Resource limitation and the biochemical composition of marine phytoplankton

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Chapter 7

General Discussion

Coastal marine systems are usually characterized by high productivity both at the base of the food web (primary production) and at higher trophic levels. But long-term perturbations of nutrient deliveries from land have led to drastic changes in nutrient availabilities, in terms of overall concentrations as well as in terms of the relative ratios of DIN, P and Si. Marine systems that were once thought to be nitrogen limited (Hecky & Kilham 1988; Howarth & Marino 2006) are now becoming more and more affected by P-limitation (Loeb et al. 2009; Ly et al. 2014).

Effects of changing DIN:P ratios cause changes at the phytoplankton community level (Philippart et al. 2000; Cadée & Hegeman 2002) but also affect the cellular composition of phytoplankton (Klausmeier et al. 2004a). For instance, under N- and/or P-deficiency C-rich storage compounds, such as carbohydrates or triglycerides, are preferably accumulated by microalgae (Granum et al. 2002; Borsheim et al. 2005). Consequently, C:N and C:P ratios of phytoplankton increase, which lead to a decline in its nutritional value and thereby alters food web interactions (Sterner et al. 1993). Further shifts in cellular composition are also expected when nutrient N:P ratios change as some components like proteins only need N whereas DNA and RNA contain both N and P.

The aim of this thesis was to advance knowledge about adaptation of the cellular composition in coastal primary phytoplankton under different resource scarcities and to gain a better understanding of how higher levels within the food web may be dealing with changes in prey quality. As a result, the following four questions were targeted.

1. Do we have the methodology to assemble a carbon budget of major biomolecules in phytoplankton?
2. What are the effects of different nutrient limitations on biomolecule concentration and synthesis in phytoplankton?
3. How does that affect phytoplankton food quality?
4. In which ways and to what extent does zooplankton adapt to “bad” food i.e. when there is a mismatch between the composition of phytoplankton and the requirements of zooplankton?

**Tracing C-flow through biomolecule pools**

Uptake studies with $^{13}$C-enriched compounds are ideal to investigate in-situ processes (Boschker and Middelburg 2002). Bulk measurements of $^{13}$C uptake are a great tool to determine system productivity but they lack information about the intracellular fate of the fixed carbon. And although the tools to investigate major biomolecules have been used for years (e.g. Van Den Meersche et al. 2004; Veuger et al. 2005) available studies primarily focus on specific biomolecule (groups) and lack a simultaneous study of all major biomolecule groups.

In this thesis I show how combining established and novel methods can be used to identify the composition and biosynthesis of individual amino acid (AA), fatty acid (FA) and carbohydrate (CH) pools in marine phytoplankton communities. All major biomolecules can be examined in detail and provide an overview of the ability of phytoplankton to allocate carbon into different functional groups of biomolecules. Earlier studies investigating multiple biomolecule groups simultaneously displayed several disadvantages. In order to trace carbon uptake earlier studies utilized radioactive $^{14}$C carbon. This required following stricter regulations regarding safety and obtaining special working permissions. The approach used in these studies (Marañón et al. 1995; Suárez & Marañón 2003) was only capable of separating entire biomolecule groups but not individual compounds. Both CH and FA are found as part of structural and storage compounds and if no distinction can be made between structural and storage compounds than a lot of information is lost regarding physiological responses to nutrient availability. Therefore, $^{13}$C is the isotope of choice and the addition of a recently newly available method that measures DNA and RNA nucleotides (Moerdijk-Poortvliet et al. 2014) allow the assembly of a carbon budget with all major biomolecule groups found in algae.

The distribution of individual compounds in the total carbon pool reveals community specific differences in biomolecule composition and relate to the long-
term history of nutrient limitation experienced by the cell over several days (biomass turnover time 3 – 12 days). Overall, the contributions of different biomolecule classes found by using LC- and GC/C-IRMS methods agreed well with previous findings (Becker 1994; Lynn et al. 2000; Mock & Kroon 2002b). However, substantial amounts of total POC concentrations could not be identified (18 ± 17% to 44 ± 1%), suggesting other biomolecules, such as pigments, phytosterols, amino sugars or acidic sugars may also be important. The contribution of detrital C can be substantial too (Andersson & Rudehall 1993). In order to access the current state of a phytoplankton community it is better to look at carbon fixation patterns instead of POC composition.

