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A sea of change

Impacts of reduced nitrogen and phosphorus loads on coastal phytoplankton communities

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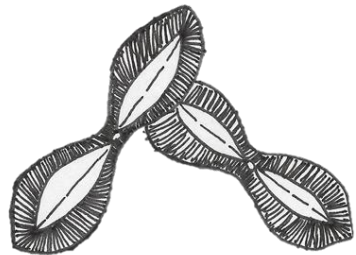
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Chapter 7

Synthesis



The aim of this thesis was to better understand the ways in which changes in nutrient loads impact phytoplankton communities. As nutrients entering coastal systems become more and more influenced by anthropogenic activities so too comes the responsibility to understand what potential consequences may result. This onus is not only limited to scenarios where eutrophication occurs, but should also be undertaken when de-eutrophication efforts are implemented. To illuminate potential impacts after de-eutrophication, I used the North Sea as a current example of a coastal system experiencing large shifts in nutrient loading.

In this chapter, I will first place my findings in the context of long-term changes in the riverine nutrient loads entering the North Sea. Then I discuss how my experimental results with phytoplankton communities sampled from the North Sea align with prevailing theories in biodiversity and community ecology. Next I discuss the impacts of nutrient load and ratio changes on the community composition and biomolecular composition of phytoplankton in the North Sea and how this may impact higher trophic levels in the food web.

Changes in the nutrient composition of the North Sea

Aquatic systems are rarely isolated, but are continuums where upstream inputs are reflected in downstream outputs (Paerl et al., 2014). Several major European rivers influence the nutrient budget of the North Sea. At the onset of the OSPAR convention in 1986, total riverine nitrogen and phosphorus loads entering the Dutch and German part of the North Sea were high due to several decades of anthropogenic eutrophication (Fig. 7.1; van Beusekom et al., 2017). Subsequent de-eutrophication efforts have led to a gradual decline of the riverine nitrogen and phosphorus loads, and this declining trend still continues today (Fig. 7.1). After correcting for freshwater discharge differences, total nitrogen concentrations from rivers decreased annually by between 2.4-2.7% from 1985 to 2014, while total phosphorus concentrations decreased more steeply by 5.3-5.5% (van Beusekom et al., 2017). This disproportionate decrease of phosphorus is reflected in the molar N:P ratios of the major riverine nutrient loads, particularly in the Dutch waters, which increased from ~25 in the 1980s to ~60 in 2014 (Fig. 7.2; van Beusekom et al., 2017).

Sampling of the Terschelling transect, from the riverine-influenced nearshore region to the central North Sea, revealed an impact of changing riverine nutrient loads (Chapter 2). The nearshore region displayed the highest total concentrations of

both nitrogen and phosphorus, especially in spring, indicating fluvial inputs are the major source of nutrients to the system (Fig. 2.2, Chapter 2). In contrast, the central North Sea displayed lower total nutrient concentrations, likely influenced predominantly by North Atlantic currents (Fig. 2.2, Chapter 2). The fluvial influence was also reflected in the N:P ratios along the transect. Extremely high DIN:DIP ratios were observed nearshore, particularly in April, indicative of severe P limitation of these coastal waters (Fig. 2.3B). P limitation of the nearshore phytoplankton community was confirmed by bioassay experiments, with co-limitation of P and Si by the diatoms in the community. Similar patterns of P limitation have also been documented in nearby waters of the Marsdiep in the western Wadden Sea (Ly et al., 2014). A spatial gradient transitioning from high N:P ratios in the riverine-dominated nearshore to low N:P ratios in the North Atlantic-dominated central North Sea is clearly visible in the spring months, both in the inorganic nutrients (Fig. 2.3) and in the organic nutrients contained by the seston (Fig. 2.4). Studies performed in the 1990s along the Terschelling transect agree with the trends found in our study, but at that time none described such extremely high nearshore N:P ratios (Peeters and Peperzak, 1990; De Vries et al., 1998). The offshore gradient from high to low N:P ratios therefore seems to be of recent origin. Thus, our findings support the hypothesis that the nutrient budget of the North Sea has experienced major reductions in nutrient loads due to de-eutrophication, which has shifted coastal waters of the North Sea towards P limitation.

