Chapter 3
Ecological Significance of Sterols in Aquatic Food Webs

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3.1 Introduction

Sterols are indispensable for a multitude of physiological processes in all eukaryotic organisms. In most eukaryotes, sterols are synthesized de novo from low molecular weight precursors. Some invertebrates (e.g., all arthropods examined to date), however, are incapable of synthesizing sterols de novo, and therefore have to acquire sterols from their diet. Here, we aim to demonstrate that such nutritional requirements not only affect the performance of an individual in its environment but may also have major consequences for the function of aquatic ecosystems. Starting from general patterns of occurrence and biosynthesis of sterols, we next explore the physiological properties and nutritional requirements of sterols. These aspects are then integrated into a more ecological perspective. We emphasize their effects on aquatic food webs in general and on herbivorous zooplankton in particular with the major aim to outline how the interplay of physiological capabilities of individual herbivores and trophic interactions in the food web will determine the effect of low dietary provision of sterols on structure and function of aquatic ecosystems.

3.2 Occurrence of Sterols

The ability to synthesize sterols de novo is a characteristic feature of eukaryotic cells. In prokaryotes, sterols are usually absent or they are found in such small amounts that contamination from other sources cannot be excluded. However, there is evidence that at least some eubacteria are capable of synthesizing sterols de novo (e.g., *Methylococcus capsulatus*, Volkman 2003, 2005). Small amounts of sterols

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have also been detected in some cyanobacterial strains, but the occurrence of sterols in cyanobacteria is controversial (Summons et al. 2006). The presence of a sterol biosynthetic pathway in cyanobacteria could not be confirmed by molecular data, which implies that sterols are usually absent in these prokaryotes (for critical reviews see: Volkman 2003, 2005; Summons et al. 2006).

Eukaryotes may be divided into three groups that differ in their sterol profiles: (a) the plant kingdom, which is characterized by a large set of different phytosterols, (b) the animal kingdom, where cholesterol tends to be the principle sterol, and (c) the fungal kingdom, which is generally characterized by the presence of ergosterol (Fig. 3.1). For reasons of convenience, not reflecting taxonomy, heterotrophic protists will be treated separately. Typical sterols of higher plants are sitosterol (stigmast-5-en-3β-ol), stigmasterol (24E-stigmasta-5,22-dien-3β-ol), and the two C-24 epimers campesterol (campest-5-en-3β-ol) and 22-dihydrobrassicasterol (ergost-5-en-3β-ol) (Fig. 3.2). Sterol profiles of algae are highly diverse so that a general pattern is hard to define and beyond the scope of this chapter (for reviews see: Nes and McKean 1977; Patterson 1991; Volkman et al. 1998; Volkman 2003). The sterol profile of animals is comparatively simple with characteristic high levels of cholesterol (cholest-5-en-3β-ol, Fig. 3.2; often >90% of total sterols) and only small amounts of other sterols. These minor sterols are either biosynthetic precursors or of dietary origin, in particular in herbivorous species, or are provided by symbiotic algae or other associated organisms such as fungi in the gut (Goad 1981). Ergosterol

