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Life history consequences of sterol availability in the aquatic keystone species *Daphnia*

Abstract The absence of essential biochemical nutrients, such as polyunsaturated fatty acids or sterols, has been considered as a mechanism determining trophic interactions between the herbivore *Daphnia* and its phytoplankton food source. Here, we experimentally quantify the sensitivity of two *Daphnia* species to decreasing amounts of dietary sterols by measuring variations in life history traits. The two species *Daphnia magna* and *D. galeata* were fed different mixtures of the sterol-containing green alga *Scenedesmus obliquus* and the sterol-free cyanobacterium *Synechococcus elongatus*; a higher proportion of *Synechococcus* in the food is equivalent to a decrease in dietary sterols. To address the significance of sterol limitation, the *Daphnia* species were also fed *Synechococcus* supplemented with cholesterol. In both species, somatic and population growth rates, maternal dry mass, the number of viable offspring, and the probability of survival were significantly reduced with the lower availability of sterols. A high correlation between the sterol content of the mixed diet and the somatic and population growth rates was found, and growth on cholesterol-supplemented *Synechococcus* fitted well into this correlation. Somatic growth of first-clutch neonates grown on 100% *Synechococcus* exhibited a pattern similar to that of somatic growth of their mothers grown on the different food regimes, which demonstrated the significance of maternal effects for sterol-limited population growth. *Daphnia galeata* had a twofold higher incipient limiting sterol level than *D. magna*, which indicated interspecific differences in sterol requirements between the two *Daphnia* species.

The results suggest a strong impact of dietary sterols on life history traits and therefore, population dynamics of the keystone species *Daphnia*.

Keywords Food quality · Sterol limitation · Maternal effects · Population growth · Survival

Introduction

The flow of energy in aquatic food webs is often constrained by the low transfer efficiency of organic carbon to higher trophic levels. In particular, interactions between primary producers and their herbivorous consumers can lead to a reduced productivity at the first consumer level and to a decoupling of primary and secondary production. Crustacean zooplankton of the genus *Daphnia* are the key organisms in aquatic ecosystems; they are effective filter-feeders with high grazing impact on the phytoplankton and serve as the most important link between primary production and the production of many fish species (Lampert 1987). Daphnids feed on a broad range of particle sizes and are unable to select particles individually with regard to their quality.

Cyanobacteria are known to be an inadequate food source for freshwater cladocerans (De Bernardi and Giussani 1990), but the reasons determining this dietary inadequacy are controversial. Grazing resistance attributed to morphological properties, toxicity, and the absence of essential elemental or biochemical nutrients are considered to be responsible for the lack of grazer control on cyanobacterial assemblages, leading to the accumulation of cyanobacterial biomass and therefore to reduced water quality (Carmichael 1994). In freshwater ecosystems, the available phosphorus (P) is often scarce, and daphnids have been shown to be P-limited when their food source exceeds a carbon-to-phosphorus ratio (C:P) of approximately 300 (Sterner and Hessen

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1994; DeMott 1998). However, C:P ratios < 300 are found in the seston of many lakes (Brett et al. 2000), and in these cases, other factors appear to be potentially limiting for *Daphnia* growth (Sundbom and Vrede 1997).

Polyunsaturated fatty acids (PUFAs) are essential biochemical nutrients, and a low availability of single PUFAs in natural seston (Müller-Navarra 1995; Müller-Navarra et al. 2000; Wacker and Von Elert 2001) and in monoalgal food (Von Elert 2002; Ravet et al. 2003) has been shown to constrain growth of *Daphnia*. The fact that cyanobacteria do not contain long-chain PUFAs (Ahlgren et al. 1992) has led to the hypothesis that the low carbon transfer efficiency at the cyanobacteria–*Daphnia* interface is due to the lack of PUFAs. However, Von Elert and Wolffrom (2001) have suggested that the absence of a non-PUFA lipid present in eukaryotic algae might be responsible for the poor assimilation of cyanobacterial carbon.

Sterols are another class of lipids that are absent in most prokaryotic cyanobacteria (see Volkman 2003 for a critical review), and recently, Von Elert et al. (2003) have shown that the absence of sterols can constrain the carbon transfer between cyanobacteria and the herbivore *D. galeata*. Like insects, crustaceans are incapable of synthesizing sterols de novo and, therefore, must acquire these essential nutrients from their diet (Goad 1981). Cholesterol, the principal sterol in crustaceans, is an indispensable structural component of cell membranes and serves as a precursor of steroid hormones, such as ecdysteroids, which are involved in the process of molting (Goad 1981). Unlike carnivorous crustacea, the herbivorous daphnids cannot rely on a dietary source of cholesterol since only trace amounts are found in phytoplankton species (Nes and McKean 1977). However, eukaryotic phytoplankton is often rich in various phytosterols, some of which are suitable precursors for the synthesis of cholesterol (Martin-Creuzburg and Von Elert 2004).

The focus of the present study was to quantify the sensitivity of the freshwater cladoceran *Daphnia* to decreasing amounts of dietary sterols by measuring variations in life history traits. For this purpose, two species of daphnids (*D. magna* and *D. galeata*) were fed with different mixtures of the sterol-containing green alga *Scenedesmus obliquus* and the sterol-free cyanobacterium *Synechococcus elongatus*. Thus, a higher proportion of *Synechococcus* in the food is equivalent to a decrease in dietary sterols. To address the significance of sterols for proper growth, the two *Daphnia* species were also fed with *Synechococcus* supplemented with cholesterol. The coccoid *S. elongatus* was chosen because it is a nontoxic, phosphorus-rich cyanobacterium that is well assimilated by *Daphnia* (Lampert 1977a, b, 1981), but is deficient in essential fatty acids (Von Elert and Wolffrom 2001) and lacks sterols (Martin-Creuzburg and Von Elert 2004). In addition to the determination of juvenile somatic growth rates, which are often used as a relative measure of fitness in *Daphnia* (Lampert and Trubetskova 1996), we applied a demographic

approach to estimate the intrinsic rate of population increase (r) and its dependence on the dietary sterol content. Furthermore, effects of maternal lipid investment on the juvenile somatic growth rates of the first-clutch offspring were investigated.

