Simultaneous Effects of Light Intensity and Phosphorus Supply on the Sterol Content of Phytoplankton

Maike Piepho¹*, Dominik Martin-Creuzburg², Alexander Wacker¹

¹ Institute of Biochemistry and Biology, Theoretical Aquatic Ecology, University of Potsdam, Potsdam, Germany, ² Limnological Institute, University of Constance, Konstanz, Germany

Abstract

Sterol profiles of microalgae and their change with environmental conditions are of great interest in ecological food web research and taxonomic studies alike. Here, we investigated effects of light intensity and phosphorus supply on the sterol content of phytoplankton and assessed potential interactive effects of these important environmental factors on the sterol composition of algae. We identified sterol contents of four common phytoplankton genera, Scenedesmus, Chlamydomonas, Cryptomonas and Cyclotella, and analysed the change in sterol content with varying light intensities in both a high-phosphorus and a low-phosphorus approach. Sterol contents increased significantly with increasing light in three out of four species. Phosphorus-limitation reversed the change of sterol content with light intensity, i.e., sterol content decreased with increasing light at low phosphorus supply. Generally sterol contents were lower in low-phosphorus cultures. In conclusion, both light and phosphorus conditions strongly affect the sterol composition of algae and hence should be considered in ecological and taxonomic studies investigating the biochemical composition of algae. Data suggest a possible sterol limitation of growth and reproduction of herbivorous crustacean zooplankton during summer when high light intensities and low phosphorus supply decrease sterol contents of algae.

Introduction

Sterols are important structural and functional molecules in eukaryotic cells. They are involved in the regulation of membrane fluidity, signal transduction and modulation of the activity of membrane-bound enzymes [1]. In animals and higher plants, sterols are precursors of steroid hormones and involved in the synthesis of a variety of secondary metabolites [2]. In contrast to animals, which predominantly contain cholesterol, plants and algae contain a great diversity of different phytosterols. These sterols differ in their number and position of double bonds in the ring structure of the tetracyclic molecule and in the structure of the side chain. Until now it is unclear if this variety in sterol composition entails an advantage for plants and if individual sterols play specific roles in plant cell metabolism [2]. Sterols are able to regulate membrane permeability and fluidity but in higher plants e.g. stigmasterol was found to be less efficient than sitosterol and 24-methylcholesterol [3]. Grandmougin-Ferjani et al. [4] revealed that cholesterol and stigmasterol are able to stimulate the H⁺ pump of the ATPase in higher plant plasma membranes. These examples suggest that specific sterols may have particular functions in plant cells apart from their general role as structural components in cell membranes.

The sterol composition of algae is of great interest for several reasons. Microalgae are an important source of sterols for higher trophic levels in the aquatic food web. Although most eukaryotic organisms are able to synthesize sterols de novo, presumably all arthropods depend on ingesting sterols with their diet [5]. The main sterol in arthropods is cholesterol, which is characterized by a double bond at position Δ⁵ in the ring structure. Most phytosterols that are ingested with food are more or less efficiently converted into this molecule [6,7]. However, not all of the great number of phytosterols in algae are suitable as cholesterol precursors in crustacean zooplankton [8–10]. For example, sterols with double bonds at positions Δ⁵, Δ⁸ or Δ¹ apparently cannot be converted into cholesterol. Food quality in terms of sterols therefore greatly depends on the sterol composition of algal species in zooplankton diet [7,8].

Sterol compositions are also used in taxonomic studies [11]. Sterol profiles might serve as chemotaxonomic markers to distinguish species or to identify sources of organic matter in sediments. However, there is still not enough information about the occurrence of sterols in some taxonomic groups. For all these reasons an understanding of differences in sterol composition between phytoplankton species and variations with environmental conditions, such as light intensity, is important. In plants and algae, research has focused on the diverse sterol composition of different species [1,2,12] but there are very few studies that deal with the influence of culture conditions on the sterol content or composition of algae. Some studies suggest that the sterol content of algae changes with environmental conditions, such as nutrient supply or light intensity [13,14]. As yet, however, simultaneous effects of nutrient and light availability have not been considered.

