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IMPACT OF 10 DIETARY STEROLS ON GROWTH AND REPRODUCTION OF *Daphnia galeata*

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Abstract—In crustaceans, cholesterol is an essential nutrient, which they must directly obtain from their food or by bioconversion from other dietary sterols. Eukaryotic phytoplankton contain a great variety of sterols that differ from cholesterol in having additional substituents or different positions and/or number of double bonds in the side chain or in the sterol nucleus. In this study, we investigated to what extent these structural features affect the growth and reproduction of *Daphnia galeata* in standardized growth experiments with the cyanobacterium *Synechococcus elongatus* supplemented with single sterols as food source. The results indicated that Δ^5 (sitosterol, stigmasterol, desmosterol) and $\Delta^{5,7}$ (7-dehydrocholesterol, ergosterol) sterols meet the nutritional requirements of the daphnids, while the Δ^7 sterol lathosterol supports somatic growth and reproduction to a significantly lower extent than cholesterol. Dihydrocholesterol (Δ^0) and lanosterol (Δ^8) did not improve the growth of *D. galeata*, and growth was adversely affected by the Δ^4 sterol allocholesterol. Sterols seem to differ in their allocation to somatic growth and reproduction. Thus, structural differences of dietary sterols have pronounced effects on life-history traits of *D. galeata*.

Key Words—Food quality, cyanobacteria, dietary sterols, cholesterol, *Daphnia galeata.*

INTRODUCTION

The transfer of energy from primary producers to higher trophic levels is an important factor that determines the trophic structure of aquatic food webs. At the phytoplankton–zooplankton interface, the efficiency of carbon transfer is highly variable. This variation can be attributed to the changing nutritional value of phytoplankton assemblages. Nutritional inadequacy can be due to toxicity (Lampert,

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1981a,b), digestive resistance (Porter and McDonough, 1984), or mineral (Elser et al., 2001) or biochemical composition of phytoplankton species, and can result in a decoupling of primary and secondary production. The biochemical composition of phytoplankton, in particular the content of polyunsaturated fatty acids (PUFAs), has been discussed as being potentially limiting for *Daphnia* growth (Ahlgren et al., 1990; Müller-Navarra, 1995; Wacker and Von Elert, 2001).

PUFAs are of special importance for freshwater zooplankton nutrition in lakes dominated by cyanobacteria, as articulated in a correlative study by Müller-Navarra et al. (2000). Cyanobacteria in general lack long-chain PUFAs (Cobelas and Lechardo, 1988; Ahlgren et al., 1992), and the well-known low carbon-transfer efficiency at the cyanobacteria–*Daphnia* interface has been suggested to be caused by a deficiency in long-chain PUFAs (Müller-Navarra et al., 2000). Supplementation of the cyanobacterium *Synechococcus elongatus* with a PUFA-rich fish oil emulsion leads to better growth and reproduction of *Daphnia* (DeMott and Müller-Navarra, 1997) and, therefore, supports the correlative evidence. However, Von Elert and Wolffrom (2001), have found that the absence of a non-PUFA lipid present in eukaryotic algae constrains assimilation of cyanobacterial carbon. Fish oil contains other lipids in addition to PUFAs, such as sterols, which are also essential for growth and reproduction of crustaceans (Goad, 1981). Cyanobacteria, as prokaryotes, lack or contain only traces of sterols (Hai et al., 1996, Volkman, 2003). In a previous study, we have shown that the low carbon-transfer efficiency of cyanobacteria to *Daphnia galeata* is caused by the lack of sterols in cyanobacteria (Von Elert et al., 2003).

Like all arthropods, crustaceans are incapable of synthesizing sterols de novo and, therefore, must acquire these essential nutrients from their diet (Goad, 1981). Crustaceans generally have a simple sterol composition with characteristic high cholesterol levels (Teshima and Kanazawa, 1971a; Yasuda, 1973). Cholesterol is an indispensable structural component of cell membranes and serves as a precursor for many bioactive molecules, such as ecdysteroids, which are involved in the process of molting (Goad, 1981; Harrison, 1990). However, the herbivorous cladoceran *Daphnia*, unlike carnivorous crustaceans, cannot rely on a dietary source of cholesterol because only trace amounts are found in many phytoplankton species (Nes and McKean, 1977). Eukaryotic phytoplankton contain a great variety of plant sterols (Nes and McKean, 1977; Volkman, 2003), which can be distinguished from cholesterol by their chemical structure. These phytosterols are often characterized by additional substituents or by the position and/or number of double bonds in the side chain or in the sterol nucleus (Piironen et al., 2000). The crustaceans examined to date are capable of converting dietary sterols to cholesterol (Teshima, 1971; Teshima and Kanazawa, 1971b; Ikekawa, 1985; Harvey et al., 1987), but not all sterols are suitable precursors for the synthesis of cholesterol (Teshima et al., 1983).

