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Carbon Assimilation and Respiration of *Daphnia magna* with Varying Algal Food Quality

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ABSTRACT: To elucidate the mechanisms by which algal food quality affect *Daphnia* growths, we measured carbon incorporation rates and respiration rates of *Daphnia magna* with Cryptomonad *Rhodomonas minuta*, green algae *Scenedesmus acutus* and cyanobacteria *Synechococcus* sp. with varying physiological states as food. Carbon assimilation rates were high with *R. minuta* and *S. acutus* and low with *Synechococcus* sp. showing a similar pattern to the growth rate pattern. There was no clear difference among respiration rates of three algal species. Carbon assimilation rates and respiration rates of *D. magna* appeared to be independent on Molar C:P ratios in algal foods. Carbon growth efficiencies (incorporated carbon per assimilated carbon amount) were lower when *D. magna* fed with *Synechococcus* sp. than fed with *R. minuta* or *S. acutus*. Analysis of variance results show that carbon assimilation rates which were sum of incorporation and respiration rates and carbon growth efficiencies were only dependant on species affiliation. Overall, our results showed that algal species with varying ω3 polyunsaturated fatty acid content led different carbon incorporation rates and overall carbon assimilation rates of *D. magna*.

Key words: Carbon assimilation, *Daphnia*, Essential fatty acids, Incorporation, Respiration, Seston food quality

INTRODUCTION

The importance of algal food quality for zooplankton production and dynamics as well as overall ecosystems behavior has become increasingly recognized during the last ten years. While many researchers agree that algal food quality is a very important parameter, there is some discussion about what is the most likely determinant of algal food quality in freshwater pelagic food webs (Brett 1993, Hessen 1993, Urabe and Watanabe 1993, Müller-Navarra 1995a, Gulati and DeMott 1997, Sterner and Schulz 1998). The two most studied and debated hypotheses are the mineral phosphorus (P) (Hessen 1992, Urabe and Watanabe 1992, Urabe et al. 1997, DeMott 1998; see Sterner and Schulz 1998 for more references) and essential fatty acid (EFA) limitation hypotheses (Ahlgren et al. 1990, Müller-Navarra 1995 ab, Brett and Müller-Navarra 1997, Müller-Navarra et al. 2000, Müller-Navarra et al. 2004), although compelling evidence also exists for nitrogen (protein) limitation (Checkley 1985, Kilham et al. 1997) and digestion resistance hypotheses (Van Donk and Hessen 1993, 1995, Van Donk et al. 1997).

Most algal food quality studies have focused on *Daphnia* growth while few studies investigated the effects of algal food quality on carbon assimilation and respiration of zooplankton. Early studies show that different algal food leads different assimilation rates and assimilation efficiencies in *Daphnia* (Lampert 1977, Lampert 1987). Richman and Dodson (1983) report that high quality foods reduce respiration rates.

Many studies have focused on the effect of P limitation on the mechanisms of carbon uptake in *Daphnia* species. Van Donk and her colleagues (Van Donk and Hessen 1993, 1995, Van Donk et al. 1997) suggest that P-limited algae become digestion-resistant via thickening their cell walls. Their hypothesis predicts that P-limited algae would reduce carbon assimilation rate of *Daphnia*. DeMott (1988) shows that *Daphnia* deal with excessive carbon with P-limited food by reducing the net assimilation of C relative to P and suggested three possible mechanisms: 1) reduction of C assimilation across the gut wall, 2) storing excessive carbon as lipids or other C-rich compounds, and 3) increasing respiration or extracellular release of organic C-compounds. DeMott and his colleagues (DeMott et al. 1998) report that *Daphnia* could reduce C assimilation rates with P-saturated algae after acclimating *Daphnia* with P-defficient algae. However, these studies did not measure respiration of *Daphnia* in conjuction with sestion P content.

This study tried to elucidate the mechanisms by which algal food quality affect *Daphnia* growths. We measured carbon incorporation and respiration of *D. magna* with Cryptomonad *Rhodomonas minuta*, green algae *Scenedesmus acutus* and cyanobacteria *Synechococcus* sp. with varying physiological states as food.