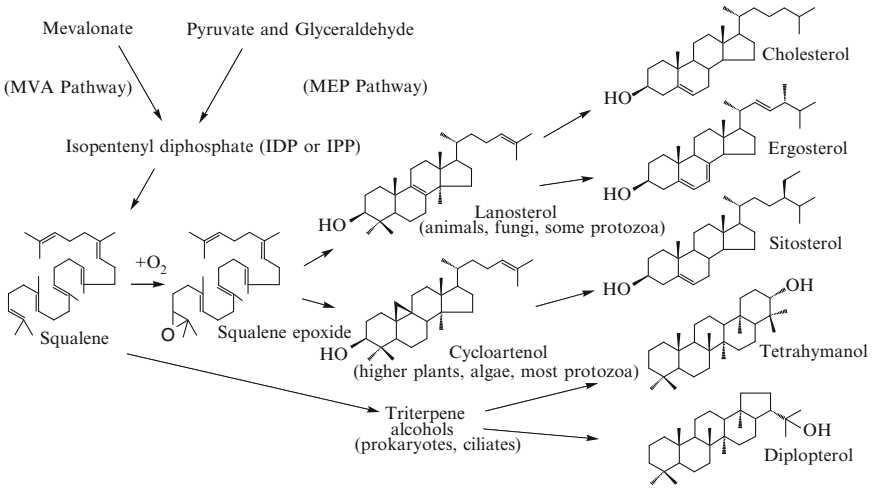

Fig. 3.1 Biosynthesis of sterols and other triterpenoid alcohols – a simplified scheme. Isopentenyl diphosphate is synthesized either via the classical mevalonate pathway (MVA) or via the more recently reported methylerythritol-phosphate pathway (MEP). Molecular oxygen is required for the cyclization of squalene via squalene epoxid. In animals, fungi, and some protozoa (e.g., dinoflagellates), the cyclization leads to lanosterol and in higher plants, algae, and most protozoa to cycloartenol, which are subsequently converted to functional products such as cholesterol, ergosterol, or sitosterol. Hopanoids (e.g., diplopterol) and the triterpenoid alcohol tetrahymanol are presumably formed nonoxidatively by direct cyclization of squalene itself (Ourisson et al. 1987)

