



The comprehensive effect of natural food quality and quantity on growth rate of herbivore consumers

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ABSTRACT

The performance of herbivore consumers in freshwater ecosystems is influenced by food quality and quantity, which are crucial factors in understanding energy flow. However, the comprehensive effects of these factors on consumer growth based on natural food research remain unclear. To address this gap, we conducted a growth experiment culturing the Cladocera *Daphnia similis* in natural lake water collected from 30 subtropical lakes. Our results showed that the seston concentration (SC), seston phosphorus (seston P), and seston carbon: phosphorus ratio (seston C:P, in moles) were the most important factors in influencing the growth rate of *D. similis* according to the Random Forest model. Specifically, the growth rate of *D. similis* was significantly positive correlated with SC and seston P, and significantly negative correlated with seston C:P. *D. similis* exhibited the optimal growth performance within the seston C:P range of 32.8 to 69.8, with a sharp decline in growth rate observed at a break point of seston C:P of 70. The combined effect of food quality and quantity on growth rate was that higher SC (≥ 0.26 mg C/L), lower seston C:P (≤ 69.80), and higher P (≥ 0.11 mmol/g) were associated with significantly higher growth rates. Additionally, the growth rate increased significantly with the biomass of cryptophyte, indicating the importance of food composition. Our study shows that the growth rate of *D. similis* is co-affected by the quality and quantity of natural food. SC, seston C:P, seston P and algae composition are reliable indicators for assessing the growth rate of consumer in freshwater ecosystems. The inhibitory effect of low C:P on consumer growth should be applied with caution when assessing the development of herbivore consumers in natural lakes.

1. Introduction

In recent decades, human activities have strongly changed the cycles of essential elements, such as phosphorus (P) and nitrogen (N) in natural systems (Tong et al., 2020; Turan et al., 2022). The altered bioavailability of the key elements (N and P) may have far-reaching effects on the interactions of biota. Generally, the element composition of primary producers is flexible and changes accordingly to the nutrient status of their environment (Paul et al., 2016). In contrast, most consumers are able to regulate and hold their elemental ratios more constant than those of primary producers (Sterner and Elser, 2002; Persson et al., 2010). This stoichiometric mismatch may affect interactions between consumers

and their food resources, and further affect the cycling of material and energy through ecosystems (Hessen et al., 2013). Ecological stoichiometry (ES) is a scientific approach that investigates the balance of multiple chemical elements in ecological interactions and processes (Sterner and Elser, 2002). An important finding of ES was the recognition of the crucial role of energy and nutrients transferring driven by pelagic consumers in aquatic food webs (Olsen et al., 1986). Investigating how the growth of consumers is influenced by the quality and quantity of their food is a crucial aspect of advancing ES.

The well-established knowledge of the effects of stoichiometric mismatch on the interface of primary consumers and primary producers in freshwater ecosystems was usually obtained through laboratory-

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based works (Park et al., 2002; Weider et al., 2004; Elser et al., 2016; Zhou et al., 2018). For example, numerous laboratory studies have demonstrated the negative effects of resource P limitation on the growth and reproduction of consumers (Sterner et al., 1993; DeMott et al., 1998; Anderson and Hessen, 2005). The growth rate hypothesis (GRH) explains the underlying mechanism by suggesting that P, as a key component of ribosomal RNA, is strongly linked to the growth of organisms (Elser et al., 2003; Acharya et al., 2004; Jeyasingh et al., 2020). In addition to the well-studied effects of P limitation, recent studies have reported that excessive P in food inhibits the growth of zooplankton consumers, coined as “stoichiometric knife-edge” (Sterner and Elser, 2002; Boersma and Elser, 2006). According to the stoichiometric knife-edge, the growth of consumers is expected to peak at the Threshold Elemental Ratio (TER), where neither carbon nor phosphorus is limiting (Anderson et al., 2005; Frost et al., 2006; Elser et al., 2016), and which by itself may be even dependent on the environmental temperature (Boersma et al., 2016; Malzahn et al., 2016; Laspoumaderes et al., 2022).

One challenge arising from these laboratory experiments is that it is always difficult to generalize the lab findings to fields given the high environmental complexity of natural ecosystems. Laboratory experiments are able to recreate field conditions to some extent, but they have limitations as they hardly ever include natural food composition of zooplankton (Hessen et al., 2013; Elser et al., 2016). Only one or two algal species are typically used to feed zooplankton in most laboratory experiment researches (DeMott et al., 1998; Bell et al., 2019), which clearly cannot represent the food composition of zooplankton in natural lakes. The natural food of zooplankton is composed of a diverse range of algal species, and the biochemical composition or morphology of algal cells plays an important role in determining their quality as food for zooplankton consumers (Müller-Navarra, 1995; Zhou et al., 2018). In addition to the importance of different algae composition of food, obviously food quantity also affects the growth performance of herbivore consumers (Müller-Navarra and Lampert, 1996; Boersma et al., 2016). Thus, especially the influence of natural food sources on zooplankton needs further study.