Biosynthesis patterns reflected a community specific response to the imminent nutrient limitation, especially when combined with bioassays where different nutrients were added. Bulk carbon fixation rates and cumulative carbon fixation rates of AA, FA and CH were mostly similar and indicating no differences between the two approaches, suggesting that C-incorporation into these three biomolecule groups is dominant. When investigating biosynthesis patterns, the incubation periods can be kept short (≤ 24 h). Hence, this approach presents an alternative to classical long-term bioassays (Beardall et al. 2001). It can guarantee field relevant results because changes in community composition and activity will be negligible. Albeit the requirements for large water volumes to meet biomass requirements and the extend lab work necessary the combination of methods provides a highly detailed and so far unique view into the fate of carbon fixed through photosynthesis.

**Phytoplankton biomolecule synthesis**

The work in this thesis revealed that clear relationships between biomolecule concentration and synthesis exist for most biomolecule groups and that minimum concentrations of structural FA and especially AA are required to maintain growth (Chapter 3 & 5) showing that clear nutrient depended regulating mechanisms are in place.

Phytoplankton growth was severely limited by P at near-shore stations, followed by a transitional zone that displayed P-limitation in early spring and N-limitation in late spring and summer. Farther offshore, N-limitation was persistent demonstrating an offshore gradient from P to N-limitation. Persisting efforts to
decrease riverine P inputs will continue to increase the imbalance between DIN and P. Additionally, P stocks are diminishing at an alarming rate to satisfy needs for manufacturing fertilizers, and this unfolding P-crisis may lead to even lower P inputs into the coastal ocean and hence increased P-limitation of coastal phytoplankton (Abelson 1999; Gilbert 2009; Vaccari 2009).

Amino acid synthesis was most affected by nitrogen availability, because it is a main component of this compound (Chapter 3 - 5; Tapia et al. 1996). N availability regulated AA synthesis at two different levels. Firstly, total AA synthesis rates decrease with decreasing DIN availability as shown in lab cultures (Chapter 5) and a sudden lack of N resulted in an immediate decrease in AA production. The opposite occurs as soon as the N-limitation is relieved; synthesis rates increase drastically up to 8-fold within 24 h even though total carbon fixation did not change. Secondly, the synthesis of essential AA from non-essential AA precursors is slowed down under N limitation. The synthesis of essential AA requires multiple steps and relies on numerous additional enzymes, proteins themselves, and it may be beneficial to decrease the production of these enzymes under N scarcity.

P-limitation affected AA synthesis as well, however, rates did not decrease to an extent found under N-limitation. Responses to P resupply took much longer and were therefore delayed compared to N-limited communities. Synthesis of RNA, especially ribosomal RNA, is a major sink for P in phytoplankton and accounts for up to half of the P uptake (Van Mooy & Devol 2008; Hessen et al. 2010). P-limited phytoplankton typically down-regulates ribosome synthesis and its overall intracellular content, causing protein synthesis to be limited by the availability of P for RNA synthesis (Elser et al. 2000; Wang et al. 2014). When P is resupplied, the content of RNA and ribosomes need to increase first thereby delaying enzyme and protein synthesis compared to N-limited phytoplankton causing an asymmetric response in AA synthesis between N- and P-limited incubations. The relative distribution of individual AA was not affected under P-limitation, further indicating P-limitation regulated the assembly of proteins from AA, contrary N-limitation where protein synthesis is regulated by a low supply of individual AA.

And although, RNA and DNA pools were not investigated, the increased deficit between bulk C-fixation and cumulative C-fixation revealed that an additional biomolecule pool was present after N-resupply to N-limited
phytoplankton. This was most likely ribosomal RNA that was being produced in response to increased availability of N for protein synthesis. It may even be so that much of the increase in AA synthesis was actually used for ribosomes. At the same time this C-pool was negligible in P-limited phytoplankton after P was resupplied (within the first 24 h), supporting the conclusions drawn above.

A lack of N or P is known to result in the build-up in storage CH (glucose) and storage lipids (Granum et al. 2002; Borsheim et al. 2005). Nutrient dependent shifts in glucose synthesis were tightly and, as expected, inversely coupled to shifts in AA synthesis. At the same time, storage FA synthesis was phytoplankton community specific and relationships with N-limitation were mainly detected in flagellate dominated summer phytoplankton in my field studies. Nutrient specific responses in overall FA synthesis were revealed after 72 h, demonstrating that they occur slower than responses in AA. The effects of N and P limitation were identical and the resupply of nutrients caused a shift from storage FA to structural FA. The profiles of individual FA were also affected and nutrient shortage resulted in an accumulation of saturated and mono-unsaturated FA, because desaturases and elongases cannot be synthesized in required amounts, causing a deficiency in polyunsaturated FA, especially in the structural FA fraction (Flynn et al. 1992).