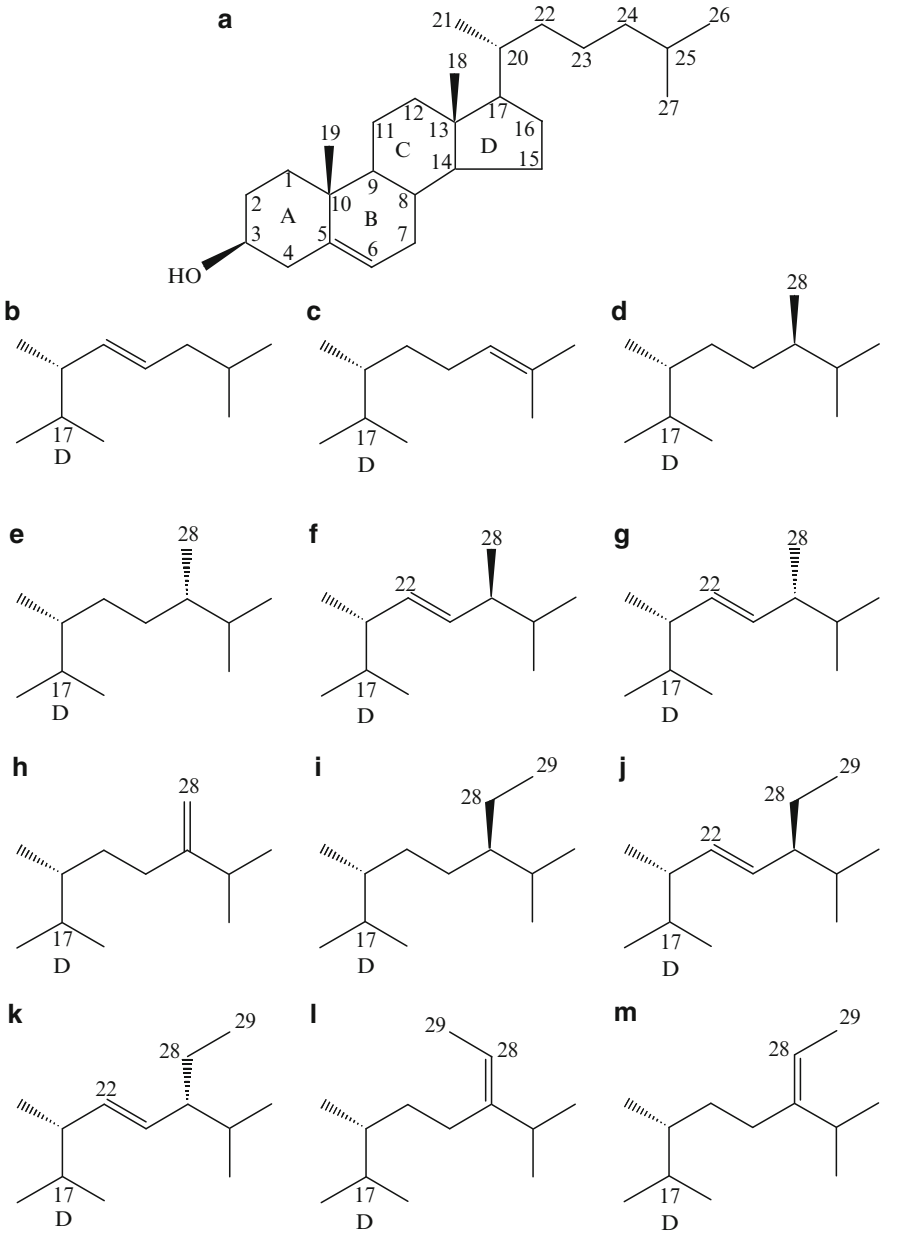

Fig. 3.2 Cholesterol (cholest-5-en-3β-ol; **a**) and other Δ^5-sterols common in aquatic food webs (the tetracyclic sterol nucleus similar to cholesterol): 22-dehydrocholesterol ((22E)-cholesta-5,22-dien-3β-ol; **b**), desmosterol (cholesta-5,24-dien-3β-ol; **c**), campesterol (campest-5-en-3β-ol; **d**), 22-dihydrobrassicasterol (ergost-5-en-3β-ol; **e**), epibrassicasterol ((22E-campesta-5,22-dien-3β-ol; **f**), brassicasterol ((22E-ergosta-5,22-dien-3β-ol; **g**), 24-methylenecholesterol (ergosta-5,24(24)-dien-3β-ol; **h**), sitosterol (stigmast-5-en-3β-ol; **i**), stigmasterol ((24E)-stigmasta-5,22-dien-3β-ol; **j**), poriferasterol (24E-poriferasta-5,22-dien-3β-ol; **k**), fucosterol ((24E)-stigmasta-5,24(24)-dien-3β-ol; **l**), isofucosterol ((24Z)-stigmasta-5,24(24)-dien-3β-ol; **m**). The carbon atoms are numbered according to the IUPAC recommendations; *capitals* indicate the four rings (A, B, C, D) of the sterol nucleus

(22E-ergosta-5,7,22-trien-3β-ol) is the predominant sterol in fungi, it is often used as a biomarker to detect and to quantify fungi in biological samples (Gessner and Chauvet 1993). However, care has to be taken in aquatic samples since other sources of ergosterol may exist (e.g., some green algae, Thompson 1996; and some diatoms, Véron et al. 1998).

Another group of organisms that may contain sterols are the heterotrophic protists; however, data on the occurrence of sterols in heterotrophic protists are scarce. Most research has been done on ciliates, which are presumably incapable of synthesizing sterols de novo, but incorporate dietary sterols (see below). In contrast, sterol biosynthesis has been documented in heterotrophic flagellates and dinoflagellates (Williams et al. 1966; Leblond and Chapman 2002; Giner et al. 2003; Bec et al. 2006), and in amoebae (Raederstorff and Rohmer 1987).

