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Supplementation with Sterols Improves Food Quality of a Ciliate for *Daphnia magna*

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Experimental results provide evidence that trophic interactions between ciliates and *Daphnia* are constrained by the comparatively low food quality of ciliates. The dietary sterol content is a crucial factor in determining food quality for *Daphnia*. Ciliates, however, presumably do not synthesize sterols de novo. We hypothesized that ciliates are nutritionally inadequate because of their lack of sterols and tested this hypothesis in growth experiments with *Daphnia magna* and the ciliate *Colpidium campylum.* The lipid content of the ciliate was altered by allowing them to feed on fluorescently labeled albumin beads supplemented with different sterols. Ciliates that preyed upon a sterol-free diet (bacteria) did not contain any sterols, and growth of *D. magna* on these ciliates was poor. Supplementation of the ciliates' food source with different sterols led to the incorporation of the supplemented sterols into the ciliates' cells and to enhanced somatic growth of *D. magna*. Sterol limitation was thereby identified as the major constraint of ciliate food quality for *Daphnia*. Furthermore, by supplementation of sterols unsuitable for supporting *Daphnia* growth, we provide evidence that ciliates as intermediary grazers biochemically upgrade unsuitable dietary sterols to sterols appropriate to meet the physiological demands of *Daphnia*.

Key words: albumin beads; cholesterol; *Colpidium campylum*; dihydrocholesterol; lathosterol; trophic upgrading.

Introduction

Reports on trophic interactions between ciliates and *Daphnia* are controversial. Field experiments have indicated that ciliates are suppressed during population peaks of *Daphnia* by facing a substantial grazing pressure (Carrick et al. 1991; Marcheassault and Mazumder 1997; Pace and Funke 1991; Wickham and Gilbert 1991; Zöllner et al. 2003), and several laboratory studies have revealed that cladocerans effectively prey on ciliates up to a certain size, which implies that daphnids are important ciliate predators (Jack and Gilbert 1993; Porter et al. 1979; Sanders et al. 1996). However, although rich in nitrogen and phosphorus, ciliates are less nutritious for daphnids than many algae (Bec et al. 2003; DeBiase et al. 1990; Sanders et al. 1996; Wickham et al. 1993).

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Recently, the dietary sterol content has been identified as a crucial factor in determining food quality for *Daphnia* (Martin-Creuzburg and Von Elert 2004, 2005a; Von Elert et al. 2003). Available data suggest that ciliates lack the ability to synthesize sterols de novo (e.g. Conner et al. 1968; Harvey and McManus 1991; Harvey et al. 1997; Klein Breteler et al. 2004; Martin-Creuzburg et al. 2005b), which led us to hypothesize that the observed nutritional inadequacy of ciliates is caused by their lack of sterols. This hypothesis is complicated by the finding that ciliates are able to incorporate exogenously supplied sterols into cell membranes and to metabolize them to various sterols (Conner et al. 1968; Harvey and McManus 1991; Harvey et al. 1997). In the absence of exogenous sterols, ciliates produce the pentacyclic triterpenoid alcohol tetrahymanol and/or hopanoids, which are functionally equivalent to sterols as structural components of cell membranes (Conner et al. 1968; Harvey and McManus 1991; Martin-Creuzburg et al. 2005b). Hence, the occurrence of sterols in ciliates, and thereby the food quality of the ciliates for higher trophic levels might depend on the food source preyed upon by the ciliates, i.e. ciliates feeding on a sterol-free diet (most prokaryotes; Volkman 2003) are expected to produce tetrahymanol and/or hopanoids, and ciliates feeding on eukaryotic food sources are expected to incorporate dietary sterols, a process that might be associated with changes in the structure of the sterols via metabolism. Since sterols differ in their suitability to support *Daphnia* growth (Martin-Creuzburg and Von Elert 2004), these structural changes might also affect the food quality of ciliates for *Daphnia*.

