Acta Oceanol. Sin., 2018, Vol. 37, No. 4, P. 58–68
DOI: 10.1007/s13131-017-1122-z
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Effects of nutrient limitations on three species of zooplankton

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Received 24 May 2017; accepted 18 September 2017

Abstract

Nutrient imbalance—a mismatch in nutrient ratios between the available food supply and the demands of consumers—has the potential to be transported up food chains, exposing zooplankton to nutrient limitations. In this study, the response of *Calanus sinicus* (copepod), *Moina mongolica* (cladocera), and *Brachionus plicatili*s (rotifer) to nutrient-limited (no-limited, P-limited, and N-limited) food were evaluated from the perspective of growth, reproduction, and stoichiometric homoeostasis. The results indicated that the growth of three species was suppressed under nutrient-limited (especially P-limited) conditions. However, the effect of nutrient limitations on their reproduction was species-specific. For *C. sinicus*, the dietary UFA (unsaturated fatty acid) as a major phospholipid component affected their egg production and total FA supporting energy promoted the hatchability of eggs. Furthermore, excess carbon in the diet promoted egg production but reduced hatching success. For *M. mongolica*, nutritional (P and UFA) and energy (total FA) support affected their fecundity. *B. plicatilis* fecundity exhibited the same pattern of growth (no-limited>N-limited>P-limited). In terms of stoichiometric homeostasis, *B. plicatilis*'s elemental compositions were less likely to be affected by nutrient limitations than *M. mongolica*. We suggest that the effects of nutrient imbalances could potentially become an evolutionary force affecting ecosystem structure and stability in eutrophic waters.

Key words: zooplankton, nutrient, growth, reproduction, stoichiometric homoeostasis

Citation: Chen Lei, Li Chaolun, Zhou Konglin, Shi Yongqiang, Liu Mengtan. 2018. Effects of nutrient limitations on three species of zooplankton. Acta Oceanologica Sinica, 37(4): 58–68, doi: 10.1007/s13131-017-1122-z

1 Introduction

Eutrophication is a major pollution problem worldwide (Howarth, et al., 2002) that is associated with increased nutrient concentrations and altered nutrient ratios (based on the Redfield ratio) (Arai, 2001; Nixon, 1995; Purcell et al., 2007). Eutrophication probably changes the nutrient content of phytoplankton (an important food resource for zooplankton) (Rhee, 1978; Elser, 2002; Dickman et al., 2008). Nutrient imbalances between food resources and consumers can cause a mismatch between nutrient supply and demand in planktonic food webs. This mismatch has the potential to affect the growth and reproduction of consumers (Sterner and Elser, 2002). As organisms have different nutrient use strategies, this effect might vary among species. Species-specific changes are likely to become an evolutionary force affecting ecosystem structure, functioning and stability (Chapin et al., 1997; Knops et al., 2002; Sterner and Elser, 2002). However, the mechanisms and approaches involved with species-specific changes in growth and reproduction caused by altered nutrient ratios are not fully understood.

Ecological stoichiometry offers a new perspective in the study of eutrophication (Sterner and Elser, 2002). It is generally accepted that the stoichiometry of primary producers is highly affected by nutrient conditions (Goldman et al., 1979; Hall et al., 2005; Klausmeier et al., 2004; Rhee, 1978; Sterner and Elser, 2002). For example, the molar N:P ratio of *Scenedesmus* spp. closely tracks variations in N:P supply ratios (Rhee, 1978). Animals usually maintain relative homeostasis (Elser et al., 2000); however, herbivores that encounter low-quality food sources experience difficulty maintaining strict homeostasis when feeding on food that is limited in P or N because of the costs of homeostasis, leading to a mismatch between supply and demand (Elser et al., 2001; Malzahn et al., 2010; Van Nieuwerburgh et al., 2004).

Nutrient supplies from food influence life-history traits such as growth, reproduction and fitness (Sterner and Elser, 2002;

Foundation item: The National Key R & D Program of China under contract No. 2017YFC1404401; the National Natural Science Foundation of China under contract No. 41476139; the National Basic Research Program of China under contract No. 2015CB452903.

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Sterner and Schulz, 1998). Studies have shown that the growth conditions of some species of herbivores are affected by nutrient limitations. In *Acartia tonsa*, P-limited food reduced its growth rate (expressed as RNA:DNA) and no compensatory growth occurred (Malzahn and Boersma, 2012). In *Cladocera galeata*, P-limited algae was a poor food resource, as the somatic growth rate was lower than N+P-saturated and N-limited algae, which were of a comparable quality (Weers and Gulati, 1997). The somatic growth of *Brachionus calyciflorus* decreased under P- or N-limited conditions, and the growth rate correlated negatively with the food C:N ratio (Hessen et al., 2007; Jensen and Verschoor, 2004). Under nutrient-limited conditions, reproduction may also be affected. Augustin and Boersma (2006) found that *A. tonsa* fed with N-limited algae produced more eggs; however, the egg hatching was not significantly affected. *B. calyciflorus* reproduction decreased when fed with P- or N-limited algae (Jensen and Verschoor, 2004).

Zooplankton have various reproductive modes. Ventura and Catalan (2005) found that some species (*Cladocera pulicaria*, *Cyclops abyssorum*, and *Diaptomus cyaneus*) with different reproductive modes had different elemental variability. The stoichiometric changes (C:P and C:N) of females and their reproductive tissues during reproduction were species specific. Therefore, the effects of nutrient limitations on these species may differ, and the effects on species with different reproductive modes are not fully understood. Therefore, we investigated the effects of food quality (no-limited, P-limited, and N-limited) on the condition of planktonic crustaceans with different reproductive modes. *Calanus sinicus* (copepod), *Moina mongolica* (cladocera), and *Brachionus plicatilis* (rotifer) were chosen as experimental subjects because (1) *C. sinicus* performs gamogenetic reproduction and the eggs hatch externally from the females, (2) the parthenogenetic reproduction of *M. mongolica* involves unfertilized eggs that grow up in the brood pouch, and (3) the parthenogenetic reproduction of *B. plicatilis* is different from that of *M. mongolica* because the eggs incubate outside the females. In this study, the main objectives were (1) to compare the different responses of three species to nutrient limitations based on their growth, reproduction, and elemental homoeostasis (just for *M. mongolica* and *B. plicatilis*) and, (2) based on dietary nutrients (C, N, and P), fatty acids, and amino acids, to explore the potential limiting factors of the growth and reproduction of the three species.

2 Materials and methods

2.1 *Food preparation*

Stock cultures of *Phaeodactylum tricornutum* (diatom) and *Skeletonema costatum* (diatom) were cultivated in an f/2 culture medium (Guillard and Ryther, 1962), and the *Chlorella vulgaris* (green algae) was cultivated in a *Chlorella* culture medium (625 μmol/L NH_4NO_3, 28.7 μmol/L K_2HPO_4 and ferric citrate). The seawater used in the experiment was collected from the Huiquan Bay in Qingdao, China. Prior to the experiment, the seawater was filtered through a sterile 0.2 μm filter, heated to a boil, cooled and stored until use. All algal treatments were maintained at (20±0.5)°C under a 12 L:12 D of photoperiod at 4 000 lx of light intensity. The salinity was 30 to 31.

The no-limited treatment consisted of culture-enriched seawater. The two limitation treatments were enriched according to the culture recipe but without the addition of the limiting nutrient (N-limited and P-limited) (Boersma et al., 2009; Malzahn et al., 2010; Schoo et al., 2014). The ionic strength was maintained by the addition of KCl. To ensure the quality and constant supply of algae, new cultures of the three treatments were inoculated every other day and harvested after 5 d. Preliminary tests indicated that the nutrient composition (percentage of dry weight, C%, N% and P%) and molar ratios (C:N, C:P and N:P) of *P. tricornutum*, *C. vulgaris*, and *S. costatum* changed significantly under experimental conditions after a growth period of 5 d (all $P<0.05$, Table 1). These conditions allowed the zooplankton to feed on algae with different nutritional contents.

2.2 *Zooplankton preparation*

The adult copepod *C. sinicus* used for the experiment was collected from R/V *Beidou* in the Yellow Sea of China during June 2014. The cladocera *M. mongolica* was obtained from the Ocean University of China. Dormant eggs of rotifer *B. plicatilis* were bought from a commercial hatchery. Before the experiment, *M. mongolica* was cultivated in seawater for about one year, and *B. plicatilis* was hatched in seawater.

