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A sea of change

Impacts of reduced nitrogen and phosphorus loads on coastal phytoplankton communities

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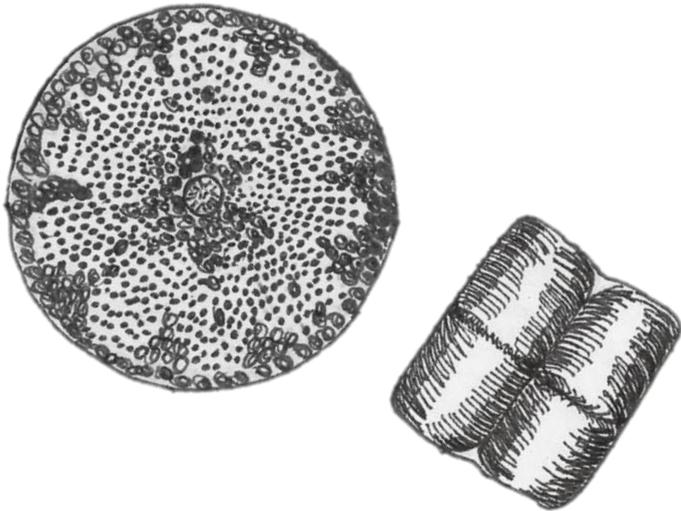
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Chapter 1

Introduction



General Introduction

Phytoplankton are the base of the food web in both freshwater and marine ecosystems and create nearly half of global net primary production (Field et al., 1998). Represented by thousands of species distributed over more than 10 phyla (Guiry, 2012), phytoplankton are diverse in structure and growth strategy. Diatoms utilize silica to build rigid and complex cell walls, dinoflagellates challenge the plankton meaning of passive “wanderer” by propelling themselves with whipping flagella, while cyanobacteria straddle the worlds of bacteria and photosynthetic organisms. All, however, possess a need for nitrogen and phosphorus, two major critical elements for phytoplankton growth. While there are notable exceptions, such as N₂-fixation or phagotrophy of organic matter, most phytoplankton acquire nitrogen and phosphorus through uptake of dissolved inorganic forms from the surrounding environment.

Given the universal need for nitrogen and phosphorus by photosynthetic phytoplankton, the paradoxical diversity of phytoplankton has long been queried by ecologists. Survival of the fittest should, in theory, lead to dominance of only a few species, i.e., the best competitors for the limiting resources. If there are only a handful of limiting resources (e.g., nitrogen, phosphorus, iron, light), why does the diversity of phytoplankton greatly exceed this number? Hutchinson (1961) famously described this mismatch between theory and reality as the “paradox of the plankton.” This thesis investigates the role that limiting resources have on the community composition of phytoplankton both in laboratory and natural systems through studies of competition, and by exploring impacts of changing nitrogen and phosphorus abundances.

Nutrients and the North Sea

Although nutrients such as nitrogen (N) and phosphorus (P) are required for growth, they can also be overly abundant leading to eutrophic conditions. Eutrophication is linked to a suite of negative environmental impacts including increases in toxic algal blooms (Heisler et al., 2008; O’Neil et al., 2012), depletion of oxygen (i.e., hypoxia; Westernhagen and Dethlefsen, 1983; Diaz and Rosenberg, 2008; Breitburg et al., 2018) and light limitation to benthic zones (Bricker et al., 2008; Hautier et al., 2009). The global increase in eutrophication is due to anthropogenic factors, especially fertilization for agriculture and the waste water of urban regions (Vitousek et al., 1997; Glibert et al., 2005; Rabalais

et al., 2009).

The North Sea is a primary example of a coastal region impacted by anthropogenically caused eutrophication. The overabundance of nutrients which entered the North Sea came predominantly from major European rivers such as the Scheldt, Maas, Rhine, Weser and Elbe. Residual currents transport fluvial-derived nutrients along the length of the Dutch coastline (Fig. 1.1).

