Absence of sterols constrains food quality of cyanobacteria for an invasive freshwater bivalve

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Abstract The accumulation of cyanobacterial biomass may severely affect the performance of aquatic consumers. Here, we investigated the role of sterols in determining the food quality of cyanobacteria for the invasive clam *Corbicula fluminea*, which has become a common benthic invertebrate in many freshwater ecosystems throughout the world. In standardized growth experiments, juvenile clams were fed mixtures of different cyanobacteria (*Anabaena variabilis*, *Aphanothece clathrata*, *Synechococcus elongatus*) or sterol-containing eukaryotic algae (*Cryptomonas* sp., *Nannochloropsis limnetica*, *Scenedesmus obliquus*). In addition, the cyanobacterial food was supplemented with different sterols. We provide evidence that somatic growth of *C. fluminea* on cyanobacterial diets is constrained by the absence of sterols, as indicated by a growth-enhancing effect of sterol supplementation. Thus, our findings contribute to our understanding of the consequences of cyanobacterial mass developments for benthic consumers and highlight the importance of considering sterols as potentially limiting nutrients in aquatic food webs.

Keywords Benthic–pelagic coupling ·
Corbicula fluminea · Cyanobacterial blooms ·
Essential lipids · Food quality

Introduction

The accumulation of cyanobacterial biomass, frequently observed in aquatic ecosystems around the globe, severely affects food web processes and is often associated with hazards to human health and livestock and reduced recreational quality of water bodies (Carmichael 1992; Codd 1995). As the frequency of cyanobacterial bloom formation is expected to increase with global warming (Paerl and Huisman 2008), it is important to investigate the consequences of cyanobacterial mass developments for ecosystem processes, e.g., the role of cyanobacterial carbon within the food web and its food quality for aquatic consumers.

In general, cyanobacteria represent a nutritionally inadequate food source for aquatic consumers, which is due to (1) morphological properties, i.e. to the formation of filaments or colonies that hamper ingestion (De Bernardi and Giussani 1990; Van Donk et al. 2011), (2) the production of toxins (Carmichael 1992; Wilson et al. 2006) or other secondary metabolites that reduce the efficiency of digestion (protease inhibitors; Schwarzenberger et al. 2010), and/or (3) a deficiency in essential biochemical nutrients, in particular sterols (Von Elert et al. 2003; Martin-Creuzburg et al. 2008). It is generally assumed that cyanobacteria, in contrast to eukaryotic algae, are unable to synthesize sterols de novo (Volkman 2003; Summons et al. 2006). Sterols are required for a multitude of physiological processes in eukaryotic consumers, e.g., they are indispensable structural components of cell membranes and serve as precursors for steroid hormones (Goad 1981; Martin-Creuzburg and Von Elert 2009). The lack of sterols has been suggested to constrain the carbon transfer efficiency from cyanobacteria to herbivorous zooplankton (Von Elert et al. 2003; Martin-Creuzburg et al. 2008, 2010).

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When blooms settle down, a huge pelagic carbon pool is transferred to the benthic food web (Nascimento et al. 2008). It has been shown that cyanobacterial carbon can be ingested and assimilated by benthic invertebrates, but the nutritional value seems to be rather low (Karlson et al. 2008; Nascimento et al. 2009). In laboratory growth experiments, bivalves showed reduced growth and reproduction rates when fed cyanobacterial food sources (Wacker and von Elert 2003; Basen et al. 2011). Despite the fact that they are capable of removing colonial and filamentous cyanobacteria from the water column, feeding on cyanobacteria usually results in low ingestion rates and in an increased production of pseudofaeces, irrespective of cyanobacterial toxin production (Pires et al. 2005; Bontes et al. 2007). In contrast to morphological properties and toxicity, the role of essential dietary compounds in determining food quality for bivalve species has been poorly investigated. Experiments with marine bivalves, primarily species relevant for aquaculture, suggest that the ability to synthesize sterols *de novo* is generally low or absent (Walton and Pennock 1972; Goad 1981). Thus, a dietary source of sterols is presumably required for growth and reproduction (Soudant et al. 2000; Park et al. 2002), which may explain why cyanobacteria are of low nutritional quality for bivalve species.