3.3 Biosynthesis of Sterols

Sterols are synthesized from low molecular weight precursors via isopentenyl diphosphate (IDP or IPP) and squalene (Fig. 3.1). The universal C_5 building block IPP is synthesized by two different pathways: the classical Bloch–Lynen pathway, where IPP is formed from three molecules of acetyl-CoA via mevalonate (MVA pathway), and the more recently reported MEP pathway (Rohmer et al. 1993), where IPP is formed from pyruvate and glyceraldehyde via methylerythritol-phosphate (MEP). Squalene, which is synthesized from 6 IPP molecules, is oxidatively converted to oxidosqualene. Cyclization of oxidosqualene leads to either lanosterol (5α-lanosta-8,24-dien-3β-ol; animals, fungi, some protozoa) or cycloartenol (5α-cycloart-24-en-3β-ol; higher plants, algae, most protozoa), which are subsequently modified to products such as cholesterol or phytosterols (Fig. 3.1). Sterol biosynthesis is an energetically expensive process and requires molecular oxygen. For further details on the biosynthesis of sterols and the occurrence of the two different pathways (MVA and MEP) the reader is referred to Volkman (2003, 2005) and Summons et al. (2006).

3.4 Physiological Properties of Sterols

Sterols are characterized by a tetracyclic fused ring skeleton, a polar head group at position 3 (3β-OH), and an alkyl side chain at position 17 (Fig. 3.2). The large number of naturally occurring sterols is defined by the number and/or position of double bonds or by additional substituents in the sterol nucleus or in the side chain. Because of the trans-configuration of the ring skeleton, the sterol molecule is planar and rigid, except for the rather flexible side chain. Sterols are indispensable structural components of eukaryotic membranes. Their amphipathic nature enables the incorporation into phospholipid bilayers: the polar 3β-OH group oriented to the aqueous phase and the nonpolar sterol nucleus and the alkyl side chain located in

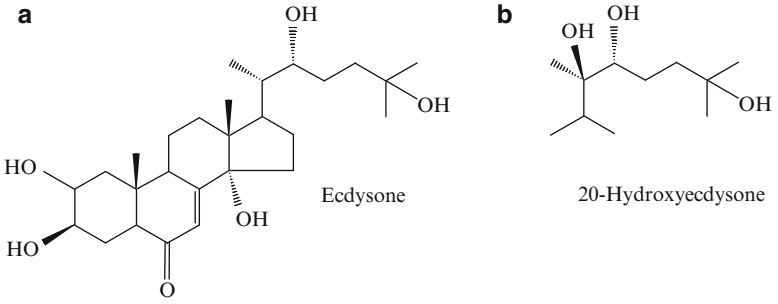

Fig. 3.3 Ecdysteroids – molting hormones of arthropods

the hydrophobic core of the phospholipid bilayer, interacting with the acyl chains of fatty acids. The sterol content is a major means by which eukaryotic cells modulate and refine membrane fluidity, permeability, and the function of various membrane proteins (Haines 2001; Ohvo-Rekilä et al. 2002; and see Chap. 10). It has been shown that the ordering capacity provided by cholesterol is of significantly greater magnitude than that of any of cholesterol's metabolic precursors (Dahl et al. 1980). Likewise, the ordering effect provided by phytosterols differs from that of cholesterol, which may limit direct substitution of cholesterol by phytosterols in animal membranes (Haines 2001).

Furthermore, sterols serve as precursors for vitamin D and for the multitude of naturally occurring steroid hormones, such as the brassinosteroids that promote growth in plants (Schaller 2003), the molt inducing ecdysteroids (Fig. 3.3) in arthropods (Grieneisen 1994), and the various steroids that are involved in the regulation of developmental processes in higher animal. Cholesterol has been found to covalently modify proteins of the "hedgehog" family, which are secreted signaling proteins that are required for developmental patterning of embryonic structures in insects, vertebrates, and other multicellular organisms (Porter et al. 1996). These examples illustrate the unique biochemical properties of sterols and their wide array of effects on biological processes.

