Long-term trends in phytoplankton biomass, composition and dynamics in the Belgian part of the North Sea

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List of abbreviations

A	AIC	Akaike information criterion
	AMO	Atlantic Multidecadal Oscillation
	AMORE	Advanced Modelling and Research on Eutrophication
	ANOVA	Analysis of variance
B	BCS	Belgian continental shelf
	BMDC	Belgian Marine Data Centre
	BPD	Belgian Phytoplankton Database
	BPNS	Belgian Part of the North Sea
C	CHEMTAX	CHEMical TAXonomy
	Chl	Chlorophyll a
	Chl P90	90^{th} percentile of Chl
	CMs	constitutive mixotrophs
	CPR	Continuous Plankton Recorder
	CRA	Concerted Research Actions
D	DCS	Dutch continental shelf
	DIN	dissolved inorganic nitrogen
	DIP	dissolced inorganic phosphorus
	DITS	Data Inventory and Tracking System
	DPNS	Dutch part of the North Sea
	DSi	dissolved silica
E	EA	East Atlantic pattern
F	FCM	Flow cytometer or flow cytometry
	FISH	fluorescent in situ hybridization
	FLO	orange fluorescence
	FLR	red fluorescence
	FLY	yellow fluorescence
G	GAMM	General Additive Mixed Model
	GMST	global mean surface temperature
	GNCM	non-constititutive mixotrophs that aquire their phototrophic capacity by ingesting general non-specific prey
H	HA	Harmful Algae
	HAB	Harmful Algae Bloom
	HPLC	High-performance liquid chromatography
	HR	Helgoland Roads
	HTS	high throughput sequencing
I	IDOD	Integrated and Dynamical Oceanographic Data management database
	IMERS	Integrated Marine Readings and Samples
	IMIS	Information system of the Flanders Marine Institute
M	MAAs	mycosporine-like amino acids
	MMH	match/mismatch hypothesis

N	N	nitrogen
	NAO	North Atlantic Oscillation
	NAO_w	winter North Atlantic Oscillation
	NCM	non-constititutive mixotrophs
	NGS	Next-Generation Sequencing
	NHT	Northern Hemisphere Temperature anomalies
	NIOZ	Royal Netherlands Institute for Sea Research
	NPP	net primary production
O	OSPAR	Convention for the Protection of the Marine Environment of the North-East Atlantic
P	P	phosphorus
	PAR	Photosynthetically active radiation
	PCA	Principal Component Analysis
	PCD	Programmed cell death
	PCI	Phytoplankton Colour index
	PMPZ	Project Sea
	PMX	permanently mixed area
	PP	primary production
	psu	Practical salinity unit
R	RCP	Repressentative Concentration Pathway
	REPHY	French Observation and Monitoring program for Phytoplankton
	ROFI	Region of Freshwater Influence
	RV	research vessel
	RWS	Rijkswaterstaat
S	SAHFOS	Sir Allister Hardy Foundation for Ocean Science
	SAR	stramenopiles, alveolates and Rhizaria
	SHA	sandwich hybridization assay
	Si	Silicate
	SNCM	non-constititutive mixotrophs that aquire their phototrophic capacity by ingesting specific prey
	SOMLIT	Service d'Observation en Milieu Littoral
	SPM	suspended particulate matter
	SST	sea surface temperature
U	UVR	ultraviolet radiation
	UWWTD	Urban Waste Water Treatment Directive
V	VLIZ	Flanders Marine Institute
W	WFD	EU Water Framework Directive
	WitOMI	Within Outlying Mean Index
	WoRMS	World Register of Marine Species
	WWT	waste water treatment

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General Introduction

1.1. Marine phytoplankton

1.1.1. Importance and general characteristics

Marine ecosystems, covering 71 % of the Earth's surface, are among the most important ecosystems in the world. They play a major role in the global carbon cycle (Behrenfeld et al., 2006; Hays et al., 2005) and function as main storage of global carbon (Falkowski et al., 2000), they play a pivotal role in global nutrient and energy cycles and have important climate regulation functions (e.g. biological carbon pump) (Ducklow et al., 2001; Falkowski and Oliver, 2007). This is for a large part due to the activity of phytoplankton. Even though marine phytoplankton constitutes less than 1 % to the Earth's biomass (Field et al., 1998), it is estimated that phytoplankton is responsible for half of the global yearly net primary production (NPP) (Winder and Sommer, 2012). The diversity of the phytoplankton is important for the functioning of the system, both with respect to which groups are present and how diverse these groups are.

'Plankton' is defined as aquatic 'plants'[1] (phytoplankton) and animals (zooplankton) which are passively driven by ocean currents and which can vary strongly in size (from small bacteria to jellyfish) (Hays et al., 2005). Phytoplankton is generally small, with a size range less than 1 µm to more than 1 mm (Finkel et al., 2007), and can be divided in different size classes, namely pico- (< 2 µm), nano- (2-20 µm), micro- (20-200 µm) and macrophytoplankton (> 200 µm).

Phytoplankton has fast turnover rates, and the phytoplankton biomass in the oceans approximately turns over on the order of one week (Falkowski et al., 1998). Due to their large population size and short generation time, the development of phytoplankton communities is tightly linked to changes in their environment and they are among the first organisms which are responding evolutionary to environmental changes (Hays et al., 2005; Lynch et al., 1991). Factors affecting phytoplankton distribution, their seasonal pattern and community composition are among others oceanic currents, nutrient contents, temperature and salinity patterns, as well as the availability of light and the exposure to chemicals.

Phytoplankton plays a major role in the functioning of the marine food web, which is controlled by complex bottom-up and top-down effects (Suikkanen et al., 2013). Because phytoplankton is capable of doing photosynthesis, it forms the basis of the marine food web and affects all upper trophic levels not only pelagic, but also benthic organisms in the aphotic zone, from copepod

[1] Referring to photosynthetic organisms, many of which do not belong to the plant lineage, but to different eukaryotic lineages, mainly the SAR (stramenopiles, alveolates and Rhizaria) clade (Burki 2014).

herbivores, over zooplankton carnivores, to pelagic fish, marine mammals and birds (Beaugrand, 2009; Turner and Tester 1997; Graf, 1992).

1.1.2. Taxonomic classification

'Phytoplankton' involves photosynthetic protists and cyanobacteria and includes about 25.000 morphologically distinct species within 8 phyla (Falkowski et al., 2004; Litchman and Klausmeier 2008). Taxonomically, protists are divided in different classes spread over different branches of the eukaryotic phylogenetic tree: SAR (stramenopiles, alveolates and Rhizaria; Bacillariophyceae (diatoms), Dinophycea (dinoflagellates) and the golden-brown algae classes Chrysophyceae and Dictyochophyceae) and Archeaplastida (the green algae classes Chlorophyceae, Prasinophyceae, Trebouxiophyceae and Ulvophyceae) (Burki 2014) (Figure 1). Prymnesiophytes and Cryptophycea (cryptophytes) have not been yet assigned to any of the supergroups (building blocks of the tree) (Burki 2014). In the past, it has been reported that three phytoplankton groups are dominating the modern oceans, viz. diatoms, dinoflagellates and haptophytes (Falkowski et al., 2004). Recent genomic studies have shown that these three groups have also the highest diversity (de Vargas et al., 2015).

1.1.2.1. Picophytoplankton

As in former times only prokaryotes were included in the picoplankton size class and microbial eukaryotes (protists) were included in the nanoplankton size class, only little is known about the contribution to the marine phytoplankton biomass of small sized phytoplankton groups, such as cyanobacteria and green algae of the picoplankton size class and it was assumed that picoeukaryotes are quantitative negligible in the ocean (Massana, 2011). Then, eukaryotic picoplankton cells were quantified by epifluorescence microscopy and flow cytometry (Massana, 2011). Eukaryotic picoplankton are today known to be ubiquitous in surface oceans and are populating surface oceans at abundances of 10^2 to 10^4 cells mL^{-1} (up to 10^5 cells mL^{-1} in coastal and nutrient-rich regions) (Massana, 2011). They are the most abundant eukaryotes in the sea (Massana, 2011). It is suggested that picoplankton contributes to about one third of the global phytoplankton biomass (Buitenhuis et al., 2012; Quere et al., 2005). It is estimated that picocyanobacteria (such as *Synechococcus* and *Prochlorococcus*) are responsible for about 25 % of the marine NPP and all picoplankton for about 60 % to 80 % and for about 80 % to 90 % of the phytoplankton biomass in the open sea (~10 % in most productive systems) (Flombaum et al. 2013; Wei et al., 2018; Massana, 2011) and are therefore important contributors to the algal biomass and NPP (Massana, 2011). Due to their effective resource and light acquisition, picophytoplankton has an advantage over larger phytoplankton cells in nutrient-poor systems

(Massana, 2011). Phototrophic picoeukaryotes (PPE) express a clear annual cycle with higher abundances during winter (study in Blanes Bay, northwestern Mediterranean) (Massana, 2011). PPE are mainly grazed by flagellates, as they are too small to be eaten by copepods (Massana, 2011). Picoplankton does not contribute much to the biological carbon pump, as small cells do not sink passively (Massana, 2011).

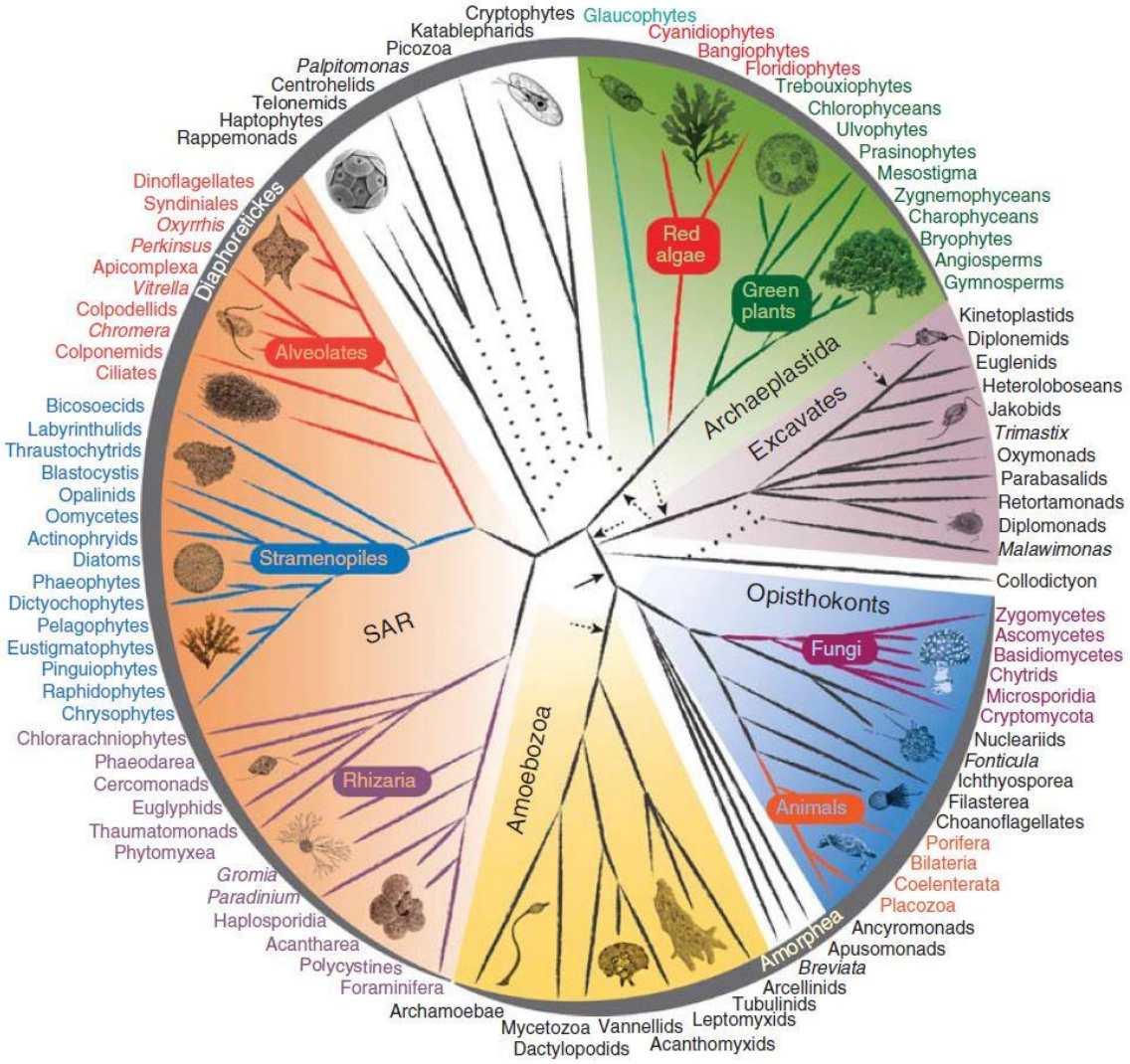

Figure 1 Eukaryotic tree based on phylogenetic evidence and morphological characteristics. The branching pattern does not necessarily represent the inferred relationships between the lineages. Dotted lines denote uncertain relationships, including conflicting positions. The solid branch leading to haptophytes illustrates the strong support for placing haptophytes as sister to SAR (stramenopiles, alveolates and Rhizaria). Taken from Burki (2014).

1.1.3. Trait-based classification approach

1.1.3.1. Physiological characteristics

Traditionally, planktonic protists have been separated in two groups, the 'phytoplankton', which is photoautotrophic, and the 'microzooplankton' being phagotrophic (Mitra et al., 2016). But,

phytoplankton is also able to use different energy sources for growth and can be either auto-, hetero- (feeding on organic carbon) or mixotrophic (heterotrophy + autotrophy) (Hansen, 1991, Jansson et al., 1996). Today it is known that these energy acquisition mechanisms are more complex and mixotrophy plays a more important role than believed earlier (Mitra et al., 2016). Therefore, the traditional classification has been updated and nowadays four energy acquisition types can be distinguished: (i) phagoheterotrophs lacking phototrophic capacity, (ii) photoautotrophs lacking phagotrophic capacity, (iii) constitutive mixotrophs (CMs) as phagotrophs with an inherent capacity for phototrophy, and (iv) non-constitutive mixotrophs (NCMs) that acquire their phototrophic capacity by ingesting specific (SNCM) or general non-specific (GNCM) prey (Mitra et al., 2016). 'Phagoheterotrophs lacking phototrophic capacity' correspond to the common concept of 'microzooplankton' and 'photoautotrophs lacking phagotrophic capacity' correspond to the common concept of 'phytoplankton' (Mitra et al., 2016). CMs represent the concept of mixotrophic unicellular algae and include many eukaryotic phytoplankton groups, almost all phototrophic protists such as diatoms, various prokaryotic groups (e.g. cyanobacteria and bacteria) and eukaryotic prey (e.g. ciliates, dinoflagellates and cryptophytes) (Mitra et al., 2016). The NCMs (about a third of the ciliates) ingest photosynthetic prey and live in symbiosis with these photosynthetic organisms or maintain parts of their prey necessary for photosynthesis (e.g. chloroplasts) and use them for some while (Mitra et al., 2016).

In general, three different nutrient acquisition strategies are distinguished. Species with high nutrient uptake and growth rates, species having high uptake, but low growth rates (storage-adapted species) and species with low nutrient uptake and growth rates (Litchman and Klausmeier, 2008). Species-specific storage-capacities can strengthen the success of a species in the community (Grover, 1991). For example, large phytoplankton cells can rapidly take up newly available nutrients (e.g. through coastal upwelling) and temporarily store them in their vacuoles for later use (Falkowski et al., 1998).

Phytoplankton is able to use the underwater light energy to perform photosynthesis (Litchman and Klausmeier 2008). The availability of light is therefore essential for food webs based on these photosynthetically active organisms (Winder and Sommer, 2012). In the water column, light is vertically attenuated due to the light absorption capacity of water. The presence of dissolved substances and suspended particles, including phytoplankton, may strongly increases the attenuation of light in the water column, resulting in a strong vertical light gradient in aquatic ecosystems (Litchman and Klausmeier, 2008; Reid et al., 1990). Under low light conditions, phytoplankton is able to increase their chlorophyll a content, either by an increase in the size of

the photosynthetic units or their number (Falkowski and Owens, 1980). Photon capture is thus enhanced and light can be used more efficiently (Litchman and Klausmeier, 2008). Phytoplankton can not only capture and use different light intensities, but also light spectra (Litchman and Klausmeier, 2008). Next to the photosynthetic pigment chlorophyll (a, b, c and d), there is a set of other accessory pigments (phycobilins and carotenoids) which are able to capture different parts of the visible light spectrum and different phytoplankton groups express a different set of pigments (Litchman and Klausmeier, 2008).

An increase in stratification will increase the exposure time to photosynthetically active radiation (400-700 nm), but also ultraviolet radiation (UVR) (280-400 nm) (Beardall et al., 2009). UVR can damage cells and cause damage to the DNA, inhibits nutrient uptake and carbon assimilation (Beardall et al., 2009). Under high light conditions some species exhibit photoprotection mechanisms by producing UV-B absorbing substances (UV-screening compounds) such as mycosporine-like amino acids (MAAs), mycosporine-glycone and shinore, which function as a UV-screen, and pigments involved in photoprotection (e.g. zeaxanthin, lutein, alloxanthin, diatoxanthin) to protect themselves from light damage (Beardall et al., 2009, Laviale and Neveux 2011; Marchant et al., 1991; Riegger and Robinson, 1997). Changes in irradiance may therefore affect the ability of some phytoplankton species to compete for nutrients and light (Hegarty and Villareal, 1998), which can alter phytoplankton community composition. However, phytoplankton can cope with changes in irradiance and underwater light field (e.g. caused by cloud passing in front of the sun or vertical mixing between bottom and surface in turbid waters) with rapid photoacclimation (MacIntyre et al., 2002).

1.1.3.2. Morphological characteristics

Morphological characteristics of phytoplankton like cell size, shape and the formation of colonies influence their sinking rate, nutrient uptake and exposure to grazing (Litchman and Klausmeier, 2008). Small phytoplankton species for example have a competitive advantage over larger cells when nutrient levels are low, due to a large surface area/volume ratio (Falkowski et al., 1998). In addition, small phytoplankton species divide more rapidly and have reduced sinking rates, which is an advantage under nutrient limited conditions (Winder and Sommer, 2012). On the other hand, small cells are more prone to grazing (Thingstad et al., 2005).

1.1.3.3. Behavioral characteristics

The ability to swim/float allows some phytoplankton to move from an environment with undesirable conditions to a more favorable one. For example, by swimming in the direction of or

away from high light conditions, they can either improve their light capture capacity or protect themselves from excessive light conditions (Litchman and Klausmeier, 2008).

1.1.3.4. Life history types

Phytoplankton dynamics are not completely externally driven by factors such as light, nutrient availability or grazing (D'Alelio et al., 2010; Eilertsen and Wyatt, 2000). Species can regulate their population dynamics by adopting different life cycle strategies (D'Alelio et al., 2010). Four phases can be distinguished: growth, sex, quiescence and cell death (von Dassow and Montresor, 2010). During the vegetative growth phase, cells reproduce asexually by mitosis leading to the increase in cell number and biomass (D'Alelio et al., 2010; von Dassow and Montresor, 2010). During the sexual life cycle stage, cells reproduce including meiosis (D'Alelio et al., 2010). Sexual reproduction increases the genetic variation in the population and can be an advantage under changing ecological conditions (Litchman and Klausmeier, 2008; Lynch et al., 1991). 'Quiescence' or 'resting stage' refers to any life cycle stage with a reduced metabolic rate, in which the cell does not divide anymore, but stays viable. This can be for example a spore or a cyst (von Dassow and Montresor, 2010). The last phase, the cell death, can be genetically controlled by programmed cell death (PCD), stimulating an intracellular biochemical reaction at which's end the cell dies (von Dassow and Montresor, 2010).

1.2. Studied phytoplankton groups

As the focus of this thesis mainly lies on three phytoplankton taxa, viz. diatoms (*Chapter 2*, *Chapter 3* and *Chapter 5*), dinoflagellates (*Chapter 2*, *Chapter 3* and *Chapter 5*) and *Phaeocystis* (*Chapter 2* and *Chapter 5*), which are also among the most important once in the Belgian part of the North Sea (BPNS) (Desmit et al., 2015; Lancelot et al., 2009; Muylaert et al., 2006), more information is given on the characteristics of these phytoplankton groups.

1.2.1. Diatoms

Diatoms are single-celled organisms with siliceous cell walls (frustules). They are a non-motile phytoplankton group with a weak buoyancy capacity (O'Neil et al., 2012). Diatom cell size decreases during vegetative cell divisions and a sexual stage is required to overcome cell death (D'Alelio et al., 2010). After cell death, they aggregate in flocks and start to sink. Through this, they contribute significantly to the biological carbon pump[2] (Bopp et al., 2005).

[2] The so-called 'biological pump', a process describing the flux of carbon to the deep ocean, is controlling the carbon flux through the pelagic system via the fixation of inorganic carbon during photosynthesis, its

The annual diatom biomass depends both on the meteorological factors, light and temperature, as well as on the nutrient availability (Gypens et al., 2007). They generally have large cells and high nutrient requirements (Beardall et al., 2009) and dominate marine communities under high nutrient and low-light conditions (Bopp et al., 2005; Litchman and Klausmeier, 2008; Malviya et al., 2016). Diatoms can rapidly take up and store excessive nutrients in their vacuoles for later use (Falkowski et al., 1998) and are therefore also favored under conditions of intensive mixing (Winder and Sommer, 2012). Diatoms are positively stimulated by the increasing availability of silicate (Si), which they need to build up their silica walls (Tréguer and Pondaven, 2000). The depletion of available Si therefore implies an advantage for other phytoplankton species to grow (Humborg et al., 2000). The comparison of three prominent diatom species revealed that the reactions to external forcing such as climatic changes, is quite species specific (Schlüter et al., 2012). Generally, diatoms may grow across a wide range of temperatures, but it is widely accepted that they prefer a cooler and well-mixed environment (Kumaran et al., 2017; Margalef, 1978; Suzuki and Takahashi, 1995; Xie et al., 2015). Warmer temperatures might especially have a negative effect on large-celled diatom species, and generally shift diatom communities to smaller sized species (Lewandowska and Sommer, 2010).

1.2.2. Dinoflagellates

Dinoflagellates are motile algae which can move within the water column to access appropriate nutrient and light conditions (Findlay et al., 2001). They are not only known to be important primary producers, but are also notorious, because many species can produce various (neuro-)toxins which can harm animals (and humans) (Wang, 2008).

Dinoflagellates can easily access low nutrient concentrations (Falkowski and Oliver, 2007; Winder and Sommer, 2012) and have an advantage over diatoms when mixing is weak (Winder and Sommer, 2012). Reduced water column mixing (or stratification) is determined by meteorological processes and result in less nutrient-rich upwelling water and nutrient-depleted conditions in surface waters (Winder and Sommer, 2012). Therefore, dinoflagellates become more abundant under stratified warm water conditions with low nutrient concentrations and react species-specific to water turbulences with reduced growth rates (Berdalet et al., 2007; Gómez and Souissi, 2008; Margalef, 1978; Widdicombe et al., 2010). In addition, many dinoflagellates are mixotrophic (Du Yoo et al., 2009; Günther et al., 2012). In low nutrient conditions phagotrophy (ingestion of

transfer in the food web, physical mixing, as well as transport and gravitational settling (Ducklow et al., 2001).

solid particles) may be an important survival mechanism of heterotrophic dinoflagellates (Legrand et al., 1998).

1.2.3. *Phaeocystis*

The marine Prymnesiophyte *Phaeocystis* has a polymorphic life cycle of solitary cells and gelatinous colonies (Wang et al., 2015). Solitary cells have a diameter of 3 to 10 µm, colonies with cells embedded in mucilaginous matrix can have a diameter up to 3 cm (Rousseau et al., 1994; Wang et al., 2015).

Phaeocystis is commonly considered as a nuisance alga (Blauw et al., 2010). The decline of the bloom is accompanied by the formation of massive foam in the surf zone and on beaches, floating slicks and a bad odour (Blauw et al., 2010; Seuront et al., 2006), which might negatively affect tourism and recreation (Blauw et al., 2010; Cadée and Hegeman, 2002). *Phaeocystis* can also inhibit the copepod's egg production during spring, which strongly affects the following copepod generations (Daro et al., 2006). In addition, the trophic efficiency of the food web is negatively correlated to *Phaeocystis* (Lancelot et al., 2009), as small copepods do not graze on *Phaeocystis* colonies (Breton et al., 1999; Gasparini et al., 2000; Rousseau et al., 1990). Finally, dense *Phaeocystis* blooms can lead to net-clogging and can even harm fish, mussels and benthic invertebrates (a.o. through anoxia and clogging of feeding apparatus) (Chang, 1983; Peperzak and Poelman, 2008; Pieters et al., 1980; Rogers and Lockwood, 2009; Seuront et al., 2006).

Various hypotheses exist about the relationship of *Phaeocystis* with its environment. In general, *Phaeocystis* occurs in spring after silica has been depleted and the reduced silica pool limits diatoms (Borkman et al., 2016; Lancelot et al., 1987; Peeters et al., 1991; Reid et al., 1990). But also a negative correlation of *Phaeocystis* to a reduced light availability and higher turbidity and suspended particulate matter (SPM) concentration have been discussed (Gómez and Souissi, 2008; Karasiewicz, 2017; Peperzak et al., 1998). Furthermore, it has been shown that *Phaeocystis* is positively correlated with sea surface temperature (SST) in the preceding months (Borkman et al., 2016; Gieskes and Kraay, 1977; Gómez and Souissi, 2008). Also eutrophication, for example a positive correlation with a high NO_3^- and PO_4^{3-} concentration in February, and nutrient ratios such as an excess of N and P over Si, seem to influence the abundance of *Phaeocystis* (Borkman et al., 2016; Cadée and Hegeman, 1986; Gómez and Souissi, 2008; Lancelot et al., 1987; Lancelot et al., 2007; Muylaert et al., 2006; Riegman et al., 1992). Other studies have shown that low concentrations of P give an advantage for *Phaeocystis* over diatoms, due to their lower P demand, their P-storage capacity and the ability of *Phaeocystis* cells to grow on inorganic P sources (Karasiewicz et al., 2018; Burson et al., 2016). Also large-scale climate patterns such as the NAO

index have been considered to affect *Phaeocystis*. In the BPNS and the English Channel, a negative correlation of the *Phaeocystis* spring bloom magnitude and their dominance over diatoms with the winter NAO index has been identified (Breton et al., 2006; Irigoien, 2000; Seuront and Souissi, 2002). Recently, a study from the French English Channel coasts suggests that a combination of the winter environmental conditions and the pre-*Phaeocystis* bloom diatom community composition is the major factor being responsible for the *Phaeocystis* bloom development (Karasiewicz et al., 2018). These hypotheses are presented and discussed in more detail in *Chapter 5* of this thesis.

1.3. Human impacts on phytoplankton

Marine ecosystems have been under human-induced pressure for decades. More than 50 % of the world's population lives within 60 km distance to the coast (Smith, 2003). The transformation of marine, brackish and freshwater habitats by humans has steadily intensified (Lotze et al., 2005) and due to a growing world population this stress is predicted to further increase during the current century. Marine coastal ecosystems are influenced and modified by anthropogenic activities (Suikkanen et al., 2013), such as eutrophication through agricultural (e.g. use of fertilizers) and industrial activities (e.g. sewage) (Billen et al., 1985), climate change (Barange et al., 2014), pollution (industrial and pharmaceutical pollutants, oil spills) (Camphuysen, 1998; Gómez et al., 2007; Shahidul Islam and Tanaka, 2004), overharvesting of fish stocks, especially of large predators and ecosystem engineers (e.g. oysters) (Lotze et al., 2005), and structural/geomorphological impacts (harbour infrastructure, exploitation of oil and gas reserves, wind farms, shipping lanes/dredging, etc.) (Bergman, 2000; Breuer et al., 2004; Hay, 1990; Henderson et al., 1999; Reubens et al., 2011; Zviely and Klein, 2003). Marine coastal areas are known for their high biodiversity (Gray, 1997), and the above-mentioned activities can have serious effects on the marine food web including biodiversity loss (Lotze et al., 2005, Worm et al., 2006), and harming of fish (Beaugrand et al., 2003; Cloern, 2001) and other biota (Gray et al., 2002).

1.3.1. Eutrophication

Marine coastal areas are naturally enriched with nutrients by upwelling of deep-water, wind-induced mixing and geological weathering from land run-off and stones within the waterbody itself (Bricker et al., 2008). Besides those natural nutrient sources, nutrient inputs are altered by human activities such as agriculture, urban runoff, inefficient wastewater treatment plants and the consumption of fossil fuels (increasing atmospheric deposition) (Bricker et al., 2008). There is

no commonly valid definition of ‘eutrophication’, but it generally refers to excessive nutrient and organic matter inputs, and its effects on aquatic ecosystems (Andersen, 2006; Nixon, 2009).

Nutrient over-enrichment has been discussed to be responsible for the increase of phytoplankton biomass and primary production, a longer bloom duration and changes in the seasonal phytoplankton dynamics (Boesch, 2002; Cloern, 2001; Edwards, 2001). In addition, eutrophication can lead to dissolved oxygen depletion (hypoxia and anoxia), biodiversity loss, habitat destruction (e.g. worldwide increase of dead zones) and increases in the frequency of harmful algae blooms (HABs) (Hoagland et al., 2002; Howarth et al., 2011; Diaz and Rosenberg, 2008). For example, increasing algal blooms through eutrophication lead to higher decomposition rates and by this may lead to anoxic conditions harming other biota (Lotze et al., 2005). Through coastal hypoxia caused by eutrophication, oceans become even more acid and acidification harms calcified organisms such as mollusks and crustaceans (Howarth et al., 2011). Finally, positive biogeochemical feedback mechanisms strengthen the eutrophication effects and can fuel phytoplankton blooms by e.g. a further increase P supply, through nutrient release from sediments during degradation processes (Carstensen et al., 2007; Howarth et al., 2011).

There is currently much discussion about the hypothesis that eutrophication has a stimulating effect on the marine phytoplankton biomass (Glibert, 2017; Gowens et al., 2012). The review by Gowens et al. (2012) discusses this hypothesis using the example of HABs. They show that the correlation of the increasing frequency of HABs with eutrophication is less clear than it is often assumed and that other factors such as climate change might play an important role.

1.3.2. Climate change

It has been shown that phytoplankton is sensitive to climate change (Reid et al., 1998). Some studies even suggest that SST is the most important factor shaping marine plankton community composition (Rühland et al., 2008; Suikkanen et al., 2013; Sunagawa et al., 2015). It is even hypothesized that climate is the main force driving long-term phytoplankton changes (Edwards, 2001). As a result, the influence of rising temperatures on terrestrial, marine and freshwater communities is an intensive field of ecological research (Bowler et al., 2017; Wiltshire and Manly, 2004).

Generally, different phytoplankton species can have very different optimal growth temperature ranges, which can even differ within the same phytoplankton group (Beardall et al., 2009). In many studies total phytoplankton biomass has been shown to exhibit a positive correlation with rising temperatures (Suikkanen et al., 2013), but also declines in biomass have been demonstrated

(Boyce et al., 2010; Lewandowska and Sommer, 2010). One explanation for these antagonistic results can be indirect interactions within complex food webs. Warming SST are supposed to alter marine food web structure and weaken the biological carbon pump due to the reduction of the biological drawdown of dissolved inorganic carbon at higher temperatures and the resulting reduced organic carbon sinking rates (O'Connor et al., 2009; Wohlers et al., 2009). They can also positively stimulate heterotrophic plankton and as such can intensify top-down control (Winder and Sommer, 2012). Experiments have shown that increasing temperatures stimulate the role of consumers in the food web and can lead to an overall reduction in total biomass unless increases in primary production are pronounced (O'Connor et al., 2009). In addition, the by humans to the atmosphere released CO_2 dissolves in the oceans, lowers the seawater pH and leads to more acid water conditions, which also effects phytoplankton community structure, as different species are favored under different pH conditions, and the functioning of the entire ecosystem (e.g. through altered bacteria and zooplankton activity) (Hays et al., 2005).

It has been shown that the onset of the bloom is correlated to temperature conditions and that the timing of the spring bloom is advanced by warming (Kromkamp and Van Engeland, 2009; Lewandowska and Sommer, 2010). As a result, the increasing temperatures caused by climate change may lead to trophic mismatches (e.g. temporal decoupling of the fish larvae production and the phytoplankton production, which serves as a food source) (Edwards and Richardson, 2004). Another possible result is a change in phytoplankton community composition due to an increase in marine stratification caused by the temperature increase (Winder and Sommer, 2012). Even human health can be threatened by climate related food web changes, as the increasing frequency of HAB events (e.g. of toxin producing species) can be related to increasing temperatures and stratification (Masó and Garces, 2006).

1.3.3. Food web changes

1.3.3.1. Overfishing

Overfishing may lead to a decline in fish biomass and recruitment (e.g. overexploitation of the Atlantic cod in the North Sea) (Beaugrand et al., 2003; Cook et al., 1997). The reduction of large predators has resulted in the homogenization and simplification of the marine food web (Lotze et al., 2005). It has been shown that anthropogenic disturbances like overfishing (in combination with climate change) cascades down the food web (top-down control) and can change the primary producer community structure (Casini et al., 2008; Jackson et al., 2001).

1.3.3.2. Introduction of invasive species

Finally, the introduction of invasive species by shipping (e.g. ballast water) can also lead to changes in the community structures through competition (Hay, 1990; Kerckhof et al., 2007). Shipping and aquaculture are the main introduction vectors (Gollasch, 2002; Kerckhof et al., 2007). For example, diatoms and dinoflagellates can be introduced in foreign ecosystems when ships' ballast water is discharged (Hallegraeff and Bolch, 1992). Introduced species can then outcompete indigenous phytoplankton species. For example, the increasing dominance of the non-indigenous diatom *Mediopyxis helysia*, which was first observed in the German Wadden Sea in 2009, changes the phytoplankton community composition and strongly diminishes the phytoplankton species richness (Meier et al., 2015). Laboratory experiments show that the growth of dinoflagellates and chlorophytes is suppressed by the presence of *M. helysia*, while the growth of diatoms was positively stimulated (Meier et al., 2015).

All these human-induced changes can result in rising economic effects through increasing expenses for public health, financial losses in commercial fisheries, recreation and tourism (Hoagland et al., 2002).

1.4. Impact of natural phenomena on phytoplankton

Also large-scale hydro-climatic cycles deriving from atmospheric pressure differences like the AMO (Atlantic Multidecadal Oscillation), the winter NAO (North Atlantic Oscillation), EA (East Atlantic) pattern or NHT (Northern Hemisphere Temperature) anomalies, influence the marine phytoplankton (Edwards, 2001; Edwards et al., 2006; Goberville et al., 2014). For example, meteorological conditions in the North Atlantic and Europe are strongly dependent on the NAO, quantified by the NAO index which reflects pressure differences between the Azores High and the Icelandic Low (Ruddick and Lacroix, 2006) (see *1.5.2.2.*).

1.5. Study area: the Belgian Part of the North Sea (BPNS)

The BPNS is an area of pronounced temporal and spatial natural variability regarding climate, freshwater input, nutrient concentration and salinity. As the variation in temperature, light and nutrient state drive the seasonal phytoplankton cycle, phytoplankton in the BPNS is strongly influenced by natural and anthropogenic caused variation.

1.5.1. Physical and chemical characteristics of the BPNS

The Belgian coastline is about 65 km long, extends about 87 km offshore and has a surface area of approximately 3,600 km² (Ruddick and Lacroix, 2006). The BPNS is situated in the Southern Bight of the North Sea and has an average depth of 20 m (maximum depth 30 to 40 m) (De Galan

et al., 2004; Ruddick and Lacroix, 2006). The bathymetry of the seafloor is characterized by a series of sandbanks, called the Flemish Banks, which are orientated more or less parallel to the coastline and can reach heights up to 20 m from the bottom of the seafloor (Figure 2) (Ruddick and Lacroix, 2006; Begische Staat, 2012). Sand and added clay, silt and gravel are the main substrates of the sea bottom (Belgische Staat, 2012).

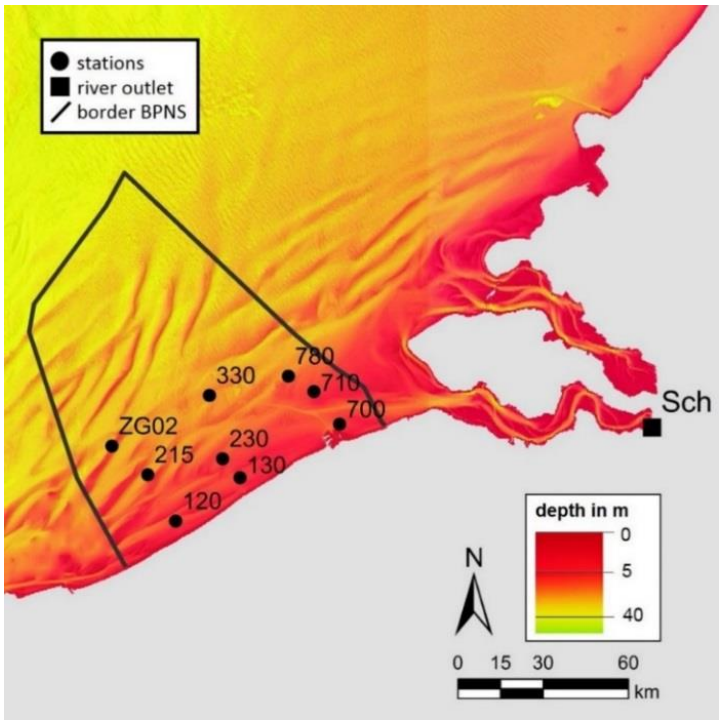

Figure 2 Bathymetry (source: EMODnet) of the BPNS and location of the marine observation stations sampled during the Flemish marine LifeWatch[3] campaigns. BPNS: Belgian part of the North Sea, Sch: Scheldt river outlet.

The BPNS is a region with strong tidal currents of often more than 1 m s^{-1}, which cause daily tides that vary from 3 m at neap tide to over 4.5 m at spring tide (Ruddick and Lacroix, 2006; Belgische Staat, 2012). Near the coast tidal currents occur parallel to the coast (Figure 3) (Belgische Staat, 2012). Sandbanks create tide channels that differ at ebb and flow (Belgische Staat, 2012). In addition, hydrodynamics are influenced by wind and storms that can cause water level rise of several meters, change water transport, salinity, nutrients and SPM concentration (Belgische Staat, 2012). Residual currents, winds and tides result in a residence time defined as 'the average time needed by a water mass within a domain to leave that domain for the first time', which lies between 0 and 7 days (Belgische Staat, 2012). Due to its shallowness and strong currents, most of the area is permanently mixed except for the north-eastern part which can be intermittently stratified (Figure 4) (Belgische Staat, 2012; van Leeuwen et al., 2015; Capuzzo et al., 2018; Capuzzo et al., 2015).

[3] European infrastructure for biodiversity research coordinated by the Flanders Marine Institute (VLIZ)

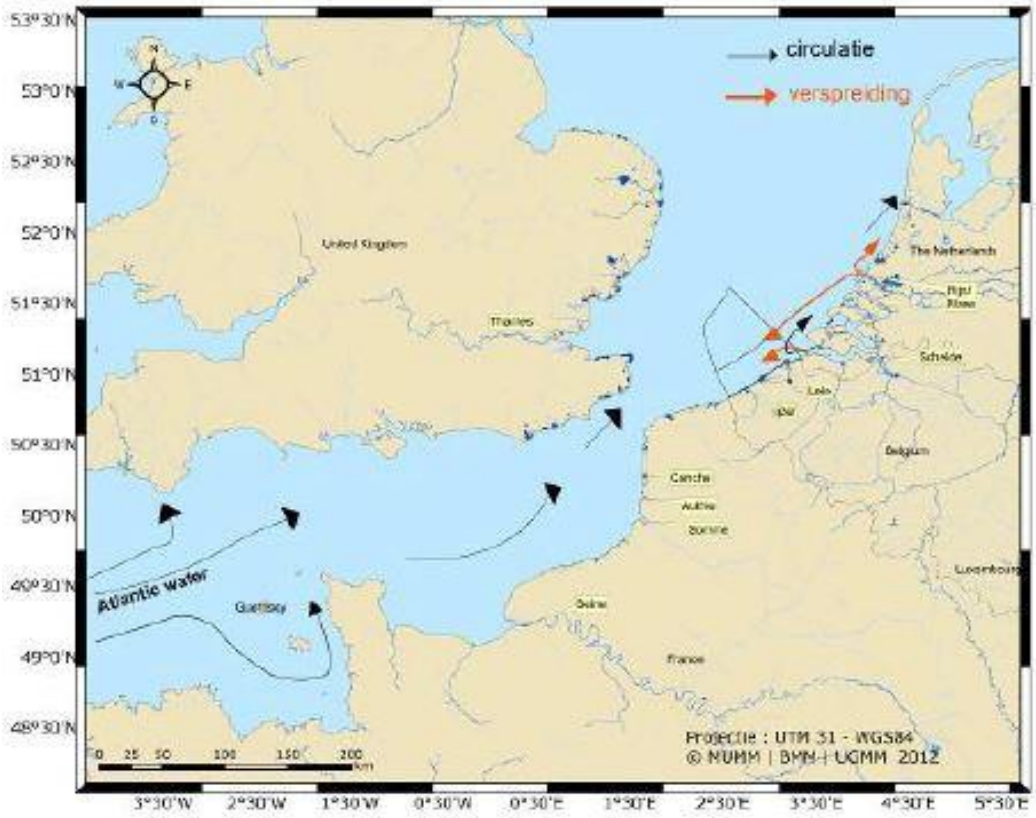

Figure 3 General water mass circulations in the English Channel and the southern part of the North Sea. Mean annual residual currents are indicated as black arrows. Horizontal dispersion caused by the tide and the Scheldt and Rhine/Meuse water mass transport are indicated as red arrows. Source: Belgische Staat (2012).

The water flowing into the Southern Bight of the North Sea via the English Channel makes up the biggest part of the water masses in the BPNS (about 95 % in areas far from the coast) (Figure 3) (Lacroix et al., 2004; Ruddick and Lacroix, 2006; Belgische Staat, 2012). The Atlantic Ocean supplies the BPNS with nutrient-rich waters (Figure 3), which also receives nutrients via freshwater input from the rivers Seine (mean annual discharge: 479.3 $m^3 s^{-1}$), Somme (mean annual discharge: 35.6 $m^3 s^{-1}$), Rhine (mean annual discharge: 1420.6 $m^3 s^{-1}$), Meuse (mean annual discharge 721.5 $m^3 s^{-1}$) and Scheldt (mean annual discharge: 132.8 $m^3 s^{-1}$) into the Southern Bight of the North Sea (Lacroix et al., 2004; for more more information on mean annual discharge see Table 1 in *Chapter 4*). Freshwater masses in the central BPNS are dominated by the Rhine river (Lacroix et al., 2004). The Scheldt water fraction is generally smaller, except during February to March (Lacroix et al., 2004). Due to strong westerly-winds, the Rhine and Scheldt water contribution in the central BPNS, are smallest during winter (Lacroix et al., 2004). Interannual variation in the river nutrient loads originates both from the upstream river nutrient concentrations, as well as from fluctuations in the river discharge (Gypens et al., 2007). Due to variability in discharge, nutrient loads to the sea vary significantly within the seasonal cycle (Gypens et al., 2007).

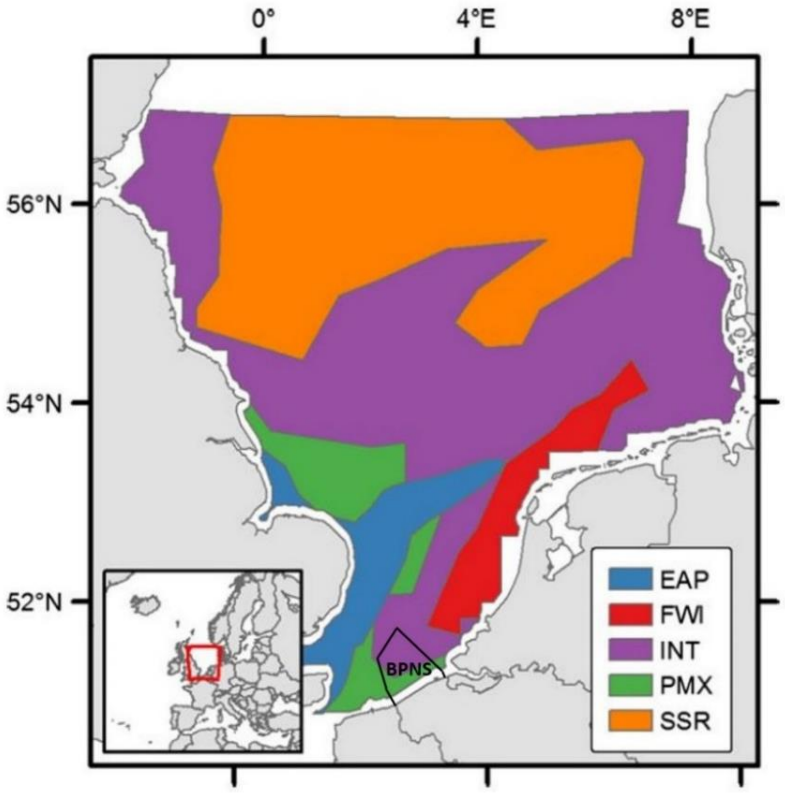

Figure 4 Hydrodynamic regions in the North Sea. The five regions were identified based on the length of mixing and stratification period (see also van Leeuwen et al., 2015). EAP: East Anglia Plume, FWI: freshwater influence, INT: intermittently stratified, PMX: permanently mixed, SSR: seasonally stratified. The BPNS is indicated. Modified from Capuzzo et al. (2015).

The freshwater input from the Scheldt estuary results in a strong on- to offshore salinity gradient (Figure 5) (De Galan et al., 2004). Areas close to the Scheldt estuary and the coast have a mean salinity of 31.6 psu (26.6-32.9 psu), while the offshore area has salinities of 34.2 psu (33.0-35.2 psu) on average (De Galan et al., 2004). Typical seawater temperatures in the BPNS in winter fluctuate between 1.2 and 6.6 °C, while summer maxima vary between 18.6 and 21.9 °C (Gypens et al., 2007). Summer photosynthetically active radiation (PAR) maxima typically range from 1100 to 1340 µmol quanta m^{-2} s^{-1} at the surface (Gypens et al., 2007).

The south-west of the North Sea is described as an area with high SPM concentrations and turbidity (Capuzzo et al., 2018; Fettweis et al., 2012; Gohin, 2011). In the BPNS, SPM is by far the first factor responsible for turbidity. The large tidal energy induces high water velocities during ebbs and flows and the shear-stress to the bottom causes a resuspension of sediments into the shallow water column. During the slacks, the SPM sinks to the bottom and flocculation accelerates this sedimentation (Fettweis et al., 2014). Therefore, the turbidity is influenced by the tidal cycle on the short time scale (Fettweis et al., 2014). It is also influenced by wind and storms and shows a seasonal pattern with higher turbidity in winter than in summer (Fettweis et al., 2014). Current studies hypothesize the influence of plankton and bacteria on flocculation through Transport

Extracellular Polymer (TEP) production, with feedback mechanisms between plankton and water clarity (Fettweis et al., 2014; Fettweis and Baeye, 2015; Shen et al., 2018).

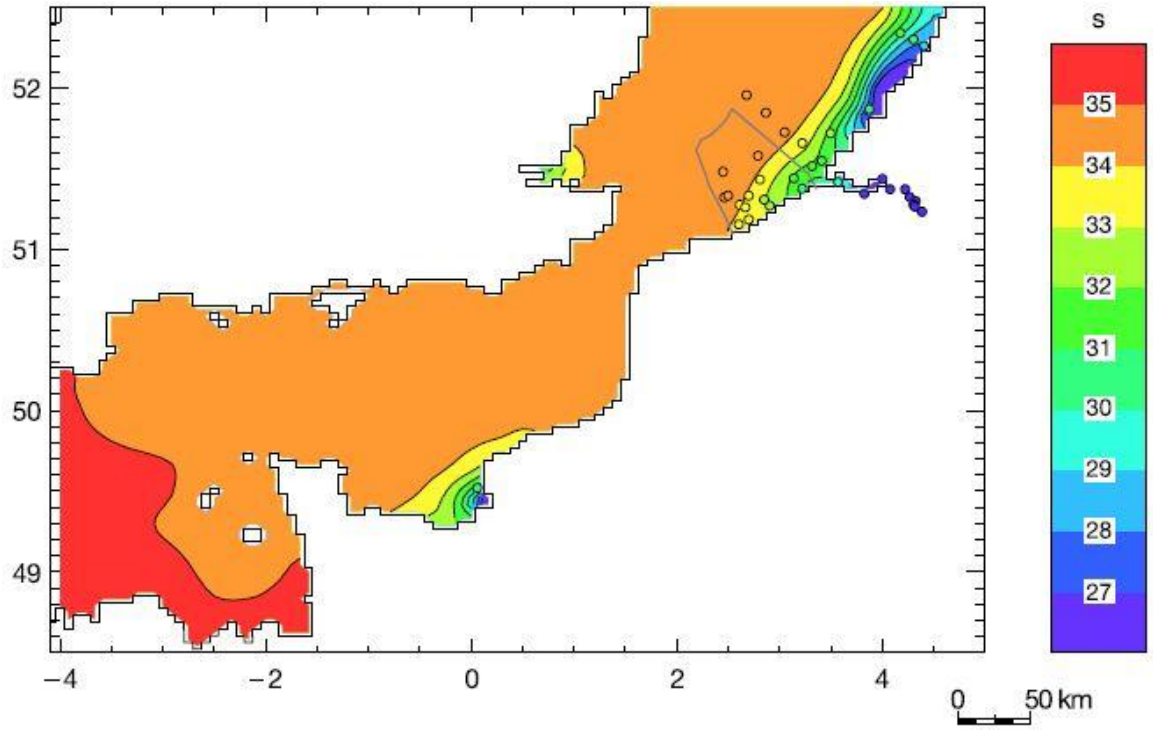

Figure 5 Average surface salinity in the period 1993-2002. Taken from Lacroix et al. (2004).

1.5.2. Human impacts in the BPNS

The BPNS is a coastal area which has been very heavily impacted by human activities during the last decades.

1.5.2.1. Direct impacts

The Scheldt watershed is an intensively anthropogenically influenced area. About 39 % of its surface is in agricultural use and 25 % of its area is urbanized (Passy et al., 2013). Through diffuse (e.g. land runoff, atmospheric deposition, precipitation) and point nutrient sources (e.g. sewage treatment plants, industrial waste water treatment (WWT) facilities) inland surface waters get charged with high nutrient loads. Via river discharge this polluted water reaches the Scheldt estuary and finally ends up in the BPNS.

After the eutrophication period which lasted until the end of the 1980s/beginning of the 1990s, a 'de-eutrophication' period started with the decrease of phosphorus (P) input to the North Sea due to the ban of polyphosphates from washing powder and more efficient P removal from domestic and industrial waste (Burson et al., 2016). Also N input to the North Sea was decreased by the improved waste water management (Burson et al., 2016). However, the reduction of N was less successful than the reduction of P, due the still remaining intense use of agricultural fertilizers

(Burson et al., 2016). The Si cycle on the other hand is more indirectly impacted by anthropogenic activities (Desmit et al., 2018). The Si biogeochemical cycle in the Scheldt is controlled by diatom Si uptake, Si retention and sedimentation (Brion et al., 2006; Desmit et al., 2018). Since the de-eutrophication the concentration of dissolved Si in rivers has increased, probably due to a reduced retention of Si by upstream diatom production (Prins et al., 2012). This resulted in an elevated Si input to the coastal North Sea (Prins et al., 2012). This unequal change in nutrient input (P, N and Si) has led to the alteration of nutrient ratios (e.g. increase in N:P ratio) (Burson et al., 2016). As different phytoplankton groups have different nutrient requirements these changes had marked consequences for the phytoplankton community structure (Burson et al., 2016).

Also the construction of offshore wind farms and dredging activities may have increased the turbidity in the BPNS. Dredging activities increase the concentration of (SPM) in coastal waters, which is the main factor controlling water column turbidity for example in the Dutch Wadden Sea (Philippart et al., 2013).

1.5.2.2. Indirect impacts and natural variability

Climate change affects environmental conditions. It directly influences the water temperature, and indirectly increases stratification and light availability due to reduced mixing and less SPM concentration (Winder and Sommer, 2012). During the past century North Sea temperatures have increased about 0.6 °C (other studies even mention an increase of 1-2 °C) with the strongest increase happening during the last 50 years (Baudron et al., 2014; Beardall et al., 2009; Beaugrand, 2009; Wiltshire et al., 2010; Wiltshire and Manly, 2004). Since 1988, the North Sea has higher temperatures in the first half of the year compared to the previous three decades (O'Brien et al., 2000).

Also large-scale climate patterns have an indirect impact on the phytoplankton community in the BPNS. For example, the NAO index, which quantifies the air pressure differences between the Azores High and the Icelandic Low, affects precipitation, wind, clouds and air temperature in the BPNS region (Ruddick and Lacroix, 2006). High NAO indices lead to stronger south-westerly winds, transporting more water from the English Channel to the Southern Bight of the North Sea, resulting in higher water temperature and turbidity, leading to diffuse freshwater nutrient input into the BPNS (due to increased rainfall) (Ruddick and Lacroix, 2006). In addition, the spreading of the Scheldt river plume is influenced by these winds as the river plume is pushed in more eastward direction and is less extended towards the center of the BPNS (Ruddick and Lacroix, 2006). The resulting environmental changes such as the changes in turbidity (and hence light availability), temperature, but also in riverine nutrient input (by change in rainfall and river runoff), have the

potential to affect the phytoplankton community, resulting in e.g. increase in phytoplankton biomass by an increase in higher temperatures (Edwards 2001, Edwards et al., 2006, Goberville et al., 2014).

1.5.3. Biological characteristics of the BPNS

1.5.3.1. Phytoplankton distribution and dynamics

1.5.3.1.1. Temporal distribution

Phenology is the study of annually recurring life cycle events such as the timing of migrations and flowering (Edwards and Richardson, 2004). Just like terrestrial vegetation, marine phytoplankton growth follows a seasonal cycle. Bloom start, timing, maximum and growth rates are useful phytoplankton parameters to evaluate the seasonal phytoplankton succession in the context of climate change and the match/mismatch hypothesis (MMH), which describes the decoupling of the former synchronized seasonal timing of the prey with its predator (Mieruch et al., 2010). Typically, an annually recurring spring bloom is formed, but the timing, peak and composition can vary strongly from year to year (Dakos et al., 2009), and single species can have different bloom patterns (Mieruch et al., 2010).

Changes in the phytoplankton biomass are caused by the difference in the phytoplankton growth rates and losses (e.g. by grazing or dilution) (Behrenfeld and Boss, 2017). A “bloom” is described as a “high concentration of phytoplankton” biomass, but as phytoplankton conditions differ between ecosystems it is not possible to put a discrete value on the biomass maximum itself or the timeframe in which the biomass should increase (e.g. if the biomass peak is achieved within 1 week or even 3 months) (Behrenfeld and Boss, 2017). Bloom development depends on the complex interplay of several factors such as the mixing of the water column, light and nutrient availability, temperature, grazing pressure and the community structure of the grazing and grazed population, but light availability and temperature increase are discussed to be the most important factors kick-starting the spring bloom (Behrenfeld and Boss, 2017; Lohmann and Wiltshire, 2012). Generally, the annual timing of the diatom spring bloom is determined by the preceding environmental conditions (Jerosch et al., 2006).

In the BPNS, the spring biomass peak is the highest, followed by a smaller peak in summer (Gypens et al., 2007). The spring phytoplankton bloom (expressed as chlorophyll a) typically occurs between March to May (Desmit et al., 2015). Typical spring peak chl a values range between 13-21 µg chl a L^{-1}, but in some years maxima of more than 40 µg chl a L^{-1} can be reached (Gypens et

al., 2007). The summer peaks are less intense and have magnitudes of about 5 to 12 µg chl a L^{-1} (Gypens et al., 2007).

The most important phytoplankton groups in the BPNS are diatoms, dinoflagellates and *Phaeocystis* (Desmit et al., 2015; Lancelot et al., 2009; Muylaert et al., 2006; Terseleer et al., 2014). The typical phytoplankton succession and dynamics in the BPNS are as follows (based on 1990s and 2000s data): a spring diatom community is 'followed' by a bloom of *Phaeocystis globosa* (with a strong degree of overlap) and a summer diatom bloom (Figure 6) (Rousseau et al., 2006; Desmit et al., 2015; Gypens et al., 2007; Tungaraza et al., 2003; Rousseau et al., 2013). This diatom-*Phaeocystis*-diatom succession typically occurs between March and July (Gypens et al., 2007). Dinoflagellates reach their biomass peak during summer months. Even though *Phaeocystis* typically constitutes the biggest part to the biomass of the spring phytoplankton bloom (Gypens et al., 2007), there are also a few years in which the spring bloom is dominated by diatoms (Breton et al., 2006).

