Microalgal primary producers and their limiting resources
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Citation for published version (APA):
Ly, J. (2013). Microalgal primary producers and their limiting resources

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CHAPTER 7

General discussion
In this thesis, I have demonstrated that P is the limiting resource for phytoplankton growth during the spring bloom in the western Dutch Wadden Sea. By using the PLFA biomarker, it was concluded that P is a limiting resource that affects phytoplankton biomass, community composition and C-uptake. Additionally, influence of freshwater discharges or nutrient fluxes from the sediment were also shown to be important factors that modified P availability in the Marsdiep basin. In this last chapter, I will discuss the results obtained from previous chapters and will integrate it into a broader context of the Wadden Sea ecology according to the following three major questions:

- Is phosphorus the main limiting resource for phytoplankton growth?
- How will a change in phytoplankton composition affect the transfer of organic matter produced by primary producers to primary consumers?
- What are the implications of this study for the interpretation of the data for monitoring program?

Phosphorus is the main limiting resource for phytoplankton growth

Availability of nutrients and light are crucial factors for the wax and wane of phytoplankton (Sverdrup, 1953; Winder and Cloern, 2010). Various studies and long-term data series of the ecology of the Wadden Sea considered the factors that could be responsible for changes in primary production (Philippart et al., 2007). Cloern et al. (1999) developed an index for coastal ecosystem sensitivity in order to diagnose the effect of nutrient enrichment, based on a phytoplankton growth model. When Cloern’s index was applied to the Marsdiep basin, it suggested that the phytoplankton community in the western Dutch Wadden Sea was limited both by light and P (Colijn and Cadée, 2003; Loebl et al., 2009). The nutrient enrichment experiments carried out in this study revealed that P (and Si for diatoms) rather than light was the limiting resource for phytoplankton growth during the spring bloom in the Marsdiep basin (chapters 3 and 4). Although the effect of light on the phytoplankton community was not experimentally studied in this thesis, from geographic information system and Secchi disk data it was concluded that it was unlikely that light was the limiting factor for phytoplankton growth in the Marsdiep basin during the period when this study was carried out (chapter 3). A depletion of Si in the water column during spring can shape the phytoplankton community and increase harmful algae blooms (Hecky and Kilham, 1988; Müller et al., 2013). However, the Marsdiep basin receives episodic freshwater discharges and also sedimentary mineralization processes occurring on tidal flats can alleviate Si or P limitation during the spring bloom (Van Bennekom et al., 1974; De Jonge, 1990; Van Raaphorst and De Jonge, 2004; chapters 2 and 4).

When soluble reactive phosphorus (SRP) concentrations are low (<0.03 μmol L⁻¹), the phytoplankton community is considered to be P-limited, while when the SRP concentrations are
intermediate in late spring (between 0.11 and 0.17 µmol L$^{-1}$), the phytoplankton is in a transition to becoming P-replete (chapters 2, 3 and 4). When inorganic P is depleted, other potential sources of dissolved P such as DOP may become available that could sustain phytoplankton growth (Karl, 2007; Duhamel et al., 2010). The concentration of DOP in the water column is often higher than that of SRP (Kolowith et al., 2001; Duhamel et al., 2010) (see DOP paragraph).

As reported in chapter 4, not all phytoplankton taxonomic groups were limited by P during the spring bloom. Shifts to small phytoplankton types and low diversity are expected under limiting nutrient conditions (Interlandi and Kilham, 2001). The results presented in this thesis confirm that Bacillariophyceae are poor competitors for phosphate when compared to flagellates (Egge, 1998; Irwin et al., 2006; Finkel et al., 2010). The surface/volume ratio of the cell affects the nutrient uptake. Large cells experience a disadvantage because their surface is small compared to their cell volume and therefore the rate with which they can take up nutrients is much slower. P limitation will therefore result in the disappearance of Bacillariophyceae from the phytoplankton community and cause a shift to non-diatom primary producers with smaller cell sizes. Small cell size species are associated with small grazers like dinoflagellates and ciliates who will increase C cycling (Barber and Hiscock, 2006).