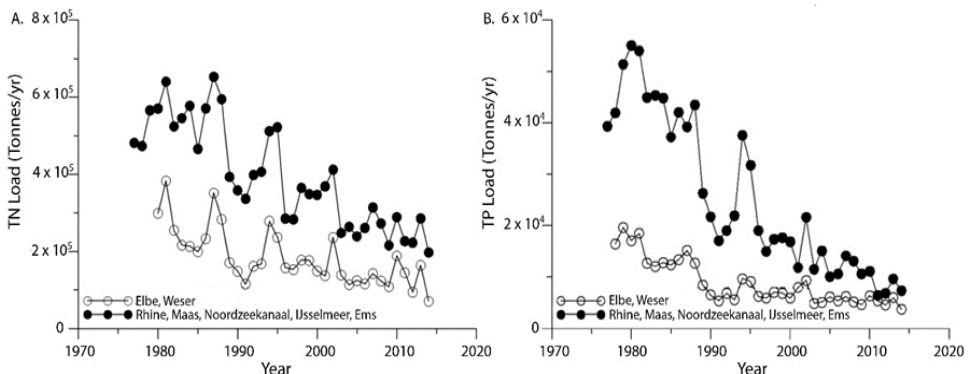


Figure 7.1 Total nitrogen (A) and total phosphorus (B) loads of the major rivers entering the Dutch part (Rhine, Meuse, Noordzeekanaal, IJsselmeer and Ems) and German part (Weser, Elbe) of the North Sea. Reproduced from van Beusekom et al. (2017).

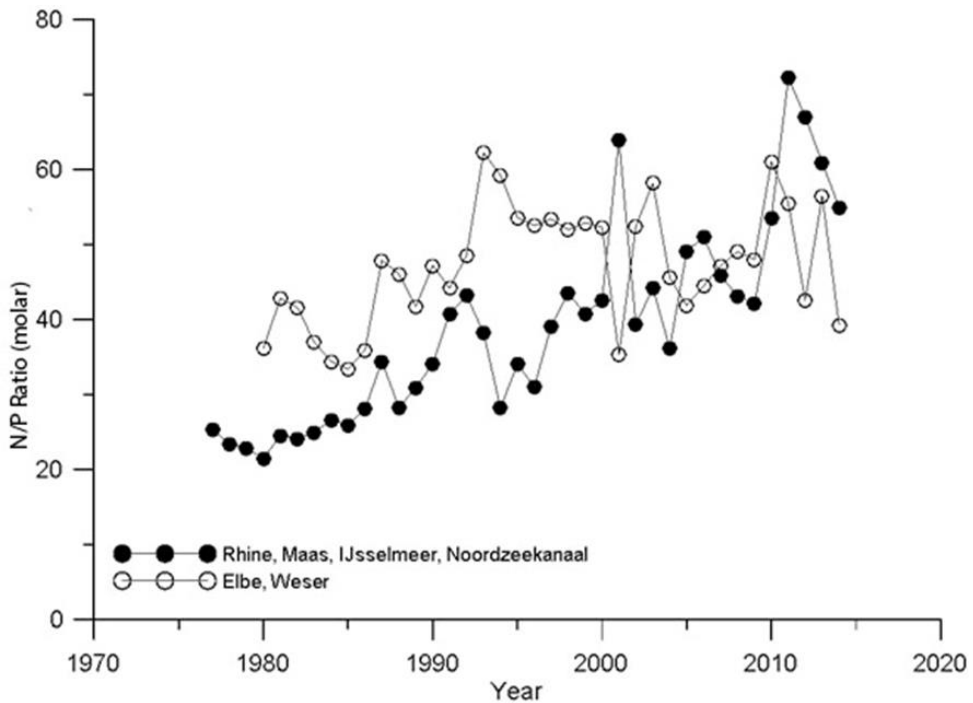


Figure 7.2 Molar N/P ratio of the major riverine loads to the Dutch part (Rhine, Meuse, Noordzeekanaal, IJsselmeer and Ems) and German part (Weser, Elbe) of the North Sea. Reproduced from van Beusekom et al. (2017).