Since the survival temperature and appropriate food concentration were different for these three species, they were cultivated at their appropriate temperature and fed with their appropriate food, respectively. The copepod *C. sinicus* was bred at 8°C, and others were at 20°C. In this study, *C. vulgaris* (6.5 μg/mL, calculated by carbon) was used as food for the rotifer *B. plicatilis*,

Table 1. Nutrient compositions (percentage of dry weight, C%, N% and P%) and molar ratios (C:N, C:P and N:P) of the combination of *P. tricornutum* and *S. costatum* (PS), *P. tricornutum* (PT), *C. vulgaris* (CV), and *S. costatum* (SC) grown under no-limited, N-limited, and P-limited conditions after 5 days

	Treatment	C%	N%	P%	C:N	C:P	N:P
PS	no-limited	11.55±1.12	2.81±0.21^{a}	0.36±0.03^{a}	4.78±0.13^{a}	98.95±5.95^{a}	20.57±0.30^{a}
	N-limited	11.33±0.66	1.99±0.04^{b}	0.51±0.01^{b}	7.10±0.61^{b}	57.64±4.11^{a}	8.53±0.06^{b}
	P-limited	12.31±0.66	2.25±0.03^{b}	0.11±0.00^{c}	6.72±0.51^{b}	449.29±93.79^{b}	61.03±5.93^{c}
PT	no-limited	11.55±2.35	2.77±0.39^{a}	0.21±0.02^{a}	4.84±0.34^{a}	139.03±17.88^{a}	28.67±1.66^{a}
	N-limited	9.76±0.82	1.36±0.21^{b}	0.47±0.03^{b}	8.43±1.06^{b}	53.54±4.06^{a}	6.38±0.54^{b}
	P-limited	12.33±0.74	1.77±0.15^{b}	0.05±0.01^{c}	8.18±1.15^{b}	704.99±189.18^{b}	85.22±11.73^{c}
CV	no-limited	13.12±1.51^{a}	2.36±0.36^{a}	0.19±0.03^{a}	6.50±0.22^{a}	178.05±40.40^{a}	27.54±6.95^{a}
	N-limited	18.04±2.01^{b}	1.57±0.19^{b}	0.30±0.05^{b}	13.64±2.98^{b}	157.33±38.74^{a}	11.49±0.81^{b}
	P-limited	20.81±1.51^{b}	1.83±0.04^{ab}	0.06±0.01^{c}	13.25±1.04^{b}	946.90±238.32^{b}	71.58±17.67^{c}
SC	no-limited	11.54±0.46	2.85±0.09	0.51±0.05^{a}	4.72±0.08^{a}	58.87±5.99^{a}	12.46±1.07^{a}
	N-limited	12.91±1.56	2.61±0.20	0.54±0.01^{a}	5.77±0.35^{b}	61.73±7.09^{a}	10.68±0.66^{a}
	P-limited	12.28±0.58	2.73±0.20	0.16±0.01^{b}	5.26±0.14^{ab}	193.58±15.42^{b}	36.84±3.41^{b}

Note: The values represent the means of three replicate samples and are listed as the mean±SD. Significant differences (Tukey's HSD test, $P<0.05$) among the three treatments are indicated by different superscripted lowercase letters (a, b, and c).

since *C. vulgaris* was a common bait for *B. plicatilis* (Chen and Wu, 2005; Yang and Ye, 2000). The cladocera *M. mongolica* fed on *P. tricornutum* (5 μg/mL). Before the experiment, *M. mongolica* was cultivated for about three months feeding on *P. tricornutum*. *S. costatum* and *P. tricornutum*, which were mixed in the same concentration, were used as the food for the copepod *C. sinicus* (0.5 μg/mL for nauplii and 2 μg/mL for adults). This mixed food could support nauplii and adults with ideal particle size scale (Li et al., 2006). The salinity of seawater used in this experiment was 30 to 31.

2.3 *Growth and reproduction experiments*

C. sinicus—Groups of 12 adult females and 4 adult males were placed into spawn bottles and eggs harvested within 24 h were used for the experiment. They were placed into 24 glass beakers at 125 eggs per beaker. Three treatments (no-limited, N-limited and P-limited)×eight replicates were established. Algae were added and refreshed every other day. After the eggs hatched, growth conditions of nauplii (total length and developmental stage) were monitored.

Fifteen groups (three treatments×five replicates) of nine adult female copepods and four adult males were placed into spawn bottles. Algae were added and refreshed every other day. After cultivation for 48 h, the egg production of three treatments was monitored for 5 d. The egg production rate represents the number of eggs produced by one female during one day. Every day, the eggs produced were transferred into 6-well microtiter plates. The hatching success was calculated as the percentage of eggs hatched in 24 h of the total eggs.

M. mongolica—Newborns within 12 h after birth were collected. They were transferred to 6-well microtiter plates with three individuals per well. Three treatments (no-limited, N-limited and P-limited)×eighteen replicates were established. Algae were added separately to the three treatments and refreshed every other day. Since there were about 6 d before subitaneous eggs production, in this study, the growth condition was monitored every 2 d. Forty-five individuals were randomly selected from each treatment and their total lengths were measured using the ocular micrometer of a dissecting microscope (Olympus SZ61). After 6 d, the subitaneous eggs were counted for fecundity determination. The fecundity was assessed as the number of subitaneous eggs produced by one female during the experiment.

B. plicatilis—Resting eggs were incubated in shallow dishes containing sterile seawater. Neonate (<2 h) were transferred to 24-well microtiter plates at two individuals per well in a 2 mL food suspension. Three treatments (no-limited, N-limited and P-limited)×forty-eight replicates were established. *C. vulgaris* were added separately and refreshed every other day. As *B. plicatilis* produced eggs after the cultivation of about 40 h, in this study, the growth condition was observed every 12 h. Twenty individuals, which were randomly selected from each treatment, were fixed in ultrapure water. Then, their bodies were turned over to measure the maximum area (including the foot and egg) from the top view using an inverted microscope (Nikon AZ100) with the software of NIS-Elements D 3.0. After cultivation of 36 h, the fecundity was assessed as the number of eggs produced by one individual during the experiment.

Comparison of growth rate between species—The variation in the species' growth rate based on their food's stoichiometry (N:P, C:P, and C:N) was compared. The growth rate was calculated as $G=(L_t\text{-}L_0)/t$, where G is the growth rate, L_t and L_0 are the total length of *C. sinicus* and *M. mongolica* or the root square of the maximum area of *B. plicatilis* after t days and at the beginning of the experiments, and t is the experimental duration. The stoichiometry of each species' food was used as the x-axis, and the corresponding growth rate was used as the y-axis.

2.4 *Homoeostasis experiments*

Newborns (within 12 h for *M. mongolica*, 2 h for *B. plicatilis* and 24 h for *C. sinicus*) were transferred to 2 L beakers at 10 ind./L for *M. mongolica*, 100 ind./L for *B. plicatilis*, and 5 ind./L for *C. sinicus*. Three treatments×three replicates were established. Since these three species had different life history traits (especially life span) (Lin and Li, 1984; Wang and He, 2001), their response time to nutrient conditions were different. In this study, *M. mongolica* were cultivated for 10 d, and *B. plicatilis* were cultivated for 5 d under different nutrient conditions. Then they were collected to analyze C, N, and P contents. For *C. sinicus*, samples collected after cultivation of 15 d were not enough to analyze C, N, and P contents.

To estimate the ability of *M. mongolica* and *B. plicatilis* to maintain homoeostasis, the homoeostatic regulation coefficient (H) was calculated according to the following equation (Sterner and Elser, 2002): $y=cx^{\frac{1}{H}}$. The H in this study was calculated to linearize the equation above using logarithms (Sterner and Elser, 2002): $\log y=\log c+\frac{\log x}{H}$, where y is the stoichiometry (e.g., N:P and C:P) of *M. mongolica* or *B. plicatilis* and x is the stoichiometry of their food. Linear regressions were fitted, and the parameters of these regressions were used to assess the homoeostatic responses of these three species.