Materials and methods

Cultivation and preparation of food organisms

The green alga *Scenedesmus obliquus* (SAG 276-3a, Sammlung von Algenkulturen Göttingen, Germany) 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 growth experiments, *S. obliquus* and the cyanobacterium *S. elongatus* (SAG 89.79) were each cultured semi-continuously in Cyano medium (20°C; illumination at 120 and 60 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively) at a dilution rate of 0.25 d^{-1} in aerated 5-l vessels; the cells from 25% of the culture were harvested every day, replacing the sampled medium with freshly prepared medium. Stock solutions of these food organisms were obtained by centrifugation and resuspension in fresh medium. The carbon concentrations of the food suspensions were estimated from photometric light extinction (800 nm) and from carbon-extinction equations determined previously.

For simplification, the following abbreviations will be used for the different food regimes: 'Scen' for *S. obliquus* and 'Syn' for *S. elongatus*. Five food suspensions containing different proportions of *Scenedesmus* and *Synechococcus* were prepared. The total carbon concentration of 2 mg C l^{-1} was represented by 100% Scen, 80% Scen + 20% Syn, 50% Scen + 50% Syn, 20% Scen + 80% Syn, and 100% Syn. In a sixth treatment, *Synechococcus* was supplemented with cholesterol (Sigma). For the supplementation, 30 mg bovine serum albumin (BSA) was dissolved in 7.5 ml of ultra-pure water, and 600 μl of an ethanolic stock solution of cholesterol (2.5 mg ml^{-1}) was added. Subsequently, the solution was brought to 60 ml with 6 mg particulate organic carbon (POC) of the *Synechococcus* suspension and Cyano medium. After incubation on a rotary shaker (100 revolutions min^{-1}) for 4 h, excess BSA and free cholesterol were removed by washing the cells three times in fresh medium according to Von Elert (2002). The resulting suspension was used as food in the growth experiments. Preliminary experiments showed that BSA did not affect the growth of *Daphnia*.

Growth experiments

Growth experiments were conducted with third-clutch juveniles (birth \pm 6 h) of a clone of *D. magna* (originally isolated from Großer Binnensee, Lampert 1991) and a clone of *Daphnia galeata* (originally isolated from Lake

Constance, Stich and Lampert 1984). The experiments were carried out at 20°C in glass beakers filled with 1-l of filtered lake water (0.45-µm pore-sized membrane filter). Each treatment consisted of three replicates with 20 (*D. magna*) or 55 (*D. galeata*) animals per beaker. The food suspensions were renewed daily until the animals released their third clutch. At day 6 of the experimental period, five *D. magna* or ten *D. galeata* individuals were sub-sampled, dried for 24 h, and weighed on an electronic balance (Mettler UMT 2; ±0.1 µg). The juvenile somatic growth rates (g) were determined as the increase in dry mass from the beginning of an experiment (W_0) to day 6 (W_t) using the equation:

$$g = \frac{\ln W_t - \ln W_0}{t}$$

The remaining animals were kept in the corresponding treatments until they reached maturity. Shortly before the release of the juveniles, five *D. magna* or 15 *D. galeata* individuals were transferred separately to 100 ml jars without adding food. After juveniles hatched, the dry mass of the mother and of the neonates was determined (within 0–3 h after release from the brood pouch). The same procedure was used for the second and third clutch. In order to estimate maternal effects of the different food regimes on the performance of the offspring, subsamples of first-clutch neonates were reared 6 days on 100% *Synechococcus* (sterol-free food conditions), and the juvenile somatic growth rates were determined as described above. Population growth rates (r) were estimated iteratively using the Euler–Lotka equation:

$$1 = \sum_{x=0}^n l_x m_x e^{-rx}$$

where l_x is the age-specific survivorship, m_x is the age-specific fecundity (number of neonates per individual), and x is the age at reproduction (in days). The probability of survival until reproduction (l_x) was estimated from the mortality that occurred in the different treatments. Growth rates were calculated as the means of each treatment.

Analyses

Sterols were extracted from approximately 0.5 mg POC of the food suspensions according to Martin-Creuzburg and Von Elert (2004). Free sterols were quantified with a gas chromatograph (HP 6890, Agilent Technologies, Waldbronn, Germany) equipped with an HP-5 capillary column (Agilent) and a flame ionization detector by comparison with an internal standard (5 α -cholestan). Sterols were identified using a gas chromatograph-mass spectrometer (Finnigan MAT GCQ) equipped with a fused silica capillary column (DB-5MS, J&W); the instrumental settings are described elsewhere (Martin-Creuzburg and Von Elert 2004). Mass spectra were

identified by comparison with mass spectra of a self-generated spectra library or mass spectra found in the literature. POC was determined with an NCS-2500 analyzer (ThermoQuest GmbH, Egelsbach, Germany).

Statistical analyses

Somatic and population growth rates of *Daphnia* were analyzed using 1-way analyses of variance (ANOVA). The dry mass of mothers and neonates, and the number of offspring were analyzed in full-factorial designs. The experimental factors were either food category (1-way) or food category and number of successive clutches (repeated design). Analyses of variance were carried out using the General Linear Model module of STATISTICA 6.0 (StatSoft Inc., Tulsa, OK, USA). Significant levels of multiple tests were adjusted after Bonferroni (Rice 1989). The functional relationships between the dietary sterol content and the somatic (g) or population growth rates (r) were expressed as Monod curves (Monod 1950) modified with a threshold S_0 for zero growth (Rothhaupt 1988):

$$g = g_{\max} \frac{c - S_0}{c - S_0 + K_s} \quad r = r_{\max} \frac{c - S_0}{c - S_0 + K_s},$$

where g_{\max} and r_{\max} are the maximum growth rates (d^{-1}), c is the resource concentration ($\mu\text{g mg C}^{-1}$), S_0 is the threshold concentration for zero growth ($\mu\text{g mg C}^{-1}$), and K_s is the half saturation constant ($\mu\text{g mg C}^{-1}$).