One of the most spreading invaders in freshwater ecosystems is the Asian clam *Corbicula fluminea*. Originating in southeast Asia, *C. fluminea* has been introduced to North and South America and Europe in recent decades and has undergone a remarkable range expansion to become an ubiquitous benthic invertebrate in lentic and lotic freshwater ecosystems (McMahon 1982; Araujo et al. 1993; Darrigran 2002). Invasive bivalves, like *C. fluminea* and *Dreissena polymorpha*, can strongly affect invaded native ecosystems. As highly efficient filter feeders, they can alter phytoplankton dynamics, influence the pelagic nutrient cycling (Cahoon and Owen 1996; Hwang et al. 2011), and affect native benthic communities. By transferring pelagic carbon into the benthic food web, bivalves improve the benthic–pelagic coupling thereby stimulating benthic productivity (Strayer et al. 1999; Sousa et al. 2008; Gergs et al. 2009).

In the present study, we investigated the consequences of a dietary sterol deficiency for the growth of *C. fluminea* in standardized growth experiments. Juvenile clams were fed different sterol-free (cyanobacteria) and sterol-containing food sources (algae). In addition, clams were fed a mixture of three different cyanobacteria supplemented with sterols to assess whether the growth of *C. fluminea* on a cyanobacterial diet is constrained by a deficiency in dietary sterols. In addition, the sterol composition of food sources and clam tissues was recorded.

Materials and methods

Cultivation of cyanobacteria and algae

Food sources for *C. fluminea* were cultivated semi-continuously in aerated 5-l vessels at a dilution rate of 0.25 day^{-1} at 20°C with illumination at $100\text{--}120 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$, and harvested in the late-exponential growth phase. The coccoid cyanobacterium *Synechococcus elongatus* (SAG 89.70; Sammlung für Algenkulturen, Göttingen, Germany), the filamentous cyanobacterium *Anabaena variabilis* (ATCC 29413; American Type Culture Collection, Manassas, USA), the gelatinous cyanobacterium *Aphanotece clathrata* (SAG 23.99), the green alga *Scenedesmus obliquus* (SAG 276-3a) and the eustigmatophyte *Nannochloropsis limnetica* (SAG 18.99) were grown in Cyano medium (Jüttner et al. 1983). The flagellate *Cryptomonas* sp. (SAG 26.80) was grown in modified Woods Hole (WC) medium enriched with vitamins (Guillard 1975), nitrogen (2 mM, final concentration), and phosphorus (100 μM , final concentration). These food organisms were used because they differ in their sterol content and composition. Food suspensions were prepared by concentrating the cells by centrifugation and resuspension in fresh media. Carbon concentrations of the food suspensions were estimated from photometric light extinctions (800 nm) and from carbon-extinction equations determined prior to the experiment.

Sterol supplementation of cyanobacteria

Cyanobacteria were enriched with a mixture of cholesterol (Sigma, C8667, purity 99%), stigmasterol (Sigma, S2424, 95%), and ergosterol (Sigma, E2000, 95%) using a modified protocol of a method described by Von Elert (2002). Sterols were dissolved in ethanol (2.5 mg ml^{-1}) to prepare ethanolic stock solutions. For supplementation, 40 mg of bovine serum albumin (BSA, Sigma A7906, 98%) was dissolved in 10 ml of ultrapure water and 266.7 μl of each sterol stock solution were added during gentle stirring. Subsequently, 10 ml of Cyano medium and 2.67 mg particulate organic carbon (POC) of the three cyanobacterial stock solutions (8 mg POC in total) were added and, after 5 min of incubation, the volume was brought to 80 ml with Cyano medium. The resulting suspension was incubated on a rotary shaker (100 rpm) for 4 h with illumination at $100 \mu\text{mol m}^{-2} \text{ s}^{-1}$. To remove excess BSA and free sterols, cyanobacterial cells were then concentrated by centrifugation and resuspended in fresh medium; this process was repeated twice. The obtained cyanobacterial food suspension ("Mix + BSA + Sterols") was then used as food for *C. fluminea* in the growth experiment. Control

food suspensions ("Mix + BSA") were prepared similarly but without adding sterols.

Clam sampling

Corbicula fluminea were collected in February 2009 in the upper basin of Lake Constance at a sampling site described by Werner and Rothhaupt (2008). The clams were collected at a water depth of 2–3 m by scuba-diving. After separation of living individuals from debris, sand and gravel, they were placed in flow-through systems with filtered (<30 µm), aerated lake water and pre-combusted sediment at an ambient temperature of 20°C. Clams were kept under these conditions for 2 weeks until the start of the growth experiment.