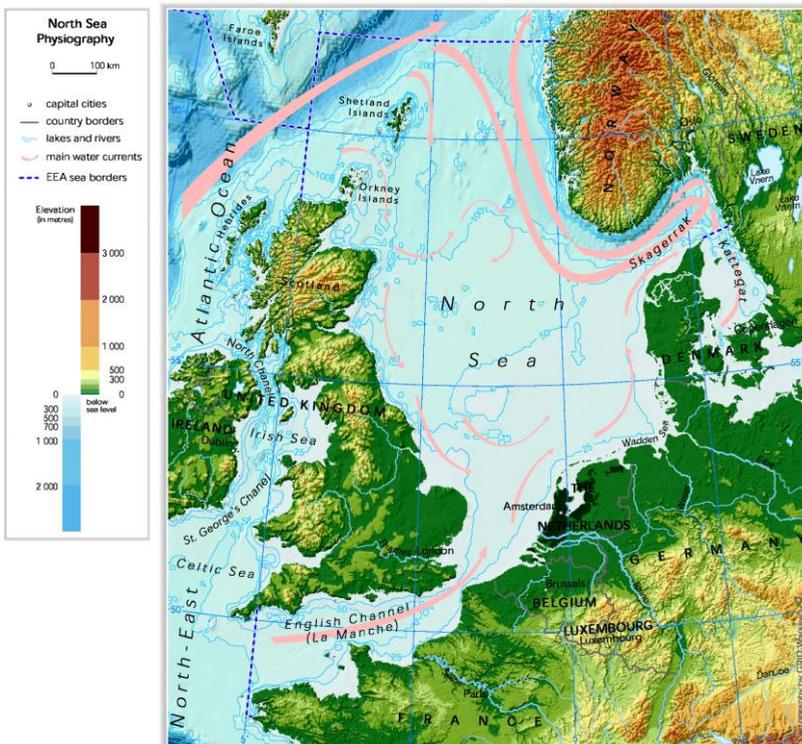


Figure 1.1 Map of North Sea with main and residual currents (pink arrows) and major rivers (from <https://www.eea.europa.eu/data-and-maps/figures/north-sea-physiography-depth-distribution-and-main-currents>).

Substantial increases of riverine P and N from the 1960's to mid-1980's (Pätsch and Radach, 1997) led to increases in primary production (Cadée and Hegeman, 2002), changes in the phytoplankton community composition (Philippart et al., 2000), hypoxia in the German Bight (Westernhagen and Dethlefsen, 1983) and an increase in the occurrence of *Phaeocystis* spp. blooms (Cadée and Hegeman, 1986; Lancelot et al., 1987). The nuisance blooms of *Phaeocystis* spp. led to large foam scums covering beaches along the Belgian, Dutch, German and Danish coast and impacted beach users (Fig. 1.2).



Figure 1.2 Foam from a bloom of *Phaeocystis* spp. on the beaches of the North Sea in the Netherlands (Reproduced from Howarth et al., 2011).

As a response to the eutrophication issue, a coalition of countries bordering the North Sea convened to develop solutions. In 1986, the OSPAR convention (Convention for the Protection of the Marine Environment of the North East Atlantic) established regulatory goals to reduce the nitrogen and phosphorus loads of the major rivers entering the North Sea to half of 1985 levels. This proved to be a mixed success. By 2002, P loads were reduced by 50-70% while N loads were down only 20-30%, thus increasing the N:P ratio of nutrients entering the North Sea (Lenhart et al., 2010; Passy et al., 2013). Traditionally, primary production in marine systems is considered limited by N (Ryther and Dunstan, 1971; Blomqvist et al., 2004; Howarth and Marino, 2006) while terrestrial waters are typically P-limited (Schindler, 1977; Hecky and Kilham, 1988; Schindler et al., 2008). However, as anthropogenic processes continue to alter the nutrients in these waters, historical limitation patterns are being altered (Elser et al., 2007; Abell et al., 2010; Paerl et al., 2014). The North Sea is not the only region experiencing elevated N:P ratios; particularly as globally the use of N-rich fertilization is increasing (Turner et al., 2003; Glibert et al., 2014) while efforts to remove P from domestic and industrial wastewater have improved (Grizzetti et al., 2012). Riverine influenced coastal zones such as the Gulf of Mexico (Sylvan et al., 2006), the Baltic and Mediterranean seas (Granéli et al., 1990; Krom et al., 2004), and the Pearl River estuary (Xu et al., 2008) have all shown a transition towards P limitation during some part of the year. How shifting N:P ratios toward P-limiting conditions will impact the phytoplankton

community composition, and nutritional quality for the rest of the food web, is of primary concern to those utilizing these systems.