Understanding phytoplankton species composition

A fundamental issue for understanding how changes in nutrient loads affect phytoplankton community composition is the role of competition. More specifically, do interspecific differences in the competitive ability for limiting resources control relative species abundances in a community, as predicted by niche-based theories? Or is competition an effectively neutral interaction and are species abundances largely driven by more stochastic processes, as predicted by neutral theory? In Chapter 3, competition for N, P, and light was clearly shown to shift community composition depending on which of the resources were limiting, which is consistent with the major predictions of niche-based models (Tilman, 1982; Huisman and Weissing, 1995; Grover, 1997; Brauer et al., 2012).

Yet, the high diversity commonly observed in phytoplankton communities, despite a low number of potentially limiting resources, is not easily explained by niche-based theories. The neutral theory of biodiversity allows for high species diversity by assuming that the competitive abilities for resources are equal among

species (Bell, 2001; Hubbell, 2001). Neutral theory argues that the abundances of species are based on random fluctuations of birth, death and emigration/immigration, not on intrinsic differences in the competitive abilities of the species for a limited set of resources (Etienne and Olff, 2004; Hubbell, 2005). These random fluctuations in individual species abundances are described as ecological drift.

Some studies have applied the neutral theory to phytoplankton community data and from this developed a “clumpy distribution” concept where species abundances seem to aggregate along trait lines such as cell size (Vergnon et al., 2009; Segura et al., 2013). This implies that species of similar cell size are assumed to have effectively equal competitive ability for limiting resources, such that ecological drift determines the abundances within size groups. However, this assumption is merely based on field observations but rarely tested experimentally, even though determining the driving forces behind natural community composition from observational studies alone can be misleading.

For example, in Chapter 5 the co-dominance of *Cyanobium* sp. and *Nannochloropsis* sp. in a multispecies community under P-limiting growth conditions suggested an explanation in terms of neutral theory, because the two species appeared to have an equal competitive ability for P. However, when these two species were isolated and grown in dual-species competition experiments, they did not show random trajectories of their species abundances as predicted by neutral models, but reached the same steady-state abundances irrespective of the starting conditions (Chapter 5). Their stable coexistence indicates that the competitive interactions between these two species were not at all neutral. Instead, it is more likely that differentiation along other niche-dimensions enabled their coexistence. In particular, our results indicate that differences in pigmentation and light use between these two species may have allowed for a stabilization of their abundances despite their nearly neutral competitive ability for P. Thus, while clumpy distributions of similar-sized species may appear to be a result of neutral competition, when scrutinized experimentally, competition along other niche-dimensions may in fact be stabilizing community structure.

Niche-based theories have emphasized that changes in environmental nutrient ratios, such as the N:P ratio, have major effects on the composition of natural communities (Tilman, 1982; Grover, 1997). The nutrient-load hypothesis of Brauer et al. (2012) extends the classic resource-ratio theory to include light

as a third limiting resource, a scenario commonly found under eutrophic conditions (Cloern, 1999; Havens et al., 2003). As pointed out by the nutrient-load hypothesis not only changes in nutrient ratios but also changes in absolute nutrient loads may affect the species composition. Obviously, resolving this debate is of high relevance if we are to understand how the phytoplankton composition will respond to de-eutrophication efforts. Therefore, I examined experimental responses of natural marine phytoplankton communities sampled from the North Sea to several N:P ratios and nutrient loads (Chapter 3). Five major predictions of the nutrient-load hypothesis were examined. Limitations (and co-limitations) of N, P, and light were successfully induced in target chemostats, resulting in significant changes in phytoplankton community structure. Although the same set of species coexisted in nearly all chemostat cultures at steady state, the relative dominance of individual species was clearly driven by nutrient limitation at low nutrient loads and co-limitation with light at moderate nutrient loads. At high nutrient loads, light limitation developed. Increasing nutrient loads induced changes in relative species dominance even when N:P ratios remained constant. These experiments confirm the prediction of the nutrient-load hypothesis that both nutrient ratios and total nutrient loads should be considered when attempting to predict the impact of nutrient management strategies on phytoplankton community structure.

