Chemical composition of phytoplankton as the determinant of food quality

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DEDICATION

To my parents,
and to Changwei
without whom this would
not have been possible.

CONTENTS

SUMMARY

The trophic transfer across phytoplankton-zooplankton interface is crucially important in aquatic food webs. The factors regulating the trophic transfer efficiency have been widely studied. There is an increasing awareness of food quality in terms of chemical composition of phytoplankton as the major control of the phytoplankton-zooplankton interaction via bottom-up processes. Nutrient availability has broad effects on chemical composition of phytoplankton. Other factors, e.g., dilution rate in continuous and semicontinuous cultures, can interact with nutrient supply and affect phytoplankton food quality, hence affecting the performance of zooplankton.

This study aimed to investigate the effects of nitrogen (N):phosphorus (P) supply ratios and growth rates (dilution rates) on elemental and biochemical composition of marine phytoplankton, as well as the effects of food quantity and quality on the trophic transfer of essential chemicals and the performance of copepods. For this purpose, laboratory experiments were firstly conducted with three species of marine phytoplankton in semicontinuous cultures to test the interactive effect of five N:P supply ratios and four growth rates. Subsequent copepod experiments were performed with one species of marine copepods (*Acartia tonsa*) feeding on one phytoplankton species (*Rhodomonas* sp.), where the factors of food quantity and quality were crossed.

The experimental works with three species of marine phytoplankton, presented in CHAPTER 2 and CHAPTER 3, examined the interactive effect of N:P supply ratios and growth rates on phytoplankton carbon (C):N:P stoichiometry and fatty acid (FA) composition. Overall, there was a significant positive relationship between N:P biomass ratios and N:P supply ratios across the entire range of growth rates, and N:P biomass ratios converged to an intermediate value at higher growth rates (CHAPTER 2). Two mathematical models were applied to phytoplankton stoichiometry. Based on the results, I concluded that Ågren's functions (based upon biochemical assumptions) might explain the underlying biochemical principle for the Droop model.

Biochemical responses of phytoplankton were discussed in CHAPTER 3. For all species, the highest saturated and monounsaturated fatty acid (SFA and MUFA) contents were observed under N deficiency at the lowest growth rate, while polyunsaturated fatty acids (PUFAs) revealed variable responses to N:P supply ratios and growth rates among the three species. Total fatty acids (and SFAs and MUFAs) in all species showed significant negative

correlations with N cell quota (Q_N) under N deficiency, while PUFAs had species-specific correlations with Q_N. Thus, I concluded that elemental and biochemical limitations of phytoplankton food quality should be considered mutually for studying the trophic interaction between phytoplankton and zooplankton.

The trophic transfer across the phytoplankton-zooplankton interface enters the picture in CHAPTER 4, where I analyzed the effects of food quantity and stoichiometric food quality on the trophic transfer of essential elements and biochemicals between phytoplankton and copepods, as well as the egg production rate of copepods (*A. tonsa*). The results showed higher relative gross growth efficiencies for C and nutrient (N and P), as well as higher relative trophic transfer efficiencies for ω3- (and ω6-) PUFAs and C, under optimized food conditions (balanced nutrient diets under higher food quantity). In addition, egg production rate was also higher under optimized food conditions. Thus, I suggested that the trophic transfer of essential elements and biochemicals across the phytoplankton-zooplankton interface might predict the performance of consumers and trophic transfers at higher trophic levels in marine systems.

Further in CHAPTER 5, ingestion rate and nucleic acid content (RNA content and RNA:DNA ratio) of *A. tonsa* were investigated in response to food quantity and food quality (as chemical composition of phytoplankton). Both ingestion rate and nucleic acid content correlated positively with food concentration and were generally higher on the balanced nutrient diet. Egg production rate correlated positively with nucleic acid content. Food quality showed no significant effect on the nucleic acid-egg production relationship. This result is in agreement with the increasing recognition that RNA-based indices can be used as good indicators of copepod egg production.

In summary, the results in this thesis highlight the importance of simultaneous consideration of elemental and biochemical food quality for understanding the trophic transfer of energy and matter in food webs. Evaluating responses of this mutual regulation to multiple ambient factors is a necessary step towards the phytoplankton-zooplankton relationship in more realistic scenarios that will allow in the future the prediction of zooplankton’s performances in changing aquatic environments.

ZUSAMMENFASSUNG

In aquatischen Nahrungsnetzen kommt dem trophischen Transfer über die Phytoplankton-Zooplankton Schnittstelle eine große Bedeutung zu. Die Faktoren, die die Effizienz des trophischen Transfers regulieren wurden bereits eingehend untersucht. Der Nahrungsqualität wird dabei eine zunehmende Bedeutung als Kontrollmechanismus der Phytoplankton-Zooplankton Interaktion im Rahmen der „Bottom-up"-Prozesse beigemessen. Die Nährstoffverfügbarkeit hat einen großen Einfluss auf die chemische Zusammensetzung des Phytoplanktons. Andere Faktoren, wie z.B. die Verdünnungsrate in kontinuielrichen und semikontinuierichen Kultureb, können mit der Nährstoffzufuhr interagieren und beeinflussen die Nahrungsqualität des Phytoplanktons und somit auch die Performance des Zooplanktons.

Ziel dieser Studie ist es die Auswirkungen von Stickstoff (N): Phosphor (P)-Verhältnissen im Medium und Wachstumsraten (Verdünnungsraten) auf die elementare und biochemische Zusammensetzung von marinem Phytoplankton zu untersuchen. Zusätzlich sollen die Effekte der Nahrungsquantität und –qualität auf den trophischen Transfer von essentiellen Substanzen und auf Fitness der Copepoden analysiert werden. Zu diesem Zweck wurden zunächst Laborexperimente mit semikontinuierlichen Kulturen von drei verschiedener Arten des marinen Phytoplanktons durchgeführt um den wechselwirkenden Effekt von fünf N:P-Verhältnissen und vier Wachstumsraten zu testen. Nachfolgende Copepoden-Experimente wurden mit einer marinen Copepodenart (*Acartia tonsa*) durchgeführt, deren einzige Nahrungsquelle die Phytoplanktonart *Rhodomonas* sp. war. Dabei wurden sowohl die Effekte der Nahrungsquantität als auch der Nahrungsqualität in einem voll faktoriellen Design untersucht.

In Kapitel 2 und 3 werden die wechselwirkenden Effekte der N:P-Verhältnisse im Nährmedium und der Wachstumsraten auf die (C):N:P-Stöchiometrie und Fettsäurezusammensetzung der drei getesteten Phytoplanktonarten untersucht. Über die gesamte Spannweite der getesteten Wachstumsraten wurde eine signifikant positive Beziehung zwischen den N:P-Verhältnissen der Biomasse und den N:P-Verhältnissen des Nährmediums festgestellt. Bei höheren Wachstumsraten konvergierte das N:P-Verhältnis im Phytoplankton zu einem mittleren Level unabhängig vom N:P-Verhältnis im Nährmedium (Kapitel 2). Anschließend wurden zwei mathematische Modelle auf die Ergebnisse zurr Phytoplankton-Stöchiometrie angewendet. Anhand dieser Ergebnisse konnte ich

schlussfolgern, dass die Ågren-Funktionen (basierend auf biochemischen Annahmen) die zugrundeliegenden biochemischen Mechanism für das Droop Modell erklären könnten.

In Kapitel 3 werden die Reaktionen der Fettsäurezusamemnsetzung auf die Kulturbedingungen behandelt. Der höchste Gehalt an gesättigten und einfach ungesättigten Fettsäuren (SFA und MUFA) wurde für alle Algenarten unter N-Limitierung und der geringsten Wachstumsrate beobachtet. Der Gehalt an mehrfach ungesättigten Fettsäuren (PUFAs) hingegen zeigte, in Reaktion auf die verschiedenen N:P-Verhältnisse im Nährmedium und die Wachstumsraten, unterschiedliche Ausprägungen bei den getesteten Arten. Der Gesamtfettsäuregehalt, sowie die SFA als auch die MUFA, zeigten unter N-Limitierung bei allen Arten eine signifikant negative Korrelation mit dem Zellquote des Stickstoffs (Q_N). Der PUFA-Gehalt wies artenspezifische Korrelationen zu Q_N auf. Daraus konnte ich den Schluss ziehen, dass elementare und biochemische Limitierungen des Phytoplanktons als Nahrungsquelle gemeinsam betrachtet werden sollten, wenn trophische Interaktionen zwischen Phytoplankton und Zooplankton untersucht werden.

Der trophische Transfer über die Phytoplankton-Zooplankton-Schnittstelle wird in Kapitel 4 betrachtet. In diesem Kapitel analysierte ich den Effekt der Nahrungsmenge und stöchiometrischen Nahrungsqualität auf den trophischen Transfer von essentiellen Elementen und Fettsäuren zwischen Phytoplankton und Copepoden sowie auf die Eiproduktionsrate der Copepoden (*A. tonsa*). Unter optimierten Nahrungsbedingungen (ausgeglichenes Nährstoffverhältnis und hohe Nahrungsmenge) zeigten sich sowohl höhere relative Wachstumseffizienzen für Kohlenstoff (C) und die Nährstoffe N und P als auch höhere relative trophische Transferraten für ω3- (und ω6-) PUFAs und C. Die Eiproduktionsrate war unter diesen optimalen Ernährungsbedingungen ebenfalls höher. Folglich nehme ich an, dass der trophische Transfer der essentiellen Elementen und Fettsäuren über die Phytoplankton-Zooplankton-Schnittstelle sowohl die Leistung der Konsumenten, als auch den trophischen Transfer zu höheren trophischen Stufen im marinen System voraussagen kann.

In Kapitel 5 wird die Aufnahmerate und der Nukleinsäuregehalt (RNA-Gehalt und RNA:DNA-Verhältnis)von *A. tonsa* als Reaktion auf Nahrungsquantität und Nahrungsqualität (chemische Zusammensetzung des Phytoplanktons) untersucht. Sowohl die Aufnahmerate als auch der Nukleinsäuregehalt zeigten eine positive Korrelation mit der Nahrungskonzentration und waren generell höher bei den ausgeglichenen Nährstoffverhältnissen. Die Eiproduktionsrate der Copepoden korrelierte positiv mit dem

Nukleinsäuregehalt. Die Nährstoffmanipulationen zeigten keinen signifikanten Einfluss auf das Verhältnis von Nukleinsäure zu Eiproduktionsrate. Dieses Ergebnis ist in Übereinstimmung mit der zunehmenden akzepeieren Erkenntnis, dass RNA-basierte Indizes als Indikatoren für die Eiproduktion der Copepoden geeignet sind.

Die Ergebnisse dieser Studie machen deutlich, dass es wichtig ist, die elementare und biochemische Nahrungsqualität gemeinsam zu betrachten, um den trophischen Transfer von Energie und Stoffen in Nahrungsnetzen zu verstehen. Es ist ein wichtiger Schritt die Reaktion dieser gemeinsamen Regulierung von multiplen Umgebungsfaktoren zu evaluieren. Nur so ist es möglich in Zukunft, die Performance natürlicher Zooplanktongemeinschaften in den von stetigen Veränderungen geprägten aquatischen Lebensräumen realistisch vorhersagen zu können.

CHAPTER 1

General introduction

Trophic transfer across the phytoplankton-zooplankton interface

The trophic interaction between phytoplankton and crustacean zooplankton (zooplankton hereafter) is of critical importance in marine food webs. This can be attributed to two reasons. First of all, marine phytoplankton is currently responsible for approximately half of global primary production (Falkowski and Raven 2007, Finkel et al. 2010), and plays an enormous role in coupling multiple nutrient cycles in marine ecosystems (Arrigo 2005). Second, zooplankton occupies a key ecological position, which provides a link between primary producers and higher trophic levels such as fish (Harris et al. 2000). This link makes energy and matter in phytoplankton available for higher trophic levels. Therefore, understanding the regulation of trophic transfer across the phytoplankton-zooplankton interface is a basic step for further exploring the energy and matter transfer in the whole food webs.

Food quantity and quality have been considered as crucial factors in regulating the trophic interaction between phytoplankton and zooplankton via bottom-up processes, especially with current mounting interest in global change effects on marine phytoplankton (Sardans et al. 2012). Food quantity is conventionally measured in terms of carbon (C) absolute concentration, because C:biomass ratios are conservative and C is closely related to energy content (Sterner and Robinson 1994). Lampert (1977a, b) conducted a series of seminal studies on the role of food quantity, based on which Sterner and Schulz (1998) introduced the hypothetical relationship between food quantity and zooplankton growth rate (Fig. 1-1). Positive responses of zooplankton to food quantity have been reported in several aspects of zooplankton's performances such as egg production (e.g., Jónasdóttir 1994, Gusmão and McKinnon 2009) and ingestion rate (e.g., Frost 1972, Zamora-Terol and Saiz

2013). In the ocean, food quantity shows spatial and temporal variations, e.g., the limitation of food quantity for zooplankton in oceanic regions but not for coastal zooplankton (Huntley and Boyd 1984). This variability suggests that a wide range of food quantity should be considered in studying marine planktonic trophic transfer.

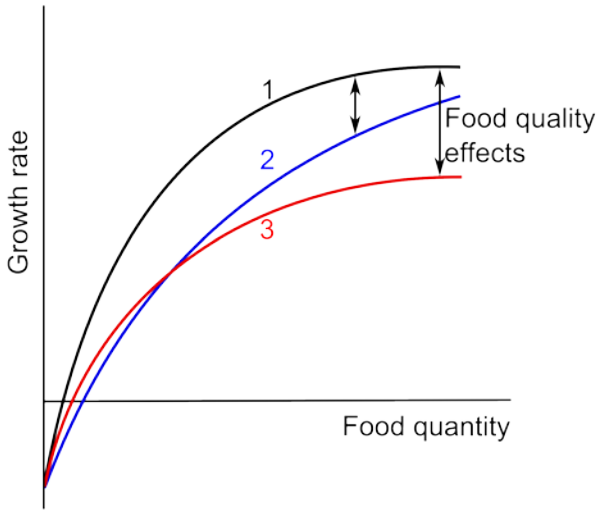

Figure 1-1. Hypothetical responses of specific growth rate of zooplankton to food quantity and food quality. Type 1 (black line) is 'ideal' or standard food; type 2 and 3 (blue and red lines) are suboptimal foods. Food type 2 can support maximal growth at higher food concentrations, while zooplankton can never reach maximal growth rate on food type 3. See the text for detail definition of food quality. Modified after Sterner and Schulz (1998).

Fig. 1-1 reveals that the response of zooplankton growth to food quantity differs under different scenarios of food quality. The importance of food quality has become increasingly recognized since the 1990s. On the individual level, food quality can be defined as "the degree to which the consumer's nutritional needs are fulfilled by quantity and composition of the accessible food" (Müller-Navarra 2008). For zooplankton, as well as other heterotrophic and phagotrophic organisms, several factors contribute to the quality of food at a given food quantity: the properties of a food particle (e.g., food detection, filtration, ingestion and assimilation), essential and semi-essential substances (e.g., elements and biochemicals), and toxins (Müller-Navarra 2008). Among these factors, elemental and biochemical limitations of food quality have received the most intense attention, on which a long-standing controversy has attracted more attention in limnology than in marine ecology (Arts et al. 2009).

Elements are essential for animals, thus the latter must depend on its dietary elemental supplies. Often, nitrogen (N) and phosphorus (P) are limited for aquatic animals (Anderson

et al. 2004). Elemental quality of food is frequently expressed as elements in relative terms, e.g., the relative content of phosphorus (P) (C:P), which is suggested as a key elemental quality measure for freshwater consumers (Hessen 2008). Biochemical quality of food includes two categories, macronutrients (e.g., proteins and lipids) and micronutrients (e.g., amino acids and fatty acids) (Anderson et al. 2004). Notably, certain fatty acids (FAs), i.e., polyunsaturated fatty acids (PUFAs), have attracted particular interest (Müller-Navarra 2008).

So, which factor limits zooplankton most, food quantity or quality, elements or biochemicals? According to Fig. 1-1 and the definition of food quality, the two aspects (i.e., food quantity and quality, and elements and biochemicals) should be considered simultaneously. In natural conditions, both food quantity and quality range between limitation and nonlimitation (Sterner and Schulz 1998). More recently, the reciprocal roles of food quantity and quality have been suggested in the flux of matter and energy in pelagic food webs (Hessen 2008). However, few scientific efforts have been put to study the interactive effect of food quantity and quality on the trophic transfer of elements and biochemicals. As an answer to the well-known debate of elemental versus biochemical food quality, several researchers have pointed out the importance of a mutual consideration of the two in phytoplankton food quality (Gulati and DeMott 1997, Boersma et al. 2001, Gladyshev et al. 2007). However, these studies were all conducted in freshwater ecosystems and few efforts have been made in marine ecosystems.

Phytoplankton food quality drivers and their effects

Nutrient availability. Nutrient elements are one of three principal resources for life requirements, alongside space to live, and energy (Moore et al. 2013). Of all elements, C, N and P are three of the most important components involved not only in biological structural functions but also in environmental nutrient cycles influenced greatly by biology (Sterner and Elser 2002). Anthropogenic activities have significantly increased the input of N and P to the oceans through different supply routes such as atmospheric deposition and fluvial fluxes (Moore et al. 2013 and references therein). These increases may enhance imbalances in nutrient supply to phytoplankton, e.g., leading to phytoplankton P limitation caused by elevated N loading from the atmosphere (Elser et al. 2009).

Responses of phytoplankton elemental composition to N:P supply ratios have been widely studied in both laboratory and field research. Classic chemostat experiments showed

a positive relationship between phytoplankton N:P biomass ratios and N:P supply ratios under certain ranges of N:P supply ratios and growth rates (Rhee 1978, Goldman et al. 1979, Ahlgren 1985). For example, Rhee (1978) found that N:P biomass ratios of *Scenedesmus* sp. matched N:P supply ratios ranging from 5 to 80 (by atoms) at a fixed growth rate. Rhee's study suggests that phytoplankton "are what they eat" at a given growth rate studied (Sterner and Elser 2002). However, this "you are what you eat" model, i.e., nonhomeostatic nature of phytoplankton stoichiometry, cannot be applied in all circumstances. Goldman et al. (1979) and Ahlgren (1985) observed a more fixed N:P biomass ratios of algae at higher growth rates independent of N:P supply ratios. Also, constraints of phytoplankton N:P stoichiometry are evident in diverse natural aquatic communities (Hall et al. 2005). This suggests that non-nutrient drivers can modify the plasticity of phytoplankton stoichiometry induced by nutrient drivers, and this plasticity is originally controlled by species-specific physiology (Hall 2009). Therefore, taxonomic comparisons of phytoplankton stoichiometric responses to the interactive effect of nutrient supply and other environmental factors could be helpful to predict algal succession in the phytoplankton community in changing oceans.

Besides elements, certain biochemicals are essential for zooplankton and thus must be obtained from food sources. The importance of two micronutrients, FAs and amino acids, in biochemical quality of phytoplankton has received intense attention (Müller-Navarra 2008), and responses of phytoplankton FA composition to nutrient availability have been best investigated. In a recent review, N limitation is explicitly suggested as the single most critical effect on lipid metabolism in algae (Hu et al. 2008). Generally, nonpolar glycerolipids, primarily triacylglycerols (TAGs), are accumulated as storage lipids in many algal species under N limitation (Guschina and Harwood 2009). TAGs mainly comprise saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs), and the latter two FA groups are suitable energy and carbon sources for the maintenance of basic metabolism under unfavourable conditions (Roessler 1990).

Compared to SFAs and MUFAs, PUFAs show more important roles in trophic interactions. This is due to the essentiality of certain PUFAs, e.g., ω3- and ω6-PUFAs, in zooplankton nutrition. Responses of PUFAs to nutrient supply vary greatly among phytoplankton species. For example, reduced PUFA contents were observed in the diatom *Thalassiosira weissflogii* under both N and P limitations (Klein-Breteler et al. 2005), and in the green alga *Scenedesmus quadricauda* and the cyanobacterium *Synechococcus* sp. (Ahlgren and Hyenstrand 2003) under N limitation. In contrast, increased PUFA contents were found in *Rhodomonas salina* under N limitation (Malzahn et al. 2010) and in *S.*

quadricauda and *Chlamydomonas globosa* under P limitation (Piepho et al. 2012). Two main reasons can explain these differences. The first reason is concerning FA biosynthesis such as characteristic biosynthetic pathways (e.g., desaturation of C16:0 to C16:1ω7 in diatoms) and different responses of desaturation enzymes to nutrient supply (e.g., unique desaturase enzymes Δ12 and Δ15 in primary producers) (Ahlgren and Hyenstrand 2003, Kelly and Scheibling 2012 and references therein). Second, physiological functions of PUFAs are variable. Certain biochemical mechanisms such as the incorporation of PUFAs to TAGs or phospholipid substitutions have been found to maintain growth in some algal species under nutrient limitation (Cohen et al. 2000, Van Mooy et al. 2009). This suggests that taxonomic comparison of PUFA responses would be of critical importance for studying physiological regulations of FA biosynthesis at the cellular scale, as well as for predicting responses of phytoplankton species composition at the community level, under variable nutrient conditions.

The usage of the term nutrient limitation varies greatly in the literature. In a recent review, Moore et al. (2013) clarified and defined the term nutrient limitation at different scales of biological and ecological processes. They further defined nutrient deficiency as "the stoichiometric lack of one element relative to another" (in the medium) and nutrient stress as "a physiological response to a nutrient shortage". Thus, the term N (and P) deficiency will be used to describe low (and high) N:P supply ratios in this thesis. To keep consistent with the original publication, the description of nutrient conditions in each citation will be expressed as the same term with those in the corresponding literature.

Growth rate (dilution rate). Besides nutrients, there are abundance of other factors impacting on phytoplankton food quality, e.g., light intensity, temperature, pCO_2, grazing, and sedimentation (Sterner and Elser 2002, Lewandowska 2011). In contrast to the effect of nutrients that is responsible for phytoplankton growth, factors such as dilution, sedimentation, physiological death and grazing result in population loss. The balance between cellular growth rate and loss rate determines the succession of phytoplankton species in natural communities (Hecky and Kilham 1988). Classic chemostat experiments have shown that phytoplankton growth rate and biomass stoichiometry is tightly coupled (Goldman et al. 1979, Ahlgren 1985). As a main theory of autotroph stoichiometry, the Droop model relates specific growth rate (μ) of phytoplankton to the intracellular concentration (cell quota) of the limiting nutrient (Droop 1973, 1983). More recently, Ågren (2004, 2008) interpreted the relationship between μ and nutrient cell quota from biochemical considerations. There is a considerable amount of empirical supports for the

Droop model, while few studies have applied Ågren's prediction to diverse phytoplankton species. This results in the lack of knowledge of the relationship between μ and biochemical composition in phytoplankton.

To summarise, nutrient availability and μ (μ = dilution rate in continuous cultures) are important in determining elemental and biochemical quality of phytoplankton, as well as phytoplankton community structure. The effects of these two phytoplankton food quality drivers can travel up the food chain and thus influence the performance of zooplankton and organisms at higher trophic levels.

The performance of zooplankton in response to food quantity and quality

Early experimental studies and models on food quantity and quality effects were mostly performed with freshwater zooplankton (e.g., *Daphnia*), and concerned with resulting effects on zooplankton growth (Sterner and Schulz 1998). Sterner and Schulz's hypothesis has been supported by abundant observations, showing positive responses of specific growth rate of zooplankton to food quantity and quality. However, somatic growth is not the only conversion of assimilated energy in zooplankton, but there is another utilization of energy, i.e., egg production (Harris et al. 2000). Therefore, besides somatic growth other aspects of zooplankton's performances should be also considered in studying zooplankton nutrition.

Egg production is suggested as a convenient indicator integrating the influences of growth limitation during all life cycle of copepods (Runge and Roff 2000). The effect of food quantity on marine zooplankton egg production has long been studied, showing increased egg production rates with increasing food quantity until the maximal egg production rate is reached (Checkley 1980 and references therein). Checkley (1980) also reported the inhibition of algal N limitation on egg production rate. Based on this finding, there is an increasing recognition of the importance of elemental and biochemical food quality in regulating egg production (Mayor et al. 2009, Chen et al. 2012 and references therein). In contrast to the results of earlier studies, increased egg production rates were also observed on nutrient limited diets (Augustin and Boersma 2006). This suggests that an alternative approach instead of simply considering dietary elemental and biochemical

composition is required to explain contradictory responses of egg production to nutritional food quality.

Feeding is the main route for energy and matter transfer from lower to higher trophic levels, and thus quantification of feeding is a key factor in studying trophic interactions (Båmstedt et al. 2000). Ingestion rate is one of the most common ways to express zooplankton feeding (Båmstedt et al. 2000). It has been well established that ingestion rates increase with increasing food concentrations up to a maximal rate, which can be illustrated by the classic Holling functional response model and alternative types of models (Gentleman et al. 2003, Wirtz 2012). However, there is no consistent response of ingestion rates to food quality. Most studies have focused on the effect of food type (different algal species), resulting in the mixture of several aspects of food quality effects, which makes it difficult to extract information at each aspect of food quality.

Nucleic acid indices such as RNA content and RNA:DNA ratio have been used to index copepod growth (e.g., egg production) and physiological condition (e.g., resting stage) (Saiz et al. 1998, Wagner et al. 1998, Holmborn et al. 2009, Ning et al. 2013). The widespread use of nucleic acid indices is due to several advantages of this method, e.g., simplicity and sensitivity, and variety of measuring techniques (Gusmão and McKinnon 2011). While most experimental studies have focused on the effect of food concentration on the nucleic acid content of copepods, the effect of food quality has received less attention. Recently, Gusmão and McKinnon (2011) first reported that food quality (as different algal species) can affect the relationship between nucleic acid indices and egg production rate. However, little is known of elemental and biochemical food quality effects on the nucleic acid-egg production relationship.

Many studies so far are directed toward examining which factor of chemical food quality (i.e., elements or biochemicals) explains the performance of copepods better. Jónasdóttir (1994) showed the first evidence of good correlations for both elemental and biochemical composition of phytoplankton with copepod reproduction. Although such statistical correlations to some extent might explain the effects of elemental and biochemical food quality on zooplankton, it is hard to distinguish correlation from causation, and the direct and indirect effects of food quality. The indirect effects of elemental limitation may include changes in biochemical composition of phytoplankton (Ravet and Brett 2006). Recently, Gladyshev et al. (2011) found that the transfer of essential PUFAs from the producers to the primary consumers was higher than that of bulk C, while nonessential PUFAs showed lower

transfer efficiency. This provides evidence of trophic transfer efficiency of essential chemicals as a better indication of zooplankton's performances.

Zooplankton community is often determined by predominant taxa, which play the main role as a crucial link between primary producers and higher trophic levels in food webs (Harris et al. 2000). Copepods dominate the zooplankton community in marine coastal zones and are the most important prey of fish larvae and other planktivores (Turner 2004, Vargas et al. 2006). Therefore, a typical calanoid copepod species (*Acartia tonsa*) is chosen as model copepods in this thesis to study its responses to variable food supply, which would be valuable for assessing it ecological roles in planktonic trophic interactions.

Aim of this study

The aim of this study is to investigate the impact of N:P supply ratios and growth rates on elemental and biochemical composition of marine phytoplankton, as well as the effects of food quantity and quality on the trophic transfer of essential chemicals and the performance of copepods. Two series of experiments were conducted under controlled laboratory conditions. First of all, I conducted phytoplankton experiments to test phytoplankton C:N:P stoichiometry and FA composition in response to N:P supply ratios and growth rates. The second series of experiments conducted with copepods focused on the effects of food quantity and quality on essential chemical trophic transfer and the performance of copepods. In this thesis, the following four aspects are addressed.

CHAPTER 2. I started in this chapter with stoichiometric responses of phytoplankton to five N:P supply ratios and four growth rates. I focused on taxonomic comparisons of phytoplankton C:N:P stoichiometry in response to N:P supply ratios and growth rates, as well as the application of mathematical models to phytoplankton stoichiometry.

CHAPTER 3. Responses of phytoplankton FA composition were investigated under the same ranges of N:P supply ratios and growth rates as set in CHAPTER 2. Interspecific differences in FA responses were considered. In order to test the link between elements and biochemicals in phytoplankton, I analyzed the relationship between FAs and N (and P) cellular quotas under N (and P) deficiency.

CHAPTER 4. The interactive effect of food quantity and stoichiometric food quality was examined on the relative gross growth efficiency for C and nutrient (N and P), as well as the relative trophic transfer efficiency for ω3- (and ω6-) PUFAs and C. Furthermore, the nutritional importance of food quantity and quality for higher trophic levels was determined

by measuring copepod egg production rate. I suspected that trophic transfer of essential elements and biochemicals might predict reproductive responses of copepods to dietary nutrient conditions.

CHAPTER 5. I further considered the effects of food quantity and quality on the performance of copepods. The emphasis in this chapter was on ingestion rate, RNA content and RNA:DNA ratio. Moreover, the relationship between egg production rate and nucleic acid indices (the RNA content and RNA:DNA ratio) was studied to test if food quality could affect the nucleic acid-egg production relationship.

CHAPTER 2

Stoichiometric responses of phytoplankton species to the interactive effect of nutrient supply ratios and growth rates[*]

Rong Bi[**], Carmen Arndt, and Ulrich Sommer

ABSTRACT

Three species of phytoplankton, *Rhodomonas* sp., *Phaeodactylum tricornutum* Bohlin, *Isochrysis galbana* Parke, were cultivated in semicontinuous culture to analyze the response of carbon (C):nitrogen (N):phosphorus (P) stoichiometry to the interactive effect of five N:P supply ratios and four growth rates (dilution rates). The relationship between cellular N and P quotas and growth rates fits well to both the Droop and Ågren's functions for all species. We observed excess uptake of both N and P in the three species. N:P biomass ratios showed a significant positive relationship with N:P supply ratios across the entire range of growth rates, and N:P biomass ratios converged to an intermediate value at higher growth rates. The effect of growth rates on N:P biomass ratios was positive at lower N:P supply ratios, but negative at higher N:P supply ratios for both *Rhodomonas* sp. and *I. galbana*, while for *P. tricornutum* this effect was negative at all N:P supply ratios. A significant interactive effect of N:P supply ratios and growth rates on N:P biomass ratios was found in both *Rhodomonas*

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sp. and *P. tricornutum*, but not in *I. galbana*. Our results suggest that Ågren's functions may explain the underlying biochemical principle for the Droop model. The parameters in the Droop and Ågren's functions can be useful indications of algal succession in phytoplankton community in changing oceans.

INTRODUCTION

Nitrogen (N) and phosphorus (P) are the most frequent limiting nutrients for primary production in marine, freshwater and terrestrial ecosystems (Hecky and Kilham 1988, Vitousek and Howarth 1991, Downing 1997, Elser et al. 2007). Marine phytoplankton, currently responsible for ca. 50% of global primary production (Falkowski and Raven 2007, Finkel et al. 2010), is nutrient limited in large parts of the world oceans and throughout the annual growth season. The ocean's nutrient-limited zones are expanding because of the spatial and temporal expansion of ocean surface stratification due to ocean warming and freshening. In contrast, coastal regions may experience an increase in nutrient supply from intensified upwelling and nutrient loads from terrestrial sources (Finkel et al. 2010). N:P concentrations and supply ratios are relatively stable in the open ocean (Redfield ratio, 16:1) (Redfield 1958, Falkowski 2000), but there is stronger spatial or temporal variability in coastal seas and in oceanic areas influenced by oxygen minimum zones (Karl et al. 1993, Cavender-Bares et al. 2001, Twomey and Thompson 2001, Ford et al. 2008, Lam and Kuypers 2011). Variation in N:P supply ratios may drive a large variability in N:P stoichiometry of primary producers (Hall 2009). In contrast to the plasticity of phytoplankton stoichiometry, the stoichiometry of consumers exhibits less variability, resulting in a mismatch between carbon (C):N:P stoichiometry of primary producers and consumers (Elser et al. 2000a, Andersen et al. 2004). This ecological imbalance has large effects on consumers in terms of food quantity and quality, which may ultimately affect top predators by bottom-up processes (Andersen et al. 2004).

The success of phytoplankton species in natural communities depends on whether the cellular growth rate exceeds or equals loss rates from dilution, sedimentation, physiological death, and grazing (Hecky and Kilham 1988). Nutrient limitation of growth rates is important for both the stoichiometry of phytoplankton biomass and the determination of phytoplankton community structure (Rhee 1973, Terry et al. 1985b, Sterner and Elser 2002, Flynn et al. 2010).

Models are useful tools to simulate and predict the response of marine phytoplankton to changing ocean conditions (Sunda et al. 2009). Nutrient-limited growth of phytoplankton can be described by three models: the Monod model (Monod 1942, 1949); the quota model, such as Droop's quota model (Droop 1983); and the mechanistic model based on the biochemical processes (Flynn 2003). The Monod model describes the steady-state growth rate of microbes as a function of environmental nutrient concentrations. Thus, its validity is questioned under nonsteady state conditions, such as batch cultures and field conditions (Droop 1983). The Droop model is suggested as an alternative quota model (Sommer 1991a). As one of the main theories of autotrophic stoichiometry (Sterner and Elser 2002), the Droop model relates the specific growth rate of phytoplankton to the intracellular concentration (cell quota) of the limiting nutrient (Droop 1973, 1983) and has been successfully applied to a considerable amount of empirical studies. If the cell quota of the limiting nutrient is related to biomass carbon, this provides a prediction of the limiting nutrient:C stoichiometry of biomass (Flynn 2008). However, it makes no direct prediction of the biomass content of a nonlimiting nutrient (e.g., N in the case of P limitation). According to Loladze and Elser (2011), the elemental composition of autotrophs is determined by their biochemical composition: constraining protein synthesis rates will result in a lower N:P biomass ratio, and constraining RNA production rates will lead to a higher ratio. Based on the assumption that autotrophic growth requires protein (N based) and protein synthesis requires ribosomes RNA (rRNA) (P based), Ågren (2004) proposed that cellular N:C ratios increase linearly, while cellular P:C ratios increase quadratically as a function of growth rates in autotrophs. Ågren's prediction has been confirmed with one freshwater alga (*Selenastrum minutum*) and one tree seedling (*Betula pendula*), but few further studies have applied this model to other freshwater or marine phytoplankton species.