Harmful Algal Blooms

The ability to predict phytoplankton community dynamics is valuable to understand, and hopefully avoid, occurrences of harmful algal blooms. Harmful algal blooms vary in environmental impact. Nuisance blooms affect recreational and economical use of beaches or waterways, such as the *Phaeocystis* blooms mentioned previously. Toxic algal species have more severe consequences. Blooms of cyanobacterial species producing microcystin, a potent hepatatoxin (MacKintosh et al., 1990), have increased globally with increased eutrophication and climate change (Huisman et al., 2005; Paerl and Huisman, 2009; Carmichael and Boyer, 2016). Several species of dinoflagellates and some diatom species produce toxins affecting human health through shellfish poisoning (Table 1.1).

Filter feeding shellfish, many of which are consumed by humans, can amplify toxin effects as they concentrate toxins within their tissue after ingestion of toxic phytoplankton cells (Anderson, 1989; Van Dolah, 2000; James et al., 2010; Fig 1.3). Marine mammals have also been affected by toxic phytoplankton with mass strandings being attributed to phytoplankton toxin poisoning (Scholin et al., 2000).

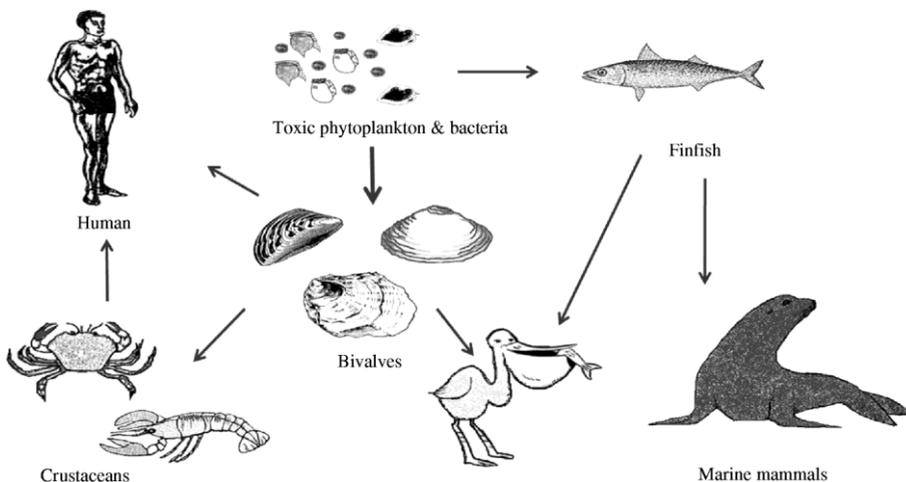


Figure 1.3 Diagram illustrating the interrelationships between harmful algae and shellfish, finfish, birds and mammals (Reproduced from James et al., 2010).

Table 1.1 Toxic syndromes caused by human consumption of marine seafood containing accumulated algal toxins (Reproduced from James et al., 2010).

Toxic syndrome	Toxins	Affected seafood	Toxic algae
Paralytic shellfish poisoning (PSP)	Saxitoxin (STX), neosaxitoxin (NEO), gonyautoxin (GTX) and 18 analogues	Bivalve shellfish, crustaceans	<i>Alexandrium</i> spp., <i>Gymnodinium</i> spp.
Diarrhetic shellfish poisoning (DSP)	Okadaic acid (OA), dinophysistoxins (DTXs), pectenotoxins (PTXs)	Bivalve shellfish	<i>Dinophysis</i> spp., <i>Prorocentrum</i> spp.
Neurotoxic shellfish poisoning (NSP)	Brevetoxins (PbTx)	Bivalve shellfish	<i>Karenia brevis</i>
Amnesic shellfish poisoning (ASP)	Domoic acid (DA) and analogues	Bivalve shellfish, finfish	<i>Pseudonitzschia</i> spp.
Azaspiracid poisoning (AZP)	Azaspiracids (AZAs) and analogues	Bivalve shellfish	<i>Protoperidinium crassipes</i> , <i>Azadinium spinosum</i>