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MATERIALS AND METHODS

Culture of Algae and *Daphnia*

Flow-through experiments were conducted using three algal species: *Rhodomonas minuta* Skuja**,** *Scenedesmus acutus* (Meyen) Chodat and *Synechococcus* sp. *Scenedesmus acutus* originated from the algal collection of the Max Planck Institute for Limnology in Plön, Germany while *Synechococcus* sp. was obtained from Dr. William DeMott at the Indiana-Purdue University, Indiana, USA. We chose these alga taxa because of their large differences in fatty acid, especially PUFA (which is defined as polyunsaturated fatty acid molecules with a chain length of 18 or more carbon atoms) composition (Table 1). HUFA are defined as a subset of PUFA molecules with 20 or more carbon atoms. All algal species were cultured using the synthetic medium L16 (Lindström 1983) modified with b_{12} and biotin vitamins (not for *S. acutus*) and earth extract. This medium is useful for culturing both algae and zooplankton since it has an ionic composition similar to that in many eutrophic lakes. P-and N-deficient algae were grown in batch culture for 4～7 days by leaving phosphorus and nitrogen out of the growth medium. The clone of *D. magna* used in these experiments was isolated from a small pond near the University of California at Davis, USA and cultured for several years on *S. acutus* in L16 medium at 20℃ with a 16L:8D h light cycle. Our results are comparable to those of the prior study using the same strains of algae that have been used in the previous study with similar objectives (Park et al. 2002).

Flow-through Experiments

We performed a series of flow-through experiments using the three algal species at four different algal physiological states (nutrient saturated, P-limited, N-limited and senescent), with *D. magna* as the test zooplankter. These treatments were intended to create a wide range of algal biochemical and mineral composition by manipulating the culture environment. Therefore, each algal food was an independent treatment and not a homogeneous replicate within a "treatment". To take into account any changes in algal fatty acid and elemental (C, N and P) composition during each experiment, we measured those parameters at the beginning and end. The experiments were performed in a flow-through culture system to keep the food concentration constant (Lampert et al. 1988). Zooplankters were maintained in 250 mL chambers suspended in a temperature controlled water bath (20 ± 0.5℃) placed in a temperature-controlled room (20 ± 0.5℃). These chambers received a constant food supply from stirred reservoirs (2-L Erlenmeyer flasks) with a multi-channel peristaltic pump. The flow rate for each chamber was kept at 1.44 L d^{-1}. Each chamber had a 243-μm mesh screen at the bottom so that algae (but not the daphniids) could pass through.

Table 1. Fatty acid (FA) profiles of three algal species: *Rhodomonas minuta* (*Rhodomonas*), *Scenedesmus acutus* (*Scenedesmus*), and *Synechococcus* sp. (*Synechococcus*). Fatty acid contents are shown in nmol mgC^{-1} (mean (SD)). ND: not detected, SAFA: saturated fatty acid, UFA: unsaturated fatty acid, ω3-PUFA: 18:3ω3 + 18:4ω3 + 20:5ω3 + 22:6ω3, ω6-PUFA: 18:2ω6 + 18:3ω6 + 20:2ω6 + 20:3ω6 + 20:4ω6, HUFA: 20:5ω3 + 22:6ω3

FA	*Rhodomonas* (n=18)	*Scenedesmus* (n=15)	*Synechococcus* (n=10)
13:0	0.28 (0.2)	0.62 (0.8)	0.09 (0.1)
14:0	16.60 (6.1)	7.81 (2.7)	21.68 (3.7)
15:0	2.98 (2.3)	1.68 (0.6)	2.01 (0.6)
16:0	121.23 (44.9)	142.55 (125.2)	90.69 (14.2)
16:1	6.79 (3.1)	5.31 (5.5)	35.27 (13.1)
17:0	4.54 (2.4)	2.70 (0.93)	4.10 (2.29)
18:0	84.31 (35.0)	84.43 (31.5)	108.5 (31.6)
18:1ω12/ω9	13.39 (9.1)	170.63 (279.9)	7.43 (4.8)
18:1ω7	5.64 (2.2)	6.76 (2.57)	6.63 (2.7)
18:2ω6	22.38 (16.0)	20.62 (27.0)	1.52 (4.0)
18:3ω6	0.66 (0.3)	0.68 (1.2)	0.13 (0.4)
19:0	1.57 (0.7)	1.43 (0.7)	1.27 (0.6)
18:3ω3	93.85 (62.2)	26.28 (32.0)	0.55 (0.6)
18:4ω3	58.43 (24.7)	4.97 (6.0)	1.03 (1.0)
20:0	1.35 (0.7)	1.41 (0.7)	1.37 (0.7)
20:1ω9	1.21 (1.4)	1.35 (1.4)	0.14 (0.1)
20:1ω7	0.51 (0.64)	1.11 (1.9)	0.08 (0.2)
20:2ω6	0.69 (0.63)	0.40 (0.2)	0.43 (0.4)
20:3ω6	ND	ND	0.17 (0.3)
20:4ω6*	ND	ND	ND
20:5ω3	57.25 (28.1)	2.27 (2.3)	0.22 (0.32)
20:1ω9*	ND	ND	ND
22:6ω3	7.01 (2.58)	2.40 (1.8)	0.90 (0.6)
FA	644.75 (215.7)	623.39 (481.2)	415.18 (65.2)
SAFA	228.19 (72.9)	242.63 (144.1)	229.76 (47.3)
UFA	416.56 (151.7)	380.76 (340.1)	185.42 (30.6)
PUFA	240.27 (127.3)	57.62 (67.7)	4.96 (3.04)
ω3-PUFA	216.54 (112.1)	35.91 (40.0)	2.71 (1.89)
ω6-PUFA	23.73 (16.4)	21.7 (28)	2.25 (1.6)
HUFA	64.26 (28.8)	4.68 (3.0)	1.12 (0.68)
ω3-PUFA(%)	33.59	5.76	0.65