Under field conditions, the diet of the nonselectively suspension-feeding *D. galeata* is complex. The diet usually consists of phytoplankton, protozoa, bacteria, and detritus in varying ratios. Depending on the composition of their diet, the cladocerans are provided with a large variety of sterols in different quantities. De Lange and Arts (1999) correlated biochemical variables of natural seston with *Daphnia* growth rates and found that the sterol content is a useful tool to predict *Daphnia* growth. However, growth of the herbivorous zooplankton might not only be limited by the total sterol content itself, but also by the absence of sterols that are suitable precursors of cholesterol. In periods when phytoplankton assemblages are dominated by species with an unsuitable sterol pattern, growth and reproduction of *Daphnia* could be constrained by the low availability of suitable sterols. The first evidence that structural differences of dietary sterols can have pronounced effects on life-history traits of arthropods has been found in terrestrial systems. Behmer and Grebenok (1998) pointed out that growth and fecundity of the moth *Plutella xylostella* was affected by dietary sterols. Further on, it was recently demonstrated that sterols with double bonds at Δ^7 and/or Δ^{22} (Figure 1) failed to support development of different grasshopper species and that survival of the grasshopper *Schistocerca americana* was constrained by the ratio of suitable to unsuitable sterols in their diet (Behmer and Elias, 2000). Consistently, the development of marine copepods was negatively affected by Δ^7 sterols, whereas Δ^5 sterols allowed a rapid development of the copepods (Klein Breteler et al., 1999). Comparable investigations on the structural requirements of freshwater zooplankton with regard to sterols are missing to date.

The aim of this study was to investigate to what extent structural features of sterols, such as the alkylation of the side chain or the presence or absence of double bonds, affect the nutritional value of single sterols for *Daphnia*. Standardized growth experiments of *D. galeata* with the cyanobacterium *S. elongatus* supplemented with single sterols as food source were conducted. *S. elongatus* is well assimilated by *Daphnia* (Lampert, 1977a,b) and does not contain any sterols. Thus, the cyanobacterium is a convenient source of carbon and a useful “transfer vehicle” for delivering sterols to the daphnids.

METHODS AND MATERIALS

Cultures and Growth Experiments. Laboratory growth experiments were conducted with a clone of *Daphnia galeata*, which was originally isolated from Lake Constance (Stich and Lampert, 1984). The green alga *Scenedesmus obliquus* (SAG 276-3a, Sammlung von Algenkulturen Göttingen, Germany) was grown in batch culture and harvested in the late-exponential phase. It was used as the food source for the stock culture of *D. galeata* and for the newborn experimental

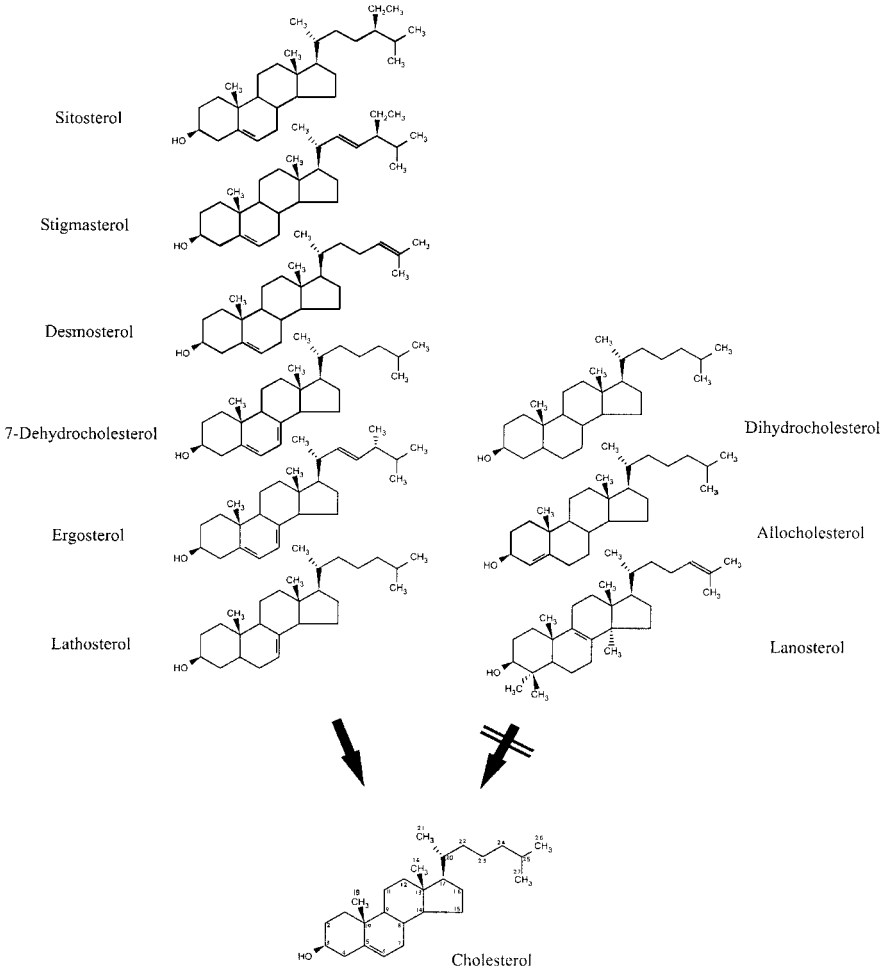

FIG. 1. Structural requirements for the conversion of dietary sterols to cholesterol in *Daphnia galeata*. Sterols on the left are suitable precursors for the synthesis of cholesterol, whereas sterols on the right are not. Potential intermediates in sterol metabolism are not shown.

animals, which were cultured to the age of 48 hr in a flow-through system prior to the growth experiments. *S. elongatus* (SAG 89.79) was grown in chemostats at a dilution rate of 0.25 d^{-1} according to Von Elert and Wolffrom (2001). *S. elongatus* and *S. obliquus* were grown in Cyano medium (Jüttner et al., 1983). Chemostat-grown cells were concentrated by centrifugation and resuspended in fresh medium. Carbon concentrations of the cyanobacterial suspensions were

estimated from photometric light extinction (800 nm) using carbon-extinction equations. *S. elongatus* had a molar C:N:P ratio of 121:23:1. Growth experiments were carried out at 20°C in glass beakers filled with 0.5 l of filtered lake water (0.45 μm pore-sized membrane filter) containing 2 mg C l^{-1} *S. elongatus*. The 48-hr-old juveniles (released from the third clutch within 10 hr) were transferred from the flow-through system into these beakers. The food suspensions were renewed daily within the 4 d of the experimental period. Somatic growth rates (g) were determined as the increase in dry weight (W) during the experiments using the equation:

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

Subsamples of the experimental animals were taken at the beginning (W_0) and at the end (W_t) of an experiment. The subsamples consisting of ~15 juveniles were dried for 12 hr and weighed on an electronic balance (Mettler UMT 2; ± 0.1 μg). Each treatment consisted of three replicates with 15 animals each, and growth rates were calculated as means for each treatment.