The only prediction of the nutrient-load hypothesis which was not confirmed by the chemostat experiments was the prediction of competitive exclusion under single resource limitation (Chapter 3). Although stable coexistence was predicted for the co-limitation experiments, we did not expect to find stable coexistence in the single limitation experiments as well. Interestingly, the light-limitation only treatment did have fewer species at steady state than the other treatments. The three species coexisting under light-limited conditions represented three different functional groups of phytoplankton; green algae (*C. marina*), diatoms (*N. agnita*), and cyanobacteria (*Synechococcus* spp.). The light absorption spectra of these three species (Chapter 3) partially overlapped but also revealed that each of the species contained pigments which allowed for specialized utilization of part of the light spectrum. These results show that light should not be interpreted as a single resource, but as a spectrum of resources, and indicate that niche differentiation in the light spectrum may have supported their coexistence. A similar stabilization of species coexistence

through niche differentiation in the light spectrum was also observed in the dual-species competition experiments in Chapter 5.

Coexistence due to differences in light absorption spectra has been convincingly demonstrated by Stomp et al. (2004) using two closely related *Synechococcus* sp. strains (BS4 and BS5) isolated from the Baltic Sea. Strain BS4 has high contents of phycocyanin and therefore appears green in color and grows well in red light. Conversely, strain BS5 contains phycoerythrin, appears red in color, and grows well in green light. When both strains were grown competitively under either green or red light, competitive exclusion occurred (Fig. 7.3c, d). However, when grown under the full spectrum of white light, coexistence occurred as each strain utilized different sections of the light spectrum (Fig. 7.3e). Further studies found similar patterns of coexistence among red and green picocyanobacteria in field data (Stomp et al., 2007b). Our results indicate that differences in light absorption spectra may contribute not only to the coexistence of closely related picocyanobacteria, but also to the coexistence of phytoplankton taxa from completely different functional groups (e.g., cyanobacteria, diatoms and green algae).

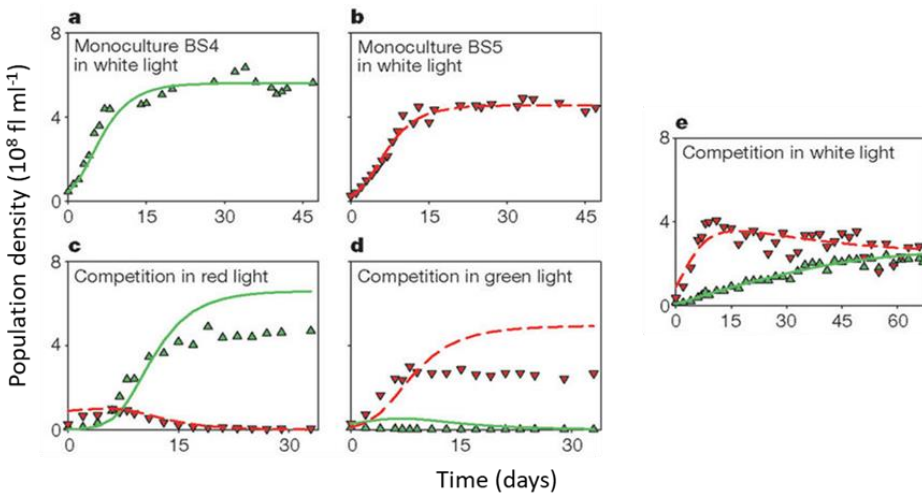


Figure 7.3 Time course of monocultures of *Synechococcus* strains BS4 (a) and BS5 (b) in white light. Competition between BS4 and BS5 in red light (c), green light (d) and white light (e). Symbols represent the observed population densities of BS4 (green triangles) and BS5 (red triangles). Lines represent the population densities predicted by the model: green solid line for BS4, red dashed line for BS5 (Reproduced from Stomp et al. 2004)