Therefore, we tested the influence of different light intensities on the sterol content and composition of phytoplankton species under both high-P and low-P conditions. We chose four phytoplankton...
genera (Senedesmus, Chlamydomonas, Cryptomonas and Cyclotella) that are widespread in freshwater lakes and so are important food components for zooplankton. High light intensities and low phosphorus availability are common in many lakes during summer. By analysing both conditions simultaneously we were able to detect interactive effects of the two factors light intensity and phosphorus supply, i.e. phosphorus supply influenced the reaction of algal sterol contents to varying light intensities.

**Methods**

The two Chlorophyceae *Senedesmus quadricauda* (Turpin) Brebisson and *Chlamydomonas globosa* J.W. Snow, the Cryptophyceae *Cryptomonas ovata* Ehrenberg (all three species from the culture collection of the Limnological Institute, University of Constance), and the Mediophyceae (Bacillariophyta) *Cyclotella meneghiniana* Kutzing (collection of algal cultures, Göttingen, SAG 1020-1a) were cultivated in WC-medium [15] with one high-P (50 μM P) and one low-P-medium per species and continuous light. To meet uniformity of variance assumptions, light intensity as continuous variable and medium phosphorus concentration as factor. The statistical test resulted in four possibilities to describe the reaction of sterol contents to experimental conditions: (1) There is a significant reaction to light but not to phosphorus concentrations, i.e. no difference between the high-P and low-P treatments; (2) there is a significant difference between the high-P and low-P treatments but no reaction to light; (3) both light and phosphorus supply have a significant influence on the contents of sterols; (4) the two factors interact, i.e. reaction to light differs depending on phosphorus supply. To meet uniformity of variance assumptions, light intensities were log transformed. All statistical calculations were carried out using the statistical software package R version 2.6.0, which is under general public licence (R Development Core Team, 2007).

**Results**

The four freshwater species *Senedesmus quadricauda* (Chlorophyceae), *Chlamydomonas globosa* (Chlorophyceae), *Cryptomonas ovata* (Cryptophyceae) and *Cyclotella meneghiniana* (Mediophyceae) possess different sterol compositions and also differ in their total sterol content. The highest sterol content (10 μg mgC⁻¹ on average) was found in *S. quadricauda*. *C. meneghiniana* and *C. ovata* had an average sterol content of 7–8 μg mgC⁻¹. In *C. globosa* the lowest sterol content was observed (4 μg mgC⁻¹ on average).

In *S. quadricauda*, fungisterol (IUPAC name: 5β-ergost-7-en-3β-ol), chondrillasterol ((22E)-5β-poriferastera-7,22-dien-3β-ol), and 22-dihydrochondrillasterol (5β-poriferast-7-en-3β-ol) were detected (Fig. 1). Statistical analyses of the effects of light and phosphorus supply on the content of each of these three sterols in *S. quadricauda* revealed significant interactions (Table 1), i.e. in the high-P treatment the sterol content increased with light intensity but decreased in the low-P treatment. This effect was most pronounced for chondrillasterol, whose content increased by 50% in the high-P treatment but decreased by the same factor in the low-P treatment. The total sterol content in *S. quadricauda* increased from 9 to 13 μg mgC⁻¹ in the high-P treatment and decreased from 11 to 8 μg mgC⁻¹ in the low-P treatment.

The only sterol we found in more than one of the analysed species was fungisterol, which was abundant in the two green algae *S. quadricauda* and *C. globosa*. In *C. globosa*, the same interaction as in *S. quadricauda* was observed for fungisterol (Table 1). Though this sterol was present in very low amounts, fungisterol doubled in content in the high-P treatment whereas the content decreased slightly in the low-P treatment (Fig. 2). Besides fungisterol we also found ergosterol ((22E)-ergosta-5,7,22-trien-3β-ol) in *C. globosa*, which in fact was present in much higher amounts than fungisterol. Ergosterol content did not change with light but increased with phosphorus availability, i.e. in the high-P treatment.