* These fatty acids were detected in few samples but were so little that the averages of them were non-detectable levels.

Food concentrations were kept well above the incipient limiting level (ILL) and ranged between 0.5～1.5 mg C L^{-1}. Algal food concentration was set using the relationship between absorbance at 800 nm and previously determined dry weights of each algal species. The calibration curve between absorbance and dry weight was determined separately for each experiment. Each flow- through chamber received 6～8 four-day-old *D. magna* which were born no more than 12 h apart and were maintained on *S. acutus* before use in the experiments. An aliquot of 20～30 juveniles was used for the initial biomass determinations.

After 3 days (standard deviation: 44 min) in the flow-through system, *D. magna* were collected from the chambers and washed in L16 media, without algae, for at least 30 min. They were then dried at 60℃ for 48 hrs before weighing. The somatic instantaneous growth rate of *D. magna* (g day^{-1}) was calculated as the dry weight accrual during the experiment according to the following exponential equation:

$$g = [\mathrm{Ln}(Wt) - \mathrm{Ln}(W_0)] / t \quad (1)$$

where W_0 and Wt are the mean individual dry weights at the beginning and end of each experiment, respectively, and t is the duration of experiment in days. We measured *D. magna* weight to the nearest 1 μg with a Perkin Elmer AD-6 microbalance.

Carbon Assimilation and Respiration of *D. magna*

Carbon incorporation rates (I) of *D. magna* were measured as carbon stable isotope signature decrease in *D. magna* during the flow-through experiment. *D. magna* individuals were labeled with ^{13}C by feeding them with *S. acutus* grown in L16 media enriched with $NaH^{13}CO_3$ for 2 days, prior to the flow-through experiments. We measured the decrease rates of $\delta^{13}C$ in *D. magna* with different algal foods. The *D. magna* carbon incorporation rates were estimated by following simple mass balance equation.

$$\begin{aligned}\triangle^{13}C_{Daphnia} &= {}^{13}C_{\mathrm{inflow}} - {}^{13}C_{\mathrm{outflow}} \\ &= k \times C_{Daphnia} \times {}^{13}C/C_{\mathrm{seston}} - R \times C_{Daphnia} \times {}^{13}C/C_{Daphnia} \quad (2)\end{aligned}$$

where C stands for carbon content, inflow stands for ^{13}C inflow into *D. magna*, outflow stands for ^{13}C outflow from *D. magna*, k stands for carbon incorporation rate (μg C·μg C individual^{-1} day^{-1}), and R stands for respiration rate of *D. magna* (μg C·μgC individual^{-1} day^{-1}). For calculations of carbon incorporation rates of *D. magna*, we used an allometric relationship between *D. magna* biomass and its respiration rate (Peters 1987) to make carbon incorporation estimates independent of respiration measurements.

D. magna respiration rates were measured by the differences between dissolved oxygen in BOD bottles (volume: 300 mL) with and without *D. magna* using the Winkler method (Strickland and Parsons 1972). Respiration rates were measured twice per experiment using algae at the beginning and at the end of each experiment. Each algal food was filled into 3 BOD bottles and 2 neonates per bottle were put into 2 BOD bottles. One BOD bottle per each algal food was incubated without *D. magna*. All BOD bottles were incubated under no light condition in the temperature controlled room (20℃) for 6～12 hrs.

We estimated carbon assimilation rates from the sum of incorporated (I) and respired (R) carbon amount.

