Good food versus bad food: the role of sterols and polyunsaturated fatty acids in determining growth and reproduction of *Daphnia magna*

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Abstract The absence of dietary sterols and polyunsaturated fatty acids (PUFAs) has been shown to affect the performance of the freshwater herbivore *Daphnia*. Here, we compared somatic growth rates and clutch sizes of *Daphnia magna* reared on a diet of low food quality (*Synechococcus elongatus*) and of high food quality (*Cryptomonas* sp.) and investigated if and to what extent the absence of sterols or PUFAs in the cyanobacterium *S. elongatus* accounts for the observed differences in food quality. The supplementation of *S. elongatus* with cell-free lipid extracts (fatty acids, sterols, total lipids) obtained from the flagellate *Cryptomonas* sp. suggested that the superior food quality of *Cryptomonas* sp. is predominantly, but not completely, a combined effect of its sterol and PUFA composition. Our laboratory study suggests that somatic growth of *D. magna* feeding on *S. elongatus* is primarily constrained by the absence of sterols, whereas egg production is primarily limited by the absence of long chain PUFAs.

Keywords Sterols · Polyunsaturated fatty acids · Liposomes · Growth · Reproduction

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Introduction

Cladocerans of the genus *Daphnia* are a key component of freshwater food webs. Due to their high grazing activity on the phytoplankton they are significantly involved in transferring mass and energy (expressed as carbon) from primary producers to higher trophic levels (e.g., Leibold 1989; Carpenter et al. 2001). However, the carbon transfer efficiency across the phytoplankton *Daphnia* interface is often constrained by the predominance of nutritionally inadequate phytoplankton. Nutritional inadequacy can be attributed to morphological properties that hamper ingestion or digestion (e.g., filaments or colony formation, digestion-resistant cell walls), toxicity, and/or to a deficiency in elemental (mostly phosphorus) or biochemical nutrients (Sturner and Elser 2002; Wilson et al. 2006; Martin-Creuzburg et al. 2008).

In the recent years, food-quality research has increasingly focused on the biochemical nutrient requirements of the major zooplankton taxa. In particular, a dietary deficiency in essential lipids, i.e., polyunsaturated fatty acids (PUFAs) and sterols, has been shown to affect the performance of crustacean grazers (Müller-Navarra et al. 2000; Von Elert 2002; Ravet et al. 2003; Martin-Creuzburg et al. 2005a). Crustaceans, like all arthropods, are incapable of synthesizing long chain PUFAs and sterols de novo, and therefore require a dietary source of these lipids to satisfy their physiological demands (Harrison 1990; Grieneisen 1994). PUFAs and sterols are both

integral parts of cell membranes and both serve as precursors of many bioactive molecules. The long chain PUFAs, arachidonic acid (ARA; 20:4 n –6) and eicosapentaenoic acid (EPA; 20:5 n –3), for instance, are precursors of prostaglandins which, among other things, may have several functions in arthropod reproduction (Harrison 1990; Stanley 2006). Sterols, on the other hand, are precursors of steroid hormones, such as ecdysteroids, which are involved in the process of molting (Grieneisen 1994; Martin-Creuzburg et al. 2007).

In previous studies, we supplemented the sterol- and PUFA-free picocyanobacterium *Synechococcus elongatus* with single purified sterols and demonstrated that the well-known nutritional inadequacy of this picocyanobacterium is primarily due to the absence of sterols, i.e., somatic growth of daphnids was significantly improved by sterol supplementation (Martin-Creuzburg and Von Elert 2004; Martin-Creuzburg et al. 2005a, b). The absence of PUFAs in *S. elongatus* was found to be of minor importance for somatic growth but may potentially affect egg production in *Daphnia*, as indicated by increased clutch sizes when a *S. elongatus* diet was supplemented with cholesterol and EPA simultaneously (Martin-Creuzburg et al. 2008).