**Spectrometry**

Particulate organic carbon (POC) was measured as the sum of particulate organic carbon (POC) and particulate organic nitrogen (PON) with a High-TOC Analyser (Elementar Analysensysteme GmbH, Hanau, Germany). To determine the composite nature of POC, a gas chromatograph-mass spectrometer (Finnigan MAT GCQ) equipped with a fused-silica capillary column (DB-5MS, Agilent; GC configurations as described in Martin-Creuzburg et al. [17]) was used. Sterols were analysed in their free form and as their trimethylsilyl derivatives, which were prepared by adding 20 ml of iso-hexane sterol extract with 10 ml of N,O-bis(trimethylsilyl)trichloroacetamid included 1 per cent trimethylcholorosilane for 1 hour at room temperature. Spectra were recorded between 50 and 600 amu in the EI ionization mode.

Sterol Contents of Phytoplankton
The last-mentioned effect was also significant in the total sterol content of *C. globosa*: in the high-P treatment the content was 4 \( \mu \text{g mgC}^{-1} \) but in the low-P treatment only 3 \( \mu \text{g mgC}^{-1} \).

In *C. ovata*, brassicasterol ((22E)-ergosta-5,22-dien-3\( \beta \)-ol) and stigmasterol ((22E)-stigmasta-5,22-dien-3\( \beta \)-ol) were the principal sterols (Fig. 3). Whereas the content of the first did not change with any of the experimental conditions, the content of the latter was higher in the high-P than in the low-P treatment (Table 1), but did not change with light intensity. No significant change in sterol content was seen when we examined the sum of sterols in *C. ovata*.

24-methylenecholesterol (ergosta-5,24(24\(^1\))-dien-3\( \beta \)-ol) and 22-dihydrobrassicasterol (ergost-5-en-3\( \beta \)-ol) were detected in *C. meneghiniana* (see discussion for details about identification of sterols). Consistent with the results obtained for *S. quadricauda* we found interactive effects of the two factors light and phosphorus supply on both sterols in *C. meneghiniana* (Table 1). 24-methylenecholesterol content increased about 2-fold with light in the high-P treatment whereas it decreased 1.5-fold in the low-P treatment (Fig. 4). The content of 22-dihydrobrassicasterol did not change with light availability in the high-P treatment but decreased with light in the low-P culture (Fig. 4). The total sterol content increased from 5 to 8 \( \mu \text{g mgC}^{-1} \) in the high-P treatment but decreased from 7 to 5 \( \mu \text{g mgC}^{-1} \) in the low-P treatment.

### Discussion

We observed significant changes of sterol contents with light intensity and phosphorus supply. Interestingly, sterols of *S. quadricauda* and *C. meneghiniana* increased with light intensity in the high-P treatment, but decreased with light intensity in the low-P treatment. In general, sterol contents tended to be lower in low-P algae, at least at medium to high light intensities. Where we observed interactions, we found higher sterol contents at low light in the low-P cultures than in the high-P treatments.

To date, there have not been many studies on the effects of changing environmental conditions on the sterol content of algae. In accordance with our results, Gordillo et al. [13] observed an increase of sterols with increasing light intensities in the halotolerant green alga *Dunaliella*. In contrast, Parrish et al. [18] found the sterols in the marine dinoflagellate *Gymnodinium* to be more abundant in low light. Since in the latter study the algae were grown in batch cultures for several days a P-limitation cannot be ruled out. If this was the case, the results of Parrish et al. [18] would be consistent with our results for low-P *S. quadricauda* and *C. meneghiniana*.