Here, we assessed the importance of sterols in determining the food quality of the ciliate *Colpidium campylum* for *Daphnia magna. D. magna* was reared on the ciliate *C. campylum,* whose lipid content was altered by allowing them to feed on fluorescently labeled albumin beads supplemented with either cholesterol, lathosterol, or dihydrocholesterol. Cholesterol, the principal sterol in *Daphnia* (Martin-Creuzburg and Von Elert 2004), was used because it is expected to meet the physiological demands of the cladocerans. As lathosterol and dihydrocholesterol are less efficiently used by daphnids (Martin-Creuzburg and Von Elert 2004), they were selected to examine the potential of *C. campylum* to biochemically upgrade dietary sterols for *Daphnia*.

Results and Discussion

The sterol-supplemented albumin beads were readily ingested by *Colpidium campylum*. The ingestions rates and the proportions of labeled cells were not affected by supplementation with sterols (ANOVA, $F_{3,8} = 2.14$; $p = 0.17$ and $F_{3,8} = 3.46$; $p = 0.07$, respectively; Table 1). In each treatment, approximately 50—60% of the ciliate cells were fluorescently labeled. Unattached sterols can also be incorporated directly from the culture medium (e.g. Harvey et al. 1997; Nes et al. 1981); this could have further increased the sterol loading of the ciliate cells.

Results obtained from experiments with the ciliate *Tetrahymena pyriformis* suggest that the incorporation of supplemented sterols into cell membranes results in a reduced volume of the ciliate cells, possibly because of an increased ordering of the membrane components (Conner et al. 1982). According to Jack and Gilbert (1993), larger ciliates are less susceptible to *Daphnia* predation, and clearance rates increase with decreasing ciliate size; hence, a reduced cell volume of *C. campylum* might facilitate the ingestion process. Although the incorporation of supplemented sterols into ciliate cell membranes slightly reduced the cell volume of the protists (Table 2), this effect was not significant (Tukey's HSD, $p > 0.05$), and therefore considered to be negligible. It should be mentioned that daphnids (*Daphnia pulex*) can ingest ciliates much larger than *C. campylum*, and among various ciliates tested, *T. pyriformis,* a ciliate similar in size to *C. campylum,* was most vulnerable to *Daphnia* predation (Jack and Gilbert 1993).

Growth rates of *D. magna* were significantly affected by the food supplied in the growth experiment (ANOVA, $F_{5,12} = 258.6$; $p < 0.001$; Fig. 1). Growth of *D. magna* on *C. campylum* did not differ from growth on *C. campylum* previously fed with unsupplemented albumin beads (Tukey's HSD, $p = 0.22$). Supplementation of the albumin beads with any one of the three sterols prior to feeding to *C. campylum* improved the growth of *D. magna* (Tukey's HSD, $p < 0.05$). Supplementation with lathosterol had a lower ameliorating effect on growth than supplementation with cholesterol or dihydrocholesterol.

Several sterols were detected in the wheat grains (dominated by sitosterol, campesterol and its stanols, which are commonly found in wheat grains, e.g. Takatsuto et al. 1999) used here to enhance bacterial growth, but no sterols were detected in the bacterial fraction of the ciliate

Table 1. Ingestion of unsupplemented or sterol-supplemented albumin beads by *Colpidium campylum*. Values are means of $n = 3$; CI in parentheses (−95%; +95%).

	Number (beads cell^{-1})	Range (beads cell^{-1})	Labeled cells (%)	Ingestion rate (beads h^{-1} cell^{-1})
C. campylum+unsupplemented beads	0.99 (0.67; 1.32)	0—5	61.11 (54.79; 67.44)	2.98 (2.00; 3.96)
C. campylum+cholesterol-supplemented beads	1.06 (0.52; 1.61)	0—4	63.89 (46.65; 81.13)	3.19 (1.57; 4.82)
C. campylum+lathosterol-supplemented beads	0.77 (0.43; 1.11)	0—4	51.67 (40.72; 62.62)	2.32 (1.29; 3.34)
C. campylum+dihydrocholesterol-supplemented beads	1.12 (0.58; 1.67)	0—6	60.56 (48.60; 72.51)	3.37 (1.73; 5.01)

cultures. *C. campylum* cells grown without sterol-supplemented beads did not contain any polycyclic alcohols except tetrahymanol (gammaceran-3β-ol) and diplopterol (hopan-22-ol), which suggests that a dietary source of sterols for *C. campylum* was not available.