During the present study, we conducted standardized growth experiments with *Daphnia magna* feeding on *S. elongatus* and the flagellate *Cryptomonas* sp., which is ranked as superior food for *Daphnia* (Weers and Gulati 1997; Martin-Creuzburg et al. 2005b, 2008). In contrast to the previous studies described above, we supplemented *S. elongatus* with lipid enriched liposomes prepared from different lipid extracts derived from *Cryptomonas* sp. (fatty acid extract, sterol extract, total lipid extract), to investigate the effect of naturally occurring mixtures of sterols and fatty acids on growth and reproduction of *D. magna*.

Materials and methods

Cultivation of the food organisms

The green alga *Scenedesmus obliquus* (SAG 276-3a) was used as food for the stock cultures of the daphnids. It was grown in batch cultures in Cyano medium (Jüttner et al. 1983) and harvested in the late-

exponential growth phase. For the growth experiments, *Synechococcus elongatus* (SAG 89.79) was cultured semi-continuously in Cyano medium (20°C; illumination at 80 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$) at a dilution rate of 0.25 day^{-1} in aerated 5-l vessels. *Cryptomonas* sp. (SAG 26.80) was grown in modified WC medium with vitamins (Guillard 1975) at 20°C with an illumination at 120 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$. Carbon concentrations of the autotrophic food suspensions were estimated from photometric light extinctions (800 nm) and from previously established regressions between carbon and light extinction determined in dilution series of each food suspension.

Preparation of lipid extracts of *Cryptomonas* sp. (total lipids, fatty acids, sterols)

Three pre-combusted GF/F filters (Whatman, 47-mm diameter, particle retention 0.7 μm) were each loaded with 20 mg particulate organic carbon (POC), i.e., three filters with 20 mg POC, one for each extract. Lipids were extracted from the filters three times using a mixture of dichloromethane/methanol (2:1, v/v). The pooled cell-free extracts were evaporated with nitrogen to dryness and resuspended in 4 ml ethanol (three total lipid extracts). The sterol extract was prepared from one total lipid extract by saponification with 0.2 mol l^{-1} methanolic KOH (70°C, 1 h) and partitioning of the sterols into *iso*-hexan:diethyl ether (9:1, v/v). The fatty acid extract was prepared from another total lipid extract after saponification and partitioning of neutral lipids into *iso*-hexan:diethyl ether as described above and by acidification (HCL, 30%, pH < 2) of the residual KOH fraction and extraction of the free fatty acids with diethyl ether. Subsequently, the lipid extracts (total lipids, fatty acids, sterols) were evaporated to dryness using a rotary evaporator and dissolved in 4 ml of chloroform.

Liposome preparation

Substantially, liposome stock suspensions were prepared by the method described by Martin-Creuzburg et al. (2008). Instead of single purified lipids, however, the lipid extracts of *Cryptomonas* sp. were used for preparation. Briefly, 3 mg of 1-palmitoyl-2-oleoyl-phosphatidylglycerol (POPG) and 7 mg of 1-palmitoyl-2-oleoyl-phosphatidylcholin (POPC, Lipoid, Germany) were dissolved in an aliquot of chloroform. Sterol or

PUFA containing liposomes were prepared by adding the total lipid extract, the sterol extract or the fatty acid extract of *Cryptomonas* sp., respectively. The resulting suspensions were dried using a rotary evaporator, dissolved in 10 ml buffer (20 mmol l⁻¹ NaP_i, 150 mmol l⁻¹ NaCl, pH 7) and incubated in a rotary shaker (100 revolutions min⁻¹) for 30 min. Subsequently, the liposome suspensions were sonicated in an ultrasonic bath, and excess free lipids were removed by washing the liposomes in fresh buffer using an ultra-speed centrifuge (150,000×g, 90 min, 4°C). The liposome stock suspensions were stored at -20°C. Prior to the addition of liposomes to the experimental beakers, the liposome stock suspensions were again sonicated (2 min). Finally, 300 µl of a liposome stock suspension ($\sim 1 \times 10^6$ liposomes ml⁻¹, mean diameter ~ 4.8 µm) was used as a food supplement in each beaker.