Supplementation of Sterols. Sterols used for supplementation are given in Table 1, they were selected according to their chemical structure and their natural occurrence. To enrich *S. elongatus* with sterols, 10 mg bovine serum albumin (BSA) was dissolved in 5 ml of ultra-pure water, and 200 μl of an ethanolic stock solution of the free sterol (2.5 mg ml^{-1}) were added. Subsequently, 4 mg particulate organic carbon (POC) of the *S. elongatus* stock solution were added to each solution, and the volume was brought to 40 ml with Cyano medium. The resulting suspension was incubated on a rotary shaker (100 revolutions min^{-1}) for 4 hr. Surplus BSA and free sterols were removed by washing the cells three times in 10 ml fresh medium according to Von Elert (2002). The resulting *S. elongatus* suspension was used as food in the growth experiments.

TABLE 1. NOMENCLATURE OF STEROLS SUPPLEMENTED TO THE *Daphnia galeata* FOOD SOURCE, *Synechococcus elongatus*

Trivial name	IUPAC name	Formula	Commercial source
Cholesterol	Cholest-5-en-3β-ol	$C_{27}H_{46}O$	Sigma C-8667
Stigmasterol	Stigmasta-5,22-dien-3β-ol	$C_{29}H_{48}O$	Sigma S-2424
Sitosterol	Stigmast-5-en-3β-ol	$C_{29}H_{50}O$	Sigma S-1270
Ergosterol	(22E)-Ergosta-5,7,22-trien-3β-ol	$C_{28}H_{44}O$	Sigma E-6510
Lathosterol	5α-Cholest-7-en-3β-ol	$C_{27}H_{46}O$	Sigma C-3652
Dihydrocholesterol	5α-Cholestan-3β-ol	$C_{27}H_{48}O$	Sigma D-6128
Lanosterol	5α-Lanosta-8,24-en-3β-ol	$C_{30}H_{50}O$	Sigma L-1504
Allocholesterol	Cholest-4-en-3β-ol	$C_{27}H_{46}O$	Steraloids C6100
7-Dehydrocholesterol	Cholesta-5,7-dien-3β-ol	$C_{27}H_{44}O$	Steraloids C3000
Desmosterol	Cholesta-5,24-dien-3β-ol	$C_{27}H_{44}O$	Steraloids C3150

Analyses. Sterols were analyzed from approximately 0.5 mg POC of the food suspensions filtered on precombusted GF/F filters or from 60 to 80 animals washed twice with ultra-pure water. Lipids were extracted three times with dichloromethane:methanol (2:1 (v/v)). After saponification with 0.2 mol l^{-1} methanolic KOH (70°C, 1 hr) and addition of ultra-pure water, the neutral lipids (sterols) were partitioned into *iso*-hexane:diethyl ether (9:1 (v/v)). The sterols were analyzed as free sterols with a gas chromatograph (HP 6890) equipped with an HP-5 capillary column (Agilent) and a flame ionization detector. The carrier gas (helium; purity 5.0) had a flow rate of 1.5 ml min^{-1}. The temperature was raised from 150 to 280°C at 15°C min^{-1} and increased to 330°C at 2°C min^{-1}. The final temperature was held for 5 min. Sterols were quantified by comparison to 5α-cholestan, which was used as an internal standard and identified using a gas chromatograph–mass spectrometer (Finnigan MAT GCQ) equipped with a fused silica capillary column (DB-5MS, J&W). Spectra were recorded between 60 and 400 amu in the EI ionization mode. POC was determined with an NCS-2500 analyzer (Carlo Erba Instruments).

Data Analysis. All data were analyzed using one-way analysis of variance (ANOVA). For growth rates and clutch sizes, raw data met the assumption of homogeneity of variance; values of the supplemented sterols and cholesterol in *D. galeata* were $\log_{10}$-transformed to meet assumptions for ANOVA. The effects of single treatments were tested by Tukey's HSD post hoc tests. A significance level of $P = 0.05$ was applied to all statistical analyses.

RESULTS

Growth Experiments. Growth of *D. galeata* on unsupplemented *S. elongatus* was in general poor (growth rate, g = 0.07 d^{-1}). Supplementation of *S. elongatus* with sterols affected somatic growth of *D. galeata* (ANOVA, $F_{10,22} = 389$; $P < 0.001$; Figure 2). Growth rates on cyanobacteria supplemented with stigmasterol ($g = 0.30\ d^{-1}$), sitosterol ($g = 0.32\ d^{-1}$), ergosterol ($g = 0.32\ d^{-1}$), and 7-dehydrocholesterol ($g = 0.30\ d^{-1}$) were highest and significantly different from growth rates with the other treatments (Tukey's HSD, $P < 0.05$). Supplementation with desmosterol also led to a high growth rate ($g = 0.28\ d^{-1}$), but was significantly lower than the growth rates obtained with sitosterol, ergosterol, and 7-dehydrocholesterol. Supplementation with cholesterol had a less-pronounced effect on growth ($g = 0.24\ d^{-1}$) than supplementation with the sterols mentioned above. Dihydrocholesterol ($g = 0.09\ d^{-1}$) and lanosterol ($g = 0.08\ d^{-1}$) did not improve growth, compared to growth of animals reared on unsupplemented *S. elongatus* (dihydrocholesterol, $P = 0.92$; lanosterol, $P = 1$). Negative growth rates were observed after supplementation with allocholesterol ($g = -0.03\ d^{-1}$).