2.5 *Analytical procedures*

For analysis of the C and N contents, the samples were coated with tin boats and measured using an elemental analyzer (Elementar Company (EL), Germany). To analyze the P content, the samples were weighed and digested with 5 mL of nitric acid and 1 mL of perchloric acid; then, the temperature of the heating plate was increased from 175°C to 220°C to dry out the acid solution. Next, the solution was cooled to room temperature and 1:1 nitric acid was added to the sample. The sample was rinsed with Milli-Q deionized water and transferred to 25 mL specimen tubes. Then, all samples with a series of standard solution (0, 1, 5, 10, 20, 50 μg/L) and with a 20 μg/L Rh internal standard solution were analyzed using an ICP plasma emission spectrometer (Thermo Fisher Scientific, iCAP-Qc).

To analyze the amino acid content, approximately 20 mg of each of the samples was placed in an ampoule and 10 mL of 6 mol/L HCl and N_2 were added; then, the ampoule was sealed and placed in a 110°C drying oven for 24 h of hydrolyzation. Subsequently, 0.02 mol/L of HCl was added to a volume of 10 mL. The amino acid content was evaluated using high-performance liquid chromatography (HPLC) with phenylisothiocyanate as the reagent. An Agilent 1100 liquid chromatograph was used with a Venusil-AA (4.6×250 mm, 5 μm) column at a temperature of 40°C, detection wavelength of 254 nm, mobile phase A of 0.1 mol/L sodium acetate (7% acetonitrile), mobile phase B of 80% acetonitrile, and gradient elution of 1 mL/min. The fatty acids were measured as fatty acid methyl esters (FAMEs) after the method described by Liu et al. (2011).

2.6 *Statistical analysis*

The nutrient composition and molar ratios of *C. vulgaris*, *P. tricornutum*, *S. costatum*, and a combination of *P. tricornutum* and *S. costatum* as well as their nutritional content (fatty acid,

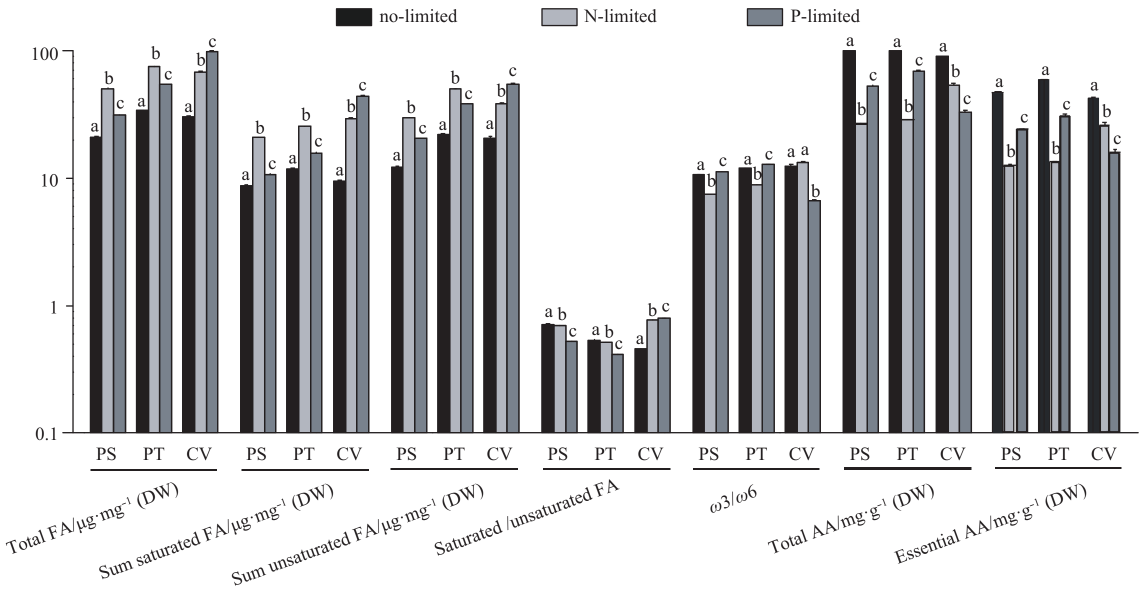

Fig. 1. Summary of several aggregative indicators of fatty acids (FA) and amino acids (AA) of the combination of *P. tricornutum* and *S. costatum* (PS), *P. tricornutum* (PT), and *C. vulgaris* (CV) grown under different nutrient conditions (no-limited, N-limited and P-limited). Error bars: standard deviation. Different lowercase letters above columns (a, b, and c) indicate a significant difference among three treatments (Tukey's HSD test, $P<0.05$).

amino acid) were statistically analyzed using a one-way ANOVA with treatment as the variable. Tukey's HSD test was used as the post hoc test. The Kruskal-Wallis test was used when the data did not approach homoscedasticity (Levene's test) or normality (Kolmogorov-Smirnov test). All statistical tests were carried out with SPSS 19.0 and Origin 9.0.

3 Results

3.1 *Food nutrition*

Figure 1 showed that, for *P. tricornutum* and the mixture of *P. tricornutum* and *S. costatum*, the lowest content of total fatty acids (FA), saturated FA (SFA), and unsaturated FA (UFA) and the highest value of SFA/UFA were detected in the no-limited treatment. However, the highest content of total FA, SFA, and UFA and the lowest value of ω3/ω6 FA were exhibited in the N-limited treatment. In terms of amino acids (AA), the no-limited treatment had the highest content and the N-limited treatment had the lowest content.

For *C. vulgaris*, the no-limited treatment had the lowest content of total FA, SFA, and UFA and the lowest value of SFA/UFA. In terms of AA, the no-limited treatment had the highest content. In contrast, the P-limited treatment had the highest content of total FA, SFA, and UFA; the highest value of SFA/UFA; the lowest value of ω3/ω6 FA; and the lowest content of AA.

3.2 *Zooplankton growth*

The total length of *C. sinicus* exhibited significant differences among the three treatments on the 11th day (Kruskal-Wallis test, $P<0.01$; Fig. 2). The no-limited treatment resulted in the highest value of total length, followed by the N-limited treatment and the P-limited treatment (no-limited: 692 μm, N-limited: 648 μm, and P-limited: 517 μm; Fig. 2). The no-limited treatment also had the highest percentage of individuals reached the stage of Copepodite 1. The P-limited treatment had the lowest percentage (no-limited: 60%, and P-limited: 20%; Fig. 2).

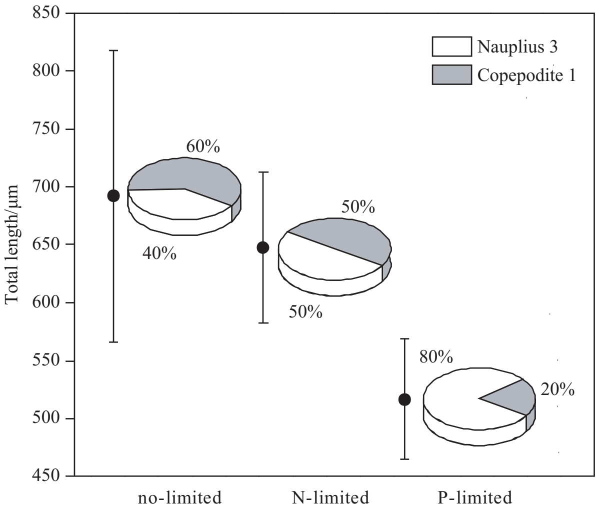

Fig. 2. On the 11th day, the total length and percentage of different developmental stages (Nauplius 3 and Copepodite 1) of *C. sinicus* reared under no-limited, N-limited, and P-limited conditions. Error bars: standard deviation.

For *M. mongolica*, the highest value of total length was observed in the no-limited treatment and the lowest value was found in the P-limited treatment during the three days after the experiment started (Fig. 3a). However, during the three days before the experiment ended, the lowest value of the total length was observed in the N-limited treatment (Fig. 3a).

For *B. plicatilis*, during 36 h after the experiment started, the highest value of the maximum area was found in the no-limited treatment and the lowest value was found in the P-limited treatment (Fig. 3b). In contrast, during 12 h before the experiment ended, individuals in the N-limited treatment had the highest value of the maximum area, followed by those in the no-limited treatment and those in the P-limited treatment.