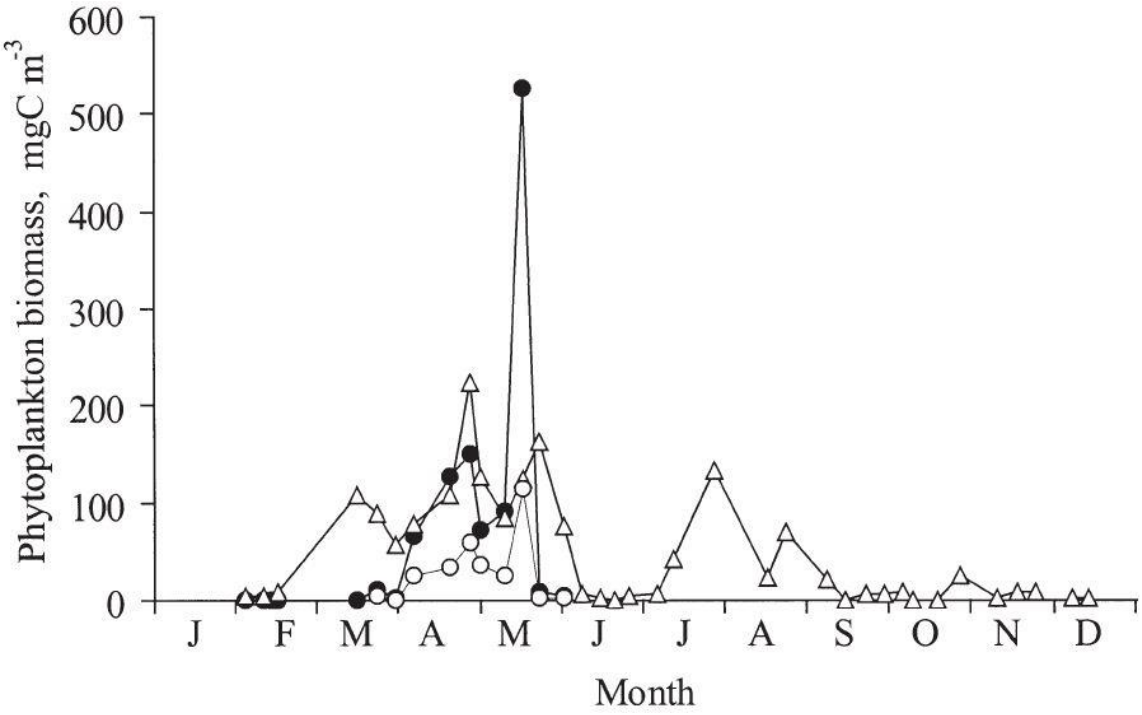

Figure 6 Seasonal development of total phytoplankton biomass (open circles), diatom biomass (triangles) and *Phaeocystis* biomass (filled circles) measured at station 330 in the BPNS (study year 1995). Source: Rousseau et al. (2002).

In the BPNS, three diatom communities can be distinguished (Figure 7), which succeed each other during the course of the year, and are characterized by distinct Si:C ratios (Muylaert et al., 2006; Rousseau et al., 2002; Rousseau et al., 2006). The first community (Community 1, typical winter community) dominating from January and March consists of diatoms with a bentho-pelagic

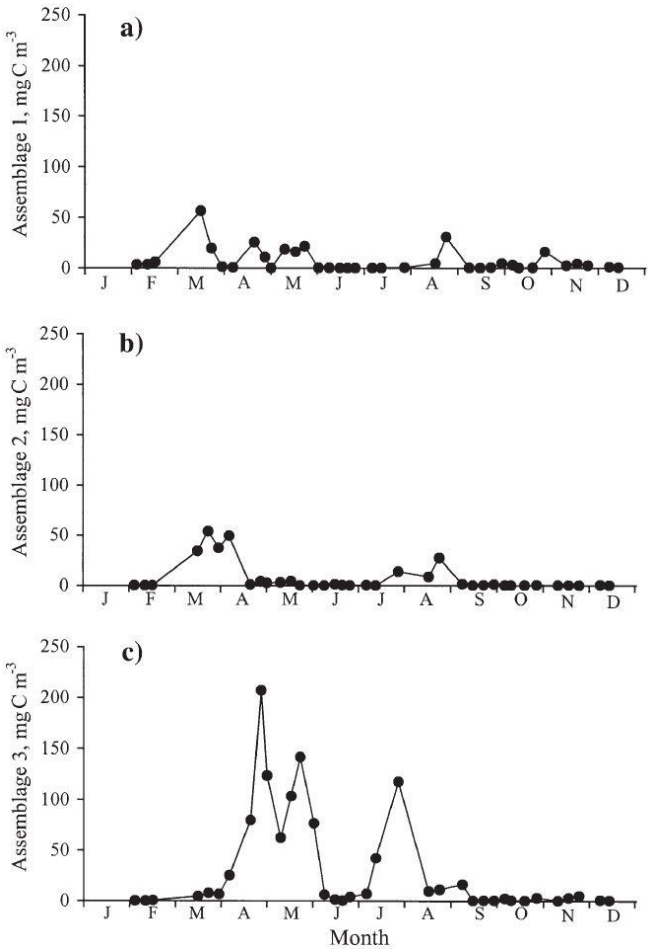

Figure 7 Seasonal development of the three diatom communities based on data of station 330 in the BPNS (study year 1995). a) Community 1, b) community 2 and c) community 3. Source: Rousseau et al. (2002).

lifestyle (*Actinoptychus senarius*, *Paralia sulcata*, *Plagiogrammopsis vanheurckii*, *Rhaphoneis amphiceros, Odontella aurita*) and small pelagic diatoms (*Thalassiosira* spp., *Thalassionema nitzschioides*) (Muylaert et al., 2006). The second community (Community 2) consists mainly of species of the genus *Chaetoceros*, as well as *Lithodesmium undulatum*, *Leptocylindricus danicus* and *Skeletonema costatum* (Muylaert et al., 2006; Rousseau et al., 2002). The last community (Community 3, typical summer community) consists of lightly silicified species, such as *Rhizosolenia* (e.g. *R. hebetata*), *Guinardia* (*G. flaccida*, *G. delicatula*, *G. striata*), *Dactyliosolen fragilissima*) and *Pseudo-nitzschia* spp. and replace community 1 from spring to summer (Muylaert et al., 2006; Rousseau et al., 2002). After community 3, community 2 returns, and after that community 1, which is dominant from July/August to December and the cycle begins again (Muylaert et al., 2006). Community 2 is more of a 'transition community' and is never dominant in the BPNS (Muylaert et al., 2006).

An analyses of the size class distribution in the BPNS by Terseleer (2014) shows that different diatom size classes are present in the BPNS during the course of the year. Small diatoms species (< $6*10^3$ µm³) are the most abundant diatom size class during about the first 100 days of the year and from about Julian day 225 until the end of the year. However, they never constitute the majority to the diatom biomass. Intermediately sized species ($6*10^3$ µm³ – $4.9 * 10^4$ µm³) start to increase early during the year (about Julian day 50) and are dominant in abundance and biomass during from about Julian day 100 until about Julian day 225. Large diatom species (> $4.9 * 10^4$ µm³) start to bloom in summer and make up the biggest part of the diatom biomass from about Julian

day 225 until the middle of the winter (Julian day 50), even though they are never the most abundant size class.

1.5.3.1.2. Spatial distribution

Phytoplankton biomass concentrations typically follow a coast to offshore gradient. For example, in the Dutch part of the North Sea a strong decreasing biomass trend from coast to offshore is observed, in which dinoflagellates only make up a small part in stations near the coast, compared to diatoms or flagellates, whereas the offshore stations are mainly dominated by dinoflagellates (Baretta-Bekker et al., 2009). In the German Bight higher proportions of small phytoplankton species were observed in more offshore areas in relation to coastal areas (Wollschläger et al., 2015). Also in the BPNS, spatial variations in the biomass distribution have been observed. For example, higher chlorophyll a concentrations are detected near the coast than in offshore areas (Rousseau et al., 2006). There are also local differences in the timing of the bloom start. In the BPNS, the bloom starts first in the less turbid south-west waters (around March) and dominates the complete coastal zone in April to May (Muylaert et al., 2006; Rousseau et al., 2006).

1.5.3.2. Seasonal zooplankton distribution

Microzooplankton (including heterotrophic protists such as dinoflagellates and ciliates, but also young metazoan stages such as nauplii, copepodites I – II and pluteus larvae), copepods and the dinoflagellate *Noctiluca scintillans* are the dominant zooplankton groups in the BPNS (Belgische Staat, 2012). Main copepod species are *Temora longicornis*, *Pseudocalanus elongates*, *Centropages hamatus* and *Acartia clausii* (Daro et al., 2012). Small copepods have a moderate biomass density during early spring and become abundant after the *Phaeocystis* peak during spring (Figure 8) (Daro et al., 2006). Copepods are the dominant zooplankton group from May until September (Daro et al., 2006). The copepod summer minimum occurs at the same time as *N. scintillans* reaches it maximum biomass density (June-July), which starts to increase in density after the decline of the *Phaeocystis* peak (Daro et al., 2006). *N. scintillans* feeds on diatoms, *Phaeocystis* aggregates, copepod eggs and various protists (Daro et al., 2012). Microzooplankton biomass is most dense during April and May and actively grazes on free-living *Phaeocystis* cells and heterotrophic nanoflagellates during that time (Daro et al., 2006). During the *Phaeocystis* bloom microzooplankton is an alternative food source for copepods (Daro et al., 2012). The diet of microzooplankton is nanophyto- and nanoprotozooplankton (Daro et al., 2012).

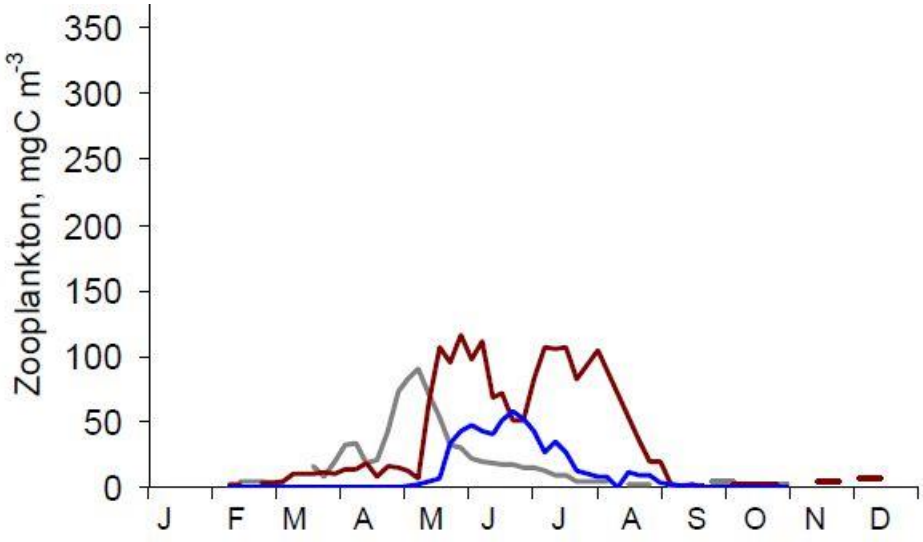

Figure 8 Seasonal biomass distribution of microzooplankton (grey), copepods (brown) and the dinoflagellate *Noctiluca scintillans* (blue) in the central BPNS (station 330) during 1988-2004. Modified from Daro et al. (2006).

1.6. Monitoring phytoplankton in-situ

Phytoplankton can be monitored using different parameters such as cell abundances, biovolumes, carbon biomass, chlorophyll a concentration, fluorescence and pigment composition. The methodologies deliver different datasets (abundances, biovolume, biomass, functional groups, taxonomic community composition) and all have advantages and drawbacks. The available methods for the detection and quantification of phytoplankton are described below.

1.6.1. Microscopic phytoplankton counts, biovolume and carbon content estimation

The conduction of microscopic phytoplankton counts by the use of the Utermöhl method (Utermöhl, 1958), is a traditional method to study the composition of the phytoplankton community. This method uses an inverted microscope to count a sub-sample of phytoplankton cells on a coverslip, settled by means of a settling chamber (Paxinos and Mitchell, 2000). Based on cell size measurements conducted during the microscopic counts, biovolumes can be estimated with the help of scientific literature reporting on geometrical cell shape of the different phytoplankton species (e.g. Hillebrand, 1999). In the literature also biovolume to carbon contents can be found, which help to estimate the carbon biomass of the phytoplankton community (see e.g. Menden-Deuer, 2000).

An advantage of light microscopy is that the resulting dataset has a high taxonomic resolution. On the other hand, microscopic phytoplankton studies are very time consuming and a lot of taxonomic knowledge is required. Also the picoplankton community cannot be quantified, which

might contribute to a significant part to the phytoplankton community (Buitenhuis et al., 2012, Quere et al., 2005). A disadvantage of the biovolume and carbon content estimation (based on size measurement) is that the fixation of the phytoplankton samples (e.g. with Lugol's solution or glutaraldehyde), results in biovolume changes of the phytoplankton cells (Menden-Deuer et al., 2001). For diatoms and dinoflagellates, both shrinkage and swelling of the cells has been observed (Menden-Deuer et al., 2001).

1.6.2. Pigment composition and concentration

Chlorophyll a is accepted as a proxy for phytoplankton biomass and is commonly used to evaluate the ecological state of marine and freshwater environments. Therefore, the determination of the chlorophyll a content is of high interest when it comes to monitoring and management of aquatic ecosystems. There exist different methodologies to quantify chlorophyll a in water samples. In the 1960s, the first accepted spectrophotometric methods were used (Lorenzen, 1967; Strickland and Parsons, 1968). High performance liquid chromatography (HPLC) was introduced later in the 1990s. In contrast to other pigment detection methodologies, HPLC allows to measure various pigments next to chlorophyll a (and chlorophyll b and their degradations products) (e.g. fucoxanthin, chlorophyll *c3*, zeaxanthin, lutein etc.) by means of their retention time. Those pigments serve as biomarkers for different phytoplankton groups. The detected pigment concentrations can be analysed e.g. by CHEMTAX (CHEMical TAXonomy) (Mackey et al., 1996), which allows to quantify the biomass contribution (as fraction of the total measured chlorophyll a concentration) of different phytoplankton groups, based on the theoretical pigment composition per phytoplankton group (see *Chapter 5*).

Spectrophotometric methods deal with determination problems induced through e.g. interference with other pigments. It has been shown that spectrophotometric methods overestimate the chlorophyll a content when compared to HPLC (Dos Santos et al., 2003; Murray et al., 1986; Pinckney et al., 1994; Sartory, 1985). The main advantage of the HPLC method is, that it physically separates the pigments and therefore only measures the real chlorophyll a content. Therefore, HPLC is supposed to be the most accurate technique, as the results are not influenced by interference with other pigments. On the other hand, this method is less affordable when a high number of samples needs to be analyzed (Pinckney et al., 1994), because it is quite time consuming and expensive. That is why HPLC did not fully replace other chlorophyll a determination methods. HPLC should be the method of choice when precise knowledge of the pigment quantity is required (Murray et al., 1986; Pinckney et al., 1994). Generally, phytoplankton methodologies depending on chlorophyll a measurements have the drawback that the chlorophyll a content is

highly variable and depends on environmental conditions (e.g. nutrient deficiency and light regime) and also varies among species (Alvarez-Fernandez and Riegman, 2014; Riemann et al., 1989).

A less widely used pigment parameter is the Phytoplankton Colour index (PCI) measured by a continuous plankton recorder (CPR) maintained by the Sir Allister Hardy Foundation for Ocean Science (SAHFOS). The CPR is towed behind 'ships of opportunity' (Raitsos et al., 2005). Water entering the CPR streams through a filtering silk (mesh size 270 µm), which is then covered by a second layer of silk and finally stored in a tank filled with 4 % formaldehyde (Edwards, 2001). The silk within the CPR is constantly moving and 10 cm silk represents 18.5 km of sailed distance and 3 m^3 of filtered seawater (Edwards, 2001). In the laboratory, the PCI is determined according to a colour chart, where the shading of the green colour serves as an estimate of the phytoplankton biomass (Edwards, 2001).

1.6.3. Flow cytometry

A flow cytometer (FCM) typically measures several parameters among which sideward scatter (SWS) and forward scatter (FWS) and three fluorescence parameters: red (FLR), orange (FLO), and yellow fluorescence (FLY) of the particles flowing through the flow cytometer (Thyssen et al., 2008). Based on these parameters, clusters can be distinguished and assigned to different phytoplankton groups.

One of the benefits of a flow cytometer is that it provides high frequency data with a high temporal and spatial resolution (Thyssen et al., 2008). On the other hand, the taxonomic resolution of the flow cytometric dataset is quite low, as phytoplankton cells are grouped in functional groups based on their morphological criteria (e.g. size and fluorescence) (Thyssen et al., 2008). A huge advantage of this methodology is that samples are measured on site, without perturbation linked to transport, preservation and storage (Thyssen et al., 2008).

1.6.4. Molecular monitoring techniques

Various molecular methodologies are applied in environmental monitoring e.g. traditional Sanger sequencing, high throughput sequencing (HTS) techniques, formerly known as Next-generation sequencing (NGS), such as Tag Pyrosequencing (e.g. Zimmermann et al., 2015; Monchy et al., 2012), sandwich hybridization assay (SHA) (e.g. Tyrrell et al., 2002) or fluorescent in situ hybridization (FISH) (e.g. Greenfield et al., 2008).

With metagenomic[4] technologies, such as HTS, it is possible to process many samples simultaneously, at low effort and costs. HTS techniques deliver many sequences and by this it is likely that also rare species are detected (Aguiar-Pulido et al., 2016; Monchy et al., 2012). In addition, molecular methods allow to analyze the picoeukaryotic phytoplankton communities, something that is not possible with traditional microscopy (Medlin et al., 2006). Therefore, a higher number of taxa are identified compared to light microscopy (Zimmermann et al., 2015). Even though the resulting datasets have a high taxonomic resolution, it is difficult to make any statement about the abundance of the taxa as the number of sequences does not correlate to the number of organisms (Monchy et al., 2012). Finally, molecular methods can be biased by PCR and sequencing errors (Monchy et al., 2012).

1.7. Long-term phytoplankton trends in the North Sea and adjoining areas

Not much is known about the phytoplankton long-term trends in the BPNS, especially before the 1990s and after the 2000s. In order to distinguish natural variability from human-induced changes on phytoplankton composition and dynamics, long-term datasets are needed (Cadée and Hegeman, 2002; Suikkanen et al., 2013). As major biotic changes occur on a timescale of 11 to 45 years or more (Lewis and Allen, 2009), Henson et al. (2010) suggested that time series should have a length of about 40 years in order to distinguish natural variability from the global warming trends.

Even though marine biota have a huge socioeconomic value, there are relatively few long-term biological monitoring programs (Hays et al., 2005), and this is especially the case for phytoplankton as cell counts are very expensive and time-consuming (Wiltshire et al., 2010) (see *1.6.1.*). As a result, long-term phytoplankton monitoring datasets are generally rare or often miss sufficient temporal and/or taxonomic resolution (Wiltshire et al., 2010). In addition, many monitoring campaigns are short-term, because they are financed in the framework of projects with a duration of maximally 3-5 years (Lewis and Allen, 2009).

1.7.1. Datasets

Fortunately, several phytoplankton 'long-term' monitoring programs have been carried out in the North Sea, the English Channel and the Northeast Atlantic Ocean, resulting in an impressive amount of publications (Figure 9, Table 1): the Continuous Plankton Recorder (CPR) dataset for the Northeast Atlantic and the North Sea, the Helgoland Roads time series (HR; Helgoland,

[4] Metagenomics is the 'study of the genomes in a microbial community'. It is used to analyze the taxonomic composition of the microbial community (Aguiar-Pulido et al., 2016).

Germany), the Marsdiep time series (Wadden Sea, The Netherlands), the Rijkswaterstaat (RWS) monitoring (Dutch part of the North Sea), station 330 (BPNS, Belgium), the French Observation and Monitoring program for Phytoplankton (REPHY; Eastern English Channel, France), the French SOMLIT (Service d'Observation en Milieu Littoral; Eastern English Channel, France) program and the Plymouth L4 time series (Western English Channel, UK).

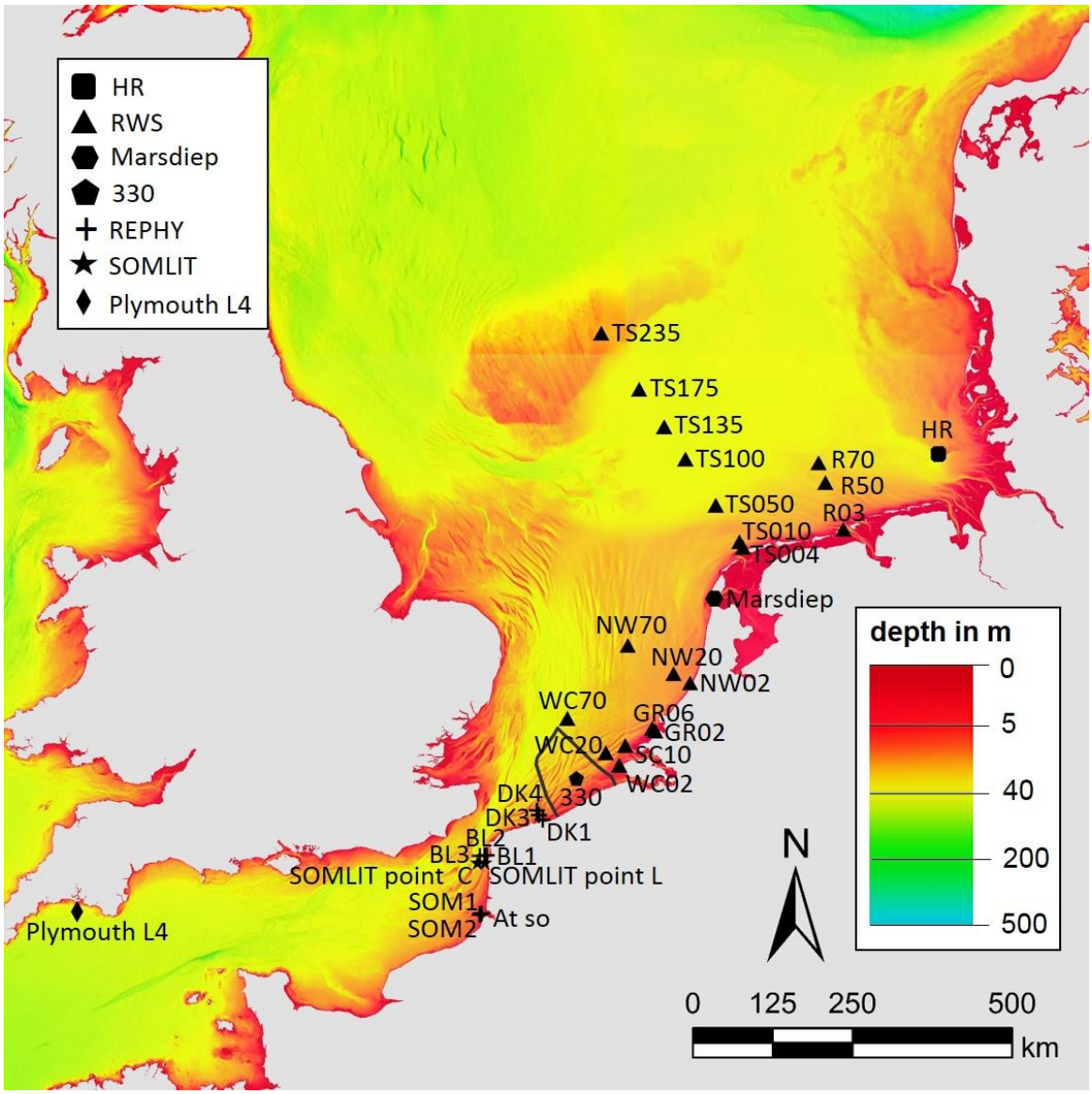

Figure 9 Bathymetry (source: EMODnet) and locations of long-term sampling stations. The Belgian part of the North Sea is indicated as a black line. BL: Boulogne transect, DK: Dunkerque transect, GR: Goeree transect, HR: Helgoland Roads, NW: Noordwijk transect, RWS: RIjkswaterstaat, R: Rottumerplaat transects, REPHY: French Observation and Monitoring program for Phytoplankton and Hydrology in coastal waters, SOM: Bay of Somme transect, SOMLIT: Service d'Observation en Milieu Litoral, TS: Terschelling transect, WC: Walcheren transect.

The CPR is the largest plankton survey of the world and already started as early as the 1930s with PCI measurements (for explanation PCI see chapter 1.6.2.) (Figure 10) (Edwards, 2001; Hays et al., 2005). It systematically covers the Northeast Atlantic Ocean and the North Sea via a complex

network of CPR transects (Figure 11) (Raitsos et al., 2014). Since 1948, phytoplankton counts are conducted as well (Gieskes et al., 2007). Diatoms and dinoflagellates were counted in the same way since 1948, while coccolitophores and silicoflagellates only have been counted since 1993 (before they were only documented as 'present' or 'absent') (Leterme et al., 2005).

Table 1 Non-exhaustive overview of important publications related to long-term phytoplankton monitoring programs

long-term series	area	selection of related articles
CPR	North Atlantic Ocean, North Sea	Gieskes & Kraay (1977), Reid et al. (1998), Edwards et al. (2001), Reid et al. (2003), Drinkwater et al. (2003), Beaugrand (2004), Edwards & Richardson (2004), Raitsos et al. (2005), Leterme et al. (2005), Leterme et al. (2006), Edwards et al. (2006), McQuatters-Gollop et al. (2007), Gieskes et al. (2007), Beaugrand (2009), Hinder et al. (2012), Alvarez-Fernandez et al. (2012), Goberville et al. (2013), Raitsos et al. (2013), Beaugrand et al. (2014), Raitsos et al. (2014), McQuatters-Gollop et al. (2015), Chivers et al. (2017)
Helgoland Roads	German Bight, North Sea	Hickel (1998), Radach et al. (1990), Wiltshire and Dürselen (2004), Wiltshire and Manly (2004), Hoppenrath (2004), Wirtz & Wiltshire (2005), Gieskes et al. (2007), Hoppenrath et al. (2007), Wiltshire et al. (2008), Raabe & Wiltshire (2009), Mieruch et al. (2010), Wiltshire et al. (2010), Günther et al. (2012), Schlüter et al. (2012), Lohmann & Wiltshire (2012)
Marsdiep	Wadden Sea, Netherlands	Cadée & Hegeman (1991), Philippart et al. (2000), Cadée & Hegeman (2002), Philippart et al. (2007), Philippart et al. (2010), Ly et al. (2014)
RWS Monitoring	Dutch part of the North Sea, Netherlands & Westerschelde	Baretta-Bekker et al. (2009), Kromkamp & Van Engeland (2010), Prins et al. (2012), Alvarez-Fernandez & Riegman (2014), Burson et al. (2016)
Station 330	st. 330, Belgian part of the North Sea, Southern Bight	Breton et al. (2006), Rousseau et al. (2002), Lancelot et al. (2009), Terseleer Lillo (2014), Gypens et al. (2007), Desmit et al. (2015)
LifeWatch campaigns	various stations, Belgian part of the North Sea, Southern Bight	Muylaert et al. (2006)
REPHY	Eastern English Channel	Lefebvre et al. (2011), Hernández-Fariñas et al. (2014), Hernández-Fariñas et al. (2015), Karasiewicz et al. (2018)
SOMLIT	Boulogne-sur-Mer, North-eastern English Channel, France	Gómez & Souissi (2007), Gómez & Souissi (2008), Goberville et al. (2010)
Plymouth L4	Western English Channel	Southward et al. (2004), Lewis & Allen (2009), Widdicombe et al. (2010), Smyth et al. (2010), Tarran & Brunn (2015), Xie et al. (2015), Irigoien et al. (2000)

CPR: Continuous Plankton Recorder, RWS: Rijkswaterstaat, REPHY: French Observation and Monitoring program for Phytoplankton and Hydrology in coastal waters, SOMLIT: Service d'Observation en Milieu Litoral.

In Helgoland, an island in the German Bight of the North Sea, a regular daily marine sampling campaign was initiated in 1962 at the 'Cable buoy site', called the 'Helgoland Roads time series' (Figure 9, Figure 10) (Wiltshire and Dürselen, 2004; Wiltshire et al., 2010). Phytoplankton community composition data (size measurements allow the transformation to biovolume and biomass), zooplankton data (since 1974) and a set of abiotic measurements are collected (Figure

10) (Hickel, 1998; Wiltshire and Dürselen, 2004; Wiltshire et al., 2010). As such, it is one of the longest phytoplankton composition time series worldwide (Wiltshire and Dürselen, 2004). The main phytoplankton group observed in Helgoland are diatoms (90 % of the spring bloom consists of diatoms) (Wiltshire et al., 2008).

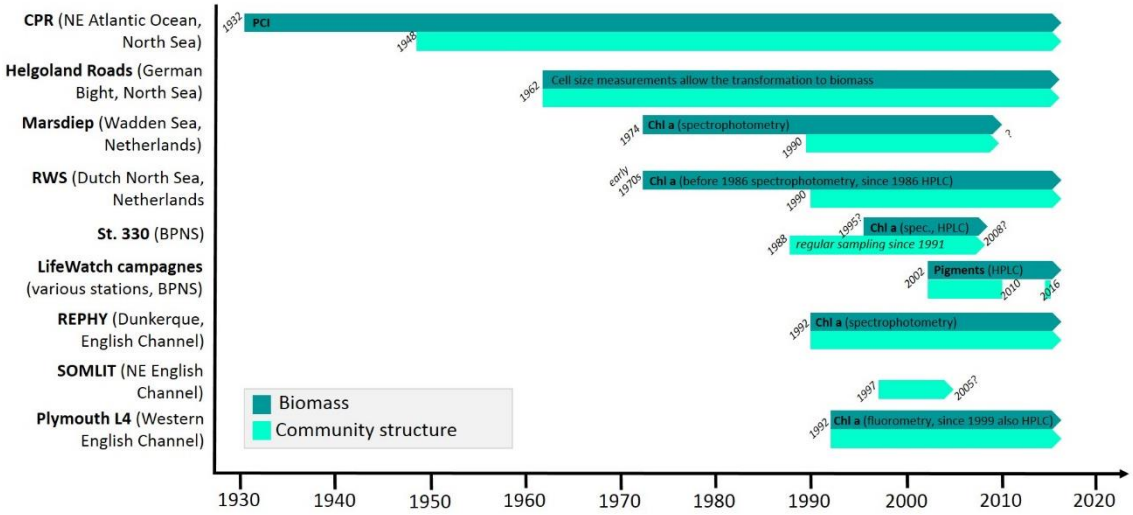

Figure 10 Overview of (long-term) monitoring programs and sampling campaigns in the North Sea, English Channel and NE Atlantic Ocean from the 1930s until now. CPR: Continuous Plankton Recorder, PCI: phytoplankton color index, RWS: Rijkswaterstaat, REPHY: French Observation and Monitoring program for Phytoplankton and Hydrology in coastal waters, SOMLIT: Service d'Observation en Milieu Litoral.

In the Marsdiep tidal inlet (Marsdiep time series, between the Dutch mainland and the island Texel), which connects the North Sea and the Dutch Wadden Sea, long-term chlorophyll a and phytoplankton composition studies were conducted (Figure 9, Figure 10) (Philippart et al., 2007; Philippart et al., 2010). Chlorophyll a sampling was conducted since the early 1970s (phytoplankton composition only since 1990) at high water conditions which in fact means that coastal North Sea water (entering the Wadden Sea) is sampled (Figure 10) (Cadée and Hegeman, 2002). Diatoms and flagellates are the dominant phytoplankton groups in this area (Philippart et al., 2007).

Since the early 1970s, chlorophyll a has been monitored along several transects in the Dutch part of the North Sea (e.g. the Walcheren, Noordwijk, Terschelling and Rottumerplaat transects), as well as at some individual stations (Goeree6 and Marsdiep Noord) (Figure 9, Figure 10) (Baretta-Bekker et al., 2009). In the beginning of the program, 76 marine stations were monitored (Baretta-Bekker et al., 2009). In 1993, this number was reduced to 18 sampling stations (Baretta-Bekker et al., 2009). The sampling frequency was mainly twice a month during summer and monthly during winter (Baretta-Bekker et al., 2009). Since 1990, also the phytoplankton community composition is determined (Figure 10) (Baretta-Bekker et al., 2009). In this area, diatoms, dinoflagellates and *Phaeocystis* are the most important phytoplankton groups (Baretta-Bekker et al., 2009).

In the central BPNS, the phytoplankton community has been monitored since 1988 at station 330 on a weekly basis except during winter and summer, when sampling was only conducted bimonthly or monthly (Figure 9, Figure 10) (Breton et al., 2006; Lancelot et al., 2009). From 1988 to 1991 sampling only took place between February mid-June (Breton et al., 2006). Secondly, within the LifeWatch program, monthly samples are being taken since 2002 to estimate the pigment composition at several stations in the BPNS (Figure 2, Figure 10). In the period from 2003 to 2010 and in 2016, also the phytoplankton community structure has been studied. The main phytoplankton groups in the BPNS are diatoms, dinoflagellates and *Phaeocystis* (Desmit et al., 2015; Lancelot et al., 2009; Muylaert et al., 2006; Rousseau et al., 2006).

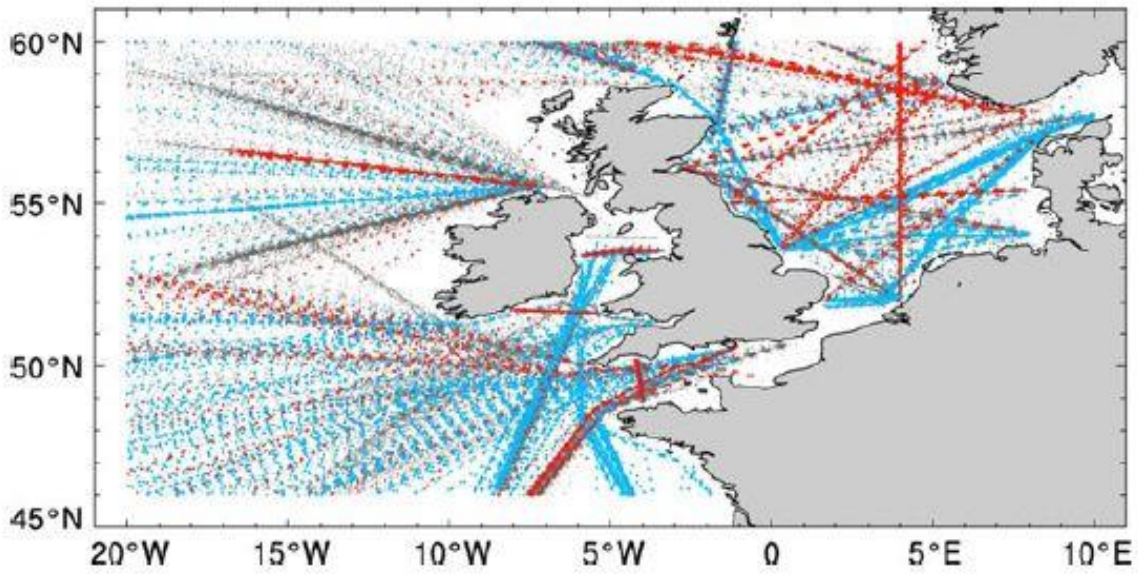

Figure 11 Example of CPR transects in the Northeast Atlantic and the North Sea (red: 1978-1987, blue: 1998-2007). Taken from Raitsos et al. (2014).

Three onshore-offshore transects in the Eastern English Channel (Bay of Somme and Boulogne) and the Southern Bight of the North Sea (Dunkerque) have been monitored since 1992 for phytoplankton community composition and biomass (chlorophyll a) (Figure 9, Figure 10) (Hernández-Fariñas et al., 2014; Lefebvre et al., 2011). Samples are collected on a two-weekly to monthly basis (Hernández-Fariñas et al,. 2014). The species composition in the Eastern English Channel is similar to the species composition observed in the BPNS with diatoms, dinoflagellates and *Phaeocystis* being the most abundant phytoplankton groups (81 % of total abundance) (Hernández-Fariñas et al., 2014; Lefebvre et al., 2011).

From 1997, the French marine monitoring program SOMLIT (1997-2005) monitoring 12 sites along the French Coast (English Channel, Atlantic Ocean and Mediterranean Sea), of which two stations were situated in the turbulent northeastern English Channel (2 km and 8km off Boulogne-sur-

Mer), an area experiencing strong tidal currents and winds (Figure 9, Figure 10) (Goberville et al., 2010; Gómez and Souissi, 2007; Salomon and Breton, 1991).

Station L4 in the western English Channel was originally established to monitor zooplankton, but in 1992 several other physical, chemical and biological parameters were added to the monitoring program (Figure 9, Figure 10) (Lewis and Allen, 2009). Also phytoplankton (counts and chlorophyll a) samples have been taken weekly since 1992 (Widdicombe et al., 2010; Xie et al., 2015). The temperate waters of station L4 are well mixed only during autumn and winter and weakly stratify during the rest of the year (Widdicombe et al., 2010). In the Western English Channel flagellates (cryptophytes and nanoeukaryotes, excluding *Phaeocystis*) dominate the community (87 %), while diatoms, *Phaeocystis*, coccolitophores, dinoflagellates and ciliates together only make up 13 % of the total abundance (1992-2007) (Tarran and Bruun, 2015; Widdicombe et al., 2010). Abundant diatoms in this area are *Guinardaa delicatula*, *Eucampia zodiacus* and *Chaeotoceros socialis* (Xie et al., 2015).

1.7.2. Overview of long-term trends

1.7.2.1. Phytoplankton biomass

In the general North Sea, an overall increase in phytoplankton biomass has been observed between 1948 and 2002, while in the Northeast Atlantic a decrease has been observed (1960-2001; CPR study) (Figure 12; Beaugrand, 2004; Beaugrand, 2009; Edwards, 2001; Edwards et al., 2006). The beginning of the 1970s is characterized by a relatively low biomass in the North Sea (Marsdiep), followed by a strong increase and even a doubling in phytoplankton biomass by the end of the 1970s (Cadée and Hegeman, 2002; Philippart et al., 2000). These findings are partly contradicting with another study (study period 1958-2002) in which an increasing biomass trend in the CPR dataset was found not only in the North Sea, but also in the Northeast Atlantic (Leterme et al., 2005). However, Leterme et al. (2005) analyses the Northeast Atlantic and North Sea dataset together which might have influenced the study results as regional differing trends are no longer considered. Also in the German Bight an increase in total phytoplankton biomass from 1962 to 1984 was found (based on biovolumes), mainly due to the increase in flagellates (Radach et al., 1990). This was confirmed by another study which showed a marked increase of the total phytoplankton biomass (based on biovolumes) by about threefold (1962-1994) in the German Bight, mainly caused by flagellates and not by an increase of diatoms or dinoflagellates (Hickel, 1998). The PCI values in the North Sea (CPR study) after 1998 decreased, especially in autumn and winter months (Alvarez-Fernandez et al., 2012). This decrease in biomass (chlorophyll a) has also

been shown in the Marsdiep time series (after 1994), as a result of the de-eutrophication actions (Cadée and Hegeman, 2002). Also in the Dunkerque transect (Southern Bight of the North Sea) chlorophyll a has decreased since the 1990s, while values increased in the Somme and Boulogne-sur-Mer transect (Eastern English Channel) (Lefebvre et al., 2011).

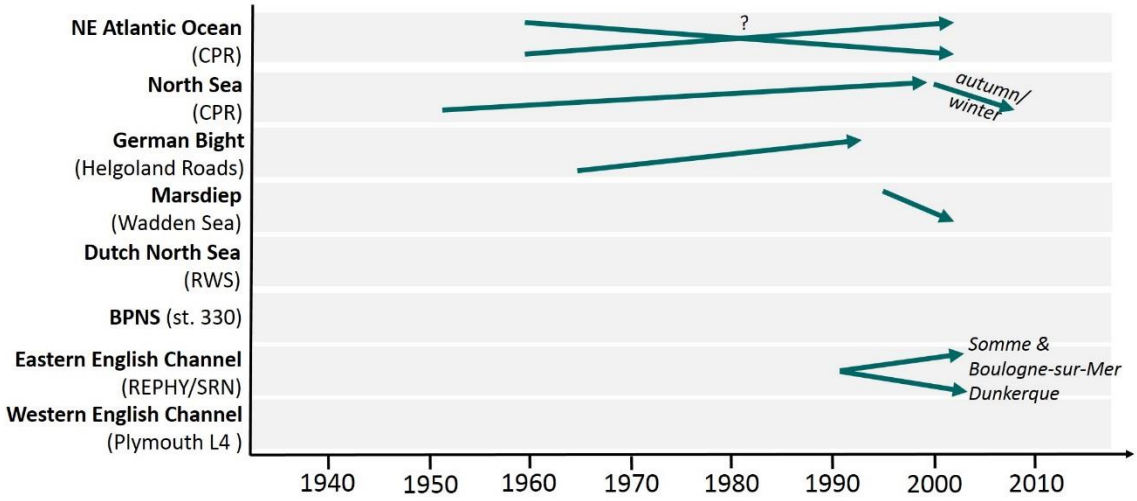

Figure 12 Overview of general (long-term) trends of the total phytoplankton biomass in the North Sea, the English Channel and the North Atlantic Ocean.

1.7.2.2. Diatoms

A CPR study revealed a decrease in diatom abundance in the central North Sea since the 1960s and an increase in winter diatom abundance since the 1990s (Edwards et al., 2006; Figure 13). Another CPR study (study period 1958-2002) also showed a decreasing contribution of diatoms to the biomass in the Northeast Atlantic (Leterme et al., 2005). In HR time series, diatoms decreased slightly from 1962 to 1984 in the German Bight (Radach et al., 1990). Beaugrand et al. (2014) (CPR study) identified a decrease in diatom abundance (1958-1982) especially at the end of the 1960s in the North Sea and two stepwise increases in diatoms at the beginning of the 1980s and the end of the 1990s. A later study on the central North Sea CPR data, has additionally shown an increase of diatom bloom maxima (abundance) after 1999 (Alvarez-Fernandez et al., 2012). In addition, a study of HAB species in the Northeast Atlantic and the North Sea (CPR, 1960-2009) revealed that diatoms did not decrease, and some HAB (e.g. *Pseudo-nitzschia*) and non-HAB (e.g. *Thalassiosira*) taxa even increased in abundance (Hinder et al., 2012). Long-term trends extracted from the Plymouth L4-time series include a decrease in diatom abundance during the period 1992-2007 (Widdicombe et al., 2010). In the Dutch part of the North Sea an increase in diatom biomass (as carbon) was found (period 1991 to 2010) (Alvarez-Fernandez and Riegman, 2014).

In Helgoland, a change in phytoplankton community composition was detected in the period between 1975 and 2005, especially a rise in large and heavily silicified diatom species (e.g. *Paralia*

sulcata) (1975-2005) (Gebühr et al., 2009; Wiltshire et al., 2008). During a study period of 9 years (1997-2005) two warm water species were detected for the first time in the northeastern English Channel, *Eucampia cornuta* and *Chaetoceros peruvianus*, as well as the proliferation of the two diatom species *Proboscia indica and Rhizosolenia hebetate* f. *semispina*, which are known to be more competitive under stratified water conditions (Gómez and Souissi, 2007). It seems that their appearance was associated with the exceptional warm water conditions and the resulting reduction in mixing in 2003 and 2005 (Gómez and Souissi, 2007).

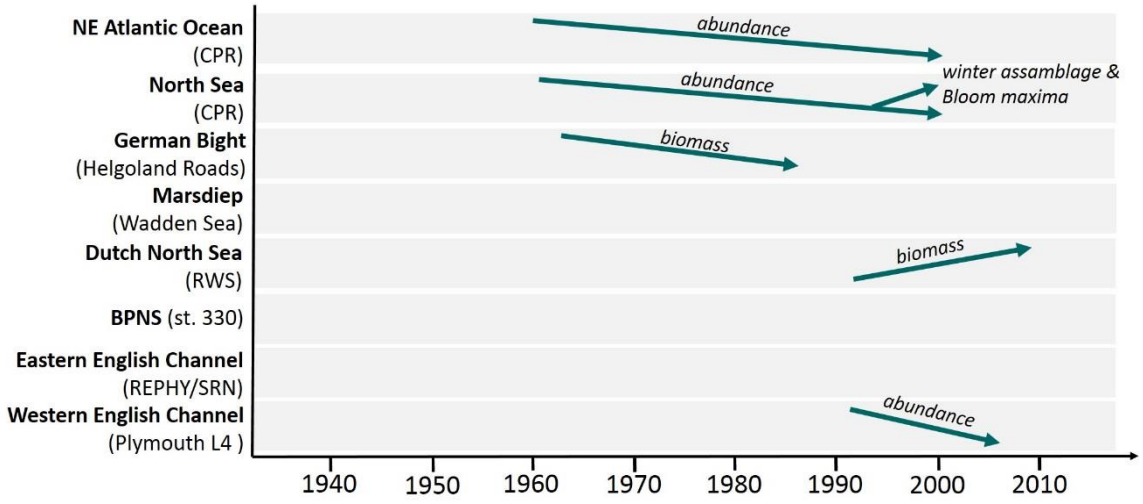

Figure 13 Overview of general (long-term) trends of the total diatom biomass/abundances in the North Sea, the English Channel and the North Atlantic Ocean.

1.7.2.3. Dinoflagellates

A CPR study (study period 1958-2002) found an increasing contribution of dinoflagellates to the phytoplankton biomass in the Northeast Atlantic (Figure 14; Leterme et al., 2005). In the northern part of the North Sea an increase of dinoflagellate abundance has been shown, while in the central and southern part of the North Sea a decrease of dinoflagellate abundance was observed (CPR study) (Beaugrand et al., 2014; Leterme et al., 2005). After the decrease of dinoflagellate abundance until around 2000 in the central North Sea, a steep increase was detected (CPR study) (Beaugrand et al., 2014). However, in a later study about the central North Sea CPR data, a decrease in dinoflagellate abundance from 1990 onwards with a pronounced decrease after 1999 has been observed (Alvarez-Fernandez et al., 2012). Long-term trends extracted from the Plymouth L4-time series are the abundance increase of the dinoflagellate *Prorocentrum minimum* and heterotrophic dinoflagellates in the period 1992-2007 (Widdicombe et al., 2010). In the Dutch part of the North Sea (RWS monitoring, period 1991 to 2010) an increase of dinoflagellate carbon biomass was found (Alvarez-Fernandez and Riegman, 2014). A study of HAB species in the Northeast Atlantic and the North Sea (1960-2009) could reveal that dinoflagellate species,

including HAB taxa (e.g. *Prorocentrum* spp.) and non-HAB taxa (e.g. *Ceratium furca*), have declined in abundance and this especially strong since 2006 (Hinder et al., 2012).

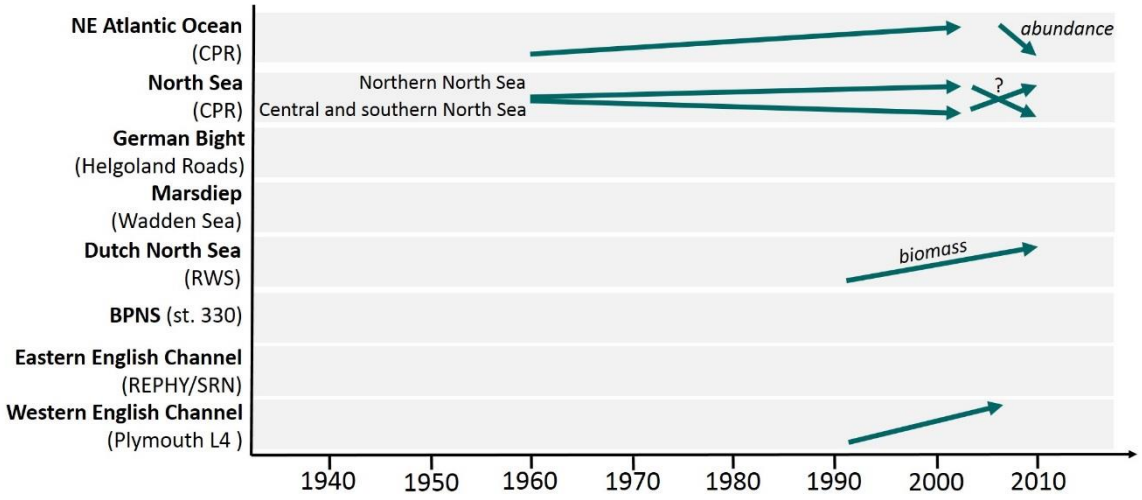

Figure 14 Overview of general (long-term) trends of the dinoflagellate biomass/abundances in the North Sea, the English Channel and the North Atlantic Ocean.

1.7.2.4. *Phaeocystis*

In some areas, such as the Marsdiep tidal inlet, summer (August) and autumn peaks (September) in *Phaeocystis* have been reported since the end of the 19th century (Cadée and Hegeman, 1991). In addition, the occurrence of *Phaeocystis* blooms are not limited to coastal waters as the CPR time series reveals (Gieskes et al., 2007). In the North Sea, the frequency of occurrence of *Phaeocystis* has been especially high during and before the 1960s, and after the 1980s (CPR study) (Figure 15) (Gieskes and Kraay, 1977; Gieskes et al., 2007). In the Dutch Wadden Sea, *Phaeocystis* bloom duration has increased from the end of the 19th century to the end of the 1980s (Cadée and Hegeman, 1991). In the North Seas, the *Phaeocystis* season was longer before 1965 and after 1995 than in between (CPR study) (Gieskes et al., 2007). The increasing trend and the longer bloom duration of *Phaeocystis* since the middle of the 20th century (Cadée and Hegeman, 2002) has reversed to a general decreasing trend in the magnitude of the spring bloom since the 1990s in the Marsdiep (Cadée and Hegeman, 2002). Also in the western English Channel, a decreasing *Phaeocystis* trend between 1992 and 2007 was observed (Widdicombe et al., 2010). The 'de-eutrophication' trend is discussed as a possible factor responsible for this change (Gómez and Souissi, 2008). A modeling study in the BPNS, identified an increasing trend in *Phaeocystis* cell numbers and primary production (PP) between 1950 and 1985, followed by a decrease in cell numbers and a stable period for PP. For the period 1988 to 2001, however, no clear trend was observed regarding the maximum cell numbers (Lancelot et al., 2009) and also in the Dutch part of the North Sea no clear trend has been observed (1990-2007) (Prins et al., 2012).

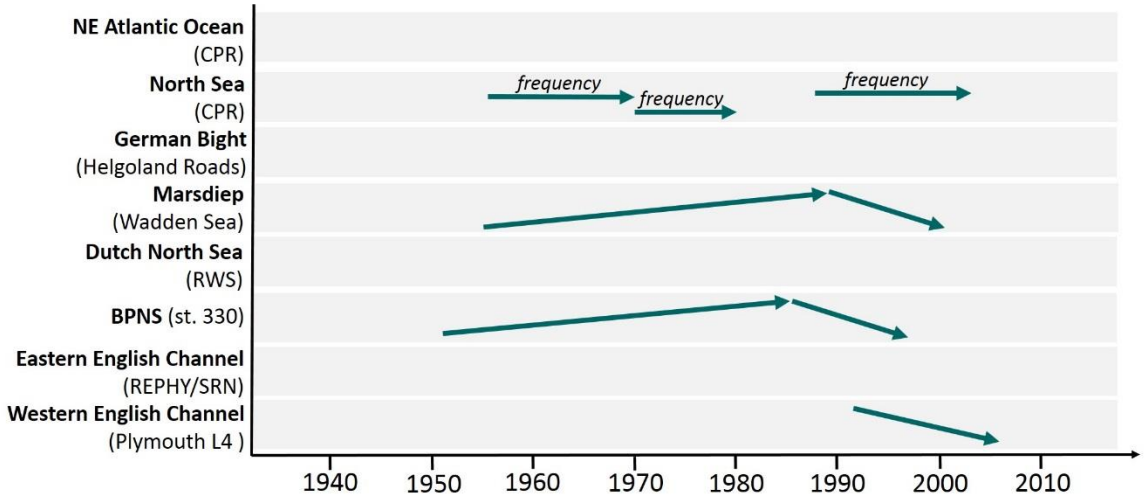

Figure 15 Overview of general (long-term) trends of the *Phaeocystis* biomass/abundances in the North Sea, the English Channel and the North Atlantic Ocean. If 'frequency' is mentioned above the arrow, the arrow refers to the frequency of blooms and not the biomass.

1.7.2.5. Regime shifts

A marine regime shift is defined as a sudden, long-lasting change between contrasting, persistent states in the marine ecosystem (deYoung et al., 2008). Three regime shifts were observed during the last decades in the CPR datasets, in the late 1970s/beginning of the 1980s, the late 1980s (1987/1988) and in the middle of the 1990s (Beaugrand, 2004; Beaugrand et al., 2015; Beaugrand et al., 2014; Raitsos et al., 2014; Raitsos et al., 2005; Reid et al., 1998; Spencer et al., 2011). The stepwise biomass increase, measured as PCI color as proxy for chlorophyll a, in the middle of the 1980s is responsible for about 60 % of the biomass increase during the period 1948 to 2002, of which 80 % of the increase took place during winter (Raitsos et al., 2005). An increase in chlorophyll a after the regime shift in the 1980s of about 13 % in the open North Sea and of about 21 % in coastal North Sea waters has been calculated (McQuatters-Gollop et al., 2007).

Also in the Marsdiep time series this regime shift resulted in biomass changes. Periods with a relative stable phytoplankton community composition were interrupted twice by two drastic compositional changes between 1976 and 1978, as well as between 1987 and 1988 (Philippart et al., 2000). During the first regime shift the system changed from a eutrophic P-controlled to a N-controlled system, giving an advantage to large diatom species, especially chain-forming species (Philippart et al., 2000). In 1988, the system shifted back to a P-controlled system with remaining high biomass values and a high proportion of large diatom species (Philippart et al., 2000). After the second regime shift (1988-2003) total diatom abundances were lower than between the two shifts whereas flagellate abundances further increased (Philippart et al., 2007).

1.7.2.6. Seasonality

In the German Bight (HR time series) the diatom *Guinardia delicatula* starts to bloom earlier and declines later (during the period 1962-2008) (Schlüter et al., 2012). However, overall the diatom spring bloom is delayed and has shifted to the end of March (1962-2002), which is probably caused by the trend towards warmer autumn months, a longer persistence of zooplankton grazers at the end of the year and the depression of the diatom biomass accumulation during winter (Wiltshire and Manly, 2004).

In the Marsdiep, analyses of chlorophyll a data (1974-2007) did also not reveal any long-term trend in the phenology of the phytoplankton spring bloom (Philippart et al., 2010), but in contrast another study reported earlier and more persistent *Phaeocystis* blooms (1973-2000) (Cadée and Hegeman, 2002). The magnitude of the autumn bloom slightly decreased since the 1990s in the Marsdiep, which resulted in an overall shortening of the growing season with consequences for primary consumers during late summer, as they rely on phytoplankton as a food source (Philippart et al., 2010).

A study on the CPR dataset showed that the extent of the phytoplankton growing season in the southern North Sea did not change (from 1960 to 1995) (Edwards, 2001). Generally, the diatom and spring and autumn peak did not shift in timing in the period from 1958 to 2002, even though there is a strong inter-taxon variability (Edwards and Richardson, 2004). The blooms of most dinoflagellates however, occur earlier (Edwards and Richardson, 2004). The bimodal phytoplankton cycle before the regime-shift in the mid-1980s changed to a unimodal cycle, resulting in an extension of the growing season and higher biomass values the whole year round (Raitsos et al., 2014).

Through the comparison of two periods (1991-1998 and 1999-2005) marked seasonal changes were observed in the RWS time series, with an earlier increase of the total phytoplankton biomass in spring, especially of the diatoms and dinoflagellate biomass, and a forwards shift of the phytoplankton peak from May to April (Baretta-Bekker et al., 2009). Gieskes et al. (2007) also observed an earlier start of the phytoplankton spring bloom between 1976 and 2004. In addition, diatoms and dinoflagellates reach higher bloom biomass maxima (Baretta-Bekker et al., 2009). This was confirmed by another study, which not only identified an increasing diatom biomass (1990-2007), but also an increasing bloom frequency and higher maximum bloom cell numbers of some diatom species (e.g. *Chaeotoceros socialis*) (Prins et al. 2012).