A classic chemostat experiment (Rhee 1978) showed the close match between the nutrient supply ratio and cellular stoichiometry in *Scenedesmus* sp., indicating the absence of homeostasis of N:P biomass ratio within the range of nutrient supply ratios studied (Sterner and Elser 2002). Rhee (1978) also reported excess uptake of N under P limitation and of P under N limitation in *Scenedesmus* sp. (luxury consumption) (Sterner and Elser 2002). The capacity of excess uptake is one important cause of stoichiometric variability in nature (Ågren 2008). The capacity of excess uptake in autotrophs varies both between and within species, and also between different elements (Ågren 2004, 2008). Thus excess uptake can also function as a competitive strategy. The nonhomeostatic nature of elemental composition in phytoplankton can be modified by environmental factors, such as pH,

temperature, light, growth rate, as well as the species composition of phytoplankton communities (Goldman et al. 1979, Ahlgren 1985, Sterner and Hessen 1994). Besides in unialgal cultures, constraints of primary producer N:P stoichiometry are also evident in diverse aquatic and terrestrial communities (Hall et al. 2005). To explain the variability of phytoplankton stoichiometry theoretically, Legović and Cruzado (1997) proposed a model of phytoplankton growth on multiple nutrients based on Michaelis-Menten-Monod uptake of nutrients, the Droop function, and Liebig's law of the minimum. This model was further analyzed by Klausmeier et al. (2004), showing that phytoplankton stoichiometry matches the nutrient supply ratio at low growth rates, but becomes less variable at higher growth rates. Generally, different algal species have similar physiological and compositional responses to nutrient limitation, such as decreases in photosynthetic pigments, storage of C, and decreases in protein content (Healey 1973, Shifrin and Sallie 1981, Hecky and Kilham 1988, Larson and Rees 1996, Lynn et al. 2000). However, some algal species do not exhibit these general responses to nutrient limitation (Van Baalen and Marler 1963, Terry et al. 1985a, Ahlgren and Hyenstrand 2003, Leonardos and Geider 2004). Species-specific differences in the response to N and P enrichments and N:P ratios are also found in natural phytoplankton communities (Lagus et al. 2004), but more empirical data are required to explore the elemental and biochemical principles of the interspecific differences in responses to the interactive effect of N:P supply ratios and other environmental conditions .

In this study, we focus on taxonomic comparisons of phytoplankton C:N:P stoichiometric responses to the interactive effect of N:P supply ratios and growth rates, as well as the application of mathematical models to phytoplankton stoichiometry. We addressed the following questions: (i) Is the response of cellular N and P contents to growth rates consistent with the predictions of both the Droop model and Ågren's functions? (ii) If so, could the Droop model be explained by the biochemical mechanisms implicit in Ågren's functions? (iii) Are there species-specific differences in phytoplankton responses to the interactive effect of growth rates and N:P supply ratios?

MATERIALS AND METHODS

Experimental setup. The selected algal species are from three different marine phytoplankton classes: *Rhodomonas* sp. (Cryptophyceae), *Isochrysis galbana* (Prymnesiophyceae) (Parke 1949), and *Phaeodactylum tricornutum* (Bacillariophyceae). The culture medium was prepared by enriching sterile filtered seawater (pore size 0.2 μm) with macronutrients and micronutrients based on the modified Provasoli's enriched

seawater medium (Provasoli 1963, Ismar et al. 2008). Macronutrients were added as sodium nitrate ($NaNO_3$) and potassium dihydrogen phosphate (KH_2PO_4), with five N:P supply ratios (Table 2-1). For the diatom (*P. tricornutum*) culture, also sodium silicate pentahydrate ($Na_2SiO_3 \cdot 5H_2O$) was added at a concentration of 880 µmol · L^{-1}. Three algal species were cultivated at 18 ^oC and salinity of 18 ± 1 psu. The light intensity was constant at 100 µmol photons · m^{-2} · s^{-1} at a light:dark cycle of 16:8h. This light intensity did not limit the growth of the three species (Beardall and Morris 1976, Hammer et al. 2002, Tzovenis et al. 2003). The cultures were kept in 1 L Erlenmeyer flask containing 500 mL culture volume. All flasks were aerated slightly with filtered air and shaken manually twice per day at a set time.

Table 2-1. N:P supply ratios and N (P) concentrations in culture media.

N:P supply ratio (mol · mol^{-1})	N concentration (µmol · L^{-1})	P concentration (µmol · L^{-1})
10:1	352	36
14:1	498	36
24:1	880	36
35:1	880	25
63:1	880	14

First, batch culture experiments were performed to obtain an estimate of the observed maximal growth rate (μ_{max}). All species were precultured until the culture reached the exponential phase. Algae from the exponential phase were used as an inoculum for the subsequent experimental batch culture, which was run in triplicate. Algae were cultivated until the culture reached to the early stationary phase. Cell density was transformed logarithmically and the linear increasing part in log plot was defined as the exponential phase. μ_{max} is defined by equation 2-1,

$$\mu_{max} = \frac{\ln N_1 - \ln N_2}{t_1 - t_2} \tag{2-1}$$

where N_1, N_2 are the population cell density at time 1 (t_1) and 2 (t_2) within exponential phase. Semicontinuous cultures were started with the algae from batch cultures when batch cultures had reached early stationary phase. For each N:P supply ratio, four specific growth rates (μ, d^{-1}) were applied, which were 20%, 40%, 60% and 80% of μ_{max}, and the equivalent daily renewal rate (D, d^{-1}) can be calculated according to equation 2-2,

$$D = 1 - e^{-\mu \cdot t} \tag{2-2}$$

where t is renewal interval (d) (here t = 1d). The steady state of cultures was assessed based on the net growth rate (r). When r was zero (at steady state), μ was equivalent to D.

Sample analysis. Cell density was counted daily using an improved Neubauer haemacytometer. At steady state, the cultures were harvested for elemental analysis. For the determination of particulate organic carbon (POC), nitrogen (PON) and phosphorus (POP), samples were filtered onto pre-combusted Whatman GF/F filters. After filtration the samples were immediately dried and stored in a desiccator. Analysis of POC and PON were carried out after Sharp (1974) by gas chromatography in an organic elemental analyzer (Thermo Flash 2000) (Thermo Fisher Scientific Inc., Schwerte, Germany), while POP was determined colorimetrically by converting organic phosphorus compounds to orthophosphate (Hansen and Koroleff 1999).

In N- or P-deficient cultures, the relationship between N:C biomass ratios (N cell quota, Q_N) or P:C biomass ratios (P cell quota, Q_P) and μ can be expressed by the Droop model (equation 2-3) (Droop 1983),

$$\mu = \mu_{max}' \cdot (1 - \frac{Q_0}{Q}) \tag{2-3}$$

where μ_{max}' is the theoretical maximal growth rate at infinite cell quotas (d^{-1}); Q_0 is the minimal quota of nutrient needed for viability (mol · mol^{-1}); Q is the actual cell quota at any finite growth rate (mol · mol^{-1}). The realized nutrient-saturated growth rate (identical to μ_{max} in equation 2-1) is attained at the saturating cell quota (Q_{max}).

Ågren (2004, 2008) interpreted the relationship between Q_N or Q_P and μ from biochemical considerations. Under stable and balanced growth, this relationship can be described as equation 2-4 and 2-5,

$$Q_N = \frac{\mu}{Ø_{CN}} + \beta_N \tag{2-4}$$

$$Q_P = \frac{\mu^2}{Ø_{CN} \cdot Ø_{NP}} + \beta_P \tag{2-5}$$

where $Ø_{CN}$ is the rate of protein-C synthesis per daily nitrogen assimilation (mol · mol^{-1} · d^{-1}). $Ø_{NP}$ is the rate of protein synthesis by ribosomes (mol · mol^{-1} · d^{-1}), based on the assumption that protein production (expressed as N content) is proportional to the P in ribosomes. N containing compounds other than protein are considered proportional to the amount of C with the factor β_N (mol · mol^{-1}). Similarly ribosomes are not the only P containing compounds but there is also additional P which is proportional to the amount of

C with the factor β_P (mol · mol^{-1}). Data for Q_N (and Q_P) and μ in the extreme N- and P-deficient cultures (N:P supply ratio = 10:1 and 63:1) were fit to the Droop and Ågren's functions.

Statistics. The normality of dependent variables was tested with a normal probability plot of residuals and Shapiro-Wilk's W-Test. Algal N:P biomass ratios were transformed using $\log_{10}$ (x). Moreover, N:P supply ratios used in the experiment were approximately logarithmically distributed; thus, the data for N:P supply ratios were also transformed using $\log_{10}$ (x). The Droop model and Ågren's functions were fitted using a nonlinear least squares method and the estimated parameters were obtained using Statistica 8. The response of N:P biomass ratios to N:P supply ratios and growth rates were analyzed by multiple regression with the second-order quadratic equation using R 2.12.0 (R Development Core Team 2010), with the predicting variables μ, N:P supply ratio, μ^2, (N:P supply ratio)^2 and μ · (N:P supply ratio).

RESULTS

The observed maximal growth rate (μ_{max}). For all species, μ_{max} values did not differ substantially between the different N:P supply ratios. The μ_{max} values (±SD) were 0.85 d^{-1} (±0.05) for *Rhodomonas* sp., 0.42 d^{-1} (±0.02) for *I. galbana*, and 1.00 d^{-1} (±0.03) for *P. tricornutum*, respectively.

Fitting to the Droop model. The relationship between μ and Q_N (and Q_P) showed good fits to the Droop model for all species ($r^2 \geq 0.705$) (Table 2-2), the regressions and the observed data are shown in Fig. 2-1 and Table A1. Both Q_N and Q_P of the three species showed highly significant correlations with μ ($p < 0.001$) (Table 2-2). In N-deficient cultures (N:P supply ratio = 10:1), the μ_{max}' value of the three species decreased in the following order: *P. tricornutum* (1.263 d^{-1}) > *Rhodomonas* sp. (1.260 d^{-1}) > *I. galbana* (0.526 d^{-1}). The rank order of Q_0 values for N was *Rhodomonas* sp. (0.101 mol · mol^{-1}) > *P. tricornutum* (0.078 mol · mol^{-1}) > *I. galbana* (0.064 mol · mol^{-1}). In P-deficient cultures (N:P supply ratio = 63:1), rank orders for both μ_{max}' and Q_0 for P were *Rhodomonas* sp. > *P. tricornutum* > *I. galbana*. Q_{max} was calculated based on equation 2-3 for all species where μ_{max} was lower than μ_{max}' (Fig. 2-1, Table 2-2), except for *P. tricornutum* in P-deficient cultures. The μ_{max} value of *P. tricornutum* in P-deficient cultures (1.00 d^{-1}) was higher than μ_{max}' (0.913 d^{-1}), and thus neither the Q_{max} value for P nor P storage capacity of *P. tricornutum* was shown in the result. Q_{max} values for N and P were different among the three

species, with the rank order of *P. tricornutum* (0.375 mol · mol^{-1}) > *I. galbana* (0.320 mol · mol^{-1}) > *Rhodomonas* sp. (0.309 mol · mol^{-1}) for Q_{max} of N, and *Rhodomonas* sp. (9.570 mmol · mol^{-1}) > *I. galbana* (7.947 mmol · mol^{-1}) for Q_{max} of P. Cellular N and P storage capacities could be calculated from values of Q_0 and Q_{max} (Q_{max} - Q_0). Thus the rank order of N storage capacities in the three species was *P. tricornutum* (0.297 mol · mol^{-1}) > *I. galbana* (0.256 mol · mol^{-1}) > *Rhodomonas* sp. (0.208 mol · mol^{-1}), and for P storage capacities the rank order was *Rhodomonas* sp. (7.742 mmol · mol^{-1}) > *I. galbana* (7.007 mmol · mol^{-1}).

Table 2-2. Estimated parameters of *Rhodomonas* sp., *Isochrysis galbana*, and *Phaeodactylum tricornutum* obtained by nonlinear least squares method according to the Droop model (equation 2-3).

Species	N:P supply ratio	μ_{max}'			Q_0			r^2
		Estimate	*t*	*p*	Estimate	*t*	*p*	
Rhodomonas sp.	10:1	1.260 (0.872, 1.648)	7.237	<0.001	0.101 (0.084, 0.117)	13.555	<0.001	0.705
	63:1	1.051 (0.940, 1.161)	21.148	<0.001	1.828 (1.691, 1.965)	29.772	<0.001	0.945
I. galbana	10:1	0.526 (0.395, 0.657)	8.923	<0.001	0.064 (0.053, 0.076)	12.975	<0.001	0.755
	63:1	0.476 (0.363, 0.590)	9.344	<0.001	0.940 (0.760, 1.121)	11.601	<0.001	0.750
P. tricornutum	10:1	1.263 (1.102, 1.424)	17.480	<0.001	0.078 (0.071, 0.085)	25.472	<0.001	0.923
	63:1	0.913 (0.815, 1.011)	21.091	<0.001	1.176 (1.042, 1.309)	19.872	<0.001	0.933

Values of Q_0 for N:P = 10:1 are for N which are expressed as mol · mol^{-1} and for N:P = 63:1 are for P which are expressed as mmol · mol^{-1}. N:P supply ratios are expressed as mol · mol^{-1}. Values of

μ_{max}' are expressed as d^{-1}. Numbers in parentheses are 95% CI. The number of observations (*n*) is 12 for all species except for *Phaeodactylum tricornutum* at N:P = 63:1, in which *n* = 11. The significance level of the full models is $p < 0.05$.

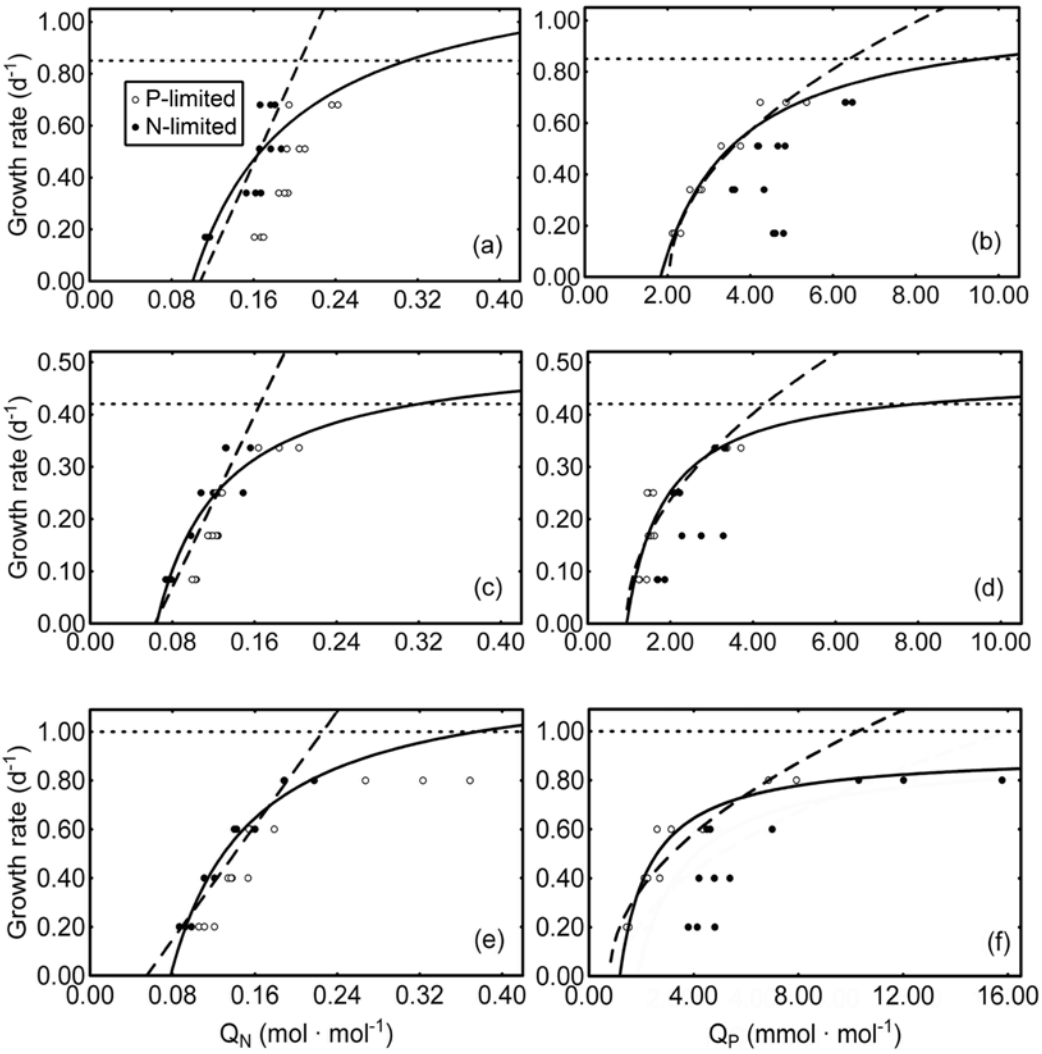

Figure 2-1. Growth rate as a function of N and P cell quotas for (a and b) *Rhodomonas* sp., (c and d) *Isochrysis galbana*, and (e and f) *Phaeodactylum tricornutum* under N- (N:P supply ratio = 10:1) and P-deficient (N:P supply ratio = 63:1) conditions. N- and P-deficient data series were fit to the Droop and Ågren's functions shown in panels (a, c, and e) and (b, d, and f), respectively. Solid lines and broken lines are least-square fits of the Droop model and Ågren's functions, respectively. The observed maximum growth rates (μ_{max}, 0.85 d^{-1} for *Rhodomonas* sp., 0.42 d^{-1} for *Isochrysis galbana*, and 1.00 d^{-1} for *Phaeodactylum tricornutum*) are shown as dotted lines.

Fitting to Ågren's functions. The Ågren's functions provided a good fit ($r^2 \geq 0.723$) to the observed data of Q_N (and Q_P) and μ for all species (Fig. 2-1, Table 2-3). The parameter estimates of Ågren's functions are given in Table 2-4, and corresponding adjusted r^2 values are shown in Table 2-3 (linear regression for Q_N, quadratic regression for Q_P). In *Rhodomonas* sp., values of estimated parameters, $Ø_{CN}$ (8.735 mol · mol^{-1} · d^{-1}), β_N (0.108

mol · mol^{-1}) and β_P (0.0021 mol · mol^{-1}), were the highest among the three species, while *P. tricornutum* had the highest value of $Ø_{NP}$ (34.409 mol · mol^{-1} · d^{-1}). *I. galbana* had the lowest values of $Ø_{CN}$ (4.104 mol · mol^{-1} · d^{-1}) and $Ø_{NP}$ (16.006 mol · mol^{-1} · d^{-1}), and *P. tricornutum* had the lowest values of β_N (0.055 mol · mol^{-1}) and β_P (0.0008 mol · mol^{-1}). All parameters had highly significant effects on the regression ($p \leq 0.001$), except β_P in *P. tricornutum* ($p = 0.064$).

Table 2-3 shows adjusted r^2 values of linear (XC = a + b · μ) and quadratic (XC = a + b · μ^2) (X = N or P) regressions of Q_N and Q_P on μ. For Q_N, the linear regression fit the data better for both *Rhodomonas* sp. and *I. galbana*, and the quadratic regression fit better for Q_P in all species.

Table 2-3. Adjusted r^2 values for linear and quadratic regression of cell N and P quotas on the growth rate.

Species	Cell N quota		Cell P quota	
	Linear	Quadratic	Linear	Quadratic
Rhodomonas sp.	**0.723** (12)	0.570 (12)	0.896 (12)	**0.912** (12)
I. galbana	**0.747** (12)	0.660 (12)	0.664 (12)	**0.787** (12)
P. tricornutum	0.911 (12)	**0.925** (12)	0.794 (11)	**0.890** (11)

Numbers in parentheses are the number of observations (*n*). The largest r^2 value for N- or P-deficient cultures is in bold.

Table 2-4. Estimated parameters of *Rhodomonas* sp., *Isochrysis galbana*, and *Phaeodactylum tricornutum* obtained by nonlinear least squares method according to Ågren's functions (equations 2-4 and 2-5).

Species	$Ø_{CN}$		$Ø_{NP}$		β_N		β_P	
	Estimate	*p*	Estimate	*p*	Estimate	*p*	Estimate	*p*
Rhodomonas sp.	8.735 (4.928, 12.542)	<0.001	18.139 (14.179, 22.099)	<0.001	0.108 (0.085, 0.131)	<0.001	0.0021 (0.0017, 0.0024)	<0.001
I. galbana	4.104 (2.419, 5.789)	<0.001	16.006 (10.141, 21.872)	<0.001	0.063 (0.040, 0.086)	<0.001	0.0009 (0.0005, 0.0014)	0.001
P. tricornutum	5.859 (4.570, 7.148)	<0.001	34.409 (25.276, 43.542)	<0.001	0.055 (0.034, 0.076)	<0.001	0.0008 (0.0000, 0.0016)	0.064

Values of $Ø_{CN}$ and $Ø_{NP}$ are expressed as mol · mol^{-1} · d^{-1}. Values of β_N and β_P are expressed as mol · mol^{-1}. Numbers in parentheses are 95% CI. The number of observations (*n*) is 12 for all

species except for *Phaeodactylum tricornutum* at N:P = 63:1, in which $n = 11$. The significance level of the full models is $p < 0.05$.

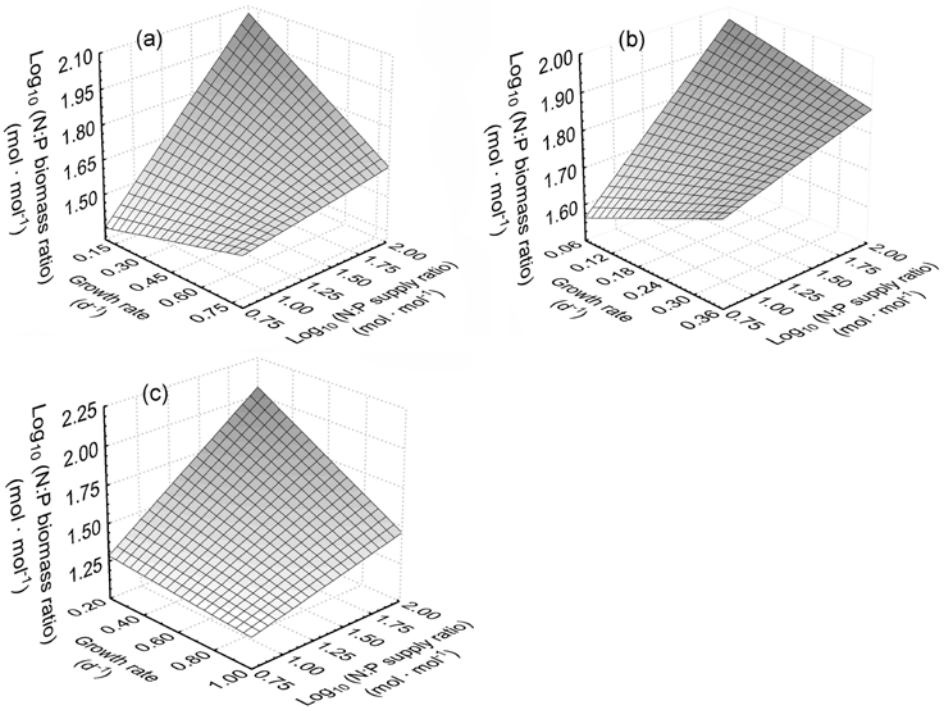

Figure 2-2. N:P biomass ratios as functions of both N:P supply ratios and growth rates. (a) *Rhodomonas* sp., (b) *Isochrysis galbana*, (c) *Phaeodactylum tricornutum*. Data of both N:P biomass ratios and N:P supply ratios were transformed using $\log_{10}(x)$.

Excess uptake of N and P. For both *Rhodomonas* sp. and *P. tricornutum*, Q_N in P-deficient cultures was higher than that in N-deficient cultures (and vice versa for Q_P) (Fig. 2-1, a, b, e, and f). For *I. galbana*, Q_P in N-deficient cultures was only higher than that in P-deficient cultures at lower growth rates, but not at the highest rate (Fig. 2-1d). However, Q_N in P-deficient cultures was slightly higher than that in N-deficient cultures (Fig. 2-1c, Table A1). For all species, both Q_N and Q_P for the replete nutrients varied with growth rate. Especially for *Rhodomonas* sp., there was the largest difference for Q_N and Q_P between limiting and nonlimiting conditions at the lowest growth rate (Fig. 2-1, a and b, Table A1).

Interactive effects of growth rates and N:P supply ratios on N:P biomass ratios. For all species, the quadratic term of either μ (μ^2) or N:P supply ratio ((N:P supply ratio)^2) was not significant (*Rhodomonas* sp., $p > 0.821$; *I. galbana*, $p > 0.469$; *P. tricornutum*, $p > 0.588$) in the multiple regression on N:P biomass ratios. N:P biomass ratios increased with increasing N:P supply ratios across the entire range of dilution rates for all species, but the positive response became weaker as dilution rates increased (Fig. 2-2). According to the multiple regression analysis, the positive relationships between N:P biomass ratios and N:P supply ratios were highly significant in all species ($p < 0.001$) (Table 2-5). Dilution rates had a

positive effect on N:P biomass ratios of both *Rhodomonas* sp. and *I. galbana* at low N:P supply ratios, but the effect became negative with increased N:P supply ratios (Fig. 2-2, a and b), while for *P. tricornutum* this effect was negative across the entire range of N:P supply ratios (Fig. 2-2c). All predictors had significant effects on N:P biomass ratios of *Rhodomonas* sp., with the combined effects of three predictors accounting for 59% (r^2) of the variability (Table 2-5). For *P. tricornutum*, both N:P supply ratios and the interactive term (μ · (N:P supply ratio)) had significant effects on N:P biomass ratios, and all predictors together explained 70% (r^2) of the variability in N:P biomass ratios. Only N:P supply ratios showed significant effects on N:P biomass ratios of *I. galbana*, with the combined effects of three predictors accounting for only 30% (r^2) of the variability.

Table 2-5. Results of multiple regression for N:P biomass ratios of *Rhodomonas* sp., *Isochrysis galbana*, and *Phaeodactylum tricornutum*.

Species	Independent variable	Parameter estimate ± SE	t value	*p*	r^2 (adj.)	*n*
Rhodomonas sp.	N:P supply ratio (NP)	0.641 ± 0.104	6.153	**<0.001**	0.59	59
	Growth rate (μ)	0.757 ± 0.314	2.412	**0.019**		
	NP × μ	-0.691 ± 0.224	-3.087	**0.003**		
I. galbana	N:P supply ratio (NP)	0.366 ± 0.105	3.474	**<0.001**	0.30	60
	Growth rate (μ)	1.118 ± 0.644	1.735	0.088		
	NP × μ	-0.731 ± 0.459	-1.592	0.117		
P. tricornutum	N:P supply ratio (NP)	0.716 ± 0.120	5.944	**<0.001**	0.70	59
	Growth rate (μ)	0.277 ± 0.308	0.897	0.374		
	NP × μ	-0.512 ± 0.220	-2.325	**0.024**		

N:P biomass ratios and N:P supply ratios were transformed using $\log_{10}(x)$. The significance level of the full models is $p < 0.05$. Significant *p* values are shown in bold; *n* is the number of observations.

DISCUSSION

The observed maximal growth rate (μ_{max}). Our result is consistent with Cherif and Loreau's hypothesis (2010) of equal observed (realized) maximal growth rates for all nutrients. This hypothesis assumes that μ_{max} should be equal in most populations under multiple nutrient limitation (essential, nonsubstitutable resources), which has been confirmed by many empirical experiments (Ahlgren 1985, Elrifi and Turpin 1985, Droop 2003, Baek et al. 2008). The μ_{max} is achieved when the cell reaches its production limit, and therefore it is unrelated to the availability of different resources (Cherif and Loreau 2010).

Fitting to the Droop model. The growth of the three phytoplankton species in both N- and P-deficient cultures was in accordance with the well-established Droop Model. This model has been widely used to predict the possible dominant species in various nutrient regimes of aquatic ecosystems (Spijkerman and Coesel 1996, Fujimoto et al. 1997, Ducobu et al. 1998, Spijkerman and Coesel 1998, Yamaguchi et al. 2008). In the Droop model, the parameters (μ_{max}' and Q_0) are used as the basis for evaluation of population dynamics. For example, Ducobu et al. (1998) found that the prediction of the Droop model is in line with the result of competition experiments of P-limited continuous cultures, showing that the prochlorophyte *Prochlorothrix hollandica* is a better competitor for P than the cyanobacterium *Planktothrix agardhii* on the basis of ecophysiological parameters, such as the maximal growth rate, the maximal cell quota for P, and the minimal cell quota for P.

The parameter μ_{max}' is determined by cellular mechanisms, and nutritional and physical factors operating at realized growth rates (Droop 1973), although μ_{max}' is a mathematical abstraction (μ for infinite Q) and it is never reached. Thus μ_{max}' may indicate environmental and physiological effects on phytoplankton. Fujimoto et al. (1997) worked on two blue-green algae, *Microcystis aeruginosa* and *Phormidium tenue*, and they found that μ_{max}' indicates the fitness of phytoplankton for a given set of environmental conditions. In their study, μ_{max}' of *M. aeruginosa* was higher at 30°C and that of *P. tenue* was higher at 20°C for both N- and P-limited growth, indicating that *M. aeruginosa* is better-adapted to high temperature than *P. tenue*. This result corresponds to the seasonal algal succession observed in Lake Kasumigaura, where *M. aeruginosa* dominates only in summer. In our study, under N-deficient conditions, the highest μ_{max}' value was found for *P. tricornutum* and the lowest value was found for *I. galbana*, showing that *P. tricornutum* is best-adapted to N-deficient conditions among the three species. However, in P-deficient conditions, the highest μ_{max}' value was found for *Rhodomonas* sp. and the lowest value was still found for *I. galbana*,

showing that *Rhodomonas* sp. is best-adapted to P-deficient conditions. Our result suggests that the variability of N:P supply ratios may lead to shifts in phytoplankton species composition.

The μ_{max}' value of algal species in N-deficient cultures is higher than the μ_{max}' value of the same species in P-deficient cultures, which has been found in many previous studies (Ahlgren 1985, Elrifi and Turpin 1985, Sommer 1991a, Fujimoto et al. 1997, Liu et al. 2001, Droop 2003), as well as in the present study (Table 2-6). Fujimoto et al. (1997) reported that the μ_{max}' value of *M. aeruginosa* was higher for N-limited growth than that for P-limited growth at both 20°C and 30°C. The μ_{max}' value of *P. tenue* for N-limited growth was higher at 20°C, while this value for P-limited growth was higher as temperature increased to 30°C. In Sommer's study (1991a), two species *Ceratium hirundinella* and *Peridinium* had a higher μ_{max}' value in N-limited cultures, while for *Stephanodiscus* the μ_{max}' value was slightly higher in P-limited cultures. Similarly, in two harmful flagellates *Chattonella antiqua* and *Chattonella ovata*, μ_{max}' values were also higher in P-limited cultures than that in N-limited cultures (Nakamura 1985, Yamaguchi et al. 2008). The comparison in Table 2-6 shows that the μ_{max}' value is higher in 12 of 16 cases under N-deficient conditions, while only in four cases the μ_{max}' value is higher under P-deficient conditions (the chi-square test with Yates' correction, $X^2 = 3.06$, df = 1, $p > 0.05$). In N-deficient conditions, P is replete, in this case most phytoplankton can accumulate P (Flynn 2010). Our study also found a lower N:P biomass ratio N-deficient cultures (Fig. 2-2). Low N:P biomass ratios can reflect increased allocation to P-rich rRNA when the growth rate is high (Elser et al. 2000b). Moreover, many studies have suggested a high correlation between rRNA and growth rate (Binder and Liu 1998, Worden and Binder 2003). Therefore, P sufficiency may allow a higher allocation to P-rich rRNA and thus explain the higher μ_{max}' value in N-deficient conditions. In the present study, we observed a common response of μ_{max}' to nutrient deficiency (a higher μ_{max}' value in N-deficient conditions); however, this finding remains to be tested in further studies.

Table 2-6. Comparison of μ_{max}' in N- and P-deficient conditions for different phytoplankton species.

Species	N or P deficiency	μ_{max}'	Reference
Aureoumbra lagunensis	N-	**1.30**	Liu et al. (2001)[a]
	P-	0.54	
Microcystis aeruginosa	N- (20°C)	**0.84**	Fujimoto et al. (1997)
	P- (20°C)	0.46	
	N- (30°C)	**1.45**	
	P- (30°C)	0.88	
Phormidium tenue	N- (20°C)	**1.31**	Fujimoto et al. (1997)
	P- (20°C)	1.08	
	N- (30°C)	0.59	
	P- (30°C)	**0.91**	
Isochrysis galbana	N-	**0.53**	This study
	P-	0.48	
Rhodomonas sp.	N-	**1.26**	This study
	P-	1.05	
Phaeodactylum tricornutum	N-	**1.26**	This study
	P-	0.91	
Oscillatoria agardhii	N- (N:P = 7)	**0.82**	Ahlgren (1985)[b]
	N- (N:P = 14)	**0.92**	
	P- (N:P = 21)	0.79	
	P- (N:P = 28)	0.59	
Selenastrum minutum	N-	**2.01**	Elrifi and Turpin (1985)
	P-	1.92	
Ceratium hirundinella	N-	**0.32**	Sommer (1991a)[a]
	P-	0.28	
Peridinium (>35μm)	N-	**0.49**	Sommer (1991a)[a]
	P-	0.42	
Stephanodiscus (>20μm)	N-	0.61	Sommer (1991a)[a]
	P-	**0.62**	
Chattonella antique	N-	0.74	Nakamura (1985)
	P-	**0.86**	
Chattonella ovata	N-	0.79	Yamaguchi et al. (2008)

	P-	**0.86**

[a]Values of μ_{max}' were calculated from the data given in references. [b]Values of μ_{max}' were calculated by Droop (2003). Values of μ_{max}' are expressed as d^{-1}. The largest μ_{max}' value in N- or P-deficient culture for each species is in bold.