Since food features in natural freshwater ecosystems are much more intricate than that in the laboratory (Elser et al., 2016), there are only a few studies that explored the effects of natural food on the performance of herbivore consumers (Brett and Müller-Navarra, 1997; Boersma et al., 2001; Wacker and von Elert, 2001; Müller-Solger et al., 2002; Currier and Elser, 2017). In natural lakes, the P content in food seston is a key determinant in population recruitment of herbivore consumers (Sterner, 1998). Herbivore consumers' abundance exhibits strong and consistent inverse relationships with the seston C:P ratio (DeMott and Gulati, 1999). Although several studies (Brett and Müller-Navarra, 1997; DeMott and Gulati, 1999) conducted laboratory experiments based on natural freshwater ecosystems and found that seston P content can affect the growth of consumers, direct observation of demographic effects in nature is still challenging (Sterner, 1998). In addition, most of the studies mentioned above are based on temperate lakes (DeMott and Gulati, 1999; Hessen, 2006), and virtually little is known about these interactions in tropical and subtropical environments. We pose that this is an important hiatus in our knowledge as food quality (seston C:P) of lakes differs significantly between subtropical and temperate zones (Hessen, 2006; Wang, 2014). Due to their climate differences, subtropical and temperate lakes have different algae compositions and seasonal dynamics (De Senerpont Domis et al., 2013). Moreover, it has been shown that temperature and food demands interact (Laspoumaderes et al., 2022), as in some cases higher temperatures can exacerbate (Persson et al., 2011) or reduce (Boersma et al., 2016) the effects of food quality (in terms of P-demand) on zooplankton growth. Given this, further research is needed on the interactive effects of food quality and quantity on consumers, especially in tropical and subtropical regions.

With this study, we aimed to: 1) analyze which of the quantity and quality factors of natural food for herbivore consumers are the most

important in determining the growth rate of zooplankton. 2) elucidate how these factors affect the growth rate of consumers and explore the optimal seston C:P that can support high growth rate in natural food-based growth experiments. We used *Daphnia similis* as a study species, as the genus *Daphnia* has a good research base, and is a well-established model organism to study the effect of food element limitation and how consumers deal with this (Sterner and Elser, 2002). Further, the genus also plays a key role in freshwater systems, which adds direct ecological relevance to our study. We collected seston from 30 subtropical lakes with different food quality (seston C, N, P, seston C:P, seston C:N, seston N:P), quantity (seston concentration, SC), and phytoplankton composition as food for *D. similis* to carry out the growth experiments. First, we built the relationship between the growth rate (GR) of *D. similis* and each single food factor. Next, we used a Random Forest model to analyze the contribution of all food features to *Daphnia* GR and visualized the trend of model-predicted GR with the screened food factors using partial dependence plots. Finally, we assessed the combined effect of food quality and quantity on the GR.

2. Materials and methods

2.1. Experimental preparation

We collected *D. similis* from Lake Taihu, China (120°10'17"E, 31°29'45"N). The *D. similis* population was maintained in artificial growth media (COMBO, Kilham, et al., 1998), which was replaced every week. *Daphnia* was fed the green algae *Tetrademus obliquus*, also cultured using COMBO. *T. obliquus* was obtained from the Freshwater Algae Culture Collection at the Institute of Hydrobiology, Chinese Academy of Sciences. To collect a large cohort of individuals for the growth experiments, one single clone of *D. similis* from the population was cultured with a high concentration (1.0 mg C/L) of *T. obliquus* for several days until maturity. Twenty-four hours before the start of the experiment, embryo bearing females of *D. similis* were transferred to COMBO medium without nitrogen and phosphorus. Neonates were collected within 24 h for the experiment.

We selected 30 shallow lakes (Table S1) in the Nanjing area in China. These lakes are located near cities or towns, and their ecosystems have all been affected by human activities. Lake water was collected in spring at three depths (50 cm below the water surface, at the middle, and 50 cm above the sediment), and then mixed to obtain a well-mixed representative sample of the lake. We took ten liters of the lake water back to the laboratory for the growth experiments. The water samples were filtered through a 75 µm mesh to remove other zooplankton and large inedible particles. The resulting filtrate, referred to as the experimental water, served as the culture medium and food source for *D. similis* in growth experiments.

2.2. Growth experiments

To test how differences in the quality and quantity of lake seston from 30 lakes affect the growth rate of zooplankton, we grew the neonates collected from the standardized lab conditions, in containers with water from each of these natural lakes and established their growth rates. There were 30 treatments (i.e., 30 lakes) with 3 replicates for each treatment. For each replicate, five neonates were cultivated in a 250 ml beaker filled with 200 ml experimental water. All the beakers were put in an incubator under the conditions of 20°C and light: dark cycle of 14:10 h, light intensity of 800 lx.

