frontiers in Marine Science

ORIGINAL RESEARCH
published: 28 April 2020
doi: 10.3389/fmars.2020.00274

Food Quantity and Quality Interactions at Phytoplankton–Zooplankton Interface: Chemical and Reproductive Responses in a Calanoid Copepod

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Specialty section:
This article was submitted to Marine Biology, a section of the journal Frontiers in Marine Science

Received: *02 December 2019*
Accepted: *06 April 2020*
Published: *28 April 2020*

Citation:
Bi R and Sommer U (2020) Food Quantity and Quality Interactions at Phytoplankton–Zooplankton Interface: Chemical and Reproductive Responses in a Calanoid Copepod. Front. Mar. Sci. 7:274. doi: 10.3389/fmars.2020.00274

Marine food webs form the major component of the biological pump and play a central role in the global carbon (C) cycle. Understanding the response of particular processes in marine food webs to changing environments is a prerequisite to predict changes in ecological functioning in the future ocean. Here, we experimentally assessed the effects of nitrogen:phosphorus (N:P) supply ratios (the molar ratios 10:1, 24:1, and 63:1) on elemental and biochemical quality of marine phytoplankton *Rhodomonas* sp., and the interactions between food quantity and quality on stoichiometric C:N:P, fatty acids (FAs) and reproductions in copepods *Acartia tonsa*. Overall, the stoichiometry of *A. tonsa* was to some extent homeostatic in response to the changing algal C:N and C:P ratios, with significant changes in C:N ratios of *A. tonsa* observed, especially under higher food quantities. The relative gross growth efficiencies (GGEs) for C and N (and P) were analyzed, revealing that copepods may achieve homeostasis by lowering the GGE for C while increasing it for the limiting nutrient. Egg production rates in *A. tonsa* were lowest on nutrient deficient diets under low food quantities. Reduced egg production rates may be attributed to the lowered GGEs for C and reduced transfer efficiency of essential FAs between phytoplankton and copepods, indicating interactive-essential effects of elements and FAs on copepod production. Our results highlight that nutrient deficiency in the environments may reduce energy transfer efficiency at the base of food webs by altering phytoplankton chemical composition, which can interact with food quantity and have implications on food web dynamics in the changing ocean.

Keywords: food webs, food concentration, nutritional quality, stoichiometry, fatty acids, egg production, nutrient limitation

INTRODUCTION

The pelagic food webs play a fundamental role in the biological pump and contribute substantially to carbon sequestration in the ocean (Passow and Carlson, 2012). Global climate change has exerted profound and complex impacts on marine food webs (Sommer et al., 2002; Edwards and Richardson, 2004; Lewandowska et al., 2014). For example, primary production, zooplankton abundance and fish stock recruitment in the North Sea have declined over 25 years and this has been driven primarily by a decrease in riverine nutrient inputs; the reduction in nutrient inputs caused a decline in primary production which was mirrored by a decline in small copepod abundance, revealing bottom–up control of herbivorous zooplankton by phytoplankton production (Capuzzo et al., 2018). In the central Baltic Sea in summer, zooplankton become more carnivorous under nutrient depletion (Loick-Wilde et al., 2019), because small (<5 μm) phytoplankton (generally favored by low nutrients) and N_2-fixing cyanobacteria are outside the optimal food size range of herbivorous zooplankton (Sommer et al., 2002). Thus, the structure of marine pelagic food webs differs at different levels of nutrient richness (Sommer et al., 2002). Understanding the responses of marine food webs to environmental changes is therefore of critical importance to predict the structure and functioning of marine ecosystems under future ocean scenarios.

Phytoplankton and crustacean zooplankton (zooplankton hereafter) occupy a key ecological position in marine food webs as they constitute the crucial link of energy and substance transport to higher trophic levels (Harris et al., 2000). Ocean environmental changes have caused pronounced changes in phytoplankton biomass (Boyce et al., 2010; Lotze et al., 2019) and chemical composition (Galbraith and Martiny, 2015; Bi et al., 2017, 2018; Ruess and Müller-Navarra, 2019), altering the quantity and quality of phytoplankton as a food for consumers. While food quantity has long time been acknowledged as a limiting factor for consumers, limitation by food quality became an issue since ca. 25 years ago (Sommer, 1992; Urabe and Watanabe, 1992; Müller-Navarra et al., 2000; Boersma et al., 2008). Sterner and Schulz (1998) pioneered the integration of food quantity and quality aspect in regulating zooplankton nutrition, showing that zooplankton growth rate in response to food quantity differs under distinct scenarios of food quality. To date, studies on the interactive effects of food quantity and quality have been mostly performed with freshwater zooplankton (e.g., Amarasinghe et al., 1997; Boersma and Kreutzer, 2002; Schälicke et al., 2019), while little is known for marine zooplankton (but see Ambler, 1986; Koski et al., 2010).

Food quantity is conventionally measured in terms of carbon (C) absolute concentration (Sterner and Robinson, 1994), and food quality is primarily determined by the chemical match between phytoplankton elemental and biochemical composition and zooplankton demands (Müller-Navarra, 2008). Elemental quality of food is frequently expressed as elemental stoichiometry (Anderson et al., 2004; Hessen, 2008). Of all biochemical indicators of food quality, fatty acids (FAs) have attracted particular interest (Müller-Navarra et al., 2004; Ruess and Müller-Navarra, 2019). Essential FAs (EFAs) cannot be synthesized by consumers, or the synthesis rate is not sufficient to meet the basic biochemical needs of consumers (Arts et al., 2001; Kainz et al., 2004), and thus must be acquired through diet. ω3- and ω6-polyunsaturated FAs (PUFAs) such as eicosapentaenoic acid (20:5n-3; EPA) and docosahexaenoic acid (22:6n-3; DHA) are essential for animals and have received intense attention (Müller-Navarra, 2008; Parrish, 2009; Taipale et al., 2011; Ilić et al., 2019). There is evidence for the significance of phytoplankton elemental stoichiometry or EFAs in regulating marine zooplankton growth (Malzahn and Boersma, 2012; Diez et al., 2013), egg production (Augustin and Boersma, 2006; Jónasdóttir et al., 2009; Franco-Santos et al., 2018), development rate (Marja et al., 1998; Malzahn et al., 2010; Mathews et al., 2018), feeding behavior (Meunier et al., 2016), and movement patterns (Herstoff et al., 2019). The relative importance of elemental *versus* biochemical quality of food is a long-standing controversy which has attracted more attention in limnology than in marine ecology (Lampert, 2009), some studies proposing the primary control of elemental quality (e.g., Hessen, 1992; Boersma, 2000; Becker and Boersma, 2005; Malzahn and Boersma, 2012) but the others emphasizing the importance of FA availability in zooplankton nutrition (e.g., Brett, 1993; Müller-Navarra, 1995; Park et al., 2002; Ravet and Brett, 2006; Li et al., 2019). So far, little effort has been invested to simultaneously deal with food quantity and quality effects, and particularly to incorporate elemental and biochemical quality of food as limiting factors in the study of zooplankton production in the changing environments.