Analyses

For the analysis of fatty acids and sterols, ca. 0.5 mg particulate organic carbon (POC) of the food suspensions was filtered separately onto precombusted GF/F filters (Whatman, 25 mm). The total lipids were extracted three times from filters with dichloromethane/methanol (2:1, v/v), and the pooled cell-free extracts were evaporated to dryness with nitrogen. The fatty acid and sterol content of the liposomes was analyzed directly in 100 µl of the liposome stock suspensions. The lipid extracts were transesterified with 3 mol l⁻¹ methanolic HCL (60°C, 15 min) for the analysis of fatty acids or saponified with 0.2 mol l⁻¹ methanolic KOH (70°C, 1 h) for the analysis of sterols. Subsequently, fatty acid methyl esters (FAMES) were extracted three times with 2 ml of *iso*-hexan; the neutral lipids were partitioned into *iso*-hexan:diethyl ether (9:1, v/v). The lipid containing fraction was evaporated to dryness under nitrogen and resuspended in 10–20 µl *iso*-hexan. Lipids were analyzed by gas chromatography on a HP 6890 GC equipped with a flame ionization detector and a DB-225 (J&W Scientific) capillary column to analyze FAMES or with a HP-5 (Agilent) capillary column to analyze sterols. Details of GC configurations are given elsewhere (Von Elert 2002 for fatty acids; Martin-Creuzburg and Von Elert 2004 for sterols). Lipids were quantified by comparing with internal standards (C17:0 ME and C23:0 ME; 5 α -Cholestan) and

identified by their retention times and their mass spectra, which were recorded with a gas chromatograph-mass spectrometer (Finnigan MAT GCQ) equipped with a fused-silica capillary column (DB-225MS, J&W for FAMES; DB-5MS, Agilent for sterols). Sterols were analyzed in their free form and as their trimethylsilyl derivatives. Spectra were recorded between 50 and 600 atomic mass units (amu) in the EI ionization mode. The detection limit was 20 ng of fatty acid or sterol. The absolute amount of each lipid was related to the POC, which was determined using a NCS-2500 analyzer (ThermoQuest GmbH, Egelsbach, Germany).

Daphnia growth experiment

Growth experiments were conducted with third-clutch juveniles (born within 8 h) of a clone of *Daphnia magna* (originally isolated from Großer Binnensee, Lampert 1991). Stock cultures of *Daphnia* were raised in filtered lake water obtained from Lake Constance (0.2 µm pore-sized membrane filter) and saturating concentrations of *S. obliquus*. The growth experiments were carried out at 20°C in glass beakers filled with 200 ml of filtered (<0.2 µm) lake water supplemented with 2 mg C l⁻¹ of algal or cyanobacterial carbon and the prepared liposomes. The treatments were as follows: *S. elongatus*, *S. elongatus* supplemented with either control liposomes or liposomes prepared with the different lipid extracts (fatty acid, sterols, total lipids), and *Cryptomonas* sp. Each treatment consisted of three beakers (replicates) with six *D. magna* per beaker. Animals were transferred daily into new beakers with freshly prepared food suspensions. The experimental animals were subsampled at the beginning and at the end of an experiment, dried for 24 h, and weighed on an electronic balance (Mettler UMT 2; ± 0.1 µg). Growth rates (*g*) were determined as the increase in dry mass from day 0 (*M*₀) to day 6 (*M*₆) of the experimental period using the equation: $g = (\ln M_6 - \ln M_0)t^{-1}$. Eggs were counted in the brood chamber of each female at the end of an experiment using a dissecting microscope.

Data analysis

The somatic growth rates and clutch sizes of *Daphnia magna* were analyzed by one-way analyses of variance

(ANOVA). ANOVAs were carried out using the General Linear Model module of STATISTICA 6.0 (StatSoft Inc., Tulsa, Okla., USA). Raw data met the assumption of homogeneity of variance; treatment effects were tested by Tukey's HSD post hoc tests.

