotrophic feeding strategies would allow *A. ostenfeldii* to reach high population densities under nutrient limited conditions, and furthermore gain advantage over strictly autotrophic growing phytoplankton. Future studies should include the assessment of organic substrates to further elucidate the role of mixotrophy in the formation of HABs.

Top-down control on the *A. ostenfeldii* population was also largely comparable between the three years. Grazer biovolumes increased in spring and sharply declined just before or at the onset of the *A. ostenfeldii* bloom (Fig. 5). Grazer biomass was likely supported by the phytoplankton spring bloom, and grazing may have caused chlorophyll-a concentrations to rapidly decline (Fig. 5). Consequently, chlorophyll-a concentrations were relatively low at the onset of the *A. ostenfeldii* blooms, and may have resulted in food limitation for zooplankton. This, in turn will reduce grazing pressure, which possibly facilitated the development of the *A. ostenfeldii* blooms. Alternatively, *A. ostenfeldii* may have reduced grazer abundances due to functional traits such as toxins and allelochemical production, which were shown to play a role in grazer deterrence (Colin and Dam, 2003; Selander et al., 2006; Sopanen et al., 2011; Teegarden, 1999; Tillmann et al., 2007; Tillmann and John, 2002; Wohlrab et al., 2010). In 2014 and 2015, concentrations of PSP toxins, as well as gymnodimines and spirolides largely followed *A. ostenfeldii* dynamics and were comparable between both years. This is remarkable since cell densities were substantially different in 2014 and 2015, which implies that cells from the population of 2015 contained more toxins. The production of these toxins as well as of allelopathic compounds by this *A. ostenfeldii* population may have benefitted bloom development (John et al., 2015; Tillmann et al., 2007; Tillmann and John, 2002).

Temperatures were generally above 15 °C during the bloom period, ranging up to 25 °C, and may very well be a requirement for bloom development, as a strong correlation was found with *A. ostenfeldii* abundances ($\rho = 0.74$, $P < 0.001$; Table 1). Temperatures of 15 °C were sufficient to allow growth rates of 0.22 d^{-1} for various isolates from the Dutch *A. ostenfeldii* population (Van de Waal et al., 2015), and higher temperatures may further promote growth. *Alexandrium ostenfeldii* isolates from other populations indeed showed higher growth rates at temperatures of 20–24° C (Bill et al., 2016; Kremp et al., 2012). However, temperatures were largely comparable between years and can therefore not explain the observed variations in the magnitude and durations of the blooms.

Salinities in the creek showed clear seasonal dynamics, presumably as a result of variations in precipitation ($\rho = -0.41$, $P < 0.05$; Table 1) and associated drainage via a pumping station. High *A. ostenfeldii* abundances corresponded to summer conditions with increased salinities, where highest cell numbers were reached at salinities above 11 (Fig. 3C). Recent experiments with a Dutch *A. ostenfeldii* strain showed a broad salinity tolerance with largely comparable growth rates over a salinity range of 10–35 (Martens et al., 2016). Their findings suggested that *A. ostenfeldii* is a euryhaline species, growing best at higher salinities, which is supported by the correlation between salinity and *A. ostenfeldii* population densities that was found ($\rho = 0.42$, $P < 0.05$; Table 1). Indeed, population densities were highest in 2013 and 2014, when salinities remained above 15 during the bloom period. Lower salinities in 2015 could have contributed to reduced bloom densities. The sharp drop in salinity at the end of the 2015 bloom period, caused by excessive rainfall (Fig. 3D) and drainage via the pumping station, was likely responsible for the sudden bloom termination. Salinities dropped to 3.7, and these low values were shown to cause mortality among several *A. ostenfeldii* strains (Gu, 2011; Jensen and Moestrup, 1997; Lim and Ogata, 2005; Maclean et al., 2003; Suikkanen et al., 2013), including a Dutch strain from the same population (Martens et al., 2016). Similarly, a large drop in salinity in October 2013, may have terminated the *A. ostenfeldii* bloom in that particular year (Fig. 3D).