**How will a change in phytoplankton composition affect the transfer of organic matter produced by primary producers to primary consumers?**

In this thesis, the phytoplankton community is described from a bottom up viewpoint, i.e. nutrients and light control phytoplankton growth. A shift in the phytoplankton species composition can shape higher trophic levels, because for instance the PLFA composition of the phytoplankton may influence the diet values (food quality) in aquatic food webs (Brett and Muller-Navarra, 1997; Kattner et al., 2007). The available food source is a critical component that determines growth and survival of marine herbivores as well as of higher trophic levels (Brett and Muller-Navarra, 1997). Organisms of the higher trophic levels lack certain enzymes ($\Delta$12 and $\Delta$15 desaturases) that are required for fatty acid synthesis, and therefore they depend on essential fatty acids that are produced by the phytoplankton (Brett et al., 2009). A decrease of polyunsaturated fatty acids (PUFA) (18:3$\omega$3, 20:5$\omega$3 and 22:6$\omega$3) that are produced abundantly by Bacillariophyceae and flagellates can decrease the growth rate of consumers considerably (Boersma, 2000; Von Elert, 2002). During the spring bloom, phytoplankton is usually dominated by Bacillariophyceae and the flagellate Phaeocystis sp. (chapter 3). With respect to the PLFA composition, Bacillariophyceae are considered to be a better quality food with higher amount of PUFA than Phaeocystis sp. (Turner et al., 2002; Kelly and Scheibling, 2012). Under P-limiting conditions, Phaeocystis sp. survives better because they form colonies that act as P reservoirs (Schoemann et al. 2005). Therefore, an increase of the intensity of Phaeocystis sp. will affect the transfer of energy to consumers (Cadée and Hegeman, 2002; Hamm and Rousseau, 2003; Schoemann et al., 2005). For example, large Phaeocystis sp. colonies (>10 µm) could not be
grazed by larvae of the bivalve *Crassostrea gigas* (Pacific oyster) in the Wadden Sea (V. Lemphful, personal communication).

Although not treated as it was outside the scope of this thesis, it is not only via the PLFA composition that phytoplankton or MPB can influence their grazers. As grazers seem to have less flexibility in their nutrient stoichiometry (or a stronger control on element stoichiometry) than the primary producers, a food source with a too low P-content can cause P-limited growth of grazers (Lukas et al., 2012, Elser et al., 2000; Sterner et al., 1998). As the P-requirement of herbivores also varies (Schulz et al., 1999), a food source with a low P-content (i.e. P-limited phytoplankton) can in principle also lead to species succession in grazers. In addition there is direct evidence that zooplankton can be directly P-limited as well. The freshwater crustacean *Daphnia magna* grew faster on a diet of heat stopped P-limited *Scenedesmus acutus* growth with added inorganic P than on a diet of P-limited *Scenedesmus acutus* alone (Urabe et al., 1997). Organisms differ in the C:N:P ratio, thus in their requirements for these elements. The nutrient stoichiometry between these elements influences several ecological processes such as competitive interaction, nutrient limitation, population stability, trophic interaction (Li et al., 2012 and references therein). There seems to be a relation between this nutrient stoichiometry and growth rate. The “growth rate hypothesis” (GRH) suggests that fast-growing organisms have low C:P and N:P ratios. Most of this research on ecological stoichiometry has been carried out in freshwater ecosystems, where P is often the limiting nutrient, with a few studies in the terrestrial environment (e.g. Li et al., 2012), confirming some of the concept of the GRH. The GRH might not be available when a co-limitation occurs in the herbivore (Lukas et al., 2011). More research in the marine field is warranted, but is not unreasonable to suggest that the growth rate hypothesis will be also valid in the marine environment.