In the Eastern English Channel, chlorophyll a concentration starts to rise in February, remains high from March to June and has its maximum in April (Lefebvre et al., 2011). Between March and May

Phaeocystis contributes to a big part of the total phytoplankton abundance with highest densities near the coast (Hernández-Fariñas et al., 2014; Lefebvre et al., 2011). Diatoms generally show more variation in their annual cycle with highest abundances from June to July in the English Channel, but maximum abundances in March in the Dunkerque transect (Hernández-Fariñas et al., 2014). *Phaeocystis* on the other hand generally blooms in April in this area, while dinoflagellates reach their highest abundance between July and September (Hernández-Fariñas et al., 2014).

Changes in the seasonal timing of *Phaeocystis* have been observed in the Marsdiep area, with the earlier occurrence (2 to 3 weeks) of the *Phaeocystis* bloom in the 1990s than in the 1970s (Cadée and Hegeman, 2002). Also in the Dutch part of the North Sea (RWS monitoring), marked seasonal changes were identified, with a forward shift of the *Phaeocystis* peak of about one month (comparison 1991-1998 and 1999-2005), but the blooms are smaller and shorter (Baretta-Bekker et al., 2009).

Pronounced discrepancies exist between the different long-term series (and even within different studies of the same time series). This may be due to a number of reasons. First, the time series are derived from different areas which have different environmental conditions (hydrodynamics, freshwater input, stratification, etc). Second, sampling and analytical methods (e.g. chl a methodology) differ and sampling parameters (especially for biomass) are very different (PCI, chl a, or biovolume deriving from microscopic phytoplankton counts). As the cellular chlorophyll a content varies among species and with the light regime (decrease of chlorophyll a going along with an increase in PAR), a discrepancy between the chlorophyll a concentration and the phytoplankton biomass (e.g. carbon) can be expected (Alvarez-Fernandez and Riegman, 2014). Finally, there is also a personal impact which cannot be excluded from the analyses (Günther et al., 2012). For example, the taxonomic skills of the taxonomists conducting microscopic phytoplankton cell counts influences the results, which even can improve during the course of the career of the taxonomist due to growing expertise, better microscopes and identification guides. However, it seems that from a general point of view, total biomass and dinoflagellate biomass have generally increased, while diatoms have decreased during the last decades. *Phaeocystis* seems to have decreased during the last 30 years.

1.8. Motivation and thesis objectives

The BPNS is a very heterogeneous and dynamic area under the influence of Atlantic waters entering through the English Channel into the North Sea on the one hand and freshwater input of various rivers, such as the Seine, the Scheldt, the Rhine/Meuse and the Somme, whose

watersheds are strongly anthropogenically disturbed, on the other hand. The BPNS can thus serve as a model area to study the impact of natural disturbances and anthropogenic activities on coastal marine ecosystems. Information on long-term trends in phytoplankton community structure, biomass, succession and phenology is generally lacking, except for one station in the 1990s/2000s (Breton et al., 2006) and isolated studies before and after that period. More specifically, there is no information on how climate change and changes in (de)eutrophication have affected phytoplankton. This information is of special concern with respect to the HAB species *Phaeocystis*. This taxon can display marked interannual variability but the underlying causes are little understood to date.

As Belgium has no long-term monitoring program that goes back in time for several decades, there are still some fundamental questions unresolved, especially regarding the following points:

- It is not known how phytoplankton biomass in the BPNS has changed since the 1970s. The only interannual trends that have been identified are at a single station (station 330) in the BPNS, and these revealed an increase of the diatom carbon biomass between 1992 and 1995, followed by a sharp decreases from 1995 to the end of 1998 (Breton et al., 2006).
- It is still unclear how the (de-)eutrophication and the resulting changing nutrient ratios have influenced the phytoplankton community in the BPNS.
- It still remains to be solved to which extent climate change has influenced phytoplankton biomass and community composition in the BPNS.
- It is also unknown if there have been any changes in the phenology or timing of the blooms since the 1970s (chlorophyll a, diatoms, dinoflagellates and *Phaeocystis*).
- Even though there has been conducted research regarding the phytoplankton community composition in the BPNS (e.g. by Terseleer (2014) and Muylaert et al. (2006)), it never has been studied if the today accepted annual phytoplankton succession is the same compared to some decades back in time.
- Many hypotheses exist regarding the factors controlling *Phaeocystis* blooms. It still remains unknown what the controlling factors are for biomass long-term trends of *Phaeocystis* in the heterogeneous BPNS.

In order to address the above mentioned knowledge gaps, the Belspo Brain-be project 4DEMON (4 Decades of Belgian Marine Monitoring) had been initiated, which aimed to ‘centralise, integrate and valorise data compiled during expeditions in the BPNS over the last four decades’. The

4DEMON project was the first attempt to integrate and analyse marine historic data and data from recent research projects in the BPNS.

1.8.1. Aims and hypotheses

The present PhD thesis aimed at inventorying, compiling, standardizing and integrating phytoplankton data from microscopic counts, chlorophyll a and other pigment data from the 1970s until now; analyzing spatial and temporal trends in this new dataset, with a focus on long-term changes in community composition, biomass and phenology, and its underlying causes. The main objective of this doctoral thesis is to obtain better insight into general spatiotemporal phytoplankton long-term changes in phytoplankton communities in the BPNS. We also want to know if the observed changes are indirectly (e.g. by climate warming) or directly (e.g. nutrient input) resulting from anthropogenic stress. The thesis has a strong focus on diatoms, dinoflagellates and *Phaeocystis*, which are as already mentioned, the most important phytoplankton groups in the BPNS (Rousseau et al., 2006; Desmit et al., 2015; Lancelot et al., 2009; Muylaert et al., 2006). We hypothesize that both natural and anthropogenically induced global change phenomena have impacted the phytoplankton community in the BPNS since the 1970s regarding abundance and biomass of the total phytoplankton, diatoms and dinoflagellates, the seasonal timing and community composition. More specifically, we hypothesize that:

Temperature effects:

1) the increasing winter SST has changed the annual timing of the spring bloom and allows an earlier bloom start of the spring diatom community (Peperzak, 1998; Desmit et al., 2015).
2) The increasing summer SST may positively stimulate summer dinoflagellates in the BPNS, due to an increase in stratification (Alvarez-Fernandez and Riegman, 2014; Falkowski and Oliver, 2007; Winder and Sommer, 2012).
3) As warmer temperatures negatively influence large-celled diatom species, we hypothesize that the community structure in the BPNS has shifted to generally smaller sized species especially during summer (Lewandowska and Sommer, 2010).
4) We expect a change (positive or negative) of the total phytoplankton biomass with rising temperatures (Suikkanen et al., 2013).

Effects of nutrient changes:

5) We hypothesize that the still decreasing nutrient (P and N) inputs generally lead to the decrease in phytoplankton biomass (Philippart et al., 2000). Under nutrient limited condition small and motile phytoplankton species such as dinoflagellates have an

advantage over larger cells (e.g. large diatoms) (Falkowski et al., 1998; Falkowski and Oliver, 2007; Winder and Sommer, 2012), which might have led to a relative increase of dinoflagellates to diatoms.

6) Since the de-eutrophication period, the Si transport to the coast has increased (Prins et al., 2012; Desmit et al., 2015), which may stimulate the diatom production positively, as they need Si to build up their siliceous cell walls (Tréguer and Pondaven, 2000). Therefore, the increase of the available Si implies an advantage for diatoms over other phytoplankton groups to grow (Humborg et al., 2000).
7) Since not only the nutrient concentrations in the BPNS have changed, but also the ratios (e.g. increase in DIN:DIP), we hypothesize that this ratio changes might be an advantage/disadvantage for some groups. We hypothesize that the increasing DIN:DIP ratios favors dinoflagellates over other phytoplankton groups (e.g. diatoms) because they are capable of using organic P-sources (through phagotrophy) (Burson et al., 2016; Legrand et al., 1998).

***Phaeocystis* hypothesis:**

8) Which external environmental conditions allow the marine Prymnesiophyte *Phaeocystis* to develop dense blooms in the BPNS? There exist many hypotheses and they are partly contradicting. Literature suggests that *Phaeocystis* is positively stimulated by rising temperatures (Borkman et al., 2016; Gieskes and Kraay, 1977; Gómez and Souissi, 2008), and by this we hypothesize that a positive NAO conditions in the BPNS may be stimulating the development of *Phaeocystis* blooms. As *Phaeocystis* is capable of using P-sources (Burson et al., 2016), we hypothesize that they are favored over other phytoplankton groups by the decreasing P concentration in the BPNS.

1.8.2. Thesis outline

The thesis is organized as follows:

- *Chapter 2* presents the phytoplankton community composition dataset which was generated during the course of the 4DEMON project and covers the data recovery, the compilation and integration of the data and the various quality control steps which were conducted.
- *Chapter 3* makes use of the phytoplankton dataset presented in *Chapter 2* to compare interannual and seasonal changes in the diatom and dinoflagellate abundance, biomass and community composition in the BPNS between the 1970s and the 2000s. Possible environmental causes for the observed changes are discussed.

- By integrating chlorophyll a and nutrient data from the 1970s onwards in the BPNS and the Dutch part of the North Sea, changes in phytoplankton bloom phenology were revealed. This change and potential underlying causes are studied in *Chapter 4*.
- *Chapter 5* analyses interannual phytoplankton changes between 2003 and 2016, a period for which an extensive and standardized HPLC pigment dataset is available. It mainly focuses on changes in the marine haptophyte *Phaeocystis*. It aims to answer the question under which environmental conditions *Phaeocystis* develops dense blooms.
- In the 'General discussion and conclusion' of this thesis (*Chapter 6*) the main results, recommendations for methodological improvements and further research possibilities are discussed.

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2

Marine phytoplankton community composition data from the Belgian part of the North Sea, 1968-2010

Modified from Nohe A., Knockaert C., Goffin A., Dewitte E., De Cauwer K., Desmit X., Vyverman W., Tyberghein L., Lagring R. and Sabbe K. Marine phytoplankton community composition data from the Belgian part of the North Sea, 1968-2010. *Scientific Data* volume 5, Article number: 180126 (2018).

2.1. Abstract

The Belgian Phytoplankton Database (BPD) is a comprehensive data collection comprising quantitative phytoplankton cell counts from multiple research projects conducted since 1968. The collection is focused on the Belgian part of the North Sea, but also includes data from the French and the Dutch part of the North Sea. The database includes almost 300 unique sampling locations and more than 3,000 sampling events resulting in more than 86,000 phytoplankton cell count records. The dataset covers two periods: 1968 to 1978 and 1994 to 2010. The BPD can be accessed online and provides high quality phytoplankton count data. The species taxonomy is updated, and the count values are quality checked and standardized. Important metadata like sampling date, sampling location, sampling depth and methodology is provided and standardized. Additionally, associated abiotic data and biovolume values are available. The dataset allows to conduct analyses of long-term temporal and spatial trends in phytoplankton community structure in the southern part of the North Sea, including changes in phytoplankton phenology and seasonality.

2.2. Background & Summary

Because of its location in a densely populated region with intensive economic activities, the North Sea has been seriously affected by anthropogenic activities, both historical and contemporary (Billen et al., 1999; Passy et al., 2013). Especially in the last 50 years it has been heavily impacted by pollution (e.g. heavy metals), eutrophication (with shifts in nutrient ratios), climate change, fisheries (with concomitant changes in food webs) and other disturbances (e.g. the construction of offshore windfarms) (Jennings et al., 1999; Kersten et al., 1988; Prins et al., 2012; Radach et al., 1990; Reubens et al., 2011).

Due to its role as the main primary producer in the ocean, phytoplankton influences almost all higher trophic levels, from copepod herbivores to zooplankton carnivores, pelagic fish, seabirds and marine mammals (Turner and Tester, 1997). Phytoplankton is sensitive to anthropogenic pressures and both its production and composition can change as a result of eutrophication and temperature changes, but also as a result of top-down effects of changes in higher trophic levels (e.g. through fisheries, shifts in zooplankton composition) (Pitt et al., 2008; Prins et al., 2012; Radach et al., 1990). Long-term data on phytoplankton community structure offer a unique opportunity to study the impact of various anthropogenic pressures on phytoplankton, and how phytoplankton may respond to future changes.

While in most North Sea countries such as the Netherlands, France, Germany and the United Kingdom long-term monitoring phytoplankton programs have been running for several decades

(Baretta-Bekker et al., 2009; Cadée and Hegeman, 2002; Hernandez-Farinas et al., 2014; Lefebvre et al., 2011; Philippart et al., 2010; Widdicombe et al., 2010; Wiltshire and Dürselen, 2004; Wiltshire et al., 2010; Wiltshire et al., 2008; Xie et al., 2015), in Belgium no such structured long-term monitoring effort exists. Nevertheless, an impressive amount of phytoplankton community structure studies have been conducted during the last decades, including the 1970's for which data are often lacking in neighbouring countries (Baretta-Bekker et al., 2009; Hernandez-Farinas et al., 2014; Lefebvre et al., 2011; Widdicombe et al., 2010; Xie et al., 2015). These historical and recent cell count data were until now scattered in technical reports or in digital form on laboratory computers only, while some data were directly incorporated in the database of the Belgian Marine Data Centre (BMDC). As a result, these quantitative phytoplankton community structure datasets, which took a lot of resources and expert knowledge to acquire, were never published or disseminated as a whole to the wider scientific community. In addition, because the data were collected by different researchers over a long time period, the data were never quality controlled or standardized in a uniform way.

This work is part of the 4DEMON project (www.4demon.be/), which has the aim to safeguard and centralize these valuable historical data for the future and make them available to the scientific community. To this end, we identified relevant data sources based on literature research, contacted researchers, digitised data values, assembled metadata, conducted quality control on the data and integrated the data in an extensive database for the BPNS, the Belgian Phytoplankton Database (BPD) (Figure 1) (Data Citation 1). The BPD is available through the Integrated Marine Information System hosted at the VLIZ (Flanders Marine Institute).

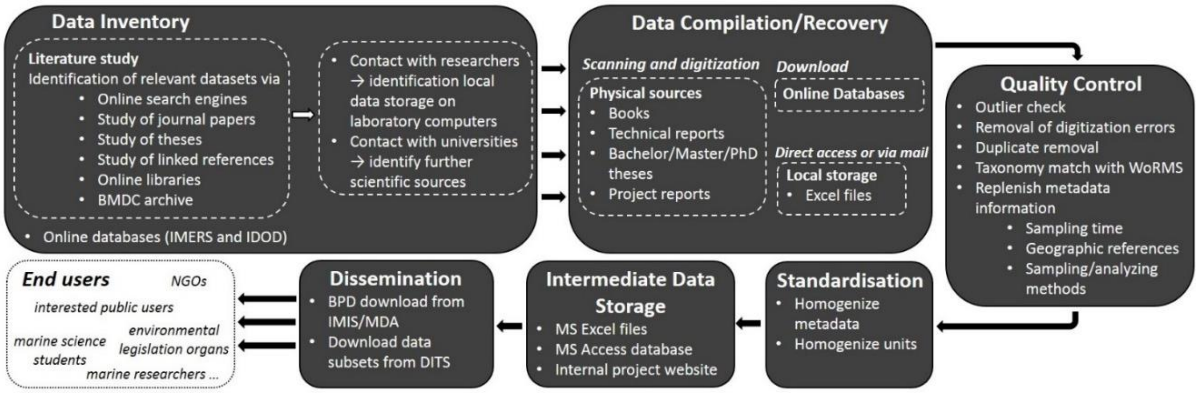

Figure 1 Representation of the workflow starting from the data source identification up to the final dissemination of the Belgian Phytoplankton Database (BPD).

2.3. Methods

2.3.1. Data inventory

Possible data sources were identified based on literature research, web-based search engines and queries in online databases such as IMIS (Integrated Marine Information System - www.vliz.be/en/imis), IMERS (Integrated Marine Environmental Readings & Samples - www.vliz.be/vmdcdata/imers) and IDOD (Integrated and Dynamical Oceanographic Data management - http://www.bmdc.be). In addition, universities and researchers were contacted by mail, phone or personally. All data sources are inventoried in the Data Inventory and Tracking System (DITS - http://dits.bmdc.be) managed by BMDC. The majority was made available through IMIS via www.vliz.be/en/imis?module=ref&SpCol=809&show=search.

2.3.2. Data compilation

After the identification of relevant data sources, all non-digitally available data sources (namely books, technical reports, Bachelor theses, Master theses, PhD theses and project reports) were scanned. The data values were manually transferred to a standard format in MS Excel. Data were also downloaded or extracted from databases. We directly accessed data already available in digital format on laboratory computers or received them from researchers. All compiled data were integrated in a MS Access database.

2.3.3. Quality Control & standardization

2.3.3.1. Metadata

A significant amount of metadata was not easily accessible via the data sources themselves. An intensive effort was made to recover all relevant metadata e.g. station information or methodological approaches from associated sources such as final project reports.

2.3.3.2. Taxonomy

During the last decades there were many extensive nomenclatural and other taxonomic revisions of phytoplankton taxa based on progress related to advances in microscopy and molecular-phylogenetic analyses. For this reason, species names needed to be referenced prior to inclusion in the database. This was done using the taxon match option available in the World Register of Marine Species (www.marinespecies.org), a universally recognized and authoritative open-access reference system for marine species managed by VLIZ and edited by more than 240 taxonomic editors world-wide. Every species name has a unique identifier known as the AphiaID (Costello et

al., 2013). This identifier enables to link the species name to an internationally accepted standardized name and associated taxonomic information, but also redirects to the most accurate information on the species taxonomy, like accepted names and synonyms.

The taxon match was conducted in November 2017. Due to spelling mistakes present in the original reports or resulting from errors during digitisation (caused by illegible or low quality handwriting in the original paper reports), many names were not recognized automatically by the World Register of Marine Species (WoRMS), but were matched manually. Finally, less than 1 % of the records could be matched neither automatically nor manually. In these cases the 'AphiaID matched' field stayed empty, but the records were not discarded.

A thorough clean-up of the species names and manual matching yielded a total of 99 % of the taxon names being referenced with an AphiaID. Some species which were not listed in the WoRMS database were, after approval of the dedicated editor, added to this register.

2.3.3.3. Geographic reference

For most of the stations geographical coordinates were available or could be deduced from the synthesis reports or publications which made use of the data. Stations with unknown coordinates, but located on a map, were georeferenced in QGIS or based on standards within Marine Regions (www.marineregions.org). Stations from the same project which had slightly different names in various paper sources were compared and a unique station name was assigned. Finally, 93.8 % of the records were assigned to stations with geographical coordinates.

2.3.3.4. Analyses and sampling methodologies

Phytoplankton was sampled using Niskin bottles, but also using other unspecified types of recipients (e.g. bottles, buckets) or a Van Dorn-sampler (Table 1). The samples were preserved with Lugol's solution, formaline or natrium acetate. They were cooled or stored at room temperature and protected from the light. Cells were consistently counted with the Utermöhl method (Utermöhl, 1958) using the inverted microscope as optical instrument; for the large dinoflagellate *Noctiluca scintillans* sometimes a stereoscopic microscope was used. Sampling techniques, preservation steps and analytical methods are described in detail in the metadata of the BPD.

Table 1 Sampling methodologies, preservation and analytical methods reported in the Belgian Phytoplankton Database (BPD).

Method	Sampling Instrument	Preservation	Analysis Instrument	Method Description
1	bucket, Niskin bottle	Lugol's solution; cool, dark	inverted microscope	Samples were fixed with Lugol's solution and stored cool at 4 °C in the dark. Samples were analysed 1-3 months after sampling with the Utermöhl method.
2	Nansen bottle	Lugol's solution	inverted microscope	Samples were fixed with Lugol's solution. They were analysed with the Utermöhl method. Sedimentation chambers of 50 ml and 100 ml were used (sometimes 10 ml). Magnification 320x.
3	Niskin bottle	formalin; room temperature	stereoscopic microscope	Samples were fixed with formalin. Samples were kept at room temperature and analysed with a stereoscopic microscope.
4	Niskin bottle	Lugol's solution	inverted microscope	Inverted microscope (magn. 40x10x, 60x10x, 100x10x). Some diatom species were analysed after oxiditation with an electron microscope (SEM) to detemine them on species level.
5	Niskin bottle	Lugol's solution	inverted microscope	Samples were fixed with Lugol's solution. Samples were analysed with the Utermöhl method. Some diatom species were analysed after oxiditation with an electron microscope (SEM) to detemine them on species level.
6	Niskin bottle	Lugol's solution	inverted microscope	Samples were fixed with Lugol's solution. Samples were analysed with Utermöhl method. Sedimentation time 24 hours. Magnification of 20x10x or 40x10x was used. Only living cells are counted.
7	not available	not available	inverted microscope	A sample volume of 250 ml was concentrated to 5 ml by decantatation. Sample were counted with an inverted microscope at 10x20 and 10x40 magnification.
8	not available	not available	inverted microscope	Sedimentation. Normally 3 subsamples were analyzed.
9	plastic bottle, glass bottle	formol, Lugol's solution	inverted microscope	Preservation with 4 % formol or Lugol's solution. Samples analysed with Utermöhl method. Samples were mixed well before transfer to sedimentation chamber. 4 hours sedimentation time. Living and dead cells were distinguished.
10	plastic bucket (surface samples), Van Dorn-sampler (depth samples)	J-JK-Na-acetaat solution	inverted microscope	Fixation with J-JK-Na-acetaat-solution. The Utermöhl method was used. 1 L of well-mixed sample was transferred to a 1-litre-measuring cylinder. 4 days sedimentation time. Supernatante was removed with a water-jet pump. Analysis with an inverted microscope, magn. 60x-1000x.
11	plastic pot	formol, Lugol's solution; dark	inverted microscope	Fixation with 2 ml 40 % formol or Lugol's solution. Samples were kept in the dark. Analysis with Utermöhl method. Sedimentation of 5 ml, 10 ml, 25 ml or 50 ml of well-mixed sample. Inverted microscope with a magnification of 200x.
12	polyethylen bottle	formol	not documented	Addition of 250 ml 40 % formol a sample volume of 25 L. Addition of destilled water to filtrate until a volume of 102.5 ml.
13	bucket, Niskin bottle	cool, dark	inverted microscope	Samples were fixed with Lugol's solution and stored cool at 4°C in the dark. Samples were analysed 1-3 months after sampling with the Utermöhl method.

2.3.3.5. Additions and changes

The data were screened for random digitisation errors (mistakes made during transfer from handwritten documents to digital format). Duplicate values, resulting from data sources reporting on the same data, were removed. All zero values were removed. All units were converted to the

common unit cells per litre. *Phaeocystis* cells associated in colonies are in the unit '10^6 coc L^{-1} (= colonial cells per litre). For 249 common phytoplankton species (168 Bacillariophyceae, 76 Dinophyceae, 4 Prymnesiophyceae and 1 Cryptophyceae) biovolume calculations based on literature values and online sources are given as additional information.

2.4. Data Records

2.4.1. Historic Projects

The BPD is a compilation of data assembled from different research projects conducted since 1968. At the end of the 1960s and in the 1970s the University of Leuven joined cruises to Iceland to investigate the pelagic environment. In 1970 the Belgian government financed an integrated research project called Projet Mer/Projekt Zee (PMPZ) to assess the quality of the marine environment of the BPNS. This project was followed by national research programs called Concerted Research Actions (CRA) from 1977 until 1981. From 1990 onwards subsequent projects such as AMORE (Advanced Modelling and Research on Eutrophication) focused on *Phaeocystis* blooms in the English Channel and the Southern Bight of the North Sea with a strong focus on the BPNS (Lancelot et al., 2003; Lancelot et al., 2007; Lancelot et al., 2012). In the years 2000, phytoplankton analyses were collected and processed in the framework of Bachelor and Master theses at Ghent University and in the framework of the EU Water Framework Directive (WFD) in order to study spatiotemporal dynamics in phytoplankton community structure in the BPNS (De Bock et al., 2009; Denayer et al., 2010; Franck, 2004; Muylaert et al., 2006; Töpke, 2009) (Table 2).

2.4.2. Record types

Data recovery resulted in a high number of biotic values and associated abiotic parameters. In total 86,746 phytoplankton records are stored in the BPD. Quantitative units are phytoplankton densities in cells per litre (95.1 % of the records) and abundance classes in cells per litre (4.4 % of the records). Abundance classes reflect a range of cell densities per litre e.g. density between 1,000 and 9,999 cells per litre. 17,342 records are tagged with a living/dead (12,114/5,228) notation. 'Dead' refers to dead cells, e.g. diatom frustules without a cell content.

2.4.3. Metadata

Each individual data record is linked to its associated metadata such as information about the data source, the sampling event, the sampling and analysis method, the project, the physical dataset origin and the phytoplankton taxonomy.

2.4.4. Spatial & temporal coverage

The database includes almost 300 individual phytoplankton sampling stations in the BPNS and adjoining areas (French, Dutch and British waters) (Figure 2) of which 137 sampling stations are situated within the BPNS resulting in a total of 51.6 % of all records deriving from samples taken in the BPNS.

Table 2 Overview of data sources integrated in the Belgian Phytoplankton Database (BPD). Additional information on the dataset source, the temporal coverage, the number of records and a link to the metadata is provided.

source	type	dataset source	temporal coverage	no. records	metadata link
AFVALWATEREN	book	paper	1970-1972	5,188	http://www.vliz.be/en/imis?module=ref&refid=13326
AMORE_ULB-ESA	project	www.bmdc.be	1997-2000	129	http://www.vliz.be/en/imis?module=project&proid=74
AMOREII_ULB-ESA	project	www.bmdc.be	2003-2006	12,305	http://www.vliz.be/en/imis?module=project&proid=1065
AMOREII_VUB-ECOL	project	www.bmdc.be	2003-2004	68	no link available
AMOREIII_ULB-ESA	project	www.bmdc.be	2007-2009	11,663	http://www.vliz.be/en/imis?module=project&proid=2084
Iceland Cruises	book	paper	1970-1971	1,095	http://www.vliz.be/en/imis?module=ref&refid=24711
IPMS-PHAEO_ULB-ESA	project	www.bmdc.be	1995-1996	68	http://www.mumm.ac.be/datacentre/Catalogues/datasets.php?proj=IPMS-PHAEO
MSc Thesis C. Vanlangedonck	MSc Thesis	paper	1976-1977	2,190	http://www.vliz.be/en/imis?module=ref&refid=216487
MSc Thesis E. de Block	MSc Thesis	paper	1977-1978	17,291	http://www.vliz.be/en/imis?module=ref&refid=216445
MSc Thesis M. Franck	MSc Thesis	Excel file	2003	791	http://www.vliz.be/en/imis?module=ref&refid=67322
MSc Thesis K. Töpke	integrated dataset	Excel file	2004-2006	1,399	http://www.vliz.be/en/imis?module=ref&refid=200644
PAE phytoplankton species dataset	integrated dataset	Excel file	2004	57	no link available
monitoring KRW	integrated dataset	Excel file	2007-2008	1,190	http://www.vliz.be/en/imis?module=ref&refid=289406
monitoring KRW	integrated dataset	Excel file	2009-2010	1,783	http://www.vliz.be/en/imis?module=ref&refid=203122
PhD Thesis A. M'harzi	PhD Thesis	Excel file	1994	440	http://www.vliz.be/en/imis?module=ref&refid=32158
PhD Thesis J. Smeets	PhD Thesis	paper	1974-1976	502	http://www.vliz.be/en/imis?module=dataset&dasid=4862
PhD Thesis M. Rabijns	PhD Thesis	paper	1971-1973	12,346	http://www.vliz.be/en/imis?module=ref&refid=226081
PhD Thesis R. Clarysse	PhD Thesis	paper	1968-1970	716	http://www.vliz.be/en/imis?module=ref&refid=69474
Project Sea Report	technical report	paper	1971	874	http://www.vliz.be/en/imis?module=ref&refid=240591
					http://www.vliz.be/en/imis?module=ref&refid=240604
					http://www.vliz.be/en/imis?module=ref&refid=240605
Project Sea Report	technical report	paper	1972	7,703	http://www.vliz.be/en/imis?module=ref&refid=240607
Project Sea Report	technical	paper	1973-1974	1,663	http://www.vliz.be/en/imis?module=ref&refid=240648
Project Sea Report	technical report	paper	1974	6,718	http://www.vliz.be/en/imis?module=ref&refid=205357
					http://www.vliz.be/en/imis?module=ref&refid=205359
TROPHOS_UGent-MARBIO	project	www.bmdc.be	2003	567	http://www.vliz.be/en/imis?module=project&proid=1074

Data are available for the years 1968 to 1978 and 1994 to 2010 (Figure 3a). In total, 3,178 sampling events took place throughout these periods of which 1,782 took place in the BPNS. The dataset has a good seasonal coverage (Figure 3b and Figure 3c). Between 1968 and 1978 2,269 sampling events took place which is on average 206 events per year. These events resulted in 56,286 records, an average of 5,117 records per year and a record to event ratio of 25. Between 1994 and

2010, 909 sampling events resulted in 30,460 records, which is on average 60 sampling events and 2,031 records per year.

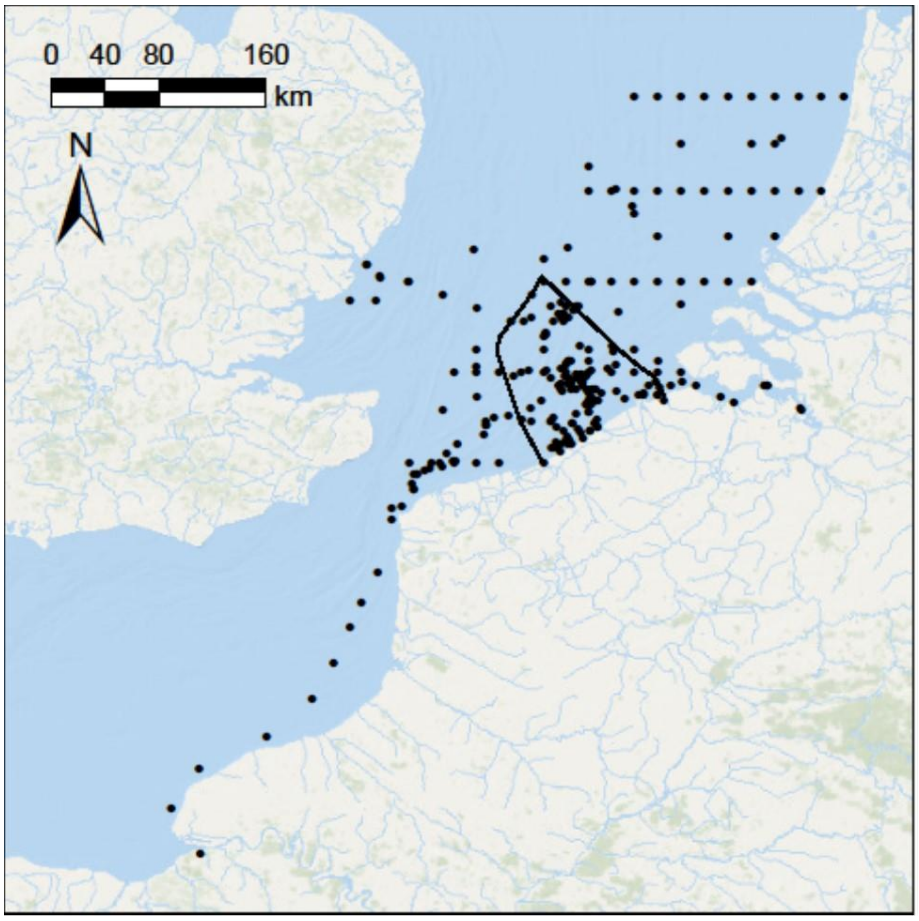

Figure 2 Locations of the sampling stations. Sampling stations are marked as black dots. The boundary of the Belgian part of the North Sea (BPNS) is indicated as a black line.

2.4.5. Taxonomic coverage

The dataset contains 681 unique AphiaIDs of which 93 % were at least identified to the genus level. The remaining 7 % were identified to a higher taxonomic level or could not be matched (1 %). Bacillariophyceae (diatoms) and Dinophyceae (dinoflagellates) are the two most counted phytoplankton groups representing 86.4 % and 6.3 % respectively of all records (Table 3).

2.4.6. Associated environmental data

The associated environmental abiotic data (15,199 environmental records) measured during the same campaigns and projects in the BPNS are included, containing *i.a*. concentrations of nutrients, chlorophyll a, temperature, salinity and pH. Similar to the phytoplankton data, this is a compilation originating from various laboratories and changes in methods have occurred over the years. The data have been quality checked and referenced in time and geographically. Duplicates, outliers and zero-values have been removed. The environmental data can be either directly linked to the

phytoplankton data via a common sampleID (1,230 samples, 10,332 environmental records) or via a combination of sampling date and station (726 samples, 4,867 environmental records). The latter do not share a common sampleID with the phytoplankton data e.g. because the exact sampling time during the day or the sampling depth may differ.

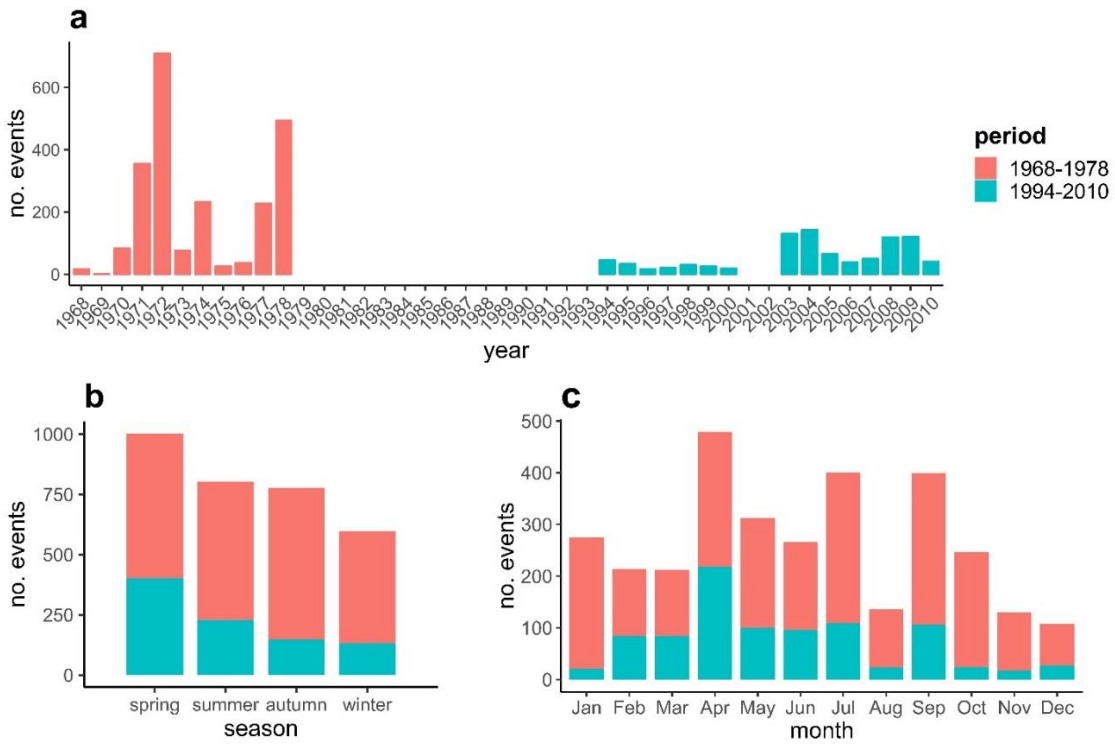

Figure 3 Amount of the phytoplankton sampling events. The two periods 1968-1978 (red) and 1994-2010 (blue) are distinguished. a: Amount of sampling events per year from 1968 to 2010. b: Amount of sampling events per season, c: Amount of sampling events per month. Winter = December-February, spring = March-May, summer = June-August, autumn = September-November.

2.4.7. Documentation and dataset dissemination

The BPD can be downloaded from IMIS (see Data Citation 1). Note that the first version of the data file (Phytoplankton_BPNS1968-2010.xlsx) does still contain zero count values, but does not yet include phytoplankton biovolume estimates or abiotic data.

2.5. Technical Validation

The BPD contains high quality phytoplankton count data of several decades and its associated abiotic data. As the BPD is a compilation of different research projects users should be aware of the following before usage. During the last decades, different protocols were used. For example, the sample collection method, the storage of the samples and the preservation methods can differ. In addition, cell counts have been performed by several researchers. In addition to variable levels of taxonomic expertise and difference in species concepts, it is a well-known fact that taxonomic skills can improve even during the careers of single taxonomists (as a result of growing

expertise, but also better analytical tools and identification guides). This personal component in microscopic taxonomic determination can never be excluded completely (Günther et al., 2012). All data present in the BPD however were obtained in well-equipped Belgian laboratories known for their high research standards and having extensive expertise in the field of phytoplankton identification and/or taxonomy. Therefore, the phytoplankton identifications and counts are considered to be generally solid.

Table 3 Taxonomic phytoplankton classes present in the Belgian Phytoplankton Database (BPD). The total number of records and relative amount of records are reported.

class	no. records	% of records
Bacillariophyceae	74,955	86.41
Dinophyceae	5,482	6.32
Prymnesiophyceae	1,046	1.21
Chlorophyceae	831	0.96
Trebouxiophyceae	431	0.50
Chrysophyceae	426	0.49
Euglenoidea	388	0.45
Dictyochophyceae	224	0.26
Cryptophyceae	147	0.17
Cyanophyceae	134	0.15
Ulvophyceae	91	0.10
Prasinophyceae	54	0.06
Cyanobacteria incertae sedis	31	0.04
Conjugatophyceae	15	0.02
Chlorodendrophyceae	9	0.01

Throughout the dataset diatom and dinoflagellate records are dominant (Table 3). Variation in taxon richness (number of accepted AphiaIDs) per sample in the BPD is shown in Figure 4a. The peaks at 1 and around 60 can be attributed to specific projects. For example, the AMORE, AMOREII_VUB-ECOL and IPMS-PHAEO projects only focused on a few specific groups (such as the Bacillariophyceae (as a whole), *Phaeocystis* and *Noctiluca*), which explains the large number of samples for which only a single group is reported. The peak around 60 is mainly due to the AMORE II and III projects. In these projects, many taxa per sample get the same (low) density of 100 cells L^{-1}. As zero values are absent for these samples, we suspect that these entries concern some indication of the fact that these species were under the detection limit. As we cannot be sure what these values mean, we have decided to leave them as they are in the dataset.

In addition, missing metadata (e.g. coordinates of sampling location) can limit the usability of some data records. Despite these caveats, the BPD is the only Belgian phytoplankton database which contains data going back almost five decades. It is a comprehensive and thoroughly quality

checked integrated data series which includes reliable data on phytoplankton in the BPNS for marine researchers and other interest groups (Figure 4b).

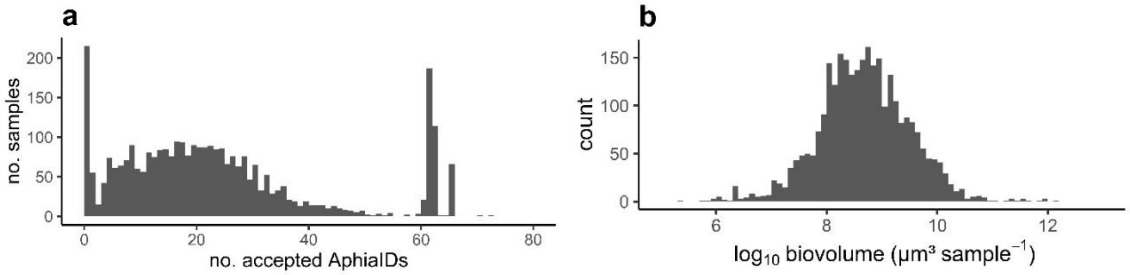

Figure 4 Summary statistics of the phytoplankton records. a: Frequency distribution of the number of accepted AphiaIDs in the BPD, b: Histogram of the logarithmically transformed biovolumes (log10 µm³ sample^{-1}) per sample of the most common phytoplankton species.

2.6. Usage Notes

The BPD can be used to study spatio-temporal changes in phytoplankton community structure in the BPNS and adjoining areas in the period 1968-2010. Inter-annual as well as seasonal patterns can be studied (Figure 5). Data can be analysed at the species level, but also aggregated data like e.g. total diatom or total dinoflagellate abundances can be analysed and community indices like diatom to dinoflagellate abundance ratios can be calculated. Furthermore, multivariate community analysis with e.g. ordination methods or general additive mixed modelling is an interesting field of study.

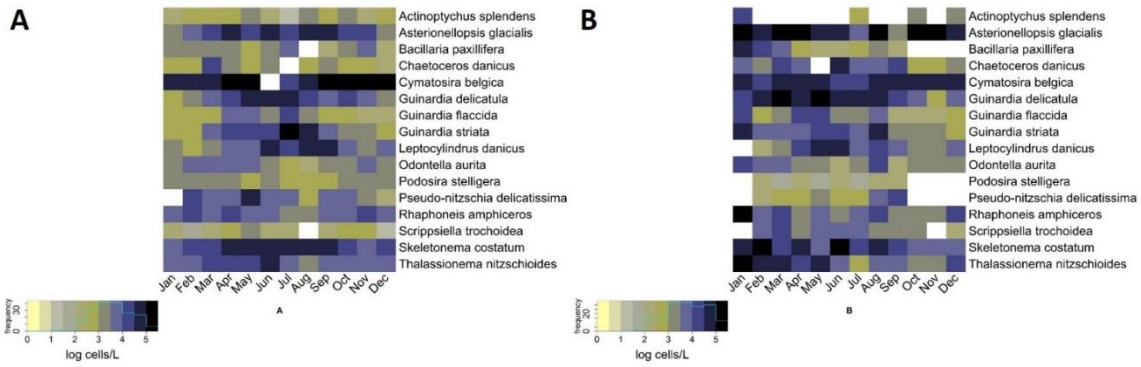

Figure 5 Heatmaps with colour key and histogram of the monthly logarithmic mean abundances of a selection of phytoplankton species reported in the Belgian Phytoplankton Database (BPD). Colours indicate the monthly mean logarithmic density (log_{10} cells L-1) of the species. a: Records dating from 1968 to 1978, b: Records dating from 1994 to 2010.

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2.8. Data citation

1. Nohe, A. et al. VLIZ https://doi.org/10.14284/320 (2018).

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Long-term phytoplankton change in a heavily impacted coastal area: evidence for diatom and dinoflagellate biomass increase and seasonal community homogenization in the southernmost part of the North Sea between the 1970s and 2000s

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3.1. Abstract

In the last decades, the North Sea has undergone important environmental changes which have led to regime shifts with noticeable changes at all trophic levels. Since the 1970s, both increases and decreases in phytoplankton biomass and production have been reported. While these conflicting observations may at least be caused by methodological differences, they may also reflect actual regional differences related to bathymetry, hydrodynamics, and riverine and Atlantic influence. We here compare biomass, seasonality and structure of diatom and dinoflagellate communities between the 1970s and 2000s, based on a newly compiled dataset from the Belgian Part of the North Sea, a hydrodynamically and bathymetrically complex area under strong human influence, which has been characterized by eutrophication (up to the 1980s) and de-eutrophication (1990s onwards), and pronounced long-term changes in turbidity and water temperature. Phytoplankton in this area is mainly dominated by diatoms, dinoflagellates and the colonial haptophyte *Phaeocystis*, but no 1970s data were available for the latter. Distinct changes were observed. Diatoms, especially large-sized taxa, showed a pronounced increase from late winter to summer, resulting in a more intense and extended growing season. Dinoflagellates increased year-round but especially in summer. The onset of both diatom and dinoflagellate blooms showed a marked forward shift (weeks to months). While in the 1970s, distinct seasonal community types were present, a striking seasonal homogenization had occurred by the 2000s. Finally, we observed a pronounced increase in harmful diatom and dinoflagellate genera. The observed changes are most likely due to an increase in sea surface temperature and water transparency (especially in winter and early spring). While nitrogen and phosphorus had decreased between the two periods, silica availability had increased, and overall nutrient ratios were more balanced, especially during winter and spring. However, other factors such as changes in grazing pressure could also have contributed and need to be further investigated.

3.2. Introduction

Global change, mainly resulting from human activities, is affecting marine ecosystems worldwide (Doney et al., 2012), with serious repercussions for the vital services that they provide to humans. Such effects will be especially pronounced in shallow coastal ecosystems, which are most directly exposed to human activities (influx of riverine nutrients, pollutants, sediments, etc.) and highly sensitive to climate warming. The Belgian Part of the North Sea (BPNS), located in the southernmost part of the North Sea, is a prime example of a heavily impacted shallow coastal zone. In the last half century, the area has been characterized by a pronounced cycle of eutrophication and de-eutrophication. Increasing riverine nutrient loads since the 1960s caused a

rise in the coastal water nutrient state which peaked in the 1980s (Billen et al., 2005). Since then, phosphorus loads have considerably decreased due to the prohibition of polyphosphate in washing detergents. Nitrogen reductions however were less successful, resulting in pronounced shifts in nutrient ratios (Brion et al., 2006; Passy et al., 2013; Burson et al., 2016). In addition, sea surface temperature (SST) has increased by more than 1 °C since the 1960s and the area has undergone significant changes in water clarity (Beaugrand, 2009; Capuzzo et al., 2015; Radach et al., 1990; Wiltshire and Manly, 2004). At the same time, climate change has resulted in shifts in the abundance and biogeographical distribution of representatives of nearly all trophic levels (McQuatters-Gollop, 2012; Hinder et al., 2012), resulting in major food web restructuring in the area.

Numerous marine ecosystem services are supported by phytoplankton which drives the ocean carbon pump and global cycles of nutrients and oxygen, and fuels marine food webs, affecting both lower and higher trophic levels from microbiota to (benthic) zooplankton to pelagic fish and seabirds (Beaugrand, 2009; Edwards and Richardson, 2004; Masó and Garces, 2006; Tréguer et al., 2017). As phytoplankton production, biomass build-up and community structure are strongly affected by nutrients, light and temperature, but also by biotic interactions with symbionts, parasites and grazers (Lima-Mendez et al., 2015), it can be expected that significant change in the phytoplankton of the BPNS must have occurred during the last decades. In order to evaluate the impact of human-induced and natural variability in factors that control phytoplankton growth, long-term data series of change in phytoplankton biomass and community structure spanning at least several decades are vital. Several highly valuable long-term datasets are available, the most important of which is the Continuous Plankton Recorder (CPR) series, which has been recording phytoplankton biomass and composition data in the North Atlantic using a consistent methodological approach since the late 1950s (McQuatters-Gollop et al., 2015). In addition, several more regional phytoplankton monitoring series exist, such as the Helgoland Roads time series (since 1962; Wiltshire et al., 2010), the Dutch monitoring program (since the early 1970s; Baretta-Bekker et al, 2009), the Dutch Marsdiep time series (since 1974; Philippart et al., 2010), and the French Observation and Monitoring program for Phytoplankton (since 1992; Lefebvre et al., 2011), all in the southern part of the North Sea.

Numerous analyses of long-term trends in phytoplankton biomass, production and community structure in the North Sea have appeared in the last decades (e.g. Cadée and Hegeman, 2002; Hinder et al., 2012). Most of these report significant changes in phytoplankton biomass and dynamics (Raitsos et al., 2014), often as part of a more comprehensive change in the whole marine ecosystem, such as the pronounced regime shift of the late 1980s, which showed a significant

increase in phytoplankton biomass in many parts of the North Sea (Edwards et al., 2006; Raitsos et al., 2014), and concomitant changes in other trophic levels from zooplankton to fish (Reid and Edwards, 2001). However, other studies reported declines in phytoplankton biomass and/or production in the North Sea area (Boyce et al., 2010; Capuzzo et al., 2018). Such discrepancies may be artefactual, related to methodological differences, but can also represent real regional differences in long-term trends. Trend analyses of phytoplankton biomass are usually based on pigment (chlorophyll a - Chl a), ocean color or CPR-derived Phytoplankton Colour Index (PCI) data, all of which have shortcomings. Chl a analyses usually do not take into account variability in C:Chl a, which can be substantial, e.g. as a result of changes in light regime and nutrient status (Alvarez-Fernandez and Riegman, 2014; Capuzzo et al., 2018). CPR-derived PCI data (e.g. Reid and Edwards, 2001; Leterme et al., 2005; Hinder et al., 2012) are incomplete as they are biased towards larger species because of the CPR silk mesh size constraint of 270 µm (Llope et al., 2012). In addition, analyses of long-term trends are often based on data with different spatial scales of aggregation [from sub-regions within the North Sea (e.g. Capuzzo et al., 2015) to the whole NE Atlantic (including the North Sea) (e.g. Leterme et al., 2005)]. This is problematic as it has been shown that trends can actually differ between subregions of the North Sea that differ in bathymetry, hydrodynamics, temperature, and freshwater and Atlantic influence (e.g. van Leeuwen et al. (2015) and Capuzzo et al. (2018)). With the exception of the Helgoland Roads and Marsdiep time series, which have been running since the 1960s and 1970s respectively, and the CPR series (which is only partly representative of community structure because of size-fractionation, cf. above, and which also is not representative of truly coastal (< 10 km from land) waters; Hinder et al., 2012), long-term datasets on phytoplankton community structure are rare. This is problematic as changes in community structure, which can be masked in biomass trends, have the potential to affect the nutritional quality of the phytoplankton and hence the transfer of organic matter and energy to higher trophic levels (Edwards and Richardson, 2004; Raitsos et al., 2014; Suikkanen et al., 2013; Burson et al., 2016). In addition, taxonomic information is needed to assess biodiversity change (which affects ecosystem functioning; e.g. Duffy et al., 2017) and the long-term dynamics of harmful algal bloom (HAB) species, and to evaluate ecosystem status (bio-indicator approach), and is therefore essential for marine policy and conservation management (McQuatters-Gollop et al., 2017).

We here present a comparison of seasonal trends in biomass and community structure of phytoplankton, and more specifically diatom and dinoflagellate communities, in the BPNS in the 1970s and 2000s. This analysis is based on a newly compiled dataset, the Belgian Phytoplankton Database (BPD) (Nohe et al., 2018), which represents a comprehensive, quality controlled and

standardized compilation of quantitative phytoplankton cell counts from the BPNS since the late 1960s, obtained from scattered published and non-published data sources. As such, this new dataset offers a unique insight into changes in phytoplankton communities during a period of intense coastal eutrophication and de-eutrophication in a heavily anthropogenically impacted coastal area. Phytoplankton in the BPNS and the southern North Sea in general is dominated by diatoms, dinoflagellates, and the colonial haptophyte *Phaeocystis* (Rousseau et al., 2008; Ford et al., 2017). However, because data on *Phaeocystis* are very incomplete for the 1970s, we have restricted our analyses to diatoms and dinoflagellates alone. Moreover, to avoid errors related to species identification issues we analyzed data at the genus level. Focus is on two periods: in the 1970s (1970-1978) and the 2000s (2003-2010) for which more or less complete datasets were available (cf. Nohe et al., 2018). Because of large data gaps and methodological inconsistencies in the environmental (physical and chemical) data from the BPNS, we used abiotic (nutrients, suspended particulate matter (SPM) and SST) and biotic (chlorophyll a) data from a monitoring transect (Walcheren) of the Dutch Rijkswaterstaat (RWS) long-term monitoring program which is situated just outside the BPNS. Our analyses revealed fundamental changes in diatom and dinoflagellate community structure, biomass and seasonal dynamics, which we hypothesize to be largely caused by increasing SST and water transparency, and altered nutrient loads and ratios.

3.3. Material and Methods

3.3.1. Study area

The physical and chemical environment of the BPNS is strongly determined by meteorological forcing, which drives seasonal and inter-annual variability in wind, currents, temperature and precipitation, and hence also suspended particulate matter (SPM) concentrations, underwater light climate, riverine discharge and nutrient dynamics (Ruddick and Lacroix, 2006). The BPNS is permanently mixed due to its shallowness (< 40 m), water influx from the Atlantic Ocean and strong tidal currents (van Leeuwen et al., 2015; De Galan et al., 2004). It receives most of its freshwater input from the Scheldt river which results in a marked east-west salinity gradient, from about 31.5 psu close to the Scheldt mouth to about 34 psu further offshore, the exact position of which depends on Scheldt discharge and Atlantic water intrusion (De Galan et al., 2004). The nutrient status of the BPNS as well is determined by this Atlantic influx (enriched by nutrients from a.o. the rivers Seine and Somme), local riverine inputs with high nutrient loads (mainly the Scheldt river, but also the Rhine and Meuse) and atmospheric deposition. As a result, as for salinity, there is a pronounced east-west gradient from high nutrient concentrations near the Scheldt river mouth to lower concentrations offshore (from > 80 to ± 10 µM for dissolved inorganic nitrogen

(DIN), 1.5-2 to < 0.5 µM for phosphate, and > 40 to < 10 µM for silicate in winter 2003) (Brion et al., 2006). SPM concentrations near the coast are always very high (from 100 mg L^{-1} up to several thousands of mg L^{-1}) and offshore always low (< 5 mg L^{-1}), while in the transition area concentrations can be high (5-50 mg L^{-1}) (Belgische Staat, 2012).

3.3.2. Datasets

All data were extracted from the Belgian Phytoplankton Database (BPD), a quality checked and standardized compilation of phytoplankton count data (cells L^{-1}) from various research projects since 1968 (Nohe et al., 2018). The data span two periods, 1970 to 1978 and 2003 to 2010 (hereafter referred to as '1970s' and '2000s') for which the datasets were most complete (Nohe et al., 2018). Samples were analysed with the Utermöhl method with the use of an inverted microscope (Utermöhl, 1958). We focused on diatom and dinoflagellate data as these represent two important phytoplankton groups in the Southern part of the North Sea (Ford et al., 2017; Lefebvre et al., 2011), and because data for the third most important taxon, the colonial haptophyte *Phaeocystis* sp., were largely lacking for the 1970s. *Noctiluca* counts were excluded, as those were not representative due to the large cell size. In addition, all data below 5 m depth were excluded. In order to ensure optimal taxonomic consistency between the 1970s and the 2000s, we aggregated all count data to the genus level (see also e.g. Beliaeff et al., 2001; Peperzak, 2010; Vyverman et al., 2007). Because benthic diatoms were often not identified to the genus level (but were sometimes reported as a single functional group), all benthic taxa were aggregated into a single unit. For some analyses, count data were converted to biovolume data. Based on geometrical shape and size data taken from literature and online sources, diatom and dinoflagellate biovolumes were calculated to the lowest taxonomic level possible (usually species) (Hillebrand et al., 1999; Hoppenrath et al., 2009; Horner, 2002; McQuoid and Nordberg, 2003; Naz et al., 2013; Throndsen et al., 2007). Following Terseleer (2014), diatoms were combined into three size/volume classes, viz. small (< 6,000 µm³), intermediate (6,000 µm³ - $4.9*10^4$ µm³) and large (> $4.9*10^4$ µm³). Because of data gaps and inconsistencies (a.o. caused by changes in analytical methods for nutrient analyses) in the environmental data available for the BPNS, environmental data [Dissolved Inorganic Nitrogen (DIN), Dissolved Inorganic Phosphorus (DIP), Dissolved Silica (DSi), nutrient ratios, sea surface temperature (SST) and SPM] were obtained from two stations in the Walcheren transect of the Dutch Rijkswaterstaat long-term monitoring program (Figure 1; note that for the 1970s data were only available for 1975-1978). For some graphs and analyses, a distinction was made between coastal and offshore stations (Figure 1), based on known gradients in salinity, nutrients, and differences in water turbidity and mixing

regimes (Baeyens et al., 1984; van Leeuwen et al., 2015; Capuzzo et al., 2018; Capuzzo et al., 2015; De Galan et al., 2004).

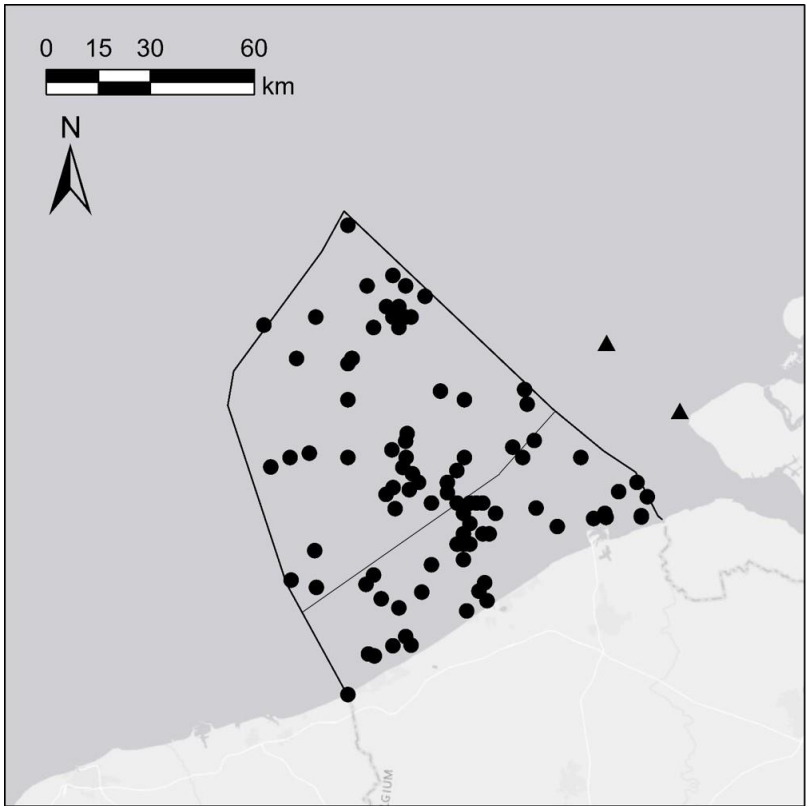

Figure 1 The Belgian Part of the North Sea (BPNS) with sampling locations and division in on- and offshore area. The Walcheren stations (RWS monitoring) in the Dutch coastal area are indicated as triangles.