In the present study, we investigated the transfer of elements [C, nitrogen (N) and phosphorus (P)] and EFAs (ω3- and ω6-PUFAs, in particular EPA and DHA) from marine phytoplankton to copepods, and egg production rates and nucleic acid ratios (RNA:DNA) of copepods under different food quantity and nutritional quality scenarios. The measurement of egg production rate is the most common technique to estimate zooplankton growth (Runge and Roff, 2000), while RNA:DNA has become a useful tool for zooplankton growth assessment, because of the primary function of RNA in protein synthesis (Saiz et al., 1998; Gorokhova, 2003; Gusmão and McKinnon, 2011; Yebra et al., 2017). We applied stoichiometric models to test the responses of the relative gross growth efficiencies (GGEs) for elements to different food quantity and nutritional quality (Anderson and Hessen, 1995), as well as the strength of limitation of elements versus EPA (and DHA) under nutrient deficient conditions (Anderson and Pond, 2000). Also, the relative trophic transfer efficiencies of EFAs versus C can be assessed by comparing the relative EFA contents of phytoplankton and copepods (Brett and Müller-Navarra, 1997; Gladyshev et al., 2011; Pommier et al., 2012). The calanoid copepod *Acartia tonsa* was chosen as the model species for our study. The adults of this species do not build up large energy storage pools but rather invest most assimilated energy directly into egg production and thus respond quickly to altered feeding conditions with changed egg production (<24 h) (Dagg, 1977; Kiørboe et al., 1985). Food quality in this study is expressed as elemental stoichiometry (C:N and C:P) and carbon-normalized contents of EFAs in the cryptophyte *Rhodomonas* sp. under a wide range of N:P supply

ratios and growth rates (dilution rates). Our study aims to test the following questions:

(1) How homeostatic is the stoichiometry of *A. tonsa* under different algal C:N and C:P? Does homeostasis depend on food quantity, and can it be achieved by increasing the GGE for the limiting nutrient or by lowering it for C?
(2) Is nutrient deficiency the ultimate cause and FA deficiency the proximate cause of changes in egg production?
(3) Do the costs of homeostasis reduce egg production under nutrient deficiency? Is this effect different under low and high food quantities?

MATERIALS AND METHODS

Phytoplankton Cultures

The cryptophyte *Rhodomonas* sp. originated from the Kiel Bight, Baltic Sea. The cultures were maintained in sterile filtered natural seawater (Sterilizing Grade Filter, Sartobran P 0.2 μm; Sartorius Stedim Biotech GmbH, Göttingen, Germany), with additional macronutrients and micronutrients based on the modified Provasoli's culture medium (Provasoli, 1963; Ismar et al., 2008). The algae were grown at 18°C and a salinity of 18 psu in a temperature-controlled room, with constant light intensity at 100 μmol photons m^{-2} s^{-1} at a light:dark cycle of 16:8 h. Algal cultures were kept in 1 L Erlenmeyer flasks with 500 mL culture volume. All flasks were aerated slightly with filtered air and agitated manually twice per day at a set time.

Around 20 days prior to the copepod feeding experiment, batch cultures of *Rhodomonas* sp. were started under three N:P supply ratios (N deficiency: N:P = 10:1 mol mol^{-1}, 352 μmol L^{-1} for N and 36 μmol L^{-1} for P; balanced nutrient condition: N:P = 24:1 mol mol^{-1}, 880 μmol L^{-1} for N and 36 μmol L^{-1} for P; P deficiency: N:P = 63:1 mol mol^{-1}, 880 μmol L^{-1} for N, and 14 μmol L^{-1} for P). Background concentrations of N and P in natural seawater were very low and thus negligible. The N:P molar ratio of 24:1 instead of canonical 16:1 was considered balanced, consistent with the setup of N:P ratio in the common and widely used f/2 medium (Guillard, 1975). The N:P ratios of 10:1 and 63:1 were chosen to cover the typical ranges of N:P in the ocean, including coastal and eutrophic regions (Downing, 1997). The observed maximal growth rate (μ_{max}, d^{-1}) was calculated from cell number changes during the exponential growth phase. Cell density was determined daily and subsequently transformed logarithmically. The linear increasing part in log plot of cell density was defined as the exponential phase. Once batch cultures reached the early stationary phase, semi-continuous cultures were started with two specific growth rates, 20% of μ_{max} for N and P deficiency, and 80% of μ_{max} for the balanced nutrient condition. Three food quality treatments were determined according to our previous studies (Bi et al., 2012, 2014). Briefly, we observed the lowest N:P biomass ratios in *Rhodomonas* sp. under N deficiency and 20% of μ_{max}, the intermediate values under the balanced nutrient condition and 80% of μ_{max}, and the highest ones under P deficiency and 20% of μ_{max} (Bi et al., 2012). Moreover, PUFA contents in *Rhodomonas* sp. were higher under N deficiency and low growth rates compared to those under P deficiency and balanced nutrient condition (Bi et al., 2014).

Semi-continuous cultures were conducted three times corresponding to the three runs of copepod chemical response experiments. Each treatment was run with two or three replicates. The outflows of semi-continuous cultures were daily supplied to copepods as food. Before feeding copepods, food suspensions from replicate flasks were pooled. Thus, copepod feeding was not influenced by variance between the algal culture replicates and copepod replicates received identical food.

General Procedure of Copepod Cultures

The copepod *A. tonsa* isolated from the Kiel Bight was obtained from the Department of Biology, Institute for Hydrobiology and Fisheries Science at the University of Hamburg and kept in our laboratory at the Helmholtz Centre for Ocean Research Kiel (GEOMAR) for more than three generations. The copepods were routinely fed *Rhodomonas* sp. grown under the balanced nutrient condition. The eggs of *A. tonsa* were stored in seawater at 4°C for later use, and those used in the experiments were not older than 3 months. It has been observed that *A. tonsa* eggs retain a high viability for up to 1 year at cold storage of 2–3°C (Drillet et al., 2006).

To initiate the experiments, a batch of eggs was incubated in filtered natural seawater. Copepod cultures were maintained under the same ambient conditions and culture medium as those in their diet throughout the experiment. The copepods were fed *ad libitum Rhodomonas* sp. until the cohort reached the copepodite V–VI stages. Developmental stage of copepods was identified using a ZEISS Discovery V.8 microscope.

Experiments of Copepod Chemical Composition Response

To test the effects of food quantity and quality on copepod chemical composition, we ran experiments using the algae from semi-continuous cultures. Six gradients of food quantities were set for each of the three food quality treatments (**Table 1**). Copepods were placed in 1 L Erlenmeyer flasks with 800 mL culture volume under different food treatments at an approximate density of 500 individuals L^{-1}. This density is within the range of observed natural copepod aggregations in the ocean (Haury and Yamazaki, 1995; Mauchline, 1998). The initial densities were set as approximately 190 individuals needed for various chemical analyses and subsequent experiments with an estimated average mortality rate of 15 to 20% d^{-1}. Three experimental runs were conducted to cover the wide range of food quantity in each food quality treatment. Duplicates of each food treatment were established. All culture flasks were aerated slightly with filtered air and carefully agitated twice per day at a set time.

Copepods were acclimated to different food treatments for 4 days prior to further measurements. During the acclimation period, the food was renewed daily to ensure that food quality remained constant. Initial food concentrations were calculated from the corresponding nominal algal concentrations and algal

TABLE 1 | Initial food concentrations (calculated from the corresponding nominal initial food concentrations and algal carbon contents; μg C L^{-1}) under different food quantity and quality treatments [the balanced and fast growth diet (80% of μ_{max}), and N- and P-deficient and slow growth diet (20% of μ_{max})] in copepod chemical response experiments and egg production rate experiments.

	Initial food concentration		
	N deficiency 20% μ_{max}	**P deficiency 20% μ_{max}**	**Balanced N:P 80% μ_{max}**
Chemical response experiments	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]
	415.8[a]	419.2[a]	314.4[a]
	916.5[b]	1059.6[b]	1257.8[a]
	2079.2[a]	2096.1[a]	1914.5[b]
Egg production rate experiments[a]	415.8	419.2	314.4
	2079.2	2096.1	1257.8

[a,b,c]*Copepods were fed the diet from experiment run 1, 2, and 3, respectively.*

C contents (**Table 1**); the mean concentrations were lower than initial values due to grazing. Food levels were adjusted once (high food), twice (medium food), or three times daily (low food). This food concentration adjustment schedules were tailored according to Campbell et al. (2001). After the acclimation period, approximately 120 individuals were harvested for chemical analyses, and the others (about 70 individuals) were picked out for egg production experiments.