Analyses of Algal Biochemical and Elemental Composition

Two hundred and fifty mL of each algal food type was filtered onto a pre-combusted glass fibre filter (Whatman GF/C for *S. acutus*; Whatman GF/F for *Synechococcus* sp.). Filters with algae were kept at −80℃ until fatty acid extractions. We used 10 μL of 21:0 (1 mg mL^{-1}) as an internal standard that was added onto the freeze-dried filter immediately before extraction. Extraction and methylation were performed according to Kattner & Fricke (1986). Algal fatty acid composition was analyzed using a gas chromatograph (Hewlett Packard 6890). Individual fatty acid methyl esters (SIGMA) were dissolved into n-hexane and used as standards to determine retention times. Fatty acid quantities were calculated using the area ratios of a sample and the internal standard of known quantity. Response factors for the single fatty acids were tested with quantitative mixes and the deviation from the internal standard used (21:0) was found to be smaller than 5%. Since conversion of fatty acid molecules occurs on a stoichiometric basis rather than by weight, we expressed algal fatty acid concentrations in molar units. Particulate carbon and nitrogen content of seston was determined using a Perkin Elmer 2400 CHN Analyser. Algal particulate phosphorus content was analysed according to Solózano & Sharp (1980).

Stable Isotope Signature

Zooplankton samples were dried at 60℃ for at least 24 hours then packed into 8 mm× 5 mm tin capsules and analyzed for carbon isotope signatures using continuous flow isotope ratio mass spectrometry (IRMS) (20-20 mass spectrometer, Europa Scientific, Sandbach, United Kingdom). Sample combustion to CO_2 occurred at 1,000℃ in an inline elemental analyzer (Europa Scientific, ANCA-GSL). The gases were separated on a Carbosieve G column (Supelco, Bellefonte, PA, USA) before introduction to the IRMS. Isotopic ratio was expressed as a per mil (‰) deviation defined by the following equations:

$$\delta^{13}C = [(^{13}C/^{12}C)_{\mathrm{sample}} / (^{13}C/^{12}C)_{\mathrm{standard}} - 1] \times 1000$$

Standard material was analyzed approximately every 20 samples for quality control. Replicate variation was less than 3% and machine analytical error is within 0.2‰.

RESULTS

ω3 Polyunsaturated Fatty Acid and Carbon Assimilation/Respiration Rates

Carbon assimilation rates per individual were high with *R. minuta* and low with *Synechococcus* sp. while carbon assimilation rates of *D. magna* fed with *S. acutus* were slightly lower than *R. minuta* (Fig. 1A). Overall, carbon assimilation rates per daphniids saturated at ω3 polyunsaturated fatty acid content of approximately 40 μg FA mg C^{-1}. This carbon assimilation pattern was similar to the pattern of *D. magna* growth rate (Park et al. 2002). Respiration rates of *D. magna* did not show any clear pattern along with ω3 polyunsaturated fatty acid content (Fig. 1B). There was no clear difference among respiration rates of three algal species.

C:P Ratio and Carbon Assimilation/Respiration Rates

Carbon assimilation rates of *D. magna* appeared to be independent on Molar C:P ratios in algal foods (Fig. 2A). Also, respiration rates of *D. magna* appeared unchanged when fed with algal foods with molar C:P ratio higher than 300 (Fig. 2B).

Carbon growth efficiencies (increase of carbon per assimilated carbon amount) were lower when *D. magna* fed with *Synechococcus* sp. than *R. minuta* or *S. acutus* (Fig. 3A). *Daphnia magna* fed with *Rhodomonas minuta* and *S. acutus* showed similar carbon growth efficiencies. As molar C:P ratios in algal food increased, carbon growth efficiencies did not show any clear pattern although carbon growth efficiencies of *D. magna* fed with *Synechococcus* sp. appeared to decrease with higher C:P ratio foods (Fig. 3B).