Sterols provided by the supplemented beads were incorporated and modified by *C. campylum* (Table 3). Moreover, the incorporation of supplemented sterols into cell membranes reduced the tetrahymanol and diplopterol content of the ciliate cells (Tukey's HSD, $p < 0.05$; ANOVA, $F_{4,10} = 31.61$ and $F_{4,10} = 40.59$; $p < 0.001$; Fig. 2). The inhibition of tetrahymanol production by exogenously supplied sterols has been extensively studied in *T. pyriformis*, an experimental model ciliate related to *C. campylum* (Class Oligohymenophorea, Subclass Hymenostomatia; e.g. Strüder-Kypke et al. 2000). Thereby, cholesterol, lathosterol, dihydrocholesterol, and several other sterols have been shown to inhibit tetrahymanol and diplopterol production (e.g. Conner et al. 1969, 1978).

Supplemented cholesterol was accumulated in high amounts in *C. campylum* cells and metabolized to (22*E*)-cholesta-5,22-dien-3β-ol and (22*E*)-cholesta-5,7,22-trien-3β-ol (Table 3). The desaturation of cholesterol to (22*E*)-cholesta-5,7,22-trien-3β-ol via (22*E*)-cholesta-5,22-dien-3β-ol has been studied in detail in *Tetrahymena* spp. (e.g. Conner et al. 1969; Nusblat et al. 2005). Sterols containing double bonds at Δ^5, $\Delta^{5,22}$, or $\Delta^{5,7,22}$ enhance the growth of *Daphnia* on a sterol-free diet (Martin-Creuzburg and von Elert 2004). Therefore, the improved growth of *D. magna* on cholesterol-supplemented *C. campylum* (Fig. 1) can be attributed to the accumulation of these sterols in the ciliate cells.

The accumulation of supplemented lathosterol in *C. campylum* was comparatively low (Table 3). In *C. campylum*, lathosterol was metabolized to (22*E*)-cholesta-7,22-dien-3β-ol and finally to (22*E*)-cholesta-5,7,22-trien-3β-ol; the same end product found for cholesterol metabolism. Lathosterol is also efficiently converted to (22*E*)-cholesta-5,7,22-trien-3β-ol in *T. pyriformis* (Mulheirn et al. 1971). Growth of *D. magna* on lathosterol-supplemented *C. campylum* was enhanced, but ranked below the growth on cholesterol-supplemented ciliates (Fig. 1). The Δ^7-sterol lathosterol has been previously shown to support *Daphnia* growth to a significantly lower extent than Δ^5-sterols (Martin-Creuzburg and von Elert 2004). Therefore, the lower growth of *D. magna* on lathosterol-supplemented than on cholesterol- or

Table 2. Size of *Colpidium campylum* grown on bacteria, bacteria and albumin beads, or bacteria and albumin beads supplemented with cholesterol, lathosterol, or dihydrocholesterol. Values are means of $n = 3$; CI in parentheses (−95%; +95%).

	Length (μm)	Width (μm)	Volume (μm^3)
C. campylum	41.8 (40.7; 43.0)	19.7 (15.5; 24.0)	8515 (5010; 12,022)
C. campylum+unsupplemented beads	40.6 (38.8; 42.4)	19.0 (17.8; 20.2)	7683 (6496; 8871)
C. campylum+cholesterol-supplemented beads	38.9 (37.3; 40.5)	17.9 (14.2; 21.6)	6544 (3717; 9371)
C. campylum+lathosterol-supplemented beads	39.8 (35.8; 43.8)	17.1 (13.8; 20.4)	6842 (4308; 7899)
C. campylum+dihydrocholesterol-supplemented beads	38.2 (35.9; 40.3)	17.5 (14.2; 19.8)	6088 (5263; 6913)