The duration of the experiment was 5 days, and the lake water in the replicates was changed daily. We used the same lake water that was kept at 4°C in the dark throughout the experiment. The body length of *D. similis* was measured at the beginning of the experiment and daily during the experiment using a microscope eyepiece scale (Olympus X31, Japan). From the fifth day of the experiment, eggs in the brood pouch of animals were detected, hence the growth rate of animals was calculated

by the change in *Daphnia* body length from day 0 to day 4 (Acharya et al., 2004). The growth rate (GR, d^{-1}) of *D. similis* was calculated from body length as Sterner (1993):

$$GR = [\ln(l_4) - \ln(l_0)]/4 \quad (1)$$

where l_0 was the initial body length of *D. similis* and l_4 was the mean body lengths of *D. similis* in each beaker on the fourth day.

2.3. Nutrients and phytoplankton of lake waters

We measured the nutrients in the lake water to get the trophic state of the lakes. The concentrations of total nitrogen (TN, mmol/L) and total phosphorus (TP, $\mu\text{mol/L}$) of the lake water were measured by the alkaline potassium persulfate method. Concentrations of dissolved nitrate ($\text{NO}_3\text{-N}$, mmol/L) and phosphate ($\text{PO}_4\text{-P}$, $\mu\text{mol/L}$) were determined with a mobile injection analyzer (Skalar, Netherlands) using lake water filtered through GF/C Whatman filters. The spectrophotometric method of nutrient measurements is described in APHA (1995) and HJ 636 (2012). The trophic status of the 30 lakes was classified based on the TP of lake water of USEPA.

We took 500 ml of the mixed lake water and added 5 ml of Lugol's solution for subsequent phytoplankton analysis. The abundance of different species of algae was microscopically determined using a 0.1-ml Sedgwick-Rafter counting chamber at x400 magnification. Biomass of phytoplankton edible ($<75 \mu\text{m}$) by *D. similis* (PPB) was calculated based on the abundance and volume of phytoplankton, assuming that 1 mm^3 of phytoplankton volume was equivalent to 1 mg of fresh-weight biomass (Hillebrand et al., 1999).

2.4. Stoichiometric measurements of seston

The experimental water was filtered through two pre-combusted GF/F Whatman filters, one of which was for C and N measurements, and the other one was for P measurements. The filters were dried at 60°C for 24 h and weighed on an electronic microbalance to measure the dry weight of seston. After drying the filters, the C (mmol of carbon per gram of dry seston, mmol/g) and N (mmol of nitrogen per gram of dry seston, mmol/g) contents were determined using an elemental analyzer (EA3000, Italy), and the P content (mmol of phosphorus per gram of dry seston, mmol/g) was determined using an inductively coupled plasma emission spectrometer (Prodigy, USA). The seston C:P, C:N, and N:P reported in this paper are all in molar ratios. The food quantity of SC was determined by multiplying the dry weight of seston by its carbon content and expressed as mg carbon dry weight per liter of experimental water (mg C/L).

2.5. Data analysis

The lake water nutrient and seston characteristics data were subjected to a \log_{10} -transformation prior to conducting correlation analysis with GR. The \log_{10} -transformation was used to normalize the data distribution, and mitigate the impact of extreme values. All the statistical analysis were conducted in R Studio unless otherwise stated.

In order to evaluate the relative importance of food quality and quantity in lakes on the GR of *D. similis*, we applied the Random Forest (RF) model (Breiman, 2001) to analyze these variable factors. The main hyperparameters for RF include the number of trees (*n*tree) and the number of variables split at each tree node (*m*try). The optimal parameters were determined by a grid search using a five repeated 10-fold cross-validation method. The *m*try parameter was tuned from 1 to 5, and the *n*tree was tuned with 50, 100, 150, 200, 250, 300, 400, and 500. All the food variables (Seston C, N, P, C:P, C:N, N:P, and SC) were used in the RF model. The index of the mean decrease in mean square-error (% incMSE) was used to evaluate the importance of the variables (the greater the value, the greater the importance of the variable). After

important variables were found, partial dependence plots (Wright, 2018) were used to visualize the relationship between the important variables and the GR based on the best model.

We further studied the combined effects of food quality and quantity on the GR of *D. similis*. We selected the three food factors with high importance in the RF model and analyzed the effects of each two factors on the GR. Firstly, we used the K-mean clustering based on the GR value to divide all the 30 samples into two categories, namely high GR and low GR. Then, the minimum thresholds of SC (Min-SC) and seston P (Min-P) and the maximum thresholds of seston C:P (Max-C:P) were found based on the high GR range. If SC was greater than Min-SC, or seston C:P was less than Max-C:P, or seston P was greater than Min-P, and if two of these factors were met, the corresponding GR sample was classified as a zone, i.e., a "high GR zone" (HGZ); the rest of GR values we call those GR in the "low GR zone" (LGZ). In the next step, we presented the trends of GR under the influence of the double factors of seston C:P and SC, seston C:P and seston P, and seston P and SC using bubble chart visualization.