Growth experiments

Adult bivalves invest most of their energy in reproduction (gametogenesis) and little in somatic growth (Soudant et al. 1999), therefore we used juveniles (initial dry mass 13.3–201.0 mg, including shells; size range 5–10 mm) which were not sexually mature to maximize somatic growth rates. The 28-day experiment (24 February to 24 March 2009) was carried out at 20°C. Glass beakers were filled with 200 ml of filtered lake water (0.45-µm pore-sized membrane filter) and about 1 cm of precombusted sediment (550°C for 5 h) to allow the clams to burrow. Clams were randomly transferred to each beaker. Each of the ten food treatments consisted of ten replicates, i.e. individual clams. Clams were fed daily with 3 mg C l⁻¹ of the food suspensions or starved without adding food. The experiment comprised the following food treatments: the three cyanobacteria *S. elongatus*, *A. variabilis*, and *A. clathrata*, the three eukaryotic algae *S. obliquus*, *N. limnetica*, and *Cryptomonas* sp., a mixture of the three cyanobacteria (1/3 POC provided by each species, "Mix"), either unsupplemented or supplemented with BSA (control, "Mix + BSA") or BSA and sterols ("Mix + BSA + Sterols"), and a mixture of the three eukaryotic algae. Water was exchanged daily to remove fecal pellets; sediment was exchanged once a week to reduce biofilm formation. Somatic growth rates (*g*) were determined as the increase in total dry mass from the beginning of the experiment (*M*₀) to day 28 (*M*_{*t*}) over time (*t*) using the equation:

$$g = \frac{(\ln M_t - \ln M_0)}{t}$$

A subsample of clams (*n* = 47) was taken at the beginning of the experiment to estimate the individual fresh and dry mass after 24 h of freeze-drying. Samples were weighed on an electronic balance (Mettler Toledo

XP2U; ±0.1 µg). The dry mass (DM, including shells) of clams at the start of each experiment was estimated from their actual fresh mass (FM, including shells) and previously established fresh-dry-mass regressions (DM = 0.625FM, *R*² = 0.994). Growth rates of clams were calculated as means (*n* = 10) ± standard deviation for each treatment (*n* = 10).

Analyses of food organisms and clam tissues

Aliquots of the food suspensions were filtered onto pre-combusted glass-fibre filters (Whatman GF/F, 25 mm diameter) and analyzed for particulate organic carbon (POC, *n* = 3 per treatment) using an NCS-2500 analyzer (ThermoQuest). The carbon content of clams was determined by analyzing freeze-dried soft-body tissues dissected from subsampled individuals at the end of the experiment (*n* = 3 per treatment).

For the analysis of sterols, glass-fiber filters loaded with ~1 mg POC of the food suspensions (*n* = 3) were sonicated and stored at -20°C in a mixture of dichloromethane/methanol (2:1, v/v). Soft-tissues of freeze-dried clams (*n* = 3 for each treatment) were separated from their shell, weighed, crushed by mechanical shearing using a mortar, sonicated, and subsequently stored at -20°C in dichloromethane/methanol (2:1, v/v). Total lipids of clam tissue or algae suspensions were extracted three times from each sample using dichloromethane/methanol (2:1, v/v) and the pooled cell-free extracts were dried under a stream of nitrogen and saponified with 0.2 M 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 iso-hexane. Sterols were analyzed by gas chromatography (GC) on a HP 6890 GC (Agilent Technologies, Waldbronn, Germany) equipped with a flame ionization detector and a HP-5 (Agilent, 30 m, 0.25 mm I.D., 0.25 µm film) capillary column. Details of GC configurations are given elsewhere (Martin-Creuzburg et al. 2009). Sterols were quantified by comparison to an internal standard (5α-cholestan). The detection limit was 20 ng of sterol. Sterols 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, 30 m, 0.25 mm I.D., 0.25 µm). Sterols were analyzed in their free form and as their trimethylsilyl derivatives, which were prepared by incubating 20 ml of iso-hexane sterol extract with 10 ml of *N,O*-bis(trimethylsilyl)trifluoroacetamide including 1% trimethylchlorosilane for 1 h at room temperature. Mass spectra were recorded between 50 and 600 amu in the EI ionization mode. Sterols were identified by comparison with mass spectra of reference substances purchased from Sigma or Steraloids and/or

mass spectra found in a self-generated spectra library or in the literature (e.g., Belanger et al. 1973; Goad and Akihisa 1997; Toyama et al. 1952). The C-24 stereochemistry and the cis-trans isomery of sterols could not be identified with certainty, and thus, if procurable, was adopted from the literature. The absolute amount of each sterol was related to the POC of the food sources or to the carbon content of clam soft-tissues and given as mean $\pm$ standard deviation.