Relative Contribution of Carbon Incorporation/Respiration Process on *D. magna* Growth

Analysis of variance results show that carbon incorporation rates of *D. magna* were dependent on both algal species and P content

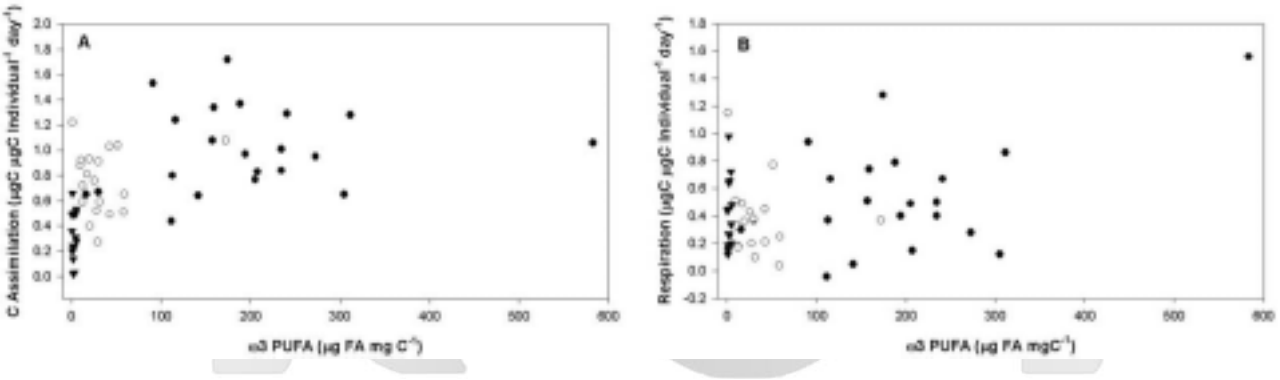

Fig. 1. Relationship between ω3 polyunsaturated fatty acid content and carbon assimilation rate (C Assimilation) (A) and respiration rate (Respiration) (B). Carbon assimilation rates were estimated from the sum of carbon incorporation rates and respiration rates. Each data point represents averages for the beginning and end of each experiment. ●: *Rhodomonas minuta*, ○: *Scenedesmus acutus*, ▼: *Synechococcus* sp.

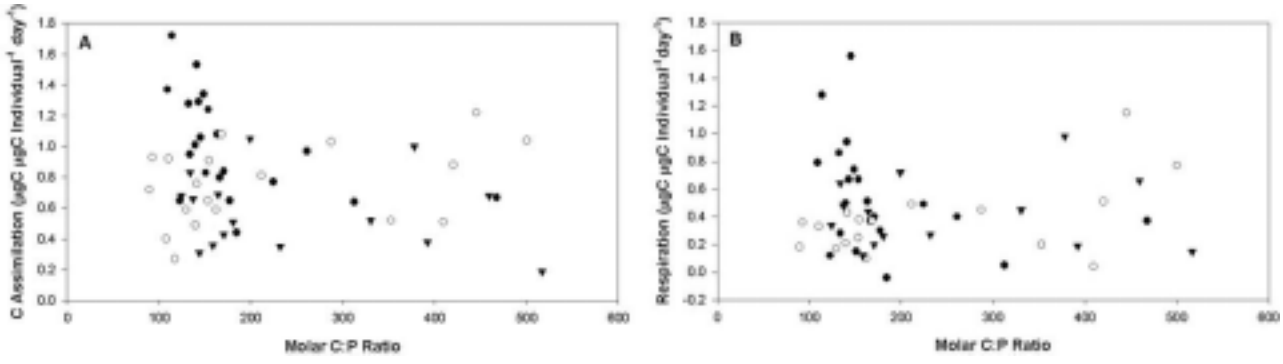

Fig. 2. Relationship between carbon to phosphorus (C:P) ratio and carbon assimilation rate (C Assimilation) (A) and respiration rate (Respiration) (B). Carbon assimilation rates were estimated from the sum of carbon incorporation rates and respiration rates. Each data point represents averages the beginning and end of each experiment. ●: *Rhodomonas minuta*, ○: *Scenedesmus acutus*, ▼: *Synechococcus* sp.