3. Results

3.1. The profile of lake nutrients and seston

The locations, sizes, depths, and levels of TP, TN, $\text{PO}_4\text{-P}$, $\text{NO}_3\text{-N}$ of each lake are shown in supplemental material Table S1. Table 1 presents the range, mean and standard deviation (SD) of concentrations of TP, TN, $\text{PO}_4\text{-P}$, $\text{NO}_3\text{-N}$ of the lake water. The trophic state of these lakes was between oligo-mesotrophic to hypereutrophic, there were a broad set of environmental variables in terms of nutrients.

The range, mean and SD of food seston C, N, P and C:P, C:N, N:P ratios and SC are shown in Table 1. The seston C:P had relatively low values, the median was 54.27 and the maximum was 138.22. Of all the lakes the highest seston N:P was 17.84. The range of SC was from 0.10 to 3.65 mg C/L. Seston C:P was negatively correlated with seston P ($r = 0.59$, $p < 0.001$) and not significantly correlated with seston C.

3.2. Phytoplankton compositions and biomass

The total PPB ranged from 0.12 to 38.37 mg/L, with a mean (SD) of 7.51 (8.77) mg/L. The biomass of these different algae in all lakes is shown in Fig. 1. The PPB classified in phyla in descending order of total biomass in the 30 lakes as Bacillariophyta, Cryptophyta, Chlorophyta, Euglenophyta, Pyrrophyta, and Chrysochyta (see supplemental material Fig. S1). The dominant species in the lakes were *Cryptomonas* sp. of Cryptophyte, and *Achnanthes* sp., *Synedra* sp., *Cyclotella* sp., *Aulacoseira* sp. of Bacillariophyta. The total PPB was significantly and positively correlated with lake TP ($r = 0.40$, $p < 0.05$), and not significantly correlated with lake TN, $\text{NO}_3\text{-N}$ and $\text{PO}_4\text{-P}$.

Table 1

The minimum, maximum, mean and standard deviation of lake water nutrients and seston.

Items	minimum	maximum	mean	standard deviation
Lake TN (mmol/L)	0.033	0.355	0.114	0.084
Lake TP ($\mu\text{mol/L}$)	0.743	39.259	4.521	7.377
Lake $\text{NO}_3\text{-N}$ (mmol/L)	0.002	0.263	0.047	0.063
Lake $\text{PO}_4\text{-P}$ ($\mu\text{mol/L}$)	0.006	0.817	0.095	0.153
Seston C (mmol/g)	4.246	16.464	8.090	3.148
Seston P (mmol/g)	0.046	0.475	0.158	0.094
Seston N (mmol/g)	0.614	3.130	1.361	0.689
Seston C:P	32.797	138.215	58.959	22.714
Seston C:N	4.559	8.793	6.241	0.940
Seston N:P	6.101	17.840	9.390	3.007
Seston concentration (mg C/L)	0.101	3.654	0.894	0.880

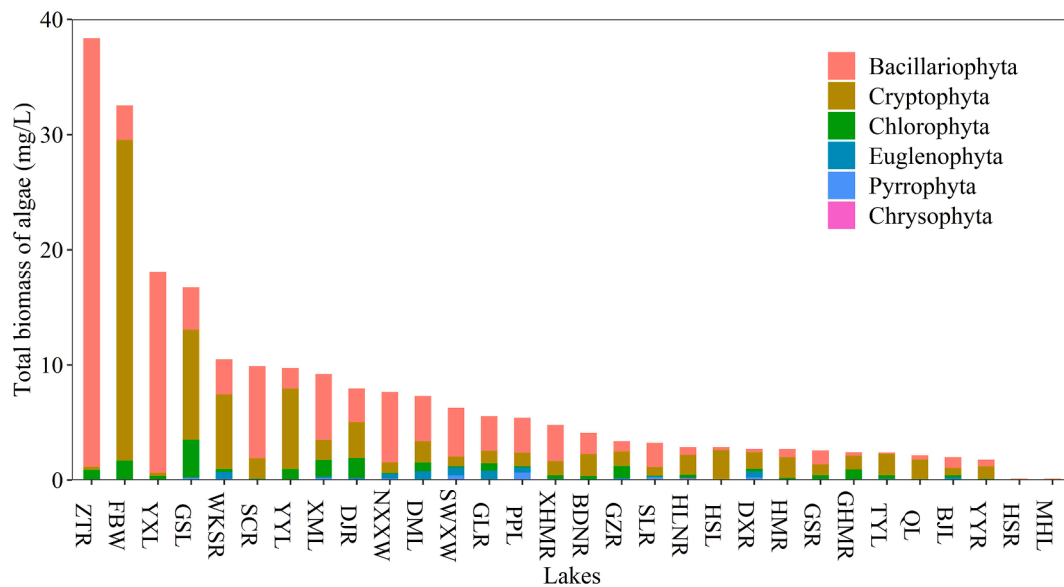


Fig. 1. Biomass of *Daphnia* edible phytoplankton (<75 μm) in natural seston. The corresponding lake abbreviations are listed in supplemental material Table S1.