Egg Production Experiments

The adults were sorted from two food levels at each food quality treatment (**Table 1**). Five females and two males were transferred into a Plexiglass chamber (10 cm in height, 5 cm diameter with a 250 μm mesh 3 cm above the bottom), which was placed within a 500 mL bottle filled with a total of 450 mL culture volume (filtered seawater and food). The 250 μm mesh allowed the eggs but not copepod adults to pass through and thus strongly reduced the possibility of egg cannibalism. Eight or ten replicates were set for each treatment except for the lowest food quantity treatment under N deficiency, in which there were only five replicates. All cultures were maintained under the same conditions as in the acclimation period. After 24 h, the eggs were collected with a 40 μm mesh and counted using a Bogorov tray. The 24 h duration is a suitable incubation period both to avoid the risk of biasing daily estimates and to be representative of the *in situ* rate (Runge and Roff, 2000).

Analytical Procedure

Algal cells were counted daily using an improved Neubauer hemacytometer (Glaswarenfabrik Karl Hecht GmbH, Rhön Mountains, Germany) under microscope (Hund, Wetzlar, Germany). For elemental and FA analysis, algae at steady state of semi-continuous cultures were harvested by filtration on pre-combusted Whatman GF/F filters (Whatman GmbH, Dassel, Germany), while copepods after the acclimation period were first filtered through a 250 μm mesh and flushed thoroughly to remove algae, and subsequently collected on pre-combusted Whatman GF/F filters. Samples for elemental analysis were immediately dried and stored in a desiccator, and those for FA analysis were frozen at −80°C. Female adults used in egg production experiment were sorted and stored in −80°C for nucleic acid analysis.

The determination of particulate organic C and N 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 P 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). Lipids were extracted with a solvent mixture of chloroform:dichloromethane:methanol (1:1:1 volume ratios). The FAME mixture of 13:0, 15:0, 17:0, 19:0 and 21:0 was added as internal standard, and tricosanoic acid (23:0) added as esterification control. Esterification was done at 50°C with a mixture of toluene and methanol which was supplemented with 1% concentrated sulphuric acid. The extract was 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. The GC was equipped with a flame ionization detector and a TR-FAME-column (10 m, 0.1 mm i.d., 0.2 μm film). Temperature programme started at 50°C for 1 min, increased by 30°C min^{-1} to 150°C, then 4°C min^{-1} to 180°C and 30°C min^{-1} to 240°C. 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. FA data are shown as carbon-normalized contents (as μg mg C^{-1}) and proportions (as a percentage of total FAs) in the two species (**Supplementary Tables 1**, **2**). In both species, ω3-PUFAs with 18–22 carbon atoms (C18–C22) were mainly represented by *a*-linolenic acid (18:3n-3; ALA), stearidonic acid (SDA; 18:4n-3), EPA and DHA, while C18-C22 ω6-PUFAs mainly included 18:2n-6, 18:3n-6 and arachidonic acid (20:4n-6; ARA).

RNA and DNA were analyzed in individual female copepods according to Malzahn et al. (2007). Samples were freeze-dried for at least 16 h before measurement. RNA and DNA were quantified fluorometrically in a microtiter fluorescence reader (Labsystems, Fluoreskan Ascent) using ethidium bromide as a fluorophore. Total nucleic acids were first 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 based on the relationship between RNA and DNA fluorescence (Le Pecq and Paoletti, 1966).

The extended stoichiometric hypothesis permits simultaneous analysis of any number of dietary components for zooplankton production (Anderson and Pond, 2000). Substrate *i* is limiting if it is in most demand relative to supply as compared to other

substrates such as *j*, and so the strength of limitation of *i* relative to *j*, $S_{i:j}$, is:

$$S_{i:j} = \frac{\phi_i \times K_j^* \times Z_{i:j}}{\phi_j \times K_i^* \times F_{i:j}} \quad (1)$$

where $F_{i:j}$ and $Z_{i:j}$ are the *i:j* ratios of components *i* and *j* in food and consumer. Parameters K_i^* and K_j^* are defined as the maximum GGE for *i* and *j*, respectively. Only the limiting substrate will be used with a maximum GGE. To take account of possible synthesis of EPA and DHA by the consumer, ϕ_i and ϕ_j are introduced as the fraction of demand for constituent *i* and *j* which is met directly by dietary intake. When the limitation potential of component *i* is higher than that of component *j*, the value of $S_{i:j}$ is higher than one and vice versa.

The GGEs for elements are defined as the efficiencies with which elements can be used for zooplankton production (Anderson, 1992; Anderson and Hessen, 1995; Anderson et al., 2004). The relative GGE for each element is governed by the stoichiometric ratio of the elements in the zooplankton versus that in the food (Anderson and Hessen, 1995), and thus can be calculated as: $K_C/K_N = Z_{C:N}/F_{C:N}$ and $K_C/K_P = Z_{C:P}/F_{C:P}$.

We show the results for $S_{N:EPA}$ and $S_{N:DHA}$ when *A. tonsa* was fed the N-deficient diet, and for $S_{P:EPA}$ and $S_{P:DHA}$ on the P-deficient diet, respectively. We assume that there is zero synthesis of EPA and DHA by *A. tonsa*, and thus parameters ϕ_{EPA} and ϕ_{DHA} are set to 1.0, as suggested by Anderson and Pond (2000). N and P must be of dietary origin, thus ϕ_N and ϕ_P are 1.0. The possible upper bound for K_N^* and K_P^* might be 0.8 (Anderson and Pond, 2000; Anderson et al., 2005; Mayor et al., 2009), thus K_N^* and K_P^* are each set at 0.8 in this study for *A. tonsa* on the N- and P-deficient diet, respectively. K_{EPA}^* and K_{DHA}^* are each set at 0.9 (Anderson and Pond, 2000).

Statistical Analysis

One-way analysis of variance (ANOVA) was conducted to test the effects of experimental run on C:N, C:P and carbon-normalized EPA and DHA contents in *Rhodomonas* sp., respectively. The same analysis was done for the effects of nutrient treatment. The effects of food quantity on the limiting strengths $S_{N:EPA}$ and $S_{N:DHA}$ in *A. tonsa* fed on the N-deficient diet, and $S_{P:EPA}$ and $S_{P:DHA}$ on the P-deficient diet were also tested by one-way ANOVA. Experimental run showed no significant effects on any response variables in *Rhodomonas* sp. (**Supplementary Table 3**). Thus, C:N, C:P and EPA and DHA contents in *Rhodomonas* sp. are shown as the average values of the three experimental runs for each food quality treatment.

Two-way ANOVA was applied to test the effects of food quantity and quality treatments on C:N, C:P, the contents of EPA and DHA, egg production rates and RNA:DNA in *A. tonsa*. If there were significant effects, a *post hoc* test [Tukey's honest significant difference (HSD) test] was applied, and the magnitude of effect was calculated [ω^2 = (effect sum of squares – effect degree of freedom × error mean square)/(total sum of squares + error mean square)] (Graham and Edwards, 2001; Hughes and Stachowicz, 2009).

Multiple regression was used to analyze the responses of K_C/K_X, with the predicting variables of algal C:X ratio, food quantity, (algal C:X ratio)^2, (food quantity)^2, and (algal C:X ratio) × (food quantity), here X = N and P, respectively. The same analysis was conducted for the ratios of carbon-normalized ω3- (and ω6-) PUFA contents between copepods and algae (ω3-PUFA Z/f and ω6-PUFA Z/f). Dependent and independent variables were Log_{10} (x) transformed to obtain approximate normality and homogeneity of variance in multiple regression analyses.

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

RESULTS

C:N:P Stoichiometry

The ratios of C:N and C:P in *Rhodomonas* sp. responded significantly to nutrient treatment changes (ANOVA; bold letters in **Table 2**). Specifically, we observed the highest C:N ratios under N deficiency at 20% of μ_{max} (Tukey's HSD test, $p < 0.001$; **Figure 1A** and **Supplementary Table 4**), and the highest C:P ratios under P deficiency at 20% of μ_{max} (Tukey's HSD test, $p \leq 0.022$; **Figure 1B**).