Effects of nutrient-load reductions on phytoplankton species composition

Our results show that the North Sea currently displays P (co-) limitation of phytoplankton growth during the spring months in coastal regions most influenced by fluvial inputs. Moving from the nearshore to the central North Sea, DIN:DIP ratios transition from P-limitation, to N and P co-limitation, to N-limitation. The distance from shore of P-limiting DIN:DIP ratios is dependent on the degree of influence of fluvial inputs, thus the furthest extension was in the month of April. In March and May the offshore gradient from P to N limitation was still observed, but by August the entire transect was exhibiting N-limiting DIN:DIP ratios. Nutrient enrichment bioassays confirm this spatial gradient from P limitation nearshore to N limitation offshore in the spring, with the addition of co-limitation by Si in nearshore regions (Chapter 2).

Phytoplankton functional groups are differentially impacted by changes in nutrient limitation. Diatoms were the most dominant group in terms of phytoplankton biomass in all months but August (Fig. 2.6), but may also be particularly susceptible to P-limitation especially in spring (Chapter 2). Their growth rate is further affected by co-limitation by Si, as confirmed by the bioassay experiments (Chapter 2; see also Ly et al., 2014). Although in Chapter 3 diatoms were dominant in both the P- and N-limiting chemostat experiments, these particular diatom species had very small cell sizes. The larger pelagic diatom species, which are indicative of the classical spring bloom, were quickly excluded in these chemostat experiments (Chapter 3). In fact, in all the chemostat experiments, it was only small species which remained. Dominance of small species under nutrient-limited conditions has been observed in several studies of phytoplankton ecology (Raven, 1998; Litchman et al., 2010; Marañón, 2015).

Bioassay results indicated that almost all functional phytoplankton groups were P-limited in the nearshore region, including the targeted *Phaeocystis* sp. and cyanobacteria (Chapter 2). However, in contrast to the diatoms, dinoflagellates and nanoflagellates appeared to be co-limited by N and P in April, despite elevated DIN:DIP ratios (Chapter 2). Several species of dinoflagellates and nanoflagellates utilize mixotrophy as a means to meet nutritional needs under inorganic nutrient limitation stress. This is a trait which may give these groups a competitive edge under stoichiometrically imbalanced DIN:DIP conditions. Dinoflagellates in particular contain many species that produce toxins which can be harmful to fish, marine mammals and humans. One possible risk of strongly

limiting diatom growth through P and Si limitation in spring is an increase in occurrence of these potentially toxic species.

The Netherlands is not immune to blooms of toxic dinoflagellates. The bloom of *Alexandrium ostenfeldii* in the Ouwkerkse Kreek proved to be particularly toxic with the species producing both saxitoxins and spirolides (Chapter 6). This event is an indication that these toxic species are not only present in Dutch coastal waters, but are capable of creating an environmental and human health risk should they become bloom forming. Many of the dinoflagellate genera identified in the sampling transect of the North Sea, such as *Alexandrium*, contain several toxin producing species. Due to the cyst forming abilities of many of these toxic species, once conditions allow for a first bloom to occur there is considerable potential for repeated bloom events (Giacobbe et al., 2007; Tang and Gobler, 2012; 2015).