Q_0 is a useful parameter to apply the Droop model to natural populations of phytoplankton. Sommer (1991a) compared Q_0 values for phytoplankton (separated by a combined technique of size fractionation and density-gradient fractionation) from Schöhsee (Northern Germany) with literature Q_0 data from freshwater algal cultures. The Schöhsee Q_0 data were within the realistic range of Q_0 obtained from literature Q_0 data, which supported the usefulness of the Droop model for field phytoplankton populations. Q_0 values for N and P in freshwater phytoplankton have wide ranges 0.014 to 0.180 mol · mol^{-1} and 0.2 to 20 mmol · mol^{-1}, respectively (Sommer 1988, 1991a, 1991b). In our study, Q_0 values for both N and P are in the ranges of those in Sommer's data compilations. Imai et al. (2006) estimated the warning levels of cell density of red-tide flagellates based on Q_0. The Q_0 values of *C. ovata* for both N and P were 20-30% lower than those of *C. antique*, suggesting that *C. ovata* has a competitive advantage over *C. antique* (Yamaguchi et al. 2008). This result can explain why *C. ovata* has become more conspicuous than *C. antique* in the Seto Inland Sea, where inorganic nutrient concentrations have decreased recently.

Fitting to Ågren's functions. The comparison of adjusted r^2 values in Table 2-3 is consistent with predictions of Ågren's theory (Ågren 2004): the relationship between N cell quota and growth rate is linear whereas that between P cell quota and growth rate is quadratic. Based on the observed rates of the catalysing capacity of Rubisco, Ågren (1985b) postulated a theoretical $Ø_{CN}$ of 23.4 mol · mol^{-1} · d^{-1}. The observed $Ø_{CN}$ value (11.817 mol · mol^{-1} · d^{-1}) for *S. minutum* (Ågren 2004) is lower than the theoretical $Ø_{CN}$ by a factor of 2. Similarly, the observed $Ø_{CN}$ values for the three species in our study are lower than the theoretical $Ø_{CN}$ by a factor of 2 to 5. An estimated value of $Ø_{NP}$ is 2.431 mol · mol^{-1} · d^{-1} calculated from the rate of protein synthesis by ribosomes (Sterner and Elser 2002, Ågren 2004), which is lower than the value of $Ø_{NP}$ for *S. minutum* (18.896 mol · mol^{-1} · d^{-1}) by a factor of 7 and for the three species in the present study by a factor of 6 to 14. The parameter β_N for *S. minutum* is 0.041 mol · mol^{-1}, which is slightly lower than the range of those for *Rhodomonas* sp., *P. tricornutum*, and *I. galbana*. The parameter β_P for *S. minutum* (0.0014 mol · mol^{-1}) is in the range of that for the three species in our study. Thus our study shows that estimated parameters of fitting to Ågren's functions for *Rhodomonas* sp., *P. tricornutum*, and *I. galbana* are consistent with his observed values for *S. minutum*.

The present study shows an agreement of parameters between the Droop model and Ågren's functions: the rank order of Q_0 for N was consistent with that of $Ø_{CN}$ for the three species. Also the lowest values of both Q_0 for P and $Ø_{NP}$ were in *I. galbana*. Klausmeier et al. (2008) interpreted the cell quota as consisting of the nutrient used in cellular structure and machinery (Q_0), and the nutrient stored for further growth (cell quota above Q_0). Furthermore, the cellular structural stoichiometry (Q_0) is described as two types of machinery: uptake machinery (representing (transport) proteins and chloroplasts) and assembly machinery (representing ribosomes) (Klausmeier et al. 2008). In order to interpret the growth of autotroph biophysically, Ågren suggested to use the rate of protein (N-rich) assimilate C ($Ø_{CN}$) and the rate of protein synthesis by ribosomes (N-rich but also a major pool of P) ($Ø_{NP}$) to denote the growth of the organism (Ågren 1985a, Ågren and Bosatta 1998, Ågren 2004). Therefore, the parameters $Ø_{CN}$ and $Ø_{NP}$ indicate the capacity of enzymes (for N) and ribosomes (for P) to convert substrates. The agreement of parameters between the Droop model and Ågren's functions shows that Ågren's functions may offer an explanation of the underlying biochemical principle for the Droop model.

Excess uptake of N and P. The observed Q_N and Q_P under nutrient replete conditions were higher than those under nutrient-deficient conditions, showing the existence of excess uptake (luxury consumption) in *Rhodomonas* sp., *P. tricornutum*, and *I. galbana*. Ågren (2004, 2008) compared observed data from several terrestrial plants and one freshwater alga. He found interspecific differences in the capacity of excess uptake. Excess uptake for N and P were also different among the three species in the present study (Fig. 2-1). For example, *I. galbana* showed a clearly lower excess uptake of P than the other two species. This observation was consistent with the indication from the parameter μ_{max}': having lowest μ_{max}' value *I. galbana* is worst-adapted to the P-deficient condition. Hence, excess uptake of nutrients could be an indication of competitive advantage. Moreover, we observed the growth rate dependency of excess uptake in *Rhodomonas* sp. and *P. tricornutum*, which has also been found in previous studies (Elrifi and Turpin 1985, Ågren 2004, 2008). At lower growth rate, there is a larger span between concentrations of limited and non-limited nutrients (Ågren 2008), and thus excess uptake is higher. At the highest growth rate, there is little or no excess uptake (Elrifi and Turpin 1985, Cherif and Loreau 2010). Therefore, a higher excess uptake at lower growth rates can be an important cause of the larger variability in autotroph stoichiometry.

Interactive effects of N:P supply ratios and growth rates on N:P biomass ratios. Several classic chemostat experiments showed that there is a positive relationship between

N:P biomass ratios of the three algal species and N:P supply ratios (Rhee 1978, Goldman et al. 1979, Ahlgren 1985). Goldman et al. (1979) found that N:P biomass ratios of *Dunaliella tertiolecta* were positively related to N:P supply ratios at any given growth rates, whereas N:P biomass ratios converged to an intermediate value at high growth rates, showing the presence of an optimal ratio required for the maximal growth. A similar result was also found for the growth of the cyanobacterium *Oscillatoria agardhii* in chemostat culture (Ahlgren 1985). The positive relationship between N:P biomass ratios and N:P supply ratios was highly significant for all species in our study. And the N:P biomass ratio converged over the range of N:P supply ratios when the growth rate approaches to the highest value. At lower growth rates, phytoplankton "are what they eat" (Sterner and Elser 2002), with considerable flexibility of N:P biomass ratios over the range of N:P supply ratios. At higher growth rates, phytoplankton "eat what they need" with an optimal nutrient uptake ratio, resulting in the convergence of N:P biomass ratios towards an optimal value independent of the N:P supply ratio (Klausmeier et al. 2004).

In addition to the similarity in the response of N:P stoichiometry to N:P supply ratios, there were also interspecific differences in the response of N:P stoichiometry to growth ratios and the interactive term of growth rates and N:P supply ratios among the three species in our study. At lower N:P supply ratios (N-deficiency), N:P biomass ratios in *P. tricornutum* correlated negatively with growth rates, however this relationship was positive in both *Rhodomonas* sp. and *I. galbana*. Growth rates alone showed significant effects on *Rhodomonas* sp., but not on *P. tricornutum* and *I. galbana*. Growth rates and N:P supply ratios together had significant interactive effects on both *Rhodomonas* sp. and *P. tricornutum*, but not on *I. galbana*.

These findings demonstrate that high dilution rates (loss rates) can explain the limited flexibility of phytoplankton stoichiometry in natural communities, and there are interspecific differences in responses to the interactive effect of N:P supply ratios and growth rates.

CONCLUSIONS

Our results suggest that the response of cellular N and P contents in *Rhodomonas* sp., *P. tricornutum*, and *I. galbana* to growth rates is consistent with the predictions of both the Droop model and Ågren's functions. Ågren's functions explain this relationship from biochemical considerations, which can be an approach to explore underlying principle for

the Droop model. The estimated parameters for both the Droop model and Ågren's functions were consistent with previous studies on freshwater and marine algae, indicating identical principles to explain the change of elemental and biochemical composition of algae across freshwater and marine ecosystems. There were species-specific differences in phytoplankton responses to the interactive effect of N:P supply ratios and growth rates. To better understand the effect of nutrient limitation on the phytoplankton community, as well as on the upper consumers in the food web, we suggest further studies should focus on the response of both elemental and biochemical composition in multiple phytoplankton species to the interactive effect of nutrient supply ratios and growth rates.

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CHAPTER 3

Linking elements to biochemicals: effects of nutrient supply ratios and growth rates on fatty acid composition of phytoplankton species[*]

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ABSTRACT

Three species of phytoplankton, *Rhodomonas* sp., *Isochrysis galbana* Parke, and *Phaeodactylum tricornutum* Bohlin, were cultivated in semicontinuous cultures to test responses of fatty acids (FAs) to five nitrogen (N):phosphorus (P) supply ratios and four growth rates (dilution rates). Characteristic FA profiles were observed for each species (representing particular algal class), which remained relatively stable across the entire ranges of N:P supply ratios and growth rates. For all species, significant direct effects of N:P supply ratios on FAs were found at lower growth rates. For all species, the highest saturated and monounsaturated fatty acid (SFA and MUFA) contents were observed under N deficiency at the lowest growth rate, while polyunsaturated fatty acids (PUFAs) revealed variable responses to N:P supply ratios and growth rates among the three species. Total fatty acids (and SFAs and MUFAs) showed significant negative correlations with N cell quota

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(Q_N) under N deficiency in all species, while PUFAs had species-specific correlations with Q_N. The result shows that characteristic FA profiles of each algal genus or species underlie fluctuations according to culture conditions. The significant correlation between FAs and Q_N under N deficiency suggests that elemental and biochemical limitations of phytoplankton should be considered mutually as determinants of food quality for zooplankton in marine ecosystems.

INTRODUCTION

The transfer of energy and matter across the plant-herbivore interface is of critical importance in aquatic food webs (Lindeman 1942, Brett and Müller-Navarra 1997). The factors regulating the trophic transfer efficiency have been widely studied. Of all limiting factors, elemental and biochemical limitations of phytoplankton have been suggested as major determinants of food quality for zooplankton (Gulati and DeMott 1997, Sterner and Schulz 1998, Anderson et al. 2004, Müller-Navarra 2008). Elemental (especially phosphorus (P)) versus biochemical (especially fatty acids (FAs)) limitation in the diet for zooplankton is a well-known controversy, which has attracted more attention in limnology than in marine ecology (Arts et al. 2009). Moreover, several studies have considered these two limiting factors as mutually non-exclusive mechanisms, especially in freshwater environments (Gulati and DeMott 1997, Lynn et al. 2000, Boersma et al. 2001, Gladyshev et al. 2007). Less is known about the interaction between elemental and biochemical limitations of phytoplankton in marine ecosystems.

Nitrogen (N):P concentrations and supply ratios reveal a strong spatio-temporal variability in coastal seas and some oceanic areas (Karl et al. 1993, Cavender-Bares et al. 2001, Twomey and Thompson 2001, Ford et al. 2008, Lam and Kuypers 2011). Under a large variation in N and P supplies, non-homeostasis of phytoplankton N:P stoichiometry was observed in several classic chemostat experiments (Rhee 1978, Goldman et al. 1979, Ahlgren 1985), as well as in our previous study (Bi et al. 2012), which analysed how the intracellular concentrations (cell quota) of N and P (Q_N and Q_P) vary in dependence of N:P supply ratios and specific growth rates (μ). The result in our previous study shows that the relationship between Q_N (and Q_P) and μ can be interpreted from biochemical considerations (Bi et al. 2012).

FAs are key biochemicals in the regulation of trophic interactions (Müller-Navarra 2008). FAs as basic constituents of lipids play an important role in cellular membrane functions,

energy storage and metabolic processes (Mourente et al. 1990, Roessler 1990, Arts et al. 2009). Certain FAs are essential, because consumers cannot synthesize them or the synthesis rate is not sufficient to meet the basic biochemical needs of consumers (Arts et al. 2001, Kainz et al. 2004, Kelly and Scheibling 2012). Thus, consumers must obtain essential FAs from their diet. Several polyunsaturated fatty acids (PUFAs) are essential for a wide array of animal taxa (Bergé and Barnathan 2005) and have received intense attention, e.g., α-linolenic acid (ALA; C18:3ω3), eicosapentaenoic acid (EPA; C20:5ω3) and docosahexaenoic acid (DHA; C22:6ω3) (Guschina and Harwood 2006, Arts et al. 2009).

The FA composition of phytoplankton is determined by both genotypic and phenotypic characteristics (Dalsgaard et al. 2003). FAs are well-known taxonomic indicators at the class but not at the species level. Dalsgaard et al. (2003) compared the patterns of FA similarities among eight classes of phytoplankton. In their study, the FA composition of each algal class was obtained by pooling FA data of different species from the same algal class. Although this comprehensive comparison shows specific FA markers for each algal class, this method omits the information about potential effects of culture conditions on phytoplankton FA composition. Laboratory studies have shown quantitative fluctuations of phytoplankton FA composition in response to one or two environmental factors (e.g. Piorreck and Pohl 1984, Cohen et al. 1988, Thompson et al. 1990, Ahlgren and Hyenstrand 2003, Piepho et al. 2012). However, little is known about qualitative fluctuations of FA composition either within or between algal species, especially under combined effects of environmental conditions (e.g., nutrient supply) and physiological states of phytoplankton (e.g., growth rate).

Mesocosm experiments conducted in marine (Hopavagen lagoon, Norway), brackish (Kiel Fjord, Germany) and freshwater (Lake Schöhsee, Germany) systems showed that N:P supply ratios influenced FA contents in phytoplankton, as well as the ratio between saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and PUFAs (Brepohl 2005). However, it has been suggested that there is no direct effect of nutrient limitation on FA synthesis of phytoplankton, but rather a direct impact of limited growth rates caused by nutrient limitation (Guschina and Harwood 2009, Piepho et al. 2012). Although Ahlgren and Hyenstrand (2003) reported the interactive effect of N concentrations and growth rates on freshwater algae, no attempts have been made to simultaneously study responses of FA composition in marine phytoplankton to wide ranges of N:P supply ratios and growth rates.

In addition, the use of different units to quantify FA composition in earlier studies makes comparisons difficult, and in some cases may even have resulted in seemingly contradictory findings. The choice of unit depends on the aim of the study. For example, FAs are best quantified on a per cell basis when focusing on cell physiology, while FA data per unit biomass (often measured in carbon content) is an ideal approach when considering food quality of algae for herbivores (Piepho et al. 2012).

In this study, we chose three marine phytoplankton species representing three algal classes, *Rhodomonas* sp. (Cryptophyceae), *Isochrysis galbana* (Prymnesiophyceae) (Parke 1949), and *Phaeodactylum tricornutum* (Bacillariophyceae). All the three species are widely used as diets in zooplankton culture because of their high particular FA contents. We tested responses of FA composition in the three species to five N:P supply ratios and four growth rates. Semicontinuous cultures were used in this study, because this culture system has been suggested as a simpler but equally effective alternative to standard continuous cultures, and thus it has been widely used to control nutritional values of microalgae to feed herbivores (Ferreira et al. 2011). The results in the present study are mainly presented as FA content per carbon, because we focus on the important role of FAs in determining food quality of phytoplankton. FA data are also expressed as a percentage of total fatty acids (TFAs) (FA proportion, % of TFAs) to compare phytoplankton FA profiles with those in previous studies. The following questions were addressed: (i) Does the characteristic FA profile of each species change in response to the large variations of N:P supply ratios and growth rates? (ii) Is there a direct effect of N:P supply ratios on FA composition at the same growth rate in all the three species? (iii) Do FA responses to these two factors vary between the three species? (iv) Do FAs correlate significantly with Q_N and Q_P in all three species?

MATERIALS AND METHODS

Study organisms and culture conditions. For *Rhodomonas* sp., *I. galbana* and *P. tricornutum,* equivalent spherical diameter (ESD), cellular C, N, and P contents are shown in Table 3-1. All cultures were set up at 18 ^{o}C and a salinity of 18 ± 1 psu in a temperature-controlled room. The light intensity was constant at 100 µmol photons · m^{-2} · s^{-1} at a light:dark cycle of 16:8 h. The culture medium was prepared with sterile filtered natural seawater from the Kiel Fjord, Baltic Sea (Sterilizing Grade Filter, Sartobran P 0.2 µm) (Sartorius Stedim Biotech GmbH, Goettingen, Germany) and enrichment nutrient solutions (macronutrients and micronutrients) based on the modified Provasoli's culture medium

(Provasoli 1963, Ismar et al. 2008). Macronutrients were added as sodium nitrate ($NaNO_3$) and potassium dihydrogen phosphate (KH_2PO_4), and dissolved background concentrations were negligible. For the diatom (*P. tricornutum*) culture, also sodium silicate pentahydrate ($Na_2SiO_3 \cdot 5H_2O$) was added at a concentration of 880 µmol · L^{-1}. Each culture was kept in a 1-L Erlenmeyer flask with 500 mL culture volume. All cultures were aerated slightly with filtered air and shaken manually twice per day at a set time. Three replicates were set up for each treatment.

Table 3-1. Equivalent spherical diameter (ESD), cellular carbon, nitrogen and phosphorus contents of *Rhodomonas* sp., *Isochrysis galbana*, and *Phaeodactylum tricornutum* in the experiments.

Species	ESD (µm)	C (ng · $cell^{-1}$)[c]	N (ng · $cell^{-1}$)[c]	P (pg · $cell^{-1}$)[c]
Rhodomonas sp.	9.2[a]	0.046 – 0.106	0.008 – 0.023	0.226 – 1.757
I. galbana	4.8[b]	0.005 – 0.012	0.001 – 0.002	0.026 – 0.081
P. tricornutum	5.4[a]	0.005 – 0.011	0.001 – 0.002	0.022 – 0.235

[a] Algal cells for the ESD calculation were harvested at the early stationary phase in batch cultures with N:P supply ratio = 24 (mol · mol^{-1}) in this study. [b] Arndt and Sommer (2013). [c] Data of cellular carbon, nitrogen and phosphorus contents are shown in a range of values from different treatments.

Table 3-2. N:P supply ratios and concentrations in culture media.

N:P supply ratio (mol · mol^{-1})	N concentration (µmol · L^{-1})	P concentration (µmol · L^{-1})
10:1	352	36
14:1	498	36
24:1	880	36
35:1	880	25
63:1	880	14

Experimental setup. First, batch culture experiments were performed for each algal species under five N:P supply ratios (Table 3-2). The observed maximal growth rate (μ_{max}) was estimated from cell number changes during the exponential growth phase (Bi et al. 2012).

Once batch cultures reached the early stationary phase, semicontinuous cultures were started with four specific growth rates (µ, d^{-1}) for each N:P supply ratio, which were 20%,

40%, 60%, and 80% of μ_{max}. The equivalent daily renewal rate (D, d^{-1}) can be estimated by $D = 1- e^{-\mu \cdot t}$, where t is renewal interval (d) (here t = 1d). Renewal of the cultures was carried out at the same hour every day. The steady state in semicontinuous cultures was assessed based on the net growth rate (r). When r was zero (at steady state), µ was equivalent to D.

Sample analysis. For each treatment replicate, one sample was taken for analysis. To avoid the effects of diel variations and subsequent variability in the data, sampling was carried out during the same hour as the daily renewal of the cultures. Algal cell density was counted daily using an improved Neubauer hemacytometer (Glaswarenfabrik Karl Hecht GmbH, Rhön, Germany). For chemical analysis, algal cells (1 to 8 mL algal culture, depending on cell density) were harvested at steady state by filtration on pre-combusted Whatman GF/F filters (Whatman GmbH, Dassel, Germany). After filtration, samples for elemental analysis were immediately dried and stored in a desiccator, and samples for FA analysis were frozen at -80 ^{o}C.

The determination of particulate organic carbon (POC) and nitrogen (PON) was carried out after Sharp (1974) by gas chromatography in an organic elemental analyzer (Thermo Flash 2000) (Thermo Fisher Scientific Inc., Schwerte, Germany). Particulate organic phosphorus (POP) was analyzed colorimetrically by converting organic phosphorus compounds to orthophosphate (Hansen and Koroleff 1999).

FAs were measured as fatty acid methyl esters (FAMEs) using a gas chromatograph (Trace GC-Ultra) (Thermo Fisher Scientific Inc., Schwerte, Germany) according to the procedure described in detail in Arndt and Sommer (2013). The FAME mixture C13:0, C15:0, C17:0, C19:0 and C21:0 was added as internal standard, and tricosanoic acid (C23:0) added as esterification control. The extracted FAs were dissolved with n-hexane to a final volume of 100 µL. Sample aliquots (1 µL) were given into the GC by splitless injection with hydrogen as the carrier gas. Individual FAs were integrated using Chromcard software (Thermo Fisher Scientific Inc., Schwerte, Germany) and identified with reference to commercially available standards, Supelco 37 component FAME mixture and Supelco Menhaden fish oil.

Statistics. Principal coordinates analysis (PCO) was performed to visualize FA composition (expressed as % of TFAs and µg · mg C^{-1}, respectively) of the three algal species under the entire range of N:P supply ratios and growth rates. PCO of FA composition was calculated from the Bray-Curtis similarity resemblance matrix. The raw data matrix was square root transformed. A vector overlay was applied on the PCO plot to

identify FA components responsible for differences among the three species based on Spearman's correlation ($r > 0.5$).

At each growth rate, the effect of N:P supply ratios on the content of each FA group (TFAs, SFAs, MUFAs or PUFAs) and main individual PUFA (ALA, EPA or DHA) was tested for each algal species using one factorial analysis of variance (ANOVA). The same analysis was done for the effect of growth rates on the content of each FA group and individual PUFA under each N:P supply ratio. In the latter analysis, data for the contents of individual PUFAs were ln (x) transformed. A post-hoc test (Tukey HSD) was applied only if there were significant effects. The magnitude of effect (ω^2 = (effect sum of squares – effect degree of freedom × error mean square) / (total sum of squares + error mean square)) was calculated only for the significant factors. This estimate can determine the variance in a response variable and relates this to the total variance in the response variable (Graham and Edwards 2001, Hughes and Stachowicz 2009).

The relationship between FA contents (FA groups and main individual PUFAs) and cell quotas (Q_N and Q_P) was tested under the extremely N- and P-deficient conditions (N:P supply ratios = 10:1 and 63:1) using linear regression analyses. Data for Q_N and Q_P were published in our previous study (Bi et al. 2012).

We compared FA profiles of the algal genus (*Rhodomonas*) and species (*I. galbana* and *P. tricornutum*) in the present study with those of the same genus and species in the literature. All FA data were expressed as % of TFAs. Multidimensional scaling (MDS) and cluster analysis were conducted based on Bray-Curtis similarity resemblance matrix. The raw data matrix was square root transformed.

PCO, MDS and cluster analysis were performed using the PERMANOVA+ add-on package to the PRIMER v6 software program (Clarke and Gorley 2006). ANOVA and linear regressions were conducted in Statistica 8 (StatSoft [Europe] GmbH, Hamburg, Germany). Significance level was set to $p < 0.05$ in all statistical tests.

RESULTS

FA profiles of the three algal species. FA profiles varied between the three algal species. Table A2, A3 and A4 show FA composition (expressed as $\mu g \cdot mg\ C^{-1}$ and % of TFAs) for *Rhodomonas* sp., *I. galbana*, and *P. tricornutum* under five N:P supply ratios and four growth rates, respectively.

Under the entire ranges of N:P supply ratios and growth rates, SFAs were most abundant in *I. galbana* (37 to 52% of TFAs) compared to *Rhodomonas* sp. (15 to 30%) and *P. tricornutum* (19 to 29%). Also, SFAs were the dominant FA group (compared to MUFAs and PUFAs) in *I. galbana*, which was mainly due to high amounts of C14:0 and C16:0. C16:0 was also the major individual SFA in *Rhodomonas* sp. The proportion of C16:0 in *P. tricornutum* was slightly higher than that of C14:0.

The proportion of MUFAs in *Rhodomonas* sp. (11 to 15%) was lower than those in the other two species (18 to 30% in *I. galbana*, 16 to 37% in *P. tricornutum*). Compared to SFAs and PUFAs, MUFAs were the least abundant FA group in *Rhodomonas* sp. The most abundant individual MUFA was different between the three species, C18:1ω7 for *Rhodomonas* sp., C18:1ω9 for *I. galbana*, and C16:1ω7 for *P. tricornutum*.

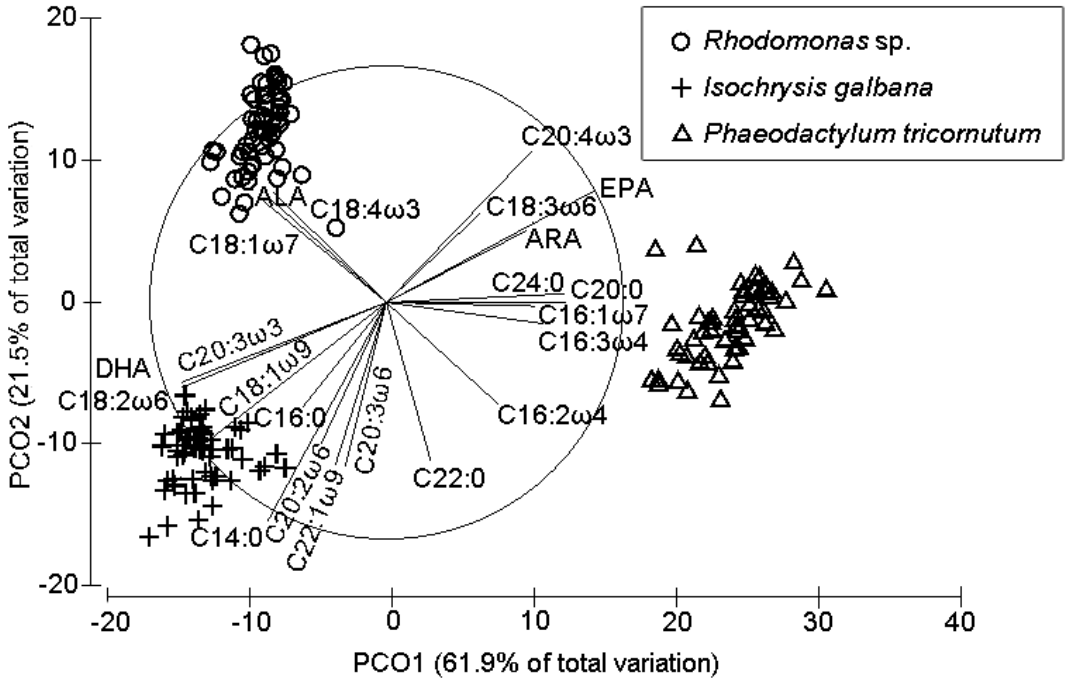

Figure 3-1. Principal coordinates analysis (PCO) of fatty acid composition (μg · mg C^{-1}) of *Rhodomonas* sp., *Isochrysis galbana*, and *Phaeodactylum tricornutum* under the entire range of growth rates and N:P supply ratios. The circle has a radius 1.0, and the length and direction of each vector indicates the strength and sign of the relationship between each FA component and the PCO axes. α-linolenic acid (ALA; C18:3ω3); arachidonic acid (ARA; C20:4ω6); eicosapentaenoic acid (EPA; C20:5ω3) and docosahexaenoic acid (DHA; C22:6ω3).

PUFAs were most abundant in *Rhodomonas* sp. (56 to 77%) compared to *I. galbana* (23 to 42%) and *P. tricornutum* (34 to 64%). The high PUFA proportion in *Rhodomonas* sp. was caused by high amounts of ALA (19 to 25%), C18:4ω3 (8 to 12%), EPA (5 to 13%), and DHA (5 to 8%). PUFAs in *I. galbana* were mainly represented by C18:2ω6 (5 to 8%)

and DHA (6 to 11%). For *P. tricornutum*, EPA (21 to 38%) was the most abundant PUFA. The ratios of both C16:1ω7/C16:0 and EPA/DHA in *P. tricornutum* were higher than one (Table A4).

Results of PCO were similar for FA content and FA proportion, and that for FA content is shown in Fig. 3-1. There was a clear separation of the three species, with two axes explaining 83.4% of the total variation. *P. tricornutum* was clearly separated from *Rhodomonas* sp. and *I. galbana* along the first axis (61.9% of the total variation). *Rhodomonas* sp. and *I. galbana* were separated from each other along the second axis (21.5% of the total variation). The vector overlay on the PCO plot shows a characteristic FA profile for each species. C16:1ω7, C16:3ω4, C20:0 and C24:0 (indicative of *P. tricornutum*) and, in the opposite direction, C18:2ω6, C20:3ω3 and DHA (indicative of *Rhodomonas* sp. and *I. galbana*) explained most of the PCO1 pattern. In addition, C18:1ω7, ALA and C18:4ω3 (indicative of *Rhodomonas* sp.) in a positive direction and C14:0, C16:0, C18:1ω9 (indicative of *I. galbana*) in a negative direction explained the PCO2 pattern to a large degree.

***Responses of the FA composition in Rhodomonas* sp.** The contents of all FA groups (TFAs, SFAs, MUFAs and PUFAs) decreased with increasing N:P supply ratios at lower growth rates (20% and 40% of μ_{max}) (Fig. 3-2a). N:P supply ratios showed significant effects on all FA groups at the lowest growth rate (20% of μ_{max}) (ANOVA, $p \leq 0.023$), with N:P supply ratios accounting for 49 to 75% of the variation (ω^2). Also, all FA groups responded significantly to growth rates under N:P = 10:1 (N deficiency) (ANOVA, $p \leq 0.011$), and growth rates explained 61 to 81% of the variation. All FA groups showed significant higher contents under N:P = 10:1 at the lowest growth rate (Tukey HSD test, $p \leq 0.024$).

ALA, EPA and DHA were considered as the most important PUFAs in *Rhodomonas* sp. due to their high abundance and nutritional values. The contents of ALA and EPA decreased with increasing N:P supply ratios at growth rates of 20%, 40% and 60% of μ_{max}, while the content of DHA showed no clear change (Fig. 3-3). N:P supply ratios had significant effects on the contents of ALA (at the lowest growth rate, 20% of μ_{max}) (ANOVA, $p = 0.020$) and EPA (at lower growth rates, 20% and 40% of μ_{max}) (ANOVA, $p \leq 0.022$), but not on DHA. N:P supply ratios explained 49 to 92% of the variation in ALA and EPA. At the lowest growth rate, the content of ALA was significantly higher under N:P = 10:1 (Tukey HSD test, $p \leq 0.039$). Similar responses were also observed for EPA (Tukey HSD test, $p \leq 0.014$).

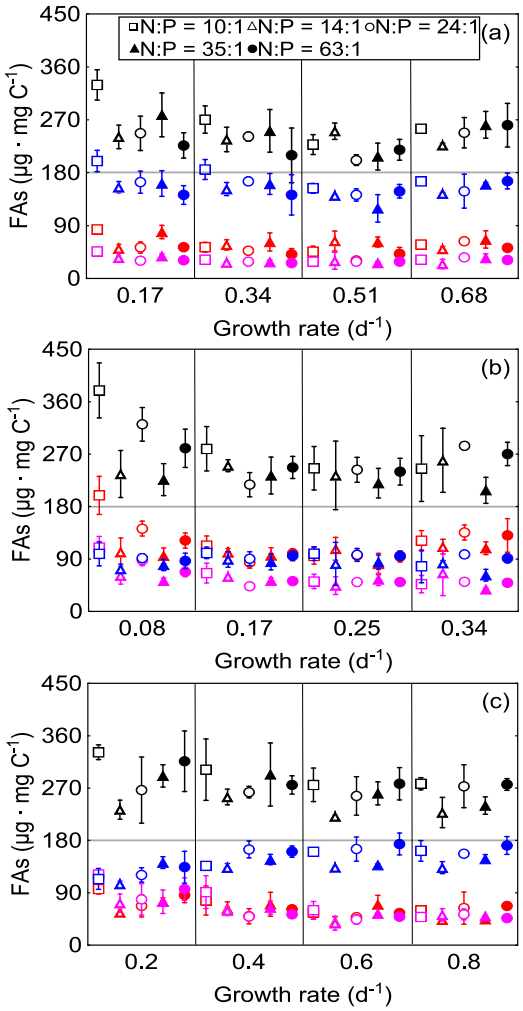

Figure 3-2. The contents of total fatty acids (black), saturated fatty acids (red), monounsaturated fatty acids (purple) and polyunsaturated fatty acids (blue) (mean ± SD) as functions of N:P supply ratios and growth rates for (a) *Rhodomonas* sp., (b) *Isochrysis galbana*, and (c) *Phaeodactylum tricornutum*. Broken lines make comparison easier.

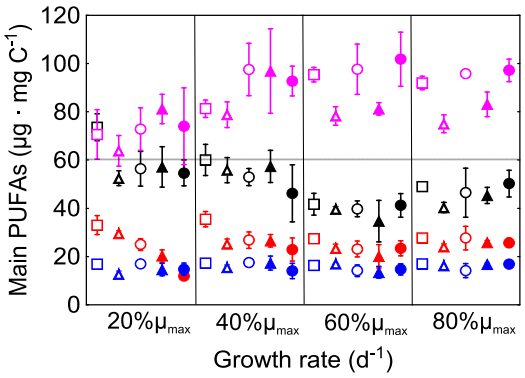

Figure 3-3. The contents of main individual polyunsaturated fatty acids (mean ± SD) as functions of N:P supply ratios and growth rates for α-linolenic acid (ALA) (black), eicosapentaenoic acid (EPA) (red), and docosahexaenoic acid (DHA) (blue) in *Rhodomonas* sp., and EPA in *Phaeodactylum tricornutum* (purple). Symbols as shown in Fig. 3-2. The broken line makes comparison easier.