In *A. tonsa*, the C:N ratios varied significantly with different food quantity and quality treatments (ANOVA; **Table 2**), showing higher values on the N-deficient diets than those in other two treatments, especially at high food quantity (>900 μg C L^{-1}) (Tukey's HSD test, $p \leq 0.031$; **Figure 1C** and **Supplementary Table 4**). The C:P ratios showed non-significant responses to food quantity and quality changes.

Fatty Acids

In *Rhodomonas* sp., carbon-normalized contents of EPA responded significantly to different nutrient treatments (ANOVA; **Table 2**), showing the highest values under N deficiency (Tukey's HSD test, $p < 0.001$; **Figure 2A** and **Supplementary Table 1**). However, carbon-normalized contents of DHA showed non-significant changes.

In *A. tonsa*, carbon-normalized EPA contents responded significantly to the changes in food quantity and quality, while DHA varied significantly only with food quality changes (ANOVA; **Table 2**). The contents of EPA were generally higher at higher food quantities and on nutrient deficient diets (Tukey's HSD test, $p < 0.006$; **Figure 2C** and **Supplementary Table 2**), while DHA was higher on the P-deficient diets than that on the balanced nutrient diets (Tukey's HSD test, $p = 0.008$; **Figure 2D**).

Gross Growth Efficiency of Elements and the Relative Trophic Transfer Efficiencies of EFAs Versus C

Multiple regression analyses show that the relative GGE of K_C/K_N responded significantly to all independent variables, while for K_C/K_P regression with only the linear term [algal C:P ratio] and the quadratic term [food quantity]^2 showed the best model fit (bold letters in **Table 3**). Specifically, K_C/K_N decreased with increasing food quality (algal C:N ratios), but

TABLE 2 | Summary of ANOVA on the responses of C:N, C:P, and the contents of EPA and DHA in *Rhodomonas* sp. to different nutrient treatments (Nut.), and C:N, C:P, EPA, DHA, the strength of limitation of deficient nutrient relative to essential fatty acids ($S_{N:EPA}$, $S_{N:DHA}$, $S_{P:EPA}$, and $S_{P:DHA}$), egg production rates and RNA:DNA in *Acartia tonsa* to different food quantity (Conc.; μg C L^{-1}) and quality (Nut.).

Species	Dependent variable	Independent variable	SS	*df*	*F*	*p*	ω^2
Rhodomonas sp.	C:N (mol mol^{-1})	Nut.	73.65	2	126.02	**<0.001**	92%
	C:P (mol mol^{-1})	Nut.	440.91	2	38.62	**<0.001**	78%
	EPA (mmol mol C^{-1})	Nut.	2.87	2	23.6	**<0.001**	68%
	DHA (mmol mol C^{-1})	Nut.	0.010	2	0.60	0.559	
Acartia tonsa	C:N (mol mol^{-1})	Nut.	0.019	2	4.83	**0.022**	12%
		Conc.	0.030	5	3.15	**0.034**	17%
		Nut. × Conc.	0.035	10	1.81	0.134	
	C:P (mol mol^{-1})	Nut.	853.7	2	0.31	0.736	
		Conc.	9471.0	5	1.39	0.283	
		Nut. × Conc.	12897.7	10	0.95	0.521	
	EPA (mmol mol C^{-1})	Nut.	0.76	2	10.8	**0.001**	23%
		Conc.	1.36	5	7.76	**<0.001**	40%
		Nut. × Conc.	0.22	10	0.61	0.781	
	DHA (mmol mol C^{-1})	Nut.	3.31	2	6.11	**0.011**	21%
		Conc.	3.85	5	2.84	0.051	
		Nut. × Conc.	1.33	10	0.49	0.872	
	$S_{N:EPA}$	Conc.	104.0	5	1.20	0.444	-
	$S_{N:DHA}$	Conc.	2.96	5	1.65	0.325	-
	$S_{P:EPA}$	Conc.	237.1	5	1.24	0.396	-
	$S_{P:DHA}$	Conc.	7.57	5	1.04	0.472	-
	Egg production	Nut.	27.84	2	8.78	**<0.001**	13%
	(eggs $female^{-1}$ d^{-1})	Conc.	84.07	1	53.0	**<0.001**	42%
		Nut. × Conc.	5.30	2	1.68	0.197	
	RNA:DNA	Nut.	1.23	2	2.76	0.156	
	(μg $μg^{-1}$)	Conc.	24.6	1	110.04	**<0.001**	85%
		Nut. × Conc.	1.59	2	3.55	0.110	

The magnitude of effect (ω^2) is shown only for significant responses. Significant p-values are shown in bold.

this negative response became weaker as food quantity increased (**Figure 3A**). K_C/K_N increased with increasing food quantity at higher algal C:N ratios (N-deficient diets), but showed a unimodal response to food quantity changes at lower C:N ratios (balanced nutrient diets). Similarly, K_C/K_P decreased with increasing food quality (algal C:P ratios), but increased with increasing food quantity (**Figure 3B**).

The strength of limitation of elements relative to EFAs ($S_{N:EPA}$, $S_{N:DHA}$, $S_{P:EPA}$, and $S_{P:DHA}$) showed non-significant responses to food quantity changes (ANOVA; **Table 2**). The values of the four parameters ranged from ca. two to 10 and one to two, respectively, and generally higher at lower food quantity (<900 μg C L^{-1}) (**Figure 4**).

The values of ω3-PUFA Z/f responded significantly to food quantity, while ω6-PUFA Z/f showed significant responses to algal C:N (and C:P) ratios, food quantity and the interactions between algal C:P and food quantity (multiple regression analyses; **Table 3**). We observed an increased in ω3-PUFA Z/f as food quantity increased (not shown in figures). Similarly, ω6-PUFA Z/f increased with increasing food quantity at higher algal C:N (and C:P) ratios, but decreased at lower algal C:N (and C:P) ratios (**Figure 5**). Moreover, ω6-PUFA Z/f generally decreased with increasing algal C:N (and C:P) ratios, but increased with increasing algal C:P ratios at high food quantities.

Egg Production Rates and RNA:DNA in *Acartia tonsa*

Egg production rates had highly significant responses to food quantity and quality changes (ANOVA; bold letters in **Table 2**), showing higher values at high food quantities and on the balanced nutrient diet (**Figure 6A** and **Supplementary Table 5**). Especially on the balanced nutrient and P-deficient diets, egg production rates were significantly higher at the high food quantity level than those at the low food quantity level (Tukey's HSD test, $p \leq 0.002$). At the high food quantity level, egg production rates were higher on the balanced nutrient diet than those on nutrient deficient diets (Tukey's HSD test, $p \leq 0.025$).

RNA:DNA in *A. tonsa* varied significantly with food quantity changes (ANOVA; **Table 2**). At each nutrient treatment, RNA:DNA was significantly higher at the high food quantity level than those at the low food quantity level (Tukey's HSD test, $p \leq 0.049$; **Figure 6B** and **Supplementary Table 5**).