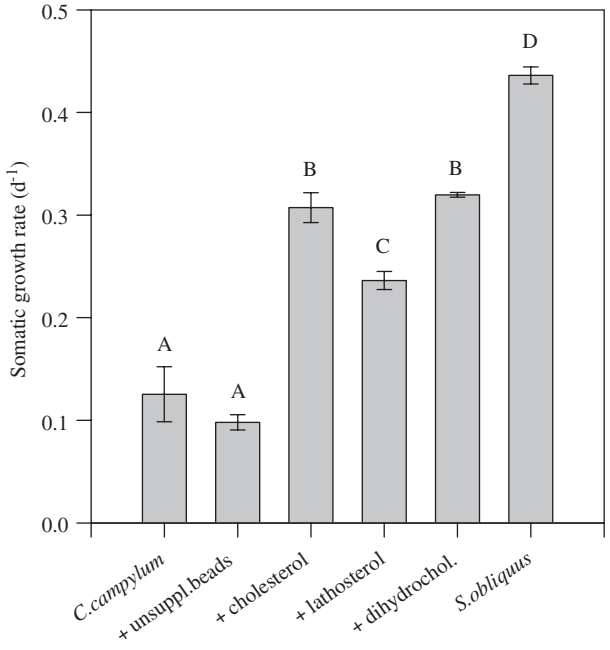

Figure 1. Juvenile somatic growth rates of *Daphnia magna* grown on *Colpidium campylum* previously fed with bacteria (bar labeled with *C. campylum*), bacteria and unsupplemented albumin beads, or bacteria and albumin beads supplemented with cholesterol, lathosterol, or dihydrocholesterol. Growth of *D. magna* on *Scenedesmus obliquus* is given as a 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).

dihydrocholesterol-supplemented *C. campylum* might be due to (1) the higher content of Δ^7-sterols, which are possibly less efficiently used by daphnids, or (2) to an overall lower sterol content of the ciliates that is insufficient in releasing *Daphnia* from sterol limitation (Table 3). Klein Breteler et al. (1999) have suggested that the heterotrophic dinoflagellate *Oxyrrhis marina*, as an intermediary grazer, might upgrade a poor-quality food source containing only Δ^7-sterols, which are not suitable for supporting the development of copepods, by producing Δ^5-sterols. Even if ciliates do not synthesize sterols de novo, they might upgrade the biochemically mediated food quality for higher trophic levels by accumulating and modifying dietary sterols, e.g. by converting Δ^7- to $\Delta^{5,7,22}$-sterols, which are highly efficient in supporting *Daphnia* growth (Martin-Creuzburg and Von Elert 2004).

High amounts of dihydrocholesterol were found in the ciliate cells supplemented with dihydrocholesterol, accompanied by even higher amounts of a sterol tentatively identified as 5α-cholest-8-en-3β-ol. This suggested an effective conversion by the ciliate of the completely saturated dihydrocholesterol to sterols unsaturated at Δ^8 in the sterol nucleus. Although lanosterol, a $\Delta^{8,24}$-sterol with additional C-4 dimethyl and C-14 methyl substituents, did not improve the growth of *Daphnia* when supplemented to a sterol-free diet (Martin-Creuzburg and Von Elert 2004), we propose that the Δ^8-sterol produced by the ciliate enhanced *Daphnia* growth. Differences in the suitability for *Daphnia* of lanosterol and the Δ^8-sterol found in the present study might be attributed to the additional methyl groups present in lanosterol, which affect important properties of the sterol molecule (Nes et al. 1978; Yeagle et al. 1977). The conversion of dietary Δ^8-sterols to cholesterol in copepods has already been proposed by Harvey et al. (1987). In contrast to *C. campylum*, *T. pyriformis* is capable of introducing a Δ^5, Δ^7, and Δ^{22} double bond into dihydrocholesterol, leading to the formation of

Table 3. Sterol content of *Colpidium campylum* grown on bacteria and albumin beads supplemented with cholesterol, lathosterol, or dihydrocholesterol and of *Scenedesmus obliquus* (values are means of $n = 3$; ± SD).