There are some possible explanations for the observed changes in sterol contents. A reason for increasing sterol contents with increasing light could be the biosynthesis pathways of different algae. Sterols can be synthesized in two possible ways: (1) The MVA (mevalonate) pathway, where the sterol precursor isopentenyl diphosphate (IPP) is synthesised in the cytosol from acetyl-CoA via MVA and (2) the MEP (methylerythritol) pathway in which IPP is synthesised in the chloroplast from glyceraldehyde-3-phosphate and pyruvate. Whereas green algae seem to use only the MEP pathway for sterol synthesis, sterols of other microalgae were found to be synthesised via the MVA pathway [1,19]. To our knowledge, the biosynthesis pathway of sterols in *Cryptomonas* is not known. In most algal classes both pathways, MEP and MVA, seem...
to be involved in isoprenoid biosynthesis. However, studies using isotopic labelling techniques [19,20] showed that in general only one of the two pathways leads to sterols as end products. For example, in one Rhodophyte and one Chrysophyte, chloroplasts contain functions that are linked with the chloroplast. This could explain the increase of sterols with increasing light in the green alga S. quadricauda and the diatom C. megniniana. Higher light intensities stimulate photosynthesis and possibly the production of sterols via the MEP-pathway.

To explain the interacting effects of light and phosphorus on sterol contents of the algae, we propose that at high light intensities the increased carbon fixation also allows an increased synthesis of sterols. On the other hand, sterol production seems to be constrained by a low phosphorus supply, as indicated by the sterol contents of the low-P treatments of our experiment. Moreover, an increase of carbon storage can also be observed under high light conditions [21]. In P-limited algae, a further increased carbon accumulation with increasing light intensities consequently would result in a decrease of sterols per carbon and could therefore explain the observed decrease in sterol contents. A more functional explanation could be that a low P-content in the cell constrains the building of plasma membranes, which contain great amounts of phospholipids, and therefore indirectly alter sterol content, since sterols are found predominantly in these membranes. On the other hand enzymes involved in sterol synthesis in plants are associated with membranes [2]. As a result, phosphorus supply might indirectly limit sterol synthesis by reducing the amount of membranes and so the enzymes needed for sterol synthesis.

However, not all of the analysed sterol contents showed the same reaction to light intensity and phosphorus supply. The content of ergosterol in C. globoa and the content of stigmasterol in C. ovata did not change with light intensity but both were higher in the high-P than in the low-P treatment. Brassicasterol content in C. ovata, on the other hand, did not change with any of the experimental conditions. The reason for these specific reactions is not clear. The sterols might have different, yet unknown, functions in the cell that result in different reactions to environmental conditions. Furthermore, the above mentioned light and nutrient dependent carbon accumulation is realized in varying degrees in different species. For example, in contrast to the other species, in C. ovata no accumulation of storage lipids was found at high light intensities or low phosphorus supply (unpubl. data). If changes in sterol content per carbon can partly be explained by changes in carbon content, species specific differences in accumulation of carbon might lead to divergent reactions of sterol contents to the experimental conditions.