Statistical analyses

All statistical analyses were carried out using the statistical software package Statistica 6.0 (StatSoft). Differences among growth rates and among sterol levels in clam tissue were analyzed using one-way analyses of variance (ANOVA) and Tukey's HSD post hoc tests. The correlation between somatic growth rates of *C. fluminea* and sterol levels in clam tissue was assessed by linear regression analyses.

Results

Sterol composition of food sources

Sterols were not detected in cyanobacterial food suspensions, i.e. whether in *A. variabilis*, *A. clathrata*, or *S. elongatus* or in the unsupplemented or BSA-treated cyanobacterial food mixtures. When enriched with sterols, the cyanobacterial food mixtures contained on average $38.3 \pm 15.3 \mu\text{g mg C}^{-1}$ of sterols in total, consisting of cholesterol (cholest-5-en-3 β -ol, $13.5 \pm 3.9 \mu\text{g mg C}^{-1}$), ergosterol [(22E)-ergosta-5,7,22-trien-3 β -ol, $7.8 \pm 4.3 \mu\text{g mg C}^{-1}$], and stigmasterol [(22E)-stigmasta-5,22-dien-3 β -ol, $17.1 \pm 7.4 \mu\text{g mg C}^{-1}$]. The green alga *S. obliquus* contained on average $13.6 \pm 5.4 \mu\text{g mg C}^{-1}$ of sterols in total, the principal sterols were fungisterol (5 α -ergost-7-en-3 β -ol, $4.6 \pm 1.8 \mu\text{g mg C}^{-1}$), chondrillasterol [(22E)-5 α -poriferasta-7,22-dien-3 β -ol, $7.8 \pm 3.4 \mu\text{g mg C}^{-1}$], and 22-dihydrochondrillasterol (5 α -poriferast-7-en-3 β -ol, $1.1 \pm 0.2 \mu\text{g mg C}^{-1}$). In *Cryptomonas* sp., total sterols averaged $8.4 \pm 4.2 \mu\text{g mg C}^{-1}$ and consisted of two principal sterols: brassicasterol [(22E)-ergosta-5,22-dien-3 β -ol, $2.7 \pm 1.3 \mu\text{g mg C}^{-1}$] and stigmasterol ($5.7 \pm 2.9 \mu\text{g mg C}^{-1}$). *N. limnetica* contained $7.6 \pm 8.9 \mu\text{g mg C}^{-1}$ of sterols in total, the principal sterols were cholesterol ($5.4 \pm 6.3 \mu\text{g mg C}^{-1}$), sitosterol (stigmast-5-en-3 β -ol, $1.2 \pm 1.4 \mu\text{g mg C}^{-1}$), and fucosterol [(24E)-stigmasta-5,24(28)-dien-3 β -ol, $1.1 \pm 1.2 \mu\text{g mg C}^{-1}$].

Sterol composition of clam tissues

Six different sterols were identified in clam tissues: cholesterol (40–72% of total sterols), brassicasterol (8–26%),

corbisterol [(22E)-stigmasta-5,7,22-trien-3 β -ol, 7–13%], campesterol (campest-5-en-3 β -ol, 2–12%), stigmasterol (5–12%), and ergosterol (0–5%) (Table 1). The total sterol content of field-collected clams at the start of the experiment was on average $9.8 \pm 2.1 \mu\text{g mg C}^{-1}$. Lowest sterol concentrations were found in clams fed *A. variabilis* ($4.8 \pm 1.6 \mu\text{g mg C}^{-1}$) and highest levels were measured in clams fed *N. limnetica* ($12.9 \pm 5.0 \mu\text{g mg C}^{-1}$) and in clams fed sterol-supplemented cyanobacteria ($12.6 \pm 0.6 \mu\text{g mg C}^{-1}$). In general, total sterol levels of clams fed eukaryotic algae ($10.5 \pm 4.0 \mu\text{g mg C}^{-1}$, $n = 9$) were significantly higher than those of clams fed cyanobacterial diets ($5.6 \pm 1.8 \mu\text{g mg C}^{-1}$, $n = 9$, ANOVA, $F_{1,16} = 11.22$, $P = 0.004$, Fig. 1) and the total sterol content of clams fed sterol-supplemented cyanobacteria was significantly higher than those of clams fed unsupplemented or merely BSA-treated cyanobacteria (ANOVA, $F_{2,6} = 17.95$, $P = 0.003$; Tukey's HSD, $P < 0.05$, Fig. 1).