Effects of nutrient-load reductions on biochemical composition of phytoplankton

In addition to changes in phytoplankton species composition, nutrient-load reductions may also affect the biochemical composition of phytoplankton (Chapters 2 and 4). The P-limited conditions in the coastal North Sea were reflected by high N:P and C:P ratios of the nearshore seston (probably mostly phytoplankton cells), which were well above the Redfield ratio in April and May and significantly higher than that of the seston from further offshore (Fig. 2.4). In the outer shore stations N:P and C:P ratios of the seston were within range of the Redfield ratio, indicating a more balanced ecological stoichiometry of phytoplankton.

While cellular C:N:P ratios are often considered in ecological stoichiometry studies (see Sterner and Elser, 2002), a major criticism is the lack of knowledge on the individual biomolecules which contribute to the overall intercellular C, N, and P pool. Using methods developed by Grosse et al. (2015, 2017), ¹³C isotopes were utilized to gain a more detailed understanding into how resource limitation impacts the accumulation and synthesis of amino acids, fatty acids and carbohydrates (Chapter 4). Both amino acid content and synthesis were lower in N-limiting chemostats when compared with P- or light-limiting chemostats. This agrees with other studies showing that N-limitation may slow amino acid synthesis (e.g., Van de Waal et al., 2010b; Loladze and Elser, 2011;

Grosse et al., 2017). Conversely, P-limitation did not reveal a major impact on amino acid synthesis. Additionally, the synthesis of essential amino acids was lower under N-limited conditions than in P- and light-limited communities (Chapter 4). Interestingly, Grosse et al. (2017) found that North Sea phytoplankton under N-limitation showed rapid recovery of amino acid synthesis when the nutrient limitation was relieved in short-term field incubations. Glucose, a storage carbohydrate, showed an opposite trend to amino acids, while structural carbohydrates and structural fatty acids did not vary significantly. Light limitation was linked with decreased accumulation of storage fatty acids. Although gaps in biomolecule composition remain (e.g., we did not measure RNA, DNA, pigments), up to 91% of the C-fixation performed by the phytoplankton was invested in the synthesis of amino acids, carbohydrates, and fatty acids. A new method to detect ^{13}C incorporation into RNA and DNA nucleotides (Moerdijk-Poortvliet et al., 2014) can further progress our understanding of how resource limitations affects the biochemical composition of phytoplankton.

The changes in biomolecular composition observed in the chemostat experiments most likely apply to the natural system as well. The DIN:DIP ratio of the nearshore North Sea in April (375:1) was in the same order of magnitude as the DIN:DIP ratio in the mineral medium supplied to the P-limited chemostat (500:1). Conversely, DIN:DIP ratios in the central North Sea (1:1) were comparably N-limited as the mineral medium supplied to the N-limited chemostat experiment (0.5:1; Chapter 2 & 3). Thus, patterns of amino acid, carbohydrate and fatty acid synthesis in the North Sea would be expected to follow the same gradient of N:P ratios as in the experiments in Chapter 4. Indeed, Grosse et al. (2017) found amino acid suppression under N-limiting conditions in the offshore waters of the North Sea and during the late summer season. Amino acid production was higher in P-limited phytoplankton nearshore, in agreement with their higher cellular N:P ratio. These results indicate that nutrient limitation not only impacts growth rates and biochemical composition of phytoplankton in experimental conditions but has a similar effect in natural systems.

Have nutrient-load reductions changed the North Sea food web?

P-deficient phytoplankton are known to be of less nutritional quality for grazers and higher trophic levels (Sterner and Elser, 2002; Plath and Boersma,

2001; Suzuki-Ohno et al., 2012; Burian et al., 2018). Traditionally, P limitation has been associated more with freshwater than with marine ecosystems, and freshwater cladocerans tend to have higher P requirements than marine copepods (Fig. 1.4; see also e.g. Sommer and Sommer, 2006; Branco et al., 2018). Therefore, P limitation of zooplankton has received much more attention in freshwater than in marine studies (e.g., Urabe and Sterner, 1996; DeMott et al., 1998; Elser et al., 2001). However, experiments show that P deficiency also affects marine grazers. For instance, P-limited phytoplankton fed to marine copepods caused a marked decline in zooplankton growth rate (Malzahn and Boersma, 2012). Subsequent experiments revealed that the resultant P deficiency of these copepods, in turn, had detrimental effects on larval growth of economically valuable species such as herring (Malzahn et al., 2007) and European lobster (Schoo et al., 2012). These experiments demonstrate that, at least in principle, P deficiency of marine phytoplankton can propagate to higher trophic levels in the food web.