3.3. Growth rates of *Daphnia similis*

The mean (SD) of GR from three replicates in each treatment ranged from 0.072 (0.022) to 0.263 (0.004) d^{-1} across the 30 lakes. The correlation analysis showed that the GR was not significantly correlated with seston C, N and seston C:N and N:P; GR was significantly and positively correlated with seston P and SC, and significantly and negatively correlated with seston C:P (Table 2). GR was significantly and positively correlated with the biomass of Cryptophytes ($r = 0.418, p < 0.05$). The relationship between GR and food factors is shown in supplemental material Fig. S2.

The result of RF model indicated that SC, seston C:P, and seston P had the highest %incMSE values, suggesting that they were the primary factors contributing to the GR among the food quality and quantity factors (Fig. 2).

The partial dependence plots displayed how the SC, seston C:P, and seston P affected the predicted GR in the RF model (Fig. 3). The GR showed a rising trend with the increase of SC and seston P. When SC < 0.56 mg C/L, the GR increased rapidly with the increasing SC, whereas it increased slower after SC greater than 0.56 mg C/L (Fig. 3a). The GR showed a downward trend with an increasing seston C:P. When C:P < 46.47, the GR decreased rapidly; between a C:P range of 46.67 and 71.62, the GR peaked at a C:P value of 59.04; and we observed a break point that the GR decreased sharply at a C:P of about 70; when the C:P >

71.62, GR remained at a low level (Fig. 3b). When seston P was less than 0.15 mmol/g, the GR increased rapidly with the increasing seston P, whereas it increased slowly after seston P was greater than 0.15 mmol/g (Fig. 3c).

3.4. The comprehensive effect of food quality and quantity on the growth rate of *Daphnia similis*

The result of the k-mean clustering of GR showed that the low GR ranged from 0.072 to 0.184 d^{-1} , and the high GR ranged from 0.219 to 0.263 d^{-1} . We found that within the high GR range, the value of Min-SC was 0.26 mg C/L, the value of Min-P was 0.11 mmol/g, and the value of Max-C:P was 69.8. The GR of *D. similis* was in the high GR zone when two of these three conditions were met: seston C:P ≤ 69.80 , SC ≥ 0.26 mg C/L, and seston P ≥ 0.11 mmol/g (HGZ, Fig. 4).

4. Discussion

Our results confirmed the importance of both natural food quality and quantity in determining the performance of zooplankton consumers. Among the food quality and quantity factors, SC, seston C:P, and seston P are the most important food factors affecting GR. The GR increased with the increase of seston P, and no inhibitory effect on GR was found for low seston C:P in food. The high quality and quantity of food can support the rapid growth of *Daphnia*. In addition, we found that the phytoplankton composition in seston also affected the GR of herbivore consumers, and the biomass of cryptophytes was significantly and positively correlated with GR.

Our results demonstrate that the GRH holds true under natural food conditions: the GR exhibited a positive correlation with seston P. We found that *D. similis* maintained a relatively high growth rate when the seston P was more than 0.11 mmol/g (Fig. 3c), which means that the higher seston P did not inhibit the growth of *Daphnia* within a certain range. This result is consistent with the findings of Sterner (1998), who observed that in natural temperate lakes with P-deficient food, the population of *Daphnia* exhibits low population recruitment. The study by Elser et al. (2001) showed that the P content in natural food is significantly related to the food quality, and that phosphate enrichment dramatically reduced the seston C:P and significantly stimulated growth of *Daphnia*. The seston P content did not hinder the growth of *D. similis* when it was as high as 0.48 mmol/g among subtropical lake seston.

With the increase of seston C:P, the GR declined (Sterner et al., 1993;

Table 2

Results of the Pearson correlation analysis between growth rate of *Daphnia similis* and food factors.

Growth rate	<i>r</i>	<i>p</i>
Seston C	0.191	0.311
Seston P	0.370	0.044
Seston N	0.222	0.239
Seston C:P	-0.377	0.040
Seston C:N	-0.205	0.276
Seston N:P	-0.338	0.068
Seston concentration	0.433	0.017
Biomass of Bacillariophyta	0.201	0.286
Biomass of Cryptophyta	0.418	0.021
Biomass of Chlorophyta	0.228	0.226
Biomass of Euglenophyta	0.225	0.369
Biomass of Pyrrophyta	0.259	0.393
Biomass of Chrysophyta	0.073	0.876
Total biomass of phytoplankton	0.294	0.115