Growth of *C. fluminea*

The dry mass of *C. fluminea* increased during the 28-day experiment in all food treatments, leading to positive somatic growth rates. In contrast, the dry mass of starving individuals slightly decreased during the experiment (Fig. 2). No clams died during the experiment. In general, clams fed one of the three cyanobacterial diets had lower growth rates than clams fed one of the three algal diets (ANOVA, $F_{1,9} = 19.44$, $P < 0.001$; Tukey's HSD, $P < 0.05$). Growth rates of clams fed the filamentous *A. variabilis*, the single-celled picocyanobacterium *S. elongatus*, the gelatinous *A. clathrata* or the unsupplemented or merely BSA-treated cyanobacterial diets (Mix, Mix + BSA) did not differ significantly (Tukey's HSD, $P > 0.05$; Fig. 2). Likewise, the significantly higher growth rates obtained with the eukaryotic algae *S. obliquus*, *N. limnetica* and *Cryptomonas* sp. did not differ significantly from each other (Tukey's HSD, $P > 0.05$). Growth rates of *C. fluminea* fed sterol-supplemented cyanobacterial food (Mix + BSA + Sterols) were significantly higher than those of clams fed unsupplemented cyanobacterial food (Mix, Mix + BSA), but did not differ from growth rates obtained with the eukaryotic food.

Discussion

Nutritional requirements of benthic invertebrates have been poorly studied, in particular with regard to essential biochemicals. We show here that somatic growth of the invasive freshwater clam *C. fluminea* on cyanobacterial diets is constrained by the absence of sterols, as indicated by a growth-enhancing effect of sterol supplementation.

Table 1 Sterol composition of soft-body tissues of *Corbicula fluminea* fed either one of the three cyanobacteria (*Synechococcus elongatus*, *Anabaena variabilis*, *Aphanotece clathrata*) or one of the three eukaryotic algae (*Scenedesmus obliquus*, *Cryptomonas* sp., *Nannochloropsis limnetica*)

	Start	Starving	<i>S. obliquus</i>	<i>A. variabilis</i>	<i>A. clathrata</i>	<i>S. elongatus</i>	<i>Cryptomonas</i> sp.	<i>N. limnetica</i>	Mix	Mix + BSA	Mix + BSA + sterols
Cholesterol	5.21 ± 1.01	5.67 ± 0.56	5.22 ± 1.29	2.98 ± 1.14	3.85 ± 0.53	3.34 ± 1.49	3.60 ± 1.36	9.28 ± 3.64	4.31 ± 1.09	2.30 ± 2.10	7.33 ± 1.48
Brassicasterol	2.35 ± 0.54	2.15 ± 0.91	0.82 ± 0.16	0.49 ± 0.16	0.63 ± 0.11	0.68 ± 0.46	2.20 ± 1.37	1.07 ± 0.78	0.69 ± 0.22	1.47 ± 1.56	1.93 ± 0.56
Ergosterol	0.52 ± 0.46	0.54 ± 0.49	n.d.	n.d.	n.d.	n.d.	0.24 ± 0.42	n.d.	0.08 ± 0.14	0.16 ± 0.28	0.55 ± 0.50
Campesterol	0.43 ± 0.09	0.44 ± 0.11	1.20 ± 0.21	0.23 ± 0.07	0.27 ± 0.01	0.27 ± 0.13	0.31 ± 0.17	0.32 ± 0.14	0.34 ± 0.10	0.29 ± 0.08	0.31 ± 0.08
Stigmasterol	0.86 ± 0.19	0.73 ± 0.29	1.73 ± 0.63	0.37 ± 0.09	0.76 ± 0.24	0.74 ± 0.43	1.84 ± 1.29	0.63 ± 0.24	0.88 ± 0.32	0.65 ± 0.14	1.16 ± 0.37
Corbisterol	n.d.	0.42 ± 0.32	0.71 ± 0.17	0.56 ± 0.10	0.80 ± 0.09	0.65 ± 0.24	0.60 ± 0.14	1.09 ± 0.58	0.75 ± 0.28	0.71 ± 0.25	0.88 ± 0.41
Unknown	0.41 ± 0.12	0.31 ± 0.30	0.13 ± 0.11	0.14 ± 0.13	0.10 ± 0.18	n.d.	n.d.	0.46 ± 0.34	n.d.	0.05 ± 0.08	0.42 ± 0.39
Total sterol content	9.78 ± 2.07	10.25 ± 3.34	9.80 ± 1.64	4.78 ± 1.62	6.40 ± 0.76	5.67 ± 2.74	8.78 ± 4.74	12.85 ± 4.95	7.06 ± 2.00	5.63 ± 1.55	12.58 ± 0.59