Field studies show that reduced P loading of the Wadden Sea since the mid-1980s was accompanied by changes in the species composition of the phytoplankton community, of the macrozoobenthic community feeding on the phytoplankton, and of the bird community feeding on the macrozoobenthos (Philippart et al., 2007). A recent study by Capuzzo et al. (2018) revealed that, in the North Sea, there has been a significant decline in primary production, abundance of small copepods, and fish recruitment averaged over seven commercially important fish stocks over the period 1988-2013. Capuzzo and colleagues found that the decline in primary production was concentrated in what they describe as the transitional east and transitional west regions of the North Sea (Fig. 7.4). They observed that the abundance of small copepods and fish recruitment were both significantly correlated with primary production, and the decline of the small copepods seemed to also be concentrated in the transitional east. These observations point at bottom-up control of small zooplankton and fish recruitment by the primary producers. However, large copepods did not show a consistent increase or decline over the investigated period. Støttrup et al. (2017) describe how large diatom blooms facilitate growth of the benthic fauna, which in turn feed demersal fish populations. They report shifts in the geographical distribution of juvenile plaice (*Pleuronectes platessa*), one of the economically most important demersal fish species, in the central and eastern North Sea since

the beginning of the 1990s, and suggest declines in overall nutrient loads may play a role (Støttrup et al., 2017).

Correlations do not provide evidence of cause-effect relationships, as acknowledged by these field studies. The North Sea has witnessed many other changes over the past decades, including changes in fisheries, global warming and ocean acidification, the placement of new wind farms and intensification of shipping activities. It is noteworthy, however, that the eastern region of the North Sea appears to show the strongest decline in primary production and coincides with the area that receives most of its nutrient inputs from the river Rhine (Capuzzo et al., 2018). These field observations are therefore consistent with the hypothesis that nutrient load reductions have affected the North Sea food web. Based on the available data, it is likely that if the current reduction of riverine nutrient loads continues, cascading effects on the productivity of fisheries will become apparent.