Toxic blooms have been linked to anthropogenic eutrophication, since N and P enrichment stimulates algal growth (Hallegraeff, 1993; Anderson et al., 2002; Heisler et al., 2008; O’Neil et al., 2012). Ultimately, reducing eutrophication would help to stem the rise in the occurrence and intensity of harmful algal blooms, but requires significant time and financial investment and is challenging to implement. Thus, water managers often use response rather than prevention tactics for chronic blooms. One such technique which has been utilized to suppress toxin-producing cyanobacterial blooms is dosing the bloom waters with hydrogen peroxide (Matthijs et al., 2012; Barrington et al., 2013). Other techniques include artificial mixing (Visser et al., 1996), flushing (Mitrovic et al., 2010), or even flocculation with clay (Sengco and Anderson, 2004). However, with all of these methods, the long-term eradication of toxic blooms is not consistently obtained.

Stoichiometry of Phytoplankton

The N and P contents of phytoplankton cells are quite plastic, and strongly affected by changes in N and P availability in their environment. The ratio of carbon (C) to N to P within phytoplankton can dramatically influence their

nutritional value to zooplankton grazers and higher trophic levels in the food web. Redfield (1934) proposed an ideal ratio of C:N:P atoms of 106:16:1. In reality, the range of ideal elemental stoichiometry is a bit broader and dependent on the species. Species with low N requirements can have optimal N:P ratios of 10:1 while those with low P requirements can be 45:1 (Geider and La Roche, 2002; Klausmeier et al., 2004). While the elemental stoichiometry of phytoplankton may be relatively broad, for example the C:P ratio of green algae can range from 100 to 2000, the nutritional needs of most animals (including zooplankton and fish) are much more constrained due to a requirement to sustain homeostasis (Fig. 1.4; Urabe and Watanabe, 1992; Sterner, 1997). Under P limited conditions, the C:P ratio of phytoplankton increases, causing problems in the ecological stoichiometry (Sterner and Elser, 2002) for higher organisms. High C:P ratios are further compounded by rising inorganic C in aquatic systems (Verspagen et al., 2014).

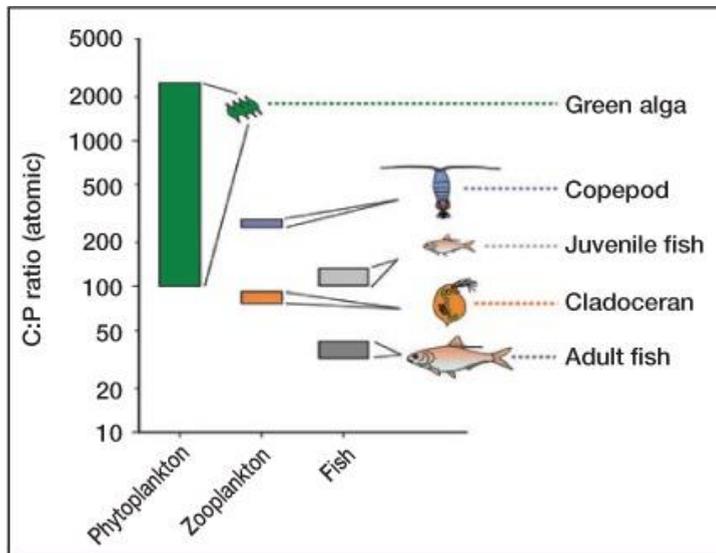


Figure 1.4 Phytoplankton display a wide range of relatively high C:P ratios. Zooplankton and fish have rather narrow stoichiometric ranges (Reproduced from Van de Waal et al., 2010a).

Changes in N and P loads of coastal waters have impacts beyond the overall C:N:P ratio of phytoplankton cells and influence the biomolecule composition of phytoplankton as well. Converting glucose, a product of photosynthesis, to other cellular biomolecules is dependent on nutrient availability (Arrigo et al., 1999). For example, nitrogen is required for amino

acid synthesis and both N and P are required for RNA and DNA synthesis. Nutrient-limited North Sea phytoplankton were shown to adjust their biosynthesis of these critical biomolecules within 24 hours of relief from nutrient limitation (Grosse et al., 2017). Understanding the variation of synthesis rates and composition of critical biomolecules under sustained nutrient stress can help to predict growth patterns of phytoplankton and their nutritional value for zooplankton under changing nutrient conditions.