Our study revealed great differences between the sterol profiles of the analysed algae. It is known that for example the fatty acid profiles of algae belonging to the same class are quite similar (e.g., [22]). In contrast, the sterols of the two Chlorophyceae S. quadricauda and C. globoa clearly differed. The sterol profile of S. quadricauda was characterised by fungisterol, chondriasterol and 24-dihydrochondriasterol, three sterols characterised by a double bond at position Δ9, which have been reported previously to occur in Scenedesmus obliquus [23]. In contrast, the second green alga C. globoa contained fungisterol and considerable amounts of the Δ5,7,22 sterol ergosterol. Though the presence of ergosterol is frequently discussed as a characteristic feature of fungi [1,24], the occurrence of ergosterol in green algae has been reported previously (e.g., [25]). The two Δ5,22 sterols stigmasterol and brassicasterol were the principal sterols detected in C. ovata. It has been reported that cryptophycean algae such as Cryptomonas and Rhodomonas contain epibrassicasterol, the 24α-epimer of brassicasterol [26,27]. Though we did not determine the side-chain stereochemistry at C-24, the presence of epibrassicasterol rather than brassicasterol in C. ovata was also assumed (cf. [28]). 24-Methylenecholesterol (Δ5,24) and a 24-methylsterol (Δ7) were detected in C. megniniana, which confirms previous studies [29]. According to Gladu et al. [29], the C-24 methyl group of the 24-methylsterol present in Cyclotella cryptica is β-oriented, indicating the presence of 22-dihydrobrassicasterol (ergost-5-en-3β-ol) rather than its 24α-epimer campesterol (campest-5-en-3β-ol).
Comparison of sterol contents of the four algae species suggests differences in their food quality for crustacean zooplankton. Martin-Creuzburg & von Elert [8] suggested that dietary phytosterols with double bonds at specific positions within the ring system of the sterol, namely $D_5$ and $D_{5,7}$ sterols, can be converted into cholesterol in the metabolism of the freshwater key herbivore *Daphnia*, whereas $D_0$, $D_8$ and $D_{4}$ sterols cannot be converted and thus are not suitable to meet the sterol requirements for *Daphnia* growth and reproduction. *C. ovata* and *C. meneghiniana* both contain exclusively sterols with a double bond at position $D_5$ in the ring system, which presumably can effectively be converted into cholesterol by *Daphnia*. The same applies to the $D_{5,7,22}$ sterol ergosterol in *C. globosa*. Martin-Creuzburg & von Elert [8] suggested that sterols unsaturated at position $D_7$ instead of position $D_5$ in the ring system can be converted into cholesterol. However, they supported growth and reproduction of *Daphnia* to a significantly lower extent than cholesterol or the other $D_5$ and $D_{5,7}$ sterols tested in their study. All three sterols we found in *S. quadricauda* as well as fungisterol in *C. globosa* possess double bonds at position $D_7$. This implies that *S. quadricauda* is a less valuable food for *Daphnia* in respect to sterols than the other investigated algae.

High light intensities and phosphorus-limited conditions are frequently observed in oligo- to mesotrophic lakes during summer. Our data suggests that at these summer conditions a sterol-limitation of the herbivore crustacean *Daphnia* is possible. The
saturation threshold of dietary cholesterol for growth of *Daphnia* was described in different studies to be in the range of 5–6 µg mgC$^{-1}$ [17,23] or 7–9 µg mgC$^{-1}$ [30], depending on the method of supplementation, temperature or presence of other food components such as polyunsaturated fatty acids [31]. Assuming that the total amount of phytosterols is converted into cholesterol, the here shown species do not reach these contents under all conditions. The species with the lowest sterol content, *C. globosa*, contained total sterol contents of <3 µg mgC$^{-1}$, which is clearly below all suggested saturation levels. In *C. meneghiniana* high light intensities together with low F-supply led to total sterol contents <5 µg mgC$^{-1}$. Adopting the higher threshold for sterol limited growth for *Daphnia* of 7–9 µg mgC$^{-1}$, total sterol content of *C. ovata* under all conditions (7–8 µg mgC$^{-1}$) and of *S. quadricauda* exposed to high light and low-P (8 µg mgC$^{-1}$) were in the range of the threshold.

On the other hand, some phytosterols might be converted more efficiently into cholesterol than others. According to the study of Martin-Creuzburg and von Elert [8] the species with the highest total amount of sterols, *S. quadricauda*, is also the species that contains the least valuable sterols for crustacean zooplankton. Consequently, sterol limitation of *Daphnia* growth and reproduction is still possible even though total sterol content of the dietary phytoplankton species is above the mentioned threshold.