DISCUSSION

Our study demonstrates the variations in C:N:P stoichiometry and FA contents of *A. tonsa* in response to different food quantity and quality treatments, showing significant effects of food quantity and quality on C:N ratios and certain essential

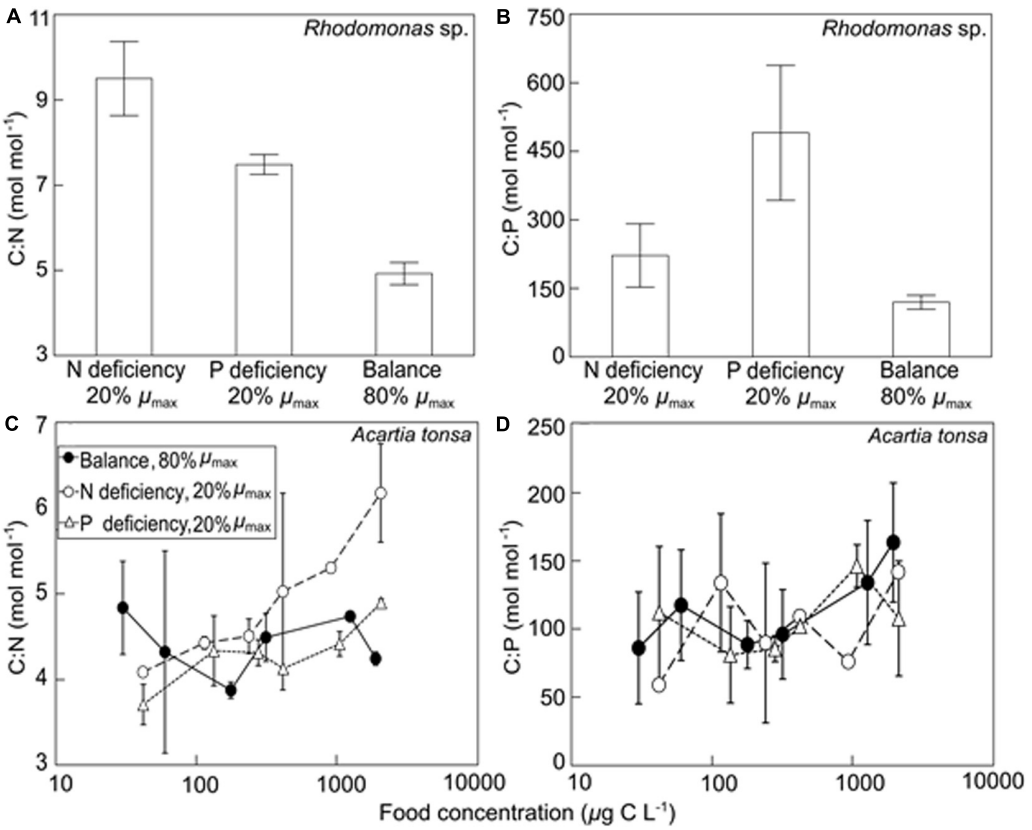

FIGURE 1 | The C:N and C:P ratios (mean ± SD) in *Rhodomonas* sp. **(A,B)** under three nutrient treatments (N deficiency and 20% of μ_{max}, P deficiency and 20% of μ_{max}, and the balanced nutrient condition and 80% of μ_{max}), and in adult *A. tonsa* **(C,D)** under different food quantity and quality treatments [N- and P-deficient and slow growing diets (20% of μ_{max}) and the balanced nutrient and fast growing diet (80% of μ_{max})]. Data presented in *Rhodomonas* sp. are mean values of the three experimental runs in each nutrient treatment. Note the log scale is used in **(C,D)**.

FA contents but not on C:P ratios. In particular, we observed stronger changes in *A. tonsa* C:N at higher food concentrations, consistent with previous observations of relaxed rather than strict homeostasis in *A. tonsa* (Malzahn et al., 2007). The limitation strength of elements and EPA (and DHA) was quantified, and the transfer efficiencies of EFAs and C were also tested to characterize the mechanisms of homeostatic controls in *A. tonsa*. We show that the GGE for C and transfer efficiency for EFAs would be reduced on N- and P-deficient diets, which can explain low egg production rates under nutrient deficient conditions.

Homeostasis of C:N:P Stoichiometry in *Acartia tonsa*

Food quality showed significant effects on the C:N ratios, but not C:P ratios in *A. tonsa* in our study (**Table 2** and **Figure 1**), consistent with early observations for *A. tonsa* feeding on *Rhodomonas salina* grown in nutrient-replete (f/2), and N and P-deplete treatments (Malzahn et al., 2010). Different responses between C:N and C:P ratios in copepods can be explained by the regulation of behavioral and physiological responses on different types of nutrient deficient phytoplankton (Burian et al., 2018). When exposed to N-deficient diets, *A. tonsa* raised amino acid (AA) retention efficiencies to compensate low dietary AA concentration, while the supply of essential FA became co-limiting and restricted the further increase in AA retention efficiencies (Burian et al., 2018). In our study, DHA concentrations in *A. tonsa* were significantly higher on N-deficient diets, while those in algae showed non-significant changes in response to nutrient concentration changes (**Table 2** and **Figure 2**), suggesting that DHA in the N-deficient diet may have become limiting for *A. tonsa*. Essential FA depletion may restrict upregulation of AA retention efficiencies and explain higher C:N ratios in copepods such as *Acartia* spp., *Temora* sp., *Centropages* sp., and *Pseudo/Paracalanus* spp. feeding on N-deficient diets in this study and previous work (Van Nieuwerburgh et al., 2004; Malzahn et al., 2007; Franco-Santos et al., 2018). The exposure of *A. tonsa* to P-deficient diets caused the increase in maximum ingestion rate (Burian et al., 2018), which may be also the case in our study and led to non-significant changes in C:P ratios in *A. tonsa*.

Food quantity showed significant effects on the C:N ratios in *A. tonsa*, causing a clear increase in C:N ratios under high food quantity (>900 μg C L^{-1}) (**Table 2** and **Figure 1**). Our results are comparable to previous studies (Malzahn et al., 2007, 2010), which showed significantly higher C:N ratios in *A. tonsa* feeding

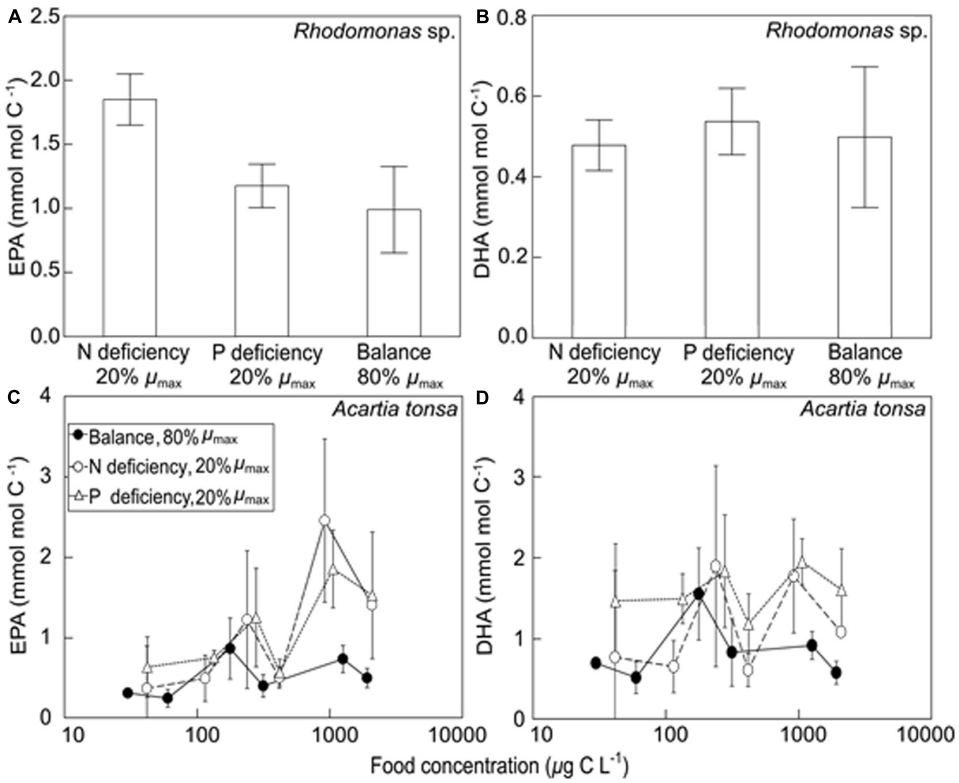

FIGURE 2 | The carbon-normalized contents (mean ± SD) of EPA (eicosapentaenoic acid; 20:5n-3) and DHA (docosahexaenoic acid; 22:6n-3) in *Rhodomonas* sp. **(A,B)** under three nutrient treatments (N deficiency and 20% of μ_{max}, P deficiency and 20% of μ_{max}, and the balanced nutrient condition and 80% of μ_{max}), and in adult *A. tonsa* **(C,D)** under different food quantity and quality treatments [N- and P-deficient and slow growing diets (20% of μ_{max}) and the balanced nutrient and fast growing diet (80% of μ_{max})]. Data presented in *Rhodomonas* sp. are mean values of the three experimental runs in each nutrient treatment. Note the log scale is used in **(C,D)**.