In addition, the sterol composition of clams fed a mixture of all three cyanobacteria (*Mix*), a cyanobacterial mixture treated merely with bovine serum albumin (*Mix* + BSA), or a cyanobacterial mixture treated with BSA and sterols (*Mix* + BSA + Sterols) is presented. Values are given as means ± standard deviations ($n = 3$). Sterols are reported as $\mu\text{g sterol mg C}^{-1}$. n.d. Not detectable

Fig. 1 a Sterol content of invasive clam *Corbicula fluminea* after 28 days of growth on a cyanobacterial diet (data obtained for the three cyanobacterial diets, *Synechococcus elongatus*, *Anabaena variabilis*, and *Aphanotece clathrata* combined, $n = 9$) and on eukaryotic algae (data obtained for the three algal diets, *Scenedesmus obliquus*, *Cryptomonas* sp., and *Nannochloropsis limnetica* combined, $n = 9$). b The sterol content of clams fed a mixture of all three cyanobacteria (*Mix*), a cyanobacterial mixture treated merely with bovine serum albumin (*Mix* + BSA), or a cyanobacterial mixture treated with BSA and sterols (*Mix* + BSA + Sterols) is shown for comparison ($n = 3$). The shaded background represents tissue sterol levels of clams at the beginning of the experiment. Data represent means and standard deviations (SD). Bars labeled with the same letters are not significantly different based on Tukey's HSD, $P < 0.05$ following ANOVA; each panel represents a separate statistical analysis

This adds to previous findings showing that the growth of zooplankton (i.e. *Daphnia*) on cyanobacterial diets is constrained by the absence of sterols (Martin-Creuzburg et al. 2005, 2008) and thus highlights the importance of considering sterols as potentially limiting nutrients in aquatic food webs.

It has been suggested that the capability of synthesizing sterols de novo is low or even absent in bivalve species, which suggests that they rely on sufficient supply with dietary sterols to cover their physiological demands (Goad 1981; Napolitano et al. 1993; Soudant et al. 1996). Total sterol levels in the soft-body of clams fed the sterol-containing eukaryotic algae (*Cryptomonas* sp., *N. limnetica* or *S. obliquus*) were significantly higher than those of clams fed cyanobacterial diets, but did not differ from sterol levels determined in clams at the beginning of the experiment. In contrast, when grown on cyanobacterial diets, total sterol levels in clam tissues decreased compared to initial sterol levels. The supplementation of a cyanobacterial diet with sterols led to significantly increased sterol levels in clam soft-bodies, indicating an incorporation of

Fig. 2 Somatic growth rates of *Corbicula fluminea* a either starved or fed different cyanobacterial (*Synechococcus elongatus*, *Anabaena variabilis*, *Aphanotece clathrata*; white bars) or eukaryotic food sources (*Scenedesmus obliquus*, *Cryptomonas* sp., *Nannochloropsis limnetica*; gray bars). In addition, b growth rates of clams fed a mixture of all three cyanobacteria (Mix), a cyanobacterial mixture treated merely with bovine serum albumin (Mix + BSA), or a cyanobacterial mixture treated with BSA and sterols (coarse bar, Mix + BSA + Sterols) are presented. Data are means $\pm$ SD, $n = 10$. Bars labeled with the same letters are not significantly different based on Tukey's HSD, $P > 0.05$ following ANOVA