	Sterol (IUPAC and trivial name)	Sterol concentration ($\mu g\,mg\,C^{-1}$)
C. campylum+cholesterol-supplemented beads	Cholest-5-en-3β-ol (cholesterol)	10.54 ± 3.27
	(22*E*)-Cholesta-5,22-dien-3β-ol (22-dehydrochol.)	18.30 ± 4.73
	(22*E*)-Cholesta-5,7,22-trien-3β-ol	trace[a]
	Total	28.84 ± 7.99
C. campylum+lathosterol-supplemented beads	5α-Cholest-7-en-3β-ol (lathosterol)	3.08 ± 1.54
	(22*E*)-Cholesta-7,22-dien-3β-ol	1.05 ± 0.38
	(22*E*)-Cholesta-5,7,22-trien-3β-ol	2.66 ± 1.68
	Total	6.79 ± 3.59
C. campylum+dihydrocholesterol-supplemented beads	5α-Cholestan-3β-ol (dihydrocholesterol)	12.83 ± 6.53
	5α-Cholest-8-en-3β-ol[b]	17.91 ± 4.91
	Total	30.74 ± 11.44
S. obliquus	(24*E*)-5α-Poriferasta-7,22-dien-3β-ol (chondrillast.)	4.08 ± 0.59
	5α-Ergost-7-en-3β-ol (fungisterol)	1.57 ± 0.32
	5α-Poriferast-7-en-3β-ol (22-dihydrochondrillast.)	0.88 ± 0.22
	Total	6.53 ± 1.13

[a]Potentially underestimated because the retention time overlapped with that of cholesterol (cp. Valcarce et al. 2000).
[b]Tentative identification.

(22*E*)-cholesta-5,7,22-trien-3β-ol (Mulheirn et al. 1971). In both cases, however, this would lead to an upgrading of food quality by the ciliate since dihydrocholesterol itself does not support the growth of *Daphnia* (Martin-Creuzburg and Von Elert 2004).

A number of physiological effects of sterol incorporation into ciliate cell membranes have also been reported, e.g. a reduction in cellular volume (as described above) and an elevation in saturated fatty acids (Conner et al. 1982; Ferguson et al. 1975). Saturated fatty acids are important energy storage molecules. In this study, however, these fatty acids are unlikely to affect the performance of daphnids since food concentrations were well above the incipient limiting level (Lampert 1978). Therefore, these changes in the fatty acid composition have been neglected.

Sterols are often considered as indispensable structural components of eukaryotic cell membranes. However, there is experimental evidence that tetrahymanol, hopanoids, and other polyterpenoids are functionally equivalent to sterols in stabilizing phospholipid bilayers (Ourisson et al. 1987; Raederstorff and Rohmer 1988). Therefore, one could speculate that also in daphnids, tetrahymanol and related compounds can be incorporated into cell membranes and, to a certain extent, replace sterols as stabilizers of cell membranes. This would explain the observed intermediate food quality of ciliates for *Daphnia* when the ciliates are cultured on prokaryotic food sources (DeBiase et al. 1990; Sanders et al. 1996). In a previous study, we provided evidence for the ability of ciliates to biochemically upgrade a sterol-free diet for subsequent use by *Daphnia* (Martin-Creuzburg et al. 2005b), most likely by producing tetrahymanol and related compounds that at least partly released *Daphnia* from sterol limitation. Ederington et al. (1995) have already reported the assimilation of tetrahymanol in tissues (mainly in eggs) of a copepod (*Acartia tonsa*) when ciliates were offered as food, and have suggested that tetrahymanol is functionally equivalent to cholesterol in the crustacean, thereby maintaining minimal egg production. In addition to their role as structural components of cell membranes, sterols also serve as precursors for many bioactive molecules, such as ecdysteroids, which are involved in the process of molting (Goad