Resource Limitation and Community Structure

In an effort to address the “paradox of the plankton,” ecologists have developed theories which attempt to encapsulate and predict the biodiversity of primary producers. Theories predominantly diverge along the key concept of the role of competition. Species either 1) display niche-based competition which results in deterministic changes in community structure, or 2) are entirely neutral and equal in their resource use which results in random species fluctuations within the community. As is common, natural patterns of species composition seem to fall somewhere in between these opposing concepts.

Niche-based competition was utilized to develop the resource ratio hypothesis of Tilman (1985). The resource ratio hypothesis posits that changes in the proportions of two critical resources, such as N and P, can lead to predictable species succession when the competitive abilities of the species for these resources are known. A species’ minimum requirement for a specific nutrient is characterized by its R^* for that nutrient, which is defined as the environmental concentration of the nutrient at which this species is at equilibrium; it is neither increasing nor decreasing in abundance. Competition theory predicts that the species with the lowest R^* for a nutrient will competitively displace all other species if this nutrient is the limiting factor in the environment (Stewart and Levin, 1973; Armstrong and McGehee, 1980). However, theory also predicts scenarios for the coexistence of multiple species, thus providing a possible solution to the “paradox of the plankton.” One explanation for coexistence is if the two species (A and B) are competing for two nutrients (R_1 and R_2), but species A is the better competitor for R_1 and species B is the better competitor for R_2 (Tilman 1982; 1985). This “trade-off” of competitive abilities allows both species to coexist at intermediate ratios of the two nutrients (Fig. 1.5). Hence, nutrient ratios (such as the N:P ratios in coastal

waters) are predicted to be a key determinant of the phytoplankton community composition.

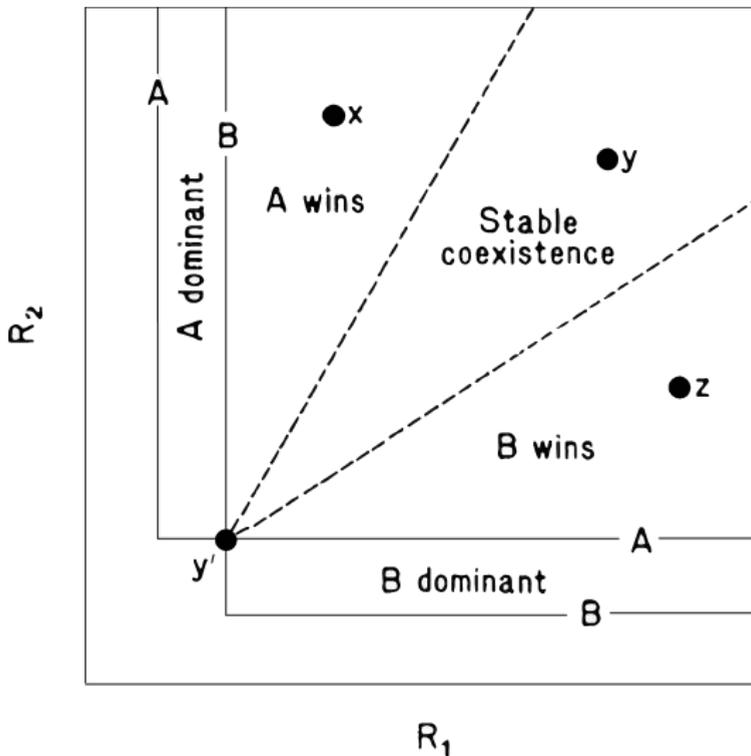


Figure 1.5 Solid lines with right-angle corners are the zero-net-growth isoclines (ZNGIs) for species A and B. The points x, y, and z represent different supply points of nutrients (R_1 and R_2). The outcome of competition is dependent on the ratio of R_1 : R_2 , where species A wins (supply point x), both species coexist (supply point y) or species B wins (supply point z). Species coexistence can occur only if there is an interspecific trade-off in nutrient requirements (i.e. if the two ZNGIs intersect). Intersection of the two ZNGIs is indicated by the two-species equilibrium point y' (Reproduced from Tilman 1985).