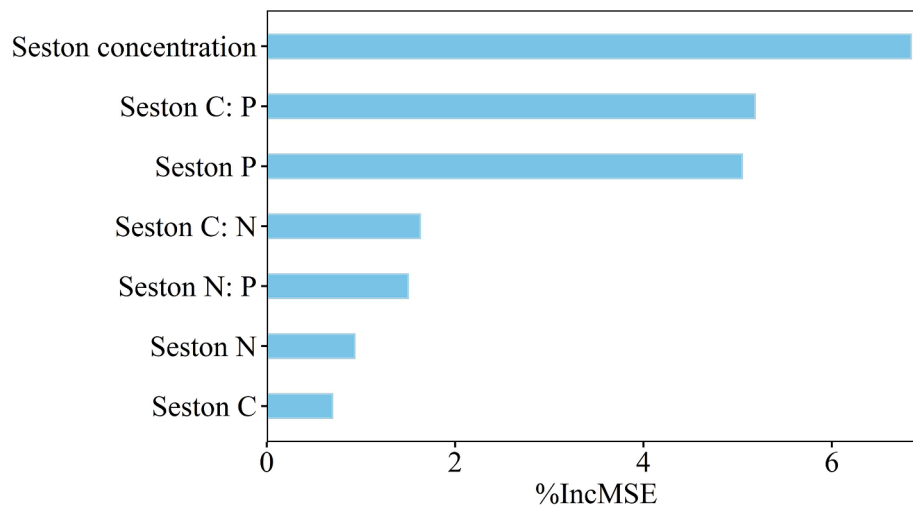


Fig. 2. The importance of the food factors for the growth rate of *Daphnia similis* in the Random Forest model denoted by the percentage increase of mean squared error (%IncMSE).

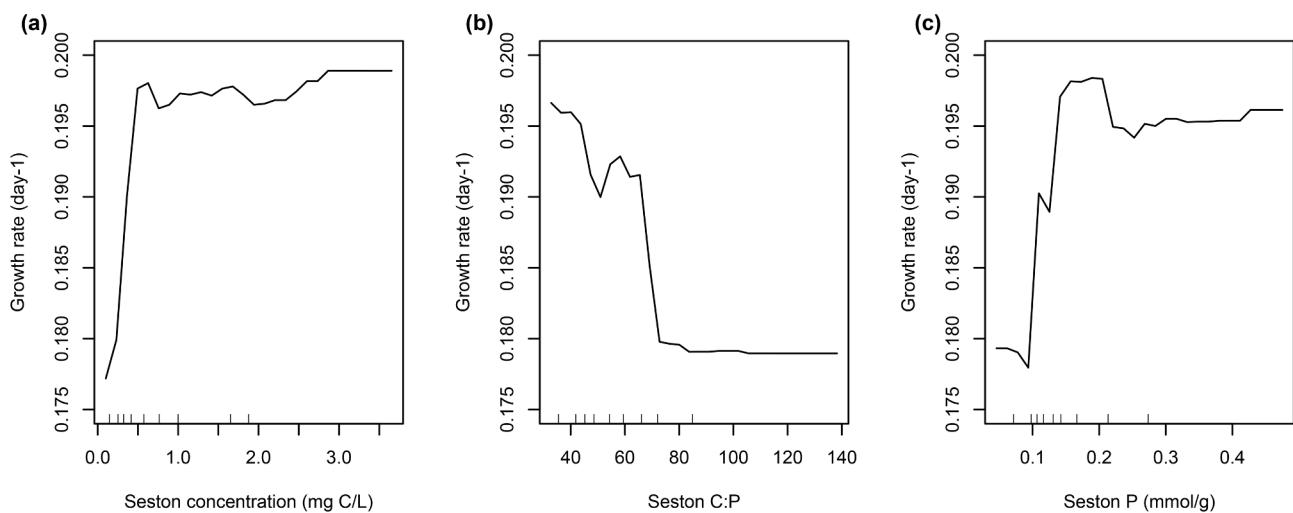


Fig. 3. Partial dependence plots of growth rate of *Daphnia similis* and food factors. Based on the Random Forest model, a changing trend of growth rate is predicted along with the range of seston concentration (a), seston C:P (b), and seston P (c). The vertical bars inside the x-axis represent the intervals between data points, with a vertical line marked for every third data along the sequence.

DeMott and Gulati, 1999; Hessen, 2006), our results also showed this tendency. Specifically, we observed a decreasing trend in GR at relatively low C:P ranges, with a rapid decline occurring at a C:P of approximately 70. High C:P can inhibit the growth of *Daphnia*, especially under the condition of higher C:P, e.g. 550–600 (DeMott and Gulati, 1999), owing to the insufficient P in the diet. However, excessive P can also hinder the growth of consumers. A study has showed that low food C:P (~60) can have deleterious impacts on *Daphnia magna* under field conditions (Currier and Elser, 2017), and studies focusing on algal food have also indicated that *Daphnia*'s growth rate was higher when the C:P ratio was 120 compared to ratios of 30 (Plath and Boersma, 2001) or 40 (Elser et al., 2016). In our study, we observed that *D. similis* growth rate was higher at very low seston C:P (<40) compared to relatively higher ratios (~100), and no knife-edge effect was observed, consistent with findings from other researches (DeMott and Gulati, 1999; Hessen, 2006). The difference in the impact of low food C:P on consumers can be attributed to three possible reasons: variations in the composition of natural food, the adaptation of the *D. similis* used in the experiment to eutrophic conditions, which allows it to cope better with very low C:P ratios, and the fact that this study focused on the lower range of seston C:P ratios.