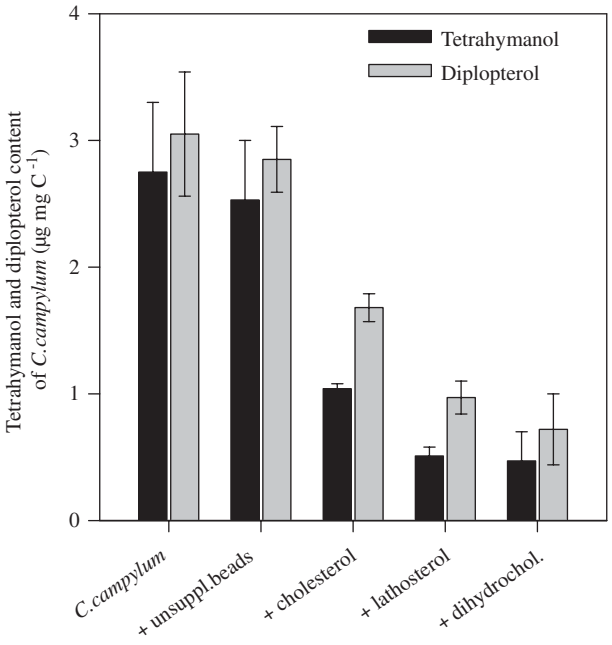

Figure 2. Tetrahymanol and diplopterol content of *Colpidium campylum* grown on bacteria (bar labeled with *C. campylum*), bacteria and unsupplemented albumin beads, or bacteria and albumin beads supplemented with cholesterol, lathosterol, or dihydrocholesterol. Data are means of three replicates per treatment; error bars indicate SD.

1981). Thus, it remains to be tested whether tetrahymanol and related compounds actually improve the performance of crustaceans, possibly by supplementation of these compounds to a sterol-free diet. However, the amounts of tetrahymanol and diplopterol found in the present study were too low to release *Daphnia* from sterol limitation, as indicated by the growth-enhancing effect of sterol supplementation.

Although the supplementation with sterols improved the food quality of *C. campylum* for *D. magna*, the achieved growth rates were significantly lower than those achieved with the green alga *Scenedesmus obliquus* (Fig. 1). According to Martin-Creuzburg et al. (2005a), chondrillasterol, fungisterol, and 22-dihydrochondrillasterol were identified as the principal sterols in *S. obliquus* (Table 3). The total sterol content of *S. obliquus* was low compared to that of the sterol-supplemented ciliates, which might indicate that *D. magna* was sufficiently supplied with sterols when grown on sterol-supplemented *C. campylum*. Moreover, this suggests that when sterol requirements are met, growth of *Daphnia* becomes limited by other factors such as n-3 polyunsaturated fatty acids (according to Von Elert et al. 2003), which are also absent in *C. campylum* (Martin-Creuzburg et al. 2005b).

Our data clearly show that the food quality of *C. campylum* for *Daphnia* is determined by the sterol availability in the diet of the ciliates. In the field, ciliates prey upon prokaryotic (bacteria, picocyanobacteria) and eukaryotic (picophytoplankton and heterotrophic nanoflagellates) food sources (e.g. Callieri and Stockner 2002; Jack and Gilbert 1993; Nakano et al. 2001; Šimek et al. 2000; Weisse 1993; Wickham et al. 1993). In the former case, ciliates do not have a dietary source of sterols since this lipid class is usually absent in prokaryotes (see the critical review of Volkman 2003). Here, we provide evidence that the food quality of a ciliate feeding on prokaryotes is comparatively low primarily because of the lack of sterols. However, in the absence of dietary sterols, ciliates produce tetrahymanol and related compounds that might functionally replace sterols as membrane reinforcers in the ciliates and even in *Daphnia*, thereby leading to an upgrading of the sterol-free food source (cf. Martin-Creuzburg et al. 2005b). Klein Breteler et al. (2004) did not find evidence for trophic upgrading of the eukaryote *Dunaliella* sp., which hardly contained any sterols, by the marine ciliate *Strombidium sulcatum* for copepods. In this ciliate, however, neither sterols nor tetrahymanol or other possible sterol surrogates were detected, which might explain its nutritional inadequacy. In contrast, feeding on sterol-containing food sources (most eukaryotes) would lead to the accumulation of dietary sterols in ciliates, and thereby to an increase in the food quality of the ciliates, as shown in the present study. Moreover, the predominance of unsuitable sterols is reduced since ciliates, as intermediary grazers, are capable of biochemically upgrading the dietary sterol composition by adding sterols to the dietary carbon which are more suitable for crustacean grazers.