Results

Lipid analyses

The lipid composition of *Synechococcus elongatus* was characterized by high amounts of short-chain saturated and monounsaturated fatty acid, and by the absence of PUFAs and sterols (Table 1). In contrast, *Cryptomonas* sp. contained high amounts of $n-3$ PUFAs, such as 18:3 $n-3$, 18:4 $n-3$, and 20:5 $n-3$. Stigmasterol (24-ethylcholesta-5,22-dien-3 β -ol) and epibrassicasterol (24-methylcholesta-5,22-dien-3 β -ol) were the principal sterols found in *Cryptomonas* sp. (Table 1). Epibrassicasterol, the 24 α -epimer of brassicasterol, has been previously found in cryptophycean

algae, such as *Cryptomonas* sp. and *Rhodomonas* sp. (Goad 1981; Gladu et al. 1990). Although we did not determine the stereochemistry of the side chain at C-24, a 24 α -configuration was also assumed.

The prepared liposomes contained significant amounts of palmitic acid (C16:0) and oleic acid (C18:1 $n-9$), which are both components of the phospholipids POPG and POPC (Table 2). In addition, liposomes prepared in the presence of the total lipid extract of *Cryptomonas* sp. contained high amounts of all major fatty acids of this flagellate (in particular 18:3 $n-3$, 18:4 $n-3$, and 20:5 $n-3$) together with the two sterols epibrassicasterol and stigmasterol. Liposomes prepared from the fatty acid or sterol extract of *Cryptomonas* sp. contained considerably lower amounts of fatty acids but hardly differed in their fatty acid composition. Sterols, however, were absent in liposomes prepared from the fatty acid extract of *Cryptomonas* sp. (Table 2).

Daphnia growth experiment

Growth of *Daphnia magna* on unsupplemented *S. elongatus* was poor (Fig. 1). Supplementation of *S. elongatus* with control liposomes or liposomes prepared with the fatty acid extract of *Cryptomonas* sp. did not affect somatic growth rates of *D. magna*. Growth was significantly improved, however, when *S. elongatus* was supplemented with liposomes prepared with the sterol extract of *Cryptomonas* sp. (Tukey's HSD, $P < 0.05$; ANOVA, $F_{5,12} = 3,499$, $P < 0.001$; Fig. 1). Supplementation of *S. elongatus* with liposomes prepared from the total lipid extract of *Cryptomonas* sp. did not further improve growth of *D. magna*, but led to significantly increased clutch sizes compared to supplementation with the sterol extract (Tukey's HSD, $P < 0.05$; ANOVA, $F_{2,6} = 147$, $P < 0.001$). *D. magna* did not produce eggs when grown on unsupplemented *S. elongatus* or *S. elongatus* supplemented with control liposomes or liposomes prepared with a fatty acid extract of the flagellate. Growth rates and clutch sizes of *D. magna* were highest when grown on *Cryptomonas* sp. (Fig. 1).

Discussion

We compared somatic growth rates and clutch sizes of *D. magna* reared on a diet of low (*S. elongatus*)

Table 1 Fatty acid and sterol content of *Synechococcus elongatus* and *Cryptomonas* sp.