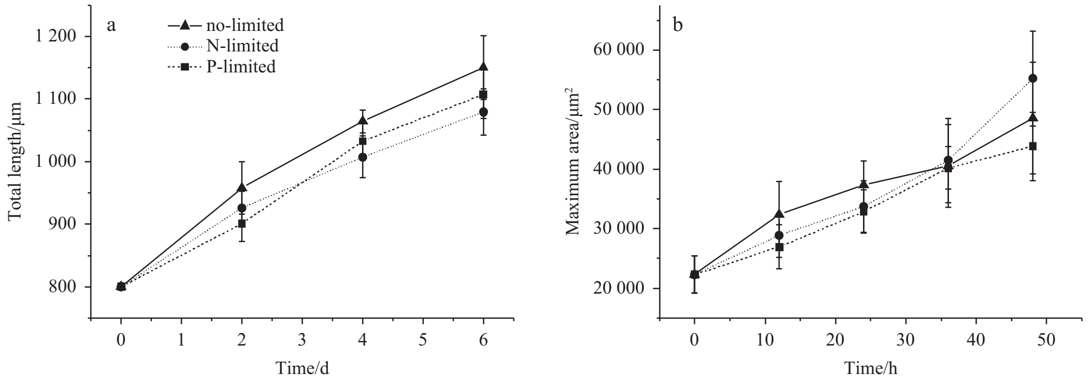

Fig. 3. Changes in the total length of *M. mongolica* (a) and the maximum area of *B. plicatilis* (b) reared under no-limited, N-limited, and P-limited conditions. Error bars: standard deviation.

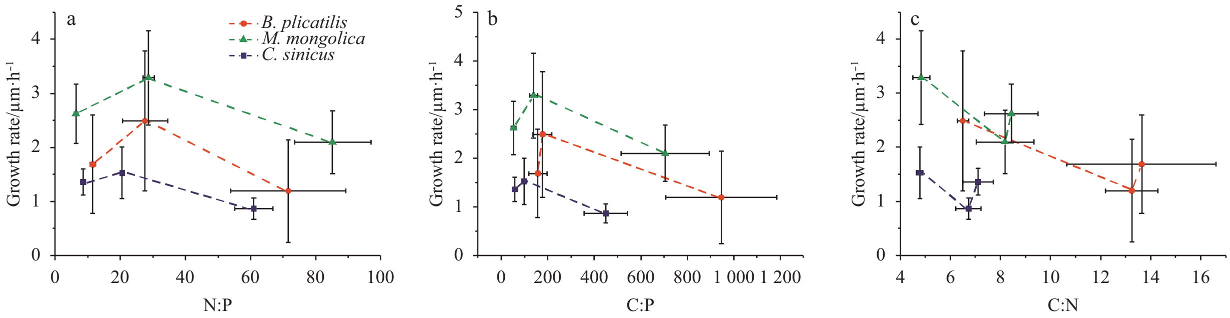

Fig. 4. Variation in the growth rate of the three species (*C. sinicus*, *M. mongolica*, and *B. plicatilis*) with the stoichiometry (N:P, C:P, and C:N) of their food. Error bars: standard deviation.

The highest growth rate of the three species (*C. sinicus*, *M. mongolica*, and *B. plicatilis*) was obtained when the dietary N:P value was 20 to 30 (Fig. 4a). This finding was in line with the Redfield ratio (N:P=16). When the N:P value deviated from 20 to 30, the growth rate of the three species declined. The extent of this decline was species specific. In general, compared with *M. mongolica* and *C. sinicus*, the growth rate of *B. plicatilis* declined more rapidly with the deviation of the N:P value (from 20 to 30) (Fig. 4a).

The highest growth rate was obtained when the C:P was 100–200, which was in line with the Redfield ratio (C:P=106). The growth rate of *B. plicatilis* exhibited a more substantial increase with the P sufficiency than that of *M. mongolica* and *C. sinicus*. With respect to the dietary C:N value, the growth rate of *B. plicatilis* decreased more slowly under N deficiency than under the other treatments.

3.3 *Zooplankton reproduction*

Both the egg production and hatching rate of *C. sinicus* were affected by nutrient conditions (no-limited, N-limited, and P-limited) (Figs 5a, b). During most of the experiment, the highest value of the egg production rate was found in the P-limited treatment and the lowest value was observed in the N-limited treatment (Fig. 5a). On the whole, the hatching success was negatively affected by P limitation. During most of the experiment, the hatching success in the P-limited treatment was lower than that in the no-limited treatment (Fig. 5b).

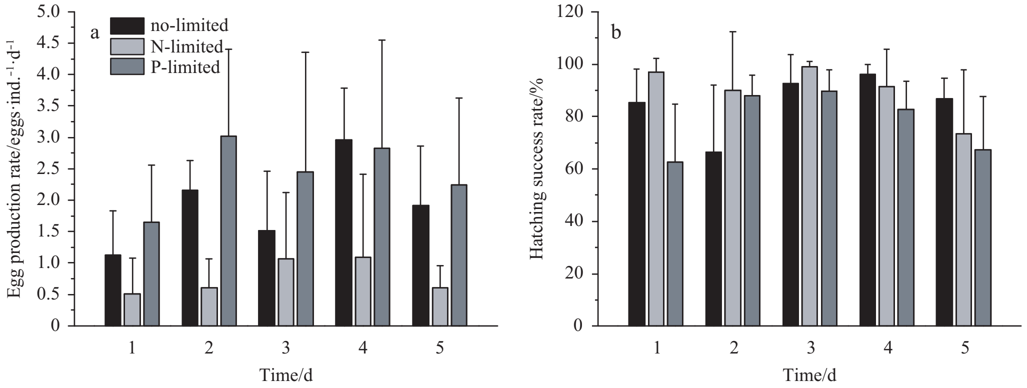

Fig. 5. Changes in the egg production rate (a) and hatching success (b) of *C. sinicus* reared under no-limited, N-limited, and P-limited conditions. Error bars: standard deviation.

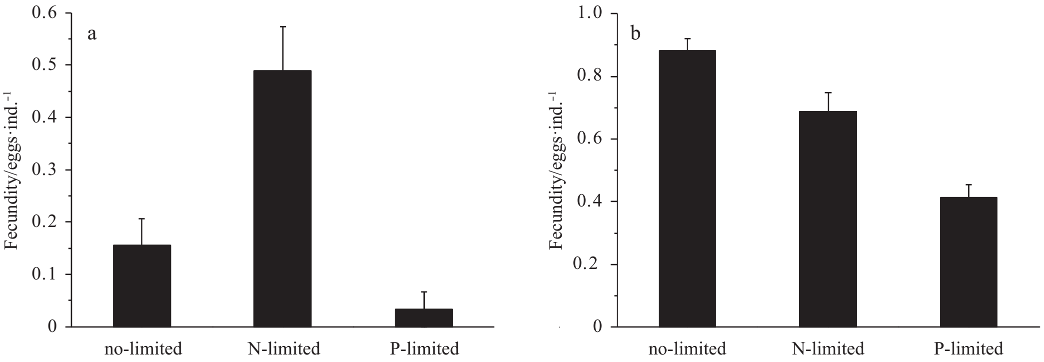

Fig. 6. Egg production rates of *M. mongolica* (a) and *B. plicatilis* (b) reared under no-limited, N-limited, and P-limited conditions. Error bars: standard deviation.

The fecundity (eggs per individual) of *M. mongolica* was significantly different among the three treatments (one-way ANOVA, $P<0.01$; Fig. 6a). Individuals in the N-limited treatment exhibited the highest fecundity, whereas those in the P-limited treatment had the lowest fecundity (N-limited: 0.49, no-limited: 0.16, and P-limited: 0.03; Fig. 6a).

The fecundity (eggs per individual) of *B. plicatilis* differed significantly among the three treatments (one-way ANOVA, $P<0.01$; Fig. 6b). The no-limited treatment exhibited the highest value of fecundity (0.88, Fig. 6b), followed by the N-limited treatment, with a value of 0.69, and the P-limited treatment, with the lowest value of 0.41 (Fig. 6b).