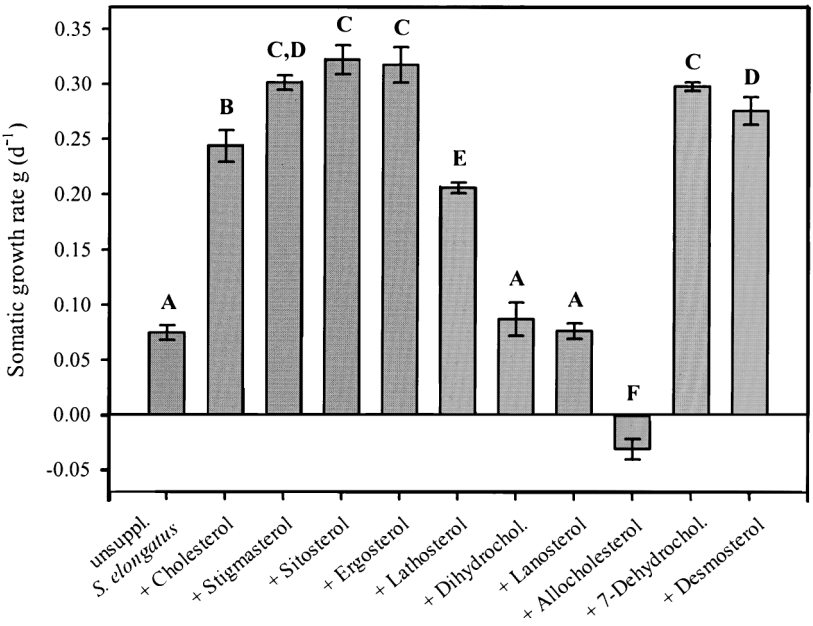

FIG. 2. Somatic growth of *Daphnia galeata* reared on *Synechococcus elongatus* unsupplemented and supplemented with single sterols. Data are means of three replicates per treatment; error bars indicate SD. Bars labeled with the same letters are not significantly different (Tukey's HSD, $P < 0.05$ following ANOVA).

Clutch sizes exhibited almost the same pattern as the growth rates (Figure 3). However, supplementation with 7-dehydrocholesterol led to the highest clutch size of 2.4 eggs per individual, whereas the growth rate obtained with 7-dehydrocholesterol did not differ from those obtained after supplementation with stigmasterol, sitosterol, and ergosterol. *D. galeata* fed on *S. elongatus* supplemented with stigmasterol produced 1.5 eggs per individual, which is significantly less than animals fed *S. elongatus* supplemented with sitosterol and ergosterol (Tukey's HSD, $P < 0.05$ following ANOVA, $F_{7,16} = 106$; $P < 0.001$). Although dihydrocholesterol did not improve growth, *D. galeata* did produce eggs in this treatment, with a clutch size of 0.2 eggs per individual. Animals kept on a diet supplemented with lanosterol or allocholesterol and animals fed pure *S. elongatus* did not produce eggs within the 4-d experiment.

Sterol Analysis. No sterols other than the supplemented sterols were detected in *S. elongatus*, which indicated that the supplemented sterols were not metabolically converted in the cyanobacterium. After feeding *D. galeata* 4 d on supplemented *S. elongatus*, all supplemented sterols could be detected in the animals (Figure 4), but the amounts per individual differed (ANOVA, $F_{9,20} = 45.5$;

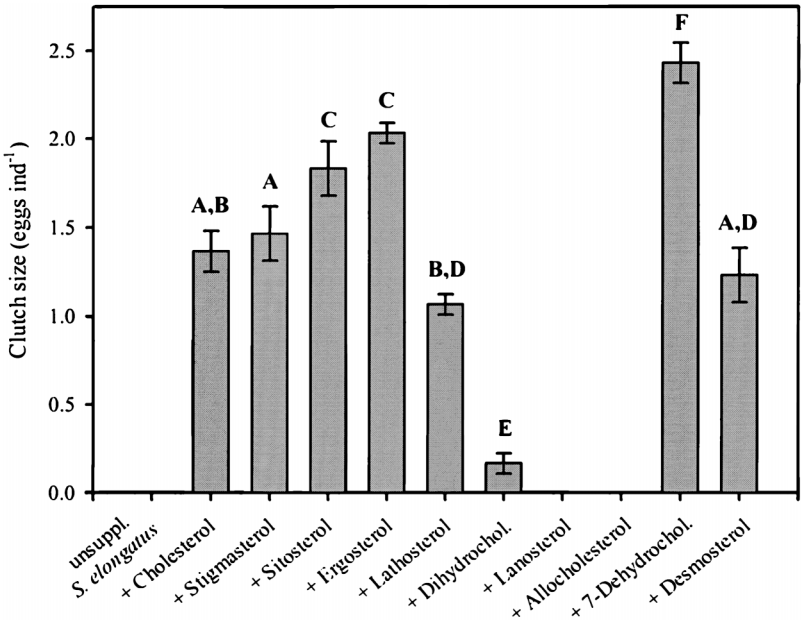

FIG. 3. Number of eggs of the first clutch of *Daphnia galeata* feeding on *Synechococcus elongatus* unsupplemented and supplemented with single sterols. Data are means of three replicates per treatment; error bars indicate SD. Bars labeled with the same letters are not significantly different (Tukey's HSD, $P < 0.05$ following ANOVA).