Under different N:P supply ratios, ALA, EPA and DHA responded significantly to growth rates: ALA under N:P = 10:1, 14:1 and 35:1 (N and P deficiency) (ANOVA, $p \leq 0.011$), EPA under N:P = 10:1, 14:1 and 63:1 (N and P deficiency) (ANOVA, $p \leq 0.026$), and DHA under N:P = 14:1 (N deficiency) (ANOVA, $p = 0.014$) (Fig. 3-3). Growth rates explained 61 to 86% of the variation in the three individual PUFAs. ALA contents were significantly higher at lower growth rates under each of the three N:P supply ratios (Tukey HSD test, $p \leq 0.022$). The response of EPA to growth rates changed with N:P supply ratios, showing significant higher contents at 20% and 40% of μ_{max} under N:P = 10:1 and 14:1 (N deficiency) (Tukey HSD test, $p \leq 0.032$) but significant lower contents at 20% of μ_{max} under N:P = 63:1 (P deficiency) (Tukey HSD test, $p \leq 0.003$). DHA contents were significantly lower at the lowest growth rate under N:P = 14:1 (Tukey HSD test, $p \leq 0.035$).

Responses of the FA composition in Isochrysis galbana. Three FA groups TFAs, SFAs and MUFAs showed reduced contents with increasing N:P supply ratios at lower growth rates (Fig. 3-2b). N:P supply ratios had significant effects on the three FA groups at the lowest growth rate (ANOVA, $p \leq 0.004$), with N:P supply ratios explaining 66 to 74% of the variation. At the lowest growth rate, the contents of the three FA groups were significantly higher under N:P = 10:1 (Tukey HSD test, $p \leq 0.046$). PUFAs and the main individual PUFA (DHA) showed no significant response to N:P supply ratios.

Growth rates had significant effects on TFAs, SFAs and MUFAs under different N:P supply ratios (ANOVA, $p \leq 0.026$), with growth rates explaining 56 to 91% of the variation. The contents of the three FA groups were significantly higher at the lowest growth rate under N:P = 10:1 and 24:1 (N deficiency and balanced nutrient condition) (Tukey HSD test, $p \leq 0.017$). MUFAs also showed significant higher contents under N:P = 63:1 (Tukey HSD test, $p \leq 0.038$). No significant effect of growth rates was observed on PUFAs or DHA.

Responses of the FA composition in Phaeodactylum tricornutum. Similar to *Rhodomonas* sp. and *I. galbana*, SFAs and MUFAs in *P. tricornutum* showed reduced contents with increasing N:P supply ratios at lower growth rates (Fig. 3-2c). N:P supply ratios showed significant effects on SFAs and MUFAs at the lowest growth rate (ANOVA, $p \leq 0.045$), with N:P supply ratios explaining 41% and 55% of the variation for SFAs and MUFAs, respectively. At the lowest growth rate, SFAs and MUFAs had significant higher contents under N:P = 10:1 (Tukey HSD test, $p < 0.05$). N:P supply ratios showed no significant effect on TFAs, PUFAs or the main individual PUFA (EPA). However, the

contents of both PUFAs and EPA showed an increasing trend with increasing N:P supply ratios at lower growth rates (Fig. 3-2c for PUFAs, Fig. 3-3 for EPA).

Growth rates showed significant impacts on SFAs, MUFAs and PUFAs under different N:P supply ratios (ANOVA, $p \leq 0.046$) (Fig. 3-2c). Around 49 to 78% of the variation was associated with growth rates. Under N:P = 10:1, the content of SFAs was significantly higher at the lowest growth rate (Tukey HSD test, $p \leq 0.044$). MUFAs showed similar responses to growth rates under N:P = 10:1, 14:1, and 63:1 (N and P deficiency) (Tukey HSD test, $p \leq 0.034$). In contrast, the PUFA content increased with increasing growth rates under each N:P supply ratios. Under N:P = 10:1, 14:1, and 24:1 (N deficiency and balanced nutrient condition), PUFAs showed significant lower contents at the lowest growth rate (Tukey HSD test, $p \leq 0.036$). EPA showed a similar response to growth rats under N:P = 10:1 (Tukey HSD test, $p \leq 0.014$) (Fig. 3-3).

FAs versus Q_N and Q_P. Linear regression analyses showed significant negative correlations between TFAs (as well as SFAs and MUFAs) and Q_N ($p \leq 0.003$) under N:P = 10:1 (Fig. 3-4, a-c for *Rhodomonas* sp., e-g for *I. galbana*, and i-k for *P. tricornutum*). However, no significant correlation was observed between any FA group and Q_P under N:P = 63:1 in all species. Correlations between PUFAs and Q_N were different among the three species, negative in *Rhodomonas* sp. ($p = 0.003$) (Fig. 3-4d), positive in *P. tricornutum* ($p = 0.008$) (Fig. 3-4l), and no significant correlation in *I. galbana* (Fig. 3-4h).

ALA and EPA in *Rhodomonas* sp. had different correlations with Q_N and Q_P, showing a negative correlation between ALA and Q_N under N:P = 10:1 ($p < 0.001$) (Fig. 3-5a), but a positive one between EPA and Q_P under N:P = 63:1 ($p = 0.020$) (Fig. 3-5b). No significant correlation was found between EPA and Q_N, ALA and Q_P, or DHA and Q_N (and Q_P) in *Rhodomonas* sp. For *I. galbana*, DHA showed no significant correlation with either Q_N or Q_P. EPA in *P. tricornutum* correlated positively with Q_N under N:P = 10:1 ($p = 0.012$) (Fig. 3-5c) but showed no significant correlation with Q_P under N:P = 63:1.

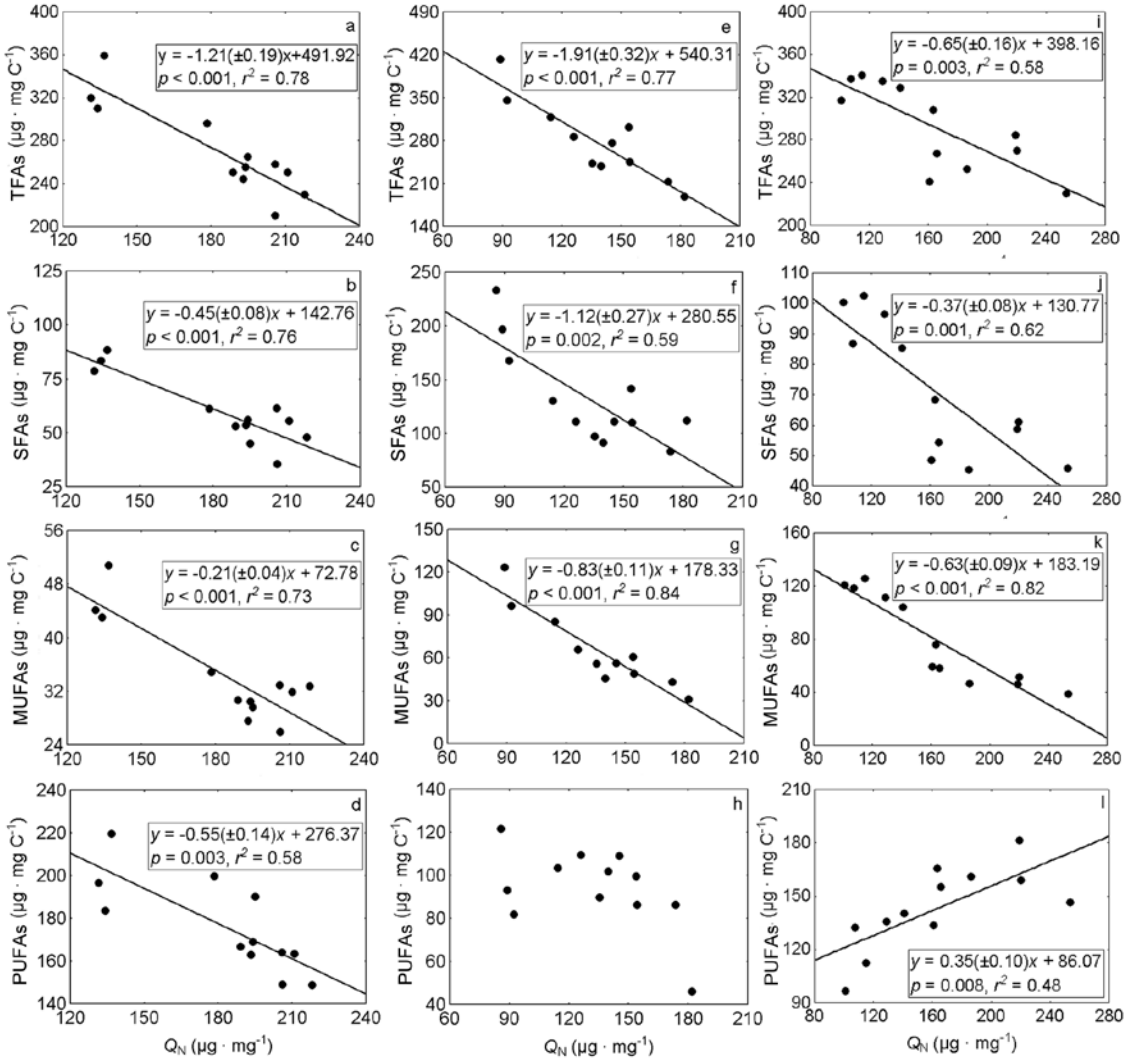

Figure 3-4. Linear regressions between fatty acid groups (total fatty acids (TFAs) saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs) (mean ± SD)) and N cell quota (Q_N) under the extremely N-deficient condition (N:P = 10:1) for (a-d) *Rhodomonas* sp., (e-h) *Isochrysis galbana*, and (i-l) *Phaeodactylum tricornutum*. Data for Q_N are from Bi et al. (2012); only significant regressions are shown.

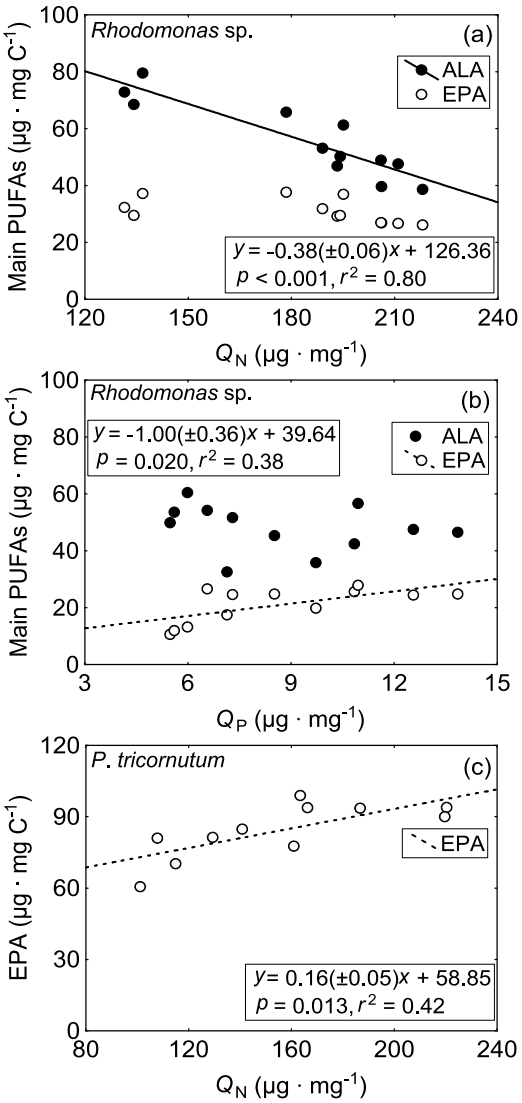

Figure 3-5. Linear regressions between main individual polyunsaturated fatty acids (PUFAs) (mean ± SD) and N and P cell quotas (Q_N and Q_P) for (a and b) α-linolenic acid (ALA) and eicosapentaenoic acid (EPA) in *Rhodomonas* sp., and (c) EPA in *Phaeodactylum tricornutum*. Data for Q_N and Q_P are from Bi et al. (2012); only significant regressions are shown.

DISCUSSION

Characteristic FA profiles of algal classes. It is well established that FA profiles are often similar between species of the same algal class but show characteristic differences between classes (Dalsgaard et al. 2003). *Rhodomonas* as a representative genus in cryptophytes is widely used as zooplankton diets in aquatic studies, e.g., *Rhodomonas lens* and *Rhodomonas* sp. (Parrish et al. 2012), and *Rhodomonas salina* (Broglio et al. 2003, Veloza et al. 2006). This is mainly due to its high PUFA content, especially ALA and EPA, which was also observed in *Rhodomonas* sp. in the present study. *I. galbana* is known as a oleaginous species with a capacity to accumulate neutral lipids, mainly triacylglycerols

(TAGs) that are generally characterized by SFAs and MUFAs in algae (Guschina and Harwood 2009). The high level of SFAs is a characteristic FA pattern in Prymnesiophytes (Brown et al. 1997), which was also shown in *I. galbana* in the present study. The presence of C16:1ω7 and EPA, as well as high ratios of C16:1ω7/C16:0 and EPA/DHA (typically >1), are considered as biomarkers for diatom-dominated plankton communities (Reuss and Poulsen 2002, Kelly and Scheibling 2012). This class-specific FA composition was also found in *P. tricornutum* in the present study.

The clear separation of three algal species in the present study demonstrates a relatively unique and stable FA composition in each species (representing particular algal class) under the wide ranges of N:P supply ratios and growth rates. Furthermore, we compared FA composition (% of TFAs) of the algal genus (*Rhodomonas*) or species (*I. galbana* or *P. tricornutum*) in the present study with those in the literature. In this comparison, most of the cited papers (nine of 12 papers) were published during the last ten years (from 2002 to 2012), and only one citation (Mourente et al. 1990) was included in the analysis in Dalsgaard et al. (2003). Culture conditions vary greatly among different studies. For example, *Rhodomonas* sp. in Renaud et al. (2002) was cultured under a light:dark cycle of 12:12h at the temperature of 25 to 35 °C and a salinity of ca. 25 psu, *R. salina* in Chen et al. (2011) under a light:dark cycle of 14:10h at 17 °C and 34 psu, and *Rhodomonas* sp. in the present study under a light:dark cycle of 16:8h at 18 °C and 18 psu. The outcome of the comparison is visualized in Fig. 3-6, which shows not only a clear separation between *Rhodomonas*, *I. galbana* and *P. tricornutum* but also great similarities (75%) within each genus or species. This result is in agreement with our suggestion above and further indicates the characteristics and relative stability of FA profile in each algal genus or species (representing particular algal class) under highly variable culture conditions.

Moreover, the comparison in Fig. 3-6 shows differences in FA composition within each algal genus or species between different studies. For example, FA composition of *P. tricornutum* in Jiang and Gao (2004) and Breuer et al. (2012) are clearly different from those in other studies (Fig. 3-6). Consistent with this, previous studies have shown that lipid or FA composition in phytoplankton varied quantitatively under different culture conditions (Ben-Amotz et al. 1985, Harrison et al. 1990, Roessler 1990, Brown et al. 1996, Malzahn et al. 2010). These findings above indicate the importance of simultaneous consideration of qualitative and quantitative fluctuations of FA composition in phytoplankton in response to variable culture conditions.

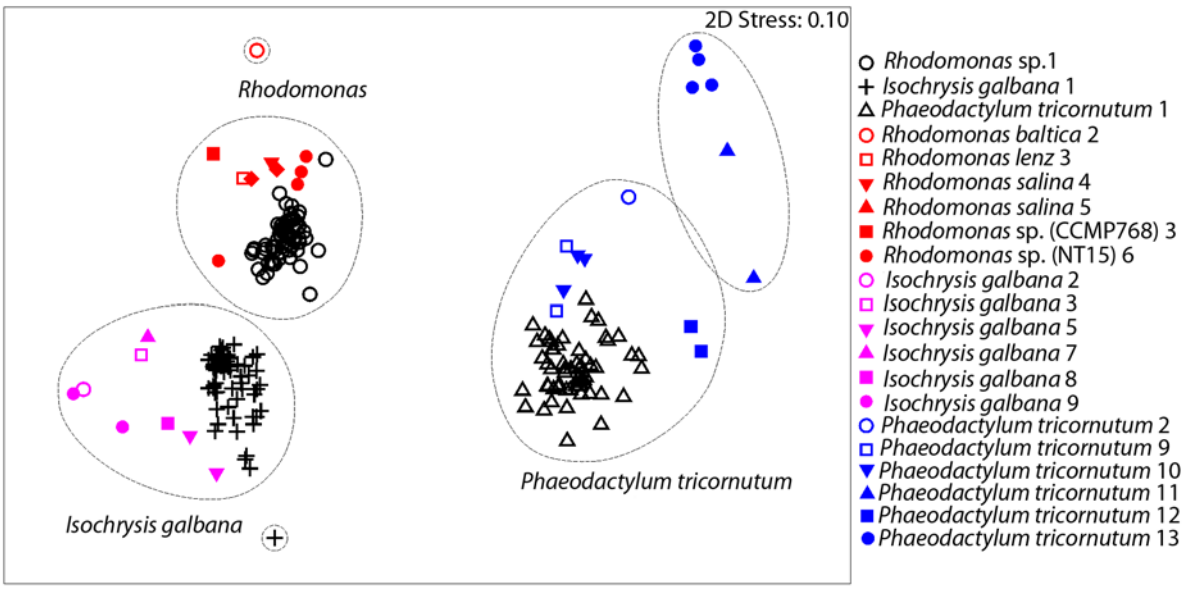

Figure 3-6. Multidimensional scaling plot with Bray-Curtis similarity obtained by cluster analysis of fatty acid composition (% of total fatty acids) in phytoplankton. The data shown were taken from both the present study and values found in the literature. The grouping marked by broken lines represents 75% similarity. Numbers following species names indicate cited publications. 1 this study, 2 Patil et al. (2007) (data are calculated from the data given in this publication), 3 Parrish et al. (2012), 4 Broglio et al. (2003), 5 Chen et al. (2011), 6 Renaud et al. (2002), 7 George et al. (2008), 8 Mourente et al. (1990), 9 Reitan et al. (1994), 10 Liang et al. (2006), 11 Breuer et al. (2012), 12 Alonso et al. (1998) and 13 Jiang and Gao (2004).

Effects of N:P supply ratios on FA composition of phytoplankton. Of all nutrients evaluated, N limitation is suggested as the single most critical effect on lipid metabolism in algae (Hu et al. 2008). In general, lipids, mainly TAGs, are accumulated under N limitation (Ben-Amotz et al. 1985, Thompson 1996). SFAs and MUFAs as major components in TAGs can be also elevated under N limitation (Roessler 1990). Malzahn et al. (2010) reported that contents of TFAs, SFAs and MUFAs in *R. salina* increased under the N-depleted condition. A similar result was also found in cyanobacterium *Synechococcus* sp. at certain growth rates (Ahlgren and Hyenstrand 2003). The results in the present study are consistent with these reports mentioned above, showing significant higher contents of SFAs and MUFAs in all three algal species under the lowest N:P supply ratio at lower growth rates. This indicates that the accumulation of SFAs and MUFAs, as well as the potential increased content of TAGs, might be triggered by the extremely N-deficient condition at lower growth rates in the three algal species, which can be used to store carbon and energy to support growth when conditions improve (Dunstan et al. 1993).

Responses of PUFAs to N deficiency revealed no consistent pattern among the three species in the present study, showing significant higher PUFA, ALA and EPA contents in *Rhodomonas* sp., relatively lower PUFA and EPA contents in *P. tricornutum*, and no clear response of PUFAs in *I. galbana* at lower growth rates. Similar to *Rhodomonas* sp., *R. salina* in Malzahn et al. (2010) also had higher PUFA contents under the N-depleted condition. In general, PUFAs are important components of cellular membrane lipids (Guschina and Harwood 2009). However, TAGs in some microalgae has been found to be a depot of PUFAs under stressful conditions (e.g., N starvation and the stationary growth phase), which can be mobilized for growth at favourable conditions (Cohen et al. 2000, Khozin-Goldberg et al. 2002). The capacity of marine phytoplankton to incorporate ω3-PUFAs into TAGs is found to vary among not only algal species but also algal growth phases (Tonon et al. 2002). This may contribute to variation in PUFA responses to N deficiency among algal species in this study. Based on our results, responses of PUFAs associated with TAGs to nutrient supply are suggested to be addressed in future studies.

Responses of PUFAs to P deficiency also varied among algal species in the present study, with markedly lower PUFA, ALA and EPA contents in *Rhodomonas* sp., relatively higher PUFA and EPA contents in *P. tricornutum*, and no clear response in *I. galbana* at lower growth rates. Harrison et al. (1990) also reported species-specific responses of PUFAs to P starvation, showing a reduced amount of DHA in both *Chaeotoceros calcitrans* and *Thalassiosira pseudonana* and a reduced EPA only in *T. pseudonana*. In contrast, a higher EPA content was observed in marine flagellate *Pavlova lutheri* under higher N:P supply ratios (P deficiency) (Carvalho et al. 2006). These findings further reveal highly variable responses of PUFAs in different phytoplankton species under P deficiency. As mentioned above, PUFAs are important membrane lipid components (Guschina and Harwood 2009). Phospholipids as a group of main membrane lipids are major biochemical reservoirs of P in marine plankton (Van Mooy et al. 2009). Thus, the inhibition of phospholipid synthesis under P deficiency may explain the reduced PUFA content in phytoplankton, e.g., the significant reduced EPA in *Rhodomonas* sp. in the present study. Furthermore, non-phosphorus lipids, phospholipid substitutions, are recently suggested as fundamental biochemical mechanisms to maintain phytoplankton growth in response to P limitation (Van Mooy et al. 2009). Van Mooy et al. (2009) found that marine phytoplankton showed different ability to substitute the non-phosphorus membrane lipids for the phospholipids. Thus, further studies concerning the regulation of phospholipid and phospholipid

substitutions are highly recommended to explore variation in PUFA responses to P deficiency among phytoplankton species.

The results discussed above suggest that the association of PUFAs with different types of lipids, e.g., TAGs, phospholipids and phospholipid substitutions, should be considered in studies of lipid biosynthesis in response to nutrient supply. Moreover, advanced analytical techniques, e.g., high performance liquid chromatography/electrospray ionization-mass spectrometry (HPLC/ESI-MS), have been recently used to improve the identification of different types of lipids in the ocean (Van Mooy et al. 2006, Van Mooy et al. 2009, Van Mooy and Fredricks 2010). In conjunction with the advent of advanced techniques, our results will provide important empirical data for further studying responses of lipid biosynthesis of phytoplankton in changing oceans.

***Effects of growth rates on FA composition of phytoplankton*.** In the present study, significant effects of N or P deficiency on FAs in the three species were only observed at lower growth rates (20% or 40% of μ_{max}). It has been suggested that nutrient limitation does not have direct effects on FA synthesis of phytoplankton, but a consequence of a limited growth rate leads to FA changes (Piepho et al. 2012). However, our study showed significant responses of FAs to N or P deficiency at the same growth rate in all three algal species, while effects of N and P deficiency became non-significant when growth rate increased. Our previous study demonstrated that high dilution rate (loss rate) could explain the limited flexibility of phytoplankton stoichiometry in natural communities (Bi et al. 2012). Thus, the optimal nutrient uptake ratio of phytoplankton at higher growth rates may explain the optimal N:P biomass ratios, as well as the relative stability of FA contents, irrespective of N:P supply ratios.

It is commonly accepted that total lipid content increases with decreasing growth rate (Borowitzka 1988, Sterner and Hessen 1994). This is probably due to the low requirement for synthesis of protein and instead a steady accumulation of lipid, mainly TAGs, when growth slows down (Siron et al. 1989, Reitan et al. 1994, Arts et al. 2009). FA accumulation at lower growth rates has been found for several algal species in previous studies (e.g. Reitan et al. 1994, Otero and Fábregas 1997, Ferreira et al. 2011, Spijkerman and Wacker 2011). Also, in the present study TFAs contents in both *Rhodomonas* sp. and *I. galbana* were relatively higher at lower growth rates. Non-significant response of TFAs in *P. tricornutum* can be explained by the decreasing PUFA content as a compensation for the increasing SFA and MUFA contents when growth rate decreased.

The relationship between FAs and Q_N (and Q_P) under N (and P) deficiency. In the present study, the relationship between FAs and Q_N (Q_P) was tested only under the extremely N (P)-deficient conditions. The reason is that we focus on the potential limitation of elemental and biochemical composition of phytoplankton as the determinant of food quality under nutrient deficiency. Our results revealed strong correlations between FAs and Q_N under N deficiency in all three species, while only EPA in *Rhodomonas* sp. correlated significantly with Q_P under P deficiency. As mentioned above, phospholipids are one of major biochemical reservoirs of P in marine plankton (Van Mooy et al. 2009). Thus, the complex regulation of membrane lipid biosynthesis (e.g., phospholipids versus phospholipid substitutions) may explain the lack of common correlation between FAs and Q_P under P deficiency in the three species in the present study. This hypothesis remains to be tested in further research.

For all species in the present study, TFAs (as well as SFAs and MUFAs) showed significant negative relationship with Q_N under N deficiency. This further indicates the increase in the protein synthesis and the decrease in the synthesis of storage lipids when Q_N increases in all three species. In contrast, the relationship between PUFAs and Q_N revealed species-specific patterns under N deficiency, i.e., negative in *Rhodomonas* sp., positive in *P. tricornutum*, and the lack of significant relationship in *I. galbana*. The significant relationship between PUFAs and Q_N in *Rhodomonas* sp. and *P. tricornutum* suggests the possible use of algal N content as the predictor of food quality. However, this relationship is species-specific, which indicates that algal N content as the predictor of food quality can be only used within each algal species but not in a mixed-species assemblage under N deficiency. This indication is in principle consistent with Müller-Navarra's suggestion (1995) of algal P content as a good predictor of food quality within one algal species. More recently, Hartwich et al. (2012) suggested that EPA concentrations can be estimated from phytoplankton biomass, while a separation of phytoplankton groups should be considered in the community with a high diversity of phytoplankton.

While algal P content was suggested as a predictor of food quality by Müller-Navarra (1995), algal N content is suggested in the present study. Müller-Navarra (1995) conducted experiments with freshwater algae *Scenedesmus acutus* and *Cyclotella meneghiniana*, while three species of marine phytoplankton were tested in the present study. Thus different aquatic systems, with distinct prevailing patterns of nutrient availability and ratios, may explain the differing roles of respective nutrients for food quality shown by Müller-Navarra (1995) and in our present study. Our knowledge of food quality for zooplankton is based

mainly on studies on *Daphnia* species, which is known to have higher P requirements, and thus be more often P-limited than other freshwater zooplankton species (Gulati and DeMott 1997). Moreover, N_2 fixation by cyanobacteria is much more likely in freshwater ecosystems than in marine ecosystems (Conley et al. 2009) (but Elser et al. 2007). These findings mentioned above may lead to a more often P-deficient than N-deficient condition and thus a good relationship between PUFAs and POP for primary producers in a lake.

The relationship between FAs and Q_N shows that elemental and biochemical properties of phytoplankton covary under N deficiency. The incorporation of two properties is important for studying the limitation of food quality on zooplankton via bottom-up processes. On the other hand, the lack of common correlation between FAs and Q_P in the present study might be an evidence of dominant non-phosphorus lipids in response to P deficiency in some species of marine phytoplankton. Although these two aspects are out of the scope of the present study, our results can be very useful for further research on lipid biosynthetic mechanisms, as well as the energy and matter transfer in food webs.

CONCLUSIONS

The present study first examined the influence of highly variable chemical conditions (N:P supply ratios) and biological conditions (growth rates) on biochemical outcome (FA composition) in three species of marine phytoplankton. The FA profile of each algal species (representing particular algal class) remained relatively unique and stable across the wide ranges of N:P supply ratios and growth rates. FA contents in all species significantly varied with N:P supply ratios at lower growth rates, while the flexibility of FA contents was constrained at higher growth rates. Moreover, our results provide the first experimental demonstration of the covariance of FAs and Q_N in three species of marine phytoplankton under N deficiency. This suggests the importance of simultaneous consideration of elemental and biochemical limitations of phytoplankton food quality in aquatic food webs.

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CHAPTER 4

Effects of food quantity and food quality on elemental and biochemical trophic transfer in marine plankton: an experimental approach*

*Rong Bi*** *and Ulrich Sommer*

ABSTRACT

We conducted laboratory experiments to examine the effects of food quantity and stoichiometric food quality on the transfer of elements and essential fatty acids between the marine phytoplankton *Rhodomonas* sp. and the calanoid copepod *Acartia tonsa*, and the reproductive response of *A. tonsa*. The relative gross growth efficiency for carbon (C) and X (K_C / K_X) (X = nitrogen (N) or phosphorus (P)) responded negatively to algal C:X ratio but positively to food concentration under nutrient deficient and food quantity limited conditions. This resulted in higher K_C / K_N and K_C / K_P under optimized food conditions (balanced nutrient diets at high food concentrations). Similar responses were observed for the relative trophic transfer efficiency of ω3- (and ω6-) polyunsaturated fatty acids (PUFAs) and C. Egg production rate of *A. tonsa* responded significantly to food quantity and stoichiometric food quality, showing higher values under optimized food conditions. These results suggest both elemental and essential PUFA trophic transfer can well predict copepod

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reproduction. Our study indicates the interactive effect of food quantity and stoichiometric food quality on essential chemical trophic transfer in marine plankton, which may predict the performance of consumers and trophic transfers at higher trophic levels in marine systems.

INTRODUCTION

Heterotrophic metazoans regulate their chemical composition more strictly than do autotrophs, while the latter usually exhibits great flexibility in chemical composition reflecting ambient conditions (Sterner and Hessen 1994, Sterner and Elser 2002). This ecological imbalance between food and consumers has great influence on the performance of consumers, which may ultimately affect the whole aquatic systems (Anderson et al. 2004, Aubert et al. 2013).

Marine and freshwater phytoplankton is frequently limited by nitrogen (N) and phosphorus (P) (Hecky and Kilham 1988, Sommer 1996, Elser et al. 2007). More recently, anthropogenic activities have been reported to induce the imbalance between carbon (C), N and P in earth's life system (Peñuelas et al. 2012). As a result, phytoplankton C:N:P stoichiometry would shift from the Redfield C:N:P ratio (Peñuelas et al. 2012). Nutrient limitation was found to travel up the food chain (Boersma et al. 2008), and thus the utilization efficiencies of phytoplankton chemical composition for zooplankton production have been considered critically in studying the flows of C and nutrients in food webs (Anderson and Hessen 1995, Kuijper et al. 2004).

The conversion efficiency by which an ingested substance is converted to predator biomass is defined as the "gross growth efficiency" (GGE) (Sterner and Elser 2002). Both empirical and modelling studies have demonstrated that GGE of zooplankton varies with prey algal C:N ratio (Checkley 1980, Kiørboe 1989, Jones et al. 2002, Kuijper et al. 2004, Anderson et al. 2012) and food concentration (Hamburger and Boētius 1987, Santer and van den Bosch 1994, Straile 1997, Anderson et al. 2004, Almeda et al. 2010). Furthermore, the variability of GGE may also be caused by other factors such as temperature, species composition, and copepod developmental stage (Straile 1997, Almeda et al. 2010). However, lack of knowledge of the interactive effect of two or more factors on GGE for multiple nutrients prevents further understanding of GGE regulation in natural conditions.

Besides elements, numerous biochemicals (e.g. certain fatty acids (FAs)) are essential because they cannot be synthesized *de novo* by consumers or the synthesis rate is not

sufficient to meet the basic biochemical needs of consumers (Wacker and von Elert 2001). Thus, phytoplankton biochemicals can be another major determinant of food quality (Sterner and Schulz 1998, Müller-Navarra et al. 2004, Sommer et al. 2012). Some polyunsaturated fatty acids (PUFAs), especially ω3- and ω6-PUFAs, are essential for all animals (Müller-Navarra 2008). Effects of PUFAs on growth and reproduction have been observed for different copepod species (Jónasdóttir et al. 1995, Müller-Navarra et al. 2000, Arendt et al. 2005, Chen et al. 2012). However, it is also evident that in some situations the availability of PUFAs in phytoplankton is high enough for fulfilling zooplankton's requirements. In this case, elemental rather than essential FA regulation can predict the limitation of egg production (Augustin and Boersma 2006, Mayor et al. 2009). Elemental and biochemical limitations have been considered mutually in regulating phytoplankton food quality, especially in freshwater environments (Gulati and DeMott 1997, Lynn et al. 2000, Boersma et al. 2001, Gladyshev et al. 2007). This non-exclusive mechanism is supported by our previous research, which showed significant covariance of elemental (N cell quota) and biochemical (FAs) composition in three marine phytoplankton species (Bi et al. unpublished data). Both elemental and biochemical composition of phytoplankton have shown good correlations with copepod reproduction (Jónasdóttir 1994). However, it is hard to distinguish correlation from causation, and the direct versus indirect effect of food quality, because indirect elemental limitation "may include factors such as changes in phytoplankton cell physical properties and/or biochemical composition" (Ravet and Brett 2006).

Recent research has found that the transfer efficiency of essential PUFAs from the producers to the primary consumers was about two times higher than that of bulk C, while a lower transfer efficiency was shown in non-essential PUFAs (Gladyshev et al. 2011). This suggests that the comparison of trophic transfer efficiencies between essential PUFAs and C might explain zooplankton performance better than simply comparing FA contents between phytoplankton and zooplankton.

In the present study, we investigated the interactive effect of food quantity and stoichiometric food quality on chemical trophic transfer between marine phytoplankton and zooplankton, and further determined the nutritional importance of these factors by measuring the egg production rate of zooplankton. *Rhodomonas* sp. (Cryptophyceae) was chosen as food source because of its high PUFA contents. We chose the calanoid copepod *Acartia tonsa* as model copepod because the adults do not build up large energy storage pools but rather invest most assimilated energy into egg production (Diekmann et al. 2009), thus making egg production a relatively fast response to feeding conditions. The objectives

of our study are to test: (i) the interactive effect of food quantity and stoichiometric food quality on the relative GGE for C and N (and P); (ii) the interactive effect of food quantity and stoichiometric food quality on the relative trophic transfer efficiency of essential PUFAs and C; (iii) whether the trophic transfer of elements and essential biochemicals could predict reproductive responses of zooplankton to dietary nutrient conditions.