3.3.3. Data analyses

Overall changes in diatom and dinoflagellate total abundance and biovolume, the dinoflagellate/diatom+dinoflagellate abundance ratio (Hernández-Fariñas et al., 2014) and environmental data between the 1970s and the 2000s were evaluated using one-way ANOVAs. Prior to analysis, data were tested on normality and transformed if needed.

To unravel changes in seasonal trends in the datasets in more detail, the total coastal diatom and total coastal dinoflagellate dataset were analysed with a general additive mixed modelling (GAMM) approach. For this, the *mgcv* package (Wood, 2017) in the open-source software R was used (RStudioTeam, 2017). Space (sampling location set as random effect) and time (seasonal smoother) were incorporated in the models. By incorporating 'method' as a random effect, a possible taxonomist dependent identification bias was excluded from the models. The data were $\log_{10}(x)$-transformed prior to analysis and a Gaussian distribution was used in the models. In addition, a log-link function was used to ensure that the fitted values are always positive (Philippart et al., 2010). One cubic regression spline (cc) *f(JulianDay)* was used to model the seasonal trend. This type of smoother ensures that the value of the smoother at the far left point

of the gradient is the same as at the far right point which is convenient to model an annual cycle (Zuur et al., 2009). The models were expanded with different residual auto-correlation structures: autoregressive correlation *corAR1*, continuous autoregressive correlation *corCAR1*, compound symmetry structure *corCompSymm*, exponential correlation *corExp*, Gaussian *corGaus* and linear correlation *corLin*, rational quadratic *corRatio* and spheric spatial correlation *corSpher*. The best model for each dataset was identified by the lowest Akaike information criterion (AIC), which takes into account the model fit versus the complexity of the model calculation (Zuur et al., 2009).

Changes in community composition and seasonality were visually evaluated using heatmaps (81 taxa) and Principal Components Analysis (PCAs), based on $\log_{10}(x+1)$ transformed relative abundance data of genera which reached a total relative abundance of 10 % in at least one sample (41 diatom and 6 dinoflagellate taxa), was carried out using Canoco 5 (http://www.canoco5.com/).

Finally, for a fulcrum analysis (Kromkamp and Van Engeland, 2010), the relative annual cumulative abundance was calculated for the total diatom and total dinoflagellate abundance data. The day of the year on which 50 % of the total annual phytoplankton abundance was reached, was identified by fitting a binomial *glm* smoother on the data.

3.4. Results

3.4.1. Environmental changes in the Walcheren transect between the 1970s and 2000s

In the 1970s, DIN but especially DIP values were significantly higher than in the 2000s, especially in summer (Figure 2, Table 1). In contrast, DSi values showed no significant overall change, except in summer and autumn when DSi values were slightly but significantly higher in the 2000s (Figure 2, Table 1). As a result, DIN:DIP and DSi:DIP ratios significantly increased from the 1970s to 2000s (except for summer DIN:DIP values which remained similar), reaching average values close to Redfield ratios (16:1 for DIN:DIP and 15:1 for DSi:DIP) in winter and early spring (Table 1). SST significantly and markedly increased between the two periods in winter and summer, with values being on average 1.16 °C and 1.3 °C higher respectively, but not in spring and autumn (note that the apparent significant overall decrease between the two periods is due to the fact that much more winter data are included in the 2000s and more summer data in the 1970s). Winter SPM values were markedly and significantly lower in winter during the 2000s, but did not differ during the rest of the year (Figure 2, Table 1). Chl a concentration was significantly higher during spring in the 2000s, but lower during winter and especially summer (Table 1).

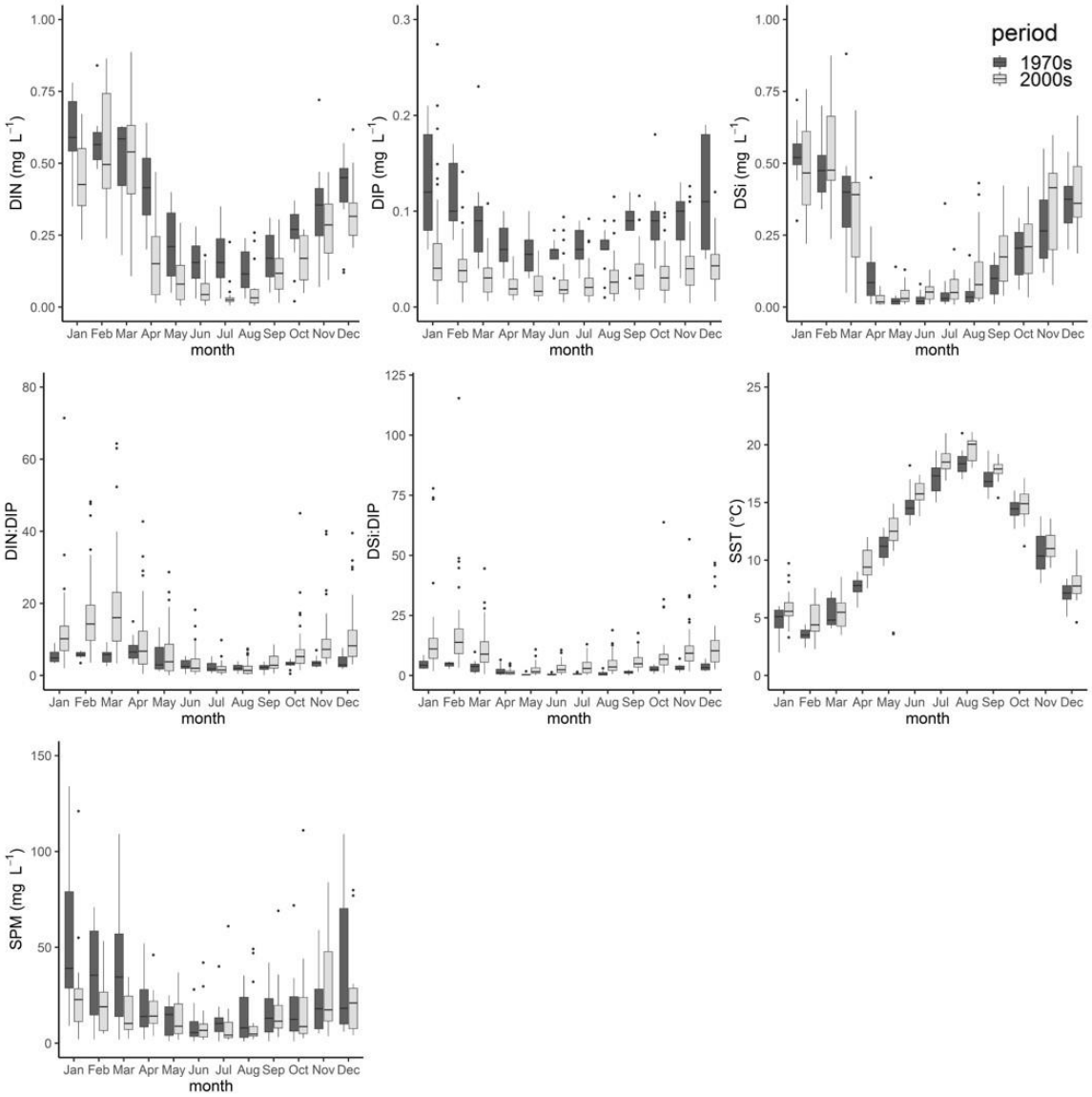

Figure 2 Seasonality of environmental parameters measured in the Walcheren transect (station Walcheren2 and Walcheren20) (Dutch North Sea coast). Data deriving from the long-term monitoring of the Rijkswaterstaat (RWS).

Table 1 Comparison of abundances, biovolumes, diatom/dinoflagellate ratios and abiotic parameters in the 1970s and 2000s. Mean values, standard deviations (sd) and number of observations (n) are given per study period. *p*-values of ANOVA analyses are given. Abiotic and chlorophyll a data derive from the Dutch long-term monitoring program conducted by the Rijkswaterstaat (RWS).

		1970s			2000s						
variable	**month/season**	**mean**	**sd**	**n**	**mean**	**sd**	**n**	**n_{1970s}/ n_{2000s}**	**$mean_{2000s}$- $mean_{1970s}$**	***p* -value**	**transformation (ANOVA)**
abundances (*10^3 cells L^{-1})											
total diatoms		**214**	**295**	**712**	**746**	**1888**	**493**	**1.44**	**532**	**<0.001**	$log_{10}(x)$
	winter	151	277	157	891	1441	70	2.24	741	<0.001	$log_{10}(x)$
	spring	353	393	200	1103	2671	209	0.96	750	<0.001	$log_{10}(x)$
	summer	186	193	163	337	552	164	0.99	151	0.81	$log_{10}(x)$
	autumn	145	199	192	392	571	50	3.84	247	<0.01	$log_{10}(x)$
total dinoflagellates		**3**	**4**	**450**	**19**	**30**	**196**	**2.30**	**16**	**<0.001**	$log_{10}(x)$
	winter	1	1	69	8	17	34	2.03	6	<0.001	$log_{10}(x)$
	spring	2	2	122	27	38	73	1.67	26	<0.001	$log_{10}(x)$
	summer	4	6	119	22	31	49	2.43	18	<0.001	$log_{10}(x)$
	autumn	3	5	136	8	16	40	3.40	5	0.27	$log_{10}(x)$
Actinoptychus		1.28	3.45	695	5.54	31.26	392	1.77	4	<0.001	$log_{10}(x+1)$
Asterionellopsis		13.12	52.51	696	132.03	832.57	452	1.54	119	<0.001	$log_{10}(x+1)$
Cerataulina		1.76	19.14	670	4.04	16.18	376	1.78	2	<0.001	$log_{10}(x+1)$
Chaetoceros		16.01	114.10	694	57.75	194.06	485	1.43	42	<0.001	$log_{10}(x+1)$
Cylindrotheca		0.65	12.02	585	15.38	41.76	214	2.73	15	<0.002	$log_{10}(x+1)$
Cymatosira		0.07	0.63	471	40.21	41.40	109	4.32	40	<0.003	$log_{10}(x+1)$
Dactyliosolen		4.40	16.43	647	3.05	22.17	371	1.74	-1	<0.004	$log_{10}(x+1)$
Delphineis		2.72	7.04	651	8.02	5.57	126	5.17	5	<0.005	$log_{10}(x+1)$
Ditylum		0.11	0.62	561	2.25	6.74	411	1.36	2	<0.006	$log_{10}(x+1)$
Guinardia		33.51	95.78	695	118.99	299.96	456	1.52	85	<0.001	$log_{10}(x+1)$
Leptocylindrus		3.98	26.65	676	20.56	85.28	394	1.72	17	<0.001	$log_{10}(x+1)$
Odontella		1.78	4.41	698	4.67	11.76	435	1.60	3	<0.001	$log_{10}(x+1)$
Paralia		22.86	36.18	706	78.50	562.63	438	1.61	56	<0.001	$log_{10}(x+1)$
Pseudo-nitzschia		5.24	21.25	673	66.16	180.05	461	1.46	61	<0.001	$log_{10}(x+1)$
Rhaphoneis		9.20	16.75	705	12.17	59.60	422	1.67	3	<0.001	$log_{10}(x+1)$
Rhizosolenia		14.01	46.92	707	83.10	629.64	450	1.57	69	<0.001	$log_{10}(x+1)$
Skeletonema		5.57	36.67	678	14.64	77.87	421	1.61	9	<0.001	$log_{10}(x+1)$
Thalassionema		14.76	45.34	690	25.34	108.63	434	1.59	11	0.77	$log_{10}(x+1)$
Thalassiosira		18.43	39.71	706	115.44	549.75	489	1.44	97	<0.001	$log_{10}(x+1)$
benthic diatoms		31.79	77.76	707	7.16	25.49	453	1.56	-25	<0.001	$log_{10}(x+1)$
biovolumes (*10^9 µm³ L^{-1})											
total diatoms		**3.99**	**6.24**	**653**	**25.44**	**65.45**	**455**	**1.44**	**21**	**<0.001**	$log_{10}(x)$
	winter	1.43	1.69	143	17.07	28.46	64	2.23	16	<0.001	$log_{10}(x)$
	spring	5.89	8.05	177	32.89	67.86	192	0.92	27	<0.001	$log_{10}(x)$
	summer	7.02	7.49	155	25.89	80.03	153	1.01	19	<0.001	$log_{10}(x)$
	autumn	1.52	2.22	178	4.48	10.48	46	3.87	3	0.15	$log_{10}(x)$
total dinoflagellates		**0.04**	**0.09**	**450**	**0.52**	**0.97**	**194**	**2.32**	**0.47**	**<0.001**	$log_{10}(x)$
	winter	0.03	0.05	69	0.23	0.51	35	1.97	0.20	<0.001	$log_{10}(x)$
	spring	0.03	0.07	122	0.81	1.35	73	1.67	0.78	<0.001	$log_{10}(x)$
	summer	0.06	0.12	123	0.50	0.63	49	2.51	0.44	<0.001	$log_{10}(x)$
	autumn	0.04	0.10	136	0.24	0.52	40	3.40	0.20	<0.001	$log_{10}(x)$
Dino/diat ratio											
	Jan	0.0115	0.0110	25	0.0098	0.0098	11	2.27	-0.0017	0.66	$log_{10}(x+1)$
	Feb	0.0120	0.0134	25	0.0044	0.0030	10	2.50	-0.0076	0.09	$log_{10}(x+1)$
	Mar	0.0138	0.0089	35	0.0125	0.0156	26	1.35	-0.0012	0.68	$log_{10}(x+1)$
	Apr	0.0106	0.0242	49	0.0159	0.0164	29	1.69	0.0052	0.28	$log_{10}(x+1)$
	May	0.0177	0.0238	41	0.0303	0.0400	25	1.64	0.0126	0.11	$log_{10}(x+1)$
	Jun	0.0255	0.0355	26	0.0225	0.0233	17	1.53	-0.0031	0.78	$log_{10}(x+1)$
	Jul	0.0146	0.0173	34	0.0493	0.0485	16	2.13	0.0347	<0,001	$log_{10}(x+1)$
	Aug	0.0297	0.0357	25	0.0824	0.1328	15	1.67	0.0527	0.06	$log_{10}(x+1)$
	Sep	0.0492	0.0505	53	0.0634	0.0533	14	3.79	0.0141	0.35	$log_{10}(x+1)$
	Oct	0.0372	0.0490	79	0.0449	0.0473	13	6.08	0.0076	0.58	$log_{10}(x+1)$
	Nov	0.0178	0.0126	31	0.0097	0.0082	6	5.17	-0.0081	0.14	$log_{10}(x+1)$
	Dec	0.0108	0.0057	23	0.0091	0.0080	14	1.64	-0.0017	0.45	$log_{10}(x+1)$

Table 1 (continued).

		1970s			2000s						
variable	month/season	mean	sd	n	mean	sd	n	n_{1970s}/n_{2000s}	$mean_{2000s}$-$mean_{1970s}$	*p* -value	transformation (ANOVA)
abiotics											
DIN (mg L^{-1})		**0.29**	**0.19**	**165**	**0.23**	**0.21**	**186**	**0.89**	**-0.06**	<0.01	-
	winter	0.53	0.16	28	0.44	0.18	44	0.64	-0.09	0.04	-
	spring	0.37	0.19	36	0.25	0.24	46	0.78	-0.12	<0.05	-
	summer	0.15	0.08	54	0.06	0.06	48	1.13	-0.09	<0.001	-
	autumn	0.26	0.13	47	0.19	0.11	48	0.98	-0.07	<0.01	-
DIP (mg L^{-1})		**0.09**	**0.07**	**141**	**0.03**	**0.03**	**540**	**0.26**	**-0.05**	<0.001	-
	winter	0.12	0.05	27	0.05	0.04	127	0.21	-0.07	<0.001	-
	spring	0.07	0.04	32	0.03	0.02	136	0.24	-0.04	<0.001	-
	summer	0.06	0.02	43	0.03	0.02	144	0.30	-0.03	<0.001	-
	autumn	0.12	0.12	39	0.04	0.03	133	0.29	-0.08	<0.001	-
DSi (mg L^{-1})		**0.19**	**0.19**	**158**	**0.23**	**0.21**	**186**	**0.85**	**0.04**	0.07	-
	winter	0.45	0.13	28	0.46	0.16	44	0.64	0.01	0.80	-
	spring	0.17	0.21	34	0.13	0.18	46	0.74	-0.04	0.33	-
	summer	0.05	0.06	49	0.08	0.09	48	1.02	0.03	<0.05	-
	autumn	0.19	0.12	47	0.25	0.15	48	0.98	0.06	<0.05	-
DIN:DIP		**3.86**	**2.60**	**139**	**8.76**	**11.76**	**538**	**0.26**	**4.90**	<0.001	-
	winter	4.95	1.89	26	15.47	16.90	127	0.20	10.52	<0.01	-
	spring	5.94	3.67	32	11.13	11.76	134	0.24	5.19	<0.05	-
	summer	2.46	1.37	43	2.47	2.69	144	0.30	0.01	0.98	-
	autumn	2.98	1.35	38	6.79	6.81	133	0.29	3.81	<0.001	-
DSi:DIP		**3.16**	**2.03**	**138**	**8.19**	**10.86**	**542**	**0.25**	**5.03**	<0.001	-
	winter	4.43	1.73	26	15.94	16.23	127	0.20	11.51	<0.001	-
	spring	1.93	2.35	32	7.75	6.81	134	0.24	5.82	<0.05	-
	summer	0.68	0.58	42	3.87	3.33	144	0.29	3.19	<0.001	-
	autumn	2.42	1.40	38	8.92	8.85	133	0.29	6.50	<0.001	-
SST (°C)		**12.27**	**5.05**	**165**	**12.02**	**5.33**	**191**	**0.86**	**-0.25**	<0.001	-
	winter	5.09	1.82	28	6.25	2.09	44	0.64	1.16	<0.05	-
	spring	8.44	2.61	35	9.05	3.25	54	0.65	0.61	0.36	-
	summer	16.9	1.96	54	18.20	1.95	48	1.13	1.30	<0.01	-
	autumn	14.04	2.91	48	14.65	2.94	45	1.07	0.61	0.32	-
SPM (mg L^{-1})		**21.39**	**23.28**	**164**	**19.02**	**23.99**	**188**	**0.87**	**-2.37**	0.42	$log_{10}(x+1)$
	winter	42.62	34.56	30	28.44	36.65	44	0.68	-14.18	0.04	$log_{10}(x+1)$
	spring	23.23	23.43	35	14.94	10.86	48	0.73	-8.29	0.25	$log_{10}(x+1)$
	summer	10.89	9.76	52	10.77	13.61	48	1.08	-0.12	0.57	$log_{10}(x+1)$
	autumn	18.1	14.94	47	22.72	23.95	48	0.98	4.62	0.49	$log_{10}(x+1)$
chl a (µg L^{-1})		**5.91**	**5.58**	**163**	**6.47**	**9.18**	**185**	**0.88**	**0.56**	0.49	-
	winter	2.07	1.58	30	1.35	0.87	43	0.70	-0.72	<0.05	-
	spring	7.23	5.91	33	14.79	14.12	48	0.69	7.56	<0.01	-
	summer	9.09	6.61	51	5.79	4.55	48	1.06	-3.30	<0.01	-
	autumn	4.05	3.20	49	3.29	2.00	46	1.07	-0.76	0.17	-

3.4.2. Changes in diatom and dinoflagellate cell numbers, biovolume and seasonal trends between the 1970s and 2000s

Both diatom and dinoflagellate cell numbers significantly increased between the 1970s and 2000s (Table 1, Figure 3), especially in the coastal zone (Supplementary Figure 1). Diatoms mainly increased during winter and spring, but not in summer, while dinoflagellates mainly increased during spring and summer (Table 1, Figure 3A, Figure 3D). Similar trends can be seen in diatom and dinoflagellate total biovolume, but here diatom values are significantly higher from winter to summer, but not in autumn, while dinoflagellate total biovolume is significantly higher in all seasons (Table 1, Figure 3G-L). The trends of 20 important (see below, derived from PCA analysis) diatom taxa underscores the general diatom trend: all genera increased in abundance between

the two periods, except for *Dactyliosolen* and benthic diatoms which significantly decrease, and *Thalassionema* which does not show any change in its annual mean abundance (Table 1).

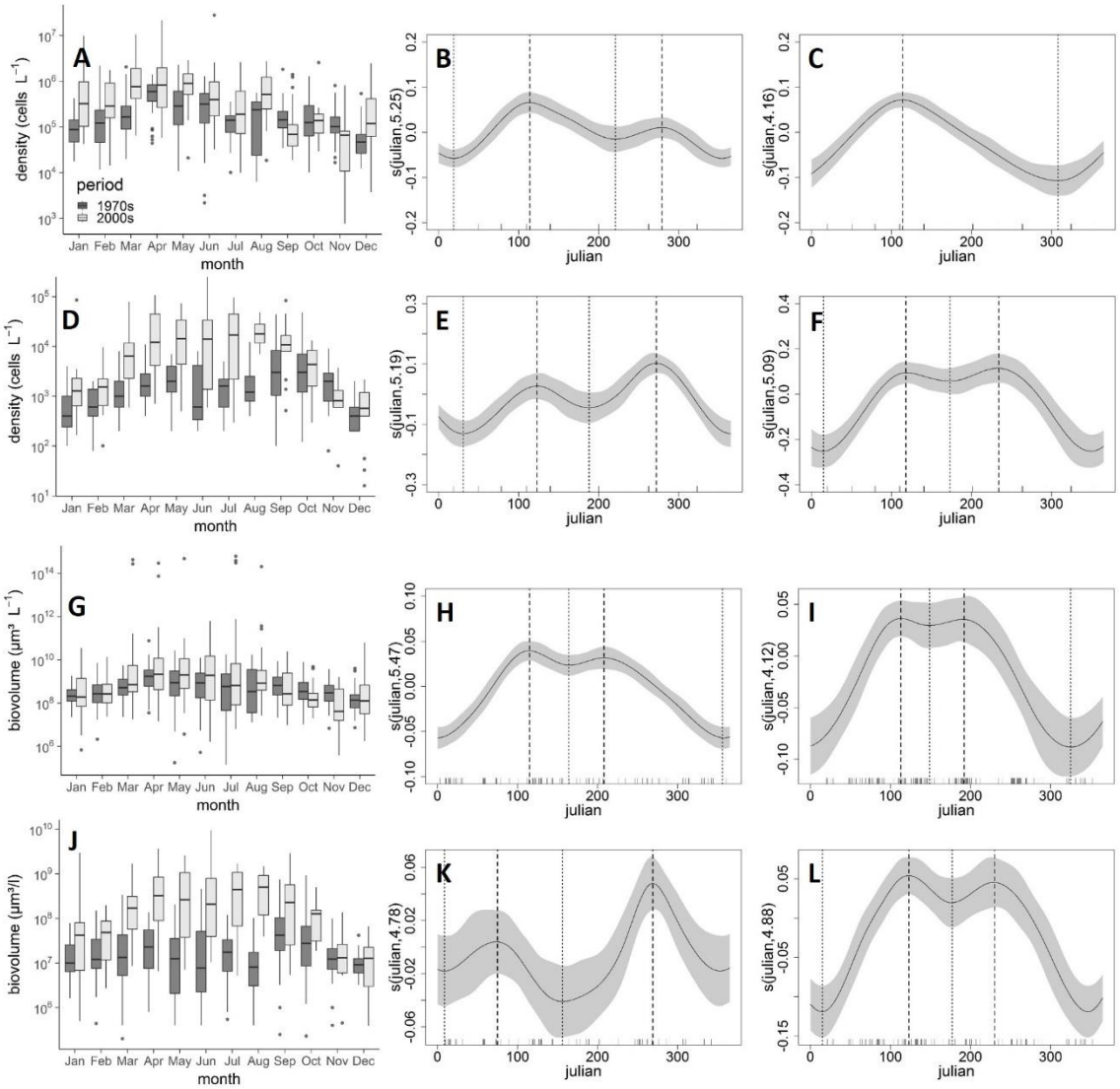

Figure 3 Seasonal trends of the diatom and dinoflagellate abundances (A-F) and biovolumes (G-L). The solid line is the smoothing curve fitted by the GAMM model and the grey area represents the 95 % confidence bands A Diatom abundances, B GAMM diatom abundance in the 1970s, C GAMM diatom abundance in the 2000s, D dinoflagellate abundances, E GAMM dinoflagellate abundance in the 1970s, F GAMM dinoflagellate abundances in the 2000s, G diatom biovolumes, H GAMM diatom biovolumes in the 1970s, I GAMM diatom biovolumes in the 2000s, J dinoflagellate biovolumes, K GAMM dinoflagellate biovolumes in the 1970s and L GAMM dinoflagellate biovolumes in the 2000s. The start of the blooms are indicated as black dotted lines. The data of the bloom peak is indicated with a black dashed line. The numbers between brackets on the y-axes indicate the estimated degrees of freedom. The higher the number the more non-linear the curve, while numbers close to 1 indicate linear functional response.

For the GAMM models, different auto-correlation structures were tested and the models were assessed through their AIC results (Supplementary Tables 1-4, Supplementary Tables 6-9). Table 5 and Table 10 summarize for each dataset the model configurations and model results of the best model fits. In the 1970s, diatom cell numbers typically display two annual peaks, in spring and autumn (Figure 3A, Figure 3B), while in the 2000s only one more extensive peak, which starts

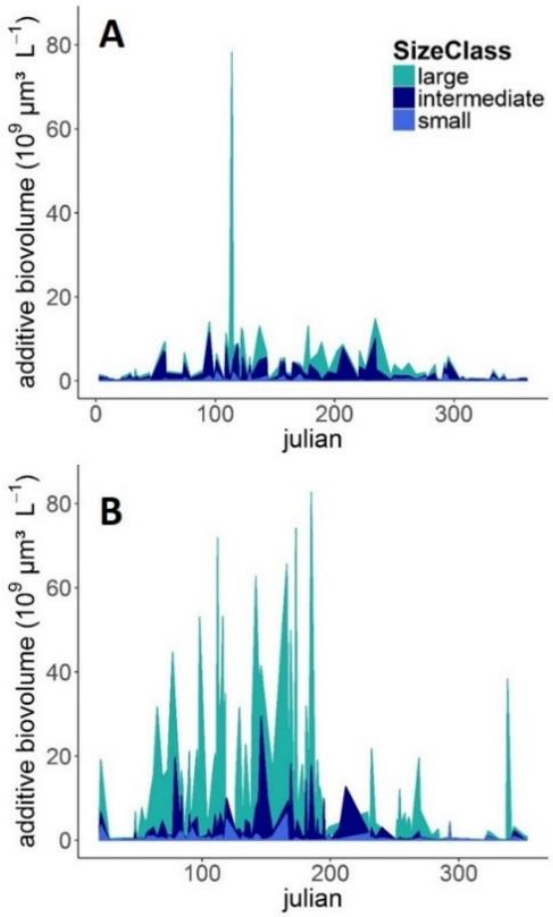

Figure 4 Annual cycle of small, intermediate and large diatoms in the onshore stations of the BPNS averaged for A 1970s and B 2000s.

earlier and is followed by a more pronounced decline in autumn, can be observed (Figure 3A,C). The total biovolume data underscore this trend, with two (albeit less distinct) peaks (spring, summer) in the 1970s (Figure 3G,H) which merge and become more pronounced in the 2000s, followed by a marked decrease in autumn (Figure 3G,I). In both dinoflagellate cell numbers and total biovolume, the spring and autumn blooms merge into one extended and pronounced spring to autumn growing season, with the highest values in the summer months (Figure 3D-F, J-L). As a result of the strong summer increase in dinoflagellate cell numbers, dinoflagellate/diatom+dinoflagellate ratios were higher from spring to autumn in the 2000s, but this increase was only significant for July (Table 1). While total biovolume of all size classes has increased between the 1970s and the 2000s, the contribution to the overall diatom biovolume increase between the two periods is mainly due to an increase in the contribution of the largest size class (a. o. *Guinardia flaccida*, *Bacteriastrum* spp., *Rhizosolenia* spp., *Rhizosolenia styliformis*, *Guinardia striata*, *Rhizosolenia setigera*, *Lauderia annulata*, *Coscinodiscus* spp., *Cerataulina pelagica*, *Coscinodiscus concinnus*), especially in early spring and summer (Figure 4, Supplementary Figure 2), resulting in one extended peak which has a prominent earlier start in late winter. The fulcrum analyses (Figure 5) confirm this forward shift in the diatom bloom, with the fulcrum being reached three weeks earlier in the 2000s than in the 1970s. For dinoflagellates this forward shift is even more pronounced, with the fulcrum being attained no less than two months earlier.

3.4.3. Changes in diatom and dinoflagellate community structure and seasonal dynamics

In total, 57 diatom and 25 dinoflagellate genera have been observed in the BPNS since the 1970s. Of these, 13 diatom and 11 dinoflagellate genera were only recorded in the 1970s (Figure 6). Most of these were rare, except for the diatoms *Cyclotella* and *Discostella*, and the dinoflagellates

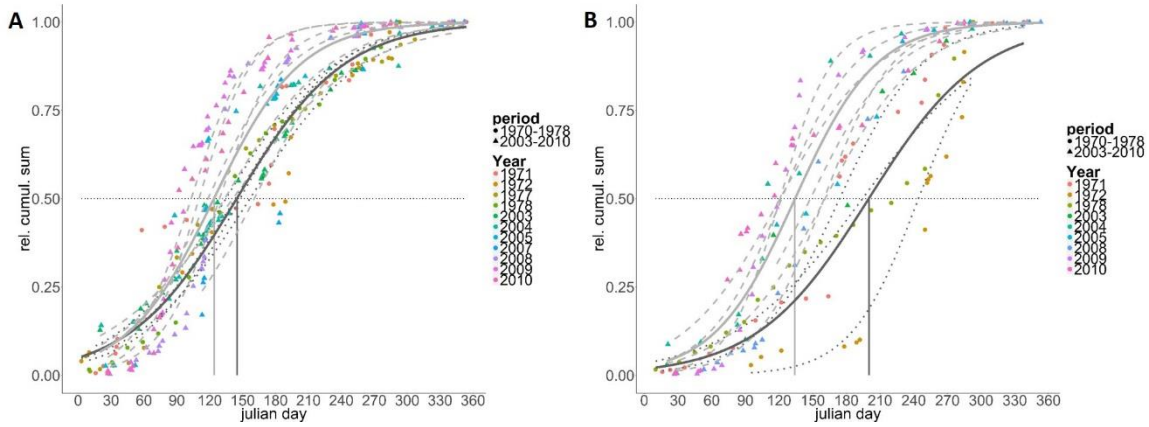

Figure 5 Fulcrum analysis of the relative cumulative sum of the annual cell abundances. A binomial *glm* smoother function is fitted on each annual data subset. In addition, a 'mean' smoother per period is added (in bold). Black: 1970s, grey: 2000s. The Julian day on which half of the annual phytoplankton cell abundance (the fulcrum) is reached is indicated. A Diatoms, B dinoflagellates.

Glenodinium and *Peridinium*. Five diatom (*Corethron*, *Detonula*, *Hemiaulus*, *Lithodesmium* and *Brockmanniella*) and dinoflagellate genera (*Diplopsalis*, *Gonyaulux*, *Heterocapsa*, *Katodinium* and *Pyrophacus*) were only observed in the 2000s. Many diatom genera have markedly increased between the 1970s and the 2000s, e.g. *Asterionellopsis*, *Chaetoceros*, *Cylindrotheca*, *Ditylum*, *Guinardia*, *Odontella*, *Pseudo-nitzschia*, *Rhizosolenia*, *Thalassiosira*, *Thalassionema* and *Plagiogrammopsis* (Figure 6, Supplementary Figure 3). Many of these (except *Pseudo-nitzschia*) also have higher winter abundances, display an extended growing season until September (without the July-August gap apparent in the 1970s), and have overall lower values in October and November, all of which corroborates the trends observed in the total diatom abundance and biovolume data (Figure 3, Figure 4 and Figure 5). The only category that seems to decline between the two periods are benthic diatoms (Figure 6). Several dinoflagellate genera as well have increased since the 1970s, such as *Alexandrium*, *Gymnodinium*, *Gyrodinium, Prorocentrum*, *Protoperidinium* and *Scrippsiella*, with especially higher values in late spring and summer, confirming the trends observed in Figures 3D-F, J-L.

Changes in community structure and seasonal dynamics were investigated using PCA (Figure 7). To avoid the analysis being heavily biased by the general increase in cell numbers between the two periods, we used the relative abundance values of the 47 most important diatom and dinoflagellate genera (cf. above). Data from both periods were used in a joint PCA (capturing about 38 % of the total variation in the dataset along the first two axes), after which samples belonging to each period were (per season) highlighted with different (light vs dark) colours to reveal shifts in community structure in autumn-winter, spring and summer respectively (Figure 7A-C). In both periods, a seasonal succession in community structure is present, with a change from an autumn-winter community (1) dominated by diatoms with a benthic to tychoplanktonic life style (e.g.

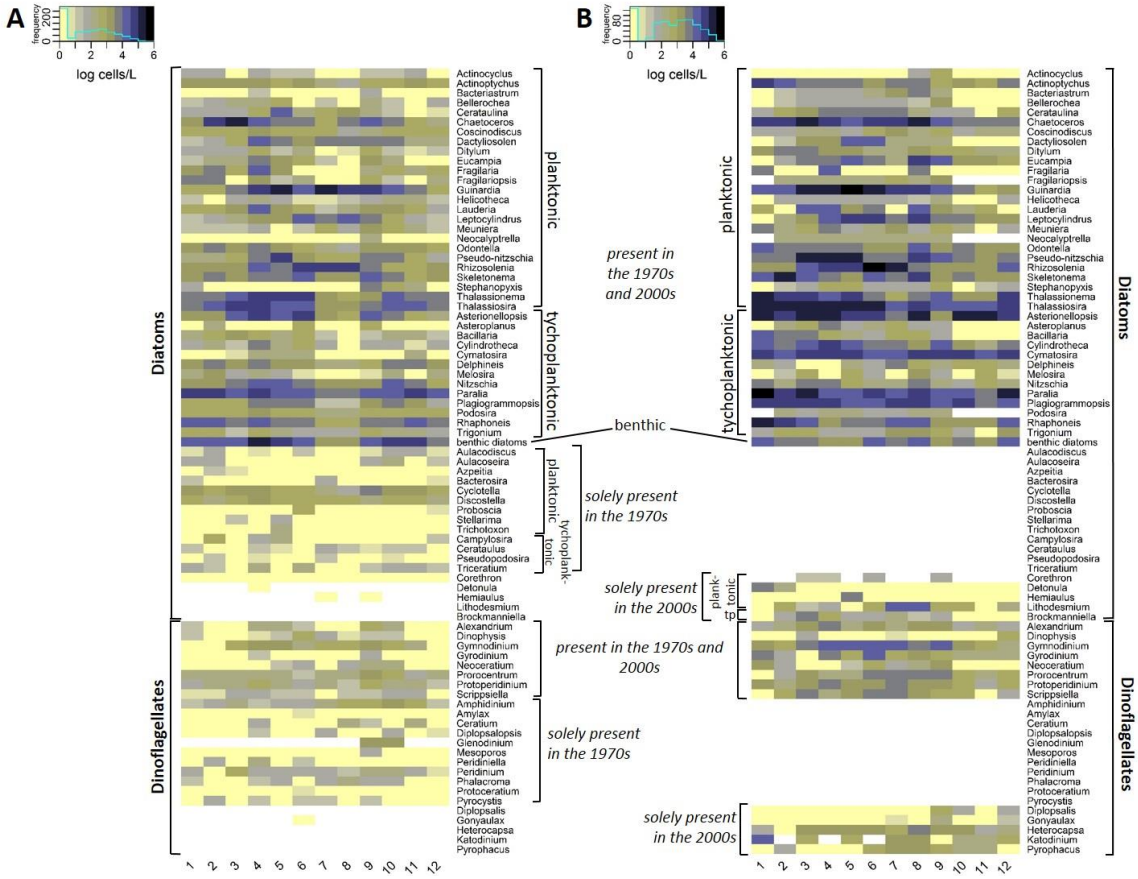

Figure 6 Heatmaps and histograms of seasonal changes in the logarithmically transformed diatom and dinoflagellate cell counts based on the monthly mean values for A 1970s and B 2000s.

benthic diatoms, *Paralia*, *Rhaphoneis*) and smaller, more heavily silicified, planktonic diatom taxa (e.g. *Actinoptychus*, *Thalassionema, Thalassiosira*), over an intermediate community (2), mainly characterized by *Asterionellopsis*, *Chaetoceros* and *Pseudo-nitzschia*, to a summer (June to August) community (3) with mainly larger, often colonial and lightly silicified species such as *Cerataulina*, *Dactyliosolen*, *Guinardia*, *Leptocylindrus* and *Rhizosolenia* (Figure 7D). Strikingly, while especially community 1 and 3 are distinct in the 1970s, with community 2 being less prevalent, there is a clear shift towards community 2 in almost all seasons in the 2000s, revealing a trend towards seasonal homogenization of the typical succession present in the BPNS in the 2000s. This is confirmed by the seasonal trends in the individual taxa (Supplementary Figure 3, Figure 7): despite significant increases in mean cell numbers between the 1970s and the 2000s in the 20 taxa best fitted in the PCA (except *Thalassionema* and benthic diatoms, Table 1), there is an overall decline in median abundance values (Supplementary Figure 3) and relative abundances (Figure 7) in some important representatives of community 1 (benthic diatoms, *Paralia*, *Rhaphoneis* and *Thalassionema*) and community 3 (*Cerataulina*, *Dactyliosolen* and *Leptocylindrus*), with other genera of community 1 displaying a shift towards earlier peak abundances (winter-early spring for *Thalassionema* and *Thalassiosira*) and important representatives of community 3 showing a clear

decline in late summer and autumn (*Guinardia*, *Rhizosolenia*). In contrast, almost all representatives of community 2 show a pronounced increase in median abundance values (Supplementary Figure 3) and relative abundances (Figure 7) throughout the year (especially *Chaetoceros*, *Ditylum* and *Pseudo-nitzschia*).

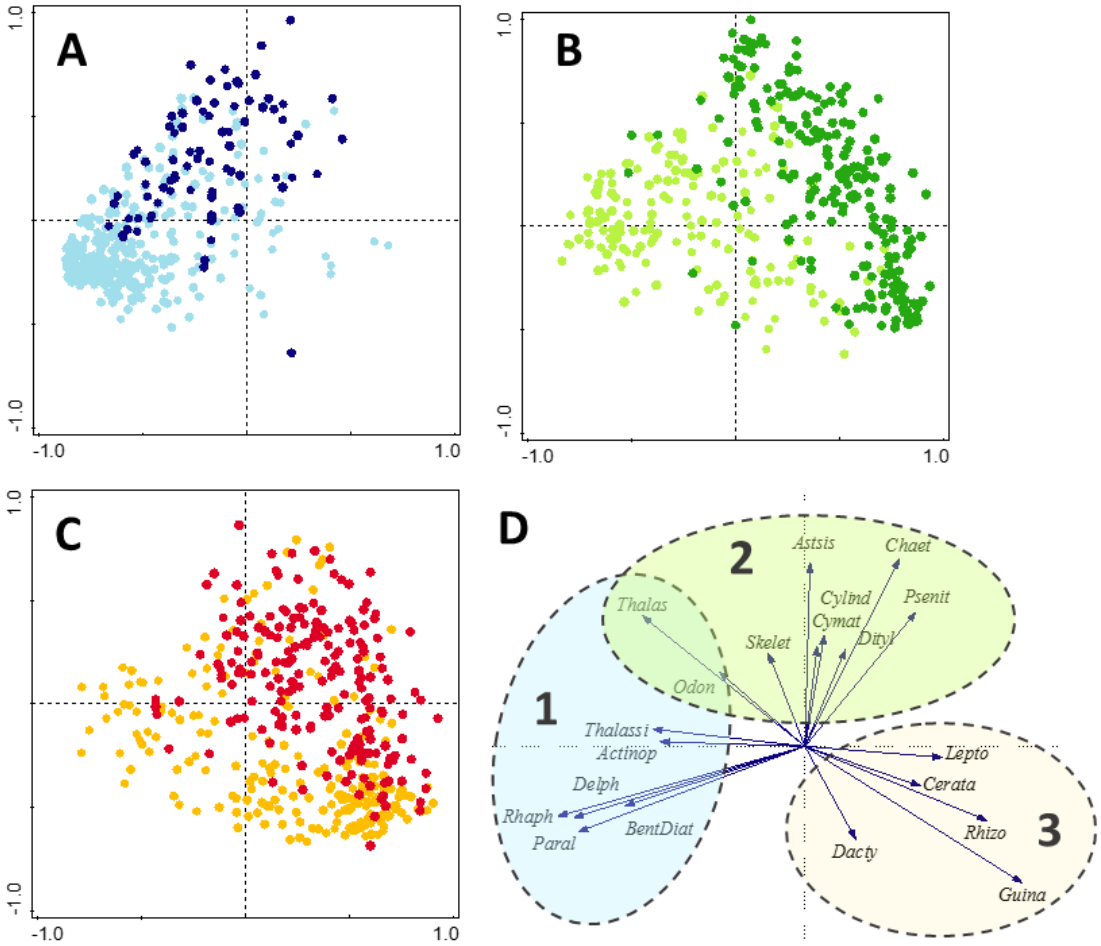

Figure 7 Principal Component Analysis (PCA) of the logarithmically transformed diatom and dinoflagellate relative abundance data. A Autumn-winter, B spring, C summer, D genera. Ellipses indicate the three typical successive communities in the BPNS. Darker colors represent the 2000s (A-C). Autumn/winter: September-February, spring: March-May, summer: June-August. Only the 20 best fitted genera are shown. Supplementary Figure 4 shows all fitted genera.

3.5. Discussion

Using a newly compiled and standardized dataset of long-term phytoplankton count data from the shallow and heavily impacted Belgian part of the North Sea, we contrasted biomass, seasonality and structure of diatom and dinoflagellate communities between the 1970s and 2000s. This revealed the following major changes: (1) a pronounced increase in diatom and dinoflagellate total biovolume respectively, with diatoms mainly increasing from winter to summer (but not in autumn), and dinoflagellates in spring and summer; (2) a shift from a bimodal annual diatom bloom pattern (spring and summer-autumn) to a single, more extended growing

season which starts 3 weeks earlier (February), ends in mid-summer (July) and is mainly dominated by large diatoms; (3) a similar shift from a bimodal annual bloom pattern in dinoflagellates to a single, extended bloom from May to September; (4) a marked change in diatom community structure, from seasonally distinct communities in the 1970s to a seasonally more homogenized community in the 2000s; and (5) a significant increase in potentially harmful algal species, such as *Pseudo-nitzschia* and several dinoflagellate genera (e.g. *Prorocentrum*). The above changes coincided with major shifts in the abiotic environment, with in the 2000s higher average winter and summer temperatures, lower winter SPM, and lower DIN and especially DIP loads but equal DSi, resulting in increased nutrient ratios more closely approximating Redfield ratios in winter and early spring. Below, we compare these trends with literature data from the BPNS and the wider North Sea area, and evaluate potential mechanisms and processes underlying these changes.

3.5.1. Diatom and dinoflagellate abundance and biomass have increased from the 1970s to 2000s

Many studies have reported an increase in overall phytoplankton biomass in the North Sea from the 1970s to the 1990s/2000s (Antoine et al., 2005; Philippart et al., 2000; Raitsos et al., 2014), especially in association with a pronounced (and almost Northern Hemisphere wide) regime shift in the late 1980s (Reid and Edwards, 2001; Beaugrand et al., 2014; Beaugrand et al., 2015). Since the late 1990s however, phytoplankton biomass and/or primary production in many regions of the North Sea appear to have stabilized (Cadée and Hegeman, 2002; Prins et al., 2012; Capuzzo et al., 2015) or even decreased (Capuzzo et al., 2018; Desmit et al. in rev., see Chapter 4). Data on diatoms and dinoflagellates alone are rare and usually more limited to the most recent decades. Most long-term data (i.e. including the 1970s) sets are based on CPR count data and report conflicting trends: while Hinder et al. (2012) and Beaugrand et al. (2014) both report increases in diatom abundance in the wider North Sea area from the 1970s to the 2000s, Leterme et al. (2005) report a decrease in diatoms and an increase in dinoflagellates in the NE Atlantic Area (including the North Sea) during the same period. More complete phytoplankton count data from the North Sea are available from time series from Germany, The Netherlands and France. Wiltshire et al. (2008, 2015) report increased winter diatom densities off Helgoland since the late 1980s, and a strong general increase in diatom cell numbers during the 2000s. Most other diatom and dinoflagellate datasets from the Southern North Sea only date back to the (early) 1990s, but here as well significant increases in both diatoms and dinoflagellates have been reported for many coastal stations in the southern Dutch part of the North Sea (Baretta-Bekker et al., 2009; Alvarez-Fernandez and Riegman, 2014), especially in the 2000s (Prins et al., 2012), while increases in

diatoms and dinoflagellates were observed on the French North Sea coast (Hernández-Fariñas et al., 2014).

Our data from the BPNS are thus in line with the overall increasing trend in phytoplankton biomass since the 1970s (even taken into account stable or slightly decreasing values since the 1990s). Increases in phytoplankton biomass can be caused by several bottom-up or top-down factors (or combinations thereof), such as eutrophication, increased light availability and SST, changes in stratification (related to climate warming or reduced wind regimes), or changes in food web structure. The observed increases in diatoms and dinoflagellates are most likely not caused by changes in nutrient concentrations in the BPNS, but rather their altered ratios. Our data (Table 1, Figure 2) and literature data (Lefebvre et al., 2011; Prins et al., 2012; Burson et al., 2016) clearly show that overall DIN and especially DIP levels have declined in the southern part of the North Sea since the 1970s. The unbalanced reduction in DIN and DIP however also affected nutrient ratios, with more balanced DIN:DIP often exceeding the Redfield ratio in the 2000s, especially in winter and spring (McQuatters-Gollop et al., 2007; Burson et al., 2016). DSi levels have remained stable or even increased (in summer and autumn, Table 1, Figure 2, see also Alvarez-Fernandez and Riegman, 2014; Philippart et al., 2007; Prins et al., 2012). This has been attributed to an increase in riverine DSi transport to the sea as a result of reduced riverine eutrophication and hence lower DSi uptake by riverine diatom blooms (Prins et al., 2012). The more balanced DIN:DIP ratios in combination with stable and more balanced DSi levels may have contributed to the observed increase in diatom biomass since the 1970s. The observed increase in diatom and dinoflagellate biomass coincides with a significant increase in winter and summer SST since the 1970s (Table 1). This shift was most pronounced in the late 1980s (Raitsos et al., 2014), and coincided with a stepwise increase in phytoplankton biomass (Reid and Edwards, 2001; Beaugrand, 2004; Raitsos et al., 2014). This warming trend has been linked with a general change in hydroclimatic conditions, including a positive phase in the North Atlantic Oscillation (NAO) and increased oceanic inflow from the Atlantic (Garcia-Soto et al., 2009; Raitsos et al., 2014; Ottersen et al., 2001; Pingree, 2005). Our data however suggest that the long-term SST trend mainly reflects the long-term global warming trend, as the 2000s are generally characterized by a negative winter NAO index which is usually associated with negative temperature anomalies (Alheit et al., 2012). The tight relationship between SST and phytoplankton biomass is supported by observations of McQuatters-Gollop et al. (2007) and Raitsos et al. (2014) who showed that North Sea ocean colour anomalies very closely tracked SST changes in the North Sea since the 1950s. The exact mechanism(s) underlying the impact of SST on phytoplankton biomass remain as yet unclear. Increased SST can enhance species-specific growth rates (Montagnes and Franklin, 2001), which

can result in shifts in community structure (see below), but indirect effects such as increased stratification and changes in top-down control related to latitudinal shifts in grazers (McQuatters-Gollop et al., 2011) can also be important. Given the fact that BPNS waters are well-mixed throughout the year, an effect of enhanced stratification seems unlikely. A long-term decline in calanoid copepods in the German Bight (Boersma et al., 2015) has been linked with a simultaneous increase in phytoplankton (Wiltshire et al., 2015). A similar decline in small copepods was also reported for the North Sea by Capuzzo et al. (2018). Copepods are also important grazers on phytoplankton in the BPNS (Daro et al., 2006), but to our knowledge no information is available on zooplankton long-term trends in the BPNS. Outside the BPNS, zooplankton long-term data are available from the CPR survey (Beaugrand, 2004). Finally, increased phytoplankton biomass may also be related to higher light availability. Light is a primary control on phytoplankton growth in the North Sea area (Wiltshire et al., 2008), and our data from the BPNS show that SPM values (which are a main determinant of underwater light climate) have significantly declined in winter (Table 1). Increases in water transparency in the North Sea since the 1970s have also been reported by McQuatters-Gollop et al. (2007) and Wiltshire et al. (2008). The lower SPM concentration in the 2000s might have been caused by the recovery of the microphytobenthic algae biofilm, which contributes to the stability of the surface layer of sediments and thereby reduces resuspension (Madsen et al., 1993; Capuzzo et al., 2015). A second factor might be changes in trawling activities, which not only alter the community of bottom filter feeders, but are also linked to changes in water clarity by disturbance and resuspension of bottom sediments (Callaway et al., 2007; Jennings et al., 1999; Capuzzo et al., 2015). In addition, changes in the weather pattern, which is effected by the NAO, alters wind, waves and residual currents, leading to changes in the hydrodynamics (Capuzzo et al., 2015; Fettweis et al., 2012). Finally, the strength of the coastal erosion and riverine sediment transport to the BPNS might have changed (Fettweis et al., 2012; Capuzzo et al., 2015).

The increase in dinoflagellate biomass between the 1970s and 2000s is striking, and corroborates reports of more recent trends in other parts of the North Sea (Baretta-Bekker et al., 2009; Prins et al., 2012; Hernández-Fariñas et al., 2014; Hickel, 1998). Dinoflagellates are generally associated with warmer and less turbulent conditions (Baretta-Bekker et al., 2009; Prins et al. 2012). In addition, many dinoflagellates are capable of mixotrophic growth, and their ability to access alternative P sources may explain their dominance during summer when nutrient levels are at their lowest (Burson et al., 2016).

3.5.2. From a bimodal to an unimodal, earlier and extended diatom and dinoflagellate growing season

In the 1970s diatom biomass showed a typical bimodal (spring and summer-autumn) bloom pattern. By the 2000s however, the diatom bloom started already in late winter and persisted until mid-summer, after which there was a strong decline. This remarkable shift in seasonal pattern has also been observed in other long-term phytoplankton datasets from the North Sea (Reid and Edwards, 2001; Gieskes et al., 2007; Philippart et al., 2010; Baretta-Bekker et al., 2012; Raitsos et al., 2014). Forward shifts in the spring bloom have also been noted in the southern bight of the North Sea (Gieskes et al., 2007; Baretta-Bekker et al., 2012; Alvarez-Fernandez and Riegman, 2014), but not further north (e.g. Wadden Sea and Helgoland, Philippart et al., 2008; Wiltshire et al., 2008), although here as well increased winter phytoplankton densities were observed (Wiltshire et al., 2008; Capuzzo et al., 2015).

The earlier start and more intense diatom spring bloom correlates well with increased water transparency, higher winter SST and more favorable nutrient ratios (albeit lower total DIN and DIP concentrations) in late winter and early spring in the 2000s (Table 1). Lohman and Wiltshire (2012) showed that atmospheric circulation patterns in winter, which increased the inflow of warmer and especially more transparent water off Helgoland, favored earlier blooms. Likewise, McQuatters-Gollop et al. (2007) and Raitsos et al. (2014) identified light and SST, but not nutrient concentrations, as the most important controls on spring bloom initiation in the North Sea area. In contrast, an increase in water clarity has been observed in the German Bight, but no shift in the phytoplankton spring bloom dynamics (Wiltshire et al., 2008). The persistently high cell numbers and total biovolume throughout spring and early summer may be related to the increased and more balanced availability of DSi. However, the observed changes in size (increased importance of large species) and community structure (increased importance of lightly silicified species) of the diatom community (see below) suggests however that other factors such as higher SST, improved water transparency, in combination with lower grazing intensity (see above) and higher grazing resistance may be more important factors in explaining the earlier and extended spring-early summer growing season. The earlier decline in diatom cell numbers and biomass in mid-summer is puzzling (also observed in Baretta-Bekker et al. (2009)), but may be related to the strongly increased biomass of dinoflagellates, especially in summer, as dinoflagellates thrive better under warm and less turbulent conditions (cf. above). Finally, it is unclear why in the 2000s autumn blooms of diatoms apparently fail to develop, but here as well competition with dinoflagellates and other phytoplankton may also be involved.

Changes in phytoplankton seasonality may lead to trophic mismatch and altered carbon transfer through the food web (Edwards and Richardson, 2004; Philippart et al., 2010; Schlüter et al., 2012; Townsend et al., 1994; Wiltshire and Manly, 2004). For example, primary consumer species rely especially in late summer and autumn on the phytoplankton carbon production (Philippart et al., 2010), and our study shows that phytoplankton biomass is especially lower in these seasons in the 2000s.

3.5.3. Seasonal homogenization of diatom community structure

One of the most striking differences between the 1970s and 2000s relates to the taxonomic and functional (size) structure of the planktonic diatom communities. Our data (Figure 7) and literature data (e.g. Rousseau et al. (2002)) show that until the 1990s, three seasonally distinct diatom communities could be observed in the BPNS: a winter to early spring community dominated by benthic, tychoplanktonic and more heavily silicified planktonic species, an intermediate spring community dominated by smaller, often colonial species (especially *Chaetoceros* spp.) and a late spring to summer community dominated by large and more lightly silicified diatoms such as *Rhizosolenia* and *Guinardia* spp. From late summer to autumn, the opposite succession was observed, but with overall lower biomass. In contrast, in the 2000s the communities have become more seasonally homogenized, i.e. from early spring to mid-summer the communities have become more similar, with both community 2 (*Chaetoceros*, *Ditylum* and *Pseudo-nitzschia* spp.) and community 3 (*Rhizosolenia* and *Guinardia*) representatives having become more important. As a result, there is a marked shift towards larger taxa in the diatom communities (Figure 4, Supplementary Figure 2), which agrees with observations in the German Bight (Wiltshire et al., 2010). Several factors maybe be related to this shift. In the past, the typical succession between seasonally distinct communities was mainly attributed to DSi availability and grazing susceptibility (Rousseau et al., 2008; Terseleer, 2014): the first community with more heavily silicified species would be dominant in winter and early spring until DSi becomes depleted, after which more lightly silicified species take over. The shift to larger species (community 2 and especially 3) would mainly be related their lower DSi requirement and to their (size-related) higher resistance to grazing (Terseleer, 2014). As DSi levels have not really changed between the two study periods, it seems unlikely that the decline of the typical winter-early spring community is related to DSi availability. Terseleer (2014) also found no link between DSi availability and the magnitude of the first spring bloom, which suggests that other factors than winter nutrient stocks control bloom formation in early spring. Rousseau et al. (2002) pointed out that the onset of the spring bloom was mainly dependent on a specific light threshold. Interestingly, Prins et al. (2012) reported a similar significant increase of *Chaetoceros* (especially *C. socialis*, which is also a dominant species in the

BPNS) since the 1990s in the Dutch part of the North Sea. *Chaetocero*s spp. have been associated with higher SST and light availability (Wiltshire et al., 2015) and appear to prefer higher nutrient availability (Prins et al., 2012). These observations are consistent with an earlier and also extended period of favorable growth conditions for this taxon. Wiltshire et al. (2015) also reported an earlier and extended growing season for *Guinardia delicatula*, and related this to its preference for higher temperatures.