**What are the implications of this study for the interpretation of the data for long term monitoring program?**

*Limitation of phytoplankton biomass measurement with Chla*

Long-term monitoring studies on phytoplankton aim to understand and assess the disturbances that have an ecological impact on the system studied. In many long term monitoring programs, Chla is used as a rapid measurement to detect inter-annual changes in phytoplankton biomass. We showed that because of this “bulk” biomass measurement, changes in the composition of the phytoplankton community as the result of different nutrient regimes were difficult to uncover (chapter 3). In this thesis, PLFA was used as a chemotaxonomy biomarker to identify different groups of primary producers (chapters 2, 4, 5 and 6). Although phytoplankton that responded to P limitation was identified, they were restricted to higher taxonomic classes. The NIOZ sampling jetty monitoring program and monitoring programs in general would benefit when they monitor genes that code for enzymes that are induced under P limitation or even better to detect their
transcripts in order to show P limitation in certain key species of phytoplankton of the Wadden Sea.

**Characterization of the dissolved organic phosphorus (DOP) pool**

Several approaches have been used to measure P limitation in phytoplankton communities. The inorganic DIN:SRP molar ratio or SRP concentrations cannot entirely assess P limitation in phytoplankton and conclusions made from the measurements should be interpreted cautiously. In the first place, in a phytoplankton community, nutrient requirements and uptake rates vary among different taxonomic groups (Geider and Roche, 2002; chapter 4). Secondly, when SRP is scarce, DOP (estimated indirectly from the differences between total dissolved P and SRP) represents an alternative source of P for the phytoplankton (Labry et al., 2005; Duhamel et al., 2010). Under P stress, marine phytoplankton synthesizes alkaline phosphatase (AP) that is capable of hydrolyzing the phosphomonoester of the DOP to inorganic phosphate (Dyhrman and Ruttenberg, 2006; Nicholson et al., 2006; Duhamel et al., 2010). The results presented in chapter 4 demonstrate that, although DOP and SRP concentrations were high in fall (respectively > 0.40 µM for DOP and 0.20 µM for SRP), ~40% of the phytoplankton cells expressed AP. This suggests that not all DOP was available for phytoplankton growth. DOP may occur in a variety of size classes and the molecular structures may exhibit different bioavailabilities. Only phytoplankton species that can utilize a large spectrum of DOP compounds will be able to survive when P becomes limiting (Dyhrman et al., 2006; Duhamel et al., 2010). In particular, cyanobacteria are known to synthesize phosphonatase, an enzyme capable to hydrolyze refractory DOP with stable C-P bonds (Dyhrman et al., 2006). In most of the monitoring programs in coastal systems, more is known about DIP than about DOP. Unfortunately, DOP has also not been considered as a parameter for nutrient limitation in the Wadden Sea (Van Beusekom and De Jonge, 2012). Therefore, more effort is needed to characterize DOP compounds and determine its bioavailability in coastal systems.

**Lack of data on microphytobenthos (MPB)**

Comparison between different regions of the Wadden Sea showed differences in the annual rate of photosynthesis in different sediment types (Billerbeck et al., 2007). The measurements reported in the chapters 5 and 6 show that the rate of C-fixation was higher in the muddy sediment than in the sandy sediment. Comparison of the MPB community, seasonality patterns, and primary production in the fine sandy locations of the western Dutch Wadden Sea (chapter 5) to the MPB of the mudflats in the eastern Wadden Sea must be done with caution (De Jonge and Van Beusekom, 1992; De Jonge and Colijn, 1994). Despite that sandy sediment is less cohesive than muddy sediment, suspension of the MPB at the sandy sites of the Marsdiep tidal flats did not occur (chapter 5). The large and heavy sand grains do probably not go so easy in suspension.

Efflux of DIP, calculated from the pore water *in situ* profiles, showed a high release to the water column during spring and summer (L. Mulder, personal communication). The phytoplankton was shown to be P-limited (chapters 3 and 4), and a supply of bioavailable P from
the sediment pore water to the overlying water can alleviate P limitation for phytoplankton after the spring bloom. In the subtidal area of the Marsdiep basin, P fluxes fueled approximately 40% of the annual pelagic primary production (Leote et al., submitted; chapter 5). In order to perform photosynthesis at the interface sediment-water, MPB obtains nutrients from the overlying water and also from the sediment (Underwood and Kromkamp, 1999). Although the overlying water P concentrations may be limiting for benthic microalgae in spring, porewater P concentration appeared to be sufficient to support MPB growth. The assessment of primary production in the Wadden Sea should include the contribution of MPB because this can be considerable.