Most structural (phospho-lipid derived) FA contain P and consequently the effects of P-limitation should be more pronounced. But several phytoplankton species are capable of substituting P- for N- or sulfur- containing polar head groups, guaranteeing synthesis of structural FA also under P- limitation (Van Mooy, et al. 2006). However, under high DIN:P ratios found in coastal areas and the prospect of increasing imbalance between DIN and P these mechanisms will not be capable to overcome long-term P-deficiencies. This may increase storage FA contributions in the future and lead to a loss of polyunsaturated FA for higher trophic levels. In addition, optimal DIN:P requirements differ between groups of phytoplankton, with optimum ratios ranging between 5 and 50 (Geider & LaRoche 2002; Quigg et al. 2003), suggesting that phytoplankton community composition will be affected as well (Tilman 1977; Tilman 1985).
CHAPTER 7

Effect on food quality for higher trophic levels

According to the theory of ecological stoichiometry, nutrient limitation leads to a specific response in the C:N:P ratios, which primarily reflects changes in the biomolecule composition (Sterner & Elser 2002). These nutrient specific responses have been studied mainly in freshwater systems and demonstrate that P-limitation in phytoplankton can lead to P-deficiencies in zooplankton causing lower performance (Boersma 2000; Elser et al. 2001). But growth and fitness of consumers respond strongly to specific molecules as well (e.g. individual AA and FA; Claybrook 1983; Müller-Navarra 1995). Results presented here indicate that P-availability also affects AA synthesis, which is usually N-dependent, demonstrating complex causalities. In order to evaluate phytoplankton food quality in more detail, knowledge of individual biomolecules, biomolecule groups (including DNA and RNA) and their dynamics would be a preferred predictor to the simple element composition.

Both low AA quality and quantity negatively affect consumer’s growth (Guisande et al. 2000) and a lack of individual essential AA was found to restrict the reproduction of *Daphnia* and lead to changes in their life cycles (Fink et al. 2011; Koch et al. 2011). In the North Sea, flagellates were of low food quality due to their strong limitation by N. But the quick increase in AA synthesis after N-resupply make the AA pool dynamic. Subsequent effects on zooplankton may therefore be observed on short time scales as well. Particularly the micro- and meso-fraction maybe affected because of their short generation times, which are for example 30 and 17 days for *Calnus helgolandicus* and *C. typicus*, respectively (Halsband-Lenk et al. 2002; Bonnet et al. 2005).

Polyunsaturated FA are crucial for zooplankton survival and the maintenance of high growth and reproductive rates (Weers et al. 1997; Burns et al. 2011). Especially, the highly unsaturated FA 20:5ω3 and 22:6ω3 are known to affect trophic transfer efficiency and food web structure (Brett & Müller-Navarra 1997), and are considered to be an indicator for food quality (Park et al. 2002). Polyunsaturated FA are decreased under both P and N-limitation. Consequently, food quality of N-limited phytoplankton is substantially lowered in terms of AA and FA concentration and composition. Food quality of P-limited phytoplankton is primarily lowered by a lack of poly-unsaturated FA and only to a lesser extend by AA. Due to shifts in nutrient availability and phytoplankton productivity, food
quality of North Sea phytoplankton was negatively affected on both seasonal and spatial scales.

Although the majority of research has been conducted on the freshwater inhabiting cladocera *Daphnia* (Boersma 2000; Plath & Boersma 2001), and lakes are mostly considered P-limited opposite to N-limited marine waters (Hecky & Kilham 1988; Howarth & Marino 2006), anthropogenically induced changes in nutrient inputs from land push coastal seas more and more from N-limited into P-limited systems. With continuing alterations in riverine DIN and P supply it is likely that smaller zooplankton species may replace larger ones (Fransz et al. 1992) and thereby redirecting primary production to species with rapid turnover, such as protozooplankton and rotifers (Viitasalo 1992) and generate changes in the structure of the entire food web in coastal systems.

**Adaptation to low food quality by higher trophic levels**

Large mismatches between the biochemical composition of primary producers and herbivores turn food quality into an important factor defining species interactions at the plant-animal interface (Hessen et al. 2004; Lukas et al. 2011). Phytoplankton is flexible in its biochemical composition, while herbivorous zooplankton often shows a strict biochemical homeostasis (Frost et al. 2005), which creates the need to mitigate dietary deficiencies with behavioural and physiological adjustments (Mitra & Flynn 2007; Clissold et al. 2010). Results in this thesis show that copepods as important grazers of marine phytoplankton use different strategies to maintain homeostasis and retain essential biomolecules from N- and P-limited primary producers (Chapter 6).