$P < 0.001$). Cholesterol was the main sterol found in *D. galeata* in all experimental treatments. *D. galeata* fed on *S. elongatus* supplemented with cholesterol had a higher cholesterol content than animals grown on unsupplemented *S. elongatus* (Figure 4). The amounts of supplemented sitosterol, dihydrocholesterol, lanosterol, and 7-dehydrocholesterol in *D. galeata* were higher than the amounts of supplemented stigmasterol, ergosterol, and allocholesterol in the animals. Only small amounts of lathosterol and desmosterol were detected in *D. galeata* reared on food supplemented with these sterols.

Immediately prior to the experiments, newborn animals were raised for 2 d on the green alga *Scenedesmus obliquus*. In addition to cholesterol, small amounts of the three major phytosterols of *S. obliquus* (Von Elert et al., 2003) were detected in these animals. Although no cholesterol was found in *S. obliquus*, the cholesterol content of *D. galeata* increased after growth for an additional 4 d on the green alga (Table 2), which indicated that the phytosterols present in *S. obliquus* were converted to cholesterol. In contrast, the cholesterol content of *D. galeata* decreased when the 2 d-old animals were fed an additional 4 d on the

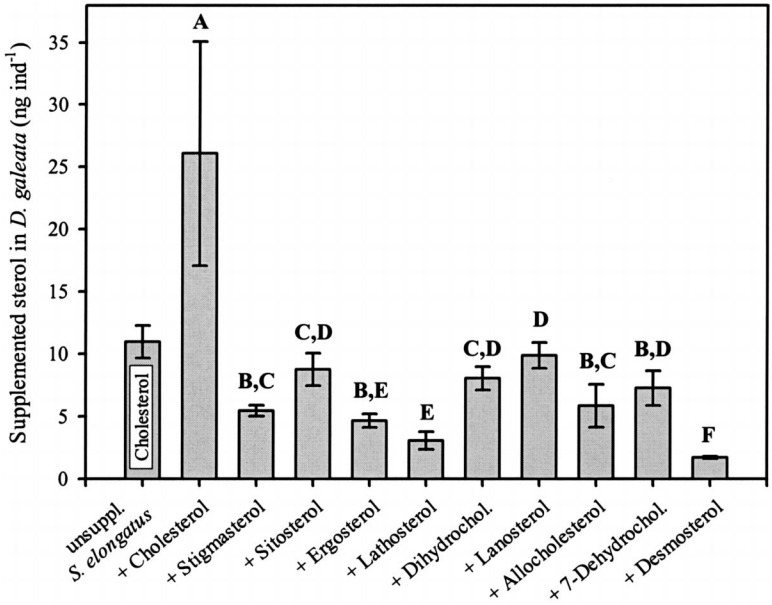

FIG. 4. Sterol content in *Daphnia galeata*, grown on *Synechococcus elongatus* unsupplemented and supplemented with single sterols. At the end of the experiment animals were analyzed for the content of the supplemented sterol. For animals grown on unsupplemented *S. elongatus*, the cholesterol content is given. 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, $F_{9,20} = 45.5$; $P < 0.001$).

unsupplemented cyanobacterium *S. elongatus* (Table 2). With the assumption that cholesterol in *D. galeata* arises from the conversion of dietary sterols, the sterol-free cyanobacterium *S. elongatus* was supplemented with single sterols, and the effect of the supplemented sterols on the cholesterol content of *D. galeata* was

TABLE 2. CHOLESTEROL CONTENT OF *Daphnia galeata* AT THE AGE OF 2 AND 6 DAYS

Food regime	Cholesterol content (ng ind^{-1}) $\pm$ SD
2 days on *Scenedesmus obliquus*	22.55 ± 0.57
6 days on *Scenedesmus obliquus*	54.52 ± 9.44
2 days on *Scenedesmus obliquus*/ 4 days on *Synechococcus elongatus*	10.96 ± 1.30

[a] The animals either were reared continuously on *Scenedesmus obliquus* or were fed with *Synechococcus elongatus* after the second day.

All cholesterol contents were significantly different (Tukey's HSD following ANOVA $F_{2,6} = 132$; $P < 0.001$). Means values of three replicates per treatment are given.

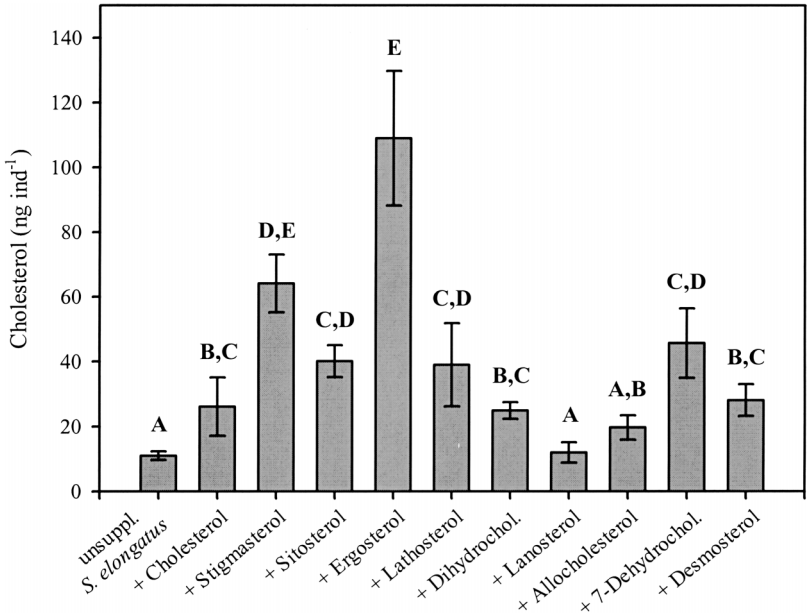

FIG. 5. Cholesterol content of *Daphnia galeata* reared on *Synechococcus elongatus* unsupplemented and supplemented with single sterols. Data are means of three replicates per treatment; error bars indicate SD. Bars labeled with the same letters are not significantly different (Tukey's HSD, $P < 0.05$ following ANOVA, $F_{10,22} = 30.6$; $P < 0.001$).