3.5.4. HAB taxa have significantly increased from the 1970s to 2000s

Several genera harbouring notorious HAB species have significantly increased from the 1970s to 2000s, such as the diatom genus *Pseudo-nitzschia* and the dinoflagellate genera *Prorocentrum* and *Alexandrium* (Figure 6). This corroborates observations and model predictions which also show that specific HAB genera such as *Pseudo-nitzschia* (Hernández-Fariñas et al., 2014) and *Alexandrium* (Gobler et al., 2017) have increased in the North Sea. These data also agree with a global increase of HAB blooms and toxic events in the last 25 years (Anderson et al., 2012). The reasons for these increases are as yet unclear, and are probably multifactorial. HABs have been linked with increased eutrophication (Anderson et al., 2012), but this would not explain why HAB genera increased in the southern North Sea during a period of de-eutrophication. Increased SST appears to be a more likely factor, especially for dinoflagellates which perform better under warmer conditions. Indeed, Gobler et al. (2017) showed that ocean warming increased the temporal and spatial availability of thermally suitable habitat for the dinoflagellate genus *Alexandrium* in the North Sea. It is clear that the increase in HAB taxa in the North Sea area is a worrying trend from a socio-economic point of view which needs to be carefully monitored in the future.

3.6. Conclusion

Comparative analyses of quantitative count data from the 1970s and 2000s has uncovered marked shifts in the biomass, community structure and seasonality of diatom and dinoflagellate communities in a heavily impacted, shallow coastal area in the southern part of the North Sea. While the 1970s represent a peak period of eutrophication, and in the 2000s de-eutrophication is well underway, the observed changes seem not to be caused by changes in nutrient loads or natural hydroclimatic oscillations. More likely, the increased biomass and extended and earlier growing seasons reflect changes in SST related to climate warming, and also regional increases in water transparency in the BPNS. However, the impact of other factors, whether or not related to broader scale global and climate change phenomena, such as changes in the trophic structure of the marine ecosystem, cannot be ruled out, but is as yet hard to evaluate giving the lack of data.

Given the central role of phytoplankton on marine food webs, the observed changes may have far-reaching environmental and socio-economic consequences.

In the last century, an impressive amount of often high-quality marine biological data was collected in the North Sea area. Many of these data are only available as non-digital, hand-written or printed tables and reports. Our study underscores the importance of recovering such data as they can offer unprecedented insights into long-term change in marine ecosystems, which are essential for properly evaluating the impact of human activities on these ecosystems.

3.7. Acknowledgements

We thank the Rijkswaterstaat (RWS) and all laboratories involved in the monitoring programs of Dutch waters for making the long-term datasets available.

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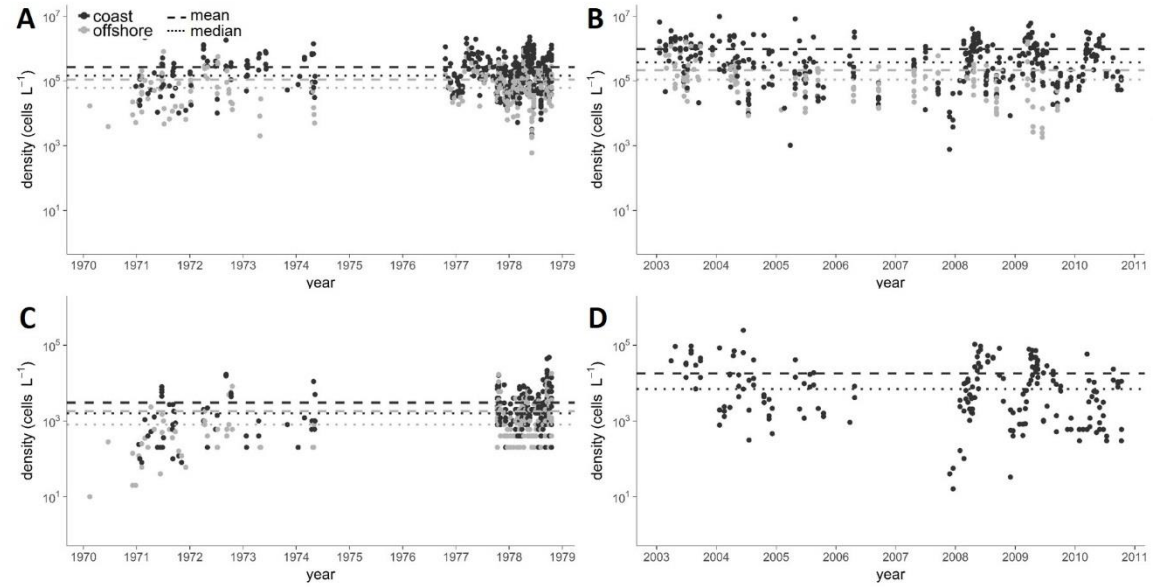
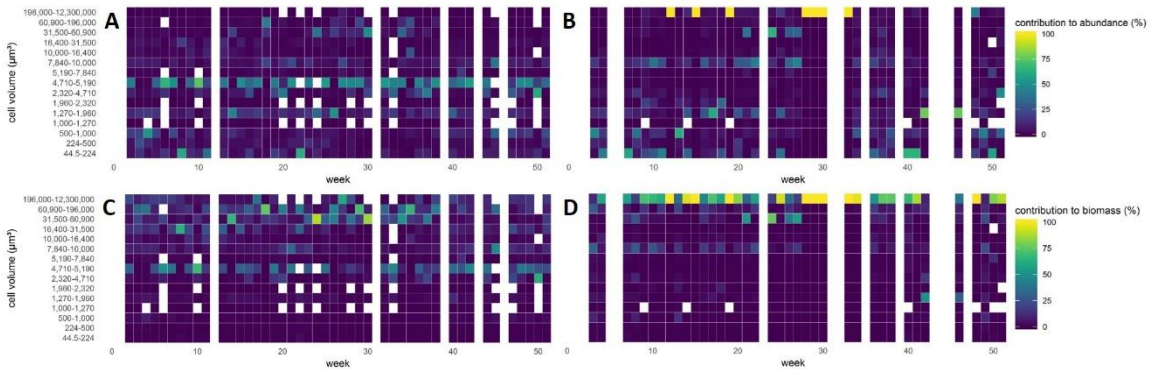
3.9. Supplementary information

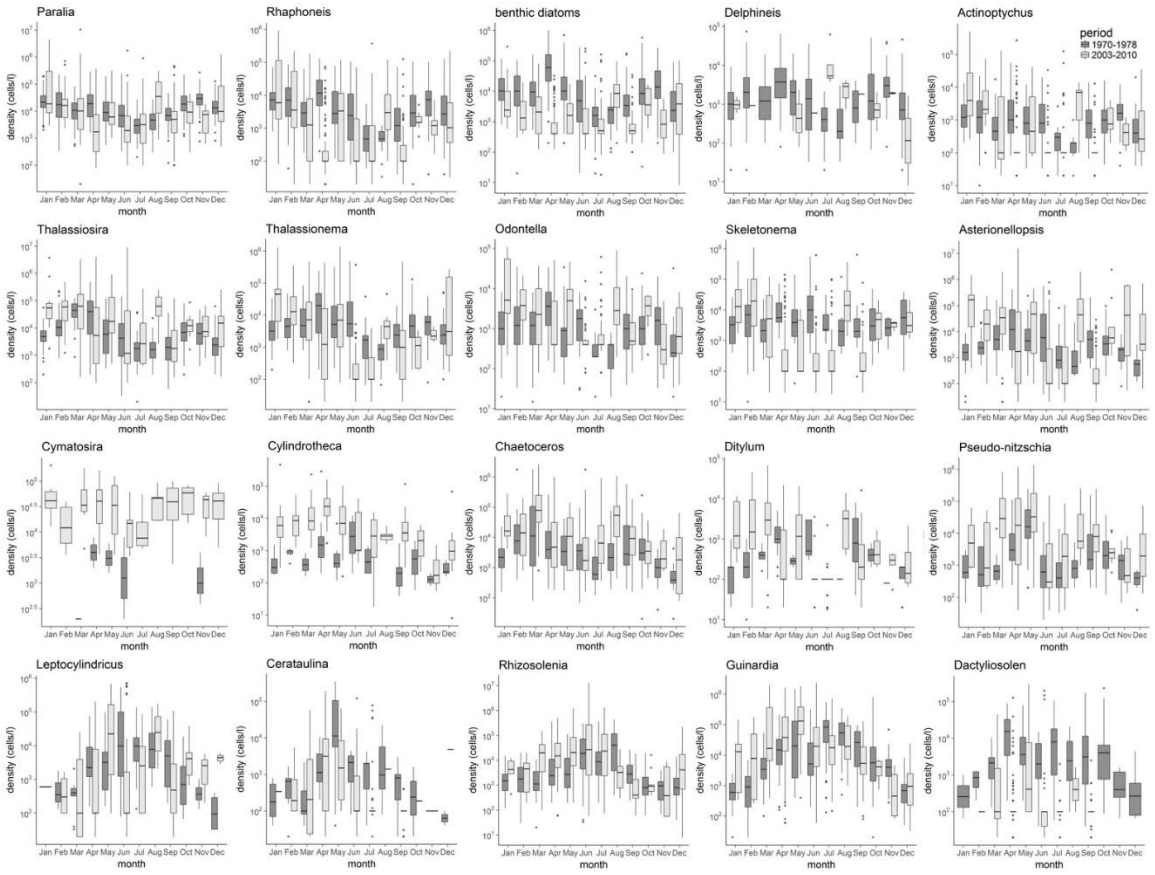
3.9.1. Supplementary Figures



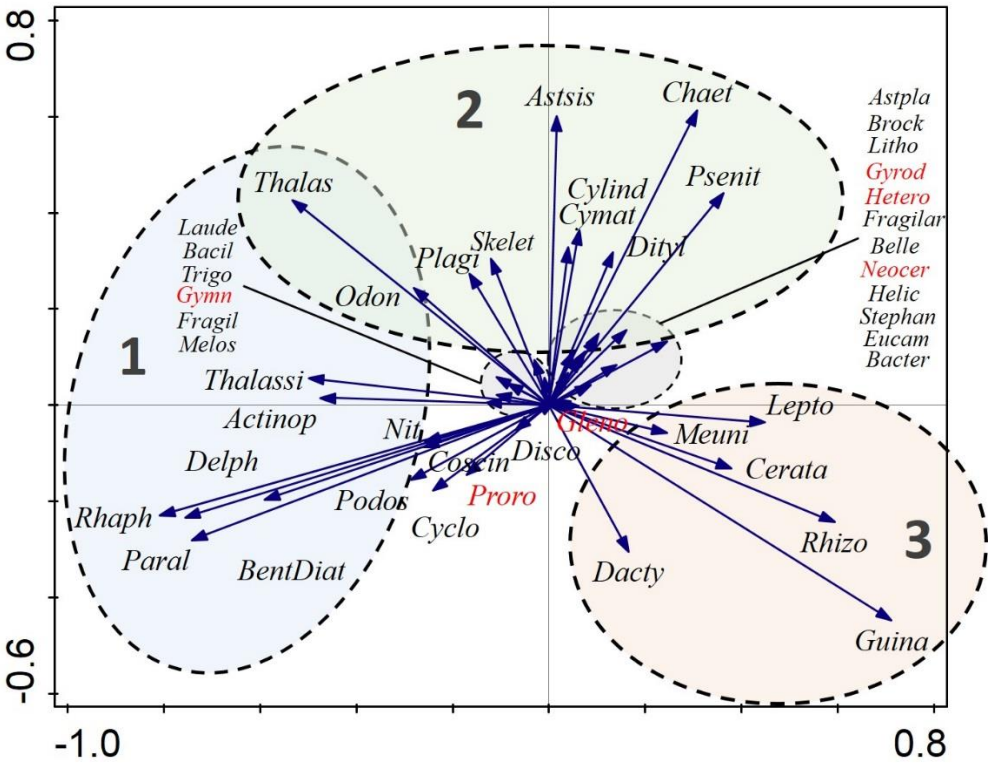
Supplementary Figure 1 Phytoplankton count data. (A) Diatom data in the 1970s and (B) 2000s, (C) Dinoflagellate data in the 1970s and (D) 2000s. Coastal (grey) and offshore (black) stations are distinguished; no offshore data are available for dinoflagellates in the 2000s.



Supplementary Figure 2 Relative contribution of diatom size classes to the total diatom density in the 1970s (A) and the 2000s (B) and to the total biovolume in the 1970s (C) and the 2000s (D).



Supplementary Figure 3 Seasonal boxplots of important phytoplankton genera of the three tradition phytoplankton communities.



Supplementary Figure 4 Principal Component Analysis (PCA) of the logarithmically transformed diatom and dinoflagellate relative abundance data. All fitted genera are shown. Dinoflagellates are indicated in red.

3.9.2. Supplementary Tables

Supplementary Table 1 Estimated AICs of the GAMMs applied on the diatom$_{1970s}$ abundance dataset. The best model is indicated in bold. The given r-squared values (R^2) determine the amount of variance explained by the model (Everaert et al., 2015).

model code	dataset	transformation	distribution	smoother (seasonal)	correlation structure	AIC	R^2
M1	diatoms$_{1970s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	no correlation	-980.5434	0.18
M2	**diatoms$_{1970s}$**	**$log_{10}(x)$**	**gaussian + log-link function**	**f(julian, bs = "cc")**	**autoregressive correlation**	**-1072.3230**	**0.18**
M3	diatoms$_{1970s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	continuous autoregressive correlation	-1072.3230	0.18
M4	diatoms$_{1970s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	compound symmetry structure correlation	-979.5613	0.18
M5	diatoms$_{1970s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	exponential correlation	-1072.3230	0.18
M6	diatoms$_{1970s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	gaussian correlation	-1054.7847	0.18
M7	diatoms$_{1970s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	linear correlation	-1052.4715	0.18
M8	diatoms$_{1970s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	rational quadratic correlation	-1061.3044	0.20
M9	diatoms$_{1970s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	spheric spatial correlation	-1054.7589	0.18

Supplementary Table 2 Estimated AICs of the GAMMs applied on the diatom$_{2000s}$ abundance dataset. The best model is indicated in bold. The given r-squared values (R^2) determine the amount of variance explained by the model (Everaert et al., 2015).

model code	dataset	transformation	distribution	smoother (seasonal)	correlation structure	AIC	R^2
M10	diatoms$_{2000s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	no correlation	-579.0604	0.22
M11	**diatoms$_{2000s}$**	**$log_{10}(x)$**	**gaussian + log-link function**	**f(julian, bs = "cc")**	**autoregressive correlation**	**-583.1803**	**0.22**
M12	diatoms$_{2000s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	continuous autoregressive correlation	-583.1803	0.22
M13	diatoms$_{2000s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	compound symmetry structure correlation	*no convergence*	*no convergence*
M14	diatoms$_{2000s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	exponential correlation	-583.1803	0.22
M15	diatoms$_{2000s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	gaussian correlation	-581.9795	0.22
M16	diatoms$_{2000s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	linear correlation	-581.9642	0.22
M17	diatoms$_{2000s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	rational quadratic correlation	-584.1617	0.22
M18	diatoms$_{2000s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	spheric spatial correlation	-581.9642	0.22

Supplementary Table 3 Estimated AICs of the GAMMs applied on the dinoflagellate$_{1970s}$ abundance dataset. The best model is indicated in bold. The given r-squared values (R^2) determine the amount of variance explained by the model (Everaert et al., 2015).

model code	dataset	transformation	distribution	smoother (seasonal)	correlation structure	AIC	R^2
M1	dinoflagellates$_{1970s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	no correlation	-297.6769	0.19
M2	**dinoflagellates$_{1970s}$**	**$log_{10}(x)$**	**gaussian + log-link function**	**f(julian, bs = "cc")**	**autoregressive correlation**	**-300.2988**	**0.19**
M3	dinoflagellates$_{1970s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	continuous autoregressive correlation	-300.2988	0.19
M4	dinoflagellates$_{1970s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	compound symmetry structure correlation	*no convergence*	*no convergence*
M5	dinoflagellates$_{1970s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	exponential correlation	-300.2988	0.19
M6	dinoflagellates$_{1970s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	gaussian correlation	-299.6500	0.19
M7	dinoflagellates$_{1970s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	linear correlation	-299.6408	0.19
M8	dinoflagellates$_{1970s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	rational quadratic correlation	-300.2347	0.19
M9	dinoflagellates$_{1970s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	spheric spatial correlation	-299.6408	0.19

Supplementary Table 4 Estimated AICs of the GAMMs applied on the dinoflagellate$_{2000s}$ abundance dataset. The best model is indicated in bold. The given r-squared values (R^2) determine the amount of variance explained by the model (Everaert et al., 2015).

model code	dataset	transformation	distribution	smoother (seasonal)	correlation structure	AIC	R^2
M10	dinoflagellates$_{2000s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	no correlation	-104.4700	0.32
M11	dinoflagellates$_{2000s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	autoregressive correlation	-115.9958	0.32
M12	dinoflagellates$_{2000s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	continuous autoregressive correlation	-115.9958	0.32
M13	dinoflagellates$_{2000s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	compound symmetry structure correlation	*no convergence*	*no convergence*
M14	dinoflagellates$_{2000s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	exponential correlation	-115.9958	0.32
M15	dinoflagellates$_{2000s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	gaussian correlation	-111.1822	0.32
M16	dinoflagellates$_{2000s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	linear correlation	-111.0638	0.32
M17	**dinoflagellates$_{2000s}$**	**$log_{10}(x)$**	**gaussian + log-link function**	**f(julian, bs = "cc")**	**rational quadratic correlation**	**-116.8606**	**0.32**
M18	dinoflagellates$_{2000s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	spheric spatial correlation	-111.0638	0.32

Supplementary Table 5 Results of the best model fits for each of the four abundance data subsets (diatoms$_{1970s}$, diatoms$_{2000s}$, dinoflagellates$_{1970s}$ and dinoflagellates$_{2000s}$). Model configuration, resulting *p*-values, r-squared values (R^2), the Akaike information criterion (AIC) and the variances of the random effects are given. The given r-squared values (R^2) determine the amount of variance explained by the model (Everaert et al., 2015).

dataset		diatoms$_{1970s}$	diatoms$_{2000s}$	dinoflagellates$_{1970s}$	dinoflagellates$_{2000s}$
data	n	468	348	289	190
	transformation	$log_{10}(x)$	$log_{10}(x)$	$log_{10}(x)$	$log_{10}(x)$
model code		M2	M11	M2	M17
model configuration	distribution	gaussian + log-link function	gaussian + log-link function	gaussian + log-link function	gaussian + log-link function
	smoother	s(days, cc)	s(days, cc)	s(days, cc)	s(days, cc)
	random effects	station, method	station, method	station, method	station, method
	correlation structure	corAR1	corAR1	corAR1	corRatio
model results	*p*-value	<0,001	<0,001	<0,001	<0,001
	R^2	0.18	0.22	0.19	0.32
	AIC	-1072.32	-583.18	-300.30	-116.86
random effects	variance $d^2_{station}$	0.0054	$9.3*10^{-11}$	$4.8197*10^{-10}$	$5.6453\text{-}10^{-11}$
	variance d^2_{method}	0.0002	0.0031	0.0131	$2.3156*10^{-13}$
	variance $d^2_{residuals}$	0.1770	0.2864	0.1740	0.4086

Supplementary Table 6 Estimated AICs of the GAMMs applied on the diatom$_{1970s}$ biovolume dataset. The best model is indicated in bold. The given r-squared values (R^2) determine the amount of variance explained by the model (Everaert et al., 2015).

model code	dataset	transformation	distribution	smoother (seasonal)	correlation structure	AIC	R^2
M1	diatoms$_{1970s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	no correlation	-1414.73	0.27
M2	**diatoms$_{1970s}$**	**$log_{10}(x)$**	**gaussian + log-link function**	**f(julian, bs = "cc")**	**autoregressive correlation**	**-1423.26**	**0.27**
M3	diatoms$_{1970s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	continuous autoregressive correlation	*no convergence*	*no convergence*
M4	diatoms$_{1970s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	compound symmetry structure correlation	*no convergence*	*no convergence*
M5	diatoms$_{1970s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	exponential correlation	*no convergence*	*no convergence*
M6	diatoms$_{1970s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	gaussian correlation	-1417.89	0.27
M7	diatoms$_{1970s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	linear correlation	-1422.33	0.27
M8	diatoms$_{1970s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	rational quadratic correlation	-1423.19	0.27
M9	diatoms$_{1970s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	spheric spatial correlation	-1422.33	0.27

Supplementary Table 7 Estimated AICs of the GAMMs applied on the diatom$_{2000s}$ biovolume dataset. The best model is indicated in bold. The given r-squared values (R^2) determine the amount of variance explained by the model (Everaert et al., 2015).

model code	dataset	transformation	distribution	smoother (seasonal)	correlation structure	AIC	R^2
M10	**diatoms$_{2000s}$**	**$log_{10}(x)$**	**gaussian + log-link function**	**f(julian, bs = "cc")**	**no correlation**	**-593.29**	**0.13**
M11	diatoms$_{2000s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	autoregressive correlation	-591.95	0.13
M12	diatoms$_{2000s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	continuous autoregressive correlation	-591.29	0.13
M13	diatoms$_{2000s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	compound symmetry structure correlation	-589.04	0.13
M14	diatoms$_{2000s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	exponential correlation	-591.29	0.13
M15	diatoms$_{2000s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	gaussian correlation	-591.29	0.13
M16	diatoms$_{2000s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	linear correlation	-590.71	0.13
M17	diatoms$_{2000s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	rational quadratic correlation	-591.29	0.13
M18	diatoms$_{2000s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	spheric spatial correlation	-591.29	0.13

Supplementary Table 8 Estimated AICs of the GAMMs applied on the dinoflagellates$_{1970s}$ biovolume dataset. The best model is indicated in bold. The given r-squared values (R^2) determine the amount of variance explained by the model (Everaert et al., 2015).

model code	dataset	transformation	distribution	smoother (seasonal)	correlation structure	AIC	R^2
M1	**dinoflagellates$_{1970s}$**	**$log_{10}(x)$**	**gaussian + log-link function**	**f(julian, bs = "cc")**	**no correlation**	**-544.06**	**0.07**
M2	dinoflagellates$_{1970s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	autoregressive correlation	-542.06	0.07
M3	dinoflagellates$_{1970s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	continuous autoregressive correlation	-542.06	0.07
M4	dinoflagellates$_{1970s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	compound symmetry structure correlation	*no convergence*	*no convergence*
M5	dinoflagellates$_{1970s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	exponential correlation	-542.06	0.07
M6	dinoflagellates$_{1970s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	gaussian correlation	-542.06	0.07
M7	dinoflagellates$_{1970s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	linear correlation	-542.06	0.07
M8	dinoflagellates$_{1970s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	rational quadratic correlation	-542.14	0.07
M9	dinoflagellates$_{1970s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	spheric spatial correlation	-542.06	0.07

Supplementary Table 9 Estimated AICs of the GAMMs applied on the dinoflagellates$_{2000s}$ biovolume dataset. The best model is indicated in bold. The given r-squared values (R^2) determine the amount of variance explained by the model (Everaert et al., 2015).

model code	dataset	transformation	distribution	smoother (seasonal)	correlation structure	AIC	R^2
M10	**dinoflagellates$_{2000s}$**	**$log_{10}(x)$**	**gaussian + log-link function**	**f(julian, bs = "cc")**	**no correlation**	**-347.16**	**0.27**
M11	dinoflagellates$_{2000s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	autoregressive correlation	-346.01	0.27
M12	dinoflagellates$_{2000s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	continuous autoregressive correlation	-346.01	0.27
M13	dinoflagellates$_{2000s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	compound symmetry structure correlation	-345.24	0.27
M14	dinoflagellates$_{2000s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	exponential correlation	-346.01	0.26
M15	dinoflagellates$_{2000s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	gaussian correlation	-345.95	0.26
M16	dinoflagellates$_{2000s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	linear correlation	-345.95	0.26
M17	dinoflagellates$_{2000s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	rational quadratic correlation	-346.26	0.26
M18	dinoflagellates$_{2000s}$	$log_{10}(x)$	gaussian + log-link function	f(julian, bs = "cc")	spheric spatial correlation	-345.95	0.26

Supplementary Table 10 Results of the best model fits for each of the four biovolume data subsets (diatoms$_{1970s}$, diatoms$_{2000s}$, dinoflagellates$_{1970s}$ and dinoflagellates$_{2000s}$). Model configuration, resulting *p*-values, r-squared values (R^2), the Akaike information criterion (AIC) and the variances of the random effects are given. The given r-squared values (R^2) determine the amount of variance explained by the model (Everaert et al., 2015).

dataset		**diatoms$_{1970s}$**	**diatoms$_{2000s}$**	**dinoflagellates$_{1970s}$**	**dinoflagellates$_{2000s}$**
data	n	465	387	289	191
	transformation	$log_{10}(x)$	$log_{10}(x)$	$log_{10}(x)$	$log_{10}(x)$
model code		M2	M10	M1	M10
model configuration	distribution	gaussian + log-link function	gaussian + log-link function	gaussian + log-link function	gaussian + log-link function
	smoother	s(days, cc)	s(days, cc)	s(days, cc)	s(days, cc)
	random effects	station, method	station, method	station, method	station, method
	correlation structure	corAR1	no correlation	no correlation	no correlation
model results	*p* -value	<0,001	<0,001	<0,001	<0,001
	R^2	0.27	0.13	0.07	0.27
	AIC	-1423.26	-593.29	-544.06	-347.16
random effects	variance $d^2_{station}$	0.0004	$1.0356*10^{-11}$	0.0005	$2.3252*10^{-12}$
	variance d^2_{method}	$7.6284*10^{-13}$	$6.3560*10^{-5}$	0.0025	0.0002
	variance $d^2_{residuals}$	0.2213	1.1475	0.6250	0.5357

4

Changes in phytoplankton biomass and phenology in the North Sea in response to increasing sea surface temperature

Modified from submitted manuscript (Limnology and Oceanography), in review: Desmit X., Nohe A., Vieira Borges A., Lagring R., Prins T., Van der Zande D. and Sabbe K. (2018).

Author contribution: AN collected and digitized the chlorophyll a data and metadata from the BPNS, conducted intercalibration exercises of the various applied chlorophyll a methodologies (finally not used in the manuscript), contributed to the study's conception and participated in the manuscript writing.

4.1. Abstract

At least two major drivers of phytoplankton production have changed in recent decades in the North Sea: sea surface temperature (SST) has increased by 1.5°C between 1988 and 2014, and the nitrogen and phosphorus loads from surrounding rivers have decreased from the mid-1980's onwards, following reduction policies. Long time series spanning four decades (1975-2015) of nutrients, chlorophyll *a* (Chl) and pH measurements in the Southern and Central North Sea were analysed to assess the impact of both the warming and the de-eutrophication trends. The de-eutrophication process resulted in a reduction of nutrient river loads to the sea, causing a decrease of marine nutrient concentrations in coastal areas under freshwater influence. A decline in annual mean Chl was observed across most sampling sites (coastal and offshore) in the period 1988-2015. Also, a shift in phytoplankton phenology was observed, with spring bloom formation occurring earlier in the year. A long time series of pH in the southern North Sea (Belgian Continental Shelf) shows an increase until the mid-1980's followed by a rapid decrease, mirroring the changes in phytoplankton production related to the processes of eutrophication/de-eutrophication and warming. Analysis of the seasonal pH signal in this dataset supports the shift in phytoplankton phenology as well. We hypothesize that (i) the decline in annual mean Chl since 1988 is most likely due to the de-eutrophication process (for coastal waters) and the SST increase (for both coastal and offshore waters) and that (ii) the shift in phytoplankton phenology is very likely due to SST increase.

4.2. Introduction

At least two major trends are currently affecting phytoplankton dynamics in the North Sea: the warming trend that started between 1982 and 1987 (Beaugrand and Reid, 2003; Edwards et al., 2006; Høyer and Karagali, 2016; van Aken, 2010) and the de-eutrophication trend, i.e. the decreasing input of nutrients and organic matter into coastal seas, that started at the end of the 1980s (Burson et al., 2016; Meyer et al., 2018; van Beusekom et al., 2009). In addition to potentially altering the stratification regime in the Central North Sea, increasing sea surface temperature (SST) may have strong effects on the physiology of marine phytoplankton, i.e. temperature may enhance phytoplankton cell division rate (Hunter-Cevera et al., 2016) or, on the contrary, negatively affect net production when it exceeds temperature optima for photosynthesis while still enhancing cell respiration (Barton et al., 2018). Such temperature effects on phytoplankton can then propagate through the food web (Beaugrand and Reid, 2003; Capuzzo et al., 2018; Richardson, 2008). Richardson and Schoeman (2004) have shown a consistent correlation between the temperature increase and the evolution of phytoplankton

abundance (counted from Continuous Plankton Recorder) in the Northeast Atlantic. They show a decreasing abundance of phytoplankton as SST increases in warmer waters and an increasing abundance of phytoplankton as SST increases in cooler waters, with a northward shift of species. The correlation was weaker for species in the North Sea. Recently, Capuzzo et al. (2018) showed a correlation between the increase in SST and a decrease in phytoplankton production in the North Sea, with a negative impact on small copepods and fish recruitment. Temperature may also have an effect on the phenology of phytoplankton blooms. Hunter-Cevera et al. (2016) already showed how increasing SST in New England coastal waters accelerated the cell division rate of phytoplankton, inducing changes in the timing and amplitude of the bloom. This supports the idea that "in a warming climate spring processes tend to occur earlier and autumn processes shift later" (Atkinson et al., 2015). Such shifts in phenology could lead to uncoupling of trophic interactions (Edwards and Richardson, 2004).

At the same time, human-induced eutrophication at a global scale has caused deleterious effects in coastal zones (e.g. oxygen minimum zones, Harmful Algal Blooms) with potentially serious consequences for ecosystem functioning, such as e.g. massive animal mortality or partial inhibition of secondary production (Daro et al., 2006; Diaz and Rosenberg, 2008; Doney, 2010; Galloway et al., 2004; Lancelot et al., 2014). The North Sea is subject to high anthropogenic riverine loads of nitrogen (N) and phosphorus (P) (Brion et al., 2006; Jickells, 1998; Lancelot et al., 1987) with an additional significant atmospheric input of N (Troost et al., 2013): 10% to 30% of the N in the Southern North Sea originates from the atmosphere (Dulière et al., 2017). This drives intense phytoplankton blooms with large biomass accumulation (usually expressed as chlorophyll *a*, Chl) in most coastal zones between March and October (Cadée and Hegeman, 2002; Desmit et al., 2018, 2015; OSPAR_Commission, 2017; Rousseau et al., 2006). Depending on the morphology and hydrology of the receiving basin and on phytoplankton community structure, eutrophication may lead to different symptoms such as foam events (caused by blooms of the colonial haptophyte *Phaeocystis*), hypoxia or even dead zones, nutrient imbalances allowing toxic species occurrence, inhibition of zooplankton egg production, and/or changes in community structure (Daro et al., 2006; Jickells, 1998; Philippart et al., 2007; Rousseau et al., 2000), and these phenomena can be further complicated by climate change (Painting et al., 2013). As eutrophication in the North Sea directly relies on N and P enrichment from rivers and, to a lesser extent, on atmospheric deposition of N (Dulière et al., 2017), mitigating eutrophication requires the reduction of nutrient inputs to the rivers (Billen et al., 2011; de Jonge et al., 2002), preferably by adopting a dual-N,P reduction strategy to avoid pronounced nutrient imbalances and undesirable consequences (Burson et al., 2016; Conley et al., 2009; Howarth and Marino, 2006).

To this end, since the late 1980s, European member states initiated various nutrient reduction policies aiming at the de-eutrophication of rivers and coastal waters, i.e. the Water Framework Directive (WFD; Directive 2000/60/EC, 2000) focusing on inland ground- and surface waters and including the near-shore coastal waters, the Marine Strategy Framework Directive (MSFD; Directive 2008/56/EC, 2008) focusing on all marine waters, and more specific policies such as the Urban Waste Water Treatment Directive (UWWTD) regulating waste waters (Directive 91/271/EEC, 1991), or the Nitrates Directive regulating the use of fertilisers (Directive 91/676/EEC, 1991).

Both the warming and de-eutrophication trends may have an effect on the timing and intensity of phytoplankton blooms in the North Sea. Our objective was to analyse long time series of Chl at sampling sites representative of typical water masses in the North Sea, from the Southern Bight to the Central North Sea, to assess patterns of long-term change in phytoplankton phenology and bloom amplitude over the past decades.

4.3. Methods

4.3.1. Study area and sampling sites

The study focuses on the North Sea and more specifically on the continuum from the Southern Bight of the North Sea (Belgium) to the Frisian coast (The Netherlands), and then to the Central North Sea at Oyster Grounds and Dogger Bank (see Figure 1 and Tables 1 and 2 for location of main river outlets and sampling sites). The Belgian continental shelf (BCS) is shallow (~20-50 m) with its coastal zone being permanently well-mixed due to the tidal energy and its offshore zone being subject to intermittent stratification in the northeast part (Ruddick and Lacroix, 2006; van Leeuwen et al., 2015). The coastal area is under freshwater influence, mainly from the Scheldt but also from the Rhine/Meuse rivers (Dulière et al., 2017), while the offshore waters are dominated by oceanic inputs including the remnant of the buoyant plumes from the Seine and Somme rivers (Ménesguen et al., 2018). In the BCS, the oceanic and freshwater end-members are well-mixed horizontally and vertically by tidal energy resulting in a decreasing coastal-offshore gradient in salinity, and nutrient and chlorophyll *a* concentrations (Desmit et al., 2015). In its southern part, coastal waters of the Dutch continental shelf (DCS) are dominated by the Rhine/Meuse Region Of Freshwater Influence (Rhine/Meuse ROFI or Delta ROFI), where intermittent salinity stratification may occur with complex spatial patterns subject to wind influence and upwelling induced by tidal straining (de Boer et al., 2009). The offshore waters show more stable circulation patterns under the influence of Atlantic waters from the English Channel. A tidal front occurs in an arc across the

Southern North Sea from Yorkshire in the United Kingdom to the Frisian coast in The Netherlands, separating relatively well-mixed waters in the south from intermittently or seasonally stratified waters in the Central North Sea (Longhurst, 2007; van Leeuwen et al., 2015).

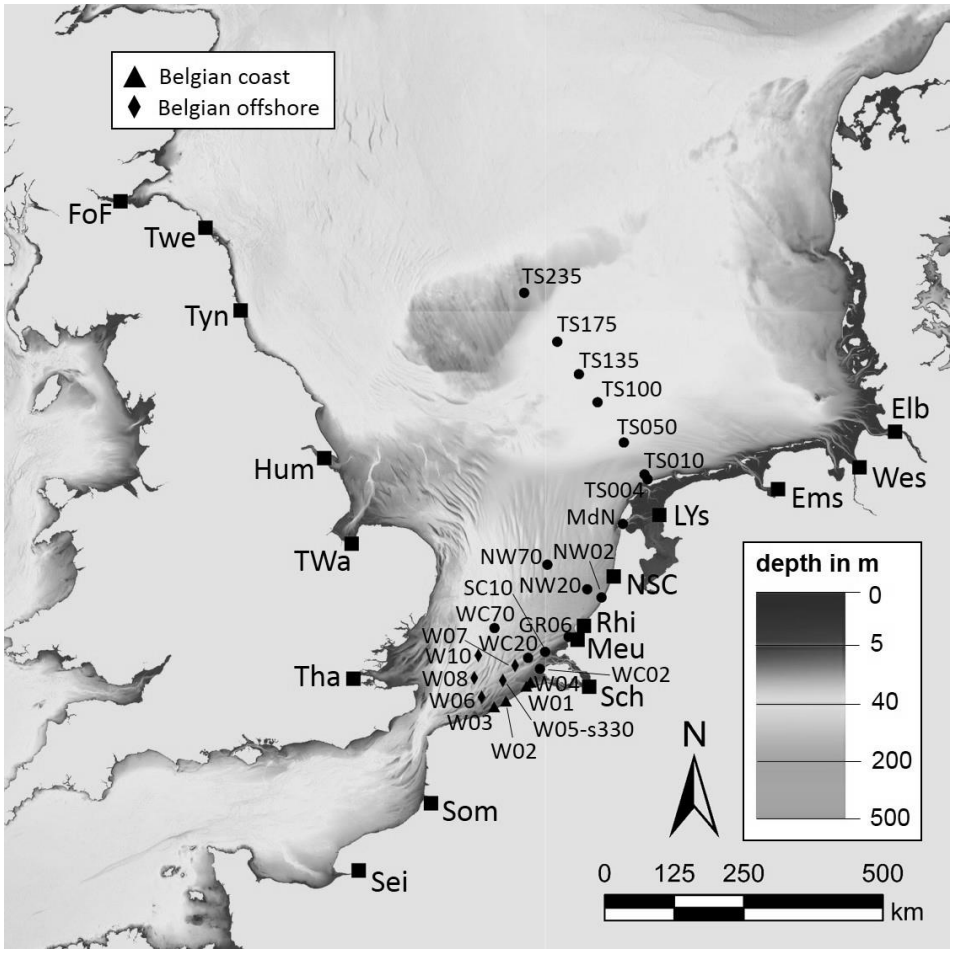


Figure 1 Bathymetry (source: EMODnet) of the North Sea with the locations of the Belgian and Dutch sampling sites and of the main river outlets (see also Tables 1 and 2 for full names of labels).

4.3.2. Dataset

A dataset of river discharges and riverine nutrient concentrations has been set up by Sonja van Leeuwen (NIOZ) in the framework of the OSPAR Intercorrespondence Group of Eutrophication MOdelling (ICG-EMO) collaboration on the basis of data available from Hamburg University (Pätsch et al., 2016; see also Section Acknowledgements). This dataset was used to force model river loads in Lenhart et al. (2010). In the present study, an updated version of the dataset (with the latest records) was used to estimate mean annual N and P loads (Table 1) and build a time series of riverine N and P concentrations in the main rivers surrounding the North Sea for the period 1975-2017.

The Belgian marine dataset includes environmental variables (e.g. SST, salinity, nutrients, Chl, dissolved oxygen, pH) since the 1970s and documents many different sampling sites in the

Southern North Sea (Nohe et al., 2018). The dataset we prepared for analysis includes data for nutrients between 1990 and 2014 and data for Chl between 1988 and 2014, as the data set showed large gaps before 1988 and a different Chl measurement methods were used (see below). In this study, we have aggregated the nine main Belgian monitoring stations into two waterbodies: Belgian coast and Belgian offshore (Figure 1, Table 2). Regarding Chl measurements, several techniques (trichromatic and monochromatic spectrophotometry, fluorimetry and HPLC) have been used by different laboratories and by different technicians since the 1970s, possibly introducing some errors that could not be corrected. The comparison between methods of Chl measurement remains a challenge (Baretta-Bekker et al., 2015; Noklegaard et al., 2005). Typically, the Chl measured by trichromatic spectrophotometry is problematic and does not compare well to Chl measured with other methods (Neveux et al., 1990). For this reason, we excluded Chl data from before 1988 from the Belgian dataset. The monochromatic spectrophotometry and the fluorimetric methods are much more comparable when they are adapted to measure phaeopigments through the acidification step and the use of Lorenzen's equations (Lorenzen, 1967). They also generally compare well to HPLC-determined Chl (Murray et al., 1986), even though Latasa et al. (1996) have shown that monochromatic spectrophotometry may overestimate Chl by 6-9% in comparison to HPLC. The Belgian dataset thus includes Chl data measured by monochromatic spectrophotometry (Lorenzen), by fluorimetry (adapted to Lorenzen) and by HPLC without any additional transformation, but acknowledging the fact that errors of 10% are possible.

The Dutch marine monitoring program has been measuring environmental variables (e.g. SST, salinity, nutrients, Chl, dissolved oxygen, pH, phytoplankton species composition) on a bi-weekly to monthly basis (depending on the station) since the 1970s along a series of transects perpendicular to the coast, from the Delta region to the Frisian coast (Figure 1, Table 2). Along the Terschelling transect, sampling sites cover the northwest Frisian coast, the tidal front, and areas in the Central North Sea, both deeper (Oyster Grounds) and shallower (Doggerbank) ones, which display thermal stratification in summer (van Leeuwen et al., 2015).

The SST data has been spatially aggregated across all available sampling sites in the North Sea, yielding the time series called SST_{NS} in this study.

4.3.3. Missing data

Twelve missing data points (out of 528 data points, i.e. 2%) in the monthly SST_{NS} dataset have been imputed with a smoothing spline function. Nutrient datasets from rivers were delivered without missing data. Monthly marine nutrient and Chl data showed missing data that have not been

replaced and were considered as NA's. For the purpose of calculating annual means, 90th percentiles and the seasonality of Chl time series, the years with too much missing monthly data of Chl have been excluded: typically when the number of months with missing data was >4 or when there was missing data in the blooming period (March-May). Missing data in annual time series were imputed for the purpose of trend analysis (see below).

4.3.4. Trend analysis

The time series of annual winter nutrient concentrations (Jan-Feb), annual mean Chl and annual 90th percentile of Chl (Chl P90) have been submitted to a trend test in eight representative sites. These time series represent a selection of water masses along the continental coast and in the central North Sea: i.e. two waterbodies in Belgian waters, each aggregating a collection of stations (Belgian coast and Belgian offshore; see Table 2) and six sampling sites in Dutch waters (NW02, NW70, GR06, TS010, TS135, TS235; see Table 2 for full names of labels). Chl P90 represents the chlorophyll *a* level such that 90% of the observations are equal to or less than this value (Gohin et al., 2008). Therefore, Chl P90 pictures the optimum tendency of the Chl time series and, in the North Sea, it is a proxy for the size of the phytoplankton biomass spring bloom, expressed in Chl units. Regarding the winter nutrients and Chl P90 time series, a Pettitt's test (Pettitt, 1979) was first applied to detect shifts in the central tendency of the time series around a single point, i.e. the cases when two periods show different averages. A trend detection test was then performed with the non-parametric Mann-Kendall's test. In the absence of a positive Pettitt's test, the trend analysis was conducted on the whole time series. In the case of a shift in central tendency, the trend analysis was conducted on the two periods identified in the time series. When a trend was detected, the parameters of the linear regression were identified with the Sen's test (Sen's slope). In the absence of a trend, no regression parameter was reported. For the annual mean Chl data set, the procedure was slightly different: a Mann-Kendall's test was systematically conducted from the year 1988 onwards, because 1988 is the year from which a more pronounced increasing trend in SST is observed (see Section Results). The Pettitt's test was only done as an indication. There was no significant autocorrelation in the time series. The whole package of trend analysis is freely available in R (packages 'kendall', 'boot', 'trend'). Default parameters for all functions were used in our analyses. These packages require continuous time series without any missing data. For the purpose of conducting the trend analyses only, the missing data in our time series have been imputed with the R function 'na.approx'. That function interpolates between existing data and allows capturing a trend with Mann-Kendall's test in spite of missing data, as was tested by Ramos and Cordeiro (2013). When no trend was detected in nutrients or Chl time series, the time

evolution was drawn with a smoothing function (LOESS with span being two third of the vector length).

Table 1 Main river outlets around the North Sea with their geographical location (country and coordinates), mean annual discharge and mean annual N, P loads. For UK rivers, Ntot is not available, and winter DIN (Jan-Feb) was used as a proxy for mean Ntot. Data made available by Sonja van Leeuwen (NIOZ) and Hermann Lenhart (UHAM), see Pätsch et al. (2016). Note that the discharges at "North Sea Canal" and "Lake Ijssel" are also Rhine water which is diverted to the north through the river Ijssel and then Lake Ijssel. Also, "Meuse" in the table is the discharge point at Haringvliet, which is also partly Rhine water mixed with water from the Meuse.

Rivers									
Country	**River name**	**Abbrev.**	**River group**	**Lat (°N)**	**Lon (°E)**	**Mean annual discharge ($m^3 s^{-1}$)**	**Ntot mean annual load (ton day^{-1})**	**Ptot mean annual load (ton day^{-1})**	**Period**
FR	Seine	Sei	Channel FR	49.480	0.486	479.3	339.1	22.3	1975-2016
FR	Somme	Som	Channel FR	50.180	1.653	35.6	16.0	0.6	1975-2016
BE	Scheldt	Sch	Delta	51.370	4.208	132.8	76.3	7.1	1977-2017
NL	Meuse	Meu	Delta	51.840	4.017	721.5	279.4	14.2	1977-2017
NL	Rhine	Rhi	Delta	51.980	4.120	1420.6	510.1	37.5	1977-2017
NL	North Sea Canal	NSC	Holland coast	52.470	4.600	90.8	31.1	3.1	1977-2017
NL	Lake IJssel	LYs	Frisian coast	53.070	5.336	507.1	164.6	7.8	1977-2017
UK	Thames (Thames, Medway)	Tha	UK South East	51.450	0.402	106.9	148.8	8.1	1975-2016
UK	The Wash (Great Ouse, Nene, Witham)	TWa	UK Mid East	52.790	0.369	108.7	232.2	3.8	1975-2016
UK	Humber	Hum	UK Mid East	53.620	-0.071	322.9	360.3	19.8	1975-2016
UK	Tyne	Tyn	UK North East	55.010	-1.422	58.2	11.0	0.5	1975-2016
UK	Tweed	Twe	UK North East	55.770	-1.991	97.1	34.4	0.8	1975-2016
UK	Firth of Forth	FoF	UK North East	56.010	-3.361	100.8	13.6	0.9	1975-2016
GE	Ems	Ems	German bight	53.320	7.254	82.0	49.1	1.6	1980-2017
GE	Weser	Wes	German bight	53.530	8.572	322.3	153.8	7.0	1980-2017
GE	Elbe	Elb	German bight	53.870	9.143	694.9	329.0	14.9	1980-2017

4.3.5. Analysis of the seasonal signal of Chl

To study the phenology of Chl, it was important to standardize the seasonal signal to allow comparing it across sites and throughout different periods. Such a standardization can be done using the multiplicative model of Cloern and Jassby (2010), based on the hypothesis that the variability of a monthly Chl time series may be explained by the multiplication of the grand mean of the time series by the annual, the seasonal and the residual effects (eq. 1).

$$Chl_{m,y} = GM * Y_y * S_m * R_{m,y} \qquad \text{eq. 1}$$

Where m and y indices respectively indicate a specific month and a specific year in the period, GM is the grand mean of Chl throughout a whole period, Y_y is the annual effect of year y, S_m is the seasonal (monthly) effect in month m and $R_{m,y}$ is the monthly residual effect. More specifically, the standardized seasonal signal S is obtained for each month of the year as shown for January (eq. 2), where A_y is the annual mean Chl of year y and N_y is the total number of years in the period.

$$Chl^{Jan}_{standardized} = \frac{1}{N_y} * \sum_y \frac{Chl^{Jan}_y}{A_y} \qquad \text{eq. 2}$$

In our case, we wanted to analyse the evolution of Chl seasonality throughout successive decades and therefore we calculated a standardized seasonal signal of Chl per decade, depending on available data.

Table 2 Marine sampling sites with their geographical location, mean salinity and period of chlorophyll a sampling. Belgian data are from BMDC, available at http://bmdc.mumm.ac.be/Interface/. Dutch data are from DONAR, available at https://waterinfo.rws.nl/#!/nav/index/.

Country	Sampling site	Abbrev.	km from coast	Lat (°N)	Lon (°E)	Marine region	Mean salinity	Range of salinity	Period of Chl sampling
BE	W03 - s120	W03	5	51.168	2.667	Belgian coast	33.6	30.3-34.8	1985-2017
BE	W02 - s130	W02	3	51.225	2.858	Belgian coast	32.3	21.0-35.4	1985-2017
BE	W01 - s700	W01	5	51.375	3.188	Belgian coast	30.8	26.7-34.9	1985-2017
BE	W04	W04	7	51.418	3.253	Belgian coast	31.6	29.3-33.7	2007-2017
BE	W06 - s315	W06	22	51.263	2.468	Belgian offshore	34.3	31.2-35.3	1985-2017
BE	W05 - s330	W05-s330	25	51.432	2.810	Belgian offshore	33.6	30.9-35.5	1983-2017
BE	W08 - s421	W08	45	51.458	2.350	Belgian offshore	34.7	31.8-35.6	1988-2017
BE	W07 - s435	W07	32	51.583	3.008	Belgian offshore	34.0	31.9-35.2	1985-2017
BE	W10	W10	64	51.683	2.417	Belgian offshore	34.9	33.9-35.3	2008-2017
NL	Walcheren 02	WC02	2	51.549	3.411	Delta ROFI	32.0	29.2-34.0	1975-2016
NL	Walcheren 20	WC20	20	51.659	3.221	Delta ROFI	33.3	30.9-35.1	1975-2016
NL	Walcheren 70	WC70	70	51.957	2.679	Delta offshore	35.0	34.2-35.5	1977-2016
NL	Noordwijk 02	NW02	2	52.261	4.406	Delta ROFI	28.9	21.5-32.1	1975-2016
NL	Noordwijk 20	NW20	20	52.342	4.175	Delta ROFI	31.9	27.9-34.2	1975-2016
NL	Noordwijk 70	NW70	70	52.583	3.531	Delta offshore	34.9	32.8-35.6	1975-2016
NL	Goeree 06	GR06	6	51.870	3.874	Delta ROFI	30.5	22.6-33.4	1975-2016
NL	Marsdiep noord	MdN	-	52.983	4.750	Wadden Sea	28.0	6.4-36.0	1976-2016
NL	Terschelling 004	TS004	4	53.415	5.151	Frisian coast	32.1	27.8-35.0	1975-2006
NL	Terschelling 010	TS010	10	53.461	5.101	Frisian coast	32.7	29.1-35.1	1975-2016
NL	Terschelling 050	TS050	50	53.768	4.767	North Sea Front	34.3	31.4-35.5	1975-2016
NL	Terschelling 100	TS100	100	54.149	4.342	Oysterground	34.6	32.3-34.9	1988-2016
NL	Terschelling 135	TS135	135	54.416	4.041	Oysterground	34.7	33.9-35.0	1988-2016
NL	Terschelling 175	TS175	175	54.719	3.692	Oysterground	34.7	34.0-35.1	1988-2016
NL	Terschelling 235	TS235	235	55.172	3.158	Doggerbank	34.9	33.9-35.2	1988-2016

4.4. Results

4.4.1. Sea surface temperature

We estimated through a linear regression (not shown) the increase of SST_{NS} over the period 1971-2014 to be 1.5°C, which is consistent with the findings of van Aken (2010) for a selection of Dutch monitoring stations. It is relevant to mention that van Aken (2010) attributed the warming trend in the North Sea starting in the early 1980s to simultaneous variation in wind circulation and cloudiness over a period of approximately 30 years, with the additional contribution of the northern hemisphere warming due to greenhouse gases over the period. In the period 1971-2014, the winter-spring values of SST_{NS} (Jan-Jun) have increased (+1.12°C) more than the summer-fall values (Jul-Dec; +0.98°C) (Figure 2, top).

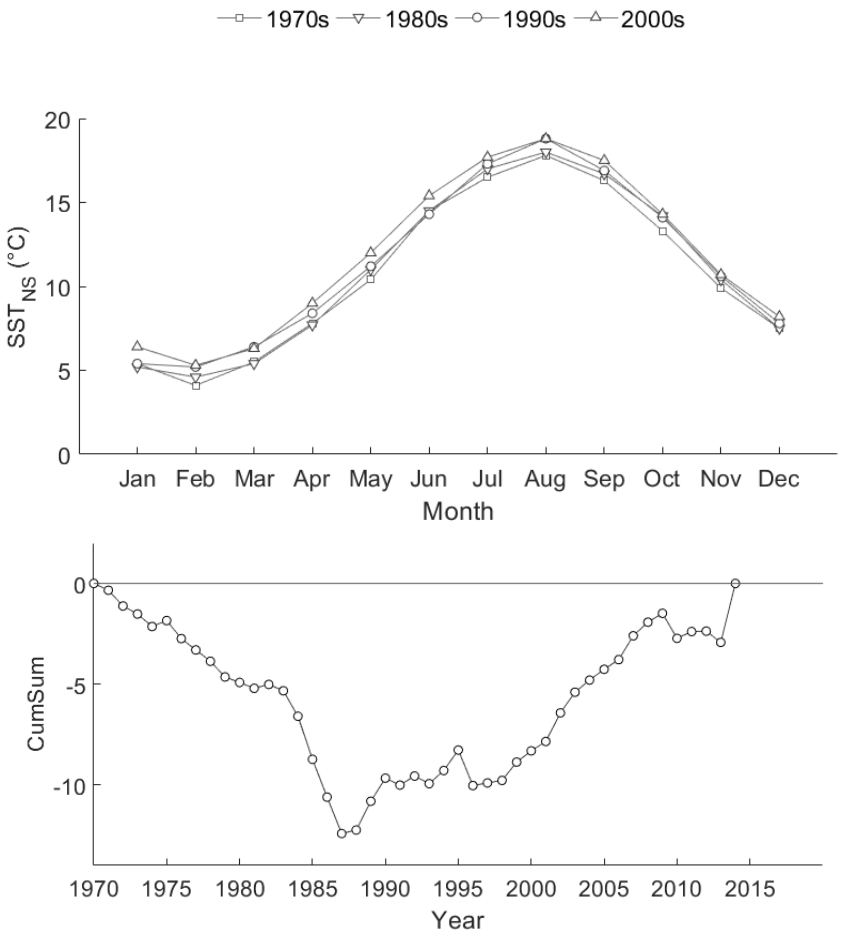


Figure 2 Top – Decadal mean of the monthly seasonal pattern of SST_{NS} in the period 1971-2014. Bottom – Cumulative sum of the annual mean SST_{NS} (z-score) in the period 1971-2014.

Monthly seasonal time series were interpolated daily (not shown) and the daily time series were averaged per decade. We estimated that the spring temperatures observed on average in the 1980s are shifted forward (i.e. to earlier days) in the 2000s by approximately 15 days. The cumulative sum of SST_{NS} is shown at Figure 2 (bottom). A cumulative sum (CumSum) is the sequential sum of the intervals between successive elements of a vector of mean 0 and standard deviation 1. Starting and ending with zero, the CumSum function is typically used for monitoring change detection, i.e. when a minimum or a maximum is detected. The CumSum of SST_{NS} suggests that an increasing trend started around 1987-1988 (as reported by Beaugrand and Reid, 2003; Edwards et al., 2006) with a dip around 1995-1996 maybe due to the NAO event (four consecutive years with negative NAO index in 1995-1998). A Pettitt's test made on SST_{NS} also shows a shift in central tendency after the year 1987: the period 1971-1987 shows a mean SST_{NS} of 10.6°C while the period 1988-2014 has a mean SST_{NS} of 11.5°C.

4.4.2. Nutrients

Table 1 shows the daily N and P loads per river, averaged over their respective period of data availability. The four most important N and P contributors are the Rhine, the Humber, the Seine

and the Elbe, together accounting for 56% and 63%, respectively, of the N and P loads from the rivers documented in Table 1 (the Rhine River loads are even underestimated in this lumped estimate as only the loads at Nieuwe Waterweg are accounted, see caption of Table 1). Riverine N and P loads to the North Sea reached a maximum in the mid-1980's and showed a decreasing trend since then (Brion et al., 2006). This decrease is related to the de-eutrophication measures that were implemented in Europe (Figure 3). The P reduction in rivers is mainly due to the ban of polyphosphate from washing powder in the early 1990s, and to waste water treatment (WWT) from urbanized and industrialized areas afterwards (Directive 91/271/EEC, 1991). The N reduction is linked to a combination of measures: the management of waste water that reduced organic matter and ammonium loads, and the increasing implementation of good agricultural practices (more moderate use of fertilizers following the Nitrate Directive, i.e. Directive 91/676/EEC, 1991). The dissolved silica concentration in some rivers (South East UK, NL Delta, NL Frisian) slightly increased, probably because of lower diatom production in the rivers as a consequence of the de-eutrophication. As a result, the export of silica to the coastal zone has increased in some areas in the growing season (Mar-Sep), potentially fostering marine diatom production (Prins et al., 2012).