Another critical resource utilized by all photosynthetic organisms is light. Due to the unidirectional nature of the light flux, modeling competition for light requires a different approach than modeling competition for nutrients (Huisman and Weissing, 1994; Huisman and Weissing, 1995; Huisman et al., 1999b). Light becomes limiting in dense phytoplankton populations where shading from co-occurring cells block the light reaching other phytoplankton cells, thus limiting their growth. This can drive competition in a similar manner to nutrient competition wherein the species with the lowest light requirement can competitively exclude other species. In order to experimentally include light as

a limiting resource, specialized growth chambers called chemostats were developed which allow for the regulation of the unidirectional light source (Huisman et al., 1999a). Chemostats of the same design were used for the experiments conducted in this thesis (Fig. 1.6).

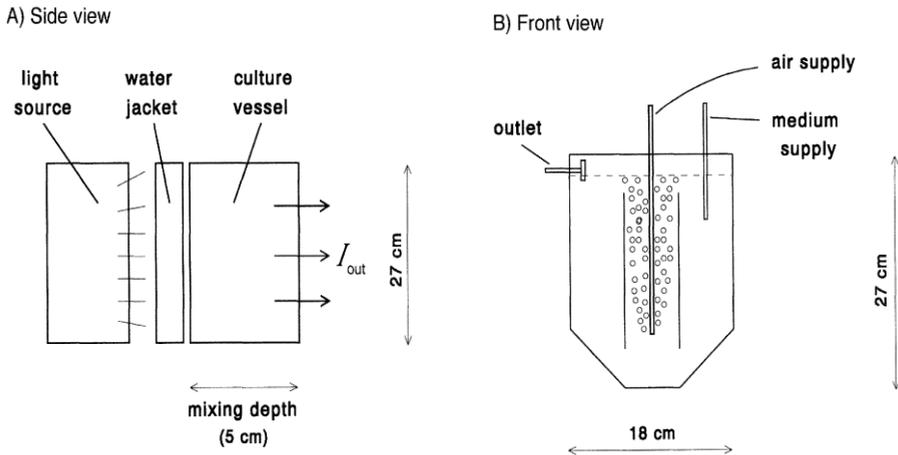


Figure 1.6 Schematic diagram of the chemostat used in this study. (A) Side-view with light source, water jacket (for temperature regulation), and culture vessel. (B) Front view of the culture vessel (Reproduced from Huisman et al., 1999a).

Including light as a limiting resource was an important step in developing competition theory. Because light limitation is linked to nutrient load, wherein high concentrations of nutrients induce high biomass and self-shading, it is a logical step to combine nutrients with light. Naturally this complicates the model analysis, but by merging the models of the resource ratio theory of two nutrients with growth under unidirectional light, Brauer et al. (2012) developed the ‘nutrient-load hypothesis’ which allows for light limitation at high nutrient loads (Fig. 1.7). By incorporating the growth characteristics of nine species under these three resources, the model of Brauer et al. (2012) predicted a high occurrence of stable coexistence of two or three species at moderate resource levels where co-limitation arises. While both the resource ratio hypothesis and nutrient-load hypothesis are niche-based models, the latter predicts that not only nutrient ratios but also the absolute loads of nutrients have a major impact on community composition.