examined (Figure 5). Animals fed *S. elongatus* supplemented with ergosterol had a tenfold higher cholesterol content (109 ng ind^{-1}) than animals grown on unsupplemented *S. elongatus* (11 ng ind^{-1}). Supplementation of cyanobacteria with ergosterol or stigmasterol led to a higher content of cholesterol in the daphnids than supplementation with cholesterol itself. Supplementation of the cyanobacterial food with sitosterol, lathosterol, dihydrocholesterol, 7-dehydrocholesterol, or desmosterol also increased the cholesterol content of *D. galeata*, which indicated that these sterols were also converted to cholesterol. Supplementation with lanosterol and allocholesterol, on the other hand, did not affect the cholesterol content of the daphnids, which suggested that neither of these sterols could be used as a cholesterol precursor by the animals.

DISCUSSION

The crustaceans examined to date are incapable of synthesizing sterols de novo—they require a dietary source of sterols to meet their basic physiological

demands. In a recently published study, we have shown that poor somatic growth of *Daphnia galeata* on *Synechococcus elongatus* is due to the lack of sterols in the cyanobacterium (Von Elert et al., 2003). Supplementation of *S. elongatus* with cholesterol improved the growth of the animals, which indicates that growth of *D. galeata* was limited by cholesterol. Since herbivorous crustaceans do not find sufficient amounts of cholesterol in their diet, they need to assimilate available dietary sterols and convert them to cholesterol (Ikekawa, 1985). Eukaryotic phytoplankton usually contain a variety of sterols that can be distinguished from cholesterol by their chemical structure. These sterols are often characterized by additional substituents or by the position and/or number of double bonds in the side chain or in the sterol nucleus (Piironen et al., 2000).

All supplemented sterols were detected in *D. galeata,* which indicates that they were assimilated by the animals. Although single sterols were found in relatively small amounts in *D. galeata*, the amounts were too high to be exclusively derived from ingested *S. elongatus* in the gut of the animals. In cases in which only small amounts of a single supplemented sterol were found in *D. galeata,* an increased cholesterol content of the animals was observed. In contrast, when a supplemented sterol was found in higher amounts in the animals, the cholesterol content was not affected. These two patterns provide evidence for which of the supplemented sterols can be converted to cholesterol by *D. galeata*.

In animals that are capable of synthesizing cholesterol de novo, the cyclization of squalene leads to lanosterol. Lanosterol differs from cholesterol by having additional C-4 dimethyl and C-14 methyl substituents and by the location of the double bond (Δ^8) in the sterol nucleus (Figure 1). Supplementation of cyanobacteria with lanosterol did not affect growth rates and clutch sizes of *D. galeata*. Furthermore, no increase in the cholesterol content of the animals was observed. The biochemical conversion of lanosterol to cholesterol involves the loss of the methyl groups, the removal of the Δ^8 double bond, and the introduction of a double bond at Δ^5. The above findings demonstrate that *D. galeata* lacks the enzymatic ability to convert Δ^8 sterols to cholesterol. Notwithstanding our findings, the conversion of Δ^8 sterols to cholesterol was hypothesized by Harvey et al. (1987) in the marine copepod *Calanus*. This suggests taxon specific differences in the structural requirements of dietary sterols for crustaceans.

The phytosterols sitosterol and stigmasterol differ from cholesterol in having an ethyl group at C-24, and stigmasterol has an additional double bond at Δ^{22} in the side chain (Figure 1). Sitosterol and stigmasterol are commonly found in higher plants and are also present in a number of microalgae (Volkman, 2003). The synthesis of cholesterol from these sterols requires a dealkylation at C-24. An efficient phytosterol C-24-dealkylating system is found in various crustacea (Ikekawa, 1985). Teshima (1971) has described the bioconversion of sitosterol to cholesterol in the prawn *Penaeus japonicus* using ^{14}C-labeled sitosterol. Our findings that food supplemented with sitosterol or stigmasterol led to an increased

cholesterol content of the animals indicates that a 24-dealkylation also occurs in *D. galeata*. Furthermore, *D. galeata* seems to be capable of saturating the additional Δ^{22} bond of stigmasterol during its transformation to cholesterol. However, sitosterol and stigmasterol improved growth more efficiently than cholesterol, which might indicate that *D. galeata* is also able to use these sterols directly without the circuitous synthesis of cholesterol and that these sterols play a yet unknown role in the metabolism of *D. galeata*.

Supplementation of cyanobacteria with desmosterol stimulated growth and egg production of *D. galeata* and increased the cholesterol content of the animals, which demonstrates that the ability to transform desmosterol to cholesterol is also present in *D. galeata*. The $\Delta^{5,24}$ diene desmosterol (Figure 1) is the terminal intermediate in the conversion of plant sterols (e.g., sitosterol and stigmasterol) to cholesterol in insects (Svoboda and Thompson, 1985). A Δ^{24} sterol reductase that reduces the double bond in the side chain, thereby converting desmosterol to cholesterol, has been found in the tobacco hornworm, *Manduca sexta* (Svoboda and Thompson, 1985). Experiments with labeled sterols have shown that the prawn *P. japonicus* also possesses the ability to use desmosterol as a precursor for the synthesis of cholesterol (Teshima and Kanazawa, 1973).