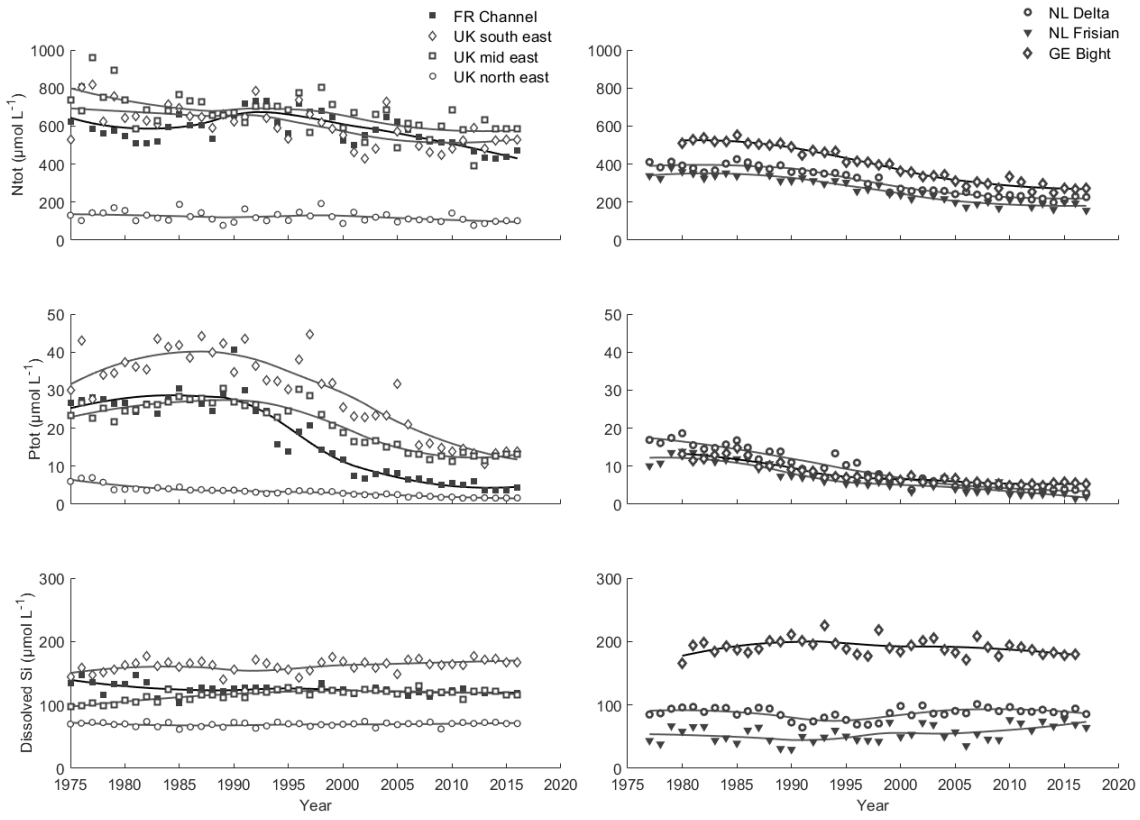


Figure 3 Annual mean nutrient concentrations in rivers (aggregated per region, see Table 1). The lines are smooth (LOESS) responses to the interannual variability of nutrient concentrations. Ntot in UK rivers is approximated by winter DIN.

Figures 4, 5, 6 and Tables 3, 4 show a selection of eight representative time series along the continental coast and in the offshore: two waterbodies in Belgian waters and six sampling sites in

Dutch waters. Among the selected series, decreasing trends in winter nutrients are observed in Noordwijk 2, Goeree 6 and Terschelling 10 (only until 1995 for winter DIN in Goeree 6) (Figure 4). These decreases in nutrient coastal concentrations, here represented by winter concentrations (Jan-Feb), follow the decrease in N and P river loads. The time series of winter DIN and DIP were subject to a trend analysis in each sampling site (see Table 3, only the data showing a trend are reported). The decrease in winter DIN depicts the constant efforts in N reduction over the period and shows an almost continuous slope. The winter DIP exhibits marked decreases until ca. 1995 within the marine sites most impacted by freshwater inputs (Noordwijk 2 and Goeree 6). The strong decreases in winter DIP are mainly linked to the ban of polyphosphates in washing powders. After 1995, a less pronounced decreasing trend is still present, probably linked to waste water treatment. In the vertically well-mixed BCZ, neither winter DIN nor DIP concentrations showed a significant trend in the period 1990-2014 in spite of a decreasing rate of Ntot in the 'NL Delta' river group of ~5 µmol L-1 year-1 on average over the period. The time series are shorter in the Belgian coastal zone and this may explain the absence of a clear trend detection. The shallow and well-mixed Belgian offshore waterbody is the only offshore area showing a slight decreasing trend in winter DIP, which might reflect perhaps a long-term propagation of the P decrease in most European continental rivers. Other offshore stations show stable winter nutrient concentrations with relatively low interannual variabilities.

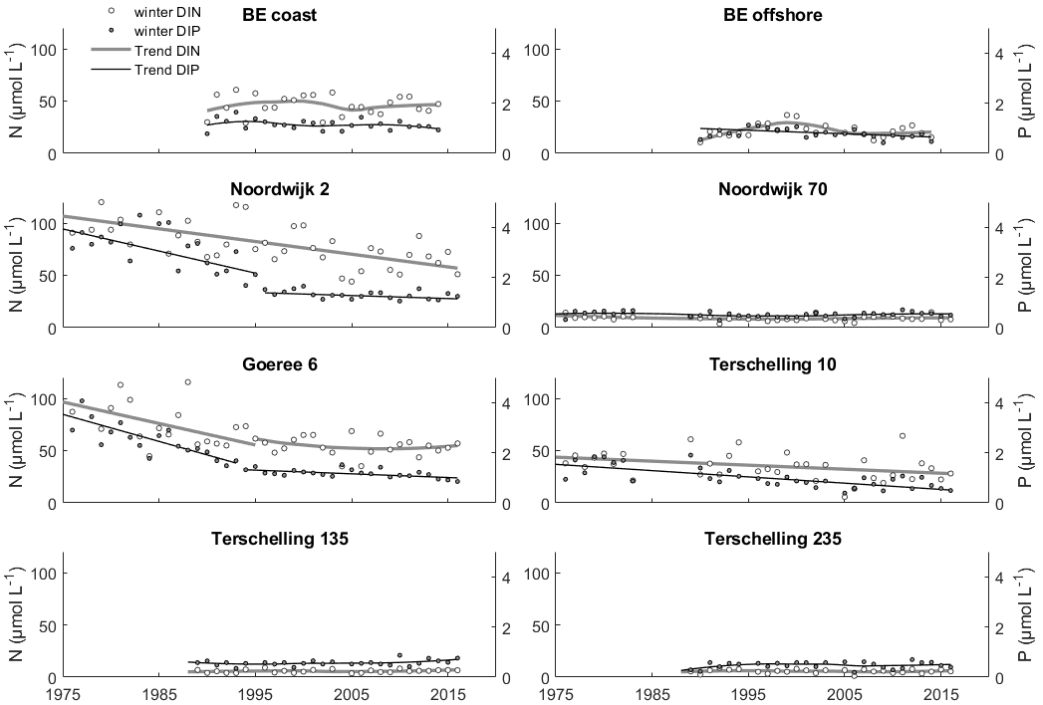


Figure 4 Annual time series of winter DIN (left axis) and DIP (right axis) concentrations (Jan-Feb) in a selection of sampling sites in the North Sea. The lines show the estimated trend regressions (see Table 3) when available or, if no trend was detected, a smooth (LOESS) response to the interannual variability of winter nutrient concentrations. The waterbodies 'BE coast' and 'BE offshore' result from the spatial aggregation of the corresponding sampling sites (see Table 2).

4.4.3. Chl P90 and annual mean Chl

The Mann-Kendall's test made on time series of Chl P90 revealed a decreasing trend at only two sites amongst the eight selected sites: Noordwijk 2 and Goeree 6 (see Table 3). While the offshore stations are mainly under the influence of Atlantic water inputs through the Channel, the coastal stations are dominated by river inputs (Dulière et al., 2017; Lenhart and Große, 2018; Ménesguen et al., 2018). The significant decrease in the Chl P90 observed at the two coastal sites is very likely due to the decrease in riverine nutrient loads during the same period (Figure 4), and more particularly the P load reduction, as the phytoplankton spring bloom is mainly limited by P (and Si) in the coastal zone of the continental shelf (Billen et al., 2011; Burson et al., 2016; Desmit et al., 2015). In other stations, like Terschelling 10, a significant decreasing trend in nutrients is detected while no trend is detected for the Chl P90. This illustrates the complexity of a system where phytoplankton shows non-linear responses to nutrient inputs (Lenhart et al., 2010).

Table 3 Statistics of trend analyses performed on time series of winter nutrient and Chl P90 concentrations in a selection of sampling stations in the North Sea (see section Methods); only sites where trends were detected are shown. The waterbody 'BE offshore' results from the spatial aggregation of the Belgian offshore sampling sites (see Table 2). 'NW02' is Noordwijk 2, 'GR06' is Goeree 6 and 'TS010' is Terschelling 10.

Statistics of Chl P90	**BE offshore**			**NW02**			**GR06**			**TS010**		
	N winter	**P winter**	**Chl P90**	**N winter**	**P winter**	**Chl P90**	**N winter**	**P winter**	**Chl P90**	**N winter**	**P winter**	**Chl P90**
Period of the whole time series	1990-2014	1990-2014	1989-2012	1976-2016	1976-2016	1977-2016	1976-2016	1976-2016	1976-2016	1976-2016	1976-2016	1976-2016
Mann-Kendall's trend (ci 0.05)	no	yes	no	yes	yes	yes	yes	yes	yes	yes	yes	no
Sen's slope	-	-0.014	-	-1.215	-0.072	-0.171	-0.958	-0.052	-0.315	-0.384	-0.025	-
Sen's intercept	-	28.905	-	2506.256	144.869	356.600	1976.095	104.536	648.294	802.612	50.910	-
Single change-point												
Pettitt's single change-point	no	no	no	yes	yes	no	yes	yes	yes	yes	yes	yes
Year of possible shift in central tendency	-	-	-	2000	1995	-	1995	1993	1997	2001	1995	2007
Period 1												
Mann-Kendall's trend (ci 0.05)	-	-	-	no	yes	-	yes	yes	no	no	no	no
Sen's slope in 1st period	-	-	-	-	-0.088	-	-2.054	-0.108	-	-	-	-
Sen's intercept in 1st period	-	-	-	-	177.731	-	4153.788	216.546	-	-	-	-
Period 2												
Mann-Kendall's trend (ci 0.05)	-	-	-	no	yes	-	no	yes	no	no	no	no
Sen's slope in 2nd period	-	-	-	-	-0.012	-	-	-0.015	-	-	-	-
Sen's intercept in 2nd period	-	-	-	-	25.213	-	-	30.705	-	-	-	-

The time series of annual mean Chl values for a selection of two Belgian waterbodies and six Dutch stations are shown in Figure 5. The Mann-Kendall's test successfully identified decreasing trends in most selected time series after 1987, the year when a shift in the central tendency of SST_{NS} occurred (see Figure 2). We used Pettitt's test to identify shifts in the central tendency of annual mean Chl and then calculated the mean Chl values before and after these shifts (Table 4). Shifts in the central tendency are observed between 1996 and 2007 at all sites except in the BE coastal waterbody and in Terschelling 235, and annual mean Chl consistently decreases by 22% to 28%.

The standardized seasonal signal of Chl per decade (1975-2015) at 8 locations (from the Southern to the Central North Sea) shows a shift in Chl phenology at all sites, with earlier spring bloom formation and termination in the last decades, especially after the 1980s (Figure 6). This earlier

bloom termination is probably due to the earlier depletion of nutrients (N, P, Si) in recent decades, as observed in the seasonal profiles of marine nutrient concentrations (not shown). Any change in the autumn phytoplankton bloom is less clear, with the exception of Terschelling 235 where the autumn bloom tends to shift later in recent years, suggesting perhaps an effect of temperature at least on maintaining the stratification regime at this site further in the season.

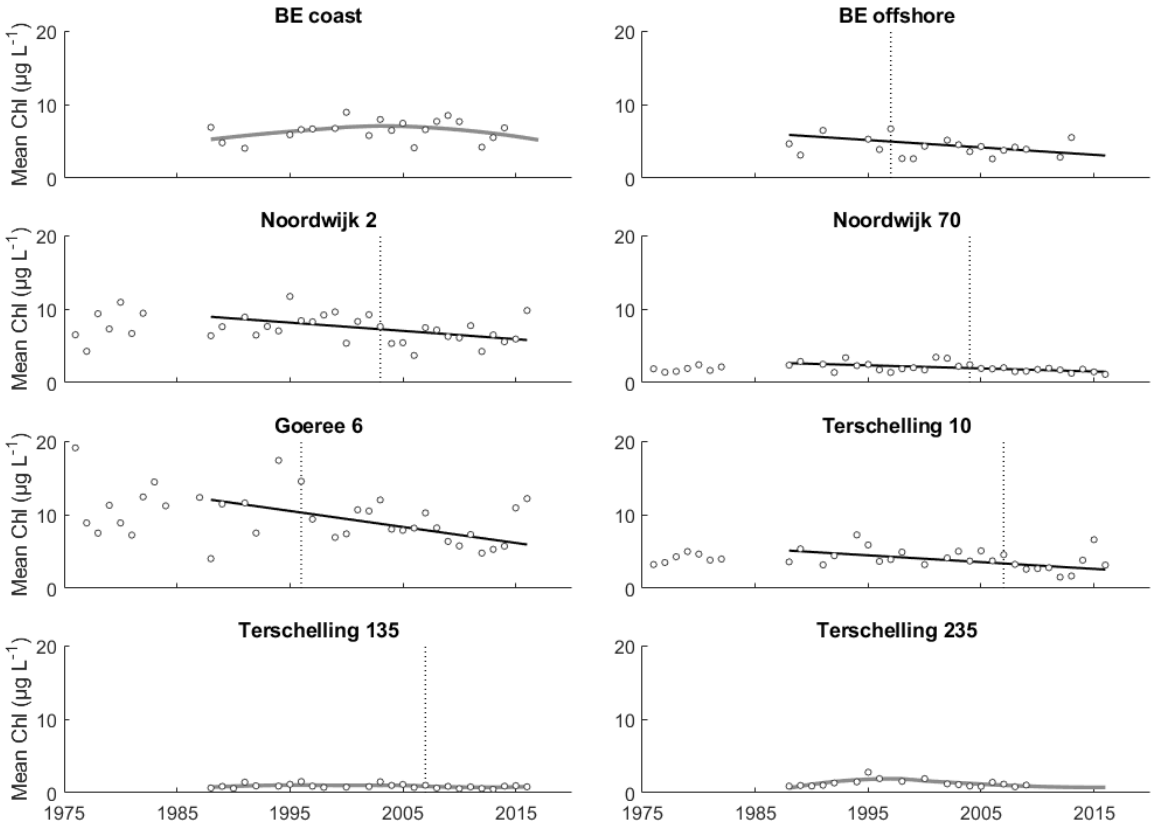


Figure 5 Time series of annual mean Chl values with their trends after 1987 (black lines), or a LOESS smoothing (grey lines) if no trend was detected, in a selection of sampling stations in the North Sea. The vertical dashed lines indicate the years where a shift in central tendency (Pettitt's test) was detected, if any.

Table 4 Statistics of trend analyses performed on time series of annual mean Chl concentrations in a selection of marine sites (see section Methods). The years of possible shift in the central tendency are indicated with the respective means of the datasets before and after the shift. The waterbodies 'BE coast' and 'BE offshore' result from the spatial aggregation of the corresponding sampling sites (see Table 2).

Statistics of annual mean Chl	BE coast	BE offshore	NW02	NW70	GR06	TS010	TS135	TS235
Period 1988-onwards								
Mann-Kendall's trend (ci 0.05)	no	yes	yes	yes	yes	yes	no	no
Sen's slope	-	-0.100	-0.113	-0.042	-0.217	-0.092	-	-
Sen's intercept	-	204.667	232.588	85.471	443.729	187.987	-	-
Single change-point								
Pettitt's single change-point	no	yes	yes	yes	yes	yes	yes	no
Year of possible shift in central tendency	-	1997	2003	2004	1996	2007	2007	-
Mean Chl in period 1	-	5.02	8.00	2.19	11.23	4.35	0.98	-
Mean Chl in period 2	-	3.85	6.24	1.67	8.30	3.12	0.74	-

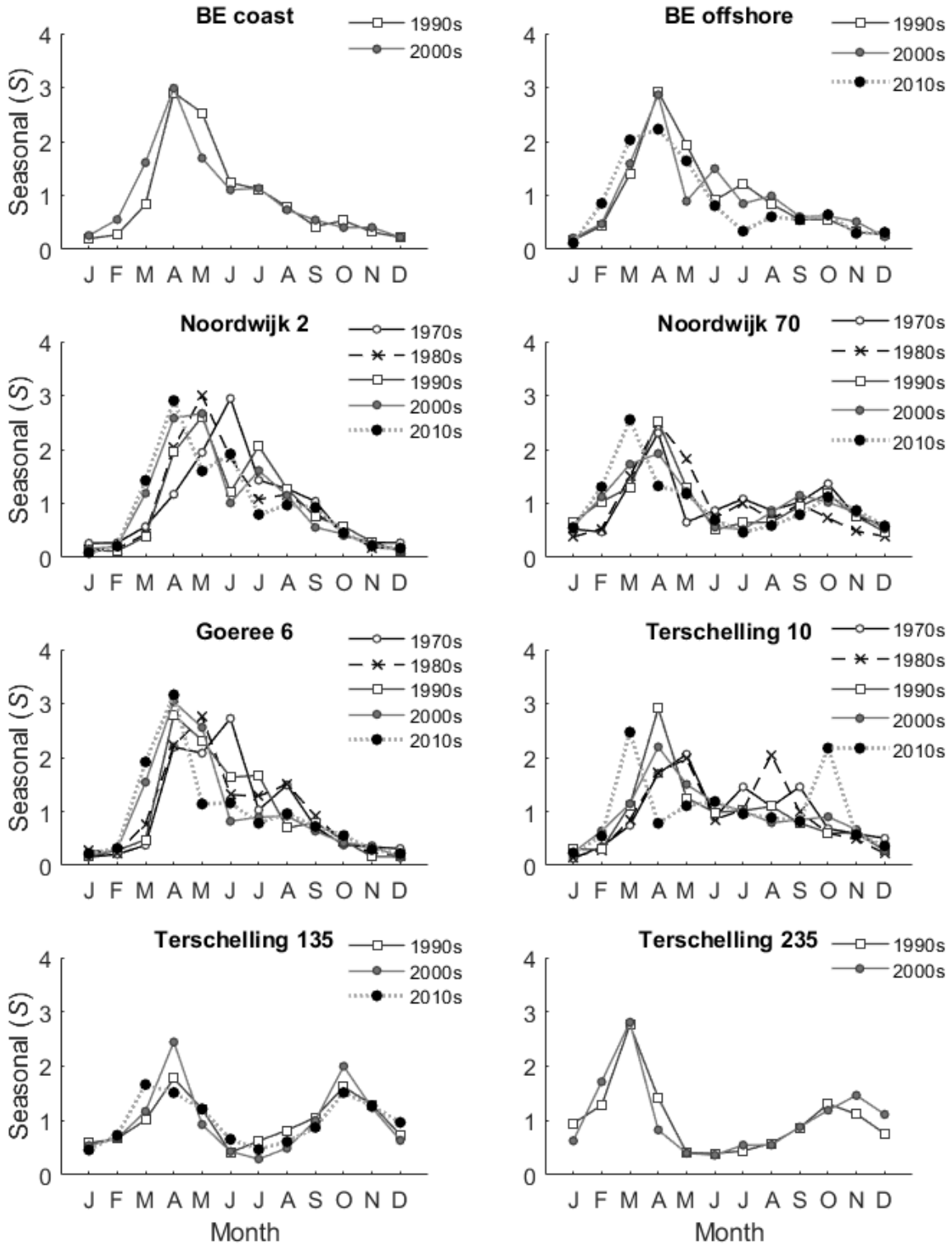


Figure 6 Seasonal component of the Chl signal (standardized according to Cloern and Jassby, 2010). Seasonal components are estimated per decade for a selection of sampling sites of the North Sea. The waterbodies 'BE coastal' and 'BE offshore' result from the aggregation of several sampling sites (see Table 2).

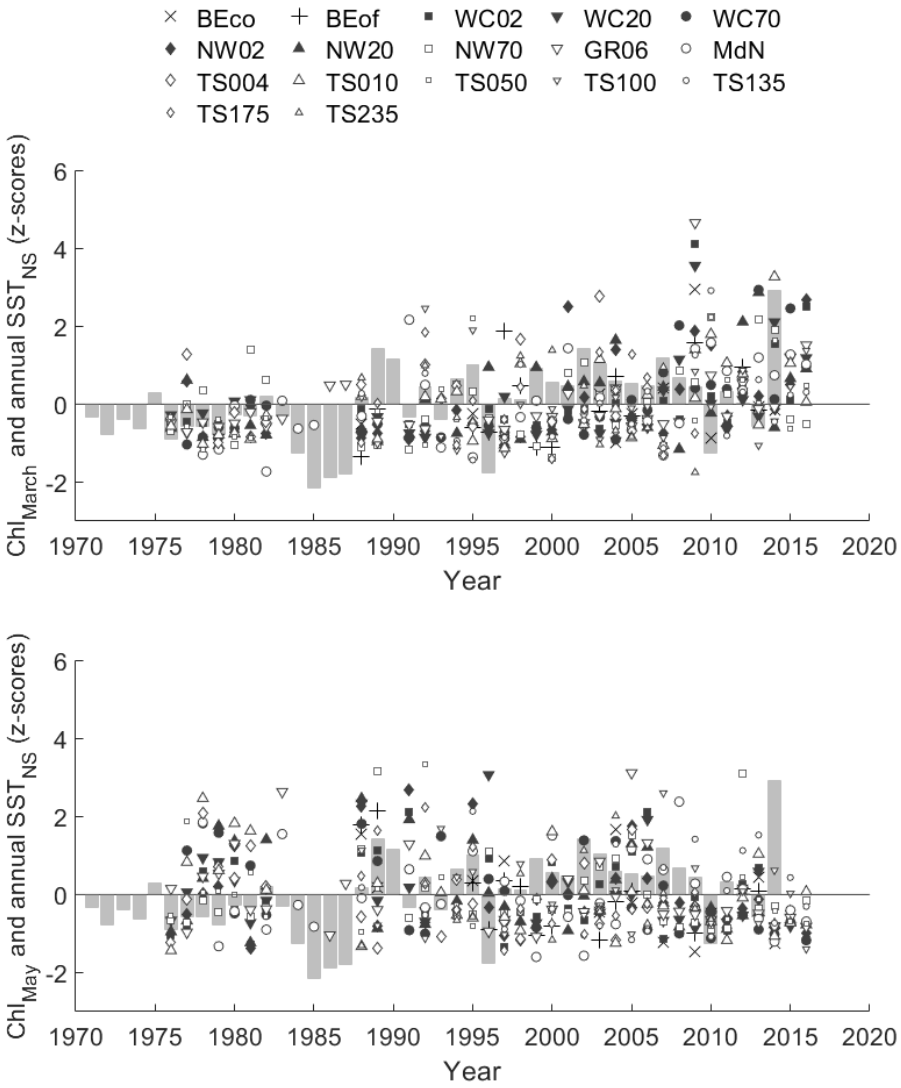


Figure 7 The bars indicate the standardized (z-score) annual mean SST_{NS}. TOP – Standardized (z-score) Chl values in March at different sampling sites for each year in the period 1975-2016. BOTTOM – Standardized (z-score) Chl values in May at different sampling sites for each year within the period 1975-2016.

A change in bloom phenology is visible in Figure 7 with the standardized Chl in March (z-score) increasing from year to year at all sampling sites, while the standardized Chl in May (z-score) decreases (especially after 1995). This further illustrates the ongoing forward shift of the spring bloom. An increasing trend (not shown) was detected over the period for the annual mean SST_{NS} (z-score, slope = 0.033; intercept = -66.33) and for Chl values in March when aggregated over all sampling sites (z-score, slope = 0.027; intercept = -54.67).

A pH time series of the Belgian dataset (aggregated across the BCS) is shown in Figure 8; a similar analysis for the DCS can be found in Provoost et al. (2010). Annual means of pH in the BCS show a gradual increase from the early 1970's to the mid 1980's, followed by a sharp decrease around 1989, after which the values more or less stabilize (Figure 8). The pH increases when the net phytoplankton photosynthesis increases and organic matter accumulates. Therefore, the pH

pattern may be related to changes in phytoplankton production resulting from eutrophication and de-eutrophication processes and/or the increase in SST after 1987.

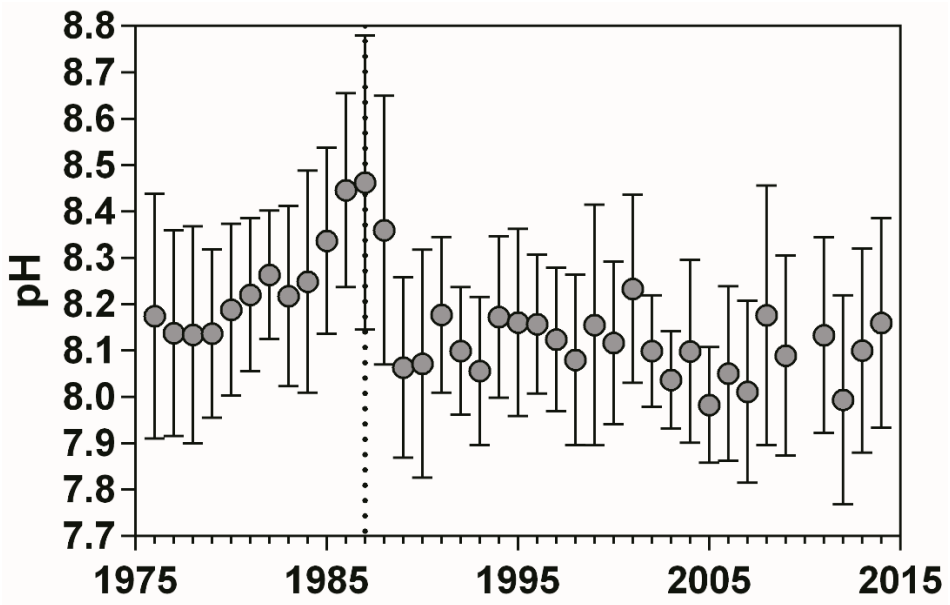


Figure 8 Time series of the pH in the Belgian continental shelf (Southern North Sea) over the period 1975-2015. The vertical dashed line indicates the year of maximum in 1987.

This trend is also visible in the seasonal amplitude of pH (calculated as the difference between the monthly minimum and maximum for a given year; Figure 9). The seasonal amplitude of the pH signal can be interpreted as a proxy of the net annual phytoplankton production. It was highest during the period 1980-1989 indicating a more intense phytoplankton production resulting in a rapid biomass accumulation. In more recent years, the seasonal amplitude of pH is lower than in any previous decade suggesting a decreasing trend in the annual phytoplankton production as a response to nutrient load reduction (Borges and Gypens, 2010; Lancelot et al., 2007), or as a response to an increase in temperature (Barton et al., 2018; Capuzzo et al., 2018) or in turbidity (Capuzzo et al., 2018). During the period 1975-2015, the seasonal signal of pH also showed a change during the phytoplankton bloom with high values shifting from late spring to early spring. This is apparent on the detrended signal of pH in April and May (Figure 9). While the pH anomaly increases in April, it globally decreases in May (starting from the 80's). This is consistent with the change observed in Chl phenology (Figure 6 and 7), with a bloom shifting towards earlier days in the spring.

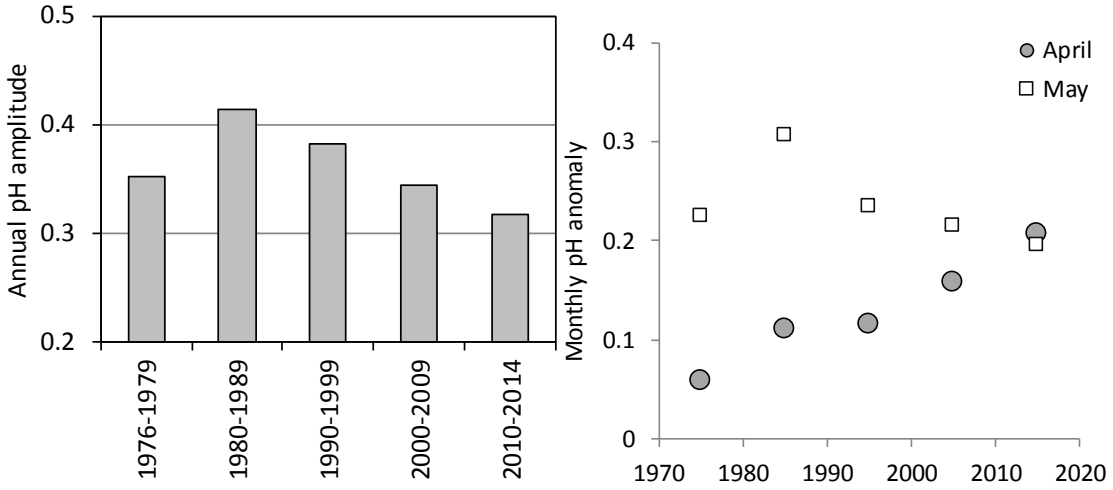


Figure 9 Left – Annual pH amplitude (calculated as the difference between the monthly maximum and minimum per yearly cycle) per decade in the BCS. Right – Monthly anomalies of pH in April and May in the BCS. This is computed as the monthly average minus the annual average; so this eliminates the long-term trends (i.e. the increasing trend of annual pH from 70's to 80's and the decreasing trend of annual pH from late 80's to nowadays).

4.5. Discussion

At least two major drivers of phytoplankton production have changed in recent decades in the North Sea: SST has increased by 1.5°C between 1988 and 2014, and the N and P loads from surrounding rivers have decreased from the mid-1980's onwards. There is still debate about the causal factors of the North Sea warming trend since the mid-1980s. Some authors attribute it mainly to an increase in Northern Hemisphere Temperature (e.g. Beaugrand and Reid, 2003), while van Aken (2010) also points out the contribution of local variations in wind circulation and cloudiness, not excluding the influence of the Northern Hemisphere Temperature. The accelerated increase in SST_{NS} in 2014 may be related to the increase in the global mean surface temperature that year at the end of a period of reduced warming, known as the hiatus (Trenberth, 2015). As the global mean surface temperature is expected to further increase, it is likely that the SST_{NS} will also rise in the future. A seasonal analysis shows that the winter-spring values of SST_{NS} have increased more than the summer-fall values in the period, which can have important repercussions for the phytoplankton spring bloom. In the same period, the N and P loads from the rivers surrounding the North Sea have been reduced as a result of de-eutrophication measures implemented by national legislations and European directives. This caused a decreasing trend in marine nutrient concentrations in the coastal stations under freshwater influence, with again potential impacts on phytoplankton production. The offshore areas (>70 km) exhibit more conservative winter nutrient concentrations as they are mostly subject to oceanic inputs of nutrients.

A decline in the annual mean Chl since 1988 was observed in most stations, while a decreasing trend in the Chl P90 was also observed in coastal stations subject to high river loads (Rhine ROFI). This is consistent with previous studies having observed that a reduction in riverine nutrient loads will impact coastal phytoplankton production through nutrient limitation (Desmit et al., 2015; Lacroix et al., 2007; Lenhart et al., 2010; Rousseau et al., 2006). However, it is less clear whether the decline in the annual mean Chl observed in most stations since 1988 can solely be attributed to reduced riverine nutrient inputs. Especially 'BE offshore', 'Noordwijk 70' and 'Terschelling 135' also exhibit a decline in annual mean Chl (at least a change in central tendency toward lower values over the period) but do not show a clear decreasing trend in nutrient concentrations (except for P in BE offshore, but it has been argued that N is limiting there; Burson et al., 2016; Desmit et al., 2015).

The increase in SST_{NS} could be another factor contributing to the decline in the annual mean Chl, as has already been shown at the scale of the northern Atlantic (Richardson and Schoeman, 2004). However, previous authors have pointed out an increase – instead of a decrease – in Chl driven by temperature (Beaugrand and Reid, 2003; Edwards et al., 2006; McQuatters-Gollop et al., 2007). Beaugrand and Reid (2003) observed that the peak of maximal variation for Northern Hemisphere Temperature anomalies was detected around 1987 and opened an exceptional period with pronounced changes in biological variables, including an increasing phytoplankton biomass. With the Continuous Plankton Recorder dataset, Edwards et al. (2006) observed an increase in phytoplankton biomass, especially from mid-1980s to 2002, with a coincident increase in SST from 1987 in the Central North Sea. McQuatters-Gollop et al. (2007) describe a general increase in Chl between 1948 and 2003 in the North Sea, and more particularly a rapid increase in Chl from the mid-1980s to the late-1990s, with a peak in 1989. The year 1989 corresponds to a known regime shift in the North Sea, i.e. "a stepwise modification in the composition and productivity of an entire ecosystem at a regional scale, reflecting substantial hydrographic change (Beaugrand, 2004; McQuatters-Gollop et al., 2007). In fact, "three very large and temporally persistent regime shifts have occurred around 1968, in the mid-1980's and post-1996" in the North Sea, according to Edwards et al. (2016). As part of these regime shifts, Edwards et al. (2016) show peaks in phytoplankton abundance in the mid-1980s and then in the late-1990s in the Greater North Sea, after which phytoplankton abundance clearly decreases. This seems to correspond to the Chl behaviour in the observations of McQuatters-Gollop et al. (2007) where Chl stabilizes or starts to decrease after the peak in the late-1990s. Our results also show an increase in annual mean Chl until the mid-1990s or early-2000s (depending on the site) followed by a decrease in annual mean Chl at most sampling sites (see Figure 5).

In the North Sea, phytoplankton dynamics follows a non-linear sequence of events punctuated by unpredictable regime shifts due to hydroclimatic variability and changing pressures (eutrophication, SST). Therefore, it is difficult to explain why phytoplankton Chl decreases after the late-1990s. More particularly, identifying what drives the Chl decline is complicated by the fact that both SST and nutrient river loads have changed in the mid-1980's. Capuzzo et al. (2018) recently reported that a decline in phytoplankton production (modeled on the basis of Chl, water clarity and surface irradiance) in the North Sea since the mid-1990s showed strong correlations with the SST increase (especially in summer and autumn) and with a decrease in N and P rivers loads. An increase in SST induces a deepening of the surface layer in stratified areas and may result in a decrease of phytoplankton production above the pycnocline, especially in summer. Capuzzo et al. (2018) propose that the decline in phytoplankton production across the North Sea in recent decades may be explained by a deepening of the stratification and a decrease in water clarity. Another recent study provides a physiological explanation for the link between rising temperatures and a decline in phytoplankton density (Barton et al., 2018). According to these authors, the rates of respiration measured in dominant lineages of marine phytoplankton seem consistently more sensitive to increasing temperature than photosynthesis. Moreover, respiration shows higher optimal temperatures than photosynthesis. Therefore, the capacity of phytoplankton to efficiently use its photosynthetic carbon to grow could be limited at higher temperatures by universal metabolic constraints, i.e. rising respiratory costs exceeding the supply of reduced carbon from photosynthesis (Barton et al., 2018).

An ubiquitous change in phytoplankton bloom phenology was also observed, with the onset of the spring bloom occurring earlier in the year. This shift in Chl phenology cannot be explained by a change in marine nutrient concentrations: even when marine nutrients followed a decreasing trend, i.e. in coastal stations, their seasonal signal did not change (except for the fact that N, P and Si are consumed at faster rates in recent years than before; not shown). Moreover, the shift in Chl phenology is also visible at offshore sampling sites (e.g. Belgian offshore, NW70, TS135, TS235) where nutrient concentrations are more conservative throughout the period. In contrast, the SST_{NS} was increasing during the period, especially in winter and spring (Figure 2, bottom). Previous authors have pointed out a shift in phenology driven by a temperature increase (see Beaugrand and Reid, 2003; Edwards et al., 2006). A mechanistic explanation was proposed by Hunter-Cevera et al. (2016): increasing temperature accelerates phytoplankton cell division rates which results in earlier bloom formation. This may explain the results of Figures 6 and 7: on the one hand, spring bloom formation tends to start earlier in recent years and, on the other hand, the bloom tends to stop earlier, after the dissolved nutrients have been depleted because of the faster growth rates.

Such a forward shift in phytoplankton blooms in recent years is also supported by the seasonal signal of the pH anomaly (Figure 9). It is commonly considered that the onset of the spring bloom in the North Sea depends on both light availability and temperature (Peperzak, 1993; Peperzak et al., 1998). Therefore, another explanation could involve a change in the underwater light field in recent decades either through a change in cloudiness and hence surface irradiance, or through a change in turbidity. Wiltshire et al. (2008) reported an increase in water transparency at Helgoland Roads but, even so, the spring bloom dynamics have hardly changed at all. They also observed a delay in the second spring bloom that was correlated to SST increase. This contradicts the hypothesis of an earlier Chl bloom onset driven by water clarity. Alvarez-Fernandez and Riegman (2014) reported an increase in the average underwater Photosynthetically Active Radiation in some coastal stations of the DCS, with an effect on the carbon-to-chlorophyll *a* ratio of local phytoplanktonic assemblages. In contrast, Capuzzo et al. (2015) showed that water clarity of the North Sea has globally decreased in the last decades driven largely by increased suspended sediment, except perhaps in the permanently mixed areas of the Southern Bight including the Belgian coast. Although water clarity may have increased locally, it seems that the general trend in the North Sea is toward a decrease in water clarity. The ubiquitous change in phytoplankton phenology that we observe is therefore unlikely explained in the first order by a change in the underwater light field. Regarding the surface irradiance, Capuzzo et al. (2018) estimated it in the North Sea between 1988 and 2013 and did not report any trend.

The decline in annual mean Chl and the shift in phytoplankton phenology may have an impact on the food web. It has been shown that the decline in phytoplankton production across the North Sea in recent years is likely the cause of a decrease in small copepod biomass and fish recruitment (Capuzzo et al., 2018). Regarding the phytoplankton phenological shift, the trophic 'match-mismatch' hypothesis predicts that a predator may miss the production peak of its prey under warming conditions as the level of response to climate change may vary across functional groups and multiple trophic levels (Edwards and Richardson, 2004). This effect may be reduced in temperate productive systems, however, as pointed out by Atkinson et al. (2015): the variability in relative timing of feeder and food is naturally high in the marine system and, thus, some compensating mechanisms exist to reduce the impact of a planktonic phenological shift on the trophic transfers, potentially reducing adverse effects. This does not mean there is no impact of a phenological shift on the food web but instead that there is a need for further studies on that topic to understand the implications of it.

As a concluding remark, the increase in SST and the reduction in riverine nutrient loads since the mid-1980s in the North Sea are potentially the dual cause of the observed decline in surface Chl

in recent decades. Also, the increase in SST is very likely responsible for the phenological forward shift in the spring phytoplankton bloom. In the last decades, phytoplankton in the North Sea responded to the observed warming and will potentially continue to do so under further warming in the future, with implications for the food web. Other temperate marine areas may potentially be subject to such effects.

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5

Long-term phytoplankton trends and variability in *Phaeocystis* blooms in the Belgian part of the North Sea

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5.1. Abstract

Colonial *Phaeocystis* is considered a nuisance species which together with diatoms regularly dominates the spring phytoplankton community in the Belgian part of the North Sea (BPNS). Many hypotheses exist concerning the abiotic and biotic factors controlling the development of *Phaeocystis* blooms but to date these are not yet fully understood. This study aims at obtaining more insight into the mechanisms affecting the occurrence and the magnitude of the *Phaeocystis* spring bloom in the BPNS. To this end, long-term interannual variation in the biomass of *Phaeocystis* and diatoms in the BPNS were estimated using HPLC-CHEMTAX pigment analysis (2003-2016) and microscopic cell counts (2003-2010). Annual anomaly (z-score) analyses, Mann-Kendall trend tests and General Additive Mixed Models (GAMMs) reveal pronounced interannual variability, an overall increase of chlorophyll a and diatom biomass during the period 2003 to 2016, but no clear long-term trend for *Phaeocystis*. *Phaeocystis* biomass showed significant interannual variation in bloom intensity, with exceptionally strong blooms in 2007, 2015 and 2016, and weak blooms in 2005, 2006 and 2011 to 2013. Correlation analyses suggest that the availability of light during the *Phaeocystis* growing period and sea surface temperature (SST) are two important factors stimulating the development of dense *Phaeocystis* blooms. Likewise, a high North Atlantic Oscillation index$_{March}$ (NAOi$_{March}$) is positively linked to the *Phaeocystis* bloom. High pre-bloom nutrient concentrations do not have a stimulating effect on *Phaeocystis*, but most nutrient concentrations in March (NO_3^-, NO_2^-+NO_3^-, dissolved inorganic phosphorus (DIP) and dissolved silica (DSi)) are negatively correlated with the magnitude of the *Phaeocystis* bloom in April.

The data suggest that competition with the preceding diatom community may play an important role in *Phaeocystis* bloom development. Under low SST, diatoms win the competition over *Phaeocystis*, because *Phaeocystis* in its flagellate form (in early spring) does not grow fast enough to accumulate a critical biomass. Eventually, diatoms bloom ends with DSi depletion and, hence, *Phaeocystis* can grow on remaining nutrients (slower and later due to low SST and with lower total biomass due to lower remaining nutrients). Under higher SST, *Phaeocystis* in its flagellate form grows fast enough to start forming colonies, while diatoms are still growing on the spring pool of nutrients. Colonial *Phaeocystis* enters into competition with diatoms for the remaining DIN and DIP and may dominate thanks to their advantage of using DIP. This allows *Phaeocystis* to develop massive blooms, which also occur earlier.

5.2. Introduction

The marine Prymnesiophyte *Phaeocystis* is commonly considered as a nuisance alga which negatively affects tourism and recreation due to foam formation and bad odours during bloom

declines (Blauw et al., 2010; Cadée and Hegeman, 2002; Seuront et al., 2006). *Phaeocystis* blooms also have far reaching ecological consequences as large colonies (typically > $4*10^6$ cell L^{-1}) become inedible to zooplankton copepods (Lancelot et al., 2009). In addition, *Phaeocystis* colonies inhibit copepod's egg production during spring, which negatively affects the following copepod generations (Daro et al., 2006). Dense *Phaeocystis* blooms can also lead to clogging of fishing nets and can harm fish, mussels and benthic invertebrates via oxygen depletion (resulting from decay of massive blooms) and clogging of their gills (Chang, 1983; Peperzak and Poelman, 2008; Pieters et al., 1980; Rogers and Lockwood, 2009; Seuront et al., 2006).

A study based on Continuous Plankton Recorder (CPR) data from the North Sea showed that the frequency of occurrence of *Phaeocystis* blooms was especially high during and before the 1960s and after the 1980s, and that the *Phaeocystis* season was also longer before 1965 and after 1995 than in the period in between (Gieskes and Kraay, 1977; Gieskes et al., 2007). In the Dutch Wadden Sea, *Phaeocystis* bloom duration had increased already from the end of the 19th century to the end of the 1980s and the increase in cell numbers since the middle of the 20th century has reversed to a general decreasing trend in the magnitude of the spring bloom since the 1990s in this area (Cadée and Hegeman, 1991; Cadée and Hegeman, 2002). Also in the western English Channel, a decreasing *Phaeocystis* trend between 1992 and 2007 was observed (Widdicombe et al., 2010). In the Belgian part of the North Sea (BPNS) no clear trend regarding the maximum *Phaeocystis* cell numbers were observed during the period 1988 to 2001 (Lancelot et al., 2009) and also in the Dutch part of the North Sea no clear trend was observed (1990-2007) (Prins et al., 2012). Despite these locally identified long-term trends in cell numbers, bloom frequency and bloom duration in various areas of the North Sea, it is generally accepted that there is strong interannual variation in *Phaeocystis* spring blooms in the Southern Bight of the North Sea and the Eastern English Channel (Schapira et al., 2008; Breton et al., 1999; Seuront et al., 2006; Lancelot et al., 1994; Gómez and Souissi, 2008; Hernández-Fariñas et al., 2014; Breton et al., 2006; Karasiewicz et al., 2018). However, why *Phaeocystis* blooms in some years, and in others not, is as yet not fully understood (Borkman et al., 2016; Karasiewicz et al., 2018).

Various hypotheses have been proposed to explain the occurrence and intensity of *Phaoecystis* blooms. According to the 'silicate-*Phaeocystis* hypothesis', *Phaeocystis* starts blooming after silica has been depleted by diatoms during spring (Borkman et al., 2016; Lancelot et al., 1987; Peeters et al., 1991; Reid et al., 1990). Other studies have suggested that instead of a single controlling factor, a combination of environmental factors and/or pre-bloom conditions can promote *Phaeocystis* blooms, such as reduced light availability and increased turbidity due to higher

Suspended Particulate Matter (SPM) concentrations in late winter, but also at the time of the *Phaeocystis* bloom (Gómez and Souissi, 2008; Karasiewicz et al., 2018; Peperzak et al., 1998); high pre-bloom sea surface temperature (SST) (Borkman et al., 2016; Gieskes and Kraay, 1977; Gómez and Souissi, 2008); elevated late winter NO_3^- and PO_4^{3-} concentrations (Borkman et al., 2016; Cadée and Hegeman, 1986; Gómez and Souissi, 2008; Lancelot et al., 1987; Muylaert et al., 2006), and elevated nutrient ratios (e.g. elevated NO_3^-: PO_4^{3-}, NO_3^-:Si and PO_4^{3-}:Si) (Borkman et al., 2016; Lancelot et al., 1987; Lancelot et al., 2007; Riegman et al., 1992). As nutrient uptake is an essential factor for phytoplankton growth, increased N and P input was for a long time considered to be the main reason for increased *Phaeocystis* growth (Lancelot et al., 1987). However, *Phaeocystis* bloom frequency was very high before the eutrophication period (i.e. before the 1960s) and has remained high since the de-eutrophication period (after the 1980s) (Gieskes et al., 2007). Large-scale climate patterns have also been considered to affect *Phaeocystis* bloom development. In the BPNS and the English Channel, a negative correlation of the *Phaeocystis* spring bloom magnitude and its dominance over diatoms with the winter NAO index has been identified (Breton et al., 2006; Irigoien, 2000; Seuront and Souissi, 2002). High NAO index values lead to the strengthening of south-westerly winds, transporting more water from the English Channel to the Southern Bight of the North Sea, resulting in higher SST, increased rainfall and diffuse nutrient input into the BPNS (Ruddick and Lacroix, 2006).

Phaeocystis also interacts with other biotic groups such as viruses and zooplankton. As a defence mechanism, *Phaeocystis* can respond to different chemical cues from copepods, ciliates and heterotrophic dinoflagellates with the enlargement of their colonies (Jakobsen and Tang, 2002; Tang, 2003; Tang et al., 2008; Wang et al., 2015). Viruses are known to cause cell lysis of *P. globosa* and are an important factor in the regulation of the bloom formation (Brussaard et al., 2005). Colony formation restricts viral infection, while the solitary cell morphotype is vulnerable to viral infection (Brussaard et al., 2005). Also grazing by zooplankton plays an important role in the control of the *Phaeocystis* bloom. However, as laboratory-derived grazing rates by crustaceans might have been overestimated compared to grazing rates in the natural environment, it is still unclear to which extent different zooplankton species graze on the different life-forms of *Phaeocystis* (Nejstgaard et al., 2007). Large copepods such as *Calanus* spp., ciliates and heterotrophic dinoflagellates are able to graze on *Phaeocystis* colonies (Nejstgaard et al., 2007; Peperzak et al., 1998), but small copepods (e.g. *Temora*, *Acartia*, *Pseudocalanus* and *Centropages*), which are among the main primary consumers in the BPNS, do not ingest larger *Phaeocystis* colonies (Breton et al., 1999; Daro et al., 2006; Gasparini et al., 2000; Nejstgaard et al., 2007;

Rousseau et al., 1990). As a result, the trophic efficiency of the food web is negatively correlated with *Phaeocystis* biomass (Lancelot et al., 2009).

A recent study from the French English Channel coast suggests that a combination of the winter environmental conditions and the composition of the diatom community before the *Phaeocystis* bloom is the major factor responsible for the *Phaeocystis* bloom development (Karasiewicz et al., 2018). In case of high winter ammonium concentrations and non-limiting Si and P conditions, diatom species with a high ammonium affinity (*Skeletonema* spp., *Thalassiosira gravida*, *Thalassionema nitzschioides* and *Pseudo-nitzschia seriata*) are stimulated and able to outcompete *Phaeocystis* until Si becomes limiting (Karasiewicz et al., 2018). Low P- and Si-concentrations on the other hand give an advantage for *Phaeocystis* over diatoms, due to their ability to use organic P-sources, their lower P demand and their P-storage capacity, and Si-limitation of diatoms (Burson et al., 2016; Riegman et al., 1992; van Boekel and Veldhuis, 1990; Veldhuis et al., 1991). Under this conditions, *Phaeocystis* is able to outcompete the diatom species with a high ammonium affinity and low silicified diatom species (*Chaeotoceros danicus*, *Ditylum brightwellii*, *Nitzschia longissimi* and *Leptocylindricus danicus*) (Karasiewicz et al., 2018).

Because *Phaeocystis* is a nuisance alga with potential ecological and economic effects, knowledge regarding the mechanisms leading to intense blooms of this taxon is essential for the development of suitable management strategies. The present study aims to achieve a better understanding of seasonal and long-term dynamics of *Phaeocystis* blooms in the BPNS. Mann-Kendall trend analyses and general additive mixed models (GAMMs) are used to investigate interannual and seasonal dynamics of *Phaeocystis* and diatom blooms, using a combination of microscopic cell counts (2003 to 2010) and High-performance liquid chromatography (HPLC) biomass estimates (2003 to 2016). We use correlation analyses to relate pre-bloom conditions of several in-situ measured environmental parameters such as nutrient concentrations, SPM and SST, the large-scale climatic index NAO and diatom biomass to *Phaeocystis* bloom magnitude.

5.3. Material and Methods

5.3.1. Study area and sampling

5.3.1.1. Research area

The BPNS is located in the Southern Bight of the North Sea (Figure 1). This area is characterized by relatively shallow waters (< 40 m depth) and a complex system of sand banks, which are orientated more or less parallel to the coast, following the direction of the residual current (De Galan et al.,

2004; Ruddick and Lacroix, 2006). The combination of strong vertical mixing and shallow depth results in a vertically homogeneous water column throughout the whole year in most parts of the region, although recent studies show that the northern part of the BPNS is intermittently stratified (van Leeuwen et al., 2015). The BPNS receives Atlantic Ocean water via the English Channel, entraining nutrients from the rivers Seine and Somme, and freshwater from the Scheldt, Meuse and the Rhine (Lancelot et al., 2007). The inflow of fresh, nutrient-rich and turbid water creates strong gradients in salinity, suspended solids and nutrients from the East towards the West.

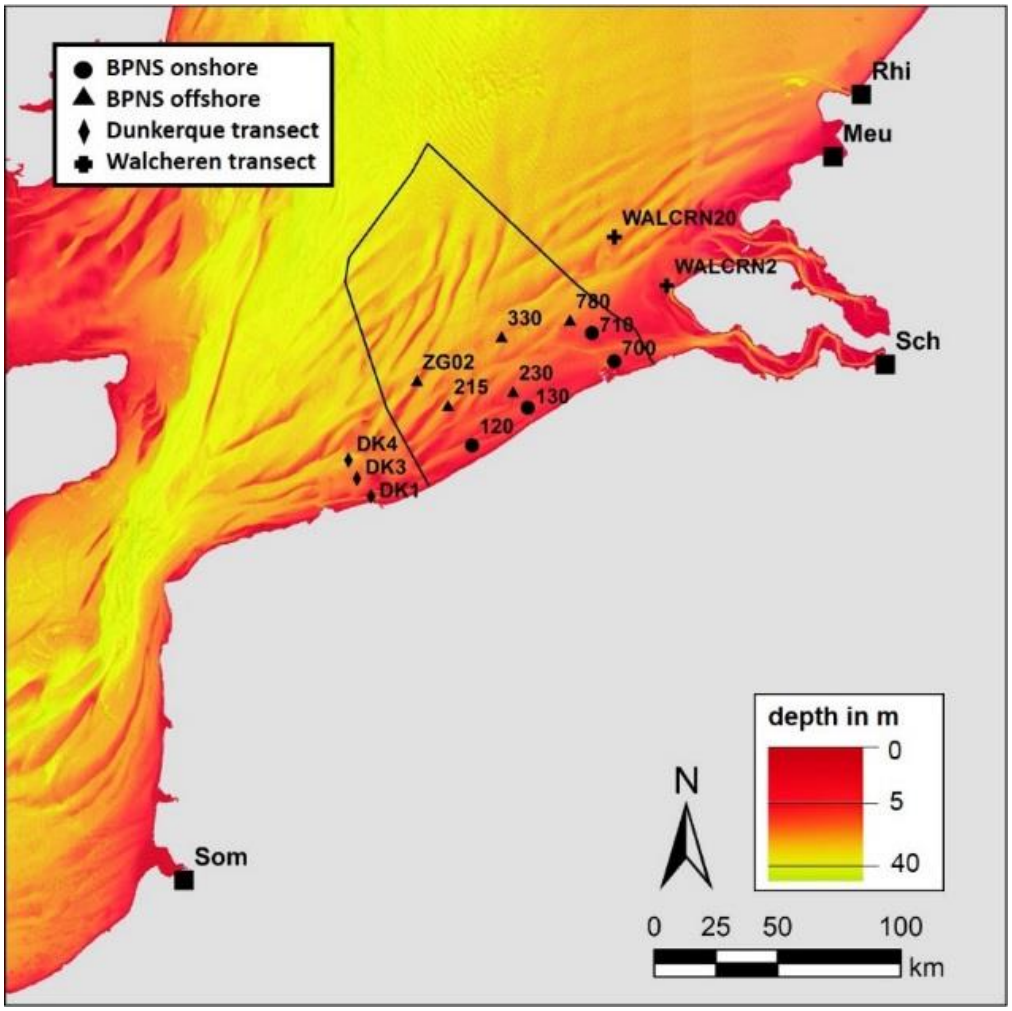

Figure 1 Bathymetry map (source: EMODnet) of the the Belgian Part of the North Sea (BPNS). Sampling locations and main river outlets are indicated. The BPNS is indicated as a black line. Meu: Meuse, Rhi: Rhine, Som: Somme, Sch: Scheldt.

5.3.1.2. Sampling campaigns

Monthly sampling cruises were performed since October 2002 by the research vessel (RV) 'Zeeleeuw' (2002-2011) and subsequently the RV 'Simon Stevin' (since 2012), exploited by the Flanders Marine Institute (VLIZ). We here analyze the data until December 2016. During each cruise, nine fixed monitoring stations were sampled, located along three transects perpendicular to the coast (Figure 1).

5.3.1.3. Sampling

Water samples were taken at 3 m water depth using the Seabird 55 ECO, a water sampler carrousel (6 bottles of 4 liters) on the CTD (Seabird 19plusV2). A subsample of seawater from the sampler carrousel was filtered using a vacuum pump until a 0.7 µm glass fiber filter (diameter 47 mm, Whatman GF/F) was saturated. The filter was then stored in a 1.5 ml Eppendorf tube, snap frozen in liquid nitrogen and kept frozen at -80 °C until analysis. For microscopic analysis, a 250 ml subsample from the sampler carrousel was fixed with Lugol's solution and stored at 4 °C until analysis (Franck, 2004).

An additional subsample was taken and stored at 4 °C for determination of SPM. For the determination of the dissolved nutrients, seawater was filtered over a 0.45 µm filter (Milllipore SLHA 025 10) and stored at -20 °C (ammonium (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-), phosphate (PO_4^{3-})) or in a refrigerator (silicate (SiO_4)). The filtrate was stored in poly-ethylene flasks of 100 ml or in 4 ml PICO vials and analyses were done within one week with the help of the continuous flow analyser system SEAL QuAAtro (Bran+Luebbe, Norderstedt, Germany) (NIOZ, 2015). In addition, SST and salinity were measured in situ with a CTD equipped with a Seabird SBE21 SeaCAT Thermosalinograph.

5.3.2. Sample analysis

5.3.2.1. Microscopic cell counts

The fixed phytoplankton samples were analysed with the Utermöhl method (Utermöhl, 1958). Samples were homogenized and a 50 ml subsample was settled for 24 hours at room temperature and in the dark before analysis. The settled sample was analysed with an inverted microscope (Zeiss Axiovert 135). A minimum of 250 cells was counted at a magnification of 200x to 400x (Muylaert et al., 2006). As *Phaeocystis* colonies disintegrate during sample storage, both colony fragments and single cells were counted (Muylaert et al., 2006). Data were expressed in cells L^{-1}. Microscopic cell counts were conducted on samples taken between 2003 and 2010.

For comparative analyses, *Phaeocystis* cell count data from two neighbouring transects on the French coast (Dunkerque transect) and the Dutch coastal zone (Walcheren transect) were used (period 2003 to 2016) (Figure 1). The Dutch phytoplankton composition data were collected by Rijkswaterstaat (RWS) and were made available via the service desk of the RWS. French phytoplankton data were collected as part of the REPHY program (French Observation and Monitoring program for Phytoplankton) (SRN, 2017) and can be downloaded from http://www.seanoe.org/data/00397/50832/.