Ciliates are abundant protists in freshwater ecosytems, and their importance as a trophic link between picoplanktonic production and higher trophic levels has often been recognized (e.g. Callieri and Stockner 2002; Gifford 1991; Porter et al. 1979; Weisse 1993). However, the data presented here suggest that the carbon transfer efficiency from ciliates to crustacean zooplankton might depend on the availability of sterols in the ciliates' food source. This might also explain the observed differences in the food quality of ciliates for metazoan grazers (Sanders and Wickham

1993) and the variability in the proposed significance of ciliates for transferring mass and energy via the microbial loop to higher trophic levels.

Methods

Cultivation and preparation of the food organisms: *Colpidium campylum* was obtained from the Laboratoire de Biologie des Protistes (Université Blaise Pascal, France). It was cultivated semi-continuously at 20 °C in mineral water (Volvic®) on an undefined bacterial assemblage. Exponential growth rates of the ciliates were maintained by exchanging 20—40% of the cultures with fresh medium every other day. Autoclaved wheat grains were added to enhance bacterial growth. Ciliate food suspensions for the *Daphnia magna* growth experiments were prepared by feeding *C. campylum* fluorescently labeled albumin beads (2 μm, Micromod, Rostock, Germany) supplemented with cholesterol, lathosterol, or dihydrocholesterol (Sigma). For the supplementation, 100 μl of a bead stock solution (2.4×10^9 beads ml^{-1} ethanol), which was previously sonicated for 10 min in an ultrasonic bath to eliminate clumping, was dissolved in 10 ml ethanol. Subsequently, 100 μl of a sterol stock solution (2.5 mg ml ethanol^{-1}) was added and incubated with beads for 30 min at 20 °C. The bead/sterol suspensions were evaporated to dryness under a stream of nitrogen. The loaded beads were resuspended in 10 ml mineral water and added to 200 ml of a ciliate suspension at a concentration of approximately 1500 cells ml^{-1}. Each *C. campylum* suspension was fed loaded beads 4 and 2 days before the ciliates were fed to *D. magna*.

For the growth experiments, ciliate cells were separated from bacteria and albumin beads by centrifugation (1600—1900 × *g*; 4 min) in special pear-shaped centrifuge tubes with a test tube projection from the bottom end. The ciliates were compressed into the test tube end; bacteria and albumin beads that remained in the fluid of the larger pear-shaped top were discarded. The ciliates that emerged from the bottom of the test tube, due to the negative geotaxis of the ciliates, were collected in a pipette and added to fresh medium. This procedure was repeated a total of 3 times. Subsequently, the ciliate suspensions were slowly filtered through a 12-μm membrane filter without vacuum, and retained cells on the filter were immediately resuspended in mineral water to obtain the protist food suspensions used in the *D. magna* growth experiments. Subsamples were taken to estimate the number of cells in the food suspensions using a Sedgewick-Rafter chamber; the carbon concentrations (particulate organic carbon, POC) were determined with an NCS-2500 analyzer (ThermoQuest GmbH, Egelsbach, Germany). The mean cell size of the ciliates was determined by measuring at least 50 unpreserved cells in length and width using an image analysis system, and the cell volume was computed using the geometric formula of a prolate spheroid (Table 2).