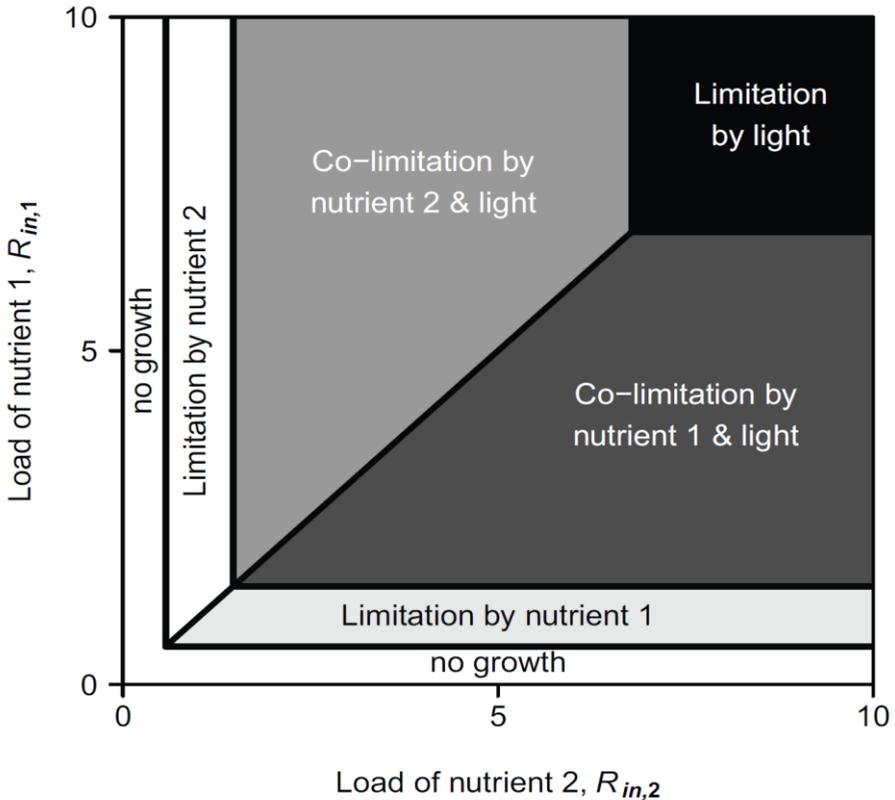


Figure 1.7 Resource limitation of a single species. In the “no growth” regions, nutrient availability is too low to support a phytoplankton population. At low nutrient loads, the growth rate is limited by either nutrient 1 or nutrient 2, depending on the ratio of the nutrient loads. At intermediate nutrient loads, growth is co-limited either by nutrient 1 and light or by nutrient 2 and light, again depending on the ratio of the nutrient loads. At high nutrient loads, growth is limited by light irrespective of the nutrient ratios (Reproduced from Brauer et al., 2012).

Further extensions of these niche-based competition models have incorporated different colors of light into resource competition (Stomp et al., 2004; 2007b). Phytoplankton species differ widely in pigment composition, by which they absorb different parts of the underwater light spectrum. Mathematical models, lab experiments and field data have shown that niche differentiation in the light spectrum may create ample opportunities for species coexistence, as illustrated by the classic experiments of Stomp et al. (2004) in which green and red picocyanobacteria coexisted in white light.

An alternative explanation for species coexistence is the neutral theory of biodiversity (Bell, 2000; Hubbell, 2001). Neutral theory proposes that all species

within the same functional group are equivalent in their competitive abilities for common resources (Hubbell, 2005; 2006). Accordingly, population abundances of the species are a result of stochastic changes in demographic properties (i.e. birth, death and migration rates) and predictions of community structure are based on probability rather than ability (Hubbell, 2001; Etienne and Olff, 2004; Fig. 1.8). As a result, the population abundances produce random ups and downs, called ecological drift (Volkov et al., 2003; Hubbell, 2005; 2006). Studies which apply the neutral theory of biodiversity to community data have found reasonable fits to observed biodiversity patterns (Bell, 2000; Alonso and McKane, 2004; Woodcock et al., 2007; De Aguiar et al., 2009), but proving its operation experimentally has been challenging (Wootton, 2005; Alonso et al., 2006).

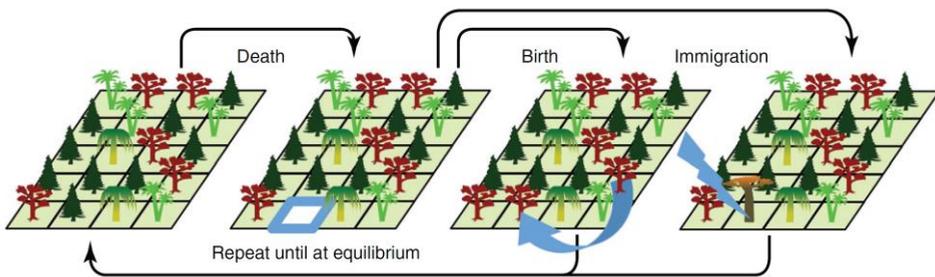


Figure 1.8 The mechanics of neutral theory in the local community. In this example, the total community population size is 20 individuals, so the probability of the indicated individual dying is $\frac{1}{20}$, the probability of birth happening within the local community, as indicated in the third panel, is $(1 - m)\frac{1}{20}$ for each living individual and the probability of immigration is m (Reproduced from Rosindell et al., 2011).