The main response of copepods feeding on N-limited algae was to increase retention efficiencies for AA and total N and to assure that resources were more efficiently used. Minimising recycling rates and increasing the selective retention of AA in copepods compensated for the lack of N in food particles. Furthermore, dietary imbalances between relative concentrations of AA in algae and consumers were balanced by the synthesis of deficient non-essential AA. However, modification of AA structure and an increase in utilisation efficiencies of biomolecules, for example by improving digestion and absorption of food particles (Secor 2009; Perhar et al. 2013) are associated with substantial metabolic cost. An increase in structural FA requirements was observed as well and caused a co-
limitation of essential AA, 20:5\(\omega\)3 and 22:6\(\omega\)3. This increase of structural lipids could have been driven by either higher cellular requirements or by an increase of the gut surface area in copepods. Increases in the size and/or surface area of the gut facilitate higher absorption efficiencies and thus a more efficient utilisation of dietary resources (Cant et al. 1996). The fact that co-limitation of essential FA and AA emerged despite high concentrations of essential FA in food algae demonstrates that biomolecule deficiencies not only depend on dietary resources but also may arise from metabolic and physiological adjustments in consumers.

The main physiological adjustment of copepods fed with P-limited food algae was the adjustment in the feeding rate. A mechanism behind surplus feeding at a low food quality is the ability of copepods to selectively increase the uptake of single biomolecule or biomolecule groups in the gut (Cowie & Hedges 1996; Clissold et al. 2010). However, increased feeding rates generally lead to a decreased absorption efficiencies as high ingestion rates are automatically linked to lower gut transition times (Mitra & Flynn 2007) and result in a less efficient utilisation of dietary resources (Schindler 1968). Increased feeding rates at low dietary P concentrations are a common response (Plath & Boersma 2001; Suzuki-Ohno et al. 2012) and in turn are probably controlled by minimum gut transition times. A lower gut transition time can prevent a complete chemical break-up of algal cells and thereby decrease the subsequent absorption of biomolecules via the gut epithelium (Briggs 1977; Secor 2009).

This thesis gives a first glance at the multi-layered, direct and indirect consequences of poor food quality. Changes in predator-prey interactions most likely have an effect on species dynamics across trophic levels and are important contributing factor in shaping aquatic food webs.

**Conclusions and outlook**

Nitrogen and phosphorus limitation lead to substantial changes in the biomolecule composition within different groups of phytoplankton. Both the distribution of individual compounds and that of total biomolecule groups were affected (Chapter 3, 4). Nutrient specific responses were found in AA synthesis, while a lack of N or P had a similar effect on FA synthesis (Chapter 4).
Even though the controlling factors were alike in different phytoplankton communities, phytoplankton group specific or even species-specific differences occurred. For example, diatoms seem to be most flexible in their biochemical composition and can maintain higher growth rates under low nutrient availabilities, giving them an advantage under a wide range of nutrient poor conditions. Other groups are better adapted to extremely low availabilities of N (cyanobacteria) or are less competitive and only thrive under nutrient replete conditions (green algae). Additional monoculture experiments are needed to determine the range at which individual species can adapt to changing nutrient and light availabilities thereby enhancing our understanding of phytoplankton community dynamics. The continuation or worsening of the imbalance between DIN and P availability in coastal waters (Burson et al. 2016) will continue to negatively influence primary producers, food quantity and quality in the future.

The dynamic changes in biomolecule synthesis translate into equally dynamic changes in food quality. But so far only a few studies have focused on the effect of C:N:P ratios in marine food webs (Walve & Larsson 1999; Malzahn et al. 2010; Schoo et al. 2010). This thesis gives first insights into the possible controlling factors that zooplankton applies in order to cope with their changing nutrient environments at the biomolecule level, opening the path for future studies investigating compound specific effects on higher trophic levels.

The presented suite of methods can be applied to other ecosystems such as estuaries, the oligotrophic ocean or sediments as well, but also to terrestrial ecosystems and other consumer organisms. Investigations into other parts of the food web should also be pursued. For example, C flow through the microbial loop has not been included in this study, even though bacterial and viral activity are tightly coupled to phytoplankton activities.