MATERIALS AND METHODS

Phytoplankton cultures. *Rhodomonas* sp. (Cryptophyceae) (equivalent spherical diameter 4.6 µm) was isolated from Kiel fjord and used as the copepod diet in this study. Cultures were maintained in sterile filtered natural seawater (Sterilizing Grade Filter, Sartobran P 0.2 µm) (Sartorius Stedim Biotech GmbH, Goettingen, Germany) with additional macronutrients and micronutrients based on the modified Provasoli's culture medium (Provasoli 1963, Ismar et al. 2008). Macronutrients were added as sodium nitrate ($NaNO_3$) and potassium dihydrogen phosphate (KH_2PO_4). Cultures were set up at 18 ^oC and a salinity of 18 ± 1 psu in a temperature controlled room. The light intensity was constant at 100 µmol photons $\cdot$ m^{-2} $\cdot$ s^{-1} at a light:dark cycle of 16:8 h. Cultures were kept in 1 L Erlenmeyer flasks with 500 mL culture volume. All flasks were aerated slightly with filtered air and shaken manually twice per day at a set time.

Table 4-1. Nutrient treatments, growth rate and N:P supply ratios and concentrations in *Rhodomonas* sp. cultures.

Nutrient treatment	Growth rate (d^{-1})	N:P supply ratio (mol $\cdot$ mol^{-1})	N concentration (µmol $\cdot$ L^{-1})	P concentration (µmol $\cdot$ L^{-1})
N deficiency at low growth rate	0.17 (20% of μ_{max})	10:1	352	36
P deficiency at low growth rate	0.17 (20% of μ_{max})	63:1	880	14
Balanced nutrient at high growth rate	0.68 (80% of μ_{max})	24:1	880	36

Around 20 days prior to the copepod feeding experiment, batch culture experiments were started under three N:P supply ratios, N deficiency (N:P = 10:1), balanced nutrient condition (N:P = 24:1), and P deficiency (N:P = 63:1) (Table 4-1). The observed maximal growth rate (μ_{max}) was calculated from cell number changes during the exponential growth phase in

batch cultures (Bi et al. 2012). Once batch cultures reached the early stationary phase, semicontinuous cultures were started with two different specific growth rates (μ, d^{-1}), 20% of μ_{max} for N and P deficiency, and 80% of μ_{max} for the balanced nutrient condition (Table 4-1). The equivalent daily renewal rate (*D*, d^{-1}) can be estimated by $D = 1 - e^{-\mu \cdot t}$, where *t* is renewal interval (d) (here *t* = 1d). The steady state in semicontinuous cultures was assessed based on the net growth rate (*r*). When *r* was zero (at steady state), μ was equivalent to *D*.

These three nutrient treatments were determined according to chemical characteristics of *Rhodomonas* sp. observed in our previous studies (Bi et al. 2012 and Bi et al. unpublished data). On the one hand, the stoichiometric N:P ratio of *Rhodomonas* sp. showed the strongest positive response to N:P supply ratios under the lowest growth rate, while N:P biomass ratios converged to an intermediate value at the highest growth rate (Bi et al. 2012). On the other hand, a significant higher PUFA content was observed in *Rhodomonas* sp. under N deficiency (N:P = 10:1) at the lowest growth rate (Bi et al., unpublished data in CHAPTER 3).

Algal semicontinuous cultures were grown three times corresponding to the three runs of copepod chemical response experiments (with different food concentration setup in each experimental run). Two replicates were set up for each treatment in the first and second semicontinuous cultures, while the last one was run in triplicate.

Algal cell density was counted daily using an improved Neubauer hemacytometer (Glaswarenfabrik Karl Hecht GmbH, Rhön, Germany). The culture suspension replaced by fresh medium every day was collected to feed copepods. Before feeding copepods, algal suspensions from replicate flasks were pooled. Thus, copepod feeding was not influenced by variance between the algal culture replicates and copepod replicates were real replicates receiving identical food.

Copepod cultures. The calanoid copepod, *Acartia tonsa*, was obtained from the Department of Biology, Institute for Hydrobiology and Fisheries Science at the University of Hamburg. *A. tonsa* eggs were hatched in filtered natural seawater (Sterilizing Grade Filter, Sartobran P 0.2 μm) (Sartorius Stedim Biotech GmbH, Goettingen, Germany) at 18 ^{o}C and a salinity of 18 ± 1 psu. Copepod cultures were maintained under the same temperature, salinity and light regime as those used in algal cultures. Before the cohort reached late copepodite stages (CV or CVI), the culture was fed with *Rhodomonas* sp. *ad libitum*.

Copepod chemical response experiment. To examine the effects of food concentration and food quality on copepod chemical composition, copepod individuals (stage CV or CVI)

were placed in 1 L Erlenmeyer flasks with 800 mL culture volume (ca. 500 individuals · L^{-1}) under different food treatments (Table 4-2). All three experimental runs were performed with N-deficient, P-deficient, and balanced nutrient food combined with different levels of food concentration. Copepod cultures were maintained under the same ambient conditions, as well as the same culture medium as those used in the cultures of their diet throughout the experiment. All experiments were run in duplicate.

Table 4-2. Actual initial food concentrations (calculated from the corresponding nominal food concentrations and algal carbon contents) (μg C · L^{-1}) in different treatments in copepod chemical response experiments and egg production rate experiments.

	N deficiency, low growth rate	P deficiency, low growth rate	Balance, high growth rate
Chemical response experiment	41.6[a]	41.9[a]	29.9[b]
	114.6[b]	133.9[b]	59.8[b]
	237.9[c]	278.0[c]	176.9[c]
	475.9[c]	419.2[a]	353.9[c]
	916.5[b]	1059.6[b]	1257.8[a]
	2079.2[a]	2096.1[a]	1914.5[b]
Egg production rate experiment[a]	415.8	419.2	314.4
	2079.2	2096.0	1257.8

[a, b, and c]Copepods were fed with diets from experiment run 1, 2, and 3, respectively.

Copepods were acclimated to different food treatments for four days prior to measuring chemical composition and egg production rate. During this acclimation period, the culture medium for copepods was renewed daily. Variations in food concentration between daily adjustments were usually less than 40% of the target level. All cultures were aerated slightly with filtered air and shaken manually twice per day at a set time. After the acclimation period, copepods were harvested for chemical analysis. Adult males and females were picked out for egg production experiment.

In multinutrient models, the gross growth efficiency (GGE) of egg production for an element is determined by the stoichiometric ratio of the elements in the zooplankton versus that in the food (Anderson and Hessen 1995). The ratio of GGE for C and N (or P) is calculated according to the modified equation suggested by Anderson and Hessen (1995):

$$\frac{K_C}{K_X} = \frac{\theta_Z}{\theta_f} \quad (4\text{-}1)$$

where K_C is gross growth efficiency for C. K_X is gross growth efficiency for N or P (K_N or K_P). For the relative GGE of K_C / K_N, θ_Z and θ_f are the ratios of C and N (mol · mol^{-1}) in the zooplankton and food biomass, respectively. For the relative GGE of K_C / K_P, θ_Z and θ_f are the ratios of C and P (mol · mol^{-1}) in the zooplankton and food biomass, respectively.

Egg production experiment. The adults used in egg production experiment were sorted from two food concentration treatments of the experimental run 1 after the acclimation period. Five females and two males were placed into a Plexiglass chamber (10 cm in height, 5 cm diameter) with a 250 μm mesh 3 cm above the bottom. Each chamber was placed within a 500 mL bottle filled with a total of 450 mL culture volume (filtered seawater and food). This resulted in ca. 137 mL of water inside each chamber. The 250 μm mesh allowed the eggs but not copepod adults to pass through and thus eliminated the possibility of egg cannibalism. The food concentration treatments are shown in Table 4-2. Eight or ten replicates were set up for each food treatment except for the lowest food concentration treatment under N deficiency, in which there were only five replicates. All cultures were maintained under the same ambient conditions, as well as the same culture medium as those used in algal cultures throughout the experiment. After 24h, the eggs in each bottle were collected with a 40 μm mesh and counted using a Bogorov tray.

Chemical analysis. One sample was taken for analysis from each replicate. Algal cells (at steady state) and adult copepods (after acclimation period) were harvested by filtration on pre-combusted Whatman GF/F filters (Whatman GmbH, Dassel, Germany). After filtration, samples for elemental analysis were immediately dried and stored in a desiccator, and samples for FA analysis were frozen at -80 ^{o}C.

The determination of particulate organic carbon (POC) and nitrogen (PON) was carried out after Sharp (1974) by gas chromatography in an organic elemental analyzer (Thermo Flash 2000) (Thermo Fisher Scientific Inc., Schwerte, Germany). Particulate organic phosphorus (POP) was analyzed colorimetrically by converting organic phosphorus compounds to orthophosphate (Hansen and Koroleff 1999).

FAs were measured as fatty acid methyl esters (FAMEs) using a gas chromatograph (Trace GC-Ultra) (Thermo Fisher Scientific Inc., Schwerte, Germany) according to the procedure described in detail in Arndt and Sommer (2013). The FAME mixture C13:0, C15:0, C17:0, C19:0 and C21:0 was added as internal standard, and tricosanoic acid (C23:0) added as esterification control. The extracted FAs were dissolved with n-hexane to a final volume of 100 μL. Sample aliquots (1 μL) were given into the GC by splitless injection

with hydrogen as the carrier gas. Individual FAs were integrated using Chromcard software (Thermo Fisher Scientific Inc., Schwerte, Germany) and identified with reference to commercially available standards, Supelco 37 component FAME mixture and Supelco Menhaden fish oil.

Statistics. Dependent variables were tested for normality and homogeneity of variances. A transformation was performed if normality and homogeneity were not fulfilled.

One-factorial analysis of variance (ANOVA) was conducted to test the effects of experimental run and nutrient treatment on stoichiometric ratios of C:N and C:P, and on the content of each FA group (total fatty acids (TFAs), saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) or PUFAs) in *Rhodomonas* sp, respectively. For *A. tonsa*, two-factorial ANOVA was used to test the effects of nutrient treatment and food concentration on stoichiometric C:N and C:P ratios, the contents of four FA groups, and egg production rates. Data for algal C:P ratio and copepod egg production rate were square root transformed, and those for copepod stoichiometric ratios and FA group contents were $\log_{10}(x)$ transformed. A post-hoc test (Tukey's honest significant difference (HSD) test) was applied only if there were significant effects. The magnitude of effect (ω^2 = (effect sum of squares – effect degree of freedom × error mean square) / (total sum of squares + error mean square)) was calculated only for the significant factors. This estimate can determine the variance in a response variable and relates this to the total variance in the response variable (Graham and Edwards 2001, Hughes and Stachowicz 2009).

The responses of the relative GGE of K_C / K_X to algal C:X ratio and food concentration were analyzed by multiple regression with the second-order quadratic equation, with the predicting variables algal C:X ratio, food concentration, (algal C:X ratio)^2, (food concentration)^2, and (algal C:X ratio) · (food concentration). The same analysis was done for the effects of algal C:X ratio and food concentration on the ratios of ω3- (and ω6-) PUFA contents between copepods and algae. In all multiple regression analyses, dependent and independent variables were $\log_{10}(x)$ transformed.

All statistic analyses were conducted in Statistica 8 (StatSoft [Europe] GmbH, Hamburg, Germany). Significance level was set to $p < 0.05$ in all statistical tests.

RESULTS

Stoichiometric composition of Rhodomonas sp. Stoichiometric ratios of C:N or C:P in *Rhodomonas* sp. showed no significant differences between the three experimental runs

(ANOVA, $p \geq 0.853$). Nutrient treatment showed highly significant effects on both C:N and C:P ratios (ANOVA, $p < 0.001$), accounting for ca. 92% of the variance.

The average C:N and C:P ratios of the three experimental runs are shown in Fig. 4-1. The C:N ratio in *Rhodomonas* sp. was highest (ca. 9.5 mol · mol^{-1}) under N deficiency at the low growth rate (Tukey HSD test, $p \leq 0.014$) (Fig. 4-1a). The lowest C:N ratio (ca. 5 mol · mol^{-1}) was observed under the balanced nutrient and high growth rate condition. The C:P ratio was highest (ca. 440 mol · mol^{-1}) under P deficiency at the low growth rate, and the lowest one (ca. 120 mol · mol^{-1}) was found under the balanced nutrient condition (Tukey HSD test, $p \leq 0.037$) (Fig. 4-1b).

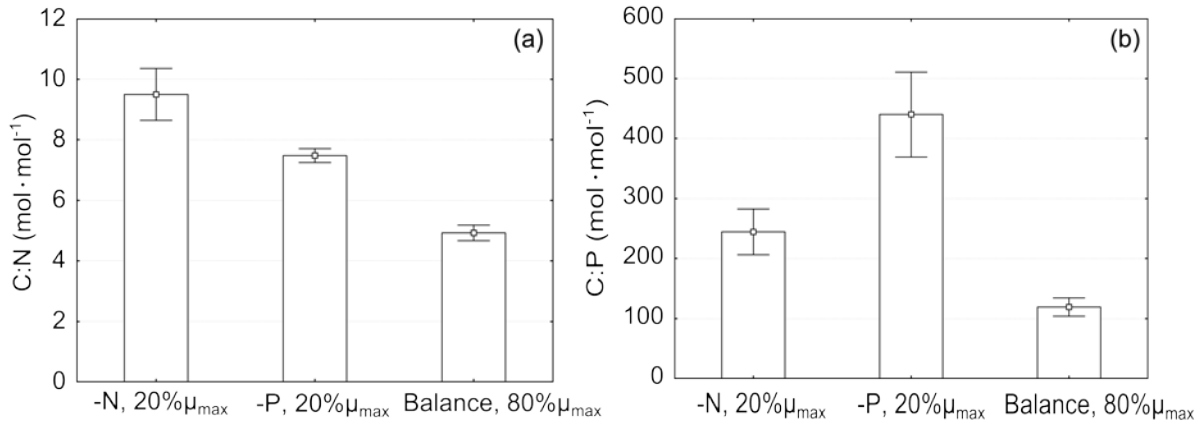

Figure 4-1. Mean (± SD) values of C:N (a) and C:P (b) ratios of *Rhodomonas* sp. under N and P deficiency (-N and -P) with low growth rate (μ = 20% of μ_{max}), and the balanced nutrient condition with high growth rate (μ = 80% of μ_{max}).

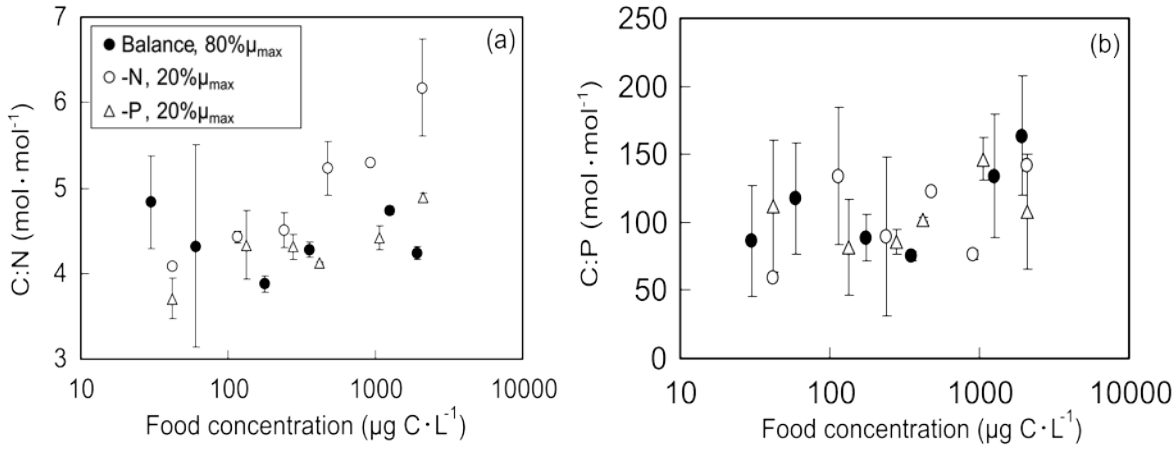

Figure 4-2. Mean (± SD) values of C:N (a) and C:P (b) ratios of adult *Acartia tonsa* feeding on *Rhodomonas* sp. under different food quantity and food quality (the balanced nutrient and fast growing diet (μ = 80% of μ_{max}), and N- and P-deficient (-N and -P) and slow growing diets (μ = 20% of μ_{max})). Note the log scale is used.

Stoichiometric response of Acartia tonsa. The C:N ratios of *A. tonsa* responded significantly to both single and interactive effects of food concentration and nutrient treatment (ANOVA, $p \leq 0.035$). Food concentration, nutrient treatment, and the interactive term ([Food concentration] · [nutrient treatment]) explained 21%, 17%, and 20% of the variance, respectively.

When feeding on the N-deficient and slow growing diet, the C:N ratios of *A. tonsa* (mean ± SD) increased with increasing food concentrations, ranging from 4.1 at the lowest food concentration to 6.2 ± 0.6 mol · mol^{-1} at the highest food concentration (Fig. 4-2a). Similarly, when feeding on the P-deficient and slow growing diet the lowest C:N ratio (3.7 ± 0.2 mol · mol^{-1}) was at the lowest food concentration, and the highest one (4.9 ± 0.04 mol · mol^{-1}) at the highest food concentration. However, no clear trend was observed for the C:N ratios of *A. tonsa* feeding on the balanced nutrient and fast growing diet, with a range of 3.8 ± 0.1 to 4.8 ± 0.5 mol · mol^{-1}. In comparison among nutrient treatments, the C:N ratios of *A. tonsa* feeding on the N-deficient and slow growing diet differed significantly from those feeding on the P-deficient and balanced nutrient diets (Tukey HSD test, $p \leq 0.004$). Especially at higher food concentrations (> 350 μg C · L^{-1}), the C:N ratios of *A. tonsa* feeding on the N-deficient diet were ca. 1.3 times higher than those feeding on other two diets.

There was no significant effect of food concentration or nutrient treatment on the C:P ratios (Fig. 4-2b). The C:P ratios of *A. tonsa* ranged from 75.3 ± 3.3 to 163.6 ± 43.7 mol · mol^{-1} when feeding on the balanced nutrient diet, from 59.2 to 142.1 mol · mol^{-1} on the N-deficient diet, and from 81.2 ± 35.4 to 146.5 ± 15.6 mol · mol^{-1} on the P-deficient diet.

In multiple regression analyses, all independent variables showed significant effects on the relative GGE of K_C / K_N (Table 4-3). K_C / K_N correlated negatively with both algal C:N ratio and food concentration. However, the quadratic terms of algal C:N ratio ([algal C:N ratio]^2) and food concentration ([food concentration]^2), and the interaction term ([algal C:N ratio] · [food concentration]) showed significant positive effects on K_C / K_N. The value of K_C / K_N decreased with increasing algal C:N ratios at lower food concentrations (Fig. 4-3a). But this negative response became weaker as food concentration increased, and unimodal responses were observed at higher food concentrations. The relationship between K_C / K_N and food concentration also changed with algal C:N ratio, showing positive at higher algal C:N ratios (P- and N-deficient diets) but unimodal at lower C:N ratios (the balanced nutrient diets).

Table 4-3. Results of multiple regression analyses for the ratio of gross growth efficiency for carbon and nitrogen (K_C / K_N), the ratio of gross growth efficiency for carbon and phosphorus (K_C / K_P), the ratio of ω3-PUFA contents between *Acartia tonsa* and food source (ω3-PUFA Z/f), and the ratio of ω6-PUFA contents between *A. tonsa* and food source (ω6-PUFA Z/f).

Dependent variable	Independent variable	Parameter estimate ± SE	*t*	*p*	r^2 (adj.)	*n*
K_C / K_N	Algal C:N ratio (C:N)	-4.84 ± 1.14	-4.26	**<0.001**	0.88	35
	Food concentration (C_f)	-0.56 ± 0.13	-4.40	**<0.001**		
	$(C:N)^2$	1.84 ± 0.67	2.75	**0.010**		
	$(C_f)^2$	0.06 ± 0.02	2.62	**0.014**		
	C:N × C_f	0.39 ± 0.11	3.51	**0.001**		
K_C / K_P	Algal C:P ratio (C:P)	-1.05 ± 0.10	-10.59	**<0.001**	0.77	34
	C_f	0.09 ± 0.04	2.05	**0.049**		
ω3-PUFA Z/f	C:N	0.24 ± 0.31	0.78	0.440	0.37	34
	C_f	0.28 ± 0.06	4.54	**<0.001**		
ω3-PUFA Z/f	C:P	0.27 ± 0.13	2.00	0.054	0.44	34
	C_f	0.27 ± 0.06	4.63	**<0.001**		
ω6-PUFA Z/f	C:N	-14.83 ± 7.08	-2.10	**0.045**	0.50	34
	C_f	-2.76 ± 0.83	-3.33	**0.002**		
	$(C:N)^2$	4.67 ± 4.15	1.12	0.270		
	C_f^2	0.21 ± 0.14	1.50	0.144		
	C:N × C_f	2.23 ± 0.70	3.19	**0.003**		
ω6-PUFA Z/f	C:P	-8.98 ± 5.08	-1.77	0.088	0.38	34
	C_f	-2.36 ± 0.94	-2.52	**0.018**		
	$(C:P)^2$	1.41 ± 1.06	1.33	0.196		
	C_f^2	0.16 ± 0.16	1.03	0.313		
	C:P × C_f	0.72 ± 0.28	2.61	**0.014**		

All dependent variables and independent variables (algal C:N ratio, algal C:P ratio and food concentration) were transformed using $\log_{10}(x)$. The significant level of the full models is $p < 0.05$. Significant *p* values are shown in bold; *n* is the number of observations.

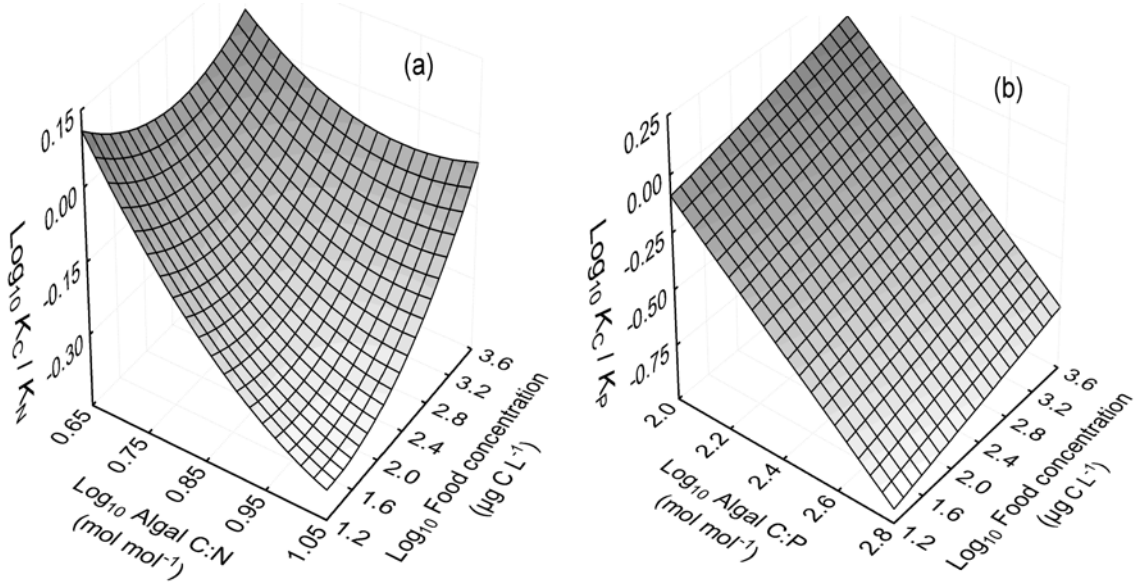

Figure 4-3. The ratios of gross growth efficiency for carbon and nutrient (K_C / K_X) (here, X = N or P) as functions of algal C:X ratio and food concentration. (a) The ratio of gross growth efficiency for carbon and nitrogen (K_C / K_N) as functions of algal C:N ratio and food concentration, and (b) The ratio of gross growth efficiency for carbon and phosphorus (K_C / K_P) as functions of algal C:P ratio and food concentration. Data for K_C / K_X, algal C:X ratio and food concentration were transformed using $\log_{10}(x)$.

For the relative GGE of K_C / K_P, multiple regression without the quadratic terms ([algal C:P ratio]^2 and [food concentration]^2) and the interaction term ([algal C:P ratio] · [food concentration]) showed the best model fit (Table 4-3). K_C / K_P correlated negatively with algal C:P ratio, but positively with food concentration (Fig. 4-3b).

Fatty acid composition of Rhodomonas sp. No FA groups (TFAs, SFAs, MUFAs or PUFAs) in *Rhodomonas* sp. showed significant differences between the three experimental runs (ANOVA, $p \geq 0.090$). Nutrient treatment showed significant effects on all FA groups except for SFAs (ANOVA, $p = 0.004$ for TFAs; $p = 0.237$ for SFAs; $p < 0.001$ for both MUFAs and PUFAs), accounting for 38 %, 56% and 47% of the variance in TFAs, MUFAs and PUFAs, respectively.

The average contents of FA components were calculated from the three experimental runs. The contents of all FA groups showed the highest values under the N-deficient and slow growth condition (Fig. 4-4). Under the balanced nutrient and fast growth condition, the contents of TFAs, MUFAs and PUFAs were significantly lower than those under nutrient deficient and slow growth conditions, respectively (Tukey HSD test, $p \leq 0.043$ for TFAs, $p \leq 0.007$ for MUFAs, $p \leq 0.006$ for PUFAs). No significant difference was observed for all FA groups between N- and P-deficient treatments.

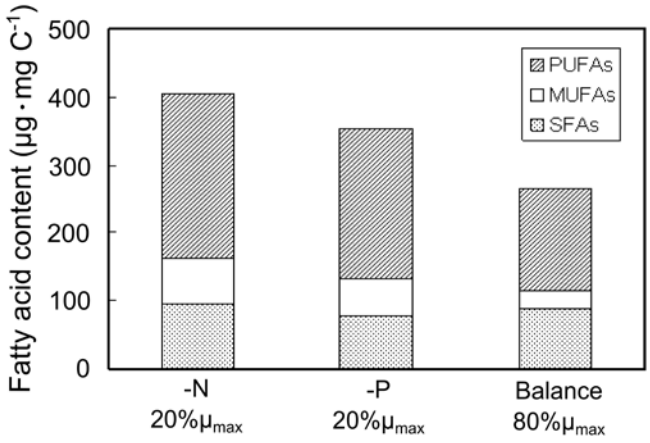

Figure 4-4. Fatty acid contents of *Rhodomonas* sp. under N and P deficiency (-N and -P) with low growth rate (μ = 20% of μ_{max}), and the balanced nutrient condition with high growth rate (μ = 80% of μ_{max}). Data presented are mean values of the three experimental runs.

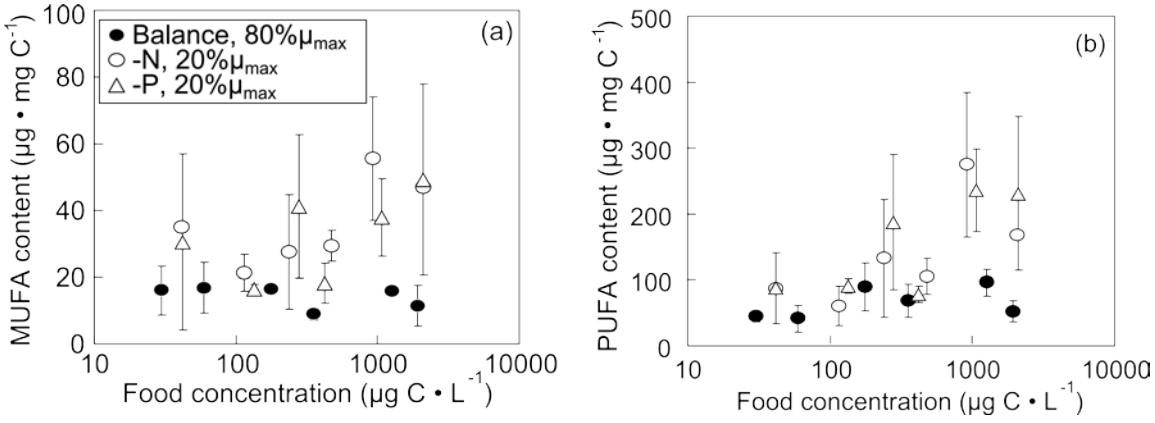

Figure 4-5. Mean (± SD) values of monounsaturated fatty acid (MUFA) content (a) and polyunsaturated fatty acid (PUFA) content (b) of adult *Acartia tonsa* feeding on *Rhodomonas* sp. under different food quantity and food quality (the balanced nutrient and fast growing diet (μ = 80% of μ_{max}), and N- and P-deficient (-N and -P) and slow growing diets (μ = 20% of μ_{max})). Note the log scale is used.

Fatty acid response of Acartia tonsa. No significant effect of nutrient treatment or food concentration was observed on either TFAs or SFAs. However, there were highly significant effects of nutrient treatment on both MUFAs and PUFAs (ANOVA, $p < 0.001$) (ω^2 = 37% and 31%, respectively).

When feeding on nutrient deficient and slow growing diets, the contents of both MUFAs and PUFAs differed significantly from those feeding on the balanced nutrient and fast growing diet (Tukey HSD test, $p \leq 0.004$), showing relatively higher values especially at higher food concentrations (Fig. 4-5). When food concentration was higher than 900 µg C · L^{-1}, MUFA contents under nutrient deficient conditions (-N: ca. 50 µg · mg C^{-1}; -P: ca. 43 µg · mg C^{-1}) were around three times higher than those under balanced nutrient

conditions (ca. 14 μg · mg C $^{-1}$) (Fig. 4-5a). Similarly, around three times differences were also found for PUFAs between nutrient deficient (-N: ca. 221 μg · mg C $^{-1}$; -P: ca. 234 μg · mg C $^{-1}$) and balanced nutrient conditions (ca. 74 μg · mg C $^{-1}$) (Fig. 4-5b).

A significant effect of food concentration was only observed on PUFAs (ANOVA, p = 0.004), with food concentration explaining 27% of the variance. When feeding on nutrient deficient diets, MUFA and PUFA contents of *A. tonsa* increased with increasing food concentrations, although the former showed no significant response to food concentration statistically. The highest MUFA and PUFA contents were observed when feeding on nutrient deficient diets at the highest food concentrations (> 900 μg C L $^{-1}$).

The ratios of ω3-PUFA contents between copepods and algae (ω3-PUFA Z/f) responded significantly to food concentration, but not to either algal C:N or C:P ratio (Table 4-3). Regression analysis showed a significant positive relationship between ω3-PUFA Z/f and food concentration.

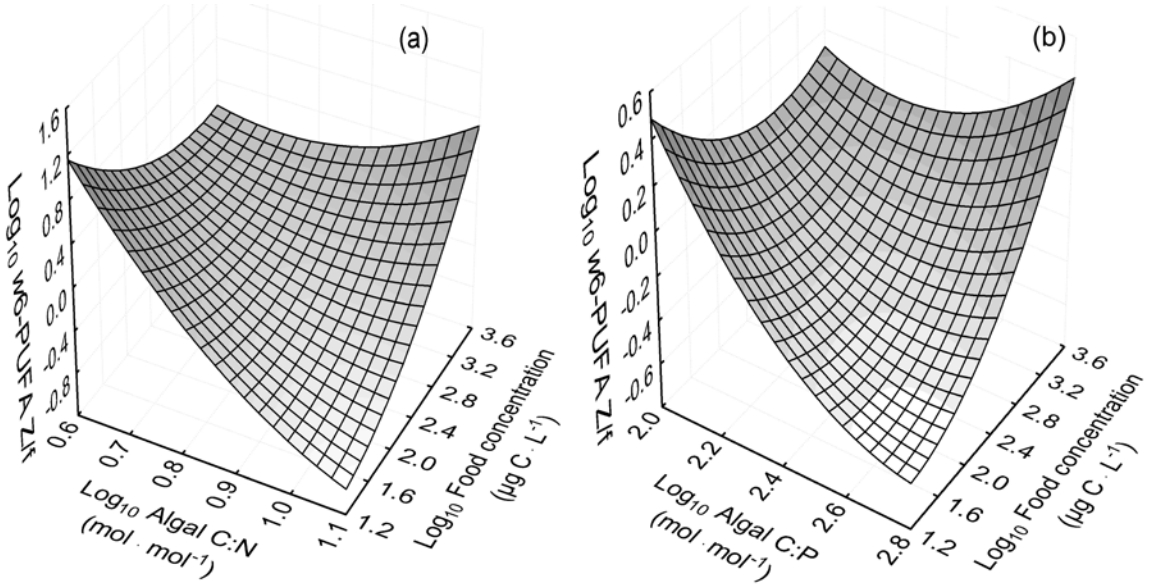

Figure 4-6. The ratios of ω6-polyunsaturated fatty acid (PUFA) contents (μg · mg C^{-1}) between *Acartia tonsa* and food source (ω6-PUFA Z/f) as functions of algal C:X ratio and food concentration (here, X = N or P). (a) ω6-PUFA Z/f as functions of algal C:N ratio and food concentration, and (b) ω6-PUFA Z/f as functions of algal C:P ratio and food concentration. Data for ω6-PUFA Z/f, algal C:X ratio and food concentration were transformed using $\log_{10}(x)$.

For the ratios of ω6-PUFA contents between copepods and algae (ω6-PUFA Z/f), multiple regression analyses showed that the single terms (algal C:N ratio and food concentration) and the interaction term ([algal C:N ratio] · [food concentration]) had significant effects (Table 4-3). When algal C:P ratio was one of the variables, only food

concentration and the interaction term ([algal C:P ratio] · [food concentration]) showed significant effects. 3D surface plots show similar responses of ω6-PUFA Z/f to the effects of food concentration and algal C:X ratio (X = N or P) (Fig. 4-6). ω6-PUFA Z/f responded negatively to algal C:N (P) ratio at lower food concentrations. This negative response became unimodal at higher food concentrations. In contrast, ω6-PUFA Z/f responded positively to food concentration at higher algal C:N (P) ratios, while this response became negative at lower algal C:N (P) ratios.

Egg production rate of Acartia tonsa. A two-factorial ANOVA showed highly significant effects of both food concentration and nutrient treatment on egg production rate ($p < 0.001$), while there was no significant interactive effect. Food concentration and nutrient treatment explained 42% and 13% of the variation, respectively.