Estimations of the incipient limiting level (ILL) were based on comparisons of growth rates using one-way ANOVAs. The sterol concentration that led to a significant decrease in growth rate with decreasing sterol supply was defined as ILL. To test statistically whether different food categories affected the mortality of *Daphnia*, a generalized linear model (GLM) with the logit function as the link function for binominal distribution was used (R 2003, Version 1.8.1).

Results

Juvenile maternal growth—Increasing proportions of *S. elongatus* in the food suspensions in which ≥50% of the total organic carbon was supplied by the cyanobacteria produced a significant decline in juvenile somatic growth rates (g) of both *Daphnia* species [Tukey's HSD following ANOVA, $F_{5,12} = 1,490$ (*D. magna*); $F_{5,12} = 1,097$ (*D. galeata*); $P < 0.001$; Fig. 1a, b]. In both species, high somatic growth rates were observed when the food consisted of 100% Scen; growth on 100% Syn was generally poor. Supplementation of *Synechococcus* with cholesterol increased the somatic growth rates to levels observed in the 20% Scen + 80% Syn treatment.

Maternal dry mass—Maternal dry mass of both *D. magna* and *D. galeata* fed 100% Scen increased significantly from first through second to third reproduction

Fig. 1 Juvenile somatic growth of *Daphnia magna* (A) and *Daphnia galeata* (B) mothers reared on 100% *S. obliquus* (100% Scen), on increasing proportions of *S. elongatus* (Syn) mixed with Scen, and on *S. elongatus* supplemented with cholesterol (100% Syn + Cholesterol). Juvenile somatic growth of the first clutch offspring of mothers grown on the corresponding food regimes was determined by feeding a 100% *S. elongatus* diet (C, *D. magna*; D, *D. galeata*). Daphnids fed on 100% *S. elongatus* did not reproduce. *D. galeata* neonates whose mothers had fed on 20% Scen + 80% Syn or on *S. elongatus* supplemented with cholesterol did not survive the 6-day experiment. 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 following ANOVA)

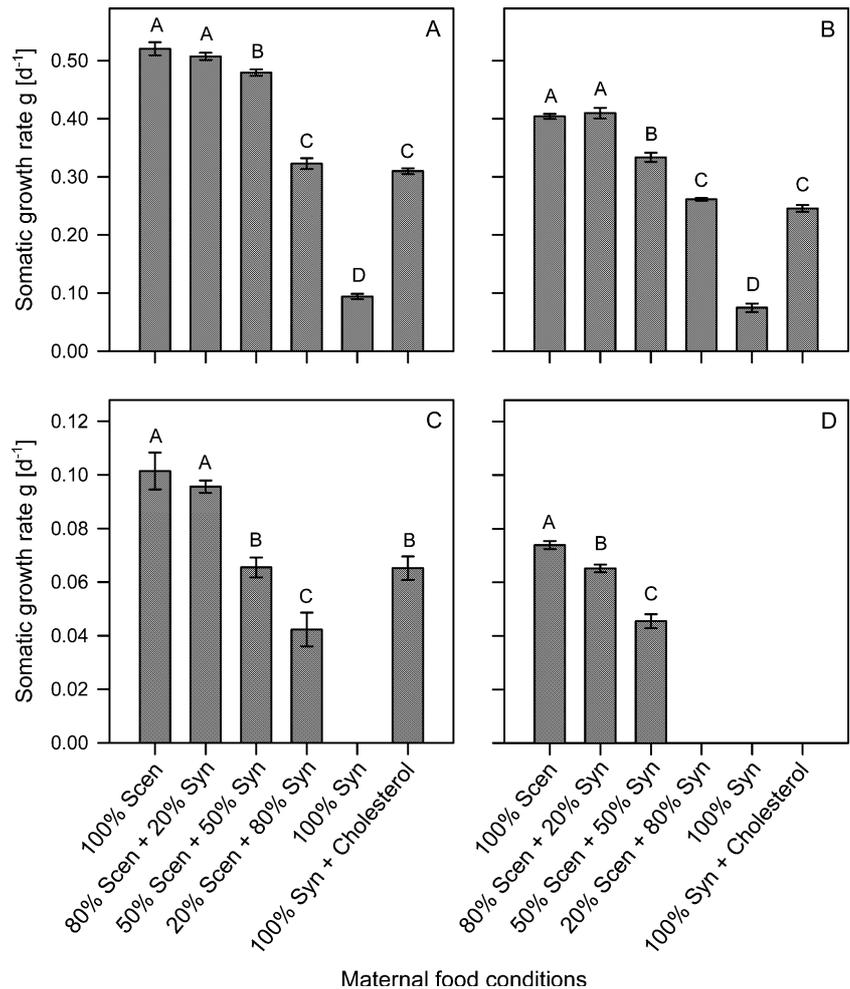
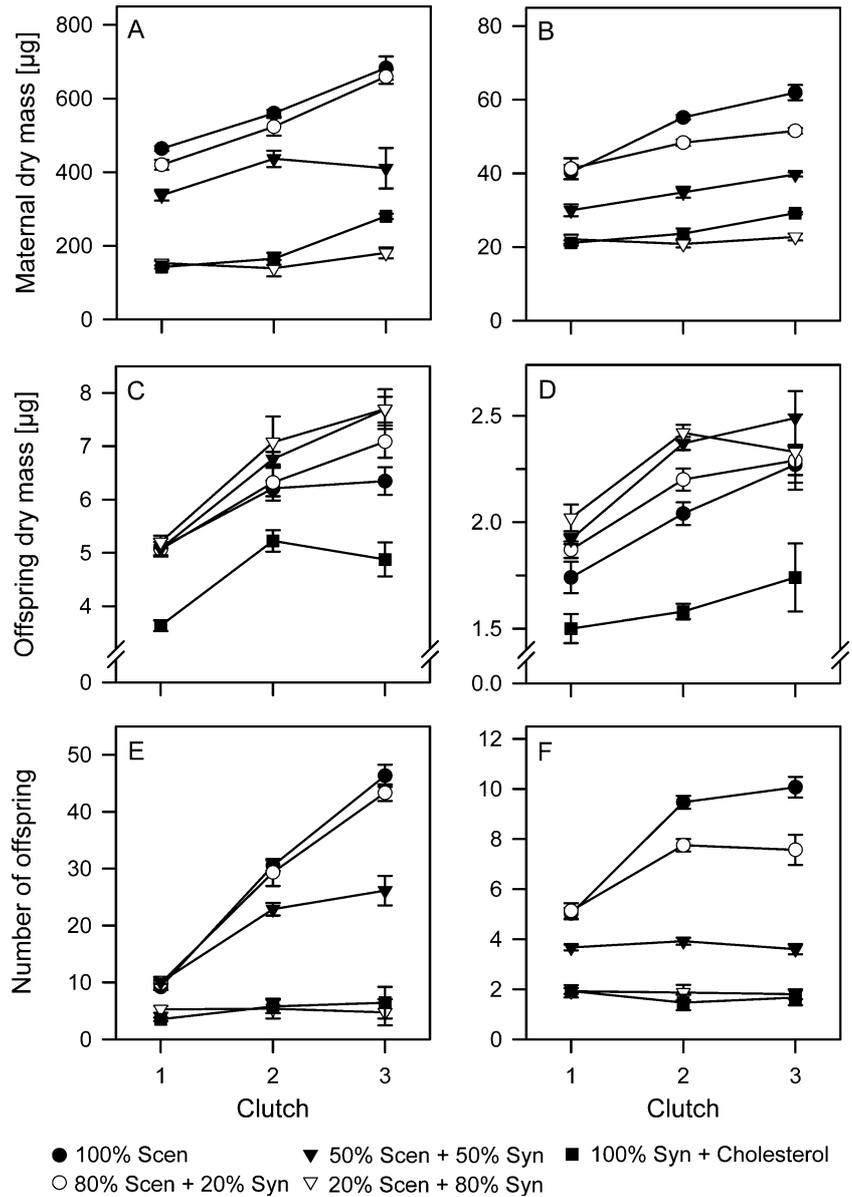