supplemented sterols. In accordance with previous studies, the sterol composition of *C. fluminea* was dominated by cholesterol, with lesser amounts of brassicasterol, campesterol, corbisterol, ergosterol, sitosterol, and stigmasterol (Duncan et al. 1987; Chijimatsu et al. 2011; Basen et al. 2011). Taking into account that bivalve species are presumably incapable of synthesising sterols de novo, sterols detected in soft-bodies of clams fed sterol-free cyanobacterial food were presumably incorporated and stored from dietary sources they had received prior to the experiment. The finding that sterols detected in clam tissues did not differ qualitatively, irrespective of the sterol composition of the food, suggests that *C. fluminea* is capable of converting dietary phytosterols to clam-specific sterols and that these sterols are functionally important in clam physiology. Besides sterols, long-chain polyunsaturated fatty acids (PUFAs) have been suggested to play an important role in bivalve nutrition (Soudant et al. 1996; Wacker et al. 2002; Basen et al. 2011). As both sterols and long-chain PUFAs are either absent or hardly represented in cyanobacteria, the growth of bivalves feeding on cyanobacteria-dominated diets might be simultaneously constrained by the availability of dietary sterols and certain PUFAs, as has been shown in laboratory experiments with *Daphnia* (Martin-Creuzburg et al. 2009). Further detailed investigations on bivalve nutrition are needed to separate effects mediated by dietary sterols from those mediated by dietary PUFAs to assess the relative importance of these essential nutrients.

In many aquatic ecosystems throughout the world, the phytoplankton is, at least seasonally, dominated by cyanobacteria (Reynolds and Walsby 1975; Oliver and Ganf 2000). Climate scenarios with rising temperatures, increased atmospheric CO₂ supplies and increased periods of thermal stratification are expected to favor cyanobacterial dominance (Jöhnk et al. 2008; Paerl and Huisman 2008), which may also affect trophic interactions in aquatic food webs as cyanobacteria are a nutritionally inadequate food source for most aquatic consumers (De Bernardi and Giussani 1990; Martin-Creuzburg et al. 2008). Recently, it has been stated that cyanobacterial carbon, deposited during bloom conditions in a marine system, did not support the benthic detritus-based food web (Karlson et al. 2008; Nascimento et al. 2009). Bivalves are significantly involved in transferring organic matter from pelagic sources to the sediment and thus provide a crucial link between pelagic and benthic food web processes (Newell 2004; Vaughn et al. 2008). Hence, it is important to understand the impact of cyanobacterial mass developments on bivalve species to more accurately assess consequences for benthic food web processes. Our data suggest that the benthic–pelagic coupling between cyanobacteria and filter-feeding bivalves is at least partially constrained by a dietary sterol deficiency.

Corbicula fluminea is considered to be one of the most important invaders in aquatic ecosystems in the last decades (Araujo et al. 1993; McMahon 2000). In contrast to some other bivalves, which are able to sort food particles according to their size, shape or surface structure (Bontes et al. 2007; Espinosa et al. 2010), *C. fluminea* is regarded as a non-selective suspension feeder (Way et al. 1990; Vaughn and Hakenkamp 2001) and thus is presumably not able to discriminate against nutritionally inadequate food particles. It remains to be tested whether *C. fluminea* is able to adjust its feeding or assimilation rate in order to gain more of a limiting nutrient (i.e. compensatory feeding). Moreover, *Corbicula* might be able to change its feeding mode from seston filtration to deposition feeding via its muscular foot (Vaughn and Hakenkamp 2001; Nichols et al. 2005), and in this way may potentially avoid the uptake of nutritionally inadequate food sources present in the water column. Thus, the predominance of cyanobacterial carbon in the water column may result in an increased utilization of benthic food sources to overcome a possible sterol limitation. However, the availability of adequate benthic food sources is potentially scarce, in particular during cyanobacterial bloom condition, as significant amounts of cyanobacterial carbon are deposited to the sediment. Considering our data, this suggests that somatic growth of *C. fluminea* and potentially other filter-feeding bivalves is constrained by a deficiency in dietary sterols when cyanobacteria dominate the phytoplankton. Consequently, the

expected increase in the frequency of cyanobacterial bloom formation in response to global warming may severely impair the growth of filter-feeding bivalves.

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