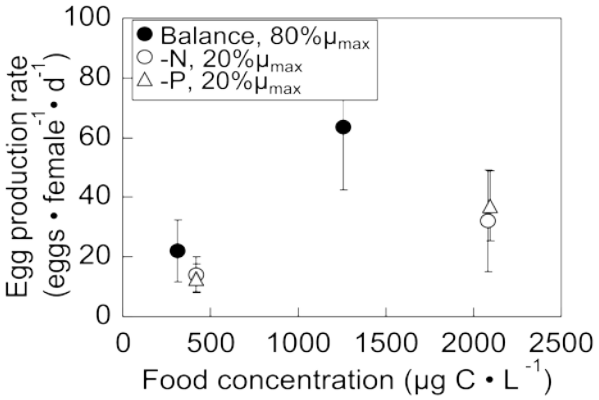

Figure 4-7. Egg production ratio (mean ± SD) of *Acartia tonsa* feeding on *Rhodomonas* sp. under different food quantity and food quality (the balanced nutrient and fast growing diet (μ = 80% of μ_{max}), and N- and P-deficient (-N and -P) and slow growing diets (μ = 20% of μ_{max})).

Egg production rates at low food concentrations were lower than those at high food concentrations irrespective of nutrient treatment (Fig. 4-7). Significant differences in egg production rate were found between two food levels when feeding on balanced nutrient and P-deficient diets (Tukey HSD test, $p < 0.001$ for the balanced nutrient diet, and $p = 0.002$ for the P-deficient diet). When feeding on the balanced nutrient diet, egg production rate at the low food concentration (22.0 ± 10.4 eggs · female^{-1} · d^{-1}) was around three times lower than that at the high food concentration (63.7 ± 21.1 eggs · female^{-1} · d^{-1}). Similarly, when feeding on nutrient deficient diets, the lower egg production rates (14.1 ± 5.8 eggs · female^{-1} · d^{-1} on the N-deficient diet, 12.7 ± 4.9 eggs · female^{-1} · d^{-1} on the P-deficient diet) were observed at low food concentrations, and the higher ones at high food concentrations (32.0

± 17.1 eggs · female^{-1} · d^{-1} on the N-deficient diet, 37.1 ± 11.6 eggs · female^{-1} · d^{-1} on the P-deficient diet).

At each food concentration level, egg production rate was higher when feeding on the balanced nutrient diet than those on nutrient deficient diets. At the high food concentration level, egg production rate differed significantly between the balanced and nutrient deficient diets (Tukey HSD test, $p \leq 0.025$), showing around two times higher when feeding on the balanced nutrient diet than those on nutrient deficient diets.

DISCUSSION

C:N:P stoichiometric trophic transfer. Although zooplankton stoichiometry is more homeostatic compared to phytoplankton (Sterner and Elser 2002), also C:N:P variability in zooplankton has been widely observed, e.g., for *Acartia* species in the present study and previous studies (Table 4-4). The C:N ratios of *A. tonsa* in the present study (4 to 6 mol · mol^{-1}) are within the range of those in the same species and other species of the same genus reported in the literature (2 to 9 mol · mol^{-1}). Compared to the C:N ratios, few data of C:P ratios have been reported for *A. tonsa*. The C:P ratios of *A. tonsa* in our study (59 to 164 mol · mol^{-1}) are consistent with those of other *Acartia* species in previous field studies (41 to 173 mol · mol^{-1}), but relatively lower than those reported by Malzahn et al. (Malzahn et al. 2007, Malzahn et al. 2010) and Schoo et al. (2010) (140 to 280 mol · mol^{-1}). In their studies, dietary C:P ratios (ca. 200 to 800 mol · mol^{-1}) were higher than those in *Rhodomonas* sp. in our study. Thus, different dietary elemental composition may explain the variation in C:N:P stoichiometry of *A. tonsa* between the present study and Malzahn and Schoo's studies.

Food quantity in terms of C to some extent influences the effect of stoichiometric food quality for zooplankton (Sterner and Robinson 1994, Boersma and Kreutzer 2002, Hessen et al. 2002, Andersen et al. 2007, Hessen 2008). In the present study, the C:N ratios of *A. tonsa* differed significantly among nutrient treatments, showing higher values when feeding on the N-deficient diet only at food concentrations > 350 μg C · L^{-1}. The higher C:N ratio in *A. tonsa* feeding on the N-limited diet was also reported at a food concentration of 1000 μg C · L^{-1} d^{-1} by Malzahn et al. (Malzahn et al. 2007, Malzahn et al. 2010). Adams and Sterner (2000) found that the C:N ratios of *Daphnia magna* correlated strongly with algal C:N ratios (*Scenedesmus acutus*) at the incipient limiting food level of 500 μg C · L^{-1}. These findings suggest that the effect of algal C:N ratio on zooplankton C:N ratio is likely more evident at higher food concentrations. This supports the stoichiometric theory and shows the

breakdown of zooplankton stoichiometric homeostasis at higher levels of food concentration (Sterner and Elser 2002).

Table 4-4. Comparison of stoichiometric C:N and C:P ratios for *Acartia* species.

Species	Food condition		C:N	C:P	Reference
Acartia tonsa	Natural particle diets	Field (East Lagoon, Galveston Bay)	3 – 5		Ambler (1985)
	A mixture of algal prey	Lab f/2 culture medium; Food concentration (C_f) = 200 g C · L^{-1}	7[a]		Jones et al. (2002)
	Rhodomonas salina	Lab f/2, -N, and –P; C_f = 1 mg C · L^{-1} · d^{-1}	ca. 5 - 6	f/2: 180 -N: 186 -P: 280	Malzahn et al. (2007)
	R. salina	Lab f/2, -N, and –P; C_f = 1 mg C · L^{-1} · d^{-1}	ca. 5 - 6	ca. 200	Malzahn et al. (2010)
	R. salina	Lab, f/2 and –P; C_f > 1 mg C · L^{-1}		f/2: 140 -P: 195	Schoo et al. (2010)
	Thalassiosira weissflogii	Lab, f/2; C_f = 300 μg C · L^{-1}	6		Saba et al. (2011)
	Rhodomonas sp.	Lab f/2, -N, and –P; C_f = 30 – 2096 μg C · L^{-1}	f/2: 4 – 5 -N: 4 – 6 -P: 4 - 5	f/2: 75 - 164 -N: 59 - 142 -P: 81 - 147	This study
Acartia bifilosa	Natural particle diets	Field (the SW coast of Finland, northern Baltic Sea)	5[a]		Koski (1999)
Acartia clausi	Natural particle diets	Field (Ebrié Lagoon, Ivory Coast)[b]	4 – 6	93 – 173[a]	Pagano and Saint-Jean (1993)
	Natural particle diets	Field (the Gulf of Trieste, Northern Adriatic Sea)	2 – 5[a]		Cataletto and Umani (1994)
	Natural particle diets	Field (the Oslofjord)	5 – 9	63 - 119	Gismervik (1997)
	Natural plankton assemblages	Field (Blanes Bay, NW Mediterranean)	5[a]		Katechakis et al.(2004)
Acartia sp.	Natural particle diets	Field (Baltic sea)	5	65, 102	Walve and Larsson (1999)
Acartia sp.	Natural particle diets	Field (Baltic sea)	6	41- 97	Pertola et al. (2002)

[a]Values were calculated from the data given in references. [b]*Acartia clausi* was the dominant species in zooplankton assemblages (70 to 100% of total mesozooplankton densities). C:N and C:P ratios are expressed as (mol · mol^{-1}). –N: N deficiency; -P: P deficiency.

According to the stoichiometric theory based on Liebig's "Law of the Minimum", only one nutrient is limiting in a given food. Moreover, C is in excess when zooplankton feeding on high C:nutrient ratio food (Hessen and Anderson 2008). Thus, N (or P) deficiency and excess C can be determined when *A. tonsa* feeding on N (or P)-deficient algae in the present study. As the limiting nutrient is utilized as efficiently as possible (Anderson and Hessen 1995), N (or P) could be used at the maximum GGE for N (or P) by *A. tonsa* feeding on N (or P)-deficient algae.

Our results showed that the relative GGE of K_C / K_N decreased with increasing algal C:N ratios (ranging from ca. 5 to 9.5 mol · mol^{-1}) under a wide range of food concentration studied. This supports the prediction of the Dynamic Energy Budget model (Kuijper et al. 2004) and the empirical results (Kiørboe 1989) which suggest a negative response of K_C but a positive response of K_N to algal C:N ratio ranging from 5 to 10 mol · mol^{-1}. On the other hand, the relative GGE of K_C / K_N increased with increasing food concentrations under higher algal C:N ratios (N- or P-deficient diets). This suggests that K_C is typically high under high food concentrations in *A. tonsa* feeding on nutrient limited algae. Contradictory responses of GGE to food concentration have been observed in previous studies, i.e. positive (Hamburger and Boētius 1987, Anderson et al. 2004) and negative (Santer and van den Bosch 1994, Almeda et al. 2010). These differences might be explained by different food nutrient quality (e.g. in the present study) and other ambient factors (Straile 1997, Almeda et al. 2010).

Similarly, the relative GGE of K_C / K_P responded negatively to algal C:P ratios (ranging from ca. 120 to 440 mol · mol^{-1}) and positively to food concentration in the present study. Thus, K_C and K_P may have similar response pattern to algal C:P ratio and food concentration compared to those of K_C and K_N to algal C:N ratio and food concentration. Our results indicate that the responses of K_C / K_X to algal C:X ratio and food concentration might to some extent be in similar patterns for different nutrients in the face of stoichiometric food quality deficiency (i.e. high dietary C:X ratios). However, there are subtle differences in response patterns for different nutrients. For example, the second-order quadratic equation fit the response of K_C / K_N best, while regressions without quadratic terms and the interactive term fit the response of K_C / K_P best. Moreover, the responses of K_C / K_N and K_C / K_P to food concentration showed different patterns when *A. tonsa* feeding

on balanced nutrient diets (low dietary C:N and C:P ratios). Thus, complex responses of the relative GGE of K_C / K_X are assumed when there is a "balanced interaction" (indicating similar chemical composition) (Sterner and Elser 2002) between zooplankton and their diet.

Excess C is evident for *A. tonsa* when feeding on nutrient limited algae under higher food concentrations in the present study. Excess C could be allocated to potential fitness-improving benefits or released as waste (leftover C) in consumers (Hessen and Anderson 2008). In most aquatic invertebrate species, e.g. *Daphnia*, excess C is commonly stored as lipids under high food abundance (Hessen and Anderson 2008), which might be a strategy for *A. tonsa* to use excess C.

Fatty acid trophic transfer. Our results showed that FA composition of *A. tonsa* to some extent reflected that of their food source. Both MUFAs and PUFAs in *Rhodomonas* sp. showed highly significant responses to nutrient treatment, with the highest contents under nutrient deficient conditions. This higher MUFA and PUFA contents under nutrient deficient treatments were reflected by those in *A. tonsa*, but only at higher food concentrations. This finding confirms our prediction above, indicating that excess C is (or partly) stored as lipid in *A. tonsa* under high food concentrations.

The correlations between FA composition of zooplankton and their diet have been widely reported in both freshwater (Brett et al. 2009) and marine (Dalsgaard et al. 2003, Brett et al. 2009) systems. PUFAs in zooplankton have been reported to reflect those of their diet, e.g., in six cladoceran species feeding on *Cryptomonas* sp. (Masclaux et al. 2012), and in *A. tonsa* feeding on *Rhodonomas salina* (Malzahn et al. 2007). However, Malzahn et al. (2010) found no correlation of FAs between *A. tonsa* and their diet (*R. salina*), although there were large differences in dietary FA contents under different nutrient treatments. Peters et al. (2006) reported that in Bornholm Basin (Central Baltic Sea), some PUFA components in the copepod *Pseudocalanus acuspes* reflected those in seston only in autumn and winter, while there was a time lag of the reflection in spring and summer. The results of our multiple regression analyses for the ratios of ω3- (and ω6-) PUFA contents between *A. tonsa* and their diet under wide ranges of algal C:N (and C:P) ratio and food concentration showed that the correlation of essential PUFAs between *A. tonsa* and their diet was food quantity and stoichiometric food quality dependent. This is in agreement with the finding in Dalsgaard et al. (2003), indicating that the applicability of FA trophic markers to higher trophic level organisms is constrained by environmental conditions, e.g., food concentration and nutrient treatment (in the present study) and seasonal dependence (Peters et al. 2006).

The ratios of ω3-PUFA contents between zooplankton and their diet have been recently used to indicate ω3-PUFA trophic transfer efficiency across the phytoplankton–zooplankton interface (Gladyshev et al. 2011). Gladyshev et al. (2011) found that ratios of ω3-PUFAs to C in zooplankton were significantly higher than those in phytoplankton. They suggested that transfer efficiency of essential PUFAs from the producers to the primary consumers was higher than that of bulk C. In the present study, the high ratios (>1) of ω3- (and ω6-) PUFAs between *A. tonsa* and their diet were found at lower algal C:N (and C:P) ratios (balanced nutrient diets), as well as at higher food concentrations. This finding suggests that the relatively high trophic transfer efficiency of ω3- (and ω6-) PUFAs is evident only when copepods feeding on balanced nutrient food irrespective of food concentration or at high food concentrations irrespective of stoichiometric food quality.

Moreover, we note that the relative trophic transfer efficiency of essential PUFAs compared to that of C is food quantity and stoichiometric food quality dependent. The positive relationship between ω3- (and ω6-) PUFAs transfer efficiency and food concentration is consistent with the result in the classic study (Lee et al. 1971), indicating that dietary PUFAs are more efficiently assimilated by zooplankton at higher food concentrations. On the other hand, the relatively low ω6-PUFA transfer efficiency compared to that of C at high algal C:N (or C:P) ratios (N- or P-deficient diets) under low food concentrations confirms the suggestions in previous studies (Kainz et al. 2004, Müller-Navarra et al. 2004, Brett et al. 2006), that for certain PUFAs, especially essential PUFAs, the trophic transfer efficiency decreases with increasing N or P deficiency in aquatic environments. However, the significant interactive effect of algal C:N (and C:P) ratio and food concentration suggests likely complex regulations of ω6-PUFA trophic transfer when copepods and their diet are in the face of a balanced or imbalanced interaction under a wide range of food concentration.

Reproductive response. Both food concentration and nutrient treatment had significant effects on the egg production of *A. tonsa*, resulting in a strong variation in egg production rate (13 to 64 eggs · female^{-1} · d^{-1}) in the present study. Kleppel et al. (1998) compared the egg production of *A. tonsa* published in the literature. They also found a strong variation in the egg production rate, ranging from 16.2 to 85.3 eggs · female^{-1} · d^{-1} at the temperature range of <2.0 to 30 °C. More recently, Gusmão and McKinnon (2009) reviewed the egg production rate of *A. tonsa* from different studies. They found that the maximum egg production rates (the asymptotic values in the sigmoid function) varied between 40.3 and

54.0 eggs · female^{-1} · d^{-1} at the temperature of 12 to 21 °C. The range of egg production rate in the present study is consistent with those in previous studies above under the similar temperature condition (ca. 18 °C).

In general, egg production rate is higher when the food is obtained from nutrient-enriched or other optimized environments (Kleppel et al. 1998). In agreement with this, the results in the present study showed that the highest egg production rate was achieved when feeding on the balanced nutrient diet at the high food concentration. The higher egg production rates of *A. tonsa* at higher food concentrations have also been reported in previous studies (Augustin and Boersma 2006, Acheampong et al. 2011). The relationship between the egg production rate in *Acartia sinjiensis* and food concentration fitted well to the Hill equation, showing that egg production rate increased with increasing food concentrations and reached an asymptotic value at the saturating food concentration (Gusmão and McKinnon 2009). This sigmoidal relationship between egg production and food concentration varies with environmental factors, e.g., temperature (Saiz et al. 1998), and algal species (Ravet and Brett 2006, Gusmão and McKinnon 2009). In the present study, food saturation levels and maximum egg production rates in different nutrient treatments could not be calculated because of too few food concentration levels. However, different egg production rates between different nutrient treatments indicate the potential influence of dietary chemical composition (C:N:P and FAs) on the relationship between egg production and food concentration. Based on these results, we assume that the maximum egg production rate would be lower, but the food saturation level would be higher for *A. tonsa* feeding on nutrient deficient diets compared to those feeding on the balanced nutrient diet.

Strong effects of dietary chemical composition on copepod reproduction have been observed in several previous studies. Jónasdóttir (1994) for the first time reported that the egg production of *Acartia* species was significantly correlated with both elemental (C and N) and biochemical composition (protein and specific FAs) of food. Often, dietary N or P limitation reduces copepod reproduction (Checkley 1980, Kiørboe 1989, Jónasdóttir 1994, Aguilera and Escribano 2012), which is also shown in the present study. However, an increased egg production rate was observed in *A. tonsa* and *Acartia clausii* when feeding on N-limited algae (Augustin and Boersma 2006). The principle regulation of dietary elemental limitation is more complex than those assumed in most studies. The indirect effect of dietary P limitation has been found to explain the majority of reduced food quality at high C:P ratios (P limitation) of phytoplankton (Ravet and Brett 2006). Indirect effects of nutrient limitation could be caused by changes in cell morphology and/or the biochemical

composition of nutrient limited phytoplankton (Brett 1993, Weers and Gulati 1997, Augustin and Boersma 2006). In agreement with this prediction, our previous study showed significant correlations between major FA groups and N cell quota (Q_N) in *Rhodomonas* sp. under N deficiency (Bi et al., unpublished data). These findings above suggest the importance of simultaneously considering elemental and biochemical limitations of phytoplankton food quality.

Therefore, biochemical limitation might be a reasonable option to explain the increased egg production rates when feeding on N-limited algae in Augustin and Boersma's study (2006). However, Augustin and Boersma (2006) suggested that the HUFA content of N-limited cryptophytes is likely high enough to meet nutritional requirements of copepods due to the very low saturation thresholds of FAs for most zooplankton species. More recently, Mayor et al. (2009) reported that PUFAs did not have the potential to limit the egg production of *Calanus finmarchicus* feeding on natural microplankton assemblages in the North Atlantic. In the present study, we found that the egg production rate of *Acartia tonsa* was higher when feeding on low unsaturated FA (UFA) diets (balanced nutrient diets with low C:N and C:P ratios). This result is in a good agreement with previous observations above, indicating that the low UFA content in *Rhodomonas* sp. under the balanced nutrient condition is high enough for nutritional requirements of *A. tonsa* in our study.

It is essential to consider the combination of multinutrient trophic processes for maintenance and reproduction of copepods. The combination of different C and N requirement explained well the higher reproduction of *Acartia* species feeding on the N-limited diet, due to the higher dietary C:N ratio (9.05) being closer to the threshold ratio (Augustin and Boersma 2006, Boersma and Elser 2006). In the present study, the relative GGEs of both K_C / K_N and K_C / K_P were higher under balanced nutrient conditions, as well as under higher food concentrations (optimized food conditions). Further, essential PUFA transfer efficiency was also higher compared to that of C under optimized food conditions. It is well known that egg production is energetically expensive (Jónasdóttir 1994, Rey-Rassat et al. 2002). The usage of lipids for copepod reproduction suggests the high requirement of C (energy) (Mayor et al. 2009). Thus, the high egg production rate under optimized food conditions in the present study demonstrates that the relative GGE for C and nutrient, as well as the relative trophic transfer efficiency for PUFA and C may well predict copepod egg production.

CONCLUSIONS

Our results for the first time provide empirical evidence that both food quantity and stoichiometric food quality can affect the relative GGE for C and nutrient (K_C / K_X) (here, X = N or P). K_C / K_X responded negatively to algal C:X ratio but positively to food concentration under nutrient deficient and food quantity limited conditions. However, different response patterns were observed for different elements under optimized food conditions. Secondly, our FA data suggested that food quantity and stoichiometric food quality could influence essential PUFA trophic transfer efficiency across the phytoplankton–zooplankton interface, showing relatively higher transfer efficiency under optimized food conditions compared to that of C. Both K_C / K_X and the relative trophic transfer efficiency for PUFA and C can well predict egg production rate of *A. tonsa* in our study. The question we should now ask is how do other environmental factors (e.g. temperature, light, and CO_2) affect the responses of K_C / K_X and essential PUFA trophic transfer to food quantity and food quality? The answer would be helpful to understand how chemical trophic transfer actually acts under natural systems.

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CHAPTER 5

Effects of food quantity and food quality on ingestion rate and nucleic acid content in the calanoid copepod *Acartia tonsa*[*]

Rong Bi and Ulrich Sommer

ABSTRACT

Laboratory experiments were conducted to evaluate the effects of food quantity and food quality (as chemical composition of phytoplankton) on ingestion rate and nucleic acid content (RNA content and RNA:DNA ratio) in the calanoid copepod *Acartia tonsa*. The functional response of ingestion rates fitted well to the Holling model (type III) on the balanced nutrient and nitrogen (N)-deficient diets. Compared to ingestion rates on the balanced nutrient diet, ingestion rates for carbon, N, total fatty acids, and ω3-polyunsaturated fatty acids (PUFAs) on the N-deficient diet were higher at lower food concentrations but became lower at higher food concentrations. Ingestion rates for ω6-PUFAs were consistently higher on the N-deficient diet independent of food concentration, suggesting that it was not a good index of food quality in this study. Nucleic acid contents correlated positively with food concentration in each food quality treatment, and were slightly higher on the balanced nutrient diet. Egg production rate correlated positively with

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nucleic acid content, while food quality showed no significant effect on the nucleic acid-egg production relationship. This result is in agreement with the increasing recognition that RNA-based indices can be used as good indicators of copepod egg production.

INTRODUCTION

Copepods are the most abundant zooplankton in the oceans (Verity and Smetacek 1996). As an important link between primary producers and organisms at higher trophic levels, copepods occupy a key ecological position in marine systems (Harris et al. 2000). Thus, the assessment of copepod performances is essential for understanding the transfer of energy and matter in pelagic food webs, especially in face to prevalent natural and human-induced perturbation in the oceans.

Feeding is the main route for energy and matter transfer from lower to higher trophic levels, and thus quantification of feeding is a key factor in studying trophic interactions (Båmstedt et al. 2000). It has been well established that ingestion rates increase with increasing food concentrations up to a maximal rate, which can be illustrated by the classic Holling functional response and alternative types of models as reviewed by Gentleman et al. (2003). However, there is no consistent response of ingestion rates to food quality, which might be explained by various characteristics of food quality such as chemical composition and shape and size of food particles. For this reason, although the effect of food type on ingestion rates has been widely studied (e.g., Støttrup and Jensen 1990, Tirelli and Mayzaud 2005, Liu et al. 2010), little is known about how chemical quality of food affects ingestion rates of copepods and the food quantity-ingestion relationship. There is evidence that the effect of food quality on ingestion rates varies greatly even within copepod species. For example, the asymptotic maximum value of ingestion rate of *Acartia tonsa* in Besiktepe and Dam (2002) was ca. 16 times higher than that in Thor and Wendt (2010). In both studies, *A. tonsa* was fed on the same algal species (*Dunaliella tertiolecta*). This suggests that chemical quality of food may contribute the bulk of the variation in ingestion rates.

The RNA content and RNA:DNA ratio have been used as indices of copepod growth and physiological condition (Saiz et al. 1998, Wagner et al. 1998, Holmborn et al. 2009, Ning et al. 2013). Compared to tranditional methods used for assessing copepod growth (e.g., the egg production method) and metabolic activity (e.g., enzymatic methods), estimation of nucleic acid indices has several advantages such as simplicity, sensitivity, and variety of techniques (Gusmão and McKinnon 2011). Food quantity has been frequently considered in

previous studies investigating the use of nucleic acid content as an index of egg production (or growth) in copepods (Gusmão and McKinnon 2011 and references therein), while the effect of food quality has received less attention. Saiz et al. (1998) suggested that the relationship between RNA content and growth rate (estimated as egg production rate) should be uniform for a particular copepod species independent of food quality and previous food history. However, a recent study has reported that food quality (as different algal species) can affect the nucleic acid-egg production relationship in copepods (Gusmão and McKinnon 2011). As mentioned above, food quality comprises various aspects. Thus, despite the known effect of food type recently reported by Gusmão and McKinnon (2011), there is still no information about the effect of chemical quality of food on the nucleic acid-egg production relationship.

In the present study, we investigated the interactive effect of food quantity and quality on ingestion rates and nucleic acid contents (RNA content and RNA:DNA ratio) in the calanoid copepod *A. tonsa*, and further determined the nucleic acid-egg production relationship in response to food quality. Food quality in this study is expressed as elemental (carbon (C): nitrogen (N):phosphorus (P) stoichiometry) and biochemical (fatty acids (FAs)) composition of phytoplankton. *Rhodomonas* sp. (Cryptophyceae) was chosen as food source due to its high polyunsaturated fatty acid (PUFA) contents. *A. tonsa* was chosen as the model copepod because the adults do not build up large energy storage pools but rather invest most assimilated energy into egg production (Diekmann et al. 2009), thus making egg production a relatively fast response to feeding conditions. The objectives of our study are to test: (i) the interactive effect of food quantity and quality on ingestion rates of copepods (C-, N-, total fatty acids (TFAs)-, ω3- (and ω6-) PUFAs-specific ingestion rates); (ii) the interactive effect of food quantity and quality on nucleic acid content (RNA content and RNA:DNA ratio) of copepods; (iii) whether food quality could affect the nucleic acid-egg production relationship.

MATERIALS AND METHODS

Phytoplankton cultures. *Rhodomonas* sp. (Cryptophyceae) (equivalent spherical diameter 4.6 μm) was isolated from Kiel fjord and used as the copepod diet in this study. Cultures were maintained in sterile filtered natural seawater from the Kiel fjord, Baltic Sea (Sterilizing Grade Filter, Sartobran P 0.2 μm) (Sartorius Stedim Biotech GmbH, Goettingen, Germany) with additional macronutrients and micronutrients based on the modified

Provasoli's culture medium (Provasoli 1963, Ismar et al. 2008). Macronutrients were added as sodium nitrate ($NaNO_3$) and potassium dihydrogen phosphate (KH_2PO_4). Cultures were set up at 18 ^oC and a salinity of 18 ± 1 psu in a temperature controlled room. The light intensity was constant at 100 µmol photons · m^{-2} · s^{-1} at a light:dark cycle of 16:8 h.

Semicontinuous cultures of *Rhodomonas* sp. were used as food source in copepod experiments. Three nutrient treatments were set up in algal semicontinuous cultures, with the combination of three N:P supply ratios and two growth rates (Table 5-1). The determination of nutrient treatment setups was based on chemical characteristics of *Rhodomonas* sp. observed in our previous studies, as described in detail in CHAPTER 4. The observed maximal growth rate (μ_{max}) was calculated from cell number changes during the exponential growth phase in batch cultures (Bi et al. 2012). Cultures were kept in 1 L Erlenmeyer flasks with 500 mL culture volume. All flasks were aerated slightly with filtered air and shaken manually twice per day at a set time. Algal semicontinuous cultures were performed twice corresponding to the two runs of copepod experiments. Two replicates were set up for each treatment in the first semicontinuous culture, while the second one was run in triplicate. Stoichiometric C:N:P composition and unsaturated fatty acid content of *Rhodomonas* sp. are shown in CHAPTER 4.

Table 5-1. Nutrient treatments setups, growth rate, N:P supply ratio and concentrations in each treatment in *Rhodomonas* sp. cultures.

Nutrient treatment	Growth rate (d^{-1})	N:P supply ratio (mol · mol^{-1})	N concentration (µmol · L^{-1})	P concentration (µmol · L^{-1})
N deficiency, low growth rate	0.17 (20% of μ_{max})	10:1	352	36
P deficiency, low growth rate	0.17 (20% of μ_{max})	63:1	880	14
Balanced nutrient, high growth rate	0.68 (80% of μ_{max})	24:1	880	36

Algal cell density was counted daily using an improved Neubauer hemacytometer (Glaswarenfabrik Karl Hecht GmbH, Rhön, Germany). The daily renewal culture medium was collected to feed copepods. Before feeding copepods, algal suspensions from replicate flasks were pooled. Thus, copepod feeding was not influenced by variance between the algal culture replicates and copepod replicates were real replicates receiving identical food.

Copepod cultures. The calanoid copepod, *A. tonsa*, was obtained from the Department of Biology, Institute for Hydrobiology and Fisheries Science at the University of Hamburg. *A. tonsa* eggs were hatched in filtered natural seawater (Sterilizing Grade Filter, Sartobran P 0.2 µm) (Sartorius Stedim Biotech GmbH, Goettingen, Germany) at 18 ^{o}C and a salinity of 18 ± 1 psu. Copepod cultures were maintained under the same temperature, salinity and light regime as those used in the cultures of their diet throughout the experiment. Before the cohort reached late copepodite stages (CV or CVI), the culture was fed *Rhodomonas* sp. *ad libitum*. Copepod individuals (stage CV or CVI) were placed in 1 L Erlenmeyer flask containing 800 mL culture volume (ca. 500 individuals · L^{-1}) under different food treatments (Table 5-2). Copepods were acclimated to each food treatment for four days prior to starting ingestion rate experiment and measuring nucleic acids.

Table 5-2. Actual initial food concentrations (measured from the corresponding nominal food concentrations and algal carbon contents) (µg C · L^{-1}) in this study.

	N deficiency, low growth rate	P deficiency, low growth rate	Balance, high growth rate
Ingestion rate experiment[b]	23.8		35.4
	59.7		88.5
	95.5		141.5
	119.3		176.9
	238.6		353.9
Nucleic acid analysis[a]	415.8	419.2	314.4
	2079.2	2096.0	1257.8

[a and b] Copepods were fed with diets from the experiment run 1 and 2, respectively.

Ingestion rate experiment. Five adult females were picked out and kept in 100 mL bottles for 6hrs. The food treatment setup is shown in Table 5-2. The experiments were started by adding the diet and ended by adding Lugol's solution. Two replications were set up for each food treatment.

Ingestion rate (I) is expressed as algal biomass ingested per copepod female per time (µg C · $female^{-1}$ · d^{-1}) and determined according to a modified Frost's equation (1972), using the following equation 5-1:

$$I = \frac{\left(\frac{\ln B_0 - \ln B_1}{t} + \mu\right) \cdot \sqrt{B_0 \cdot B_1}}{N_C} \qquad (5\text{-}1)$$

where B_0 and B_1 (µg C · L^{-1}) are food concentrations at the start and end of the ingestion rate experiment during the incubation time t (here t = 0.25), respectively. µ is dietary growth rate in semicontinuous cultures. N_c is the number of adult females (here N_c = 5). Ingestion rates for N, TFAs and ω3- (and ω6-) PUFAs were estimated by multiplying B_0 and B_1 with N or FA contents (measured upon C content, µg · µg C^{-1}).

Responses of ingestion rates to food concentration can be described by the Hill functional response model (Real 1977, Thor and Wendt 2010):

$$I = \frac{I_{max} \cdot B^h}{K^h + B^h} \tag{5-2}$$

where I_{max} is the maximum ingestion rate (µg C · $female^{-1}$ · d^{-1}). B is food concentration (µg C · L^{-1}). K is the food concentration at which I = I_{max}/2. The h exponent indicates the possibility for adaptation by copepods. The equation gives a hyperbolic type II functional response when h = 1, while the functional response becomes sigmoidal (type III) at h >1. Ingestion rates for N, TFAs and ω3- (and ω6-) PUFAs were also fitted to the Hill model.

Nucleic acid samples collected from egg production experiment. Five females and two males were used in each food treatment in egg production experiment as the detail described in CHAPTER 4. The food treatment setup is shown in Table 5-2. Female adults used in egg production experiment were sorted and stored in -80 °C for nucleic acid analysis.

Chemical analysis. Algal cells (at steady state) were harvested by filtering onto pre-combusted Whatman GF/F filters (Whatman GmbH, Dassel, Germany). After filtration, samples were immediately dried and stored in a desiccator. The determination of particulate organic carbon (POC) and nitrogen (PON) was carried out after Sharp (1974) by gas chromatography in an organic elemental analyzer (Thermo Flash 2000) (Thermo Fisher Scientific Inc., Schwerte, Germany).

RNA and DNA contents were analyzed in individual female copepods according to Malzahn et al. (2007) and Hauss et al. (2013). Nucleic acids were quantified fluorometrically in a microtiter fluorescence reader (Labsystems, Fluoreskan Ascent) using ethidium bromide as a fluorophore. At first total nucleic acids were measured, and subsequently RNase (Serva, Ribonuclease A) was applied to digest RNA to measure the remaining DNA. Nucleic acid standards were Lambda DNA (Boehringer 745782) and 16S and 23S rRNA (Boehringer 206938). DNA amounts were calculated using the relationship between RNA and DNA fluorescence described by Le Pecq and Paoletti (1966).

Statistics. The effect of food concentration on ingestion rates for C, N, TFAs and ω3- (and ω6-) PUFAs was tested in each food quality treatment using one factorial analysis of variance (ANOVA). The Holling functional response model was fitted to the relationship between ingestion rates and food concentration using a nonlinear least-squares method. Two-factorial ANOVA was used to test the effects of food concentration and food quality on the RNA content and RNA:DNA ratio. A post-hoc test (Tukey's honest significant difference (HSD) test) was applied only if there were significant effects. The magnitude of effect (ω^2 = (effect sum of squares – effect degree of freedom × error mean square) / (total sum of squares + error mean square)) was calculated only for the significant factors. The relationship between egg production rate and nucleic acid indices (i.e., RNA content and RNA:DNA ratio) was tested using general linear models (GLM) with nucleic acid indices as continuous predictors and food quality treatment as categorical predictors. Linear regression analyses were applied to test the nucleic acid-egg production relationship when data from different food quality treatments were pooled. Data for egg production rates were from CHAPTER 4. The normality and homogeneity of variances were tested prior to statistical analyses and data transformation was not necessary. All statistical tests were conducted in Statistica 8 (StatSoft [Europe] GmbH, Hamburg, Germany). Significance level was set to $p < 0.05$.