Table 1 Results of analysis of variance (ANOVA, repeated measures designs with clutch as repeated factor) of maternal dry mass, offspring dry mass, and the number of offspring in the first, second, and third clutch of *Daphnia magna* and *D. galeata* reared on various food regimes

	<i>D. magna</i>				<i>D. galeata</i>			
	SS	df	F	P	SS	df	F	P
<i>Maternal dry mass</i>								
Food regime	1283,464	4	336.44	***	6,511.22	4	990.31	***
Error	9,537	10			16.44	10		
Clutch	146,309	2	313.90	***	762.79	2	184.65	***
Clutch×Food regime	63,546	8	34.08	***	385.92	8	23.36	***
Error	4,661	20			41.31	20		
<i>Offspring dry mass</i>								
Food regime	24.69	4	71.79	***	2.57	4	75.56	***
Error	0.86	10			0.09	10		
Clutch	30.59	2	283.98	***	1.42	2	105.22	***
Clutch×Food regime	3.20	8	7.42	***	0.22	8	4.09	***
Error	1.08	20			0.13	20		
<i>Number of offspring</i>								
Food regime	4,794.49	4	253.01	***	309.48	4	1,389.87	***
Error	47.38	10			0.56	10		
Clutch	2,431.98	2	757.63	***	19.15	2	95.84	***
Clutch×Food regime	1,793.94	8	139.72	***	39.83	8	49.83	***
Error	32.10	20			2.00	20		

Raw data met assumptions for ANOVA (***) indicate significance after Bonferroni adjustment $P < 0.0083$)

Fig. 2 Maternal and individual offspring dry mass of *D. magna* (A and C) and *D. galeata* (B and D) at first, second, and third reproduction grown on 100% *S. obliquus* (100% Scen), on increasing proportions of *S. elongatus* (Syn) mixed with Scen, and on cholesterol-supplemented *S. elongatus* (100% Syn + Cholesterol). The number of viable offspring in the first, second, and third clutch is given in (E) (*D. magna*) and (F) (*D. galeata*). Daphnids fed on 100% Syn did not reproduce (not shown). Error bars indicate SD



(Tukey's HSD following ANOVA, Table 1) and were in general, lower with decreasing sterol availability (higher percentage of Syn; Fig. 2a, b). Neither species showed an increase in maternal dry mass with successive reproduction cycles when sterols were severely limiting (20% Scen + 80% Syn). Both species responded to sterol supplementation of the otherwise sterol-free diet (100% Syn) with reproduction and maternal dry masses that were equal or even higher (in the third clutch) than those observed with animals feeding on 20% Scen + 80% Syn (Fig. 2a, b). These similar responses to growth with severely limiting sterol concentrations were contrasted by the responses observed with a less severe limitation in dietary sterols, which, in general, indicated a higher susceptibility of mothers of *D. galeata* than of *D. magna* to low sterol supply. Under moderately limiting sterol concentrations (80% Scen + 20% Syn), maternal dry mass increased with successive reproduc-

tion cycles in *D. magna*, but not in *D. galeata* in the second to third reproduction cycle (Fig. 2a, b). During growth with intermediate sterol concentrations (50% Scen + 50% Syn), the dry mass of *D. magna* was reduced at the third reproduction cycle, whereas the dry mass of *D. galeata* was already reduced at the first reproduction cycle in comparison to dry masses achieved on less limiting growth conditions (Tukey's HSD following ANOVA, Table 1).

Offspring dry mass—The dry mass of individual neonates of *D. magna* (Fig. 2c) was higher in the second clutch than in the first clutch under all growth conditions (Tukey's HSD following ANOVA, Table 1). The dry mass of individuals was higher in the third clutch than in the second clutch when the maternal diet contained 80 and 50% Scen. Between treatments, no differences in the individual dry mass of first, second, and third clutch neonates were found, except for the neonates fed on

cholesterol-supplemented *Synechococcus*, which were significantly lighter in all clutches. The dry mass of second-clutch *D. galeata* neonates was higher than the dry mass of first-clutch neonates under all growth conditions (Fig. 2d), but the dry mass did not increase further in third-clutch individuals (Tukey's HSD following ANOVA, Table 1). First-, second-, and third-clutch neonates of *D. galeata* whose mothers fed on cholesterol-supplemented *Synechococcus* were also significantly lighter in all clutches.