	<i>Synechococcus elongatus</i> ($\mu\text{g mg C}^{-1}$)	<i>Cryptomonas</i> sp. ($\mu\text{g mg C}^{-1}$)
C16:0	22.13 $\pm$ 0.41	20.41 $\pm$ 0.98
C16:1 n 7	40.89 $\pm$ 0.12	5.48 $\pm$ 0.64
C17:1 n 7	0.62 $\pm$ 0.08	0.94 $\pm$ 0.04
C18:0	4.36 $\pm$ 0.68	4.13 $\pm$ 0.18
C18:1 n 9	1.44 $\pm$ 0.11	4.32 $\pm$ 0.21
C18:1 n 7	2.29 $\pm$ 0.17	8.91 $\pm$ 0.28
C18:2 n 6	ND	18.68 $\pm$ 0.22
C18:3 n 6	ND	0.89 $\pm$ 0.06
C18:3 n 3	ND	74.96 $\pm$ 0.58
C18:4 n 3	ND	39.24 $\pm$ 0.51
C20:1 n 9	ND	0.52 $\pm$ 0.09
C20:4 n 6	ND	0.60 $\pm$ 0.18
C20:3 n 3	ND	0.96 $\pm$ 0.01
C20:5 n 3	ND	34.48 $\pm$ 0.20
C22:6 n 3	ND	4.28 $\pm$ 0.16
(n 3)/(n 6)	ND	7.63
Epibrassicasterol	ND	2.43 $\pm$ 0.24
Stigmasterol	ND	3.81 $\pm$ 0.17

Data are means of three replicates $\pm$ SD

ND not detectable

Table 2 Fatty acid and sterol content of the liposomes prepared from lipid extracts of *Cryptomonas* sp. (fatty acids, sterols, total lipids)

	Liposomes (control) [μg]	Liposomes + fatty acids [μg]	Liposomes + sterols [μg]	Liposomes + total lipids [μg]
C16:0	108.34 $\pm$ 3.64	83.63 $\pm$ 0.47	55.85 $\pm$ 0.60	81.44 $\pm$ 1.43
C16:1n 7	ND	0.95 $\pm$ 0.09	0.73 $\pm$ 0.01	2.30 $\pm$ 0.03
C17:1n 7	ND	ND	ND	ND
C18:0	ND	3.10 $\pm$ 0.05	2.44 $\pm$ 0.02	3.13 $\pm$ 0.03
C18:1n 9	101.23 $\pm$ 1.88	70.90 $\pm$ 1.78	60.58 $\pm$ 0.51	67.93 $\pm$ 2.33
C18:1n 7	ND	0.65 $\pm$ 0.01	0.71 $\pm$ 0.00	1.64 $\pm$ 0.06
C18:2n 6	ND	2.82 $\pm$ 0.05	2.68 $\pm$ 0.00	7.65 $\pm$ 0.17
C18:3n 6	ND	ND	ND	ND
C18:3n 3	ND	15.78 $\pm$ 0.21	14.40 $\pm$ 0.01	50.24 $\pm$ 0.74
C18:4n 3	ND	13.23 $\pm$ 0.13	11.91 $\pm$ 0.01	45.61 $\pm$ 0.86
C20:1n 9	ND	ND	ND	ND
C20:4n 6	ND	ND	ND	ND
C20:3n 3	ND	ND	ND	ND
C20:5n 3	ND	10.36 $\pm$ 0.30	10.01 $\pm$ 0.00	33.48 $\pm$ 0.93
C22:6n 3	ND	1.40 $\pm$ 0.20	1.06 $\pm$ 0.01	2.28 $\pm$ 0.12
(n 3)/(n 6)	ND	14.41	13.95	17.20
Epibrassicasterol	ND	ND	2.34 $\pm$ 0.52	3.63 $\pm$ 0.53
Stigmasterol	ND	ND	5.04 $\pm$ 1.28	8.05 $\pm$ 1.43

Data indicate the amount that was supplied daily to *D. magna* with the different liposome suspensions (300 μl in 200 ml lake water, containing 2 mg C l⁻¹ of *S. elongatus*). Data are means of three replicates $\pm$ SD