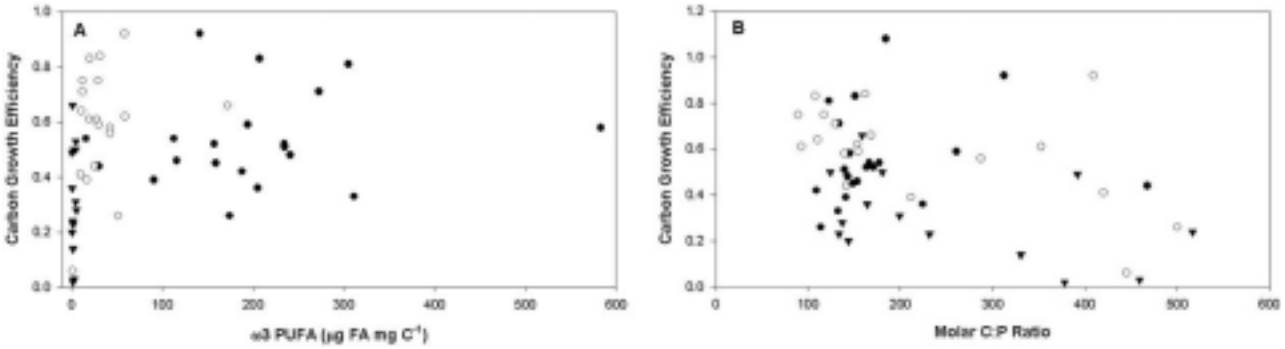

Fig. 3. Relationship between carbon growth efficiency (carbon incorporated per carbon assimilated) and (A) ω3 polyunsaturated fatty acid content and (B) carbon to phosphorus (C:P) ratio in algal foods. ●: *Rhodomonas minuta*, ○: *Scenedesmus acutus*, ▼: *Synechococcus* sp.

Table 2. ANOVA table for the effects of species affiliation and phosphorus on carbon incorporation rates, respiration rates, assimilation rates and carbon growth efficiencies of *Daphnia magna*

Independent variable	Source	SS	*F*	*p*	Variance explained (%)
C incorporation (*I*)	Species	0.934	65.41	0.0000	51.3
	P	0.182	12.77	0.0008	10.0
	Species × P	0.019	1.33	0.2542	1.0
	Error	0.686			37.7
Respiration (*R*)	Species	0.222	2.226	0.1419	4.0
	P	0.036	0.358	0.5523	0.6
	Species × P	0.271	2.722	0.1051	4.8
	Error	5.076			90.6
C assimilation (*I*+*R*)	Species	1.680	22.23	0.0000	29.6
	P	0.041	0.54	0.4646	0.7
	Species × P	0.099	1.31	0.2583	1.7
	Error	3.855			67.9
C Growth Efficiency (*Kc*=*I*/(*I*+*R*))	Species	0.411	10.30	0.0024	15.7
	P	0.100	2.50	0.1203	3.8
	Species × P	0.195	4.90	0.0316	0.2
	Error	1.915			45.6

(Table 2). Species difference explained 51% of the variation in carbon incorporation rates while P content did only 10%. As for respiration rates of *D. magna*, both species affiliation and P content did not explain with significance. The error explained 91% of the variation in respiration rates of *D. magna*. Carbon assimilation rates which were sum of incorporation and respiration rates and carbon growth efficiencies were only dependant on species affiliation.

DISCUSSION

Our results indicate that carbon assimilation rather than respiration is affected by algal food quality indices such as essential fatty acid content and P content. Traditional explanation has been that excess carbon in food with low P content (high C:P ratio over 300) would be lost in the form of carbon dioxide during the respiration and not incorporated in zooplankton body (Urabe and Watanabe 1992). However, we could not detect any trends in respiration of *D. magna* associated with species affiliation, ω3 polyunsaturated fatty acid content or C:P ratios. P content in algal foods explained only carbon incorporation rates of *D. magna* in our study.

Readers should note that carbon assimilation rates in our study were estimated based on carbon incorporation rates and respiration rates. Respiration rates were measured using Winkler method in the present study and the portion of variation in respiration not explained by species affiliation and P content was more than 90%, indicating that respiration measurements might be inaccurate. Increase of uncertainty in respiration measurements appears to have affected carbon assimilation and carbon growth efficiency patterns (Table 1).

Daphnia magna fed with *Synechococcus* sp. showed lower carbon assimilation rates, incorporation rates and carbon growth efficiencies than *S. acutus* and *R. minuta*, indicating that both assimilation and respiration affect growths of *D. magna* fed with *Synechococcus* sp. *Scenedesmus acutus* showed somewhat lower carbon assimilation rates than *Rhodomonas minuta*. However, *S. acutus* led *D. magna* to lower respiration than *R. minuta*, which made overall carbon incorporation of *D. magna* fed with *S. acutus* almost as much as *D. magna* fed with *R. minuta*.

Our study has relatively fewer observations on P limited algal foods (C:P ratios above 300) than P saturated ones, which might decrease the statistical power to detect the impacts of P content on carbon assimilation and carbon growth efficiency patterns.

Overall, our results showed that algal species with varying ω3 polyunsaturated fatty acid content led different carbon incorporation

and overall carbon assimilation rates of *D. magna*. To examine *D. magna* feeding physiology, we need further investigations on algal P content and respiration processes.

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