Number of offspring—*D. magna* and *D. galeata* showed similar dietary effects on numbers of offspring during growth in the absence of sterol limitation (highest numbers of offspring on 100% Scen) and under severe sterol limitation (lowest numbers of offspring on 20% Scen + 80% Syn; Fig. 2e, f). Neither *D. galeata* nor *D. magna* reproduced on a diet of 100% Syn. Reproduction on cholesterol-supplemented *Synechococcus* was similar to that during growth with severe sterol limitation (20% Scen + 80% Syn; Fig. 2e, f), in which no increase in the number of offspring with successive reproduction cycles was observed. With moderate sterol reduction (80% Scen + 20% Syn), the number of *D. magna* offspring increased with successive reproduction cycles, but numbers of *D. galeata* did not increase from the second to third reproduction cycle (Tukey's HSD following ANOVA, Table 1). With intermediate sterol reduction (50% Scen + 50% Syn), the number of *D. magna* offspring was higher in the second clutch than in the first clutch, whereas there was no significant difference in the number of *D. galeata* offspring (Fig. 2e, f).

Offspring growth—In order to assess the maternal effects of sterol-limited growth on offspring performance, mothers were raised on various mixtures of *Scenedesmus* and *Synechococcus* and juvenile somatic growth rates of first clutch neonates on a 100% Syn diet were determined. *D. magna* and *D. galeata* offspring exhibited the same pattern of growth as their experimental mothers (Fig. 1c, d). The growth of offspring of both species declined with decreasing sterol content of the maternal diet. However, a significant decrease in growth of *D. galeata* offspring occurred already with moderate sterol reduction (80% Scen + 20% Syn) in the maternal diet (Tukey's HSD following ANOVA, $F_{2,6}=173$; $P<0.001$), whereas the same maternal diet had no effect on the growth of *D. magna* offspring. A significant decline in *D. magna* offspring growth was observed first under intermediate sterol reduction (50% Scen + 50% Syn) in the maternal diet (Tukey's HSD following ANOVA, $F_{4,10}=71$; $P<0.001$; Fig. 1c, d). *D. galeata* offspring whose mothers had been fed with 80% Syn or with cholesterol-supplemented *Synechococcus* did not survive the 6-day experiment, whereas *D. magna* offspring performed well under these conditions, which provided further evidence for more pronounced maternal effects in *D. magna* than in *D. galeata*.

General mortality—The probability of survival, used to estimate population growth, was calculated from the mortality that occurred in the different treatments.

Neither *Daphnia* species reached maturity when grown on 100% Syn and the mortality was comparatively high (Fig. 3); *D. galeata* survival was significantly lower than that of *D. magna* (GLM, $P<0.001$). The mortality of both species grown on 20% Scen + 80% Syn and on cholesterol-supplemented *Synechococcus* was significantly lower than when grown on 100% Syn (GLM, $P<0.001$). The mortality of the two species during growth on 20% Scen + 80% Syn and on cholesterol-supplemented *Synechococcus* did not differ (GLM, *D. magna*, $P=0.131$; *D. galeata*, $P=0.410$). No mortality was observed in either species on food regimes containing $\geq 50\%$ Scen.

Population growth—The effect of increasing proportions of cyanobacterial carbon in the diet on population growth (Fig. 4) reflected the results observed for somatic growth. Population growth rates were significantly lower when the *Synechococcus* content reached 50% (*D. magna*) or 20% (*D. galeata*) [Tukey's HSD following ANOVA, $F_{4,10}=314$ (*D. magna*), $F_{4,10}=1,123$ (*D. galeata*); $P<0.001$]. The daphnids were not able to reproduce on a 100% Syn diet, and thus, no positive population growth occurred. In contrast, cholesterol supplementation of *Synechococcus* led to population growth rates of 0.16 d^{-1} for *D. magna* and 0.11 d^{-1} for *D. galeata*. However, these population growth rates were significantly lower than those observed on the 20% Scen + 80% Syn diet in both species.

Growth responses to sterol supply—The total sterol content of the food suspensions was calculated by adding up the individual amounts of the three major phytosterols detected in the green alga *Scenedesmus*. Chondrillasterol [(24E)-5 α -poriferasta-7,22-dien-3 β -ol, 60%], fungisterol (5 α -ergost-7-en-3 β -ol, 26%), and 22-dihydrochondrillasterol (5 α -poriferast-7-en-3 β -ol, 13%) were identified as the principal sterols in *Scenedesmus*, which is in accordance with published data (Wright et al. 1980; Cranwell et al. 1990; Rzama et al. 1994). It was assumed that these sterols can be converted to cholesterol by *Daphnia* as has been shown by Martin-