3.4 *Zooplankton elemental homoeostasis*

From the perspective of the homoeostatic regulation coefficient (H), for each stoichiometry (N:P, P%, etc.), the H value of *B. plicatilis* was higher than that of *M. mongolica* (Table 2). Furthermore, the absolute value of $1/H$ (slope) of *B. plicatilis* was closer to zero than that of *M. mongolica* (Table 2). For example, the $1/H_{\mathrm{C:P}}$ value of *M. mongolica* differed by 0.27 from zero, whereas the value of *B. plicatilis* differed by just 0.08; the absolute value of $1/H_{\mathrm{C\%}}$ was 9.62 for *M. mongolica* and was only 0.57 for *B. plicatilis* (Table 2).

4 Discussion

4.1 *Growth*

In our study, the growth of three species was suppressed by nutrient limitations (especially P limitation) (Figs 2 and 3). The P limitation was strongly coupled to the RNA synthesis (Elser et al., 1996; Sterner and Hessen, 1994), which has been detected in copepods (Malzahn and Boersma, 2012), cladocera (Vrede et al., 2002), and rotifers (Hessen et al., 2007). As ribosomes are the machinery of proteins synthesis, there was a close association between the P supply and protein synthesis. In P-limited treatment, protein synthesis of three species could be suppressed, and then, their growth may be negatively affected.

N-limitation also had effect on the growth of organisms and could be associated with AA content. In this study, under the N-limited condition, dietary essential AA (EAA) content was significantly lower than that in the no-limited treatment (Fig. 1 and Table 2). It was reported that non-essential AA synthesis could also be suppressed under the N-limited condition (Sterner and Elser, 2002). Lack of essential and unessential AA under N-limited condition could negatively affect protein synthesis. And then, the growth was potentially suppressed.

4.2 *Reproduction*

Under nutrient-limited conditions, three species exhibited different performance in terms of reproduction (Figs 5 and 6). And different mechanism how nutrient limitation affected the reproduction of three species was detected.

4.2.1 *Dietary stoichiometry and reproduction*

This study indicated that the main factor of dietary stoichiometry affecting the reproduction of three species was C:P ratio.

For copepods, eggs had a higher value of C:P than tissues (females without eggs) (Becker and Boersma, 2005). Females should balance the gain and loss of egg production from the perspective of nutrient output. In this study, the dietary C:P ratio in the P-limited treatment (449, Table 1) was far above the Redfield ratio (106), suggesting the carbon excess. For copepods, it was an

Table 2. The homoeostatic regulation coefficient (H) and parameters calculated from the linear regression for each stoichiometry (P%, N:P, etc.) of *B. plicatilis* and *M. mongolica*

	Stoichiometry	Absolute value of slope ($1/H$)	logc	R^2	H
B. plicatilis	C:P	0.08	2.40	0.63	12.50
	C%	0.57	2.28	0.98	1.75
	N%	0.50	0.73	0.14	2.00
	P%	0.16	3.26	0.33	6.25
M. mongolica	C:P	0.27	3.10	0.08	3.70
	C%	9.62	−9.24	0.96	0.10
	N%	5.09	−1.37	0.67	0.20
	P%	0.48	4.58	0.52	2.08
	C:N	0.82	0.40	0.64	1.22

Note: Results with $R^2<0$ were excluded.

effective strategy to produce more eggs to relieve the carbon surplus, since eggs had a higher C:P ratio than tissues (Becker and Boersma, 2005). Accordingly, the highest egg production rate (on the whole) was observed in the P-limited treatment (Fig. 5a). Inversely, the N-limited treatment, which was relatively carbon deficient (dietary C:P=58), exhibited the lowest egg production rate (Fig. 5a).

For cladocera, the P concentration in eggs was reported to be constant (Becker and Boersma, 2005; Færøvig and Hessen, 2003). Thus, the number of eggs would be suppressed if the dietary nutrient was limited. Under P-limited condition, females which were phosphorus deficient (dietary C:P, 750; $\text{TER}_{\text{C:P}}$, 250) could not support high egg production (Fig. 6a). Inversely, in N-limited treatment, females with relative excess of phosphorus (dietary C:P=53.5) tended to produce more eggs (Fig. 6a).

In this study, newly born rotifers were cultured under nutrient-limited conditions until the fecundity was detected (36 h of cultivation). It can be speculated that these rotifers reached maturity later and had shorter reproductive period than those in the no-limited treatment. Meanwhile, it was reported that, under nutrient-limited conditions, reproduction of the rotifer *Brachionus calyciflorus* reduced (Jensen and Verschoor, 2004). Thus, shorter reproductive period and lower daily reproduction may be the reasons for the lower fecundity in nutrient-limited treatments (Fig. 6b).

4.2.2 *Dietary biochemical composition and reproduction*

For *C. sinicus*, dietary EAA content in the N-limited treatment was significantly lower than that of others (P<0.05, Fig. 1 and Table 2). Helland et al. (2003) reported that EAA content of *Calanus finmarchicus* eggs was quite constant irrespective of their maternal diet. In this study, dietary EAA in the N-limited treatment (Fig. 1 and Table 2) could meet the needs of fewest eggs, leading to the lower egg production rate (Fig. 5). Besides that, Phospholipids were known to be used to form gonads (Kattner et al., 2007; Lee et al., 2006) and cell constituents (e.g., membranes) in the process of gonadogenesis and oogenesis (Kattner et al., 2007; Lee et al., 2006). PUFA, as major components of phospholipids (Chen et al., 2012; Harrison, 1990), had effect on the egg production (Sargent and Falk-Petersen, 1988). In our study, the N-limited treatment had the lowest proportion (total FA content percentage) of UFA (Fig. 1 and Table 3), which may cause PUFA deficiency and restrained the egg production (Fig. 5).

Meanwhile, as fatty acids were used to provide energy for embryonic development (Kattner et al., 2007), the highest storage of total FA in the N-limited treatment (Fig. 1 and Table 3) could facilitate the higher hatching success (Fig. 5).

For *M. mongolica*, in the N-limited treatment, significantly higher concentration of total FA and UFA (Fig. 1 and Table 3) may promote higher fecundity (Fig. 6), since fatty acids are essential to egg development as major energy support and PUFA play an important role in the structural and complex physiological development of cladocera (Weers and Gulati, 1997).

For *B. plicatilis*, the lowest quality of dietary FA and AA in P-limited treatment (Fig. 1, Tables 2 and 3) could cause the worst growth and reproduction, since dietary FA and AA have been shown to be important for zooplankton growth and reproduction (e.g., Jensen and Verschoor, 2004; Müller-Navarra, 1995; Urabe et al., 2002).

4.3 ***Stoichiometric homoeostasis***

B. plicatilis and *M. mongolica* had different values of H, implying different abilities in terms of maintaining constant chemical compositions. For each stoichiometry (N:P, P%, etc.), the $1/H$ (slope) of *B. plicatilis* was much closer to zero than that of *M. mongolica* (Table 2). As the slope ($1/H$) of zero represents the condition of homeostasis (Sterner and Elser, 2002), the above results implied that *B. plicatilis* exhibited a greater ability than *M. mongolica* to maintain constant elemental compositions. In other words, compared with *M. mongolica*, *B. plicatilis*'s elemental compositions were less likely to be affected by nutrient limitations.

4.4 ***Relationship with eutrophication***

Exploring different response of species to nutrient limitations could provide valuable insights into the mechanism of population succession under the condition of eutrophication. Changes in N:P stoichiometry over time have been observed in coastal areas (Wang, 2006). It was reported that, in the Changjiang (Yangtze River) Estuary, China, the N:P ratio has increased sharply approximately from 50 to 125 in ten years (Wang, 2006). This changing nutrient stoichiometry could be transported up the food chain and caused a mismatch between nutrient supply and demand in planktonic food webs, leading to nutrient limitation (Sterner and Elser, 2002). Our study found that effect of this nutrient limitation on the growth, reproduction, and stoichiometric homoeostasis varied with species. In this study, three species with different reproductive modes exhibited different patterns of reproduction among three treatments and different mechanism how nutrient limitation affected their reproduction was detected. It could be speculated that populations with different reproductive modes might be affected by nutrient limitations differently. In the long run, this process could become an evolutionary driving force and likely change the process and direction of population succession in coastal areas.