TABLE 3 | Results of multiple regression analyses for the relative gross growth efficiency for carbon and nitrogen (K_C/K_N), the relative gross growth efficiency for carbon and phosphorus (K_C/K_P), the ratio of ω3-PUFA contents between *Acartia tonsa* and food (ω3-PUFA Z/f), and the ratio of ω6-PUFA contents between *A. tonsa* and food (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)	−4.74 ± 1.30	−3.68	**0.001**	0.86	35
	Food concentration (C_f)	−0.53 ± 0.14	−3.70	**<0.001**		
	$C:N^2$	1.76 ± 0.76	2.31	**0.028**		
	C_f^2	0.05 ± 0.03	2.13	**0.042**		
	C:N × C_f	0.38 ± 0.13	3.07	**0.005**		
K_C/K_P	Algal C:P ratio (C:P)	−1.08 ± 0.10	−10.93	**<0.001**	0.79	33
	C_f^2	0.02 ± 0.01	2.17	**0.038**		
ω3-PUFA Z/f	C:N	0.16 ± 0.34	0.47	0.641	0.35	34
	C_f	0.28 ± 0.06	4.38	**<0.001**		
ω3-PUFA Z/f	C:P	0.24 ± 0.14	1.72	0.096	0.40	34
	C_f	0.27 ± 0.06	4.41	**<0.001**		
ω6-PUFA Z/f	C:N	−4.55 ± 1.67	−2.73	**0.011**	0.29	34
	C_f	−0.93 ± 0.52	−1.78	0.085		
	C:N × C_f	1.22 ± 0.63	1.93	0.064		
ω6-PUFA Z/f	C:P	−2.50 ± 0.71	−3.52	**0.001**	0.28	34
	C_f	−1.88 ± 0.66	−2.83	**0.008**		
	C:P × C_f	0.83 ± 0.28	2.95	**0.006**		

All dependent variables and independent variables (algal C:N ratio, algal C:P ratio and food concentration) were transformed using Log_{10} (x). Significant p-values are shown in bold; n is the number of observations.

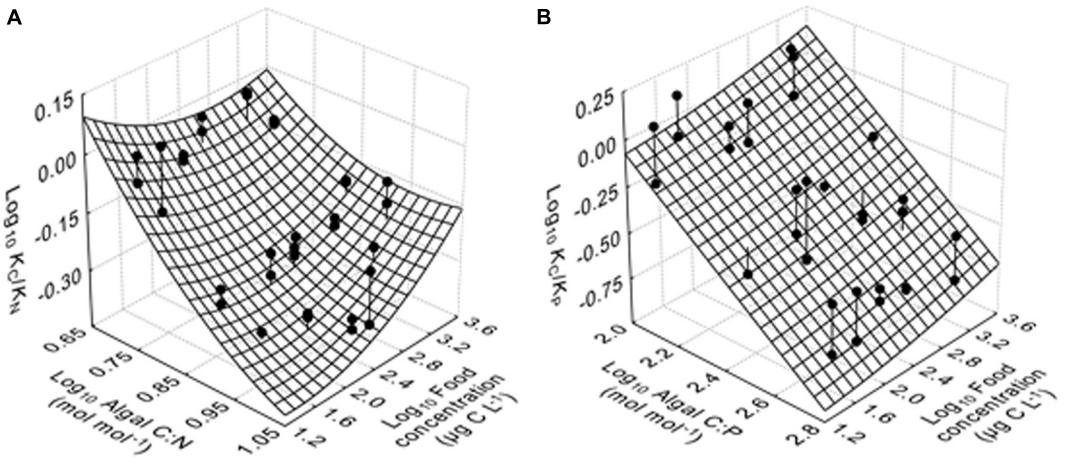

FIGURE 3 | The relative gross growth efficiency (GGE) for carbon and element X (K_C/K_X) (here, X = N or P) as functions of algal C:X ratio and food quantity. **(A)** The relative GGE for carbon and nitrogen (K_C/K_N) as a function of algal C:N ratio and food quantity, fitted to the second-order quadratic equation, and **(B)** The relative GGE for carbon and phosphorus (K_C/K_P) as a function of algal C:P ratio and food quantity, fitted to the second-order quadratic equation with only the linear term [algal C:P ratio] and the quadratic term [food quantity]^2. Data of K_C/K_X, algal C:X and food quantity were transformed using Log_{10} (x). The mesh grid is the 3D surface plot of the model prediction. Observations are represented by ball-and-stick symbols where the stick is the projection of the observation onto the surface plot.

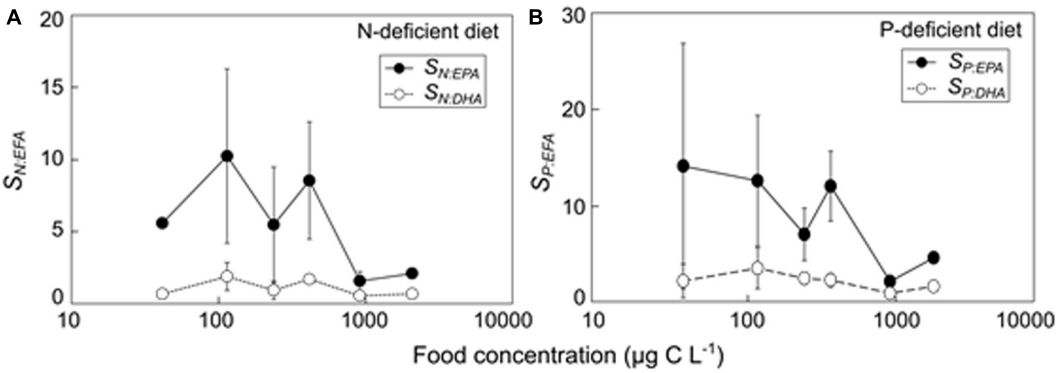

FIGURE 4 | The strength of limitation of deficient nutrient relative to essential fatty acid (EFA: EPA and DHA) on zooplankton production in response to different food quantity: **(A)** the strength of limitation of N relative to EPA and DHA ($S_{N:EPA}$ and $S_{N:DHA}$) as a function of food concentrations under N deficiency, and **(B)** the strength of limitation of P relative to EPA and DHA ($S_{P:EPA}$ and $S_{P:DHA}$) as a function of food concentrations under P deficiency. Data are shown as mean values ± SD. Note the log scale is used.

on the N-limited diets at food quantity of 1000 µg C L^{-1} d^{-1}. Therefore, the effects of algal C:N ratios on zooplankton C:N ratios are likely more evident at higher food quantity, showing the breakdown of zooplankton stoichiometric homeostasis at higher levels of food quantity (Sterner and Elser, 2002).

To summarize, our results suggest that stoichiometric variability is element dependent, showing that the C:P was more homeostatic than C:N in *A. tonsa* in response to different food quantity and quality treatments. Although the C:N ratios in *A. tonsa* showed a strong increase on N-deficient diets at high food quantity, they were still lower than those in food. This result suggests that stoichiometry in *A. tonsa* varies with food C:N:P stoichiometric ratios, while it to some extent dampens the stoichiometric variation in their food (Malzahn et al., 2007). In this regard, it is an important issue to be discussed below, whether stoichiometric homeostasis in *A. tonsa* is achieved by increasing GGEs for limiting nutrients or by lowering it for C.