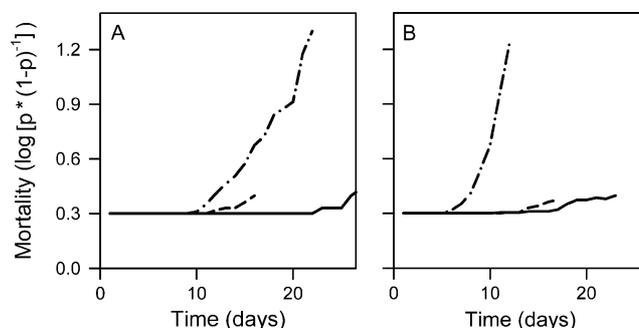
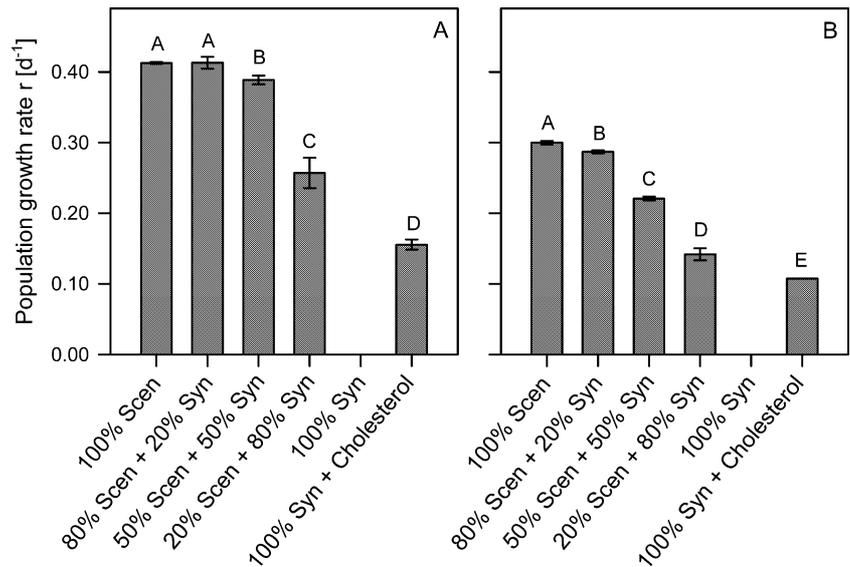


Fig. 3 Mortality of *D. magna* (A) and *D. galeata* (B) grown on 20% Scen + 80% Syn (dashed line), 100% Syn (dashed-dotted line), and cholesterol-supplemented *S. elongatus* (solid line) until the release of the third clutch. Animals grown on 100% Syn did not reach maturity. No mortality was observed in food regimes containing $\geq 50\%$ Scen (data not shown). P = Probability of mortality

Fig. 4 Estimated population growth of *D. magna* (A) and *D. galeata* (B) grown on 100% *S. obliquus* (100% Scen), on increasing proportions of *S. elongatus* (Syn) mixed with Scen, and on *S. elongatus* supplemented with cholesterol (100% Syn + Cholesterol). 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 following ANOVA)



Creuzburg and Von Elert (2004), and therefore, they were considered as cholesterol equivalents (according to Von Elert et al. 2003). Thus, *Scenedesmus* contained $6.8 \pm 0.6 \mu\text{g}$ (mean \pm SD, $n = 3$) cholesterol equivalents per mg carbon. *Synechococcus* did not contribute to the total sterol content of the food suspensions since no sterols were found in the cyanobacterium. The amount of sterols found in the 20% Scen + 80% Syn food regime and in the sterol-supplemented diet did not differ. The kinetics of sterol-limited growth, expressed as a saturation curve, was applied to a modified Monod model. The somatic growth rates and the estimated population growth rates of both *Daphnia* species were highly correlated to the dietary sterol content (Fig. 5). Albeit not considered in the Monod model, somatic growth rates, and to a lesser extent population growth rates, on the sterol-supplemented diet fitted well to the correlation of dietary sterol content versus growth rate. If the data from the sterol supplementation treatment were included in the model, the model parameters would lie within the 95% confidence interval of the values calculated without the supplemented treatment. This further corroborates the correlative evidence for growth limitation by sterols. Differences in the extent with which somatic and population growth rates on the sterol-supplemented diet fitted to the correlation of dietary sterol content versus growth rate suggest that reproduction is less sensitive to sterol limitation and that other factors (e.g. PUFAs) may become limiting in later life stages.

In both species, a significant decline in the somatic growth rates with decreasing sterol supply was observed for sterol levels $< 5.4 \mu\text{g}$ per mg of dietary carbon (Tukey's HSD following ANOVAs). Population growth rates of *D. magna* were reduced at sterol levels $< 3.4 \mu\text{g}$ per mg of dietary carbon, whereas population growth rates of *D. galeata* were reduced at sterol levels $< 6.8 \mu\text{g}$ per mg of dietary carbon (Tukey's HSD following ANOVAs).

Discussion

The trophic structure of aquatic food webs can be determined by the availability of essential nutrients. Recently, the absence of sterols was considered as one potentially important factor that is able to constrain the carbon transfer efficiency between cyanobacteria as primary producers and herbivore consumers (Von Elert et al. 2003). In the present study, we confronted two *Daphnia* species (*D. magna* and *D. galeata*) with increasing proportions of dietary cyanobacterial carbon in order to assess the relevance of dietary sterols for life history traits of *Daphnia*.

The somatic growth rates (g) of both *Daphnia* species were significantly lower during growth on increasing proportions of *Synechococcus* in the food, which indicated the inadequacy of cyanobacterial carbon to support *Daphnia* growth. A higher proportion of *Synechococcus* in the food is equivalent to a lower amount of dietary sterols since no sterols were found in the cyanobacterium. On a diet of 100% Syn, somatic growth of both *Daphnia* species was poor. However, supplementation of the cyanobacterium with cholesterol enhanced the somatic growth rates, which indicated that the animals were limited by the availability of sterols. Although supplementary cholesterol significantly improved the food quality of *Synechococcus*, the resultant somatic growth rates were still lower than on 100% Scen. These differences might be the consequence of an insufficient sterol supply, which is favored by the finding that somatic growth on sterol-supplemented *Synechococcus* fitted well to the correlation of dietary sterol content versus growth rate. We could not entirely exclude that, to some extent, PUFA limitation also constrained *Daphnia* growth, since both sterol and PUFA content of the food mixtures decreased with increasing amounts of cyanobacterial carbon. However, previous work has shown that daphnids are primarily sterol-