The range of seston C:P had relatively low values of 32.80 to 138.22, and the median was 54.27 in our study. Food with a low C:P ratio of less than 40 was to be thought unlikely to occur in nature, and an analysis based on 281 small temperate lakes showed that the average seston C:P ratio is 192 (Sterner et al., 2008). As a result, the effects of high seston C:P ratio on growth of *Daphnia* are well established due to extensive prior work, whereas the impacts of low seston C:P ratio (P-rich food) have been studied to a much lower extent. The maximum of seston C:P was 138.22 in our studied eutrophic lakes, which was lower than the TER of derived from previous studies (Elser et al., 2016; Zhou and Declerck, 2019), i.e., previous studies have suggested that such high concentrations of P in food can be detrimental to the GR of consumers. Our work extends knowledge of the effects of low seston C:P ratio of natural foods on zooplankton growth. Even though the value of C:P was as low as 32.80, we did not observe a decrease in the GR of *D. similis*. The Max-C:P that can support high GR was 69.80. The threshold we determined for the optimal C:P ratio that could support high growth rates in *Daphnia* was 32.80 to 69.8, rather than 100 to 120 in previous studies (Plath and Boersma, 2001; Elser et al., 2016). Previous studies explained the inhibitory effect of low C:P as a result of inadequate carbon intake, with the concomitant need for *Daphnia* to excrete excess P, something that

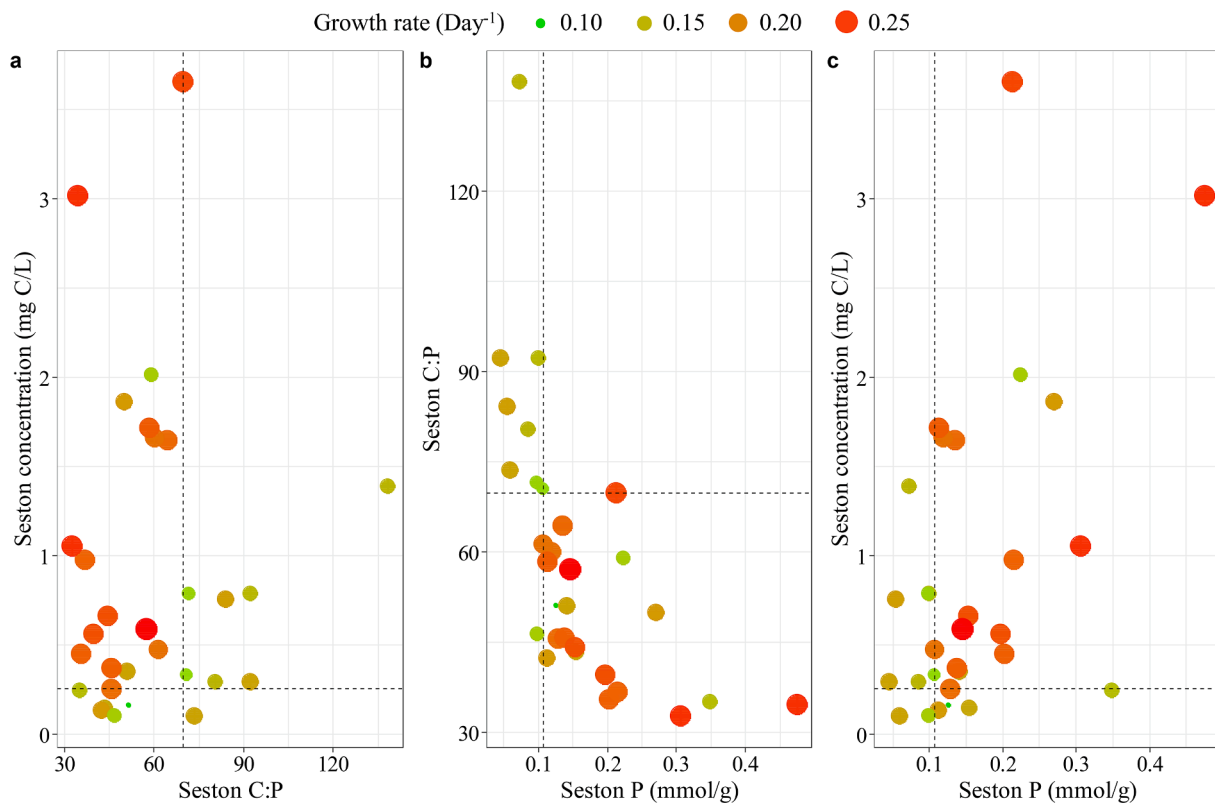


Fig. 4. Changes in growth rate under the interactive influence of two factors. a: seston concentration and seston C:P; b: seston C:P and seston P; c: seston concentration and seston P; “High GR Zone” (HGZ) was classified by two factors, and the dashed line indicates the classification boundary. HGZ appears at higher seston P (≥ 0.11 mmol/g) and seston concentration (≥ 0.26 mg C/L), and lower seston C:P (≤ 69.80). The shaded area indicates the HGZ.