5 Conclusions

Our study indicated that the nutrient imbalance associated with eutrophication influenced zooplankton growth, reproduction and stoichiometric homeostasis. The growth of three species was suppressed under nutrient-limited (especially P-limited) conditions. However, the effect of nutrient limitations on their reproduction was species-specific. For example, the highest fecundity was detected in no-limited treatment for *B. plicatilis*, while in N-limited treatment for *M. mongolica*. Dietary C:P ratio, FA and AA content played important roles on this species-specific response. As for the stoichiometric homeostasis, *B. plicatilis* exhibited a greater ability to maintain a constant chemical composition in the bodies of *M. mongolica*. These effects of nutrient imbalance could potentially become an evolutionary force affecting ecosystem structure and stability in water bodies undergoing eutrophication. Further studies examining the physiological processes underlying these species-specific responses will deepen our understanding of the ecological consequences of eutrophication in marine ecosystems.

Acknowledgements

The authors thank the crew on R/V *Beidou* for their assistance in the field. We are grateful to Jin Xin and Ji Peng for their invaluable help during shipboard research activities.

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Appendix:

Table A1. Amino acid (AA) composition of *Chlorella vulgaris* (CV), *Phaeodactylum tricornutum* (PT), a combination of *Phaeodactylum tricornutum* and *Skeletonema costatum* (PS), and *Skeletonema costatum* (SC) grown under no-limited, N-limited, and P-limited conditions after 5 d

Amino acid	CV						PT					
	No-limited		N-limited		P-limited		No-limited		N-limited		P-limited	
	Composition /mg·g^{-1}	Composition /%	Composition /mg·g^{-1}	Composition /%	Composition /mg·g^{-1}	Composition /%	Composition /mg·g^{-1}	Composition /%	Composition /mg·g^{-1}	Composition /%	Composition /mg·g^{-1}	Composition /%
Aspartic acid (asp)	8.022±0.100	8.819±0.092	4.909±0.128	9.171±0.183	2.739±0.072	8.307±0.037	14.111±0.010	11.166±0.010	2.905±0.004	10.009±0.013	7.197±0.213	10.395±0.053
Threonine (thr)	4.355±0.052	4.787±0.048	2.821±0.253	5.262±0.230	1.568±0.089	4.755±0.125	6.653±0.008	5.264±0.005	1.584±0.032	5.458±0.124	3.584±0.085	5.176±0.004
Serine (ser)	4.045±0.135	4.447±0.140	2.314±0.122	4.320±0.028	1.384±0.096	4.203±0.420	6.623±0.035	5.241±0.029	1.664±0.008	5.733±0.011	3.524±0.001	5.092±0.124
Glutamic acid (glu)	13.715±0.034	15.078±0.007	7.049±0.070	13.179±0.737	4.760±0.041	14.443±0.317	16.975±0.048	13.433±0.035	3.511±0.091	12.098±0.348	10.377±0.362	14.988±0.156
Glycine (gly)	5.157±0.070	5.670±0.066	3.307±0.061	6.179±0.171	1.677±0.138	5.094±0.575	7.343±0.402	5.811±0.317	1.743±0.048	6.007±0.147	3.801±0.031	5.492±0.090
Alanine (ala)	6.816±0.032	7.493±0.020	3.943±0.081	7.367±0.189	2.286±0.116	6.932±0.140	9.457±0.019	7.483±0.017	2.059±0.019	7.096±0.084	5.283±0.132	7.630±0.005
Cysteine (cys)	1.947±0.003	2.140±0.007	1.829±0.242	3.408±0.295	1.861±0.047	5.646±0.029	2.714±0.072	2.148±0.057	1.665±0.083	5.736±0.269	2.166±0.107	3.132±0.231
Valine (val)	5.588±0.083	6.143±0.079	3.839±0.227	7.167±0.094	2.525±0.225	7.651±0.449	8.173±0.088	6.468±0.071	2.271±0.013	7.824±0.066	4.416±0.175	6.377±0.097
Methionine (met)	1.312±0.081	1.442±0.092	0.760±0.269	1.409±0.437	0.292±0.033	0.885±0.074	1.642±0.160	1.300±0.126	0.369±0.065	1.271±0.226	0.773±0.006	1.116±0.019
Isoleucine (ile)	4.150±0.014	4.563±0.024	2.569±0.115	4.796±0.006	1.462±0.166	4.427±0.368	6.622±0.119	5.240±0.095	1.294±0.002	4.458±0.020	3.242±0.146	4.682±0.096
Leucine (leu)	7.986±0.026	8.779±0.046	4.631±0.027	8.654±0.347	2.659±0.035	8.068±0.140	10.797±0.288	8.543±0.229	2.192±0.022	7.553±0.056	5.406±0.126	7.809±0.009
Tyrosine (tyr)	3.090±0.023	3.397±0.032	1.905±0.276	3.549±0.352	0.665±0.028	2.016±0.022	4.178±0.169	3.306±0.133	0.898±0.024	3.095±0.076	2.192±0.110	3.165±0.082
Phenylalanine (phe)	4.402±0.104	4.840±0.124	2.796±0.217	5.217±0.166	1.595±0.051	4.839±0.007	6.859±0.283	5.427±0.225	1.479±0.040	5.097±0.125	3.460±0.091	4.997±0.010
Histidine (his)	2.812±0.104	3.091±0.109	2.008±0.201	3.744±0.204	1.596±0.099	4.849±0.449	3.342±0.068	2.645±0.053	1.374±0.037	4.733±0.116	2.258±0.068	3.264±0.178
Lysine (lys)	5.929±0.044	6.518±0.035	3.408±0.130	6.365±0.050	2.047±0.082	6.208±0.059	6.922±0.056	5.477±0.043	1.505±0.004	5.187±0.002	3.490±0.134	5.040±0.070
Argnine (arg)	5.121±0.056	5.630±0.073	3.028±0.032	5.658±0.201	1.941±0.084	5.885±0.074	7.279±0.021	5.760±0.018	1.321±0.036	4.551±0.111	3.923±0.112	5.666±0.023
Proline (pro)	6.517±0.167	7.165±0.198	2.441±0.154	4.556±0.079	1.913±0.272	5.792±0.648	6.683±0.051	5.288±0.039	1.188±0.005	4.094±0.029	4.139±0.145	5.977±0.064
Total AA	90.964±0.180		53.557±2.465		32.971±1.008		126.373±0.028		29.021±0.082		69.232±1.695	

Amino acid	PS						SC					
	No-limited		N-limited		P-limited		No-limited		N-limited		P-limited	
	Composition /mg·g^{-1}	Composition /%	Composition /mg·g^{-1}	Composition /%	Composition /mg·g^{-1}	Composition /%	Composition /mg·g^{-1}	Composition /%	Composition /mg·g^{-1}	Composition /%	Composition /mg·g^{-1}	Composition /%
Aspartic acid (asp)	11.054±0.171	11.059±0.063	2.656±0.071	10.053±0.142	5.527±0.084	10.381±0.074	6.833±0.090	10.771±0.238	2.378±0.063	10.151±0.385	3.589±0.127	10.368±0.367
Threonine (thr)	5.188±0.031	5.191±0.020	1.399±0.021	5.299±0.147	2.735±0.020	5.137±0.079	3.163±0.096	4.985±0.107	1.187±0.073	5.064±0.252	1.742±0.099	5.033±0.285
Serine (ser)	5.224±0.082	5.226±0.031	1.587±0.069	6.008±0.186	2.956±0.077	5.553±0.100	3.295±0.091	5.195±0.190	1.509±0.114	6.444±0.560	2.296±0.064	6.631±0.187
Glutamic acid (glu)	13.889±0.126	13.895±0.010	3.327±0.032	12.596±0.282	7.973±0.042	14.978±0.199	9.621±0.101	15.164±0.024	3.123±0.028	13.331±0.034	5.175±0.106	14.948±0.303
Glycine (gly)	6.007±0.245	6.009±0.187	1.665±0.004	6.304±0.066	3.087±0.046	5.798±0.040	4.149±0.116	6.539±0.124	1.567±0.074	6.685±0.241	2.254±0.005	6.510±0.017
Alanine (ala)	7.438±0.101	7.441±0.029	1.806±0.027	6.836±0.016	3.934±0.078	7.390±0.088	4.651±0.050	7.331±0.145	1.519±0.007	6.483±0.045	2.369±0.079	6.842±0.231
Cysteine (cys)	1.574±0.010	1.575±0.005	0.888±0.168	3.358±0.594	1.169±0.142	2.195±0.249	0	0	0	0	0	0
Valine (val)	6.174±0.010	6.176±0.051	1.737±0.035	6.576±0.050	3.181±0.036	5.977±0.116	3.411±0.072	5.376±0.065	1.125±0.092	4.800±0.339	1.742±0.099	5.033±0.285