ND not detectable

Fig. 1 Juvenile somatic growth rates of *Daphnia magna* grown on *Synechococcus elongatus* and on *S. elongatus* supplemented with liposomes prepared from lipid extracts of *Cryptomonas* sp. (fatty acids, sterols, total lipids). Growth on *Cryptomonas* sp. is given for reference. Data are means of three replicates per treatment; error bars indicate SD. Bars labeled with the same letters are not significantly different (Tukey's HSD, $P < 0.05$ following ANOVA)

and of high (*Cryptomonas* sp.) food quality. By supplementation of the cyanobacterium *S. elongatus* with cell-free lipid extracts (fatty acids, sterols, total lipids) obtained from the flagellate *Cryptomonas* sp., we analyzed if and to what extent the absence of certain fatty acids and sterols in *S. elongatus* accounts for the observed differences in food quality. In previous studies, the supplementation of *S. elongatus* with single PUFAs (Martin-Creuzburg et al. 2008) or with deliberate mixtures of PUFAs (Von Elert and Wolffrom 2001; Von Elert et al. 2003) failed to improve the growth of *Daphnia* sp., which has been interpreted to indicate that the low quality of *S. elongatus* was not due to the absence of these PUFAs. Here, to exclude that the PUFAs previously supplemented to *S. elongatus* were inadequate to support *Daphnia* sp. growth, we used a natural mixture of PUFAs from a high quality food organism (*Cryptomonas* sp.) for supplementation. The supplementation of *S. elongatus* with the fatty acid extract of *Cryptomonas* sp., containing all major fatty acids

of the flagellate, including the $n-3$ PUFAs 18:3 $n-3$, 18:4 $n-3$, 20:5 $n-3$, and 22:6 $n-3$, did not improve somatic growth of *D. magna*, indicating that the absence of PUFAs in *S. elongatus* is not the predominant food quality constraint.

In contrast to the supplementation with the fatty acid extract, the supplementation of *S. elongatus* with the sterol extract of *Cryptomonas* sp., containing stigmasterol and epibrassicasterol as the major sterols, significantly improved somatic growth of *D. magna*. Thus, our study clearly shows a prevailing sterol limitation of *D. magna* feeding on the cyanobacterium *S. elongatus*. Such a primary sterol limitation of *Daphnia* sp. on *S. elongatus* has also been demonstrated during our previous studies (cp. Von Elert et al. 2003; Martin-Creuzburg and Von Elert 2004; Martin-Creuzburg et al. 2005a, 2008) by supplementing single purified sterols, mostly cholesterol. However, cholesterol is the end-product of sterol assimilation in *Daphnia* sp., and it has not been tested before whether naturally occurring mixtures of sterols from autotrophic phytoplankton can reduce sterol limitation in *Daphnia* sp., by serving as precursors for the synthesis of cholesterol (cp. Martin-Creuzburg and Von Elert 2004). In this respect, our data suggest that at least one of the two principal sterols present in *Cryptomonas* sp. (stigmasterol, epibrassicasterol) can be converted into cholesterol, which had released *D. magna* from sterol limitation.

The supplementation of *S. elongatus* with the total lipid extract of *Cryptomonas* sp., containing PUFAs and sterols, did not increase the growth rate of *D. magna* beyond 0.4 day⁻¹. This implies that 0.4 day⁻¹ is the maximum growth rate that can be obtained by lipid supplementation of the cyanobacterium *S. elongatus*. Interestingly, a similar somatic growth rate was observed by simultaneously supplementing the cyanobacterium with cholesterol and EPA (Martin-Creuzburg et al. 2008), which suggests that the food quality of the cyanobacterium is constrained only by cholesterol and EPA (and potential precursors) but not by additional lipids. However, the addition of the total lipid extract of *Cryptomonas* sp., to the *S. elongatus* food suspension led to significantly increased clutch sizes compared with the addition of the sterol extract. The liposomes prepared from the total lipid extract of *Cryptomonas* sp. contained significantly more PUFAs, in particular