most species were probably not adapted to do and which may therefore be costly (Plath and Boersma, 2001; Elser et al., 2016).

The effects of food quality and quantity on consumers are neither independent nor interchangeable (Bukovinszky et al., 2012). The GR of *D. similis* increases with increasing SC in our study, and this is consistent with the previous lab and field research (Urabe and Sterner, 2001; Zhang et al., 2009). In our study, where food quality had a significant effect on GR at high food concentrations, but when the SC was lower than 0.26 mg C/L, GR did not show a tendency with the change of the seston C:P or seston P. Our study showed that the promoting effect of SC appeared at the low seston C:P and high seston P. When C:P was between 32.80 and 69.80, *Daphnia* had higher GR with an increase in food concentration, which showed that the promoting effect of SC appeared at the high food quality. While in the case of low food quality, GR didn't change obviously with the change of SC. We could speculate that the threshold of the optimal C:P ratio of *Daphnia* food in natural water could be lower than the threshold concluded by a growth experiment using sole cultured food due to the overlay effect of lower food concentration.

Natural food has different quality and quantity characteristics that differ significantly from food with single or several algal species with modified stoichiometry. Firstly, the composition of natural food is complex, and various food should be considered for comparison to responses from the laboratory (Elser et al., 2016). Acharya et al. (2004) even suggested that the stoichiometric heterogeneity of food can favor *Daphnia* growth. For filter feeding zooplankton, their food is composed of various algae, bacteria, protozoa and detritus, which means the difference in nutritive value in spite of overall stoichiometry heterogeneity. Both bacteria with their high phosphorus content (Gliwicz, 1969; Hessen, 1990) and detritus from cyanobacteria (de Kluijver et al., 2012) serve as vital food resources for zooplankton in eutrophic lakes. Secondly, although the stoichiometric ratios of algae can be modified in controlled experiments, there is a seasonal succession of algae in natural

lakes. Changes in lake nutrients are more likely to have an effect on the composition of the algae than on the biochemical characteristics of the same algae species. We found that the GR and cryptophyte biomass were significantly and positively correlated. Compared to green algae, cryptophyte is a higher quality food for *Daphnia* owing to its high content of polyunsaturated fatty acids (Brett et al., 2006) and lower C:P (Taipale et al., 2014). A recent study revealed that cryptophytes, as effective bacterivores, can play a key role in the transfer of carbon from bacteria to a higher trophic level (Grujic et al., 2018). *Daphnia* has a higher growth rate fed cryptophytes than green algae or ciliates (Martin-Creuzburg et al., 2005; Taipale et al., 2014). Thus, a cryptophyte dominance in phytoplankton would benefit the growth of *Daphnia*. Our results suggest that any TER computed from laboratory studies may be biased towards higher values due to the difference between natural food and algae food. Using semi-natural conditions, we found that the optimal seston C:P was between 32.80 and 69.80, which can support high growth rates in *Daphnia*. At a seston C:P lower than break point of about 70, the growth rate of *Daphnia* remained at a low level (Fig. 3b).

In conclusion, our results support GRH when applied to a diet of natural food. High quantity and quality of food will promote the growth of herbivore consumers in eutrophic lakes. Seston concentration, seston P content, seston C:P and algal composition are good indicators for the growth of herbivore consumers in lakes. A lower seston C:P ratio (32.80 ~ 69.80) can support a higher growth rate in *Daphnia*. When food molar C:P exceeds 70, growth rate of *Daphnia* decreasing sharply due to the negative effects of P deficiency. Moreover, we observed no knife-edge effects, and as a result, lower seston C:P (< 40) had no inhibitory effects on consumers when fed a natural food diet. The observed discrepancies may be related to the fact that the excess P content of food may antagonistically interact or even overruled by other factors such as food composition and food quantity (e.g. in a multiple stressor perspective, Zhou and Wang, 2023). We could not exclude the

possibility that low food C:P may still show growth reductions under food with a lower C:P ratios. Therefore, future studies are needed to address those interactive effects, especially in water bodies that are highly influenced by human activities. Answers to these questions will provide valuable insights into the factors influencing the growth of herbivore consumers in lakes and help us better understand the implications of human-induced eutrophication on energy transfer within freshwater ecosystems.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ecolind.2023.111129>.

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