5.3.2.2. HPLC phytoplankton pigment composition

Pigments were extracted in 90 % HPLC grade acetone and sonicated for 30 seconds at 40 Hertz. Extracts were immediately analysed by means of reverse phase high-performance liquid chromatography (HPLC) following the protocol as described in Van Heukelem and Thomas (2001) on an Agilent 1100 series HPLC system, using an Agilent Eclipse XDB-C8 column. Pigments were identified based on their retention time and absorption spectra in comparison with pure pigment standards (supplied by DHI Denmark) (Muylaert et al., 2006). During the sampling period, three different HPLC protocols were applied: Wright et al. (1991) (2002-2003, 2005-2008), Zapata et al. (2000) (2004) and Van Heukelem and Thomas (2001) (2008-2016). Because the focus of this study was the phytoplankton spring bloom, the 2002 and 2008 data were not used in the statistical analyses, due to missing spring samples. Finally, the 2004 HPLC data were not used in the statistical analyses, as the Zapata et al. (2000) methodology used in this year measured unrealistically low chlorophyll a concentrations (never higher than 7 µg chl a L^{-1}).

5.3.2.3. Environmental data

Dissolved inorganic nutrients (silica (DSi), phosphate (DIP), nitrite (NO_2^-), nitrate (NO_3^-) and ammonium (NH_4^+)) were measured following the methods described in Grasshoff et al. (1983). Ammonium was measured with the indophenol blue technique according to Koroleff (1969). The amount of NO_2^-, NO_3^- and NH_4^+ were summed up to dissolved inorganic nitrogen (DIN) (Muylaert et al., 2006) and nutrient ratios (DIN:DIP, DSi:DIP and DSi:DIN) were calculated. The SPM concentration was determined after the filtration of a water sample over a glass fibre filter (filter density of 50 to 100 g m^{-2}) and drying of the filter for at least 1 to 2 hours in the oven at 105 °C (unknown, 2011). The Secchi depth was determined by using a Secchi disk. LifeWatch observatory data (nutrients, SPM and Secchi depth) (Flanders Marine Institute (VLIZ), 2018) in the BPNS are available via http://www.vliz.be/en/imis?dasid=4685&doiid=310.

Daily NAO index data were downloaded from the website of the Climate Prediction Center: http://www.cpc.ncep.noaa.gov/products/precip/CWlink/pna/nao.shtml.

5.3.3. Data analysis and statistics

The relative contribution of each phytoplankton group to total phytoplankton biomass was estimated using CHEMical TAXonomy (CHEMTAX v1.95) software (Mackey et al., 1996). CHEMTAX requires three matrices, the first matrix contains the marker pigments to chlorophyll a ratios from the sampled stations. In this study the following marker pigments were used: fucoxanthin, chlorophyll *c3*, peridinin, zeaxanthin, chlorophyll b, diatoxanthin + diadinoxanthin (DDX) and lutein. As biomarkers for diatoms fucoxanthin and DDX were used. The biomarkers for *Phaeocystis*

are fucoxanthin, chlorophyll *c3* and DDX. The second matrix contains the theoretical values of the marker pigments to chlorophyll a ratios per phytoplankton group. For this study the matrix published by Muylaert et al. (2006) was used, which was based on published accessory pigment to chlorophyll a ratios (Antajan et al., 2004; Schlüter et al., 2000). The third matrix sets the limits on the theoretical marker pigment to chlorophyll a ratios. CHEMTAX optimizes the contribution of the phytoplankton groups using a steepest descent algorithm to find the lowest pigment content unexplained (lowest root mean square) of the theoretical and the limit matrix on the natural sample matrix.

Sampling stations were clustered into an onshore (stations 120, 130, 700 and 710) and an offshore (stations 215, 230, 330, 780 and ZG02) group (Table 1, Supplementary Figure 1, Figure 1), reflecting differences in salinity, nutrients and SPM concentration. Onshore stations are strongly influenced by freshwater inflow of the Scheldt and IJzer rivers and are characterized by a lower salinity, but higher nutrient and SPM concentrations. The offshore stations are mainly influenced by Atlantic Ocean water entering through the English Channel, have a higher salinity, and lower SPM and nutrient concentrations.

Table 1 Overview of sampling sites, including geographical coordinates, depth and median (P10, P90) for SST, salinity, Secchi depth, SPM and nutrient concentrations.

area	station	lat	lon	depth (m)	SST (°C)	salinity (psu)	secchi (cm)	SPM (mg L^{-1})	DIP (µM L^{-1})	DIN (µM L^{-1})	DSi (µM L^{-1})
onshore	120	51° 11' N	2° 42' E	14.5	11.4 (4.6, 20.0)	33.8 (30.6, 34.6)	120 (40, 280)	19.7 (6.9, 100.7)	0.41 (0.09, 1.34)	14.06 (1.99, 54.78)	5.07 (0.59, 27.23)
	130	51° 16' N	2° 54' E	10.6	11.0 (5.4, 19.0)	32.6 (30.4, 34.3)	60 (20, 160)	41.8 (14.4, 104.2)	0.62 (0.16, 1.43)	16.52 (2.92, 54.77)	7.62 (0.48, 26.02)
	700	51° 22' N	3° 13' E	12.1	11.0 (5.4, 19.1)	30.9 (29.7, 32.3)	40 (20, 100)	59.7 (22.9, 153.3)	1.03 (0.30, 1.44)	23.08 (9.77, 55.42)	11.23 (3.00, 29.39)
	710	51° 26' N	3° 08' E	11.1	11.0 (5.4, 19.1)	32.2 (31.1, 33.4)	80 (40, 160)	39 (12.6, 105.0)	0.76 (0.13, 1.16)	16.47 (2.29, 38.8)	8.30 (1.32, 19.02)
offshore	215	51° 16' N	2° 36' E	28.5	12.4 (6.8, 18.3)	34.0 (32.2, 34.7)	310 (100, 550)	10.4 (3.7, 28.1)	0.27 (0.08, 0.94)	7.99 (0.91, 33.09)	2.89 (0.37, 14.69)
	230	51° 18' N	2° 51' E	13.2	11.3 (5.9, 18.7)	32.6 (31, 34.5)	90 (40, 300)	25.9 (7.7, 83.1)	0.54 (0.12, 1.20)	13.03 (2.78, 43.61)	5.77 (0.45, 21.45)
	330	51° 26' N	2° 48' E	24.3	13.3 (6.6, 19.1)	33.9 (32.6, 34.7)	360 (174, 584)	7.8 (3.8, 22.3)	0.40 (0.08, 0.90)	5.19 (1.35, 24.13)	2.58 (0.42, 12.88)
	780	51° 28' N	3° 03' E	23	12.1 (5.7, 19.3)	32.6 (31.5, 33.8)	160 (78, 340)	16.7 (5.7, 43.8)	0.63 (0.10, 1.11)	11.23 (2.52, 35.49)	7.31 (0.88, 17.27)
	ZG02	51° 20' N	2° 30' E	17.5	12.3 (6.7, 18.3)	34.4 (33.2, 34.8)	400 (200, 700)	8.2 (2.9, 23.9)	0.21 (0.06, 0.64)	3.4 (0.94, 20.84)	2.21 (0.35, 8.05)

Mann-Kendall trend tests were conducted in order to identify monotonic up- or downward trends (separately for the on- and offshore group) in abiotic and biotic parameters: chlorophyll a, diatom and *Phaeocystis* biomass (as determined by HPLC-CHEMTAX), DIN, DIP, DSi, nutrient ratios, SPM, SST and salinity (for the whole time series and for the winter values (December to February) alone). The *tau*-coefficient indicates if there is a decreasing (negative *tau*) or increasing (positive *tau*) trend. The *p*-values indicate if the observed trend is significant at $p<0.05$.

Annual anomalies in the diatom and *Phaeocystis* bloom were tested for the months February (diatoms), March (diatoms) and April (*Phaeocystis*), by centering and scaling the data. For this, the *scale* function in R was used (RStudioTeam, 2017). Centering is done by subtracting the overall mean from the data. Scaling is done by dividing by the standard deviation *sd*:

$$z - score = \frac{x - mean(x)}{sd(x)}$$

By testing the April *Phaeocystis* anomalies, exceptional 'low' (L) and 'high' (H) *Phaeocystis* years could be identified.

To unravel seasonal and long-term trends, *Phaeocystis* and diatom biomass were analysed with an additive mixed modelling approach. In a first approach GAMMs were built to study the long-term trends in the dataset, in a second approach GAMMs were applied to study the seasonal trends in 'high' (H; 2007, 2015 and 2016) and 'low' (L; 2005, 2006, 2011-2013) *Phaeocystis* years. The *mgcv* package (Wood, 2017) in the open-source software R (RStudioTeam, 2017) was used for the analyses.

Prior to the modelling diatom data were $\log_{10}(x)$-transformed and *Phaeocystis* data were $\sqrt[4]{x}$-transfomed. A Gaussian data distribution was observed and indicated in the models. Space (sampling location) and HPLC methodology were set as random effects. By incorporating 'method' as a random effect, a possible HPLC method bias was excluded from the models. In the first approach a long-term smoother (cubic regression spline (cr)) was used and in the second approach a seasonal smoother was used for the 'low' (L) and 'high' (H) *Phaeocystis* dataset. This smoother is a cyclic cubic regression spline (cc) *f(JulianDay)*, which ensures that the value of the smoother at the far left point of the gradient is the same as at the far right point which is convenient to model an annual cycle (Zuur et al., 2009).

In order to analyse the intensity of the *Phaeocystis* peak in April in more detail, a correlation matrix was built using Spearman rank correlation tests. First, the mean of the onshore (120, 130, 700 and 710) and offshore (215, 230, 330, 780 and ZG02) HPLC-CHEMTAX-based *Phaeocystis* biomass values was calculated. We then correlated April *Phaeocystis* values with the environmental parameters (nutrient concentrations and nutrient ratios, SPM and Secchi depth, SST, salinity and NAO index) in the same month (April), the previous months (March and February), the two previous months (February and March), the three previous months (January to March) and the preceding autumn (September to November) conditions.

The environmental conditions before the bloom (February to March) in the on- and offshore area were analysed. This analysis was done for high (H; 2007, 2015 and 2016) and low (L; 2005, 2006, 2011-2013) *Phaeocystis* years separately. The conditions during these two periods (separately for onshore and offshore data) were analysed with a two-way ANOVA. Data were $\log_{10}(x+1)$-transformed prior to the analyses and normality was tested visually.

5.4. Results

5.4.1. Long-term biomass pattern and environmental trends

There are pronounced geographical differences in the magnitude of the *Phaeocystis* bloom between the French, Dutch and Belgian waters (Figure 2A). Among all regions, highest cell numbers have been observed in the BPNS (Figure 2A). The HPLC-CHEMTAX data revealed the existence of years with high (H; 2007, 2015 and 2016) and low (L; 2005, 2006, 2011-2013) *Phaeocystis* blooms (Figure 2B and Figure 3A). Diatoms as well have years in which the bloom is more intense than in others (Figure 2C, Figure 3B and Figure 3C). Diatom biomass anomalies are either negative/positive in March or in April, but never show the same anomaly (neg. and pos. z-score) in both months (Figure 3B and Figure 3C).

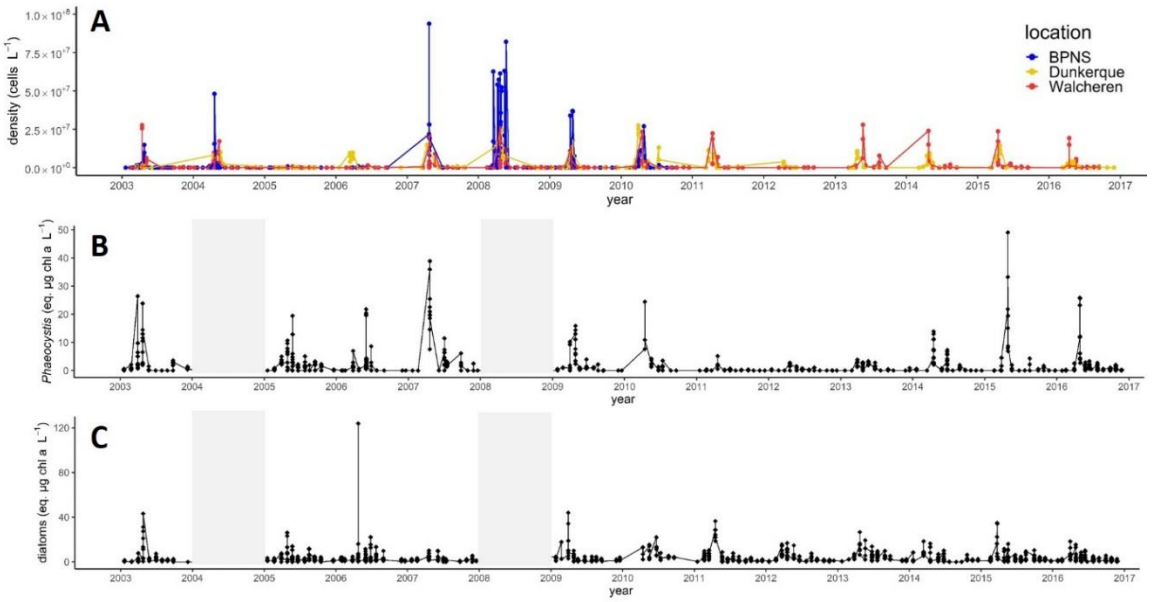

Figure 2 Long-term trends *Phaeocystis* and diatom cell numbers and/or biomass. A Single sample enumerations of *Phaeocystis* cells in the BPNS (onshore and offshore), the Walcheren transect (WALCRN2 and WALCRN20) and the Dunkerque transect (DK1, DK3 and DK4), B single sample enumerations of *Phaeocystis* biomass based on HPLC-CHEMTAX analyses in the BPNS (onshore and offshore), C single sample enumerations of diatom biomass based on HPLC-CHEMTAX analyses in the BPNS (onshore and offshore). Walcheren data were collected by Rijkswaterstaat (RWS) and are available on request. Dunkerque data collected in the frame of the REPHY program (French Observation and Monitoring program for Phytoplankton), download from http://www.seanoe.org/data/00397/50832/.

Between 2003 and 2016, considerable changes in the dominant phytoplankton group (composition) took place. Chlorophyll a biomass significantly increased in the onshore area (Table 2A) and the overall chlorophyll a long-term trend in the BPNS shows an increase until 2010, followed by a weak decrease (see Supplementary Figure 2 and *Chapter 4*). Also diatom abundances have significantly increased between 2003 and 2016, onshore and offshore (Table 2A, Figure 4A). The long-term *Phaeocystis* biomass trend is more variable (Figure 4B) and no significant

up- or downward-trend can be observed during the study period (Table 2A). The GAMM long-term smoothers (Figure 4B) confirm the annual anomalies shown in Figure 3A.

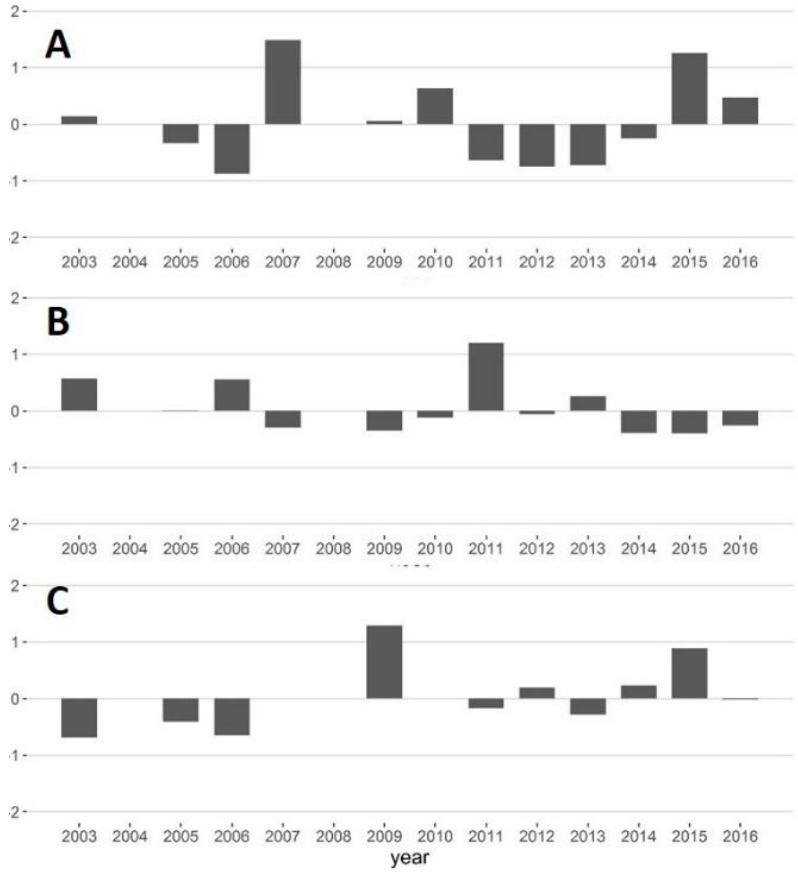

Figure 3 Winter/spring phytoplankton anomalies (z-score) during the period 2003-2016. A *Phaeocystis* in April, B diatoms in April and C diatoms in March.

The mean annual and winter DIN and DIP concentrations have decreased during the study period in the BPNS (Table 2B). DSi only has decreased offshore in the winter months (Table 2C). Consequently, nutrient ratios in the BPNS have changed since 2003. Specifically, mean annual DSi:DIP and DSi:DIN ratios have increased both in onshore and offshore locations (Table 2B), but only the onshore winter values of DSi:DIN have increased (Table 2C). SPM concentrations in the offshore BPNS increased since 2003, both mean annual as well as winter values (Table 2B and Table 2C).

Table 2 Mann-Kendall trend tests of the biotic and abiotic variables (2003-2016). A HPLC chlorophyll a and HPLC-CHEMTAX (without 2004), B abiotic variables, C winter abiotic variables (December-February). *p*-values are sign. <0.05.

A

variable	location	tau	*p-value*
chl a	onshore	0.91	**<0.05**
chl a	offshore	0.01	0.70
Phaeocystis	onshore	-0.05	0.14
Phaeocystis	offshore	-0.05	0.11
diatoms	onshore	0.12	**<0.05**
diatoms	offshore	0.07	**<0.05**

B

parameter	location	tau	*p-value*
DIP	onshore	-0.01	**<0.05**
DIP	offshore	-0.09	**<0.05**
DIN	onshore	-0.09	**<0.05**
DIN	offshore	-0.10	**<0.05**
DSi	onshore	-0.03	0.48
DSi	offshore	-0.02	0.48
DIN:DIP	onshore	0.03	0.47
DIN:DIP	offshore	-0.03	0.48
DSi:DIP	onshore	0.13	**<0.05**
DSi:DIP	offshore	0.09	**<0.05**
DSi:DIN	onshore	0.08	**<0.05**
DSi:DIN	offshore	0.09	**<0.05**
SPM	onshore	0.05	0.09
SPM	offshore	0.10	**<0.05**
secchi	onshore	0.05	0.11
secchi	offshore	0.02	0.47
SST	onshore	0.02	0.57
SST	offshore	0.01	0.78
salinity	onshore	-0.002	0.95
salinity	offshore	-0.01	0.60

C

parameter	location	tau	*p-value*
DIP	onshore	-0.29	**<0.05**
DIP	offshore	-0.36	**<0.05**
DIN	onshore	-0.24	**<0.05**
DIN	offshore	-0.27	**<0.05**
DSi	onshore	-0.11	0.08
DSi	offshore	-0.19	**<0.05**
DIN:DIP	onshore	-0.07	0.33
DIN:DIP	offshore	0.06	0.48
DSi:DIP	onshore	0.11	0.07
DSi:DIP	offshore	0.04	0.54
DSi:DIN	onshore	0.21	**<0.05**
DSi:DIN	offshore	-0.002	0.99
SPM	onshore	0.02	0.76
SPM	offshore	0.16	**<0.05**
secchi	onshore	0.04	0.51
secchi	offshore	0.02	0.72
SST	onshore	0.09	0.10
SST	offshore	0.01	0.83
salinity	onshore	0.10	0.05
salinity	offshore	-0.10	0.08

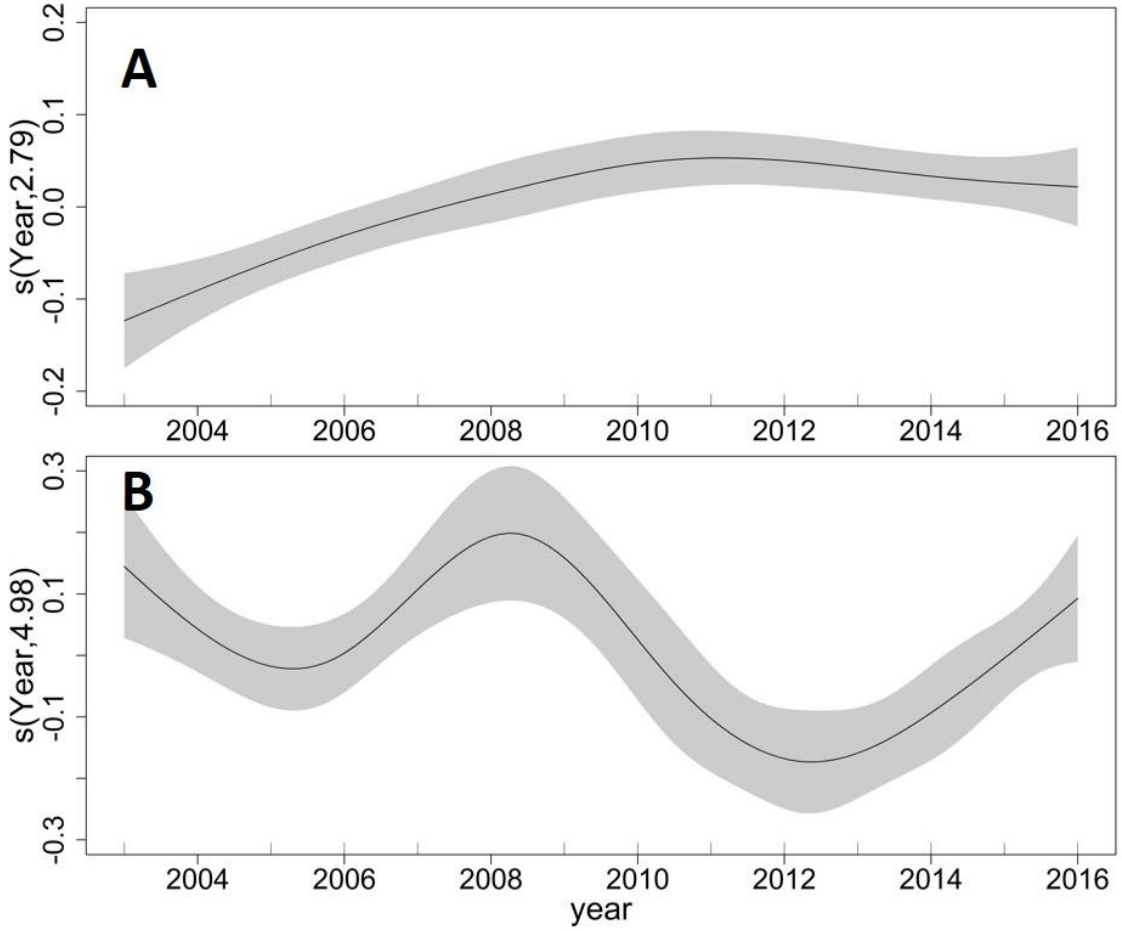

Figure 4 Interannual GAMM smoothers (on- and offshore area together) of the phytoplankton biomass determined by HPLC-CHEMTAX. A Diatoms, B *Phaeoycstis.* The numbers between brackets on the y-axes indicate the estimated degrees of freedom. The higher the number the more non-linear the curve, while numbers close to 1 indicate linear functional response.

The annual monthly relative contribution of diatoms and *Phaeocystis* biomass shows that in years with low *Phaeocystis* blooms, *Phaeocystis* cells fail to outcompete diatoms, while in years with high *Phaeocystis* blooms, *Phaeocystis* cells have outcompeted diatoms by April (Figure 5).

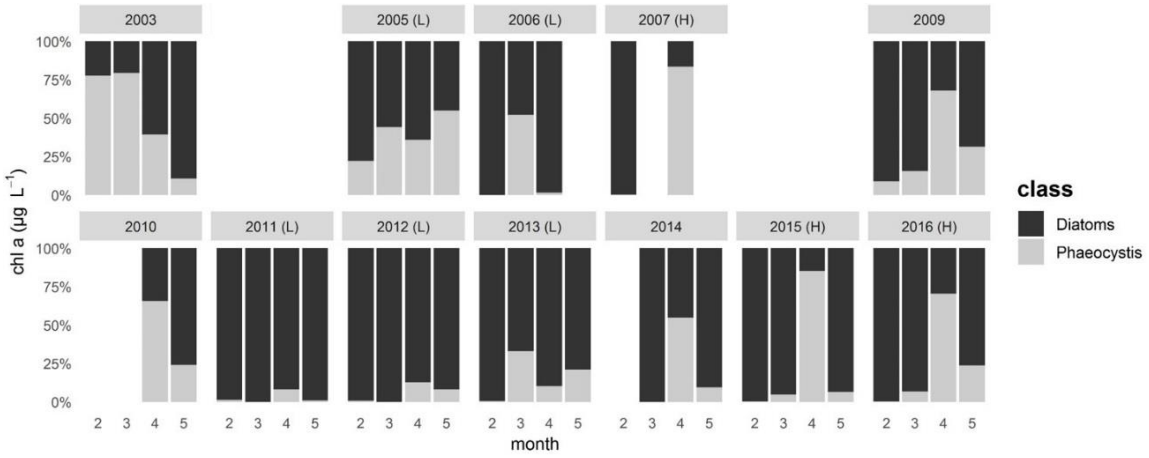

Figure 5 Monthly relative contribution of diatom and *Phaeocystis* biomass per year (February to May). L: years with low *Phaeocystis* blooms, H: years with dense *Phaeocystis* blooms.

5.4.2. *Phaeocystis* pre-bloom conditions

Regression analyses reveal a significantly negative relationship of *Phaeocystis* biomass in April with the February to March DIP (Figure 6B) and SPM (Figure 6F), while the negative relation between DSi and *Phaeocystis* biomass in April is just not significant (Figure 6C). Correlation analyses show a significantly negative relationship of *Phaeocystis* biomass in April with diatom biomass and NO_3^- concentration in the same month, but a significantly positive correlation with DSi and DSi:DIP ratio (Table 3). Concentrations of NO_3^-, $NO_2^-NO_3^-$, DIP, DSi, and the DSi:DIN ratio in March were negatively correlated with the *Phaeocystis* bloom in April (Table 3). When also the January and February nutrient concentrations and ratios are considered, these correlations are no longer significant (Table 3).

There is a negative correlation between April *Phaeocystis* biomass and February to March SPM concentration (Table 3). Pre-bloom SST values (March, February to March and January to March) are always significantly positively correlated to *Phaeocystis* biomass in April, but only the NAO_{March} index is significantly positively correlated to the *Phaeocystis* biomass in April (Table 3).

We further compared the February to March environmental conditions in the on- and offshore area in years with low (L) and high (H) *Phaeocystis* blooms by means of two-way ANOVAs (Table 4). H years were characterized by significantly lower NO_3^-, DIN, DIP and DSi concentrations both on- and offshore, and lower NO_2^- and NH_4^+ values in the onshore stations. In years with more

intense *Phaeocystis* blooms, SST values in February and March were significantly higher, while SPM values were higher in the offshore stations and salinity in the onshore ones.

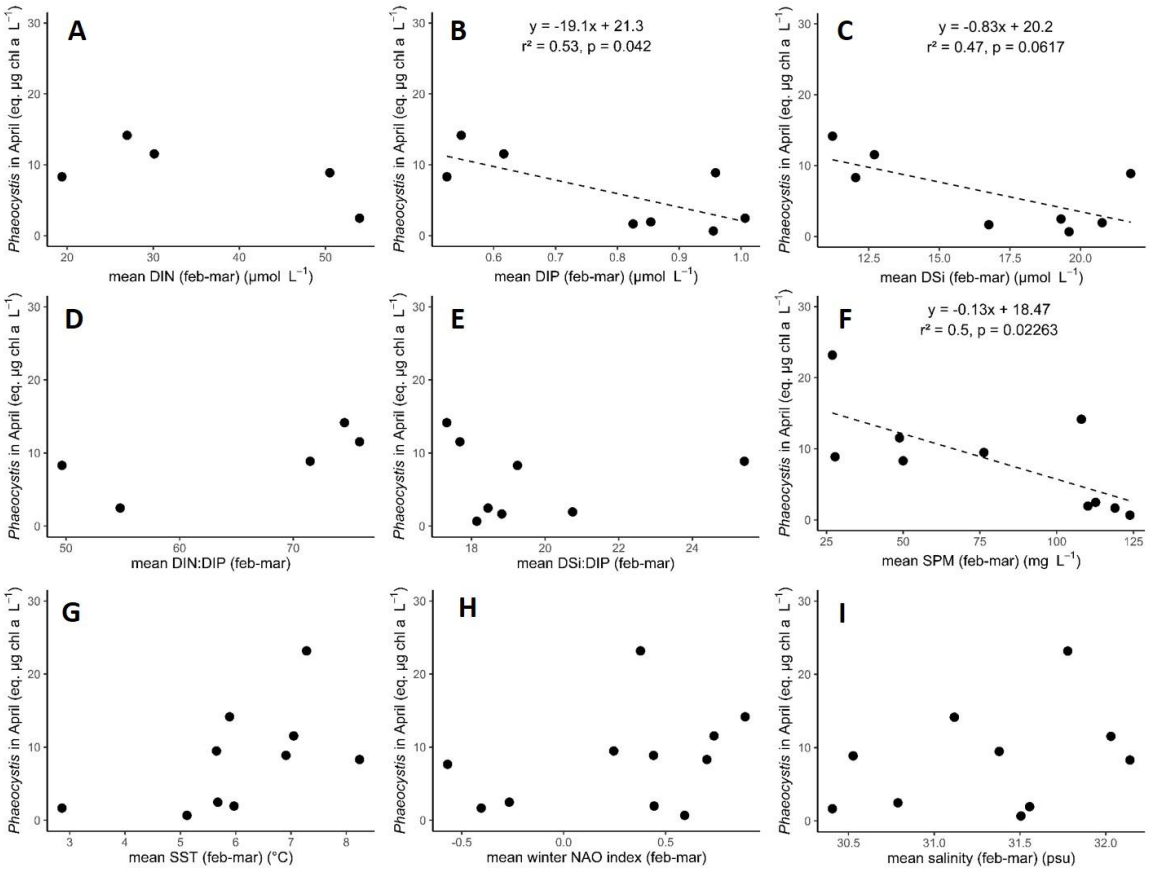

Figure 6 Correlation of *Phaeocystis* biomass magnitude April determined by HPLC-CHEMTAX and mean winter/spring environmental parameters (February-March) in the onshore area. A DIN, B DIP, C DSi, D DIN:DIP, E DSi:DIP, F SPM, G SST, H NAO index and I salinity.

Table 3 Correlation of April *Phaeocystis* biomass (HPLC-CHEMTAX, 2003-2016, without 2004, 2006 and 2008) with the April, March, February, February-March, January-March and September to November (of previous year) means of diatom biomass, abiotic variables and NAO index. Spearman rank correlation is used. * sign. level <0.05, ** sign. level <0.01, *** sign. level <0.001.

	$variables_{Apr}$	$variables_{Mar}$	$variables_{Feb}$	$variables_{Feb-Mar}$	$variables_{Jan-Mar}$	$variables_{Sep-Nov}$
diatoms	**-0.54****	0.29	-0.24	-0.05	-0.01	
NO_2^-	-0.12	-0.42	-0.02	-0.14	-0.18	0.34
NO_3^-	**-0.54***	**-0.67****	-0.02	-0.48	-0.33	0.1
$NO_2^-NO_3^-$	-0.49	**-0.65****	-0.02	-0.48	-0.33	0.08
NH_4^+	0.36	0.22	-0.5	0.12	0.1	-0.26
DIN	-0.52	-0.49	-0.6	-0.3	-0.2	-0.66
DIP	-0.11	**-0.67****	-0.27	-0.44	-0.39	0.09
DSi	**0.61***	**-0.71****	-0.17	-0.4	-0.37	0.19
DIN:DIP	-0.32	0.52	-0.24	0.08	0.62	-0.49
DSi:DIP	**0.81*****	-0.16	-0.02	-0.11	-0.03	-0.04
DSi:DIN	0.45	**-0.73***	0.21	-0.58	-0.44	-0.31
chl a	**0.78*****					
SPM	0.12	-0.43	-0.34	**-0.47***	-0.32	0.15
Secchi depth	0.01	0.31	0.14	0.22	0.13	0.11
SST	0.4	**0.54***	**0.75****	**0.61****	**0.57****	0.31
salinity	-0.1	0.28	-0.05	0.1	0.11	-0.29
NAO index	-0.31	**0.51***	0.04	0.24	0.31	

Table 4 Mean environmental winter conditions and diatom biomass (February to March) in the on- and offshore area in low (L) and high (H) *Phaeocystis* years. Conditions were compared by two-way ANOVA tests with post-hoc tests.

	onshore		offshore		*p* value			*(significant post test differences)*	
	L	H	L	H	LH	area	LH:area	onshore L:H	offshore L:H
NO_2^- (µmol L^{-1})	0.47	0.27	0.28	0.24	**<0.01**	**<0.001**	0.79	0.06	
NO_3^- (µmol L^{-1})	44.89	25.99	26.55	16.48	**<0.001**	**<0.001**	0.59	0.27	0.22
$NO_2^-NO_3^-$ (µmol L^{-1})	45.36	26.26	26.82	16.72	**<0.001**	**<0.001**	0.58	0.27	0.22
NH_4^+ (µmol L^{-1})	3.41	2.29	2.08	1.81	**<0.01**	**<0.001**	0.29	0.15	
DIN (µmol L^{-1})	51.81	28.55	32.61	18.53	**<0.001**	**<0.001**	0.81	0.30	0.28
DIP (µmol L^{-1})	1.01	0.58	0.72	0.43	**<0.001**	**<0.001**	0.36	0.11	0.08
DSi (µmol L^{-1})	19.92	11.96	12.28	7.01	**<0.001**	**<0.001**	0.78	0.33	0.29
DIN:DIP	47.36	75.18	39.15	58.44	**<0.01**	**<0.05**	0.91		
DSi:DIP	18.68	17.51	15.54	14.59	**<0.01**	**<0.05**	0.91		
SPM (mg L^{-1})	101.42	68.14	34.32	24.21	0.28	**<0.05**	0.84		
Secchi depth	48.16	69.55	141.37	250.00	**<0.01**	**<0.001**	0.24		-0.24
SST (°C)	4.78	6.97	5.01	7.50	**<0.001**	0.12	0.73	-0.15	-0.16
salinity (psu)	30.95	31.80	32.61	33.06	**<0.01**	**<0.001**	0.32	-0.01	
diatoms (µg chl a L^{-1})	3.21	7.24	3.19	3.84	0.08	0.3164	0.08		

5.4.3. Diatom and *Phaeocystis* seasonality in the BPNS

The seasonal pattern in diatom and *Phaeocystis* biomass was modelled for years with high and low *Phaeocystis* biomass (Figure 7). In low *Phaeocystis* years, diatom biomass starts to increase at the beginning of January (Julian day 8) and reaches its peak value by mid-April (Julian day 112) (Figure 7A). *Phaeocystis* biomass also starts to increase in early January (Julian day 12) and reaches

its peak at the beginning of May (Julian day 128) (Figure 7C). In high *Phaeocystis* years, the diatom biomass increase starts slightly later (Julian day 19) but the biomass peak is already reached in the second half of March (Julian day 83) (Figure 7B). In high *Phaeocystis* years, *Phaeocystis* biomass only starts to rise on Julian day 38, then increases very rapidly and reaches its maximum already by mid-April (Julian day 110), about 18 days earlier than in low *Phaeocystis* years (Figure 7D).

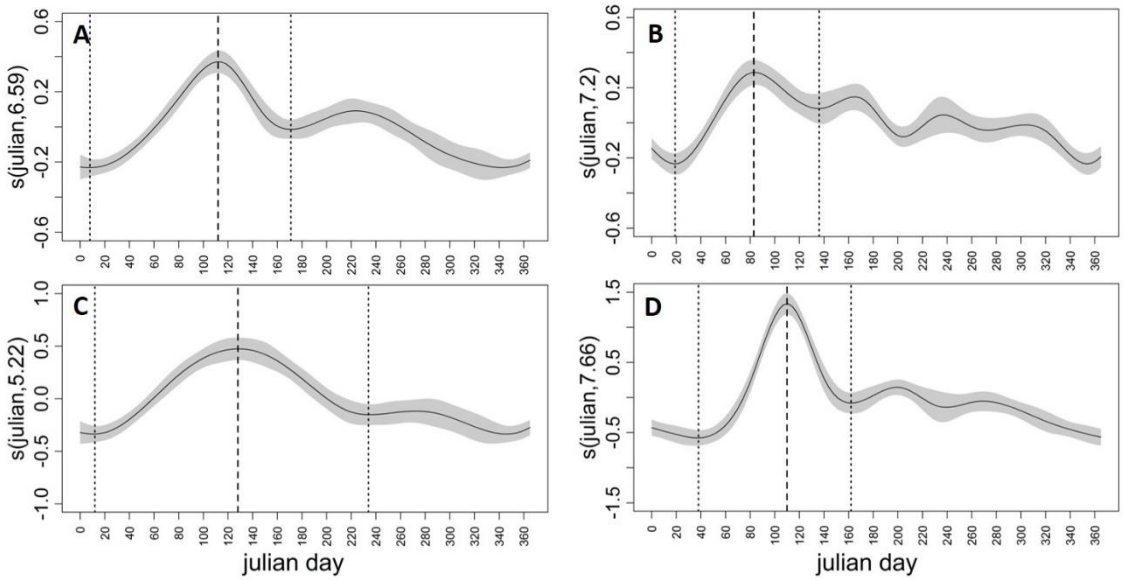

Figure 7 Seasonal GAMM smoothers of the phytoplankton groups determined by HPLC-CHEMTAX for high (2007, 2015 and 2016) and low (2005, 2006, 2011, 2012 and 2013) *Phaeocystis* biomass years. A: diatoms in low *Phaeocystis* years, B diatoms in high *Phaeocystis* years, C *Phaeocystis* in low *Phaeocystis* years, D: *Phaeocystis* in high *Phaeocystis* years. The start and the end of the spring blooms are indicated as black dotted lines. The data of the spring biomass peak is indicated with a black dashed line. The numbers between brackets on the y-axes indicate the estimated degrees of freedom. The higher the number the more non-linear the curve, while numbers close to 1 indicate linear functional response.

5.5. Discussion

The regression and correlation analyses show that the intensity of the *Phaeocystis* spring bloom in the BPNS is related to both nutrient values and physical properties of late winter-early spring water masses. Specifically, (February to) March nutrient (NO_3^-, $NO_2^-NO_3^-$, DIP and DSi) values and the DSi:DIN ratio (Figure 6 and Table 3) are significantly negatively correlated with *Phaeocystis* bloom intensity. In addition, SPM concentration (Figure 6 and Table 3) is lower, and water transparency and SST higher in February-March in high *Phaeocystis* biomass years (Table 3 and Table 4). To some extent, this is in line with the positive correlation of the April *Phaeocystis* biomass to the NAO_{March} index. In general, a positive NAO index has been related to warmer air and SST, stronger winds and more frequent storm events, more precipitation, a higher mixed layer depth, overall reduced light availability (due to an increased cloud cover) and higher salinity due to an increase in Atlantic Ocean water entering the Southern Bight of the North Sea (Hurrell and Deser, 2010; Irigoien, 2000; Trigo et al., 2002; Trigo et al., 2004). However, in coastal regions, NAO

effects can be more complex. In the BPNS, a higher NAO leads to an increase of the freshwater runoff from the Scheldt river due to an intensification of rainfall (Breton et al., 2006). This can enhance nutrient input into the BPNS, but at the same time stronger westerly winds can push the Scheldt river plume further to the east, which can ultimately lead to less nutrients in the central and offshore part of the BPNS (Breton et al., 2006).

The negative correlation between the intensity of the *Phaeocysti*s bloom with February-March nutrient concentrations suggests that biotic interactions, such as the competition with other phytoplankton groups, may play an important role in the *Phaeocysti*s bloom dynamics, a hypothesis which was first put forward by Karasiewicz et al. (2018). We suggest that under low SST, diatoms win the competition over *Phaeocystis*, as *Phaeocystis* in its flagellate form does not grow fast enough to accumulate a critical biomass. Following, the steeper diatom biomass increase leads to a faster depletion of many nutrients, until diatoms get limited by the availability of DSi (Figure 8). *Phaeocystis* can grow on remaining nutrients, but slower and later due to low SST and with lower total biomass due to lower remaining nutrients. In the case of higher SST, *Phaeocystis* in its flagellate form grows fast enough to start forming colonies. Even before DSi is depleted, colonial *Phaeocystis* enters into competition with diatoms for the remaining DIN and DIP and may dominate, as *Phaeocystis* has low P-requirements, is capable of storing P and is able to grow on organic P-sources (Riegman et al., 1992; van Boekel and Veldhuis, 1990; Veldhuis et al., 1991). The earlier diatom bloom thus opens up the opportunity for *Phaeocystis* to build more intense blooms (Figure 7D and Figure 8).

5.5.1. *Phaeocystis* - abiotic constraints

The alternation of *Phaeocystis* bloom and non-bloom years observed in the BPNS and adjacent coastal regions (the French North Sea coast (Dunkerque site); Lefebvre et al., 2011) (Figure 3A) has also been reported in other global regions (e.g. Massachusetts Bay; Borkman et al., 2016; Lefebvre et al., 2011). In the North Sea, *Phaeocystis* is generally thought to start blooming when the diatom bloom becomes limited by silica depletion (Peeters et al., 1991; Reid et al., 1990). However, diatom and *Phaeocystis* blooms can also co-occur and diatoms can be outcompeted by *Phaeocystis* even under sufficient silica concentration (Peperzak et al., 1998; Tungaraza et al., 2003). In contrast, in Massachusetts Bay the spring community is either dominated by diatoms or by *Phaeocystis* (Borkman et al., 2016). In the BPNS, both groups can be successful in the same year, but not within the same months (Figure 3A and Figure 3B). During the *Phaeocystis* peak in April, diatoms have already deceased (Table 3).

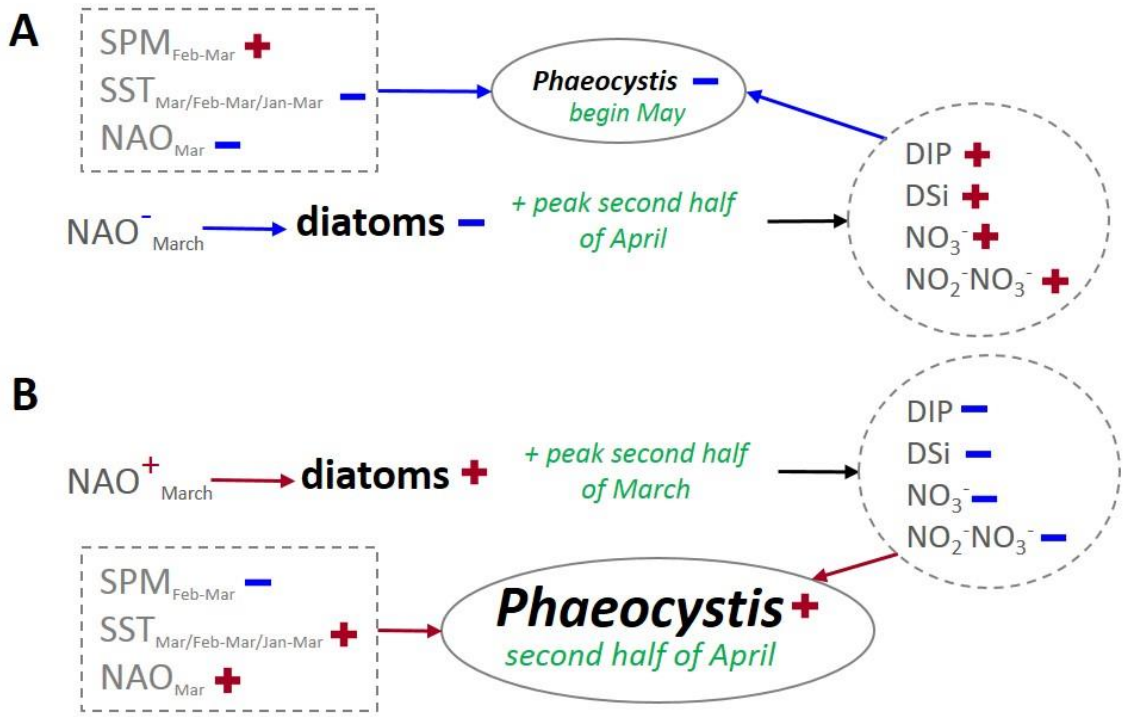

Figure 8 Scheme of the identified diatom-*Phaeocystis* succession mechanisms and direct abiotic-*Phaeocystis* relationships in years with low *Phaeocystis* blooms (A) and years with high *Phaeocystis* blooms (B). An inhibiting effect is indicated as blue arrow, a stimulation is indicated as red arrow. + and – signs indicate an in- or decrease of the phytoplankton group or parameter. The timing of the bloom peaks of diatoms and *Phaeocystis* is given in green.

Many studies have related the occurrence and magnitude of *Phaeocystis* blooms to eutrophication (Cadée and Hegeman, 1986; Gómez and Souissi, 2008; Lancelot et al., 1987), especially the inorganic N and P supply prior to the bloom start (Borkman et al., 2016; Breton et al., 2006; Desmit et al., 2015; Muylaert et al., 2006). For example, Lancelot et al. (2009) found a positive correlation between *Phaeocystis* cell numbers and annual DIN and DIP loads to the BPNS. Other studies however have not found a relation between higher nutrient levels and bloom occurrence (Bakker et al., 1990; Gieskes et al., 2007). Gieskes et al. (2007) even states that eutrophication is definitely not the main reason for changes in the *Phaeocystis* occurrence, as their bloom frequency was especially high already in the pre-eutrophication (< 1960s) and the post-eutrophication period (>1980s). In the BPNS, we observed a negative relationship between March nutrient concentrations (NO_3^-, $NO_2^-NO_3^-$ and DIP) (Table 3) and February-March DIP concentration, and *Phaeocystis* bloom intensity (Figure 6B).

Several studies suggest that nutrient ratios rather than nutrient concentrations affect *Phaeocystis* blooms (Borkman et al., 2016, Desmit et al., 2015; Gypens et al., 2007; Lancelot et al., 2009; Riegman et al., 1992). Pre-bloom nutrient ratios (e.g. elevated NO_3^-:PO_4^{3-} and PO_4^{3-}:Si) might stimulate the development of blooms (Borkman et al., 2016; Riegman et al., 1992). Following the

gradual de-eutrophication of the North Sea since the beginning of the 1990s (decrease in P and N loading), there have been remarkable changes in nutrient ratios (e.g. increase in N:P ratio), as P has been reduced much more efficiently than N (Burson et al., 2016). An increasing N:P ratio is favourable for *Phaeocystis* growth (Prins et al., 2012). In the present study, however, we did not find a significant link of the pre-bloom DIN:DIP ratio to the magnitude of the *Phaeocystis* bloom (Table 3).

As there is a negative correlation with the preceding March and February-March DIP concentrations, the hypothesis is supported that a further decrease in DIP will not negatively affect *Phaeocystis*. Lacroix et al. (2007) and Lancelot et al. (2009) already mentioned the importance of a simultaneous reduction in N and P inputs, as the decrease in *Phaeocystis* since the de-eutrophication is less strong than expected due to a lack of a proportional decrease of N inputs. They suggest that a further reduction of the N input into the BPNS will help to reduce coastal *Phaeocystis*, while the further reduction in P will more likely have a negative effect on diatoms (Desmit et al., 2015; Gypens et al., 2007; Lacroix et al., 2007). However, the long-term reduction of DIP in the BPNS did not seem to have a negative effect on diatoms as they have further increased in the period (Table 2A and Figure 4A).

The intensity of the *Phaeocystis* spring bloom appears to be negative correlated with the preceding SPM concentrations (Figure 6F and Table 3). Light availability is believed to be an important abiotic factor triggering *Phaeocystis* blooms and high turbidity during winter can delay and even harm their development (Gómez and Souissi, 2008; Karasiewicz, 2017; Peperzak et al., 1998).

In several studies SST has been mentioned to be an important factor in the regulation of *Phaeocystis* blooms. Gieskes and Kraay (1977) related the decreasing trend of *Phaeocystis* between 1948 and the 1970s to lower SSTs in this period. Cadée and Hegeman (1986) found no correlation between the onset of the *Phaeocystis* spring bloom and SST, while in a later study Cadée and Hegeman (2002) mentioned the possibility that the increased spring SST in the Marsdiep area might be responsible for the earlier start of *Phaeocystis* growth (comparison 1970s and 1990s). In the north-eastern English Channel exceptionally low February SST in 2005 (during the study period 1997-2005) led for the first time to the absence of the *Phaeocystis globosa* spring bloom (Gómez and Souissi, 2008). In the BPNS, the strong and significant positive correlation of the preceding SST to *Phaeocystis* leads to the assumption that SST is a second important abiotic driver in the development of the *Phaeocystis* spring bloom (Table 3). Colder waters early in the year (February) might harm the development of blooms (Borkman et al., 2016; Gómez and Souissi,

2008) and also in the present study high *Phaeocystis* years were characterized by higher SST (Table 4).

Data from the western English Channel reported the absence of a *Phaeocystis* bloom in 2005 (Widdicombe et al., 2010). In the same year, a dominance of tychoplanktonic diatoms was observed due to stronger water column mixing (Gómez and Souissi, 2008). The lower light availability due to the high turbidity, together with the competition with tychoplanktonic diatoms, led to a delay in phytoplankton spring bloom onset and the suppression of *Phaeocystis* (Gómez and Souissi, 2008). Also the present study, microscopic counts revealed the absence of the *Phaeocystis* bloom in the BPNS, Dunkerque and Walcheren, and a (weak) negative z-score in the *Phaeocystis* biomass in this year (Figure 2A and Figure 3A).

In some studies from the BPNS and the English Channel, a negative correlation of the *Phaeocystis* spring bloom magnitude (and their dominance over diatoms) with the winter NAO (NAO_w) index has been identified (Breton et al., 2006; Irigoien, 2000; Seuront and Souissi, 2002), while in other studies the opposite was observed (Borkman et al., 2016; Desmit et al., 2015; Karasiewicz et al., 2018). In the BPNS, high winter salinity (caused by a positive NAO_w) correlated with lower *Phaeocystis* abundance (Desmit et al., 2015), while Gieskes et al. (2007) mentioned a positive link between the abundance of *Phaeocystis* in the North Sea and the amount of Atlantic water entering through the English Channel. In the present study as well we observed a positive relationship between the April *Phaeocystis* peak and the NAO_{March} index (Table 3). Breton et al. (2006) states that *Phaeocystis* bloom biomass and the NAO_{winter} index are negatively and non-linearly linked via the river-based nitrate pulse, which is in itself also non-linearly linked to the NAO. These contradicting findings are most likely explained by regional differences of how wind and rainfall affect hydrodynamics and nutrient loads supplied by freshwater runoff (Breton et al., 2006; Drinkwater et al., 2003).

5.5.2. Biotic interactions

Our data suggest that the interaction with the other dominant phytoplankton group in the BPNS, the diatoms, is an important driver of *Phaeocystis* bloom development. This interaction has already been suggested in other studies that observed a different diatom spring community composition in low and high *Phaeocystis* years (Gómez and Souissi, 2008; Karasiewicz et al., 2018). In subset L, *Phaeocystis* reaches its spring biomass peak more than one month later than in subset H (Figure 7B and Figure 7D). A positive NAO_{March} index leads to an earlier diatom peak (Figure 8B). As a result, nutrients (and especially DSi) get depleted more rapidly. This decline in nutrient

concentration intensifies competition, but *Phaeocystis* wins over diatoms, as diatoms are limited by DSi and *Phaeocystis* can perform better at low DIP concentrations, which leads to more intense *Phaeocystis* blooms (Figure 7D and Figure 8B).

Due to a lack of available data, biotic interactions with viruses, bacteria or zooplankton have not been part of this study. However, it can be expected that the infection with viruses and the grazing by zooplankton played a role in the regulation of the bloom formation especially during the wane of the *Phaeocystis* bloom (Brussaard et al., 2005; Nejstgaard et al., 2007).

5.6. Conclusion

We present long-term *Phaeocystis*, diatom and chlorophyll a biomass trends, as well as the *Phaeocystis* bloom magnitude in the BPNS, based on microscopic count data and HPLC-CHEMTAX data. Many hypotheses exist about the controls on *Phaeocystis* growth, showing that bloom development might be quite complex and that a variety of factors can control the start and magnitude of the blooms (e.g. Borkman et al., 2016). The present study shows that SST and light availability seem to be involved in the *Phaeocystis* spring bloom development. Next to these environmental constraints, we suggest that biotic interactions with the preceding spring diatom community play an important role in this process. As the study by Karasiewicz et al. (2018) shows a different diatom community composition in low and high *Phaeocystis* years, the analysis of the diatom species composition should be an inherent part of future *Phaeocystis* studies.

We further suggest, that a sampling frequency of one time per month might be not enough to capture the *Phaeocystis* bloom development (Lancelot et al., 2009), as the peak might easily be missed. This can lead to imprecise bloom magnitude estimations and weak abiotic-biotic relationships, as well as imprecise bloom timing estimations. Therefore, we recommend a sampling frequency of at least once a week, in order to make more precise statements about the wax and the wane of the bloom, as well as the peak's magnitude. In addition, Remote Sensing data could be used to study the spatial and temporal phytoplankton biomass in the BPNS. Furthermore, European Regional Seas Ecosystem Model (ERSEM) model output could be used to get additional data integrating the spatial variability in the BPNS.