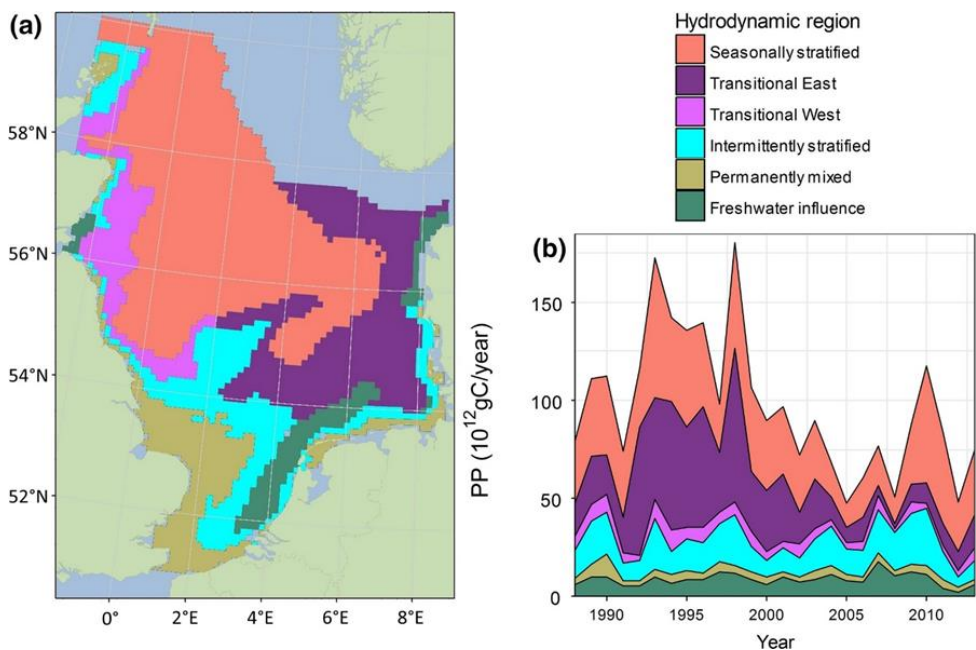


Figure 7.4 (a) Map of the North Sea showing the six hydrodynamic regions examined by Capuzzo et al. (2018). (b) Changes in total annual primary production, PP (10^{12} gC/year), in each hydrodynamic region. Reproduced from Capuzzo et al. (2018).

Recommendations for the future

Nutrient management:

While some may suggest that our results (Chapter 2) and the findings of later studies (Støttrup et al., 2017; Capuzzo et al., 2018) are arguments to avoid nutrient reduction efforts, I would strongly disagree. A return to eutrophic conditions will bring back the risk of hypoxia and nuisance algal blooms. However, the success of de-eutrophication efforts lies not only in the absolute reduction of nutrients, but in a stoichiometrically balanced nutrient reduction plan. This suggestion, of course, is based on the underlying assumption that the stoichiometry of nutrients is just as critical to consider as the total amount when battling eutrophication. Although this debate has been ongoing among scientists and water managers for some time (Philippart et al., 2007; Schindler and Hecky, 2009; Paerl et al., 2014), our results clearly point at the need to consider nutrient stoichiometry in the overall assessment of water quality and marine ecosystem functioning. Overall, the management of anthropogenically derived eutrophication is critical to promote healthy, productive coastal zones and reductions of nutrient loading are a key step in this process. The reductions will have a greater chance of success, and avoid the ecological issues identified in this thesis, if done so with N and P ratios kept in a biologically relevant stoichiometric balance.

Monitoring:

A clear conclusion from this thesis is the need to improve and continue monitoring in the North Sea. Monitoring should not be limited to inorganic nutrients and chlorophyll *a* alone, but should include the underwater light spectrum, phytoplankton community composition, seston stoichiometry and biomolecule composition of phytoplankton. This thesis has demonstrated that understanding the impact of de-eutrophication on the phytoplankton community requires analysis from all of these perspectives. Furthermore, as climate change and in particular warming and acidification increase, additional impacts are expected for the North Sea. Increased monitoring, particularly that which incorporates *in situ* flow-through nutrient and phytoplankton identification have proven invaluable to better understand patterns of primary production in these systems (Blauw et al., 2012; Aardema et al., in review).

Similar monitoring should be extended to the zooplankton community, to enable predictions of possible effects on higher trophic levels. Investigations

should include links to not only total primary production measurements, but also with respect to the community and biochemical composition of the phytoplankton. The latter having implications for the nutritional quality of phytoplankton for higher trophic levels and ultimately impacting recruitment of economically important species.

Future research:

One of the key questions emerging from our study is to what extent the cascading effects of reduced nutrient loading on higher trophic levels (e.g., zooplankton, fish) are driven by a reduction of the total primary production per se and to what extent they are driven by shifts in the species composition and nutritional quality of the primary producers. Controlled experiments at the ecosystem level, for example in large laboratory mesocosms or field enclosures including zooplankton and fish populations, exposed to different nutrient levels and nutrient ratios may shed more light on this important question.

Finally, models of nutrient budgets should be extended to understand the fate of riverine nutrients in the greater North Sea and to include changes in the nutritional quality of primary producers and their potential impacts at higher trophic levels. Ideally, these models will help to calculate and predict impacts of future riverine nutrient input scenarios. These calculations can guide management towards more stoichiometrically balanced nutrient reductions, not only in the North Sea system but in any coastal region where such efforts are being attempted.