D. galeata is able to convert $\Delta^{5,7}$ sterols to Δ^{5} sterols, as evidenced by the large increase in the cholesterol content of the animals after supplementation of the food with 7-dehydrocholesterol ($\Delta^{5,7}$). 7-Dehyrocholesterol is found in the hemolymph and in particular in Y-organs of crustaceans, where molting hormones are synthesized (Lachaise et al., 1989; Rudolph et al., 1992). In many insects, 7-dehydrocholesterol is an intermediate in the transformation of cholesterol to ecdysteroids (Rees, 1985). Several studies suggest that 7-dehydrocholesterol is formed irreversibly from cholesterol in isolated prothoracic glands (Grieneisen, 1994). Here, we showed that a transformation of 7-dehydrocholesterol to cholesterol occurs in the cladoceran *D. galeata*. Assuming that cholesterol is the key sterol in crustaceans, it is surprising that 7-dehydrocholesterol improved the growth of the daphnids more efficiently than cholesterol. Synthesis of ecdysteroids from cholesterol requires the introduction of a Δ^{7} bond into the sterol nucleus, which is not necessary in the direct conversion of 7-dehydrocholesterol to ecdysteroids. The conversion of labeled 7-dehydrocholesterol to labeled ecdysteroids has been demonstrated by injection experiments with various insect species (see Grieneisen, 1994) and by incubation of fractionated Y-organs of the crab *Menippe mercenaria* with the sterol (Rudolph and Spaziani, 1992). Presumably, *D. galeata* is also capable of utilizing 7-dehydrocholesterol as a direct precursor of ecdysteroids. Increased clutch sizes relative to the growth rates showed that 7-dehydrocholesterol effectively supported egg production. Although it is generally assumed that effects of food quantity on somatic growth and on reproduction are highly correlated in juvenile *Daphnia* (Lampert and Trubetskova, 1996), it has been suggested that limitation by food quality might affect somatic growth and reproduction differently.

This has been shown for mineral (Urabe and Sterner, 2001) and biochemical (Becker and Boersma, 2003) aspects of food limitation. In accordance with these findings, sterols seem to differ in their allocation to somatic growth or reproduction. Further detailed investigations of sterol effects on life history are needed to reveal how these differences in allocation lead to differences in effects on fitness.

Supplementation of cyanobacteria with ergosterol resulted in a tenfold higher cholesterol content of *D. galeata* than in animals fed unsupplemented food. *D. galeata* is, therefore, capable of converting dietary ergosterol to cholesterol. Ergosterol, a $\Delta^{5,7,22}$ sterol, is found in most fungi, yeast, and in some species of green algae (Nes and McKean, 1977; Akihisa et al., 1992; Petkov and Kim, 1999). Ergosterol differs from 7-dehydrocholesterol in having an additional double bond at Δ^{22} in the side chain (Figure 1). Growth rates on food supplemented with ergosterol were as high as the growth rates reached with 7-dehydrocholesterol, sitosterol, and stigmasterol. The conversion of ergosterol to cholesterol requires the saturation at Δ^{7} in the sterol nucleus, as described for 7-dehydrocholesterol, as well as saturation at Δ^{22} in the side chain, as described for stigmasterol. Only small amounts of ergosterol were detected in animals reared on ergosterol-supplemented food, which points to high metabolic transformation rates. Teshima and Kanazawa (1971b) have described the bioconversion of ergosterol to cholesterol in *Artemia salina* fed on ^{14}C-labeled *Euglena gracilis*. The ability to saturate the Δ^{5} bond of a $\Delta^{5,7}$ diene, as discussed for 7-dehydrocholesterol, might also enable the direct conversion of ergosterol to ecdysteroids.

Supplementation with dihydrocholesterol, a completely saturated molecule (Δ^{0}), did not affect somatic growth of *D. galeata*, as compared with unsupplemented cyanobacteria, which indicates that a double bond in ring B is required for the conversion of dietary sterols to cholesterol (Figure 1). In contrast to somatic growth, egg production of the daphnids was positively affected by supplementation with dihydrocholesterol, which indicated the potential significance of sterols for reproduction. We are aware of only one example of the oxidation of a Δ^{0} sterol to a Δ^{5} sterol in arthropods: the firebrat, *Thermobia domestica*, is capable of synthesizing cholesterol from dihydrocholesterol (Svoboda and Thompson, 1985). Harvey et al. (1987) documented that ring-saturated stanols are poorly assimilated and that they pass unaltered through the gut of the marine copepod *Calanus*. In this study, we found significant amounts of the supplemented dihydrocholesterol in daphnid tissues, which indicates the assimilation of this stanol.