to be continued

Continued from Table A1

Amino acid	PS						SC					
	No-limited		N-limited		P-limited		No-limited		N-limited		P-limited	
	Composition /mg·g⁻¹	Composition /%	Composition /mg·g⁻¹	Composition /%	Composition /mg·g⁻¹	Composition /%	Composition /mg·g⁻¹	Composition /%	Composition /mg·g⁻¹	Composition /%	Composition /mg·g⁻¹	Composition /%
Methionine (met)	1.369±0.097	1.369±0.084	0.562±0.083	2.129±0.340	0.842±0.062	1.582±0.129	0.986±0.019	1.555±0.017	0.783±0.037	3.342±0.120	0.918±0.116	2.652±0.334
Isoleucine (ile)	5.170±0.005	5.173±0.045	1.165±0.001	4.412±0.061	2.486±0.027	4.669±0.088	3.168±0.021	4.994±0.077	1.016±0.045	4.335±0.142	1.607±0.026	4.641±0.073
Leucine (leu)	8.555±0.157	8.560±0.241	1.947±0.051	7.368±0.099	4.147±0.034	7.791±0.001	5.460±0.219	8.605±0.269	1.666±0.001	7.111±0.078	2.682±0.008	7.746±0.023
Tyrosine (tyr)	3.288±0.077	3.289±0.045	0.766±0.030	2.898±0.076	1.574±0.020	2.957±0.062	2.050±0.148	3.230±0.204	0.612±0.010	2.613±0.011	0.856±0.027	2.471±0.079
Phenylalanine (phe)	5.514±0.074	5.517±0.128	1.403±0.019	5.310±0.003	2.756±0.053	5.176±0.057	3.664±0.069	5.777±0.161	1.310±0.033	5.591±0.076	1.941±0.093	5.606±0.270
Histidine (his)	3.529±0.041	3.530±0.006	2.005±0.077	7.594±0.387	2.658±0.088	4.993±0.125	3.786±0.023	5.967±0.090	2.720±0.008	11.609±0.166	3.130±0.184	9.042±0.535
Lysine (lys)	5.501±0.096	5.504±0.043	1.403±0.019	5.310±0.003	2.765±0.063	5.195±0.161	3.538±0.001	5.576±0.048	1.286±0.001	5.491±0.066	1.920±0.114	5.545±0.327
Arginine (arg)	5.535±0.049	5.537±0.005	1.059±0.059	4.009±0.174	2.704±0.023	5.080±0.002	3.124±0.042	4.924±0.022	0.759±0.003	3.242±0.022	1.283±0.041	3.707±0.118
Proline (pro)	4.947±0.067	4.949±0.019	1.040±0.003	3.939±0.060	2.740±0.074	5.147±0.098	2.546±0.099	4.012±0.120	0.869±0.051	3.707±0.175	1.117±0.076	3.225±0.221
Total AA	99.957±0.976		26.415±0.335		53.234±0.427		63.444±0.569		23.430±0.268		34.620±0.007	

Note: The values represent the means of three replicate samples and are given as mg/g (dry weight) (means±SD) and percentage (%) of the total AA content (means±SD).

Table A2. Fatty acid (FA) composition (means±SD) of *Chlorella vulgaris* (CV), *Phaeodactylum tricornutum* (PT), a combination of *Phaeodactylum tricornutum* and *Skeletonema costatum* (PS), and *Skeletonema costatum* (SC) grown under three different nutrient conditions (no-limited, N-limited and P-limited)

Fatty acid	CV						PT					
	No-limited		N-limited		P-limited		No-limited		N-limited		P-limited	
	Composition /μg·mg⁻¹	Composition /%	Composition /μg·mg⁻¹	Composition /%	Composition /μg·mg⁻¹	Composition /%	Composition /μg·mg⁻¹	Composition /%	Composition /μg·mg⁻¹	Composition /%	Composition /μg·mg⁻¹	Composition /%
C12:0	0.064±0.003	0.213±0.007	0.108±0.003	0.160±0.000	0.142±0.005	0.143±0.007	–	–	–	–	–	–
C14:0	1.352±0.030	4.470±0.026	2.425±0.080	3.589±0.038	3.657±0.053	3.690±0.002	2.550±0.041	7.541±0.009	4.942±0.021	6.537±0.014	3.685±0.006	6.789±0.001
C15:0	0.137±0.004	0.452±0.007	0.368±0.016	0.545±0.012	0.336±0.015	0.339±0.017	0.077±0.001	0.229±0.002	0.205±0.002	0.271±0.004	0.182±0.000	0.335±0.001
C15:1	–	–	–	–	–	–	0.146±0.002	0.432±0.001	0.077±0.003	0.101±0.004	0.113±0.000	0.208±0.001
C16:0	6.993±0.114	23.119±0.102	24.575±0.650	36.373±0.218	36.271±0.696	36.600±0.214	4.803±0.086	14.205±0.036	18.485±0.092	24.451±0.052	10.186±0.026	18.769±0.023
C16:1	–	–	–	–	–	–	8.275±0.125	24.474±0.033	34.833±0.181	46.076±0.067	24.288±0.030	44.753±0.033
C16:1ω7	7.417±0.128	24.520±0.135	19.156±0.596	28.352±0.350	32.132±0.494	32.425±0.070	–	–	–	–	–	–
C16:1ω5	0.044±0.005	0.144±0.013	0.087±0.002	0.128±0.000	0.215±0.004	0.217±0.007	–	–	–	–	–	–
C17:0	0.147±0.008	0.486±0.018	0.358±0.015	0.531±0.017	0.381±0.012	0.384±0.012	3.031±0.036	8.965±0.033	0.022±0.003	0.029±0.004	0.017±0.000	0.031±0.001
C17:1	–	–	–	–	–	–	0.589±0.016	1.742±0.022	0.143±0.200	0.188±0.263	0.478±0.002	0.881±0.003
C18:0	0.764±0.057	2.526±0.176	1.530±0.256	2.267±0.401	3.224±0.099	3.253±0.068	0.135±0.004	0.399±0.006	0.445±0.006	0.589±0.010	0.373±0.011	0.687±0.019
C18:1	–	–	–	–	–	–	0.196±0.004	0.580±0.005	2.170±0.008	2.870±0.009	0.939±0.006	1.730±0.013
C18:1ω9	2.650±0.044	8.762±0.072	6.565±0.189	9.716±0.108	10.219±0.099	10.312±0.077	–	–	–	–	–	–
C18:1ω7	0.306±0.015	1.010±0.040	0.411±0.003	0.609±0.018	0.575±0.008	0.580±0.007	–	–	–	–	–	–
C18:2ω6	0.611±0.029	2.021±0.089	0.582±0.028	0.862±0.049	1.053±0.025	1.062±0.010	0.806±0.014	2.383±0.004	1.023±0.001	1.353±0.007	0.785±0.001	1.446±0.001
C18:3ω6	0.071±0.001	0.235±0.007	0.106±0.006	0.157±0.006	0.204±0.005	0.206±0.007	–	–	–	–	–	–
C18:3ω3	0.082±0.007	0.270±0.024	0.125±0.009	0.185±0.016	0.253±0.004	0.255±0.008	0.142±0.003	0.420±0.005	0.127±0.003	0.168±0.004	0.094±0.002	0.174±0.003
C20:0	–	–	–	–	–	–	0.015±0.001	0.043±0.002	0.037±0.002	0.050±0.003	0.039±0.001	0.073±0.002
C20:2	–	–	–	–	–	–	0.044±0.005	0.130±0.014	0.106±0.003	0.141±0.005	0.099±0.001	0.183±0.002