RESULTS

Responses of ingestion rates to food quantity and quality. Data for ingestion rates in the food quality treatment of P deficiency at low growth rate were lost. Thus, ingestion rates on balanced nutrient (at high growth rate) and N-deficient (at low growth rate) diets were analysed. C-specific ingestion rates increased with increasing food concentrations in both food quality treatments, with the range of 0.3 to 21.6 ± 4.8 (mean ± SD) μg C · ind^{-1} · d^{-1} and 0.4 to 11.7 μg C · ind^{-1} · d^{-1} on the balanced nutrient and N-deficient diets, respectively (Fig. 5-1a). Food concentration showed significant effects on C-specific ingestion rates in both food quality treatments (ANOVA, $p = 0.027$ in the balanced nutrient treatment, $p = 0.007$ in the N-deficient treatment). At lower food concentrations (< ca. 150 μg C · L^{-1}), ingestion rates on the balanced nutrient diet were lower than those on the N-deficient diet. At higher food concentrations (> ca. 150 μg C · L^{-1}), higher ingestion rates were observed on the balanced nutrient diet.

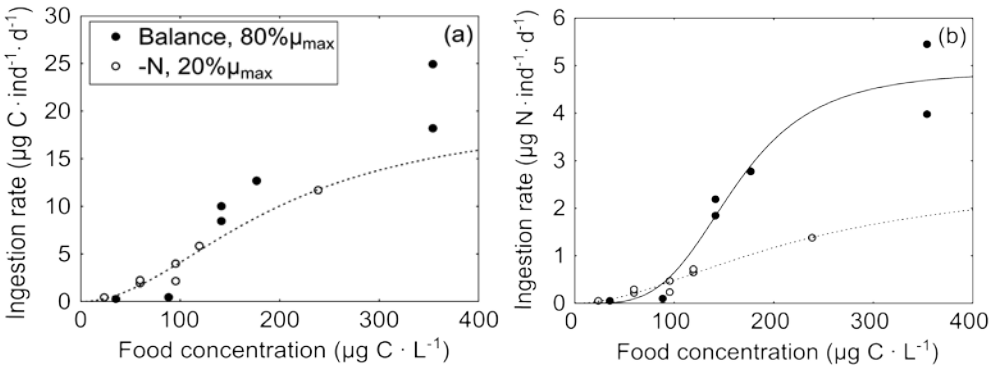

Figure 5-1. Ingestion rates of *Acartia tonsa* as functions of food concentration (μg C · L^{-1}) under different food quality treatments: balanced nutrient with high growth rate (N:P = 24:1, μ = 80% of μ_{max}), and nitrogen deficiency (-N) with low growth rate (N:P = 10:1, μ = 20% of μ_{max}). Ingestion rates are expressed as (a) carbon-specific ingestion (μg C · $female^{-1}$ · d^{-1}) and (b) nitrogen-specific ingestion (μg N · $female^{-1}$ · d^{-1}). The solid line and broken lines depict the regression of the Hill equation on the balanced nutrient and the N-deficient diet, respectively.

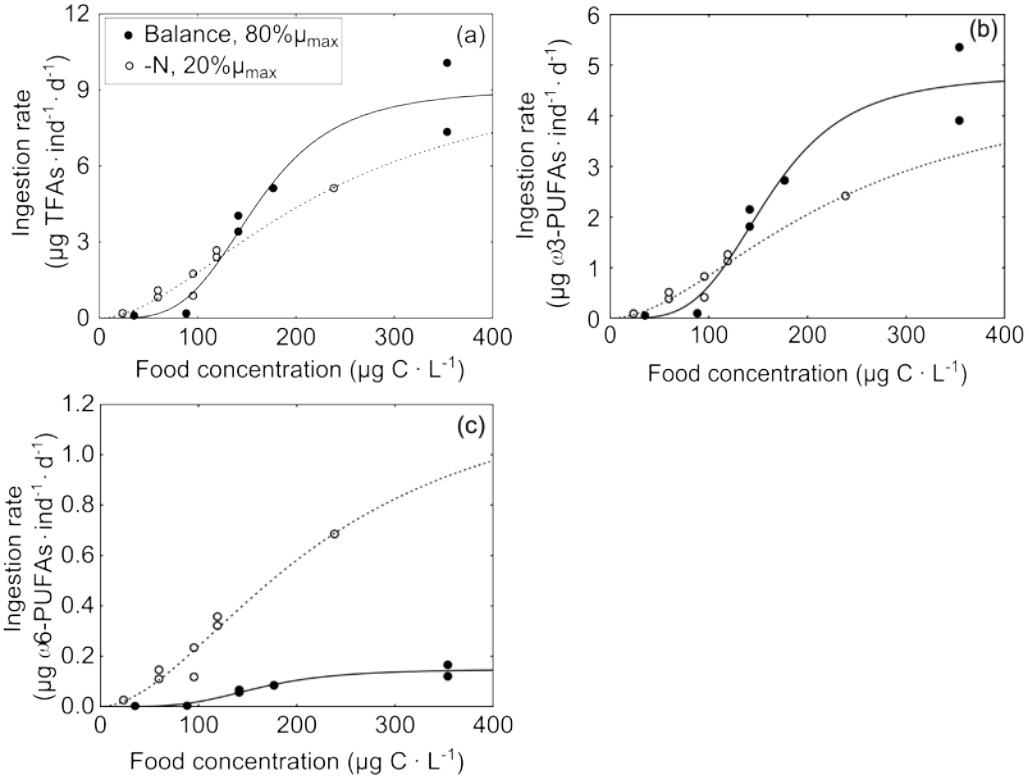

Figure 5-2. Ingestion rates of *Acartia tonsa* as functions of food concentration (μg C · L^{-1}) under different food quality treatments: balanced nutrient with high growth rate (N:P = 24:1, μ = 80% of μ_{max}), and nitrogen deficiency (-N) with low growth rate (N:P = 10:1, μ = 20% of μ_{max}). Ingestion rates are expressed as (a) total fatty acids (TFAs)-specific ingestion (μg TFAs · ind^{-1} · d^{-1}) and (b) ω3-polyunsaturated fatty acids (PUFAs)-specific ingestion (μg ω3-PUFAs · ind^{-1} · d^{-1}), and (c) ω6-PUFAs-specific ingestion (μg ω6-PUFAs · ind^{-1} · d^{-1}). The solid line and broken lines depict the regression of the Hill equation on the balanced nutrient and the N-deficient diet, respectively.

Table 5-3. Estimated parameters (±SE) obtained by nonlinear least-square regression of ingestion rates versus food concentration (μg C · L^{-1}) according to the Hill equation under two food quality treatments: balanced nutrient with high growth rate (N:P = 24:1, μ = 80% of μ_{max}), and nitrogen deficiency (-N) with low growth rate (N:P = 10:1, μ = 20% of μ_{max}).

Specific ingestion	Food quality treatment	I_{max}	K	h
Carbon (C)	Balance [a]	-	-	-
	-N	19.9±12.4	198.1±133.7	2.0±0.8
Nitrogen (N)	Balance	4.9±0.6*	161.1±14.9*	3.9±1.7
	-N	2.7±2.5	230.8±237.1	1.8±0.8
TFAs	Balance	9.1±1.1*	161.1±14.9*	3.9±1.7
	-N	10.0±9.4	230.8±237.0	1.8±0.8
ω3-PUFAs	Balance	4.8±0.6*	161.1±14.9*	3.9±1.7
	-N	4.7±4.5	231.0±238.0	1.8±0.8
ω6-PUFAs	Balance	0.1±0.02*	161.1±14.9*	3.9±1.7
	-N	1.3±1.3	230.8±237.8	1.8±0.8

[a]The relationship of C-specific ingestion and food concentration on the balanced nutrient diet could not follow the Hill model due to the presence of multi-collinearity. The parameter I_{max} and ingestion rates are expressed as μg C · ind^{-1} · d^{-1} for C-specific ingestion, μg N · ind^{-1} · d^{-1} for N-specific ingestion, μg TFAs · ind^{-1} · d^{-1} for TFAs-specific ingestion, and μg ω3- (and ω6-) PUFAs · ind^{-1} · d^{-1} for ω3- (and ω6-) PUFAs-specific ingestion, respectively. The parameter K is expressed as μg C · L^{-1} for C-specific ingestion, μg N · L^{-1} for N-specific ingestion, μg TFAs · L^{-1} for TFAs-specific ingestion, and μg ω3- (and ω6-) PUFAs · L^{-1} for ω3- (and ω6-) PUFAs-specific ingestion, respectively. Asterisk marks significant estimates ($p < 0.05$); the number of observations (n) is 8 in both food quality treatments.

A. tonsa also ingested different concentrations of N, TFAs, and ω3- (and ω6-) PUFAs under different food concentrations in both food quality treatments (ANOVA, $p < 0.05$) (N-specific ingestion (Fig. 5-1b) and FAs-specific ingestion (Fig. 5-2)). Ingestion rates for N and FAs increased with increasing food concentrations in both food quality treatments. In each food quality treatment, ingestion rates for N, TFAs and ω3-PUFAs were lower on the balanced nutrient diet than those on the N-deficient diet at lower food concentrations (< ca. 60 μg C · L^{-1} for N-specific ingestion (Fig. 5-1b) and < ca. 120 μg C · L^{-1} for TFAs- and ω3-PUFAs-specific ingestion (Fig. 5-2, a and b)), but ingestion rates became higher on the balanced nutrient diet at higher food concentrations. ω6-PUFAs-specific ingestion were higher on the N-deficient diet across the entire range of food concentration (Fig. 5-2c).

The Hill functional response model provided good fits (r^2 = 0.94 to 0.96) to observed ingestion rates in both balanced nutrient and N-deficient food quality treatments (Fig. 5-1 and 5-2), except for the relationship of C-specific ingestion and food concentration in the balanced nutrient treatment, which could not follow the Hill model due to the presence of multi-collinearity. The estimation of parameters I_{max} and K were significant in the balanced nutrient treatment but not in the N-deficient treatment (Table 5-3). The relationship between ingestion rates and food concentration could be described by the type III functional response with the parameter h>1, while this estimation was not significant in either of food quality treatments. Moreover, functional responses also differed in shape among food quality treatments. Ingestion rates for C, N, TFAs and ω3-PUFAs increased slower in the N-deficient treatment, while ω6-PUFAs-specific ingestion increased faster in the N-deficient treatment.

Responses of RNA content and RNA:DNA ratio to food quantity and quality. A two-factorial ANOVA showed significant effects of food concentration on both nucleic acid indices (i.e., the RNA content (p = 0.005) and RNA:DNA ratio (p < 0.001)). Food concentration explained 68% and 85% of the variation for the RNA content and RNA:DNA ratio, respectively. There was no significant effect of food quality or interactive effect of food concentration and quality on nucleic acid indices.

The RNA content and RNA:DNA ratio at higher food concentrations were ca. two times higher than those at lower food concentrations in each food quality treatment (Fig. 5-3). RNA:DNA ratios showed significant differences between two food levels in each food quality treatment (Tukey HSD test, p = 0.049 in the balanced nutrient treatment, p = 0.011 in the N-deficient treatment, and p = 0.004 in the P-deficient treatment). At lower food concentrations, the RNA content and RNA:DNA ratio on the balanced nutrient diet were higher than those on nutrient deficient diets. At higher food concentrations, both nucleic acid indices on the balanced nutrient diet were similar with or slightly higher than those on nutrient deficient diets.

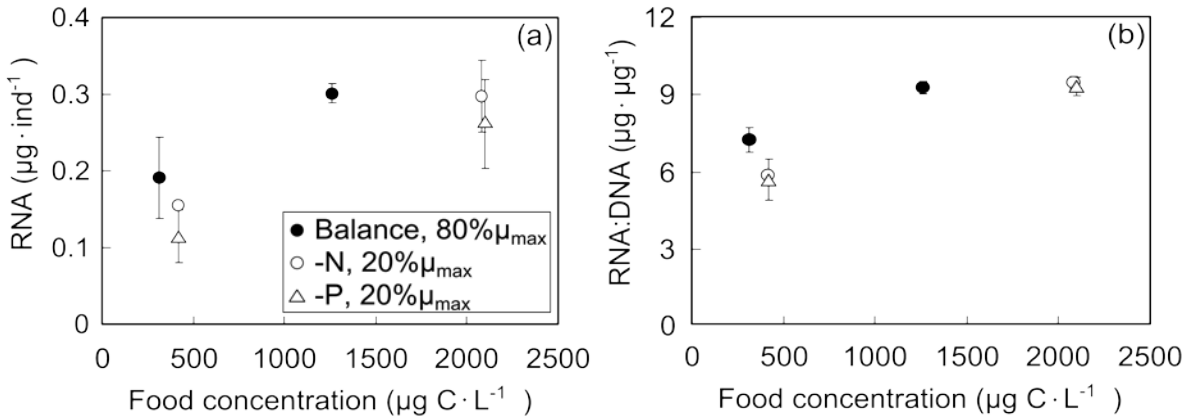

Figure 5-3. RNA content (a) and RNA:DNA ratio (b) (mean ± SD) of *Acartia tonsa* as functions of food concentration under different food quality treatments: balanced nutrient with high growth rate (N:P = 24:1, μ = 80% of μ_{max}), nitrogen deficiency (-N) with low growth rate (N:P = 10:1, μ = 20% of μ_{max}), and phosphorus deficiency (-P) with low growth rate (N:P = 63:1, μ = 20% of μ_{max}).

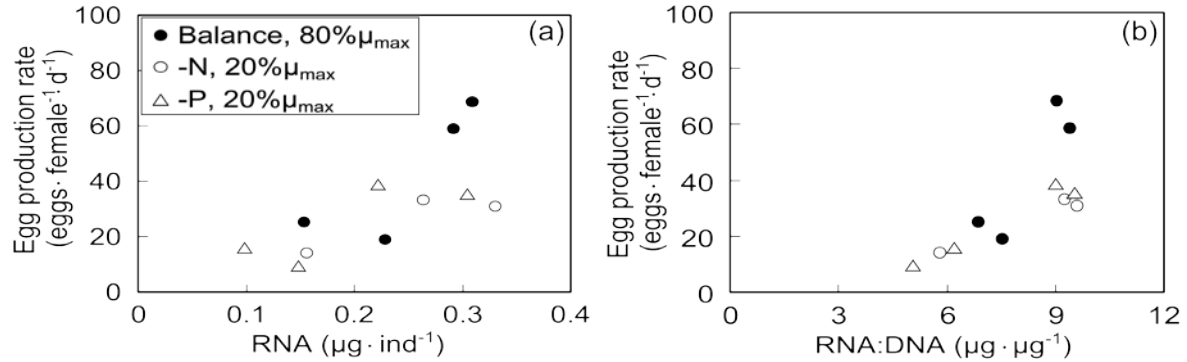

Figure 5-4. Egg production rate as a function of RNA content (a) and RNA:DNA ratio (b) in *Acartia tonsa* under different food quality treatments: balanced nutrient with high growth rate (N:P = 24:1, μ = 80% of μ_{max}), nitrogen deficiency (-N) with low growth rate (N:P = 10:1, μ = 20% of μ_{max}), and phosphorus deficiency (-P) with low growth rate (N:P = 63:1, μ = 20% of μ_{max}). Data for egg production rate were from CHAPTER 4.

Table 5-4. Linear regression of egg production rate (eggs · female^{-1} · d^{-1}) versus nucleic acid indices (RNA content (µg · ind^{-1}) and RNA:DNA ratio (µg · µg $^{-1}$)) in *Acartia tonsa* under the entire range of food quantity and quality.

Nucleic acid indices	Slope (±SE)	*p*	r^2 (adj.)	*n*
RNA	169.0±54.0	**0.012**	0.47	11
RNA:DNA	8.2±2.4	**0.008**	0.52	11

Data from different food quality treatments were pooled. The significant level is $p < 0.05$. Significant *p* values are shown in bold; *n* is the number of observations.

The relationship between egg production rate and nucleic acid indices. The RNA content and RNA:DNA ratio had significant effects on egg production rate (GLM, $p = 0.017$ for RNA content, $p = 0.009$ for RNA:DNA ratio), accounting for 41% and 47% of the variation, respectively. Food quality showed no significant effect on either the RNA-egg production relationship or the RNA:DNA-egg production relationship. Under the entire range of food quantity and quality, egg production rate showed significant positive correlations with the RNA content and RNA:DNA ratio (Fig. 5-4) (Table 5-4).

DISCUSSION

Ingestion rate of Acartia tonsa. C-specific ingestion rate of *A. tonsa* varies greatly in the literature. A recent review shows that ingestion rates of *A. tonsa* range between 2.0 and 10.5 µg C · ind^{-1} · d^{-1} in field studies, and the maximum ingestion rates range between 4.4 and 13.8 µg C · ind^{-1} · d^{-1} when feeding on algae diets in laboratory studies (Saiz and Calbet 2007). In the present study, ingestion rates of *A. tonsa* (0.3 to 24.9 µg C · ind^{-1} · d^{-1} and 0.4 to 11.7 µg C · ind^{-1} · d^{-1} on the balanced nutrient and N-deficient diets, respectively) are within the range of compiled data above, expect for the highest one (24.9 µg C · ind^{-1} · d^{-1}) at the highest food concentration on the balanced nutrient diet. The higher ingestion rate under optimized food conditions (balanced nutrient diets at high food concentrations) in the present study indicates food quantity and quality as important factors resulting in the high variability of ingestion rates.

The literature review by Saiz and Calbet (2007) was restricted on the effects of body size, temperature and food concentration and omitted other important variables such as prey species and food quality. Therefore, we compiled newly published data for C-specific ingestion rates of *A. tonsa* fed on the algal genus *Rhodomonas* (Table 5-5). Most publications listed in the data compilation are not included in Saiz and Calbet (2007). Maximum ingestion rates were obtained either from model fittings or single-point data at the highest food concentrations. Table 5-5 shows that the parameter K in the present study (160 and 198 µg C · L^{-1}) is within the range in previous research (98 to 412 µg C · L^{-1}), while the parameter I_{max} is higher than those in the literature. Different K and I_{max} are also evident when *A. tonsa* fed on another flagellate species *D. tertiolecta* and on mixed plankton assemblage. Moreover, Table 5-5 shows that different models have been used in different studies. In the present study, ingestion rates of *A. tonsa* on the N-deficient diet showed the type III functional response to food concentration. This is consistent with the result in Thor

and Wendt (2010), where *A. tonsa* fed on *Rhodomonas baltica*. However, I_{max} in the present study is around five times higher than that in Thor and Wendt (2010).

Table 5-5. Comparison of parameters in the functional response of *Acartia tonsa* in laboratory studies.

Food type	Algal medium	Food Conc.	K	I_{max}	Model	References
Rhodomonas baltica	Continuous culture	ca. 0 - 1500	225.3[a]	5.2[a]	$I = I_{max} e^{-k/C}$, (k = a constant)	Kiørboe et al. (1985)
	Walne medium	1780	ca. 250[b]	ca. 6.9[a]	-	Støttrup and Jensen (1990)
	f/2 medium	ca. 50 - 500	98.4	4.8[a]	Holling model (type III)	Thor and Wendt (2010)
Rhodomonas lens	f/2 medium	1500	ca. 400[b]	ca. 6.2	-	Jiang et al. (2009)
Rhodomonas salina	L-medium	ca. 500	ca. 200[b]	ca. 6	-	Tang et al. (2001)
	L/1 or f/2 medium	897.8[a]	ca. 412[b]	8.4[a]	-	Broglio et al. (2003)
Rhodomonas sp.	f/2 medium					This study
	Balanced	354	ca. 160	24.9	-	
	N deficiency	24 – 239	198	19.9	Holling model (type III)	
Dunaliella tertiolecta	f/2 medium	45 - 1132	693[a]	32.5	Ivlev model	Besiktepe and Dam (2002)
	f/2 medium	ca. 30 - 550	128	2.4[a]	Holling model (type III)	Thor and Wendt (2010)
Plankton assemblage from the Kiel Fjord		0 - 5600	1577	14[a]	Michaelis–Menten model	Sommer (2009)

[a] The value was calculated according to the data in the reference; [b] The value was estimated from the graphical representations; -: model fitting not possible or not provided in the literature. Food concentration (Food Conc.), food concentration at 50% of I_{max} (K) and maximum ingestion rates (I_{max}) are expressed as $\mu g\ C \cdot L^{-1}$, $\mu g\ C \cdot L^{-1}$ and $\mu g\ C \cdot ind^{-1} \cdot d^{-1}$, respectively.

Several sources of uncertainty in the compiled data such as food quality, grazing history and physical factors (e.g., turbulence intensity and container-bottle volume) (Wirtz 2013), may cause differences in functional responses of ingestion rates. In the comparison in Table 5-5, algae were mostly cultivated in f/2 medium at the exponential growth phase. However, algae in the present study were cultivated in semicontinuous cultures under wide ranges of

N:P supply ratios and growth rates. Previous chapters in this thesis have showed that N:P supply ratios and growth rates can influence elemental and biochemical food quality of *Rhodomonas* sp., which might explain different functional responses of ingestion rates between the present study and previous research.

Responses of ingestion rates to food quantity and quality. Previous research has shown different feeding responses of zooplankton to food quality. Some studies showed reduced feeding of herbivores on nutrient limited algae at certain food concentrations, e.g., for *Daphnia obtusa* on the P-limited *Scenedesmus acutus* at food concentrations 450 to 1800 µg $C \cdot L^{-1}$ (Sterner et al. 1993), and for *A. tonsa* and *A. clausii* on the N-depleted *Rhodomonas* sp. at the food concentration of 250 µg $C \cdot L^{-1}$ (Augustin and Boersma 2006). Similarly, ingestion rates of *A. tonsa* in the present study were lower on the N-deficient algae at higher food concentrations (> 150 µg $C \cdot L^{-1}$). However, at lower food concentrations (< 150 µg $C \cdot L^{-1}$), ingestion rates of *A. tonsa* were higher on the N-deficient diet in the present study. Such an increase in ingestion rates on nutrient limited diets has been also found in *Daphnia magna* (Plath and Boersma 2001), and in *A. tonsa* and *A. clausii* (Augustin and Boersma 2006) at the food concentration of 500 µg $C \cdot L^{-1}$. This increase in feeding rate is referred to as compensatory feeding that compensates for shortages of dietary essential compounds (Fink and Von Elert 2006).

Several parameters have been considered critically in studying responses of herbivores feeding to food quality. For example, ingestion rate was related to gut transit time to examine the effect of food quality on feeding strategies of copepods (e.g., Tirelli and Mayzaud 2005). It is suggested that predators may respond to low quality of food in two opposite ways: decreasing throughput of ingested material to allow more time for food digestion, or increasing throughput and the ingestion of the limiting nutrients (Mitra and Flynn 2005). Moreover, food quantity may also interact with food quality. This complicated regulation of food quantity and quality on feeding responses has recently discussed in a modelling study (Suzuki-Ohno et al. 2012). This model shows that variable feeding responses can be attributed to stoichiometric characteristics, digestive traits of herbivores, and the assimilability of a given food. In the present study, the effects of food quantity and food quality (as chemical composition of phytoplankton) were evaluated, but other parameters such as gut transit time were not accounted for. Nevertheless, the present study shows variable responses of ingestion rates in *A. tonsa* to food quantity and quality, which contributes to the studies of feeding strategies of copepods under different food conditions.

The limitation of ingestion rates as an indicator of egg production rates. Ingestion rate is commonly used to indicate metabolic activity (Runge and Roff 2000). Recently, ingested PUFAs have been suggested as indicators of food quality for egg production (Chen et al. 2012). In CHAPTER 4, egg production rates of *A. tonsa* were higher when feeding on the balanced nutrient diet at food concentrations of 250 to 2500 µg C · L^{-1}. In the present chapter, higher ingestion rates for C, N, TFAs and ω3-PUFAs were also observed when feeding on the balanced nutrient diet at higher food concentrations (> ca. 150 µg C · L^{-1}). However, ingestion rates for ω6-PUFAs were lower on the balanced nutrient diet, which was probably caused by lower ω6-PUFA contents in the balanced nutrient diet rather than a lower metabolic activity of copepods. Moreover, opposite responses of ingestion rates and egg production rates to food quality were reported for both *A. tonsa* and *A. clausii* (at the food concentration of 250 C · L^{-1}), showing lower ingestion rates for C but higher egg production rates when copepods fed on the N-depleted diet (Augustin and Boersma 2006). These findings show the limitation of not only ingested elements (e.g., C) but also certain PUFAs (e.g., ω6-PUFAs) as indicators of food quality for egg production.

Reponses of nucleic acid contents to food quantity and quality. In the present study, the RNA content and RNA:DNA ratio of *A. tonsa* were higher at higher food concentrations (ca. 1000 to 2000 µg C · L^{-1}) in each food quality treatment. This result is consistent with those in previous research (reviewed by Gusmão and McKinnon 2011). Furthermore, hyperbolic functional responses of RNA content and RNA:DNA ratio were recently observed in *Acartia bifilosa* under different food concentrations (0 to 1200 µg C · L^{-1}) (Holmborn et al. 2009). In their study, the RNA content and RNA:DNA ratio increased with increasing food concentrations under non-saturating food concentrations (0 to 200 µg C · L^{-1}), while both response variables showed slight decreases at the highest food concentration (1200 µg C · L^{-1}) compared to the second highest one (600 µg C · L^{-1}). However, the present study had too few food concentration treatments to make precise correlations between nucleic acid indices and food concentration.

The present study shows that the RNA content and RNA:DNA ratio in *A. tonsa* were higher on the balanced nutrient diet at food concentrations of ca. 300 to 400 µg C · L^{-1}. At similar food concentrations (ca. 400 µg C · L^{-1}), Malzahn and Boersma (2012) found that P-limited prey (*Rhodomonas salina*) significantly reduced RNA:DNA ratios in *A. tonsa*. In CHAPTER 4, the relative gross growth efficiency for C and N (and P), as well as the relative trophic transfer efficiency of ω3- (and ω6-) PUFAs and C, were higher on the balanced nutrient diet. Thus, the results in the present study and in Malzahn and Boersma

(2012) support the prediction in CHAPTER 4 that the trophic transfer of elements and essential biochemicals may predict the performance of consumers (in this case, RNA contents and RNA:DNA ratios).

Nucleic acid contents of *A. tonsa* were measured at two food concentration levels in the present study, which precludes the possibility to predict maximum RNA-based indices and corresponding food saturation levels. This disadvantage makes it difficult to explain the similarity of RNA content or RNA:DNA ratio between food quality treatments at higher food concentrations. Further work is recommended to study the effect of food quality on RNA-based indices in copepods under a wider range of food concentration.

RNA-based indices of egg production. In the present study, food quality in terms of chemical composition of phytoplankton showed no significant effect on the relationship between egg production and nucleic acid indices (the RNA content and RNA:DNA ratio). This result agrees in theory with Saiz et al. (1998), who hypothesized that the slope of the relationship between RNA content and growth rate (estimated as egg production rate) should be uniform for a particular copepod species independent of (nutritional) food quality and previous food history.

Overall, the RNA content and RNA:DNA ratio in the present study showed significant positive correlations with egg production rate under the entire ranges of food quantity and quality. This is in agreement with the increasing recognition that RNA-based indices can be good indicators of copepod egg production (Saiz et al. 1998, Gorokhova 2003, Holmborn and Gorokhova 2008).

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CHAPTER 6

General discussion and outlooks

Elements and biochemicals as mutual regulators of food quality

Overall, this thesis has for the first time provided empirical evidence that elemental and biochemical limitation of phytoplankton food quality shows a mutually non-exclusive regulation in the trophic transfer across the phytoplankton-copepods interface. This result agrees with the recent suggestion of Winfried Lampert, who wrote “we are now on the way to a concept incorporating both groups of resources as limiting factors” (Arts et al. 2009), where “both groups of resources” indicates inorganic nutrient stoichiometry and essential fatty acids.

From the stoichiometric viewpoint, the Droop model has been successfully applied to numerous empirical data and the results in this thesis (CHAPTER 2). From biochemical considerations, this thesis has first successfully applied Ågren’s functions to marine phytoplankton species (CHAPTER 2). Further analyses in CHAPTER 2 have shown the agreement of parameters between the Droop and Ågren’s functions, which indicates a potential incorporation of elemental and biochemical food quality in marine phytoplankton. This indication has been confirmed by the results in CHAPTER 3, showing the covariance of nitrogen (N) cell quota (Q_N) and fatty acid (FA) contents under N deficiency. Therefore, the studies in CHAPTER 2 and CHAPTER 3 are the first step to explore the mutual regulation of elemental and biochemical food quality in the trophic interaction between phytoplankton and copepods.

Both elemental and biochemical composition of phytoplankton have previously shown good correlations with copepod reproduction (Jónasdóttir 1994). In CHAPTER 4, via a different approach rather than simply comparing statistical correlations I have found that the

transfer of essential elements and biochemicals between phytoplankton and copepods may predict well the egg production rate of *Acartia tonsa*. This finding is in agreement with that in Augustin and Boersma (2006), suggesting the importance of essential chemical trophic processes for copepod reproduction. Moreover, higher RNA contents and RNA:DNA ratios on the balanced nutrient diet have been found in CHAPTER 5 and in Malzahn and Boersma (2012), which further indicates the importance of essential chemical trophic processes for the assessment of copepod condition.

Effects of N:P supply ratios and growth rates on elemental and biochemical composition of phytoplankton

This thesis has simultaneously considered elemental and biochemical responses of phytoplankton to the interactive effect of N:P supply ratios and growth rates. The results have shown species-specific responses of both elemental and biochemical composition of phytoplankton under wide ranges of N:P supply ratios and growth rates.

For all three species of phytoplankton, N:P biomass ratios in CHAPTER 2 showed significant positive correlations with N:P supply ratios and converged over the range of N:P supply ratios when growth rates approached the highest value. The results indicate that phytoplankton "are what they eat" (nonhomeostatic nature of phytoplankton stoichiometry) (Sterner and Elser 2002) at lower growth rates, with considerable flexibility of N:P biomass ratios over the range of N:P supply ratios. At lower growth rates, fatty acid (FA) contents in all three species also showed significant differences among different N:P supply ratios (CHAPTER 3).

However, phytoplankton "eat what they want" with an optimal nutrient uptake ratio at higher growth rates, resulting in the convergence of N:P biomass ratios toward an optimal value independent of N:P supply ratios (Klausmeier et al. 2004). The limited flexibility of phytoplankton stoichiometry and FA content at higher growth rates has been shown in CHAPTER 2 and CHAPTER 3, respectively. This finding suggests that high dilution rates (loss rates) can explain constraints of phytoplankton stoichiometry that is reported in diverse natural aquatic communities (Hall et al. 2005).

Species-specific responses of phytoplankton stoichiometry were observed in CHAPTER 2. First, the parameters in the Droop and Ågren's functions differed among the three species. Second, there were species-specific responses of N:P biomass ratios to growth rates, as well

as to the interactive effect of N:P supply ratios and growth rates. Also in CHAPTER 3, polyunsaturated fatty acids (PUFAs) showed species-specific responses to N:P supply ratios and growth rates. These results indicate that (i) phytoplankton has species-specific adaption to nutrient deficiency, and the parameters in the Droop and Ågren's functions can be useful indications of algal succession in the phytoplankton community; (ii) responses of elemental and biochemical food quality to nutrient deficiency differ among phytoplankton species, which may further affect consumers at higher trophic levels.

Effects of food quantity and quality on the performance of copepods

As shown in CHAPTER 1, Sterner and Schulz (1998) hypothesized that growth rate of zooplankton responds positively to food quantity, and food quality effects can lead to certain changes in this growth-food quantity functional response such as reducing or increasing the maximum growth rate (Fig. 1-1). The results in this thesis are to some extent in a good agreement with Sterner and Schulz's hypothesis, i.e., responses of egg production, ingestion and nucleic acids to food quantity and quality.

Two commonly used indices of zooplankton growth, egg production rate and nucleic acid content (RNA content and RNA:DNA ratio), showed higher values at higher food concentrations (CHAPTER 4 and CHAPTER 5). Recent studies have reported hyperbolic functional responses of egg production rates or RNA-based nucleic acids in *Acartia* to food concentration (Gusmão and McKinnon 2009, Holmborn et al. 2009). However, food concentration levels in this thesis were too few to fit either of two indices with a mathematical model. Egg production rate and nucleic acid content of *A. tonsa* were relatively higher on the balanced nutrient diet compared to those on nutrient deficient diets. CHAPTER 4 has shown that the balanced nutrient diet at higher food concentrations is the optimized food conditions, i.e., the 'ideal' food (food type 1 in Fig. 1-1), for *A. tonsa* in this thesis. Nutrient deficient diets are food type 2 or 3, while extensive data would be required to investigate how the response curves actually develop at higher food concentrations.

Also, the relationship between ingestion rates and food concentration under different food quality (CHAPTER 5) generally follows the hypothetical relationship for growth rate in Fig. 1-1. However, differences in functional response curves were observed at lower food

quantity, which can be caused by compensatory feeding that results in higher ingestion rates on nutrient deficient diets at lower food concentrations.

Outlooks

Several aspects related to this thesis were not addressed and remain poorly understood, which I believe is worthy of further research.

(i) Effects of multiple abiotic factors and resources on the phytoplankton-zooplankton relationship via bottom-up control. In this thesis, the effects of N:P supply ratios and growth rates on chemical composition of phytoplankton were studied in laboratory conditions. This approach focuses on the evaluation of these two factors in regulating phytoplankton food quality. However, phytoplankton in natural conditions has to face interactive effects of multiple abiotic factors and resources, e.g., temperature, light, and CO_2. For example, light supply is identified as a dominant trigger of the phytoplankton spring bloom in the plankton ecology group (PEG) model (Sommer et al. 2012). A recent study observed interactive effects of P supply and light intensity (and temperature) on algal FA concentrations (Piepho et al. 2012). Thus, other abiotic factors and resources may influence the effects of N:P supply ratios and growth rates on chemical composition of phytoplankton, on which further studies are recommended for better understanding responses of chemical composition of phytoplankton in more realistic scenarios.

As the model copepod species, *A. tonsa* in this thesis was cultivated in different food quantity and quality treatments with other ambient factors remaining constant. *A. tonsa* is one of the most cosmopolitan calanoid copepod species and has been reported to have high reproductive success over wide ranges of temperature and salinities (Holste and Peck 2006). Therefore, experiments with factorial combinations of multiple abiotic factors and resources might be valuable to test if there is regional limitation for using the trophic transfer of essential elements to predict the performance of copepods.