Higher temperatures are also related to reductions in wind speed ($\rho = -0.56$, $P < 0.001$; Table 1) and together both factors contribute to a more stable water column, which may benefit *A. ostenfeldii* bloom formation. In addition, *A. ostenfeldii* is sensitive to turbulence, and minimal disturbances of the water column by wind may affect its growth and bloom development (Berdalet et al., 2007). A negative correlation between *A. ostenfeldii* abundances and wind speed was indeed observed ($\rho = -0.51$, $P < 0.001$; Table 1). Specifically, in 2013 and 2014 blooms of *A. ostenfeldii* developed when wind speed was low. In the middle of the bloom period in 2014, wind speed drastically increased, which may have attributed the subsequent drop in cell densities at the end of July. In 2015, wind speed was generally not lower compared to the rest of the year. Consequently, the combination of relatively high wind speed and excessive precipitation with resulting reduction in salinity presumably led to the lower bloom densities as well as the shorter bloom period in 2015. However, a temporary disturbance in the water column may be crucial for *A. ostenfeldii* bloom initiation. Oxygen and light are considered to play a key role in cyst germination (Anderson et al., 1987), but values are low near the sediment of the creek (Fig. S1; Appendix A). Thus, in order to germinate, cysts need to be re-suspended in the water column. Influences from tides, upwelling and stream flows are absent within the creek, and resuspension of the sediment is therefore mainly depended on wind events. Indeed, higher wind speed was observed just before the start of the bloom during all three years (Fig. 3B).

Overall, changes in the physical environment over the years, particularly wind speed and salinity, may largely explain the observed differences in magnitude and duration of the *A. ostenfeldii* blooms. Various laboratory experiments showed that temperature, salinity and turbulence can substantially affect *Alexandrium* growth (Bill et al., 2016; Grzebyk et al., 2003; Jensen and Moestrup, 1997; Juhl et al., 2001; Laabir et al., 2011; Lim and Ogata, 2005; White, 1976), and thereby form important constraints on bloom development. Our results indeed indicate that the most beneficial combination of physical factors for *A. ostenfeldii* blooms includes higher temperatures and salinities (above 15° C and salinities above 10), and calm and stable weather conditions with reduced wind speed, and indirectly precipitation. These conditions were observed in 2013, which also corresponded to highest population densities reaching up to 4500 cells mL^{-1}. Several field studies also found that physical factors, which promote water column stability,

play a crucial role in the development of *Alexandrium* blooms. For instance, a correlation between higher temperatures and *A. ostenfeldii* bloom development was found in the Baltic Sea (Hakanen et al., 2012). Similarly, both salinity and wind speed were strongly related to *Alexandrium* bloom development in the Estuary and Gulf of St. Lawrence, Canada (Weise et al., 2002). In addition, a study from Thau Lagoon found that particularly wind speed and temperature play an important role in *Alexandrium* bloom development, where wind events allowed bloom initiation by resuspension of cysts, and low wind speed in combination with high and stable temperatures facilitated subsequent vegetative growth (Laanaia et al., 2013).

Even though we only studied one system, our results do indicate how the physical environment may shape HABs. This not only supports our current understanding, but also provides an example of how climate-driven changes in the physical environment may favor HAB development. Earlier studies have suggested that global rising temperatures may lead to a range expansion of HAB species (Thomas et al., 2012; Wells et al., 2015). Other consequences of global temperature increases are lower wind speeds (McVicar et al., 2012), which have already decreased globally by 5–15% over the last decades (Vautard et al., 2010). Such low wind speeds in combination with higher temperatures will enhance water column stability, which is generally favorable for HAB development (Hallegraeff, 2010; Hallegraeff et al., 1995). Furthermore, salt water intrusion is becoming an increasing global threat through sea level rise (Werner and Simmons, 2009). This allows euryhaline HAB species, such as *A. ostenfeldii* to disperse into these waters imposing an increasing health risk particularly to densely populated areas such as the Netherlands. Our results suggest that predicted changes in the physical environment may enhance bloom development in future coastal waters and embayments.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.hal.2017.02.004.

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