18:3 $n-3$, 20:5 $n-3$, and 22:6 $n-3$, than those prepared from the sterol extract, implying that the availability of dietary $n-3$ PUFAs enhances egg production in *D. magna*. This is in good agreement with the finding that *D. magna* allocates dietary 20:5 $n-3$ (EPA) preferentially into the eggs (Becker and Boersma 2005; Wacker and Martin-Creuzburg 2007), and the implication that EPA plays a crucial role in *Daphnia* reproduction (Martin-Creuzburg et al. 2008). Notably, however, the threshold levels for saturation with 18:3 $n-3$ and 20:5 $n-3$ are presumably rather low (Müller-Navarra 1995; Wacker and Von Elert 2001; Becker and Boersma 2005; Gladyshev et al. 2008). Hence, the fatty acid requirements of *D. magna* might have been satisfied already by the fatty acids present in the sterol extract, suggesting that the significant increase in clutch sizes obtained by the addition of the total lipid extract is due to other unknown lipids or lipid-like compounds present in *Cryptomonas* sp. and absent in *S. elongatus*.

Although the addition of sterols and total lipids (sterols included) improved the food quality of *S. elongatus* for *D. magna*, growth rates observed on the supplemented diets were much lower than those achieved on a sole diet of *Cryptomonas* sp. (Fig. 1). *Cryptomonas* sp. contained considerably smaller amounts of sterols and $n-3$ PUFAs (18:3 $n-3$, 18:4 $n-3$, 20:5 $n-3$, 22:6 $n-3$) than were provided with the liposomes prepared with the total lipid extract, which suggests that *D. magna* was sufficiently supplied with sterols and $n-3$ PUFAs. In addition to $n-3$ PUFAs, *Cryptomonas* sp. contained small amounts of the $n-6$ PUFA 20:4 $n-6$ (ARA), which was absent in the liposome supplemented diets. Becker and Boersma (2005) reported that even small amounts of ARA supplemented to an ARA-free diet significantly improved the growth of *D. magna*. Moreover, ARA is a precursor of prostaglandins, which have been shown to be important in the reproduction of arthropods (Harrison 1990; Stanley 2006). Thus, even the minute amounts of ARA present in *Cryptomonas* sp. may have contributed to the high food quality of this flagellate. It is also possible that the fitness of daphnids feeding on *Cryptomonas* sp. is positively affected by the $n-3/n-6$ ratio of PUFAs. Likewise, the ratio of saturated to unsaturated fatty acids may be important, because saturated fatty acids provided in excess (such as C16:0 in the liposome

supplemented diets) may have detrimental effects on daphnids (Wacker and Martin-Creuzburg 2007). It should also be noted that liposomes are a considerable source of carbon and phosphorus and that a supplementation with liposomes will provide a grazer with extra energy (Martin-Creuzburg et al. 2008). However, because the supplementation with control liposomes (prepared without lipids from *Cryptomonas* sp.) and with liposomes prepared from the fatty acid extract of *Cryptomonas* sp. did not improve somatic growth, a carbon and phosphorus limitation of *D. magna* is unlikely. In general, *S. elongatus* is very rich in phosphorus and nitrogen and thus already provides *Daphnia* with high amounts of these elements, certainly with higher amounts than are provided by *Cryptomonas* sp. (e.g., DeMott et al. 2004; Martin-Creuzburg et al. 2008). Hence, in the present study, a limitation of *D. magna* by nitrogen is also improbable (but see Gladyshev et al. 2006).

The fact that the daphnids fed *Cryptomonas* sp. performed significantly better than those animals fed with *S. elongatus* which was supplemented with the total lipid extract from the flagellate, suggests that other factors, e.g., essential amino acids or vitamins, become limiting once the sterol and PUFA requirements are satiated. Nevertheless, the main conclusions of our study are: (1) the superior food quality of *Cryptomonas* sp. for *D. magna* when compared with *S. elongatus* is predominantly a combined effect of its sterol and PUFA composition; and (2) that the absence of sterols in a *S. elongatus* diet primarily constrains somatic growth of *D. magna*, whereas the absence of long chain PUFAs primarily limits egg production. Further laboratory and field studies are required to reveal the significance of these findings under field conditions and to disentangle the complex patterns of nutritional challenges daphnids face in natural environments and their potential consequences for food web processes.

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