to be continued

Continued from Table A2

Fatty acid	CV						PT					
	No-limited		N-limited		P-limited		No-limited		N-limited		P-limited	
	Composition /μg·mg^{-1}	Composition /%	Composition /μg·mg^{-1}	Composition /%	Composition /μg·mg^{-1}	Composition /%	Composition /μg·mg^{-1}	Composition /%	Composition /μg·mg^{-1}	Composition /%	Composition /μg·mg^{-1}	Composition /%
C20:3ω6	0.084±0.007	0.277±0.017	0.147±0.004	0.217±0.006	0.277±0.004	0.280±0.008	0.170±0.007	0.502±0.021	0.249±0.002	0.330±0.004	0.109±0.001	0.201±0.002
C20:5ω3EPA	9.402±0.217	31.080±0.297	10.887±0.311	16.114±0.264	9.959±0.117	10.050±0.128	10.833±0.177	32.038±0.055	10.177±0.029	13.462±0.071	10.711±0.011	19.736±0.017
C22:0	–	–	–	–	–	–	0.106±0.002	0.313±0.006	0.135±0.001	0.179±0.001	0.121±0.005	0.223±0.010
C22:1ω9	0.125±0.009	0.414±0.029	0.132±0.009	0.196±0.016	0.201±0.008	0.203±0.005	0.138±0.008	0.408±0.029	0.084±0.003	0.112±0.004	0.137±0.005	0.253±0.010
C24:0	–	–	–	–	–	–	1.093±0.009	3.232±0.050	1.326±0.008	1.754±0.005	1.242±0.002	2.288±0.003
C22:6ω3DHA	–	–	–	–	–	–	0.664±0.013	1.964±0.022	1.013±0.005	1.341±0.015	0.673±0.002	1.240±0.005
Total FA	30.250±0.574		67.561±1.637		99.097±1.473		33.813±0.516		75.599±0.470		54.270±0.078	
Sum saturated	9.458±0.180	31.266±0.066	29.364±0.659	43.465±0.128	44.011±0.809	44.410±0.170	11.810±0.170	34.927±0.037	25.597±0.115	33.860±0.083	15.844±0.044	29.195±0.040
Sum unsaturated	20.792±0.396	68.734±0.066	38.197±0.983	56.535±0.128	55.086±0.672	55.590±0.170	22.004±0.346	65.073±0.037	50.002±0.364	66.140±0.083	38.426±0.034	70.805±0.040
Sat/Unsat	0.455±0.001	0.455±0.001	0.769±0.004	0.769±0.004	0.799±0.006	0.799±0.006	0.537±0.001	0.537±0.001	0.512±0.002	0.512±0.002	0.412±0.001	0.412±0.001
ω3	9.483±0.215	31.349±0.273	11.012±0.312	16.299±0.277	10.211±0.113	10.305±0.130	11.639±0.190	34.422±0.044	11.317±0.026	14.971±0.088	11.478±0.012	21.150±0.019
ω6	0.766±0.029	2.533±0.082	0.834±0.027	1.235±0.048	1.534±0.019	1.548±0.005	0.976±0.015	2.885±0.018	1.272±0.001	1.683±0.011	0.894±0.002	1.647±0.002
ω3/ω6	12.385±0.500	12.385±0.500	13.202±0.335	13.202±0.335	6.657±0.065	6.657±0.065	11.931±0.084	11.931±0.084	8.897±0.024	8.897±0.024	12.840±0.023	12.840±0.023

Fatty acid	PS						SC					
	No-limited		N-limited		P-limited		No-limited		N-limited		P-limited	
	Composition /μg·mg^{-1}	Composition /%	Composition /μg·mg^{-1}	Composition /%	Composition /μg·mg^{-1}	Composition /%	Composition /μg·mg^{-1}	Composition /%	Composition /μg·mg^{-1}	Composition /%	Composition /μg·mg^{-1}	Composition /%
C14:0	2.645±0.021	12.622±0.113	6.326±0.041	12.495±0.023	2.951±0.007	9.374±0.008	2.741±0.050	33.815±0.392	7.710±0.062	30.049±0.034	2.217±0.010	25.523±0.053
C15:0	0.121±0.001	0.579±0.012	0.414±0.002	0.818±0.002	0.201±0.001	0.639±0.002	0.165±0.004	2.041±0.052	0.623±0.005	2.430±0.002	0.221±0.002	2.541±0.019
C15:1	0.098±0.002	0.468±0.010	0.088±0.000	0.173±0.002	0.073±0.002	0.231±0.007	0.050±0.004	0.620±0.060	0.099±0.002	0.385±0.005	0.033±0.004	0.377±0.048
C16:0	3.200±0.037	15.267±0.022	12.283±0.073	24.261±0.036	6.056±0.010	19.238±0.008	1.597±0.027	19.698±0.233	6.081±0.056	23.701±0.040	1.926±0.012	22.172±0.089
C16:1	4.729±0.044	22.563±0.112	21.416±0.117	42.301±0.046	13.176±0.018	41.859±0.055	1.183±0.04	14.592±0.420	8.000±0.063	31.179±0.027	2.065±0.006	23.776±0.143
C17:0	1.607±0.079	7.664±0.297	0.210±0.001	0.414±0.004	0.049±0.01	0.155±0.033	0.183±0.141	2.230±1.713	0.397±0.003	1.548±0.004	0.081±0.020	0.927±0.235
C17:1	0.314±0.007	1.496±0.017	0.076±0.100	0.150±0.196	0.245±0.001	0.778±0.003	0.038±0.001	0.471±0.025	0.010±0.001	0.038±0.005	0.012±0.002	0.133±0.019
C18:0	0.330±0.006	1.576±0.019	0.537±0.005	1.061±0.014	0.534±0.013	1.697±0.039	0.526±0.010	6.486±0.088	0.629±0.004	2.450±0.027	0.695±0.018	8.005±0.165
C18:1	0.286±0.001	1.366±0.018	1.316±0.006	2.599±0.005	0.655±0.008	2.081±0.024	0.376±0.005	4.642±0.114	0.462±0.005	1.801±0.004	0.371±0.018	4.276±0.191
C18:2ω6	0.475±0.006	2.267±0.012	0.653±0.002	1.290±0.005	0.451±0.003	1.434±0.008	0.144±0.002	1.782±0.039	0.283±0.002	1.104±0.003	0.118±0.006	1.359±0.061
C18:3ω3	0.088±0.002	0.421±0.010	0.078±0.001	0.153±0.002	0.072±0.001	0.228±0.002	0.034±0.001	0.424±0.027	0.028±0.002	0.110±0.008	0.049±0.003	0.569±0.029
C20:0	0.059±0.002	0.280±0.011	0.120±0.002	0.237±0.004	0.081±0.002	0.256±0.005	0.103±0.005	1.270±0.089	0.202±0.005	0.789±0.018	0.122±0.003	1.401±0.042
C20:2	0.041±0.003	0.193±0.010	0.065±0.002	0.128±0.004	0.061±0.001	0.192±0.004	0.037±0.001	0.457±0.027	0.023±0.001	0.090±0.003	0.022±0.001	0.254±0.014
C20:3ω6	0.096±0.003	0.459±0.014	0.137±0.002	0.271±0.005	0.075±0.002	0.237±0.005	0.023±0.001	0.278±0.018	0.025±0.002	0.098±0.008	0.040±0.002	0.464±0.025
C20:5ω3EPA	5.489±0.088	26.189±0.205	5.201±0.014	10.273±0.048	5.376±0.006	17.079±0.008	0.146±0.002	1.796±0.042	0.225±0.001	0.878±0.009	0.041±0.001	0.475±0.014
C22:0	0.078±0.001	0.370±0.004	0.093±0.000	0.183±0.001	0.071±0.003	0.225±0.009	0.049±0.001	0.607±0.024	0.050±0.001	0.194±0.004	0.020±0.000	0.236±0.005
C22:1ω9	0.119±0.004	0.569±0.028	0.115±0.001	0.226±0.003	0.121±0.003	0.385±0.010	0.100±0.001	1.240±0.030	0.145±0.002	0.564±0.012	0.105±0.001	1.206±0.014
C24:0	0.702±0.010	3.351±0.074	0.836±0.004	1.651±0.013	0.813±0.001	2.582±0.005	0.312±0.018	3.843±0.198	0.346±0.010	1.347±0.044	0.384±0.001	4.419±0.024

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