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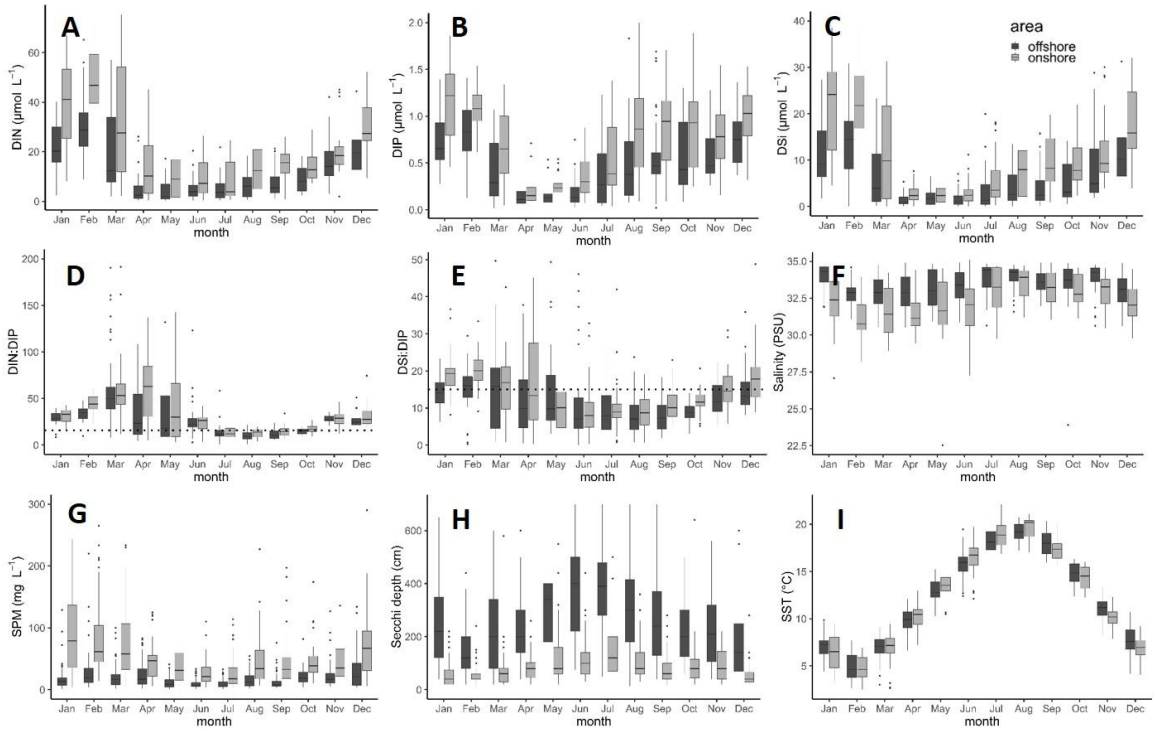
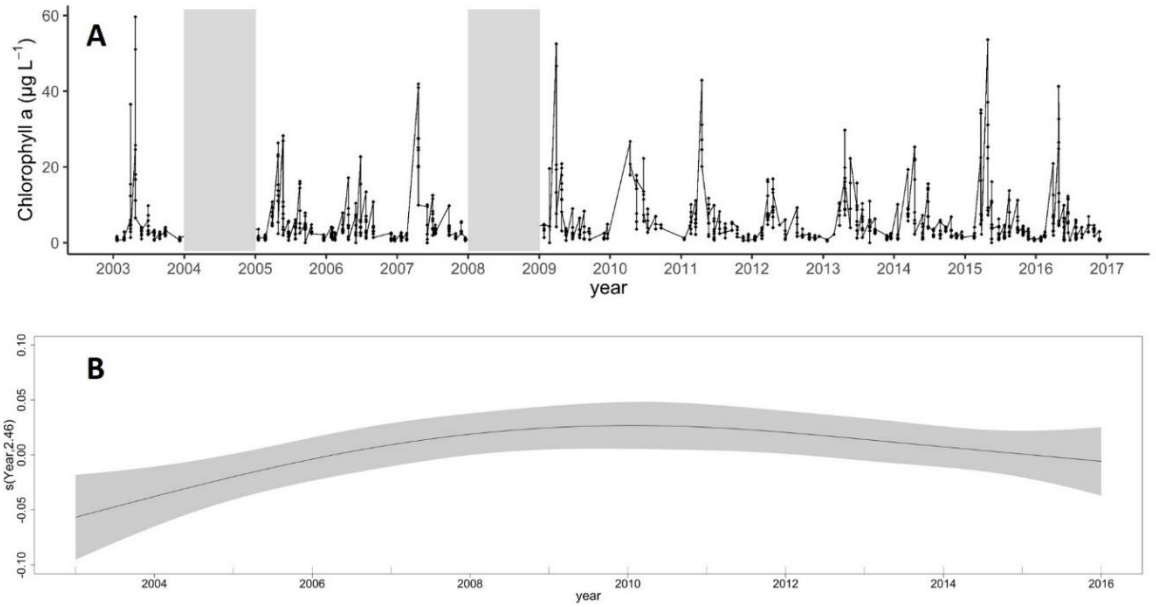
5.9. Supplementary information

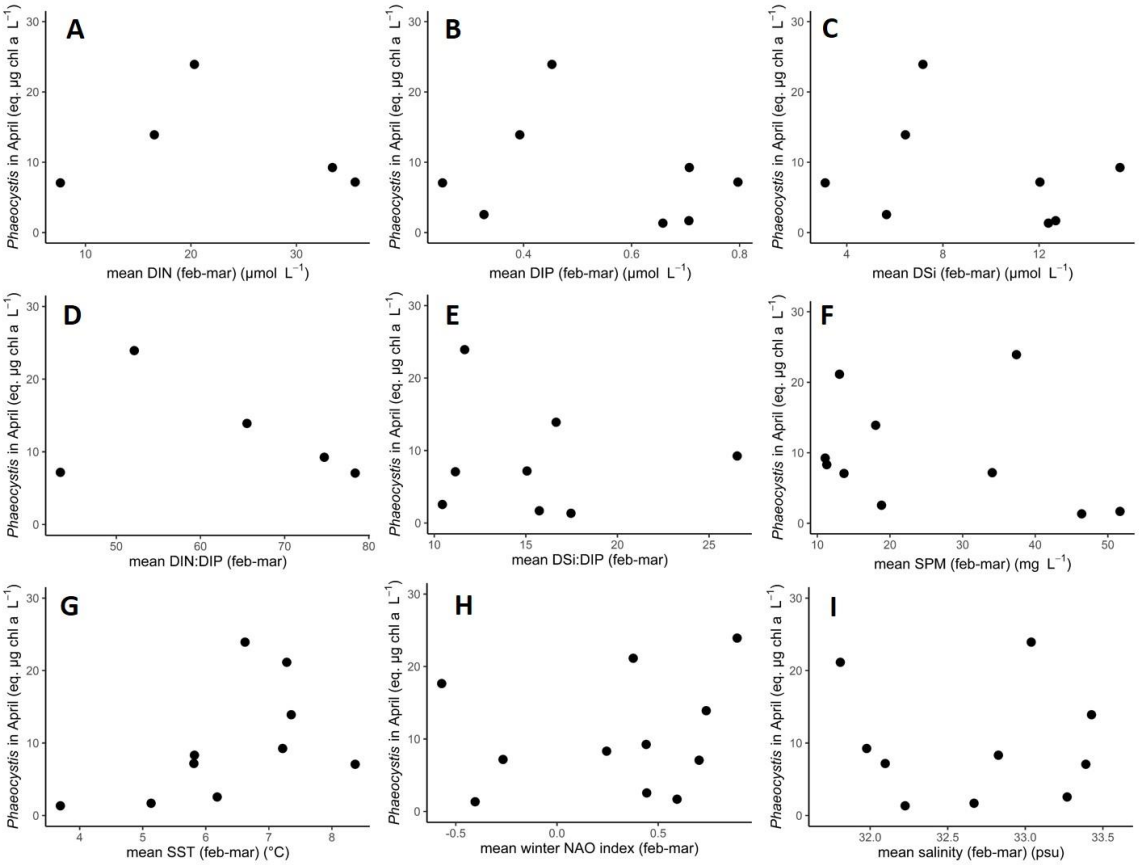
5.9.1. Supplementary Figures



Supplementary Figure 1 Seasonal boxplots of abiotic parameters in the onshore and offshore area in the BPNS. A DIN, B DIP, C DSi, D DIN:DIP, E DSi:DIP, F salinity, G SPM, H secci depth and I SST. The N:P and Si:P Redfield ratios are indicated as black dotted lines.



Supplementary Figure 2 Chlorophyll a measured by HPLC. A Long-term plot, B GAMM long-term smoother. The number between brackets on the y-axis indicate the estimated degrees of freedom. The higher the number the more non-linear the curve, while numbers close to 1 indicate linear functional response.



Supplementary Figure 3 Correlation of *Phaeocystis* in April determined by HPLC-CHEMTAX and mean winter/spring environmental parameters (February-March) in the offshore area. A DIN, B DIP, C DSi, D DIN:DIP, E DSi:DIP, F SPM, G SST, H winter NAO index and I salinity.

5.9.2. Supplementary Tables

Supplementary Table 1 Correlation of March diatom biomass (HPLC-CHEMTAX, 2003-2016, without 2004) with means of March, February and January-February of, in-situ abiotics and NAO index. Spearman rank correlation is used. * sign. level <0.05, ** sign. level <0.01, *** sign. level <0.001.

	variables$_{Mar}$	**variables$_{Feb}$**	**variables$_{Jan}$**	**variables$_{Jan-Feb}$**
NO_2^-	**-0.60***	-0.16	-0.35	-0.35
NO_3^-	-0.38	-0.01	-0.06	0.01
$NO_2^-NO_3^-$	-0.39	-0.01	-0.09	-0.03
NH_4^+	0.14	-0.45	-0.62	-0.42
DIN	-0.47	-0.24	-0.4	-0.18
DIP	-0.34	-0.09	-0.27	-0.29
DSi	-0.27	0.02	-0.09	-0.06
DIN:DIP	0.12	0.02	-0.33	0.02
DSi:DIP	**-0.66****	0.09	0.13	0.15
DSi:DIN	-0.35	-0.19	0.21	-0.07
SPM	0.23	0.33	0.24	0.26
secci	-0.06	-0.13	-0.02	-0.16
temp	0.31	-0.14	0.17	-0.04
salinity	0.23	-0.25	0	-0.13
NAO index	**0.54***	0.35	0.05	0.24

6

General discussion and conclusion

Given the overall lack of phytoplankton and environmental datasets in the BPNS, knowledge of long-term trends in phytoplankton biomass and biodiversity in the BPNS is limited. Long-term studies on phytoplankton trends from the Dutch, French, British and German part of the North Sea (see *1.7* in this thesis) may give some indications about long-term changes that might have taken place in the BPNS. However, the North Sea is spatially very heterogeneous (e.g. regarding the influence of Atlantic water influx, freshwater inflow, bathymetry and stratification regimes inducing varying horizontal and vertical gradients of salinity, turbidity and nutrients), and long-term changes found in other areas may therefore not always be the same (Capuzzo et al., 2015; Capuzzo et al., 2018), can even be contradicting or cannot be simply extrapolated to the specific conditions and changes in the BPNS. In addition, not many datasets stretch back to the 1970s, or have shortcomings such as e.g. the mesh size of the CPR dataset.

As Belgium is lacking a coordinated, long-term marine phytoplankton biodiversity monitoring program, the aim of this thesis (as part of the 4DEMON project, see *1.8*) was to recover 'hidden' marine data since the 1970s and use them to evaluate long-term changes in phytoplankton community structure, biomass and seasonality in the BPNS in relation to the changing marine environment. At the start of this PhD project an intensive effort was made to recover historical phytoplankton data from the BPNS (and adjoining areas), as well as available environmental data. The result of this effort has been presented in *Chapter 2*. In addition, a more recent HPLC pigment dataset (2003 to 2016) was used to assess the environmental constraints affecting the occurrence of the harmful alga (HA) genus *Phaeocystis* in the BPNS (*Chapter 5*). Data analyses specifically focused on changes in total phytoplankton biomass (*Chapter 4* and *Chapter 5*), and the three major phytoplankton groups in the BPNS, viz. diatoms (*Chapter 3* and *Chapter 5*), dinoflagellates (*Chapter 3*) and *Phaeocystis* (*Chapter 5*). Major changes in phytoplankton biomass and seasonality (*Chapter 3*, *Chapter 4* and *Chapter 5*) and community composition (*Chapter 3* and *Chapter 5*) were observed and discussed. This last chapter addresses the challenges of historical data integration (*6.1*), a synthesis of the main findings (*6.2*), suggestions for methodological improvements and future research perspectives (*6.3*), as well as a general conclusion (*6.4*).

6.1. Rediscovery of historical datasets

Historical data, when interpreted carefully, can provide a huge benefit to various scientific research areas such as meteorology, astronomy and also marine sciences (Kwok, 2017). These data can contribute to current scientific insights by including a systematic, long-term perspective of ecosystem change and the interaction of anthropogenic pressure with natural environmental change (Engelhard et al., 2016). The study of the ecological history of marine habitats can help to

reveal food web changes across trophic levels from phytoplankton to mammals and seabirds, and can help to evaluate whether human-induced ecosystem changes (e.g. species extinction, overexploitation, coastline transformation, pollution, eutrophication and climate warming) are responsible (Lotze and Milewski, 2004; Lotze et al., 2005). Such information is essential to guide appropriate and effective conservation, management and restoration strategies.

In this PhD thesis, a considerable effort was made to gather and integrate historical and more recent marine research data on phytoplankton community composition and biomass, and make these data available to the public in the form of one combined database (Belgian Phytoplankton Database - BPD) (*Chapter 2*). One of the main challenges we faced during the integration process was the very time-consuming digitization process. As the original documents were often only available as handwritten notes, it was not possible to digitize the documents automatically. In addition, due to the often poor quality of the handwriting many writing errors were introduced in the taxon names of the first digitized version of the database. This in turn led to the problem that it was often not possible to conduct automatic taxonomic matches, and taxonomy had to be updated manually. A second major difficulty was that metadata were often very poorly documented. Here, additional efforts had to be made to recover the metadata via e.g. related project reports or theses.

As we did not know beforehand which data we would recover during the process, the huge amount, quality and detail (e.g. identification to a low taxonomic level) of the data was a positive surprise. Unfortunately, virtually no phytoplankton compositional data exist from the end of the 1970s until the mid-1990s, resulting in a big gap in the dataset. As a result, our analysis of the oldest data (1970s) had to focus on a comparison between the 1970s and 2000s. Fortunately, these two periods represent two different states of the coastal ecosystem, one before (1970s) the documented regime shifts in the North Sea in the 1980s and 1990s (Beaugrand, 2004; Beaugrand et al., 2015; Beaugrand et al. 2014a; Raitsos et al., 2014; Raitsos et al., 2005; Reid et al., 1998; Spencer et al., 2011), marked by high nutrient concentrations in the BPNS, and a second period (2003 to 2010), a decade after the start of the de-eutrophication trend and also characterized by higher SST. This complicates the identification of environmental drivers of change in phytoplankton community.

6.2. Results synthesis

The analyses in this thesis have been conducted on different datasets (pigments, cell counts, biovolume), measured by different methodologies (e.g. inverted light microscopy,

spectrophotometry, HPLC etc.), which might influence the research results. For example, picophytoplankton species are not detected by cell counts conducted by inverted light microscopy (*Chapter 3* and *Chapter 5*). On the other hand, picophytoplankton contributes to the outcome of pigment measurement methods such as spectrophotometry and HPLC (*Chapter 4* and *Chapter 5*). However, as it is estimated that picophytoplankton contributes to less than 10 % to the coastal phytoplankton biomass (Massana, 2011), it can be argued that the influence on the analyses results might be not too high.

Depending on the analyses, different time periods were considered (e.g. 1970s (1970-1978) vs. 2000s (2003-2010) (*Chapter 3*), 1990-2014 (*Chapter 4*) or 2003-2016 (*Chapter 5*)), or a different spatial aggregation of sampling locations has been applied (e.g. see different on- and offshore aggregation in *Chapter 3* and *Chapter 5*). In addition, various statistical approaches have been used (e.g. ANOVAs, GAMMs, Mann-Kendall trend tests or multiplicative models according to Cloern and Jassby (2010)). Despite these different foci, we observed a high consistency in the main research results, which are integrated in Figure 1.

6.2.1. Long-term changes in the BPNS

The present study shows an overall increase in spring phytoplankton biomass in the BPNS from the 1970s to the 2000s, while winter and summer values decreased between these two periods (*Chapter 3*). This increase in phytoplankton biomass is supported by other studies, which also report an increase during this time period (Edwards, 2001; Beaugrand, 2004; Beaugrand, 2009; Raitsos et al., 2005; McQuatters-Gollop et al., 2007; all studies based on Continuous Plankton Recorder (CPR)-derived phytoplankton colour index (PCI)) and the Helgoland Roads (HR) time series in the German Bight (based on biovolumes) in two separate studies from 1962 until 1984 and 1962 until 1994 (Hickel, 1998; Radach et al., 1990). McQuatters-Gollop et al. (2007) calculated a biomass increase (PCI) of about 21 % after the regime shift in the 1980s in the coastal North Sea. However, Raitsos et al. (2005) mentioned that most of the biomass increase took place during winter, which contradicts with our study in which a decrease in winter chlorophyll a between the 1970s and the 2000s can be observed (*Chapter 3*). However, an increase in diatom and dinoflagellate biovolume has been observed also in winter between the 1970s and the 2000s (*Chapter 3*), a contradiction that we cannot explain at this stage.

In the offshore area of the BPNS annual mean chlorophyll decreased from 1990 until 2014 (*Chapter 4*). Alvarez-Fernandez et al. (2012) also observed a decrease in the PCI data of the CPR after 1998 in the North Sea, and this especially during autumn and winter. However, there is no

significant trend present in the onshore area of the BPNS. An increase of the onshore chlorophyll a between 2003 and 2016 was observed (*Chapter 5*), but this increase is not a consistent trend as GAMM modelling shows an increase until 2010 only followed by a slight decrease afterwards (*Chapter 5*). It can be argued that on- and offshore chlorophyll have undergone different long-

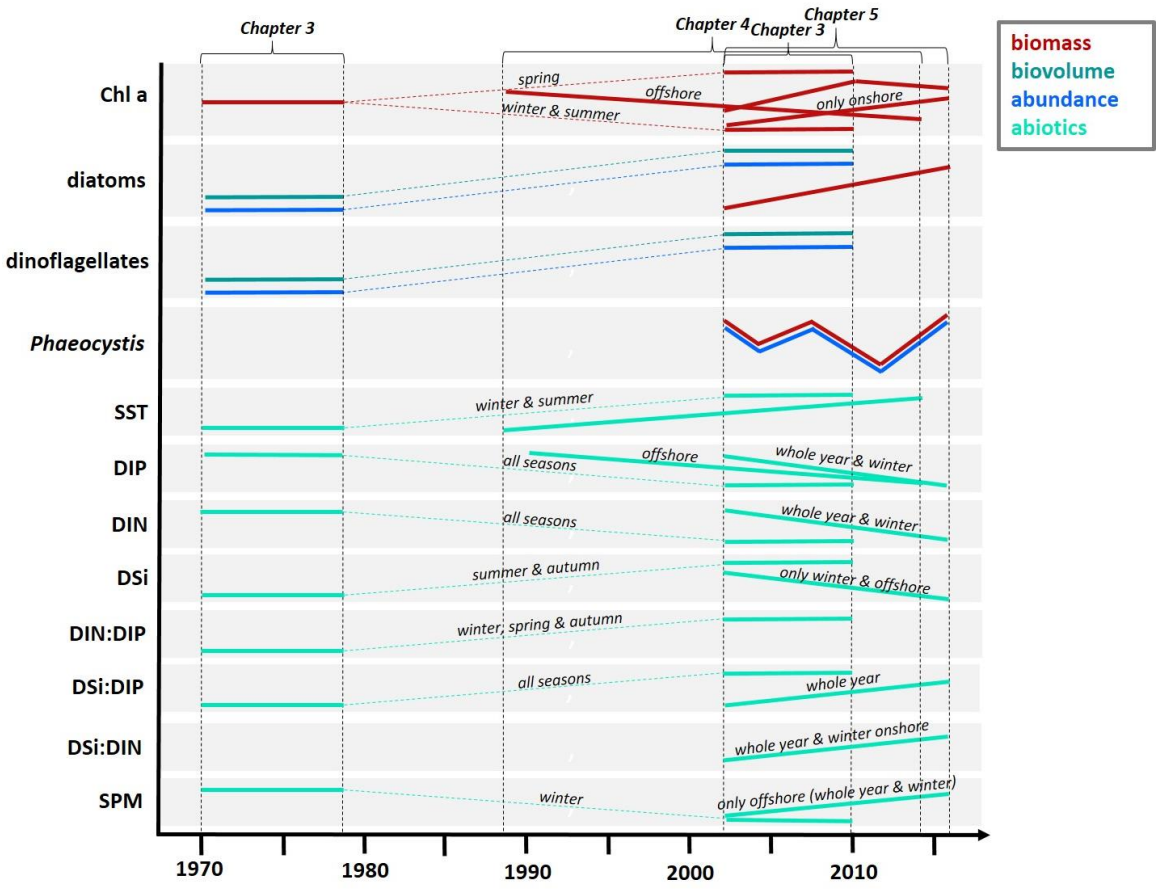


Figure 1 Summary scheme of the significant phytoplankton and abiotic long-term trends observed in the BPNS from 1970 until 2016. Note that changes are only relative changes within the different studies and no absolute values and that abundance and biovolume analyses (*Chapter 3*) exclude picophytoplankton. Abiotic data used in *Chapter 3* were collected by the Dutch Rijkswaterstaat (RWS) monitoring program. Belgian abiotic data used in *Chapter 4* derive from the Belgian Marine Data Center (BMDC), available at http://bmdc.mumm.ac.be/Interface/. Abiotic data used in *Chapter 5* are LifeWatch observatory data (Flanders Marine Institute (VLIZ), 2018), available at http://www.vliz.be/en/imis?dasid=4685&doiid=310.

term trends. That on- and offshore phytoplankton trends can be different has also been observed in the Dutch part of the North Sea (DPNS) (Baretta-Bekker et al., 2009). Different trends can be observed even in areas relatively close to each other (Lefebvre et al., 2011). They describe a decrease of chlorophyll a in coastal waters of the Dunkerque transect (Southern Bight of the North Sea), but an increase in coastal waters of the Somme and Boulogne-sur-Mer transect (Eastern English Channel) from 1992 until 2007. The study by Capuzzo et al. (2015) suggests a slight increase in chlorophyll in the PMX (permanently mixed area of the North Sea (which includes the southern part (onshore) of the BPNS) from 1988 until 2011, an area for which no significant monotonic up-

or downward trend between 1990 and 2016 was identified in the present study, but a bell shaped curve with an increase until the 2000s, followed by a decrease in recent years (*Chapter 4* and *Chapter 5*).

By comparing the 1970s with the 2000s, it could be shown that both diatom and dinoflagellate cell abundances and total biovolume increased between these two periods (*Chapter 3*). In the 2000s/2010s, the diatom biomass upward trend (pigment based) continued in both the on- and offshore area of the BPNS (*Chapter 5*). Several other studies identified a long-term increase in diatoms, such as an increase in winter diatom abundance in the CPR dataset since the 1980s/1990s (Beaugrand et al., 2014b; Edwards et al., 2006) and an increase of diatom bloom maxima after 1999 (Alvarez-Fernandez et al., 2012). Baretta-Bekker et al. (2009) also revealed an increase in the diatom spring maxima in the period 1999-2005 vs. 1991-1998 in the DPNS and an increase in dinoflagellate biomass in the second period. Leterme et al. (2005) (CPR study, 1958-2002) found a decrease of dinoflagellate abundance in the NE Atlantic (including the North Sea), while in the North Sea CPR dataset a pronounced increase in dinoflagellate abundance has been observed since the 1990s until 2007 (after a slight decrease between 1958 and the mid-1990s) (Beaugrand et al., 2014b).

It was shown that many diatom and dinoflagellate genera increased in abundance between the 1970s and 2000s (e.g. *Rhaphoneis*, *Thalassiosira*, *Thalassionema*, *Odontella*, *Skeletonema*, *Chaetoceros*, *Pseudo-nitzschia*, *Guinardia*, *Cylindrotheca*, *Alexandrium*, *Gymnodinium*, *Gyrodinium* and *Prorocentrum*). Diatoms especially increased during winter/spring, but that many of these genera decreased in abundance during autumn, while dinoflagellates mainly increased in number during spring/summer (*Chapter 3*). Hinder et al. (2012) (CPR data) showed an increase in several diatoms such as *Pseudo-nitzschia* and *Thalassiosira*. An increase of *Paralia sulcata* in the HR time series has been reported by Gebühr et al. (2009) from 1968 until 2005. Also in Dutch coastal waters it has been shown that among others the diatom species *Chaetoceros socialis* increased from 1990 onwards (Prins et al., 2012). The present study also shows that the three 'typical' phytoplankton communities that were present in the BPNS during the 1990s have become less distinct in the 2000s ('seasonal homogenization') (*Chapter 3*). In addition, it was also shown that especially large diatom genera increased (*Chapter 3*). Wiltshire et al. (2010) as well showed that especially large and heavily silicified species such as *Coscinodiscus wailesii* and *Guinardia delicatula* increased in the German Bight since the 1960s. The increase of many important phytoplankton genera and the increase in the large diatoms fits very well to the observation of a general increase in diatom abundance and biomass.

6.2.2. Seasonal changes in the BPNS

The spring chlorophyll a increases earlier and the peak occurs earlier in the 2000s than in the 1990s (Figure 2; *Chapter 4*). This pattern already has been described in a study of the Dutch RWS dataset (total phytoplankton carbon biomass) by Baretta-Bekker et al. (2009) in which they showed that in the period 1999 to 2005 the biomass increase starts earlier and the peak is already reached in April compared to the 1991-1998 time period when the spring bloom peak only occurs in May. The change in our data could have theoretically been caused either or both by the spring diatom community and/or *Phaeocystis*. As there are no diatom or *Phaeocystis* data for the 1990s, this cannot be confirmed. However, the study on *Phaeocystis* (*Chapter 5*) shows that there is a difference in bloom timing in years with high and low *Phaeocystis* blooms, both for diatoms and *Phaeocystis* which is possibly related to changes in the bloom-preceding environmental conditions. As SST_{winter} is higher on average in the 2000s than in the 1990s, it can be argued that this is one factor that affected the occurrence of earlier diatom and *Phaeocystis* blooms (and consequently earlier chlorophyll a peaks) in the 2000s. Baretta-Bekker et al. (2009) showed that diatom biomass increased earlier and reaches higher biomass in the 2000s (1999-2005) than in the 1990s (1991-1998). In addition, *Phaeocystis* reaches the peak biomass in April in the second period compared to May in the first period. The study of Baretta-Bekker et al. (2009) also shows a pronounced earlier summer peak of the dinoflagellates, which fits very well to our observations.

The described change from a bimodal diatom seasonal cycle to a unimodal cycle with one extended growing season (*Chapter 3*), has also been described by Raitsos et al. (2014) based on the CPR dataset (PCI). They showed the same change before and after the regime shift in the mid-1980s, which resulted in the extension of the growing season and higher biomass values the whole year round (Raitsos et al., 2014). In addition, our dataset shows considerable forward shifts in abundance and biovolume of both diatoms and dinoflagellates (exception: disappearance of diatom autumn abundance peak) (*Chapter 3*).

There are many studies supporting the findings of a pronounced forward shift of the phytoplankton spring bloom, there are also studies which lack such an observation. For example, the study by Wiltshire and Manly (2004) of the HR dataset in the German Bight shows a delay of the spring bloom between 1962 and 2000 and related this observation to an increase in autumn SST. They hypothesize that an increase in autumn SST results in a "longer persistence of zooplankton grazers in autumn and early winter which may depress the crucial biomass building phase", which then results in a delay of the spring bloom. It can be argued that there might be a latitude trend in the seasonal changes as there is a forward shift of the spring bloom both in the

chlorophyll a (*Chapter 4*) and the diatom dataset (*Chapter 3*), and the same shift has also been described in studies of the Dutch RWS dataset (Baretta-Bekker et al., 2009).

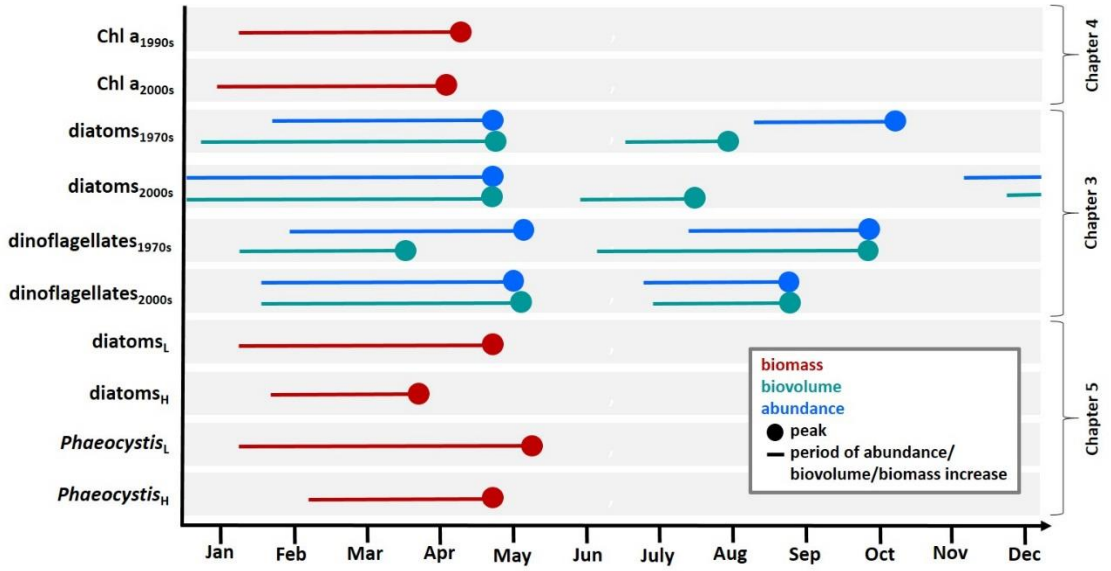


Figure 2 Summary of the observed long-term changes in seasonality of chlorophyll a biomass (*Chapter 4*), diatoms and dinoflagellates (*Chapter 3*) and seasonal differences during low (L) and high (H) *Phaeocystis* years (*Chapter 5*) in the BPNS.

6.2.3. Environmental causes

Considerable environmental changes have been identified in the BPNS during the last decades (Figure 1). The abiotic trend analyses show predominantly similar trends in all studies. SST increased from the 1970s until the 2010s (*Chapter 3* and *Chapter 4*). The decrease in DIP and DIN between the 1970s and the 2000s and from 2003 until 2016 (*Chapter 3* and *Chapter 5*), has only been significant in the period from 1990 until 2014 for DIP in the offshore area (*Chapter 4*). There is an increase in DSi between the 1970s and the 2000s (*Chapter 3*). Between 2003 and 2016 a significant decrease in the offshore area during the winter months was observed (*Chapter 5*). Due to the relatively stronger decrease in DIP vs. DIN concentration, the DIN:DIP ratio has increased between the 1970s and the 2000s (*Chapter 3*). This is also the case for the DSi:DIP ratio (*Chapter 3*). This uneven decline in nutrient concentrations has also been described by Burson et al. (2016) in the Dutch part of the North Sea and McQuatters-Gollop et al. (2007) in coastal North Sea waters. The SPM concentration in the BPNS significantly decreased in winter (1970s vs. 2000s, *Chapter 3*). However, between 2003 and 2016 an increase has been observed in the offshore area (*Chapter 5*). These environmental trends are supported by other studies, such as the HR time series in which SST and Secchi depth increased since 1968 (until 2005) and P increased until the 1980s, followed by a sharp decrease (Gebühr et al., 2009; Wiltshire et al., 2008). McQuatters-Gollop et al. (2007) described a decrease in turbidity in central and coastal North Sea waters between 1948 and 2003.

However, the study by Capuzzo et al. (2015) identified a decrease in Secchi depth in the pre-1950s compared to post-1950 in summer and winter. Capuzzo et al. (2015) states that this decrease can be most likely explained by an increase in SPM, almost no trend can be found in the SPM concentrations since 1988 until 2011 in the PMX area in all seasons.

These long-term environmental changes can be linked either directly or indirectly to the observed changes in phytoplankton abundance/biovolume/biomass/biodiversity and seasonality. SST seems to be a major factor influencing the phytoplankton community. Especially the bloom-preceding SST_{winter} seem to stimulate the forward shift of the spring diatom and *Phaeocystis* growing season and biomass, and phytoplankton (chl a) in general. The increase in SST_{summer} is most likely affecting the summer dinoflagellate community leading to an increase in abundance and biovolume, as dinoflagellates prefer less turbulent conditions which may be related to warmer summer conditions (Winder and Sommer, 2012). Another important factor is light availability, which is linked to SPM concentration, but also the concentration of other particles such as phytoplankton cells. SPM concentration, Secchi depth and PAR (Photosynthetically active radiation) can give a hint about the light availability in the water column. For the comparison between the 1970s and the 2000s the long-term SPM measurements of the Walcheren stations of the RWS monitoring program were used. The SPM concentration has decreased from the 1970s until the 2000s (*Chapter 3*). The clearer water conditions in the 2000s probably allow a more efficient use of the available nutrients by diatoms and dinoflagellates and hence an increase in abundance/biovolume. The magnitude of the *Phaeocystis* bloom has been shown to be negatively linked to high SPM concentrations (*Chapter 5*). Lewandowska and Sommer (2010) showed that warming and high light intensities may lead to an advanced bloom start and McQuatters-Gollop et al. (2007) argues that warmer and clearer water conditions in the North Sea have led to a considerable increase of the phytoplankton biomass in the southern coastal North Sea (from 1948 until 2003).

The increase of nutrient concentrations in the eutrophication period (1960s-1980s) and the subsequent de-eutrophication since the late 1980s, with a decrease in P and N input to the North Sea, has considerably impacted the marine ecosystem, and especially coastal waters under the influence of riverine nutrient input. As the chlorophyll a trends in the BPNS are less clear (increase of spring chlorophyll a 1970s vs. 2000s (*Chapter 3*), decrease of winter and summer chlorophyll a 1970s vs. 2000s (*Chapter 3*), decrease of offshore mean annual chlorophyll a from 1990 until 2014 (*Chapter 4*), but increase of onshore chlorophyll a from 2003 until 2016 (*Chapter 5*)), it is not easy to evaluate the exact relationship between nutrients and phytoplankton biomass in the BPNS.

However, eutrophication has been discussed for a long time to be the main factor driving the increase in marine phytoplankton biomass (Cloern, 2001), also other North Sea studies have not found a positive relationship between these parameters (e.g. McQuatters-Gollop et al., 2007). It can be argued that the total P and N concentrations play a less important role in phytoplankton biomass accumulation than it has been assumed in many earlier studies (Cadée and Hegeman, 1986; Lancelot et al., 1987). On the other hand, DSi input to the BPNS has increased in summer and autumn months from 1970 until 2010, but no significant trend was observed for the other seasons. Between 2003 until 2016 however, DSi decreased in the offshore zone during winter, while no trend could be identified in the onshore area.

McQuatters-Gollop et al. (2007) suggests that nutrient ratios play a more important role in the increase of phytoplankton biomass than the total concentrations, as N and P concentrations are decreasing in coastal North Sea waters, but DIN:DIP and DSI:DIP ratios are nowadays above the Redfield ratio of 16:1, respectively 15:1. This observation is supported by the present study in which especially winter and spring DIN:DIP and DSi:DIP ratios have considerably increased from the 1970s until the 2000s (*Chapter 3*). Therefore, the increase in diatoms (abundance and biovolume) especially in winter and spring could have been caused by the uneven decline in the nutrients N and P and the resulting alteration of nutrient ratios. In addition, diatoms profit from the increase in DSi:DIP (all seasons, 1970s vs. 2000s) ratios, as this offers a competitive advantage over other phytoplankton groups, a trend that continues in the 2000s/2010s. As DIP is generally limiting in the coastal zone (DIN:DIP ratio above 16:1), special effort should be undertaken to reduce DIN input to the BPNS, especially in the case of *Phaeocystis* (Desmit et al., 2012). As spring diatoms are limited by DSi, *Phaeocystis* is able to grow on the remaining DIN, even under low DIP concentrations (Schoemann et al., 2005; Desmit et al., 2012). However, the *Phaeocystis* study presented in this thesis (*Chapter 5*), diatom biomass and *Phaeocystis* biomass show no significant relationship with the DIN:DIP ratio. It can be argued that most likely a competition effect of the preceding diatom spring community with the spring *Phaeocystis* community is responsible for changes in *Phaeocystis* bloom magnitude, a relationship that needs to be further investigation (see *6.3.3.*).

As the NAO influences the weather pattern in Northern Europe and the hydrodynamics in the southern North Sea, it influences i.a. SST, SPM concentration and nutrient loads to the BPNS. By this, the NAO index effects the phytoplankton biomass indirectly and in complex ways. The effect on diatoms and *Phaeocystis* has been studied in *Chapter 5*. There is only a significant correlation of *Phaeocystis* biomass in April and the diatom biomass in March with the NAO index in March,

which shows that the relationships are quite complex and might vary strongly spatially, due to the complex hydrodynamics in the BPNS.

6.3. Future research perspectives

6.3.1. Suggestions for methodological improvements and monitoring

As blooms of some taxa might be quite short-lived (days to weeks) frequent monitoring is necessary to realistically capture seasonal patterns and the wax and the wane of blooms (Tarran and Bruun, 2015) in order to compare these between years. This is especially important in the case of harmful algae blooms (HABs). Therefore, we recommend at least a weekly sampling frequency.

In addition, it is known that phytoplankton biomass distribution and community structure can be highly spatially heterogeneous and patchy (Aardema et al., 2018). As the phytoplankton samples analysed in this thesis were sampled at low spatial resolution (point samples), more complex spatial patterns could easily be missed. Underway systems providing continuous or high frequency data, such as underway fluorometry and flow cytometry (FCM) data, can help to make more reliable statements or give complementary insight into the real spatial phytoplankton distribution (see *1.6.3.*) (Aardema et al., 2018; Thyssen et al., 2008). In the future, such (semi)continuous techniques could be used for live measurements in routine monitoring, and when deviant values are observed (e.g. a specific biomass value as a bloom threshold), additional samples can be taken for more in depth analyses afterwards (biomass and composition using pigments, DNA and/or counts).

As the interaction of phytoplankton with its environment can be quite species-specific (Schlüter et al., 2012), it is recommended to identify phytoplankton below genus level. In addition, phytoplankton enumeration should ideally be conducted by the same person to improve the quality of the counts in order to reduce the bias introduced by different taxonomists (Günther et al., 2012; Nohe et al., 2018). Furthermore, when conducting cell counts, it is strongly recommended to conduct phytoplankton size measurements in parallel, otherwise a high bias can be introduced in phytoplankton biovolume or carbon biomass estimations. In addition, FCM and molecular methods such as Next-generation sequencing (NGS) can be additionally applied to get more detailed insight in the real functional and/or taxonomic biodiversity of the phytoplankton community (see *1.6.4.*). For example, as traditional phytoplankton detection methods (such as light microscopy and epifluorescence microscopy) are not able to detect picoplankton cells or only display a few features (such as size, shape or the presence of a flagellum), molecular

methodologies can provide new insight into the biodiversity of this small-sized phytoplankton group (Massana, 2011; see also *Chapter 1.6*).

Finally, zooplankton plays an important role in the marine food-web and is just like phytoplankton one major component of the biological carbon pump, as it is the main grazer in the marine food-web (Van Ginderdeuren et al., 2014). It is not only impacting the phytoplankton community, but is at the same time sensitive to environmental (long-term) changes such as climate change (Richardson, 2008; Capuzzo et al., 2018). Therefore, also long-term changes and changes in the seasonality of the zooplankton community can be expected. Due to the scarce availability of zooplankton data, top-down effects on phytoplankton have not been a part of this study, but should be included in continuative research activities. However, in the framework of the 4DEMON project relevant zooplankton data have been identified, digitized and made publicly available:

- http://www.vliz.be/nl/imis?module=dataset&dasid=4695 (1971-1972)
- http://www.vliz.be/nl/imis?module=dataset&dasid=4668 (1971-1974)
- http://www.vliz.be/nl/imis?module=dataset&dasid=5048 (1971 and 1974)
- http://www.vliz.be/nl/imis?module=dataset&dasid=4697 (18th and 19th of April 1978)
- http://www.vliz.be/nl/imis?module=dataset&dasid=4696 (1977-1979)

In addition, there is a biannual study (2009-2010) on mesozooplankton (0.2-2 mm) in the BPNS by Van Ginderdeuren (2013) and Van Ginderdeuren et al. (2014) (accessibel via http://www.vliz.be/en/imis?module=dataset&dasid=4456). Since 2012, VLIZ generates a zooplankton monitoring time series using a zooSCAN device (http://www.vliz.be/en/zooscan-en). Zooscan data can be accessed via http://rshiny.lifewatch.be/ZooScan%20data/ (2012-2017). As the time series becomes longer, these data can be used to link phytoplankton and zooplankton long-term trends.

Also viruses are known to affect many phytoplankton species, among which also species of the genus *Phaeocystis* (Brussaard et al., 2007). Their importance in the microbial food web has been acknowledged for a long time, as the release of cell content by viral induced cell lysis has major effects on the marine carbon and energy cycle (Brussaard et al., 2007; Brussaard et al., 2005). This also positively stimulates bacterial activity (Bratbak et al., 1998; Brussaard et al., 2007; Brussaard et al., 1995). Viral infection can affect phytoplankton community dynamics and composition (Baudoux and Brussaard, 2005; Brussaard et al., 2007). Therefore, it is recommended that in the future also viral and bacterial structural and activity data are collected in order to link them to the phytoplankton community data.

6.3.2. Physiological studies of key phytoplankton species

In *Chapter 3*, it was shown that the phytoplankton community changed considerably, regarding abundance, biomass and seasonality between the 1970s and the 2000s. It was discussed how the environmental conditions dominating in the two periods might have contributed to those changes. As there was too little environmental data to match them directly with the phytoplankton observations and conduct statistical analyses, we suggest to conduct laboratory experiments using phytoplankton key species which have shown considerable changes in biomass and seasonality, such as *Actinoptychus* spp., *Thalassiosira* spp. and *Guinardia* spp. Recently, Burson et al. (2018) conducted multispecies experiments using natural phytoplankton samples from the North Sea, in order to study how resource limitation (N, P and light) influences this community. We suggest to use in a first approach monocultures and cultivate them under typical environmental conditions present in the 1970s and the 2000s in order to measure the effect on the phytoplankton species. The 1970s were characterized by higher nutrient concentrations and different nutrient ratios, lower temperatures and higher turbidity. Therefore, especially the impact of light, temperature, nutrient concentrations and nutrient ratios should be studied. In a second step then, multispecies experiments could be conducted to test if the phytoplankton species still react in the same way in the presence of biotic competition.

6.3.3. Competition experiments diatom-*Phaeocystis* interaction

The "diatom-*Phaeocystis* competition" hypothesis was first introduced by Karasiewicz et al. (2018) and supported in *Chapter 5* of the present thesis. Karasiewicz et al. (2018) applied a WitOMI (Within Outlying Mean Index) analysis, which identifies the realized subniche ("species's niche occupation within subset habitat conditions") of a species, in phytoplankton community datasets. The results suggest the presence of a different diatom community compositions in subset L and subset H, resulting in low or high *Phaeocystis* spring bloom magnitudes. Whereas Karasiewicz et al. (2018) mentions the importance of the diatom community composition, the hypothesis presented in *Chapter 5* is focused on the total diatom biomass.

The hypothesis should be studied in more detail by conducting laboratory experiments. Natural phytoplankton communities should be grown in bioreactors in the laboratory under environmental conditions found in high (H) and low (L) *Phaeocystis* years. Subset H was characterized by depleted nutrient concentrations, lower SPM concentration and higher Secchi depth, higher SST and higher salinity. Environmental parameters should be tested one by one. E.g. SST should be tested, by growing the algae under conditions typical of the ones in L and H *Phaeocystis* years, with all other parameters being equal.

Regarding the findings of Karasiewicz et al. (2018) special attention should be paid to the diatom community composition in L and H years. Daily samples should be analysed by light microscopy

(including size measurements and biovolume calculations). The data can be analysed with correlation analyses to test the diatom-*Phaeocystis* competition hypothesis. In addition, a WitOMI analyses could be conducted to discover possible community composition effects.

6.4. General Conclusion

In this PhD thesis, long-term changes in phytoplankton biomass, diatoms, dinoflagellates and *Phaeocystis*, have been investigated on the basis of traditional light microscopic phytoplankton counts, biovolume estimations and HPLC pigment measurements. The results show clearly that the phytoplankton community in the BPNS has changed considerably since the 1970s, with marked changes in seasonality, especially in the earlier bloom of the diatom spring community, earlier and more pronounced blooms of the dinoflagellate community and an earlier and sharper spring chlorophyll a peak (related to diatoms and/or *Phaeocystis*). The changes in diatom and dinoflagellate abundance, biomass and seasonality seem to be mainly linked to the increase in SST and water transparency in the 2000s compared to the 1970s, as well as altered nutrient ratios. There were too few 1970s *Phaeocystis* data available to draw any conclusions about the long-term changes in *Phaeocystis* in the BPNS, but it was shown that *Phaeocystis* biomass and seasonality varies considerably during the period 2003-2016. An earlier and higher *Phaeocystis* bloom seems to be mainly linked to higher SST and light availability in the preceding winter months, and an earlier occurrence of the diatom spring community and the fast depletion (and limitation) of the available nutrients by the diatoms.

SST has been identified as one important factor influencing the phytoplankton biomass, seasonality and community composition. If the global CO_2 emissions stay at the present level, marine SST will further increase in the future by approximately 3.2 °C by 2100 in comparison with the pre-industrial period (Representative Concentration Pathway 8.5, RCP8.5), possibly resulting in a decreasing frequency of cold winters and an increasing frequency of high summer SST (Gattuso et al., 2015; Philippart et al., 2011). It can be argued that the total biomass, especially of winter-spring diatoms and summer dinoflagellates will further increase under increasing SST. Also the occurrence of more intense *Phaeocystis* spring blooms can be expected due to a further increase in winter-spring SST, as well as the occurrence of stronger and earlier spring diatom blooms. In addition, if the forward shift of the diatom and dinoflagellate blooms further continues, an intensification of trophic mismatch scenarios can be expected with long-lasting effects for the whole marine ecosystem.

This thesis proves that the often hidden and/or undigitized historical data have a huge value especially if there is a lack of reliable long-term monitoring programs.

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Summary

Phytoplankton expresses long-term trends in many seas of the world, including biomass changes, composition changes and changes in seasonality. Phytoplankton in the Belgian part of the North Sea (BPNS) has been studied since many decades by means of different methodologies e.g. light microscopy, spectrophotometry or High-performance liquid chromatography (HPLC) pigment analyses. However, our understanding of the long-term trends in the BPNS is limited as data have often been collected in the framework of different projects and have never been integrated. Therefore, this PhD thesis aimed to improve our knowledge on phytoplankton long-term changes in the BPNS, including their environmental drivers.

Chapter 2 describes the compilation of the Belgian Phytoplankton Database (BPD), a comprehensive data collection comprising quantitative phytoplankton cell counts from multiple research projects conducted since 1968. Historical phytoplankton and environmental data have been collected, digitized and quality checked (e.g. species taxonomy update). Important metadata like sampling date, sampling location, sampling depth and sampling methodology, have been collected and standardized. An additional effort has been put in compiling species biovolume estimations. This database has been made available online (http://www.vliz.be/en/imis?dasid=5717&doiid=320) and is ready to be used by other marine researchers.

The BPD has been used in the first study in this thesis on long-term changes of the diatom and dinoflagellate community in the BPNS (*Chapter 3*). It was assessed how environmental conditions in the 1970s and the 2000s, co-vary with changes in community composition, biovolume, abundance and seasonality. To achieve this, ANOVA analyses, ordination analyses and General Additive Mixed Models (GAMMs) were performed. The results show that diatoms and dinoflagellates increased in abundance and biovolume. It can be argued, that this partly results from the overall increase in sea surface temperature (SST) and the improvement of the water clarity between the two study periods. The GAMM analyses reveal a changing phenology for both diatoms and dinoflagellates, with an earlier start of the growing season in the most recent study period. Diatoms are most likely influenced by changes in environmental winter conditions (clearer and warmer water with increased DIN:DIP and DSi:DIP ratios), while dinoflagellates most likely profit from the increased summer SST and a rise in the DIN:DIP ratio. While in the 1970s, distinct seasonal communities were present, a seasonal homogenization had occurred by the 2000s. Finally, we observed a pronounced increase in harmful diatom and dinoflagellate genera.

In a second study (*Chapter 4*), it was explored how chlorophyll a changed during the last decades. The spatial focus was broadened by also taking chlorophyll a long-term data from the Dutch part of the North Sea into account. A decline in annual mean chlorophyll was observed across most waterbodies (coastal and offshore) in the period 1988-2015. However, in the BPNS this decrease in chlorophyll a was only significant in the offshore area. In addition, a shift in phytoplankton phenology was observed with the spring bloom formation occurring earlier in the year. Most likely shifts in phytoplankton phenology are caused by the increase of SST during winter.

The last study (*Chapter 5*) analyses changes in *Phaeocystis*, a nuisance algae regularly dominating the spring phytoplankton community in the BPNS. Even though there exist many theories regarding the development of intense *Phaeocystis* blooms, there is still much dubiety about the abiotic and biotic constraints. To increase the knowledge of *Phaeocystis* bloom development in the BPNS, *Phaeocystis* and diatom biomass were estimated using HPLC-CHEMTAX pigment analysis (2003-2016) and microscopic cell counts (2003-2010). Annual anomaly analyses, Mann-Kendall trend tests and GAMMs reveal an overall increase of diatom biomass during the period 2003 to 2016, but no clear long-term trend for *Phaeocystis*. However, *Phaeocystis* biomass showed strong interannual variation in bloom intensity, with exceptionally strong blooms in some years. To investigate the abiotic-*Phaeocystis* relationship further, the dataset was separated in high and low *Phaeocystis* years and correlation analyses were conducted. The results suggest that the availability of light during the *Phaeocystis* growing period and a warmer SST are two important abiotic factors stimulating the development of dense *Phaeocystis* blooms. Also a positive North Atlantic Oscillation (NAO) index in March is positively linked to the *Phaeocystis* bloom. A remarkable finding was the negative correlation with many nutrient concentrations in March, which supports the "diatom-*Phaeocystis* competition"-hypothesis already proposed by Karasiewicz et al. (2018). Under low SST conditions, diatoms win the competition over *Phaeocystis*, as the flagellated form of *Phaeocystis* does not grow fast enough to accumulate a critical biomass. Diatoms get limited by the depleted DSi concentration and *Phaeocystis* can grow on the remaining pool of nutrients, delayed though and with a less steep and lower biomass accumulation, due to lower remaining nutrient concentrations. Under high SST conditions, flagellated *Phaeocystis* cells grow fast enough to start forming colonies. Colonial *Phaeocystis* enters into competition with diatoms for the remaining DIN and DIP and may dominate thanks to their better performance under low phosphate concentrations. This allows *Phaeocystis* to develop massive blooms, which also occur earlier. This hypothesis was supported by seasonal GAMMs showing an earlier and steeper increase in the spring diatom biomass in years with dense *Phaeocystis* blooms.

In conclusion, the results of this PhD thesis clearly show the existence of phytoplankton long-term trends in the BPNS, regarding biovolume, abundance, biomass, composition and seasonality. Important factors shaping the phytoplankton community are SST, suspended particulate matter (SPM) and nutrient ratios, and in this especially during winter and spring.

Samenvatting

In vele zeeën op aarde vertoont fytoplankton lange termijn trends qua biomassa, soortensamenstelling en veranderingen in seizoenaliteit. In het Belgisch gedeelte van de Noordzee (BPNS) wordt reeds een aantal decennia onderzoek gevoerd op fytoplankton op basis van uiteenlopende methodes, zoals lichtmicroscopie, spectrofotometrie of High-performance liquid chromatography (HPLC) pigment analyse. Desondanks is de kennis over lange termijn trends in het BPNS beperkt omdat gegevens vaak werden verzameld in het kader van verschillende projecten en nooit samen werden geanalyseerd. Met deze doctoraatsthesis wordt kennis vergaard omtrent lange termijn veranderingen van fytoplankton in het BPNS, inclusief kennis omtrent milieuparameters die deze trends beïnvloeden.

In *Hoofdstuk 2* wordt het oprichten van de Belgische Fytoplankton Databank (BPD) beschreven, een uitgebreide dataverzameling welke kwantitatieve fytoplanktonceltellingen, afkomstig uit verschillende onderzoeksprojecten die sinds 1968 werden gevoerd, omvat. Historische fytoplankton- en milieugegevens werden verzameld, gedigitaliseerd en ondergingen een kwaliteitscontrole (bijv. bijwerken van de taxonomie van de soorten). Belangrijke metadata zoals de datum van bemonstering, de bemonsteringslocatie, de bemonsteringsdiepte en de bemonsteringsmethode werden samengebracht en gestandaardiseerd. Daarnaast werd ook gefocust op het samenstellen van biovolumeschattingen. De databank werd online toegankelijk gemaakt (http://www.vliz.be/en/imis?dasid=5717&doiid=320) en kan worden gebruikt door andere mariene onderzoekers.

De BPD werd gebruikt in de eerste studie van dit doctoraat over de lange termijnveranderingen van diatomeeën- en dinoflagellatengemeenschappen in het BPNS (*Hoofdstuk 3*). Hierin werd onderzocht hoe veranderende milieuomstandigheden in de jaren 70 en 2000 in verband staan met veranderingen in de gemeenschapssamenstelling, biovolumes, abundanties en de seizoenaliteit. Om dit aan te tonen werden ANOVA-analyses, ordinatie-analyses en General Additive Mixed Models (GAMM's) uitgevoerd. De resultaten tonen aan dat diatomeeën en dinoflagellaten toenemen in abundantie en biovolume. Er kan worden aangenomen dat dit deels het resultaat is van een algemene toename in temperatuur van het zeeoppervlak (SST) en een verbetering van de waterhelderheid tussen de twee onderzoeksperiodes. De GAMM analyses tonen een veranderende fenologie aan, zowel voor diatomeeën als voor dinoflagellaten, met een vroegere start van het groeiseizoen in de recentste onderzoeksperiode. Diatomeeën werden hoogstwaarschijnlijk door veranderende milieuomstandigheden tijdens de winter beïnvloed

(helderder en warmer water met toenemende DIN:DIP en DSi:DIP ratio's), terwijl dinoflagellaten hoogstwaarschijnlijk profiteren van de toename in zomer SST en een stijging van de DIN:DIP ratio. In de jaren 70 waren verschillende seizoensgemeenschappen aanwezig, in de jaren 2000 zijn de gemeenschappen daarentegen door het jaar heen homogener geworden. Er kon ook een duidelijke toename van schadelijke diatomeeën en dinoflagellaten worden vastgesteld.

In de tweede studie van dit doctoraat (*Hoofdstuk 4*) werd onderzocht hoe chlorofyl a concentraties veranderden tijdens de laatste decennia. De ruimtelijke focus werd uitgebreid door ook rekening te houden met lange termijn data van chlorofyl a die afkomstig zijn van het Nederlandse deel van de Noordzee. Er werd een daling van de het jaarlijks gemiddelde aan chlorofyl waargenomen in de meeste waterlichamen (bij de kust en offshore) in de periode 1988-2015. In het BPNS was deze daling in chlorofyl a echter alleen significant in het offshore gebied. Daarenboven werd een verschuiving van de fenologie van het fytoplankton waargenomen waarbij de lentebloei vroeger in het jaar optreedt. Vermoedelijk is deze verschuiving het gevolg van een SST-toename tijdens de winter.

De laatste studie (*Hoofdstuk 5*) analyseert veranderingen in *Phaeocystis*, een plaagalg welke in de lente regelmatig de fytoplanktongemeenschappen in het BPNS domineert. Hoewel er veel theorieën bestaan omtrent de ontwikkeling van intense *Phaeocystis*-bloei, is er nog steeds veel onzekerheid over de abiotische en biotische omstandigheden welke deze beïnvloeden. Om de kennis over de bloeiontwikkeling van *Phaeocystis* in het BNPS uit te breiden, werden de biomassa's van *Phaeocystis* en diatomeeën berekend door middel van HPLC-CHEMTAX pigmentanalyses (2003-2016) en microscopische celtellingen (2003-2010). Jaarlijkse afwijkingsanalyses, Mann-Kendall trend tests en GAMM's onthullen een globale toename van de biomassa van diatomeeën in de periode van 2003 tot 2016, maar geen duidelijke lange termijn trend voor *Phaeocystis*. Nochtans vertoont de biomassa van *Phaeocystis* sterke jaarlijkse variaties qua bloeiintensiteit, met in sommige jaren uitzonderlijk sterke bloei. Om de relatie tussen *Phaeocystis* en abiotische factoren diepgaander te onderzoeken, werd de dataset opgedeeld in jaren met hoge en jaren met lage bloeiintensiteiten en werden hierop correlatieanalyses uitgevoerd. De resultaten wijzen aan dat de beschikbaarheid van licht gedurende de groeiperiode van *Phaeocystis* en een hogere SST twee belangrijke abiotische factoren zijn die de ontwikkeling van dense *Phaeocystis*-bloei stimuleren. Ook een positieve Noord-Atlantische Oscillatie (NAO)-index in maart is positief gecorreleerd aan de bloei van *Phaeocystis*. Een opmerkelijk resultaat was de negatieve correlatie van *Phaeocystis* met verschillende nutriëntenconcentraties in maart, wat de "diatomeeën-*Phaeocystis* competitie"-hypothese voorgesteld door Karasiewicz et al. (2018)

onderbouwt. Onder lage SST condities domineren diatomeeën *Phaeocystis*, omdat de geflagelleerde vorm van *Phaeocystis* niet snel genoeg groeit om een kritische biomassa te bereiken. Diatomeeën worden gelimiteerd door de uitgeputte DSi concentratie en *Phaeocystis* kan blijven groeien op de overgebleven pool aan nutriënten. Dit gebeurt wel met vertraging, een minder snelle toename en een lagere biomassa-accumulatie, omwille van de lagere nutriëntenconcentraties. Onder hoge SST condities groeien geflagelleerde *Phaeocystis*-cellen snel genoeg om kolonies te vormen. Koloniale *Phaeocystis* gaat in competitie met diatomeeën voor de overblijvende DIN en DIP, en kan domineren, omdat ze het beter doen onder lage fosfaatconcentraties. Dit zorgt ervoor dat *Phaeocystis* dense bloeien kan ontwikkelen die ook vroeger plaatsvinden. Deze hypothese wordt ondersteund door seizoenale GAMM's die een vroegere en snellere toename van diatomeeënbiomassa tijdens de lente aantonen in jaren met hoge bloei van *Phaeocystis*.

De resultaten van dit doctoraat tonen duidelijk het bestaan van lange termijn trends van fytoplankton in het BPNS aan, namelijk op het vlak van biovolume, abundantie, biomassa, samenstelling en seizoenaliteit. Belangrijke factoren die een fytoplanktongemeenschap vormgeven zijn SST, SPM en nutriëntenratio's, en dit in het bijzonder tijdens de winter en lente.

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