Sperfeld and Wacker [30] furthermore stated that the demand of *Daphnia* for sterols increases with increasing temperature. A sterol limitation of *Daphnia* in the field could therefore be increased in summer by high light intensities and low phosphorus supply, which reduce sterol contents in algae, and at the same time by higher temperatures, which increase the demand of *Daphnia* for sterols.

**Conclusion**

With our investigation on the change in sterol content of four species of algae with increasing light intensity, simultaneously in a high-phosphorus and a low-phosphorus approach, we demonstrated that phosphorus supply is of great importance when discussing the effect of light intensity on the sterol content. In three out of four species we found contrary reactions to light depending on phosphorus supply. The diverse sterol composition of the investigated algae indicated possible variations in the availability of adequate cholesterol precursors for crustacean zooplankton. Furthermore, the data suggest that a sterol limitation of consumers is most likely in lakes during summer conditions.

**Acknowledgments**

We thank Silvia Heim and Petra Merkel for assistance in experiments and analyses and two anonymous reviewers, who helped to improve the manuscript.

**Author Contributions**

Conceived and designed the experiments: MP AW. Performed the experiments: MP AW. Analyzed the data: MP DM-C AW. Contributed reagents/materials/analysis tools: MP DM-C AW. Wrote the paper: MP DM-C AW.