Limitation Strength of Elements and EFAs in *Acartia tonsa*

The relative GGE, K_C/K_N and K_C/K_P, decreased with increasing algal C:N (5–9.5 mol mol^{-1}) and C:P ratios (119–440 mol mol^{-1}), respectively (**Table 3** and **Figure 3**). The response patterns of K_C/K_N are in agreement with previous observations on *A. tonsa* feeding the diatom *Thalassiosira weissflogii* (Kiørboe, 1989), and model predictions (Kuijper et al., 2004; Acheampong et al., 2014; Anderson et al., 2017) which showed that K_C declined hyperbolically but K_N slightly increased with increasing algal C:N ratios between 5 and 10 mol mol^{-1}. Such changes in K_C and K_N

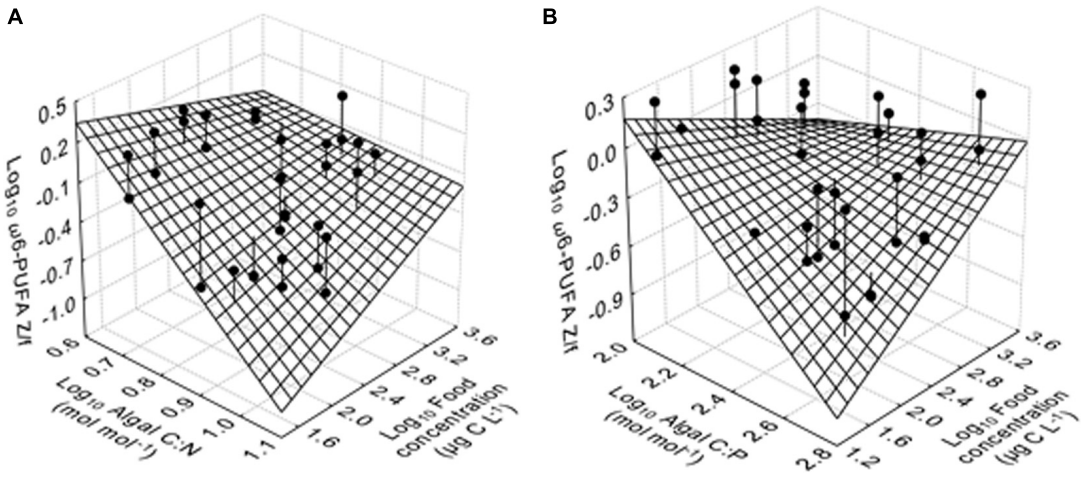

FIGURE 5 | The ratio of ω6-polyunsaturated fatty acid (PUFA) contents (μg mg C^{-1}) between *A. tonsa* and algae (ω6-PUFA Z/f) as a function of algal C:X ratio (here, X = N or P) and food quantity, fitted to the multiple regression model with the linear term algal C:X ratios, food quantity and the interactions between the two. **(A)** ω6-PUFA Z/f as a function of algal C:N and food quantity, and **(B)** ω6-PUFA Z/f as a function of algal C:P and food quantity. Data of ω6-PUFA Z/f, algal C:X and food quantity were transformed using Log_{10} (*x*). The mesh grid is the 3D surface plot of the model prediction. Observations are represented by ball-and-stick symbols where the stick is the projection of the observation onto the surface plot.

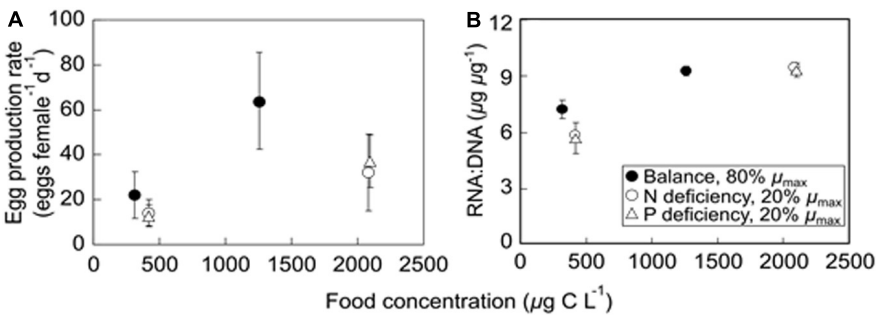

FIGURE 6 | Egg production rate **(A)** and RNA:DNA **(B)** (mean ± SD) in *A. tonsa* feeding on *Rhodomonas* sp. under different food quantity and quality treatments [N- and P-deficient and slow growing diets (20% of μ_{max}) and the balanced nutrient and fast growing diet (80% of μ_{max})].

reveal the differential allocations of C and N to egg production, indicating a strong decrease in the fraction of C but a less change in that of N allocated to egg production with increasing algal C:N ratios in *A. tonsa* (Kuijper et al., 2004).

As food concentration increased, K_C/K_N increased at higher algal C:N ratios (N-deficient diets) and showed a unimodal response at lower algal C:N ratios, while K_C/K_P increased over the entire range of algal C:P ratios in our study (**Table 3** and **Figure 3**). Previous studies have shown variable response patterns of GGE to increasing food concentrations in zooplankton, e.g., positive responses in the copepod *Eudiaptomus graciloides* (Hamburger and Boëtius, 1987), *Cyclops vicinus* (Santer and van den Bosch, 1994) and *Daphnia* (Anderson et al., 2005), negative responses in *A. tonsa* (Kiørboe et al., 1985; Wendt and Thor, 2015), the cladoceran *Penilia avirostris* (Atienza et al., 2007) and the copepod *Oithona davisae* (Almeda et al., 2010b), and non-significant changes in *A. tonsa* (Wendt and Thor, 2015). Such variations in zooplankton GGE responses to food concentrations can be explained by differences in nutrient quality of food (e.g., algal C:N and C:P ratios in the present study; Straile, 1997; Bukovinszky et al., 2012), prey species (Wendt and Thor, 2015), development stages of zooplankton (Almeda et al., 2010a), methodological protocols (Straile, 1997), as well as within-population genetic variance in the metabolic rate (Einum et al., 2019). In our study, K_C/K_N and K_C/K_P in *A. tonsa* were overall higher at higher food concentrations, especially when feeding on N- and P-deficient diets (**Figure 3**). This result is generally consistent with the predictions of the metabolic stoichiometric model (Anderson et al., 2005). Metabolism has a high C:N ratio (Anderson et al., 2017) and accounts for a lower fraction of intake at high intake (food concentrations), while more substrates are available for egg production (Anderson et al., 2005), indicating higher K_C/K_N at higher intake.

The values of $S_{N:EPA}$, $S_{N:DHA}$, $S_{P:EPA}$, and $S_{P:DHA}$ were higher than one in our study (**Figure 4**), suggesting that the limitation potential of N (and P) was higher than that of EPA and DHA on the N- (and P-) deficient diet. Similarly, Mayor et al. (2009) suggested that limiting potentials for C and N were higher than

those of EPA and DHA for egg production in the copepod *Calanus finmarchicus* in the north east Atlantic. Furthermore, Mayor et al. (2011) reported variable basal turnover rates and absorption efficiencies for essential PUFAs and N in *Calanus* spp., and showed that maintenance of essential PUFAs and absorbed N is a prerequisite for growth, as substantial post-absorptive losses of these substrates are associated with routine tissue maintenance. It can thus be expected that compared to EPA and DHA, the basal turnover rates of N (and P) in *A. tonsa* in the present study may be higher, especially at low food concentrations, causing a relatively high proportion of N (and P) utilized for basal metabolism instead of egg production on the N- (and P-) deficient diet. This assumption is in correspondence with the results in Malzahn et al. (2007), which suggested that mineral nutrient requirements have to be satisfied first, and FAs can further promote growth of larval herring in experimental tri-trophic food chains with P-deficient phytoplankton. While the primary roles of elemental versus biochemical limitation on zooplankton have long been discussed (Hessen, 1992; Brett, 1993; Müller-Navarra, 1995; Brett et al., 2000; Boersma et al., 2008), our results highlight the necessary to incorporate both elemental and biochemical approaches in the study of food webs (Anderson and Pond, 2000; Sterner and Elser, 2002; Lampert, 2009; Hessen et al., 2013).