To estimate the contamination of the food suspension with bacteria and albumin beads, subsamples were stained with DAPI, and cells were enumerated by epifluorescence microscopy. Contamination of the food suspensions with bacteria was negligible in all treatments (<0.83% of total carbon; bacterial carbon was estimated according to Bratbak 1985). Free albumin beads were only occasionally observed in the ciliate food suspensions used to feed *D. magna*, and never exceeded a concentration of 2.5×10^2 beads ml^{-1}.

The green alga *Scenedesmus obliquus* (SAG 276-3a) was used as food for the stock cultures of *D. magna* and as a reference food in the growth experiments. Culture conditions and preparation of algal stock solutions are described elsewhere (Martin-Creuzburg et al. 2005a).

Ciliate uptake of fluorescently labeled albumin beads: The uptake of sterol-supplemented albumin beads by *C. campylum* was assessed by incubating ciliate cells (1500 cells ml^{-1}) for 20 min with loaded beads (1.2×10^6 beads ml^{-1}) at 20 °C without mixing. Preliminary experiments revealed an incubation time of 20 min as the best compromise between bead uptake, countability in the food vacuoles, and digestion-egestion processes. Ingestion was stopped by adding ice-cold glutaraldehyde (final concentration 2%). Subsamples were stained with DAPI (1 μg ml^{-1}, 8 min) and gently filtered onto 0.2-μm black nucleopore filters. The uptake of the fluorescent beads by ciliates was determined using epifluorescence microscopy. In each replicate, beads were counted in at least 50 ciliate cells at × 1000 magnification.

Daphnia growth experiments: Third clutch juveniles (born within 8 h) of a clone of *D. magna* (isolated by Lampert 1991) were used in growth experiments. The experiments were carried out at 20 °C in glass beakers filled with 100 ml of filtered lake water (0.45-μm pore sized). Each treatment consisted of three replicates with four animals each. The food suspensions contained 1.5 mg

$C\,l^{-1}$ of *S. obliquus* and at least $1\,mg\,C\,l^{-1}$ ($\sim$900 cells ml^{-1}) of *C. campylum* and were renewed daily within the 6-day experiments. Juvenile somatic growth rates were determined according to Martin-Creuzburg et al. (2005a).

Lipid analyses: Lipids were extracted three times from pre-combusted GF/F filters (Whatman, 25-mm diameter) loaded with approximately 0.5 mg particulate organic carbon (POC) of the protist food suspensions using a mixture of dichloromethane/methanol (2:1, v/v). For the analysis of sterols and sterol-like compounds (tetrahymanol, diplopterol), the pooled cell-free extracts were dried under a stream of nitrogen and saponified with $0.2\,mol\,l^{-1}$ methanolic KOH (70 °C, 1 h). Subsequently, sterols were partitioned into *iso*-hexane:diethyl ether (9:1, v/v), dried under a stream of nitrogen, and resuspended in a volume of 10—20 µl *iso*-hexane. Sterols, tetrahymanol, and diplopterol were analyzed with a gas chromatograph (HP 6890, Agilent Technologies, Waldbronn, Germany) equipped with an HP-5 capillary column (Agilent) and a flame ionization detector. Details of the GC configurations are given elsewhere (Martin-Creuzburg and Von Elert 2004). Lipids were quantified as cholesterol equivalents by comparison to an internal standard (5α-cholestan). The detection limit was approximately 20 ng of sterol. Lipids were 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-5MS, Agilent). Sterols were analyzed in their free form and as their acetate and trimethylsilyl derivatives. Mass spectra were recorded between 50 and 600 amu in the EI ionization mode, and lipids were identified by comparison with mass spectra of reference substances purchased from Sigma or Steraloids (see also the Acknowledgments) and/or mass spectra found in the literature (e.g. Harvey and McManus 1991; Ten Haven et al. 1989; Venkatesan 1989). The absolute amount of each lipid was related to the POC, which was determined from pre-combusted GF/F filters (Whatman, 25-mm diameter) loaded with another aliquot of the food suspensions used for lipid analysis.

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