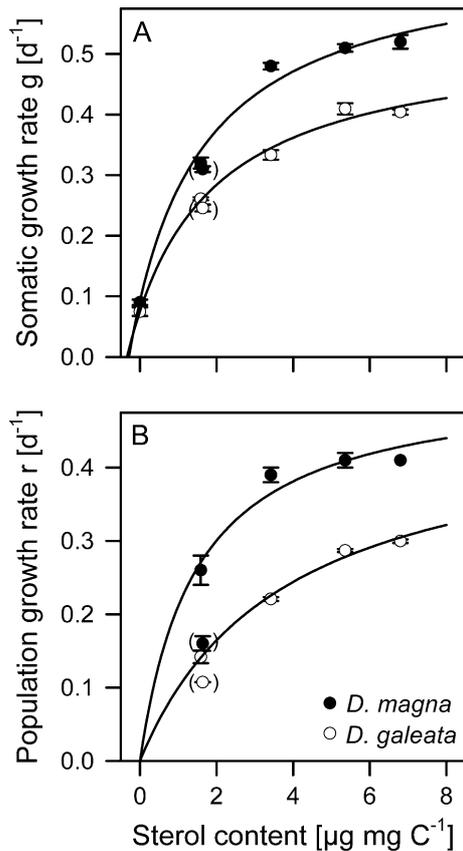


Fig. 5 Somatic (A) and population growth (B) of *D. magna* and *D. galeata* in response to the dietary sterol content. The regression line was calculated using a modified Monod model (Nonlinear estimations after Gauss-Newton: somatic growth $F_{(3,14)} = 31.00$ *D. magna*; $F_{(3,14)} = 31.52$ *D. galeata*; population growth $F_{(3,14)} = 20.72$ *D. magna*; $F_{(3,14)} = 18.47$ *D. galeata*; $P < 0.001$). The growth of animals reared on sterol-supplemented *S. elongatus* was not considered in the Monod model; symbols are given in parentheses. Error bars indicate SD

limited when grown on *Synechococcus* and that growth of *Daphnia* is enhanced by PUFAs only when the shortage of sterols has been overcome by sterol supplementation (Von Elert et al. 2003). This is corroborated by the supplementation treatment which clearly shows sterol limitation as the major constraint. Von Elert et al. (2003) estimated that *Daphnia galeata* requires 20% *Scenedesmus* in the diet to compensate for the low sterol content of a cyanobacterial food. Here we showed that sterol limitation occurs even on a diet containing 50% of the green algae. This discrepancy in the observed sensitivity to sterol limitation might be due to the lower sterol content of the green algae in the present study (6.8 vs. 10 µg per mg C; Von Elert et al. 2003).

Examination of the factors that limit somatic growth is useful to ascertain individual physiological demands, but is potentially insufficient to predict population dynamics. Therefore, we determined life history parameters of *Daphnia* from birth through the third reproductive cycle to estimate the role of dietary sterols in population growth.

Size at maturity is a key parameter often considered in studies dealing with variations of life history traits and their effects on fitness. In *Daphnia*, size at maturity is determined by the size at birth, the number of juvenile instars (e.g., Tessier and Consolatti 1989), and environmental factors (e.g., Porter et al. 1983; Pace et al. 1984). Deteriorating environmental conditions reduce the size at maturity (e.g., Lynch 1989). In the present study, the dry mass (which is correlated with body size) at maturity of both *Daphnia* species was lower with higher proportions of dietary cyanobacterial carbon. Relative to the dry mass of animals fed a 100% *Scen* diet, this effect increased with the reproductive state and was most pronounced at the third reproduction cycle. The dry mass of animals reared on a 80% *Syn* diet did not increase in successive reproduction cycles. This might be caused by differing metabolic demands for growth and survival. Maintenance metabolism is based primarily on the uptake of energy (in terms of carbon), while somatic growth requires energy and a variety of essential nutrients (Sterner and Robinson 1994). The food quantity provided in the present study was well above the incipient limiting level, and *Synechococcus* is well-assimilated by *Daphnia* (Lampert 1977a, b), which makes energy limitation rather unlikely. The lower amount of sterols in the 80% *Syn* diet is apparently not sufficient to support body growth; instead, sterols might be allocated to reproduction or used to replace degraded structural material, such as cell membranes.

While effects of food quantity on somatic growth and reproduction are highly correlated in juvenile *Daphnia* (Lampert and Trubetskova 1996), effects of food quality on growth and reproduction can differ (Urabe and Sterner 2001; Becker and Boersma 2003). In the present study, the number of neonates decreased with increasing proportions of dietary *Synechococcus*. This might be partly due to the decreasing size of the experimental mothers since clutch sizes are known to be size-dependent (e.g., Lampert 1993), but it might also be a consequence of the reduced availability of sterols, which are not only needed for somatic growth, but also for reproduction (Martin-Creuzburg and Von Elert 2004). In contrast to the lower numbers of offspring, the dry weights of the neonates were slightly, but not significantly, enhanced with increasing proportions of *Synechococcus* in the maternal food. This might be considered as an adaptive response to lower food quality that gives *Daphnia* offspring an advantage to survive poor food conditions. Gliwicz and Guisande (1992) have demonstrated that *Daphnia* mothers are capable of assessing food levels and accordingly adjust their fractional per-offspring allocation of reproductive resources. They have shown that high food levels lead to the production of large clutches with smaller eggs and low food levels lead to the production of small clutches with larger eggs that are more resistant to starvation. In the present study, where food quantity was constant throughout experiments, such a trade-off between clutch size and body mass of the hatched neonates (which is strongly

related to egg size, Guisande and Gliwicz 1992) is conceivably a response to the decreasing dietary sterol content.