**References**

1. Volkman JK (2003) Sterols in microorganisms. Applied Microbiology and Biotechnology 60: 495–506.
2. Hartmann MA (1998) Plant sterols and the membrane environment. Trends in Plant Science 3: 170–175.
3. Schuler I, Milon A, Nakatani Y, Ourisson G, Albrecht AM, et al. (1991) Differential-effects of plant sterols on water permeability and on acyl chain ordering of soybean phosphatidylycholine bilayers. Proceedings of the National Academy of Sciences of the United States of America 88: 6926–6930.
4. Grandmougin-Ferjani A, Schuler-Muller I, Hartmann MA (1997) Sterol modulation of the plasma membrane H$^{+}$-ATPase activity from corn roots reconstituted into soybean lipids. Plant Physiology 113: 163–174.
5. Goad LJ (1981) Sterol biosynthesis and metabolism in marine-invertebrates. Pure and Applied Chemistry 53: 837–852.
6. Behmer, ST, Nes, D W (2003) Insect sterol nutrition and physiology: A global overview. London: Academic Press LTD. 72 p.
7. Martin-Creuzburg D, von Elert E (2009) Ecological significance of sterols in aquatic food webs. In: Arts MT, Brett MT, Kainz MJ, eds. Lipids in Aquatic Ecosystems. New York: Springer. pp 43–64.
8. Martin-Creuzburg D, Von Elert E (2004) Impact of 10 dietary sterols on growth and reproduction of *Daphnia galeata*. Journal of Chemical Ecology 30: 483–500.
9. Prahl FG, Eglington G, Corner EDS, Ohara SCM (1984) Copepod fecal pellets as a source of dihydrophytol in marine-sediments. Science 224: 1253–1257.
10. Klein Breteler WCM, Schoot N, Baas M, Schouten S, Kraay GW (1999) Trophic upgrading of food quality by protozoans enhancing copepod growth: role of essential lipids. Marine Biology 135: 191–198.
11. Patterson GW, Tatsutaards E, Wikfors GH, Gladu PK, Chitwood DJ, et al. (1994) Sterols and alkenones of Isochrysis. Phytochemistry 35: 1233–1236.
12. Patterson GW (1991) Sterols of algae. In: Patterson GW, Nea WD, eds. Physiology and biochemistry of sterols. ChampaignIL: American Oil Chemists’ Society. pp 118–157.
13. Gordillo EJL, Geurts M, Figueras FL, Niell FX (1998) Effects of light intensity, CO2 and nitrogen supply on lipid class composition of Dunaliella viridis. Journal of Applied Phycology 10: 738–747.
14. Klein Breteler WCM, Schogt N, Rampen S (2005) Effect of diatom nutrient limitation on copepod development: role of essential lipids. Marine Ecology-Progress Series 291: 125–133.
15. Nichols HW (1973) Growth media - freshwater. In: Stein JR, ed. Handbook of phycological methods: Culture methods and growth measurements. Cambridge: Cambridge University Press. pp 7–24.
16. Wacker A, Martin-Creuzburg D (2007) Allocation of essential lipids in Daphnia magna during exposure to poor food quality. Functional Ecology 21: 738–747.
17. Martin-Creuzburg D, Sperfeld E, Wacker A (2009) Colimitation of a freshwater herbivore by sterols and polyunsaturated fatty acids. Proceedings of the Royal Society B-Biological Sciences 276: 1805–1814.
18. Parrish CC, Bodenmee G, Gentien P (1994) Time courses of intracellular and extracellular lipid classes in batch cultures of the toxic dinoflagellate, Gymnodinium cf. nagasakiense. Marine Chemistry 48: 71–82.
19. Disch A, Schwender J, Muller C, Lichtenthaler HK, Rohmer M (1998) Distribution of the mevalonate and glyceraldehyde phosphate/pyruvate pathways for isoprenoid biosynthesis in unicellular algae and the cyanobacterium Synechocystis PCC 6714. Biochemical Journal 333: 381–388.
20. Masse G, Belt ST, Rowland SJ, Rohmer M (2004) Isoprenoid biosynthesis in the diatoms Rhizosolenia setigera (Brightwell) and Habia eutritonia (Simonsen). Proceedings of the National Academy of Sciences of the United States of America 101: 4413–4418.
21. Guschina IA, Harwood JL (2009) Algal lipids and effect of the environment on their biochemistry. In: Arts MT, Brett MT, Kainz MJ, eds. Lipids in aquatic ecosystems. New York: Springer. pp 1–24.
22. Volkman JK, Barrett SM, Blackburn SI, Mansour MP, Sikes EL, et al. (1998) Microalgal biomarkers: A review of recent research developments. Organic Geochemistry 29: 1163–1179.
23. Martin-Creuzburg D, Wacker A, von Elert E (2005) Life history consequences of sterol availability in the aquatic keystone species Daphnia. Oecologia 144: 362–372.
24. Greuner MO, Chauvet E (1993) Ergosterol-to-biomass conversion factors for aquatic Hyphomycetes. Applied and Environmental Microbiology 59: 502–507.
25. Thompson GA (1996) Lipids and membrane-function in green-algae. Biochimica et Biophysica Acta-Lipids and Lipid Metabolism 1302: 17–45.
26. Goad LJ, Holz GG, Beach DH (1983) Identification of (24S)-24-methylcholesta-5,22-dien-3-beta-ol as the major sterol of a marine Cryptophyte and a marine Prymnesiophyte. Phytochemistry 22: 475–476.
27. Gladu PK, Patterson GW, Wikfors GH, Chitwood DJ, Lusby WR (1990) The occurrence of brassicasterol and epibrassicasterol in the Chromophycota. Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology 97: 491–494.
28. Martin-Creuzburg D, Bec A, von Elert E (2005) Trophic upgrading of picocyanobacterial carbon by ciliates for nutrition of Daphnia magna. Aquatic Microbial Ecology 41: 271–280.
29. Gladu PK, Patterson GW, Wikfors GH, Chitwood DJ, Lusby WR (1991) Sterols of some diatoms. Phytochemistry 30: 2301–2303.
30. Sperfeld E, Wacker A (2009) Effects of temperature and dietary sterol availability on growth and cholesterol allocation of the aquatic keystone species Daphnia. Journal of Experimental Biology 212: 3051–3059.
31. Martin-Creuzburg D, Wacker A, Basen T (2010) Interactions between limiting nutrients: Consequences for somatic and population growth of Daphnia magna. Limnology and Oceanography 55: 2597–2607.