Neutral theory has recently been applied to phytoplankton communities to explain seemingly random variation in the relative abundances of species (Chust et al., 2013; Mutshinda et al., 2016). Some studies have applied neutral theory to so-called “clumpy” distributions of plankton based on traits such as cell size (Vergnon et al., 2009; Segura et al., 2013). This is an interesting adaptation of the neutral theory, suggesting within each ‘clump’ the species have similar traits and are functionally neutral (Segura et al., 2013; Sakavara et al., 2018). While this may explain the relative distribution of species to a particular aggregation, niche-based theory may still be applied to predict the abundances of the clumps themselves. Testing these ideas experimentally is required to further define

where and when each respective theory may be operating to drive diversity of phytoplankton, or if more complex dimensions of competition are at play.

Thesis Outline

This thesis has three related aims, which collectively serve to better our understanding of the influence of changing N, P and light conditions on the productivity and phytoplankton species composition of coastal waters. I have used the North Sea as the primary ecosystem for this research, given its major changes in nutrient loads over the past several decades.

- The first aim is to determine if multi-decadal de-eutrophication efforts have changed the nutrient stoichiometry of the North Sea and therefore the nutrients limiting the primary production of these waters.
- The second aim is to investigate how observed shifts in resource limitation and nutrient stoichiometry may have influenced the species and biochemical composition of phytoplankton communities in the North Sea.
- The third aim is to use the North Sea phytoplankton as a model system to contribute to an improved conceptual understanding of how competition between species affects the species composition of natural communities.

In **Chapter 2** spatial and temporal variation in nutrient limitation of the North Sea is investigated. Multiple research cruises over a 450 km transect revealed a gradient from P and silicate (Si) limitation nearshore to N limitation in central North Sea waters. Additionally, nutrient enrichment bioassays performed on-board linked *in situ* N:P ratios of dissolved inorganic nutrients with realized nutrient limitation of phytoplankton growth. In-depth analysis of the growth responses of different functional groups to nutrient enrichment provides insights into which types of phytoplankton are expected to thrive under the range of nutrient limitations. The phytoplankton community composition is discussed with respect to potentially harmful species and nutritional deficiency trends under increasing N:P ratios.

In **Chapter 3** controlled chemostat experiments were utilized to test predictions of resource competition theory using phytoplankton communities

collected from the North Sea. As demonstrated in Chapter 2, the North Sea system experiences a range of N:P ratios as well as varying nutrient loads, thus raising the question how these changes in N and P availability may have affected phytoplankton community composition. Seven chemostats, each with a unique combination of N and P loads, were used to experimentally compare predictions of the resource-ratio hypothesis (Tilman, 1985) and nutrient-load hypothesis (Brauer et al., 2012).

In **Chapter 4** the phytoplankton communities grown in the seven experiments of Chapter 3 were further analyzed to determine how different N and P loads affect the biomolecule composition of algal biomass and corresponding biosynthesis rates of structural and storage biomolecules under controlled conditions.

Chapter 5 presents further chemostat experiments designed to investigate whether neutral theory may (at least partially) explain the species diversity in phytoplankton communities. First, phytoplankton communities sampled from the North Sea were grown under N and P limiting conditions. The two most abundant species in these multispecies experiments were isolated to measure their competitive traits (including their R^* values for N and P). Subsequently, the two species were put together in pairwise competition experiments starting from different initial abundances to investigate whether their co-occurrence reflects the randomness predicted by neutral theory (Bell, 2000; Hubbell, 2001) or the stable coexistence predicted by resource competition models (Tilman, 1985; Stomp et al., 2004; Brauer et al., 2012).

Chapter 6 discusses the treatment of an unprecedented and highly toxic *Alexandrium ostenfeldii* bloom in Zeeland, The Netherlands, using hydrogen peroxide additions. While the treatment was successful and the threat to the local shellfish industry was mitigated, the occurrence of the bloom itself speaks to the potential of future HAB events in Dutch coastal and estuarine waters. This chapter serves as a reminder of the critical importance of well-considered nutrient management strategies.

Finally, in **Chapter 7** the results of all chapters are synthesized and recommendations are presented for future nutrient management strategies as well as thoughts on further development of competition theory.