Although size at birth is an important fitness determinant (Tessier and Consolatti 1989; Lampert 1993), maternal effects on subsequent life stages should be considered when offspring performance and population dynamics are analyzed since offspring quality is not necessarily reflected in size at birth (Sakwinska 2004). Therefore, we reared first-clutch neonates of experimental mothers grown with the various food regimes on a 100% Syn diet. In this way, the pattern of growth of the offspring was, at least partly, a reflection of that of their mothers on an overall lower level. Somatic growth rates of the neonates were lower with increasing proportions of *Synechococcus* in the maternal food. This is in accordance with previous data, which showed that maternal consumption of a high quality food (*Rhodomonas*) increased neonate fitness relative to a low quality maternal food (*Microcystis*) when neonates were reared on *Microcystis* (Brett 1993). The data presented here imply a limited ability of daphnids to adjust the amount of sterols allocated to reproduction since low sterol levels in the maternal food should lead to increased sterol allocation to offspring tissues to enhance offspring survival. Instead, the results rather suggested a per-offspring allocation of sterols proportional to the availability of dietary sterols. As a consequence, insufficient amounts of sterols resulted in a limitation of both maternal and offspring growth (on a sterol-free diet). *Daphnia galeata* neonates whose mothers had fed on 80% Syn or on sterol-supplemented *Synechococcus* did not survive the experimental period (6 days), which showed that limited amounts of sterols, adequate for low maternal growth and reproduction, are not sufficient to support offspring survival. In contrast, no mortality of *D. magna* neonates occurred within the 6-day experiment, which pointed to differences in sterol requirements between the two species. This was corroborated by the finding that the survival of *D. galeata* mothers on a 100% Syn diet was significantly lower than that of *D. magna*, which seems to be less sensitive to sterol limitation. These differences in the sensitivity to sterol limitation might be owing to differences in body size and in the storage capacity for sterols.

Effects of the diminishing availability of sterols on somatic and population growth followed more or less the same pattern. Differences in the growth response to sterol supplementation between somatic (g) and population growth rates (r) were found. Somatic growth on sterol-supplemented *Synechococcus* was as good as growth on a 20% Scen diet, whereas the estimated population growth on sterol-supplemented *Synechococcus* was significantly lower than on a 20% Scen diet. This is mainly caused by the prolonged time needed in the sterol-supplemented food regime to reach the third reproductive state (a parameter considered in the Euler–Lotka equation), which might indicate that other factors become limiting in subsequent life stages. However, it

should be recognized that sterol supplementation of *Synechococcus* led to a positive population growth rate, whereas animals grown on unsupplemented *Synechococcus* did not even reproduce.

The analysis of the functional response of sterol-limited growth revealed a high correlation between the sterol content of the diet and the somatic and population growth rates. Growth rates (g and r) achieved on the cholesterol-supplemented *Synechococcus* diet were also highly correlated to the amount of sterol found in the supplemented cyanobacterium, which emphasizes the role of sterols as the principal limiting factor in this system. In the field, the performance of *Daphnia* populations is subject to multiple factors, including food availability and predation. However, the dietary sterol content should be considered when analyzing *Daphnia* growth in natural environments. In the past, the food thresholds concept (Lampert 1977c) was often used to predict *Daphnia* growth on limiting resources. Two types of threshold then have to be distinguished: The individual threshold (S_0 for g) is the amount of food at which assimilation equals respiration, resulting in a zero mass change of an individual. The population threshold (S_0 for r) is the amount of food at which reproduction compensates mortality, resulting in a zero change of population size. This concept has also been used to describe qualitative aspects of food limitation (Gliwicz and Lampert 1990; Sterner and Robinson 1994). In the present study, values of S_0 for juvenile somatic growth were negative, since positive growth rates were observed even on a sterol-free diet. This indicates sterol allocation to offspring and a limited storage capacity of sterols, but it does not allow the determination of an individual threshold for sterol-limited body growth. In contrast, the sterol thresholds for population growth were close to zero in both species. Although unlikely to occur in the field, such low sterol levels would not allow positive population growth and should finally lead to the extinction of the population.

Furthermore, we estimated the ILL at which maximum growth passes into sterol-limited growth—a value potentially important to predict the fate of *Daphnia* populations in the field. The presented data suggest that, in both species, a sterol content of $< 5.4 \mu\text{g}$ per mg of dietary carbon results in reduced somatic growth. At the population level, population growth rates of *D. magna* will be lower at sterol contents of $< 3.4 \mu\text{g}$ per mg of dietary carbon, whereas population growth rates of *D. galeata* will be lower already at sterol contents of $< 6.8 \mu\text{g}$ per mg of dietary carbon (or even higher since no maximum growth rate plateau was reached). Differences between *D. magna* and *D. galeata* in their ability to grow on *Synechococcus* have previously been reported (DeMott and Müller-Navarra 1997; DeMott 1998). The authors suggest a reduced ability of *D. galeata* to utilize a PUFA-deficient diet. Data provided here point to interspecific differences in sterol requirements between *D. magna* and *D. galeata*. In *D. magna*, a lower incremental increase in dietary sterol content is required for

the same increase in growth as in *D. galeata* (slope of regression line, Fig. 5), which suggested a lower sterol requirement of *D. magna*. The ability to cope with the low availability of sterols might be an adaptation of *D. magna* for living in eutrophic ponds (Lampert 1991), which are often dominated by cyanobacterial assemblages. Furthermore, the data presented suggested that interspecific differences in sterol requirements potentially affect the outcome of competition between coexisting *Daphnia* species when sterols are in short supply. To verify the suitability of the dietary sterol content to predict *Daphnia* growth in natural environments, further studies are needed to link growth responses of *Daphnia* to seston characteristics (e.g., sterol content).

The concept presented implies that populations decline with decreasing availability of sterols and vice versa, a process that might occur during phytoplankton species succession. In particular, it might explain the often-observed absence of *Daphnia* species during cyanobacterial blooms. In the field, effects of sterol limitation on population growth are probably even more evident since maternal effects on later life stages are not considered in the Euler–Lotka equation used here to estimate effects on population growth. In the present study, we documented that decreasing amounts of dietary sterols in the maternal food reduce the potential of neonates to grow under poor food conditions.

In summary, we showed that the dietary sterol content unambiguously affects life history traits of *Daphnia*. Somatic and population growth rates, maternal dry weights, the number of viable offspring, and the probability of survival were significantly reduced with the diminishing availability of sterols. This suggested a strong impact of dietary sterols on population dynamics of the herbivorous grazer. Sterol-limited growth might lead to a reduced energy transfer at the plant–animal interface and therefore to a decoupling of primary and secondary production. We are aware that herbivores like *Daphnia* face a complex pattern of nutritional challenges in natural environments, but sterol limitation might be one important factor with the potential to determine the structure of aquatic food webs.

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