Moreover, lower values of $S_{N:EPA}$, $S_{N:DHA}$, $S_{P:EPA}$, and $S_{P:DHA}$ at higher food concentrations indicate that the strength of limitation of N (P) relative to EFAs was reduced as food concentrations increased (**Figure 4**). It is well-known that ingestion and assimilation of zooplankton such as *Daphnia* increase with increasing food concentrations, and at threshold food concentrations the metabolic expenditure is balanced with net food intake and production equals zero (Lampert, 1977; Lampert and Sommer, 2007). According to the metabolic stoichiometric model, the proportions of C, N and P used for growth increase at high intake (Anderson et al., 2005). This positive relationship between growth efficiencies and intake can be attributed to the diminishing relative cost of maintenance (Anderson et al., 2005), which may explain the reduced limitation strength of N (and P) relative to EFAs at high food concentrations in *A. tonsa* in this study.

Altogether, we suggest that homeostatic stoichiometry in *A. tonsa* may be maintained by the reduced K_C and increased K_N (and K_P) when feeding on N- (and P-) deficient diets, which underlies differential mechanisms of C and N (and P) in controlling zooplankton production, and indicates that N and P are retained more efficiently than C in zooplankton. Significant interactions between food quantity and quality on K_C/K_N indicate that differences in dietary C:N ratios can result in variable responses of GGE to food concentration changes in *A. tonsa*. Our results indicate a higher limitation potential of N (and P) than that of EPA and DHA for *A. tonsa* feeding on the N- (and P-) deficient diet, while the limitation potential of N (and P) was reduced as food concentration increased.

Egg Production Rates in *Acartia tonsa*

Our results show that egg production rates in *A. tonsa* responded significantly to the changes of food quantity and quality, showing overall higher values on the balanced nutrient diets at the high food quantity level, and the lower ones on nutrient deficiency diets at the low food quantity (**Table 2** and **Figure 6**). It is well-established that egg production rate is higher when the food is obtained from nutrient-enriched or other optimized environments (Kleppel et al., 1998). Our results are generally in agreement with early observations which showed higher egg production rates in *A. tonsa* at higher food quantities (Jónasdóttir, 1994; Gusmão and McKinnon, 2009; Acheampong et al., 2011; Wendt and Thor, 2015), or at higher food qualities (lower food C:N ratios) (Kiørboe, 1989). In contrast, non-significant changes or a decrease in egg production rates were also found with increasing dietary C:N in *A. tonsa* (Jónasdóttir, 1994; Augustin and Boersma, 2006). For example, at similar food quantity and elemental food quality using the same algal species with our study, Augustin and Boersma (2006) reported that egg production rates in *A. tonsa* were about 10 eggs female^{-1} d^{-1} on N-sufficient diets, which was higher than that on N-depleted diets (about 4 eggs female^{-1} d^{-1}) at 15°C and a salinity of 32–33. These values of egg production rates were lower than that in our study (14 and 22 eggs female^{-1} d^{-1} on N deficient and balanced nutrient diets, respectively; **Supplementary Table 5**), where *A. tonsa* was cultured at 18°C and a salinity of 18. It has been observed that egg production rates in copepods vary strongly with the changes in temperature (Koski and Kuosa, 1999; Holste and Peck, 2006; Neila et al., 2012) and salinity (Augustin, 2006; Peck and Holste, 2006; Devreker et al., 2009); therefore, differences in temperature and salinity may potentially explain the differential results in our study and Augustin and Boersma (2006). Future studies should consider the interactions between environmental factors and food quality to better understand the responses of copepod production to the changing environments.

Biochemical limitation may be one of reasonable options to explain the low egg production rates when feeding on nutrient deficient diets in our study, because nutrient deficiency of phytoplankton can influence its biochemical contents such as FAs (Bi et al., 2014, 2017). Also, it has been observed that higher EFA contents have positive effects on egg production rates in *A. tonsa* (Amin et al., 2011; Rossoll et al., 2012). Thus, nutrient deficiency might be the ultimate cause and FA-limitation the proximate cause of lowered egg production rate. This, however, is not the case in our study, as PUFA contents in *Rhodomonas* sp. were even higher under N- and P-deficient conditions (**Supplementary Table 1**). This result is in agreement with stoichiometric analysis above which showed that N (and P) had a limitation potential higher than that of EFAs for *A. tonsa* on the N- (and P-) deficient diet.

Furthermore, we observed that the ratios of ω3- (and ω6-) PUFA contents between *A. tonsa* and food (PUFA Z/f) varied with food concentrations and nutrient treatments (**Table 3** and **Figure 5**). For example, we observed significant negative responses of ω6-PUFA Z/f to increasing food C:N and C:P ratios, suggesting that the relative transfer efficiency of ω6-PUFA to carbon may reduce with increasing food C:N and C:P. Because K_C in *A. tonsa* may decrease on N- and P-deficient diets, it is reasonable to assume that transfer efficiency of ω6-PUFAs may also decrease, and even to a larger extent compared to

carbon, in such food conditions. Co-limitation of food quantity (in terms of carbon) and quality (in terms of N, P and FAs) has been observed to influence the egg production of the North Sea copepod *Pseudocalanus elongatus* (Koski et al., 2010). Also, our results suggest that a lowered GGE for C might also lower the GGE of EFAs, which would mean that elements and EFAs are interactive-essential and not Liebig-type essential resources for the egg production in *A. tonsa*.

CONCLUSION

Our study provides new insight into trophic interactions between marine phytoplankton and copepods, with emphasis on trophic transfer of elements and EFAs, as well as the regulations of zooplankton reproduction. Overall, C:N ratios in *A. tonsa* changed significantly; however, the copepod C:P was more homeostatic than C:N and independent from algal C:P, but still showed some degree of variability. We also showed that C:N:P in *A. tonsa* was more homeostatic than its diets. Homeostasis in *A. tonsa* can be maintained via regulating GGEs for elements, with a reduced GGE for C and enhanced GGEs for N (and P) on N- (and P-) deficient diets assumed based on our results. Moreover, we first conducted experimental and mathematical analysis on elemental and FA limitation of food quality, showing a higher limitation potential of N (and P) relative to EPA and DHA for *A. tonsa* egg production when feeding on the N- (and P-) deficient diet. Egg production rates in *A. tonsa* were considerably reduced as the costs associated with homeostatic regulations on nutrient deficient diets. Along with the lowered GGE for C, reduced transfer efficiency of ω3- and ω6-PUFAs may also contribute to low egg production rates on nutrient deficient diets. Therefore, N and P deficiency in the environments can alter phytoplankton chemical composition, reduce trophic transfer efficiencies of C and EFAs from phytoplankton to zooplankton, decrease zooplankton production, and eventually change the structure and functions of marine food webs.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

RB and US planning of experiments and writing. RB conduction of experiments, sample analysis, data analysis and figures.

FUNDING

This study was supported by the National Natural Science Foundation of China (Grant Nos. 41876118 and 41630966), the State Sponsored Graduate Scholarship Program, China Scholarship Council (CSC), and the Youth Talent Support Program of the Laboratory for Marine Ecology and Environmental Science, Pilot National Laboratory for Marine Science and Technology (Qingdao) (Grant No. LMEES-YTSP-2018-02-01).

ACKNOWLEDGMENTS

We wish to thank Thomas Hansen, Cordula Meyer, and Bente Gardeler for technical assistance, and Alexander Raschke, Tanya Schchipkova, Hui Zhang, and Ashley Cipriano for their help during the experiments. We thank Martina Wichmann, Berenike Diekmann and Myron Peck for providing *Acartia tonsa* eggs, Dennis Rossoll for introducing the protocol for assessing copepod egg production, and Dennis Brennecke for helping with FA analysis. We are also grateful for comments from Stefanie Ismar, Meixun Zhao and three reviewers that greatly improved this manuscript. This work is modified after the Ph.D. thesis of Bi (2013). This is MCTL contribution 217.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2020.00274/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.