Allocholesterol and lathosterol differ from cholesterol in the position of the double bond in the sterol nucleus (Figure 1). Somatic growth of *D. galeata* was negatively affected by the supplementation with allocholesterol. A relocation of a double bond from Δ^{4} to Δ^{5}, as required for the conversion of allocholesterol to cholesterol, seems improbable; however, we cannot exclude that a toxic effect of allocholesterol masked the enzymatic abilities of the animals. Supplementation with lathosterol increased the cholesterol content of the animals, which indicated

that lathosterol was converted to cholesterol. The conversion of lathosterol to cholesterol requires a shift of a double bond from Δ^7 to Δ^5, possibly via a $\Delta^{5,7}$ intermediate, as work with mammals has shown (Nes and McKean, 1977). Prahl et al. (1984) found that, compared with Δ^5 and $\Delta^{5,7}$ sterols, Δ^7 sterols were not readily removed during passage through the gut of the copepod *Calanus*. They speculated that dietary Δ^7 sterols can be used as precursors of ecdysteroids and that the poor assimilation of these sterols provides a mechanism to avoid a haphazard production of molting hormones. Alternatively, Prahl et al. (1984) suggested that *Calanus* simply lacks the ability to convert Δ^7 to $\Delta^{5,7}$ sterols and, therefore, the Δ^7 components are only poorly assimilated. The results of this study indicate that the Δ^7 sterol lathosterol was assimilated by *D. galeata* and converted to cholesterol. However, the observed growth rates were lower than those reached with food supplemented with cholesterol. The step Δ^7 to $\Delta^{5,7}$ involves the introduction of a double bond at Δ^5, which might be costly in terms of energy and, therefore, might be responsible for the lower growth rates as compared to those reached with supplementation with cholesterol.

Although this study shows that certain dietary sterols improve the somatic growth of *D. galeata*, there must be other factors that become limiting for the growth and reproduction of the herbivore, when the animals are released from sterol limitation. The maximal growth rates on sterol-supplemented *Synechococcus* ($g = 0.32\ \mathrm{d}^{-1}$) were below the almost maximal possible growth rate ($g = 0.5\ \mathrm{d}^{-1}$) of *D. galeata* fed on the green alga *Scenedesmus obliquus* (Wacker and Von Elert, 2001). Von Elert et al. (2003) already showed that the growth on cholesterol-supplemented *Synechococcus* was further improved by additional supplementation with PUFAs. Beside sterols and PUFAs there might be additional factors that determine the nutritional value of this coccal cyanobacterium to a lower extent.

Results derived from laboratory experiments are indispensable for determining the requirements of zooplankton species for single biochemical compounds, such as sterols, and provide a first step toward assessing the ecological relevance of these compounds under field conditions. During cyanobacterial blooms, the sterol content of the food will be low since only traces of sterols are found in prokaryotes (Hai et al., 1996; Volkman, 2003). This is corroborated by the observation that the total lipid levels (with sterols as a dominant lipid class) of *Daphnia pulex* from a hypereutrophic lake are at their lowest concentration during the height of the yearly *Aphanizomenon flos-aquae* bloom (Arts et al., 1992). In a previous laboratory study, we have shown that the absence of sterols constrains the carbon transfer between cyanobacteria and *D. galeata* (Von Elert et al., 2003). Compared to cyanobacteria, which do not provide sterols in sufficient amounts, eukaryotic phytoplankton contain a large variety of sterols (Nes and McKean, 1977; Volkman, 2003). However, specific phytoplankton classes or even single species could still be deficient in sterols suitable for supporting zooplankton growth.

If such species dominate the phytoplankton, sterol limitation of growth of *Daphnia* is possible. Thus, high levels of unsuitable sterols could adversely affect growth and reproduction of *Daphnia*, and can, therefore, be responsible for reduced fecundity and, projected at the population level, for reduced population growth.

In the field, sterols of phytoplankton can be subjected to transformation prior to their ingestion by the herbivorous crustaceans. Klein Breteler et al. (1999) have suggested that the poor quality of the chlorophycean *Dunaliella* for the development of marine copepods is due to a sterol deficiency of the alga. Furthermore, they have demonstrated that the chlorophycean food is biochemically upgraded by the heterotrophic dinoflagellate *Oxyrrhis marina* to high-quality copepod food. This trophic upgrading of food quality by an intermediary protozoan is attributed to sterol production in the dinoflagellate. The Δ^7 sterols present in *Dunaliella* do not support development of the copepods, whereas a rapid development of the copepods to the adult stage is observed when fed on *Oxyrrhis marina*, which contains primarily Δ^5 sterols. This example shows that unsuitable sterols in eukaryotic algae can constrain the development of herbivorous crustaceans. Intermediary grazers, such as protozoa, might biochemically upgrade such unsuitable phytoplankton species by adding more suitable sterols to the dietary carbon, thus determining the transfer efficiency of carbon from the microbial loop to metazoan grazers in natural systems.

In summary, this study provides evidence that sterols are essential dietary compounds that significantly affect growth and reproduction of *D. galeata*. Furthermore, the results showed that *D. galeata* is capable of converting dietary sterols to cholesterol, depending on their chemical structure. Particularly, Δ^5 and $\Delta^{5,7}$ sterols met the nutritional requirements of the animals, while the Δ^7 sterol lathosterol supported growth to a significantly lower extent than cholesterol. Dihydrocholesterol (Δ^0) and lanosterol (Δ^8) did not improve the growth of *D. galeata*, and growth was adversely affected by the Δ^4 sterol allocholesterol. Hence, structural features, particularly the configuration of the sterol nucleus, determine the nutritional value of dietary sterols. In insects, the pattern of sterol metabolism is by no means ubiquitous, and the nutritional dependency on specific sterols described for *D. galeata* might not be valid for crustaceans in general. In order to assess the ecological significance of certain sterols as potentially limiting biochemical resources, further detailed studies are required to reveal pathways and potential intermediates of sterol synthesis with regard to the nutritional requirements of freshwater zooplankton species. Von Elert et al. (2003) have already suggested that sterols could play a key role in determining carbon transfer efficiency from primary producers to herbivorous zooplankton. Here, we suggest that, in addition to low dietary sterol levels, the quality of dietary sterols could strongly affect the assimilation of dietary carbon.

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