(ii) The assessment of copepod recruitment under variable food quantity and quality. Egg production rate is one of the most widely applied techniques for the estimation of copepod growth. Other factors such as hatching success and early naupliar survival should be also considered for the assessment of copepod recruitment. Responses of hatching success and early naupliar survival to food supply have appeared to obtain less attention compared to egg production rate. For example, hatching success might be controlled by food quality and/or egg quality, on which observations vary among previous studies (Koski

et al. 2012 and references therein). The transfer of essential chemicals (e.g., N, P, and PUFAs) from food via female to egg is suggested to be investigated under wide enough ranges of food quantity and quality in further studies, which would be helpful to explore the regulation of copepod recruitment.

(iii) Taxon-specific responses of phytoplankton and zooplankton. This thesis has considered taxonomic comparison of phytoplankton C:N:P stoichiometry and FA composition in response to N:P supply ratios and growth rates, but only one copepod species has been included in studying the phytoplankton-copepods relationship. As shown in previous research and this thesis, there are species-specific responses in phytoplankton to various ambient factors. Moreover, taxonomy and trophic position have been suggested as the determinant of the FA composition of zooplankton, and differences in the latter could affect both food web structure and function (Persson and Vrede 2006). Therefore, understanding taxon-specific responses of phytoplankton and zooplankton would be critical to predict changes in trophic interactions between phytoplankton and zooplankton, as well as among higher trophic levels in aquatic ecosystems.

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Appendix

Table A1. N and P cell quotas (Q_N and Q_P) (mean ± SD) of *Rhodomonas* sp., *Isochrysis galbana*, and *Phaeodactylum tricornutum* under five N:P supply ratios (10:1, 14:1, 24:1, 35:1, and 63:1 mol · mol^{-1}) and four growth rates (20%, 40%, 60%, and 80% of μ_{max}, d^{-1}).

Species	μ (d^{-1})	Q_N (mol · mol^{-1})					Q_P (mmol · mol^{-1})				
		10:1	14:1	24:1	35:1	63:1	10:1	14:1	24:1	35:1	63:1
R. sp.	0.17	0.115 ±0.002	0.149 ±0.021	0.130 ±0.009	0.163 ±0.014	0.166 ±0.005	4.655 ±0.129	3.072 ±0.076	3.952 ±0.298	1.939 ±0.125	2.203 ±0.103
	0.34	0.161 ±0.007	0.152 ±0.021	0.199 ±0.015	0.178 ±0.019	0.189 ±0.005	3.842 ±0.426	4.754 ±0.535	4.782 ±0.739	2.949 ±0.203	2.709 ±0.150
	0.51	0.176 ±0.011	0.217 ±0.011	0.191 ±0.019	0.222 ±0.015	0.203 ±0.009	4.561 ±0.347	6.662 ±0.970	5.690 ±0.617	4.457 ±0.339	3.752 ±0.451
	0.68	0.175 ±0.007	0.212 ±0.015	0.203 ±0.007	0.228 ±0.026	0.224 ±0.026	6.356 ±0.097	4.455 ±0.392	6.131 ±1.499	4.423 ±0.048	4.821 ±0.563
I. galbana	0.08	0.076 ±0.003	0.084 ±0.013	0.089 ±0.003	0.116 ±0.003	0.102 ±0.002	1.750 ±0.101	1.363 ±0.213	1.902 ±0.162	1.472 ±0.170	1.308 ±0.105
	0.17	0.113 ±0.014	0.119 ±0.013	0.127 ±0.005	0.127 ±0.021	0.119 ±0.004	2.770 ±0.500	2.009 ±0.281	2.421 ±0.223	2.271 ±0.227	1.538 ±0.075
	0.25	0.126 ±0.021	0.132 ±0.006	0.126 ±0.004	0.150 ±0.015	0.126 ±0.003	2.134 ±0.083	2.677 ±0.321	1.802 ±0.244	2.321 ±0.198	1.502 ±0.078
	0.34	0.140 ±0.014	0.147 ±0.005	0.184 ±0.014	0.205 ±0.019	0.184 ±0.020	3.238 ±0.140	1.819 ±0.157	3.642 ±0.209	2.213 ±0.315	3.386 ±0.317
P. tricornutum	0.20	0.093 ±0.006	0.121 ±0.012	0.105 ±0.016	0.132 ±0.013	0.113 ±0.008	4.246 ±0.515	3.074 ±0.170	2.625 ±0.258	1.510 ±0.060	1.488 ±0.046
	0.40	0.123 ±0.014)	0.148 ±0.008	0.137 ±0.001	0.158 ±0.001	0.142 ±0.010	4.793 ±0.589	6.098 ±0.563	2.851 ±0.181	4.384 ±1.117	2.353 ±0.312
	0.60	0.148 ±0.011	0.169 ±0.005	0.158 ±0.004	0.151 ±0.025	0.163 ±0.014	5.379 ±1.410	5.608 ±0.098	5.501 ±0.023	3.756 ±0.724	3.367 ±0.897
	0.80	0.198 ±0.017	0.203 ±0.010	0.206 ±0.018	0.195 ±0.008	0.320 ±0.051	12.701 ±2.802	7.326 ±0.733	15.209 ±1.053	5.259 ±0.593	7.394 ±0.756

Table A2. Summary of the fatty acid profile of *Rhodomonas* sp. under five N:P supply ratios (N:P = 10, 14, 24, 35, and 63 mol · mol^{-1}) and four growth rates (20%, 40%, 60%, and 80% of μ_{max}, d^{-1}).

	N:P = 10								N:P = 14								N:P = 24							
	20%		40%		60%		80%		20%		40%		60%		80%		20%		40%		60%		80%	
	Content	%	Content	%	Content	%	Content	%	Content	%	Content	%	Content	%	Content	%	Content	%	Content	%	Content	%	Content	%
C14:0	19±1	6	10±1	4	9±2	4	8±0	3	11±1	5	15±1	6	12±2	5	9±2	4	1±1	5	11±0	4	6±1	3	8±1	3
C14:1	3±1	1	3±0	1	2±0	1	1±0	0	1±0	0	0±0	0	3±1	1	2±0	1	1±0	1	1±1	1	2±1	1	1±1	1
C16:0	57±5	17	36±5	13	29±6	13	48±3	19	38±7	16	42±8	18	56±16	22	41±1	18	34±7	15	28±5	12	19±2	9	54±5	22
C16:1ω7	5±0	2	5±0	2	4±1	2	5±0	2	2±0	1	2±1	1	5±1	2	4±0	2	4±2	2	5±0	2	5±0	3	4±0	2
C18:0	7±1	2	6±1	2	8±3	3	1±1	0	1±0	0	0±0	0	2±1	1	1±0	0	8±2	3	8±3	3	5±1	2	1±1	0
C18:1ω9	23±2	7	6±1	2	4±1	2	6±0	2	16±4	6	7±1	3	7±3	3	4±1	2	9±5	4	5±1	2	2±0	1	8±3	3
C18:1ω7	15±1	4	17±1	6	18±3	8	20±2	8	15±0	6	16±1	7	21±2	8	18±0	8	15±1	7	16±3	7	19±4	9	21±5	8
C18:2ω6	20±2	6	10±1	4	9±1	4	9±0	4	21±3	9	17±4	7	7±0	3	6±1	3	12±3	5	7±2	3	6±1	3	10±2	4
C18:3ω6	2±0	1	2±0	1	2±0	1	1±0	0	2±0	1	2±0	1	1±0	0	1±0	0	2±0	1	2±0	1	1±0	1	1±0	0
C18:3ω3	74±6	22	60±6	22	42±4	18	49±1	19	52±3	22	56±5	24	40±1	16	40±2	18	56±7	25	53±4	23	40±3	20	46±10	19
C18:4ω3	51±5	15	57±5	21	55±1	24	58±1	23	33±1	14	32±6	13	47±1	19	53±2	23	48±10	21	56±1	25	55±4	28	45±9	18
C20:4ω6	1±0	0	1±0	0	1±0	0	1±0	0	2±0	1	1±0	1	2±2	1	1±0	0	1±1	1	1±0	0	0±0	0	1±0	0
C20:4ω3	1±0	0	2±0	1	1±0	1	1±0	0	2±0	1	2±0	1	1±1	1	1±0	1	1±0	1	1±0	0	1±0	0	1±0	1
C20:5ω3	33±4	10	36±3	13	27±2	12	28±2	11	29±1	12	25±2	11	23±2	9	24±1	11	25±2	11	27±3	12	23±3	12	28±5	11
C22:6ω3	17±2	5	17±1	6	16±1	7	17±0	7	13±1	5	16±1	7	17±0	7	16±0	7	17±1	7	18±1	8	14±2	7	14±3	6
∑SFAs	83±5	25	53±8	20	46±9	20	57±3	23	51±7	21	57±9	24	64±5	25	53±1	23	53±10	23	47±6	21	31±4	15	63±6	26
∑MUFAs	46±4	14	32±3	12	29±4	13	32±1	12	35±4	15	27±2	11	30±0	12	29±1	13	30±6	13	29±3	13	29±5	14	36±3	15
∑PUFAs	200±18	61	186±17	69	153±8	67	165±3	65	155±10	64	152±11	64	141±1	56	144±1	64	164±19	72	165±7	73	142±10	71	148±29	60
∑TFAs	329±26	100	270±23	100	228±17	100	255±4	100	241±20	100	236±21	100	250±14	100	226±4	100	247±29	109	241±8	107	201±9	100	248±26	100
∑ω3	176±16	53	172±16	64	141±7	62	153±3	60	130±6	54	130±12	55	128±0	51	134±1	59	148±20	65	155±7	68	133±10	66	135±28	55
∑ω6	24±3	7	13±1	5	11±1	5	12±0	5	25±3	10	21±3	9	9±3	4	8±1	4	16±3	7	10±2	4	8±1	4	13±1	5
C16:1ω7/ C16:0	0		0		0		0		0		0		0		0		0		0		0		0	
C20:5ω3/ C22:6ω3	2		2		2		2		2		2		1		1		1		2		2		2	

Abbreviations: SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; TFAs, total fatty acids; ∑ω3, sum of ω3-PUFAs; ∑ω6, sum of ω6-PUFAs. Data are expressed as fatty acid contents (mean ± SD) (μg · mg C^{-1}) and percentages of total fatty acids (% of TFAs). Trace amount of fatty acid individuals are not shown.

Table A2. Continued.

	N:P = 35								N:P = 63							
	20%		40%		60%		80%		20%		40%		60%		80%	
	Content	%	Content	%	Content	%	Content	%	Content	%	Content	%	Content	%	Content	%
C14:0	20±2	7	15±3	6	7±1	4	10±2	4	12±1	5	9±1	4	7±1	3	11±5	4
C14:1	1±0	0	1±0	0	2±1	1	2±1	1	0±0	0	1±0	0	2±1	1	1±0	0
C16:0	57±9	21	46±13	18	52±8	25	54±13	21	35±2	15	25±6	12	24±5	11	50±13	19
C16:1ω7	3±1	1	2±0	1	3±1	2	6±1	2	3±0	1	4±0	2	5±0	2	5±0	2
C18:0	1±1	0	1±0	0	2±1	1	1±0	0	6±0	3	7±4	3	10±5	5	2±1	1
C18:1ω9	16±3	6	8±2	3	4±1	2	6±6	2	11±1	5	5±2	2	3±0	1	5±2	2
C18:1ω7	16±2	6	15±3	6	16±4	8	19±0	7	16±3	7	16±3	8	19±2	9	20±1	8
C18:2ω6	3±2	11	16±5	6	6±2	3	7±0	3	15±2	7	7±2	3	6±1	3	11±2	4
C18:3ω6	3±0	1	1±0	1	1±1	1	1±1	0	3±1	1	2±0	1	2±0	1	1±0	0
C18:3ω3	57±8	21	57±7	23	35±9	17	45±3	17	55±5	24	46±12	22	41±5	19	50±6	19
C18:4ω3	32±6	12	37±4	15	41±8	20	58±3	22	40±4	18	48±12	23	58±2	27	57±4	22
C20:4ω6	2±0	1	2±0	1	1±0	0	1±0	0	1±0	0	1±0	0	0±0	0	1±0	0
C20:4ω3	1±0	0	2±0	1	1±1	0	1±0	0	1±0	0	1±0	0	1±0	1	2±0	1
C20:5ω3	20±3	7	27±3	11	20±5	10	26±1	10	12±1	5	23±5	11	23±3	11	26±2	10
C22:6ω3	15±3	5	17±3	7	13±2	6	17±1	6	15±3	7	14±3	7	15±2	7	17±0	6
∑SFAs	79±11	28	62±15	25	62±8	30	66±15	25	53±4	23	46±7	22	42±11	19	64±20	24
∑MUFAs	38±5	14	28±3	11	26±3	13	35±7	13	31±4	14	28±0	14	29±1	13	32±3	12
∑PUFAs	162±22	58	161±18	64	129±23	62	160±3	61	142±16	63	162±4	77	148±11	68	166±14	64
∑TFAs	278±37	100	251±36	100	207±23	100	261±23	100	226±22	100	209±46	100	219±18	100	261±37	100
∑ω3	126±19	45	140±15	56	110±24	53	147±4	56	122±13	54	132±31	63	138±12	63	154±12	59
∑ω6	35±3	13	20±5	8	9±1	4	12±2	4	19±3	9	10±3	5	9±1	4	13±2	5
C16:1ω7/ C16:0	0		0		0		0		0		0		0		0	
C20:5ω3/ C22:6ω3	1		2		1		2		1		2		2		2	

Table A3. Summary of the fatty acid profile of *Isochrysis galbana* under five N:P supply ratios (N:P = 10, 14, 24, 35, and 63 mol · mol^{-1}) and four growth rates (20%, 40%, 60%, and 80% of μ_{max}, d^{-1}).

	N:P = 10								N:P = 14								N:P = 24							
	20%		40%		60%		80%		20%		40%		60%		80%		20%		40%		60%		80%	
	Content	%	Content	%	Content	%	Content	%	Content	%	Content	%	Content	%	Content	%	Content	%	Content	%	Content	%	Content	%
C14:0	107±17	25	63±7	23	57±8	23	50±9	20	51±9	22	46±9	18	48±13	21	42±1	16	81±5	25	51±5	23	56±6	23	49±1	17
C14:1	1±0	0	1±0	0	1±0	0	1±0	0	1±0	0	1±0	1	1±0	1	1±0	0	1±0	0	1±0	0	1±0	0	2±0	1
C16:0	79±14	19	42±9	15	33±6	13	67±12	27	48±16	21	53±9	21	57±12	24	66±12	26	54±7	17	28±4	13	35±1	14	82±13	29
C16:1ω7	4±1	1	5±0	2	4±1	2	5±3	2	5±0	2	5±0	2	5±2	2	4±0	1	5±0	1	4±0	2	4±0	2	5±0	2
C16:2ω4	2±0	1	3±1	1	4±0	2	4±1	2	2±0	1	3±1	1	3±2	1	3±0	1	2±0	1	4±1	2	4±1	2	5±0	2
C18:0	10±2	2	6±1	2	5±2	2	3±2	1	1±0	0	1±0	0	1±1	1	2±1	1	5±1	2	4±1	2	4±3	1	3±0	1
C18:1ω9	85±76	20	48±17	17	35±12	14	30±10	12	48±15	20	41±2	16	28±10	12	43±26	17	73±9	23	29±2	13	34±1	14	31±4	11
C18:1ω7	37±47	9	11±2	4	11±1	4	10±3	4	7±1	3	9±2	4	9±4	4	15±11	6	8±1	3	9±1	4	10±0	4	10±0	4
C18:2ω6	29±7	7	22±3	8	15±4	6	12±4	5	14±4	6	14±2	6	12±4	5	13±1	5	20±2	6	14±1	6	14±1	6	16±0	6
C18:3ω6	1±0	0	1±0	0	1±0	0	1±0	0	0±0	0	1±1	0	1±0	0	1±1	0	1±0	0	1±0	0	1±0	1	1±1	0
C18:3ω3	16±2	4	25±7	9	32±5	13	14±5	6	13±2	5	19±3	8	17±8	7	16±1	6	18±2	6	26±7	12	26±7	11	19±1	7
C18:4ω3	21±4	5	19±2	7	18±5	7	17±6	7	24±3	10	26±5	10	22±9	10	18±1	7	24±4	8	18±2	8	22±1	9	23±6	8
C20:2ω6	2±1	0	1±0	0	0±0	0	0±0	0	1±0	0	1±1	0	1±0	0	1±1	0	1±0	0	0±0	0	0±0	0	2±1	1
C22:0	3±0	1	1±0	0	0±0	0	1±0	0	1±0	0	1±1	0	1±0	0	1±0	0	1±0	0	0±0	0	0±0	0	1±0	0
C20:5ω3	1±0	0	1±0	1	2±0	1	2±1	1	1±0	0	1±0	1	2±1	1	2±1	1	1±0	0	1±1	1	2±0	1	3±0	1
C22:1ω9	1±0	0	1±0	0	0±0	0	1±0	0	1±0	0	1±0	0	1±0	0	1±0	0	1±0	0	1±1	0	1±0	0	1±0	0
C22:6ω3	24±5	6	26±3	9	26±4	10	24±11	10	16±3	7	21±6	8	22±12	9	26±0	10	22±1	7	24±5	11	26±3	11	28±1	10
∑SFAs	199±33	47	113±17	40	95±14	39	121±18	49	102±24	43	101±7	40	107±20	46	111±13	43	142±13	44	84±9	39	96±9	39	135±13	48
∑MUFAs	128±35	30	66±17	24	51±12	21	47±15	19	61±14	26	60±2	24	44±15	19	66±39	25	88±9	27	43±4	20	50±2	21	51±4	18
∑PUFAs	99±21	23	101±10	36	99±12	40	77±28	32	73±8	31	89±13	36	82±37	35	83±5	32	91±8	28	90±12	42	97±11	40	98±5	34
∑TFAs	426±88	100	279±38	100	246±37	100	245±56	100	236±40	100	250±10	100	233±59	100	259±55	100	321±29	100	217±21	100	243±21	100	284±6	100
∑ω3	54±25	13	59±17	21	64±22	26	59±23	24	55±8	23	67±14	27	64±31	27	62±3	24	56±23	17	59±27	27	65±27	27	74±5	26
∑ω6	85±44	20	50±25	18	31±19	13	11±3	4	27±4	11	15±2	6	13±7	6	12±2	5	54±23	17	31±5	14	22±7	9	10±3	4
C16:1ω7/ C16:0	0		0		0		0		0		0		0		0		0		0		0		0	
C20:5ω3/ C22:6ω3	0		0		0		0		0		0		0		0		0		0		0		0	

Abbreviations: SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; TFAs, total fatty acids; ∑ω3, sum of ω3-PUFAs; ∑ω6, sum of ω6-PUFAs. Data are expressed as fatty acid contents (mean ± SD) (μg · mg C^{-1}) and percentages of total fatty acids (% of TFAs). Trace amount of fatty acid individuals are not shown.

Table A3. Continued.

	N:P = 35								N:P = 63							
	20%		40%		60%		80%		20%		40%		60%		80%	
	Content	%	Content	%	Content	%	Content	%	Content	%	Content	%	Content	%	Content	%
C14:0	50±7	22	47±8	20	35±18	16	29±2	14	74±9	26	60±6	24	56±8	23	41±3	15
C14:1	1±0	0	1±0	1	3±2	1	1±0	0	1±0	0	1±0	0	1±0	0	3±1	1
C16:0	43±6	19	47±6	20	43±5	20	75±8	36	42±5	15	34±3	14	32±3	13	83±29	31
C16:1ω7	4±1	2	5±1	2	4±1	2	3±0	1	5±0	2	5±0	2	4±1	2	4±1	2
C16:2ω4	2±0	1	2±1	1	3±1	1	2±0	1	3±0	1	4±0	2	4±0	2	4±0	1
C18:0	1±1	0	1±1	0	1±0	0	3±1	1	4±1	2	5±2	2	5±2	2	3±2	1
C18:1ω9	38±3	17	36±3	15	35±9	16	26±4	13	58±8	21	37±5	15	34±5	14	32±0	12
C18:1ω7	7±1	3	8±2	4	10±1	5	5±2	2	7±1	2	9±2	4	10±0	4	8±2	3
C18:2ω6	11±0	5	12±2	5	13±1	6	11±1	6	17±2	6	16±1	7	14±1	6	15±2	6
C18:3ω6	1±1	0	1±1	0	1±1	0	1±0	0	1±0	0	1±0	0	1±0	0	1±1	0
C18:3ω3	13±2	6	16±4	7	13±8	6	11±3	5	19±4	7	23±1	9	27±1	11	16±1	6
C18:4ω3	29±4	13	30±3	13	24±4	11	14±3	7	21±3	8	21±3	8	20±3	8	22±4	8
C20:2ω6	0±0	0	1±1	0	1±0	0	1±1	1	1±0	0	1±0	0	0±0	0	2±2	1
C22:0	1±0	0	1±0	0	1±0	0	1±1	1	1±0	0	1±0	0	0±0	0	1±0	1
C20:5ω3	1±0	0	1±0	0	2±1	1	2±1	1	1±0	0	1±0	1	2±0	1	3±1	1
C22:1ω9	1±0	0	1±1	1	1±1	0	2±1	1	1±0	0	1±0	0	1±0	0	1±0	0
C22:6ω3	19±2	9	19±6	8	24±4	11	16±4	8	22±4	8	27±3	11	26±3	11	24±7	9
∑SFAs	95±13	42	96±13	41	81±18	37	108±11	52	122±13	43	99±8	40	94±9	39	130±29	48
∑MUFAs	52±5	23	53±5	23	55±11	25	38±3	18	72±9	26	52±6	21	50±6	21	49±2	18
∑PUFAs	80±10	35	85±14	36	85±12	38	62±10	30	87±13	31	95±8	39	95±8	40	91±8	34
∑TFAs	226±27	100	233±32	100	220±25	100	208±22	100	280±33	100	247±19	100	240±23	100	270±20	100
∑ω3	64±8	28	68±12	29	64±14	29	44±7	21	53±22	19	61±23	25	63±24	26	67±10	25
∑ω6	15±1	7	11±2	5	16±2	7	9±1	5	43±22	15	36±19	14	25±10	10	8±2	3
C16:1ω7/ C16:0	0		0		0		0		0		0		0		0	
C20:5ω3/ C22:6ω3	0		0		0		0		0		0		0		0	

Table A4. Summary of the fatty acid profile of *Phaeodactylum tricornutum* under five N:P supply ratios (N:P = 10, 14, 24, 35, and 63 mol · mol^{-1}) and four growth rates (20%, 40%, 60%, and 80% of μ_{max}, d^{-1}).

	N:P = 10								N:P = 14								N:P = 24							
	20%		40%		60%		80%		20%		40%		60%		80%		20%		40%		60%		80%	
	Content	%	Content	%	Content	%	Content	%	Content	%	Content	%	Content	%	Content	%	Content	%	Content	%	Content	%	Content	%
C14:0	24±2	7	24±5	8	21±3	8	16±4	6	18±2	8	17±1	6	14±2	7	18±2	8	23±5	8	21±1	8	18±3	7	19±2	8
C14:1	1±0	0	1±0	0	1±0	0	2±0	1	1±0	0	1±1	0	1±0	0	1±0	0	0±0	0	0±0	0	0±0	0	1±0	1
C16:0	63±10	19	44±19	15	27±9	10	31±7	12	36±8	15	43±12	17	21±12	11	20±3	9	39±14	15	21±11	8	23±2	9	31±23	14
C16:1ω7	104±3	31	77±23	26	48±14	17	32±7	12	63±11	27	48±5	19	30±9	15	41±9	18	69±24	26	44±6	17	37±5	14	36±12	16
C16:2ω4	5±1	2	8±0	3	9±0	3	18±11	7	7±0	3	10±2	4	9±3	5	12±1	5	6±0	2	9±1	3	10±1	4	10±4	4
C16:3ω4	12±4	4	22±5	7	34±2	12	30±2	12	17±3	7	26±2	10	22±9	11	25±1	11	18±1	7	34±6	13	34±5	13	22±19	10
C18:0	5±1	1	4±2	1	5±1	2	5±2	2	1±0	0	1±0	0	3±2	1	3±1	1	2±1	1	3±1	1	4±1	2	4±1	2
C18:1ω9	10±1	3	4±2	1	2±1	1	4±1	2	3±1	1	4±1	2	3±2	2	5±0	2	6±2	2	2±2	1	2±0	1	5±4	2
C18:1ω7	5±1	2	6±1	2	8±1	3	2±1	1	3±3	1	4±1	2	2±1	1	3±1	1	2±1	1	2±0	1	4±3	1	2±1	1
C18:2ω6	7±1	2	7±1	2	5±1	2	4±2	1	5±1	2	4±1	2	4±1	2	6±1	3	6±1	2	5±1	2	4±1	2	3±1	1
C18:3ω6	2±0	1	3±0	1	2±0	1	2±2	1	1±0	0	1±1	0	2±1	1	1±0	0	3±1	1	3±0	1	3±1	1	1±1	0
C18:3ω3	1±0	0	1±0	0	0±0	0	3±2	1	3±1	1	2±1	1	1±1	1	4±1	2	1±0	0	1±0	0	1±0	0	2±1	1
C18:4ω3	4±1	1	5±1	2	3±0	1	2±0	1	2±0	1	2±0	1	2±1	1	2±0	1	4±1	1	3±2	1	4±1	2	2±0	1
C20:0	1±0	0	1±0	0	0±0	0	1±0	0	0±0	0	0±0	0	0±1	0	1±0	0	1±0	0	0±0	0	0±0	0	1±0	0
C20:4ω6	2±1	0	1±0	0	2±1	1	1±0	0	1±0	0	2±1	1	1±0	1	1±1	0	2±0	1	2±0	1	2±1	1	1±1	0
C20:4ω3	1±0	0	1±0	0	1±0	0	1±1	0	1±0	0	1±0	0	1±0	0	1±0	0	2±0	1	2±1	1	1±0	0	1±0	0
C22:0	1±0	0	1±0	0	1±0	0	1±0	0	1±0	0	1±0	0	1±0	0	1±0	0	1±0	0	1±0	0	1±0	0	1±0	0
C20:5ω3	71±10	21	81±4	27	95±3	35	92±2	35	64±6	27	79±5	31	62±29	32	75±4	33	73±9	27	98±11	37	98±10	38	78±31	34
C22:1ω9	0±0	0	0±0	0	0±0	0	2±1	1	1±0	0	1±0	0	0±0	0	1±0	1	0±0	0	0±0	0	0±0	0	1±0	0
C24:0	2±1	1	3±2	1	2±0	1	2±1	1	1±0	0	1±1	0	1±0	1	1±0	0	2±0	1	3±0	1	2±0	1	1±1	0
C22:6ω3	9±2	3	7±2	2	6±0	2	6±4	2	5±2	2	5±1	2	4±1	2	5±0	2	7±1	3	6±0	2	7±1	3	4±0	2
∑SFAs	96±9	29	77±25	25	56±12	20	55±8	21	56±6	24	63±12	25	40±8	21	43±5	19	67±19	25	50±13	19	48±4	19	57±22	25
∑MUFAs	121±4	37	91±28	30	60±15	22	45±6	17	72±16	31	59±7	23	38±12	20	52±10	23	78±27	29	49±8	19	44±8	17	45±15	20
∑PUFAs	114±18	34	136±4	45	160±5	58	162±18	62	105±5	45	133±7	52	115±32	60	133±10	58	121±12	45	165±15	63	165±21	64	126±55	55
∑TFAs	331±13	100	301±53	100	275±29	100	261±29	100	233±16	100	254±13	100	192±50	100	228±26	100	266±57	100	263±10	100	256±33	100	227±83	100
∑ω3	84±11	25	96±7	32	107±4	39	103±5	40	74±4	32	89±4	35	70±32	37	58±51	26	86±11	32	111±9	42	111±12	43	88±32	39
∑ω6	30±12	9	23±7	7	17±8	6	3±1	1	11±3	5	6±1	2	7±6	4	5±1	2	27±10	10	26±6	10	16±8	6	4±2	2
C16:1ω7/ C16:0	2		2		2		1		2		1		2		2		2		2		2		1	
C20:5ω3/ C22:6ω3	8		11		15		16		14		17		16		16		11		15		14		19	

Abbreviations: SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; TFAs, total fatty acids; ∑ω3, sum of ω3-PUFAs; ∑ω6, sum of ω6-PUFAs. Data are expressed as fatty acid contents (mean ± SD) (μg · mg C^{-1}) and percentages of total fatty acids (% of TFAs). Trace amount of fatty acid individuals are not shown.

Table A4. Continued.

	N:P = 35								N:P = 63							
	20%		40%		60%		80%		20%		40%		60%		80%	
	Content	%	Content	%	Content	%	Content	%	Content	%	Content	%	Content	%	Content	%
C14:0	22±3	7	18±3	6	16±3	6	19±3	7	28±5	9	22±2	8	22±2	8	22±2	8
C14:1	1±0	0	2±1	1	1±0	0	2±1	1	0±0	0	0±0	0	0±0	0	3±1	1
C16:0	49±5	17	48±17	16	46±7	18	44±39	16	51±10	16	31±2	11	27±1	10	30±5	10
C16:1ω7	65±21	23	51±6	17	47±3	18	46±16	17	88±18	28	46±4	17	43±5	16	33±1	11
C16:2ω4	11±1	4	9±0	3	12±0	5	11±1	4	8±2	3	9±0	3	11±1	4	12±1	4
C16:3ω4	29±2	10	30±3	10	28±1	11	26±3	10	22±6	7	33±1	12	36±3	13	30±5	10
C18:0	1±1	0	2±1	1	4±5	2	4±2	1	2±2	1	4±4	1	3±0	1	12±8	4
C18:1ω9	3±0	1	6±4	2	2±0	1	6±3	2	5±1	1	3±0	1	2±0	1	6±2	2
C18:1ω7	3±0	1	4±1	1	3±1	1	4±0	2	2±0	1	2±0	1	3±0	1	3±2	1
C18:2ω6	6±1	2	5±1	2	4±1	1	5±1	2	7±1	2	5±0	2	5±1	2	7±3	2
C18:3ω6	1±0	0	2±1	1	1±0	1	2±1	1	2±0	1	3±3	1	3±0	1	2±0	1
C18:3ω3	2±0	1	2±1	1	1±0	0	3±2	1	1±0	0	1±0	0	1±0	0	6±2	2
C18:4ω3	2±2	1	3±1	1	3±0	1	3±1	1	11±4	4	6±2	2	6±1	2	3±0	1
C20:0	1±2	0	1±0	0	1±1	0	1±0	0	1±0	0	1±0	0	0±0	0	2±1	1
C20:4ω6	1±0	0	2±0	1	2±0	1	1±0	0	1±0	0	2±0	1	1±0	1	2±1	1
C20:4ω3	2±0	1	1±0	0	1±0	0	1±1	0	4±1	1	3±1	1	2±0	1	1±1	0
C22:0	1±0	0	1±0	0	1±0	0	1±0	0	2±0	0	1±0	0	1±0	0	2±0	1
C20:5ω3	81±6	28	97±18	33	81±3	31	86±6	31	74±16	23	93±6	34	102±11	37	98±4	33
C22:1ω9	1±0	0	1±0	0	0±0	0	1±1	0	1±0	0	0±0	0	0±0	0	3±2	1
C24:0	1±0	0	1±2	0	1±0	0	1±1	0	2±0	1	3±1	1	2±1	1	2±1	1
C22:6ω3	5±0	2	6±1	2	4±0	2	6±0	2	4±1	1	5±0	2	7±1	3	6±2	2
∑SFAs	75±6	26	70±21	24	70±16	27	69±43	25	86±13	27	62±6	22	55±4	20	67±6	23
∑MUFAs	75±20	26	64±11	22	54±1	21	60±19	22	96±19	31	53±4	19	49±4	18	46±4	16
∑PUFAs	142±10	49	159±22	54	137±2	53	148±7	53	134±27	42	161±9	58	174±19	63	164±12	55
∑TFAs	291±19	100	293±54	100	261±20	100	277±65	100	316±52	100	275±16	100	277±28	100	296±35	100
∑ω3	93±9	32	109±18	37	60±52	23	100±6	36	94±19	30	109±8	40	118±14	43	116±7	39
∑ω6	14±5	5	10±1	3	4±3	1	6±1	2	24±9	8	18±6	6	15±8	5	3±1	1
C16:1ω7/ C16:0	1		1		1		1		2		1		2		1	
C20:5ω3/ C22:6ω3	17		17		19		14		20		17		14		16	

Contributions of authors

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CHAPTER 2: Stoichiometric responses of phytoplankton species to the interactive effect of nutrient supply ratios and growth rates

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Planning of experiments: RB, CA and US. Conduction of experiments and sample analysis: RB and CA. Data analysis and figures: RB. Writing: RB, with assistance of CA and US.

CHAPTER 3: Linking elements to biochemicals: effects of nutrient supply ratios and growth rates on fatty acid composition of phytoplankton species

Under review. *Resubmitted to Journal of Phycology*.

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Planning of experiments: RB, CA and US. Conduction of experiments and sample analysis: RB and CA. Data analysis and figures: RB. Writing: RB, with assistance of CA and US.

CHAPTER 4: Effects of food quantity and food quality on elemental and biochemical trophic transfer in marine plankton: an experimental approach

Under revision. Submitted to Journal of Plankton Research.

Authors: *Rong Bi (RB) and Ulrich Sommer (US)*

Planning of experiments: RB and US. Conduction of experiments, sample analysis, data analysis and figures: RB. Writing: RB, with assistance of US.

CHAPTER 5: Effects of food quantity and food quality on ingestion rate and nucleic acid content in the calanoid copepod *Acartia tonsa*

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Declaration

The content and design of this thesis, apart from the supervisor's guidance, is my own work. The thesis has not been submitted either partially or wholly as a part of a doctoral degree to another examining body. The thesis has not been published or submitted for publication, while one chapter (CHAPTER 2) has been published in a scientific journal (Journal of Phycology) and two chapters (CHAPTER 3 and CHAPTER 4) have been submitted to scientific journals (Journal of Phycology and Journal of Plankton Research, respectively) for peer review. The thesis has been prepared respecting the Rules of Good Scientific Practice of the German Research Foundation.

Kiel

Rong Bi