3.5 Nutritional Requirements

3.5.1 Heterotrophic Protists

Nutritional requirements for sterols in heterotrophic protozoa are poorly investigated. The sterol composition of the freshwater heterotrophic nanoflagellate *Paraphysomonas* sp. was shown not to be affected by the sterol composition of its food source, i.e. *Paraphysomonas* sp. grown on a sterol-free food source exhibited the same sterol pattern as *Paraphysomonas* sp. grown on a sterol-containing food

source, which indicates that *Paraphysomonas* sp. is capable of synthesizing sterols de novo (Bec et al. 2006). De novo biosynthesis of sterols has also been reported in some other heterotrophic flagellates and dinoflagellates (e.g. Williams et al. 1966; Klein Breteler et al. 1999; Leblond and Chapman 2002; Giner et al. 2003).

In contrast, ciliates presumably lack the ability to synthesize sterols de novo (Conner et al. 1968; Harvey and McManus 1991; Harvey et al. 1997; Klein Breteler et al. 2004; Martin-Creuzburg et al. 2005b). However, exogenously supplied sterols can be incorporated into the ciliates' cell membranes and further metabolized into various sterols (Conner et al. 1968; Harvey and McManus 1991; Harvey et al. 1997; Martin-Creuzburg et al. 2006; Boëchat et al. 2007). In the absence of exogenous sterols, many ciliates produce the pentacyclic triterpenoid alcohol tetrahymanol (gammaceran-3β-ol, Fig. 3.1) and/or hopanoids (e.g. diplopterol; hopan-22-ol, Fig. 3.1), which, in ciliates, are functionally equivalent to sterols as structural components of cell membranes (Raederstorff and Rohmer 1988; Martin-Creuzburg et al. 2006). Hence, the capability to produce tetrahymanol and/or hopanoids renders ciliates independent of a dietary sterol supply. However, the synthesis of pentacyclic triterpenoids seems to be associated with physiological costs, as suggested by the observation that the synthesis is down-regulated in the presence of dietary sterols. A few ciliates, however, require a dietary source of sterols, and this sterol requirement can be very specific; e.g. the sterol requirements of *Paramecium* are best fulfilled by adding the phytosterol stigmasterol to the growth medium but not by adding cholesterol (VanWagtendonk 1974). Other taxa may satisfy their sterol requirements by hosting symbiotic algae, which further complicates the investigation of the sterol metabolism of ciliates.

3.5.2 Invertebrates

The inability to synthesize sterols from low molecular weight precursors such as acetate or mevalonate is widespread among invertebrates. Nematodes, for instance, are incapable of synthesizing sterols de novo and require a dietary source of sterols for normal growth and development (Lozano et al. 1987; Shim et al. 2002; Merris et al. 2004). A group where biosynthesis has been demonstrated is the sponges (marine: Silva et al. 1991; freshwater: Dembitsky et al. 2003). In molluscs, reports on sterol requirements are controversial. Many gastropods have been shown to biosynthesize cholesterol, and therefore a dietary source of sterols is presumably not required (Kanazawa 2001). In contrast, experiments with marine bivalves used for aquaculture (e.g. oysters) suggest that the ability to synthesize sterols de novo is generally low or absent among bivalve species, which implies that a dietary supply of sterols is necessary for somatic growth (Voogt 1975; Trider and Castell 1980; Soudant et al. 1998). Data on sterol requirements of freshwater bivalves are scarce. Feeding experiments with radioactive-labeled acetate suggest that at least some freshwater bivalves are capable of synthesizing sterols de novo, even though the rate of incorporation of labeled acetate into sterols was found to be very low (Popov et al. 1981).

It is generally accepted that all arthropods are incapable of synthesizing sterols de novo (Goad 1981). Cholesterol is the predominant sterol found in arthropods, as in most other animals (Goad 1981). Carnivorous species are readily supplied with cholesterol, while herbivorous species cannot rely on a dietary source of cholesterol since this sterol is often hardly represented in plant material (appreciable amounts are found only in some algal classes, e.g. in eustigmatophytes and some diatoms; Véron et al. 1998). Instead, plants and algae contain several types of phytosterols that differ from cholesterol by additional substituents (e.g., methyl or ethyl groups at C-24) or by the position and/or number of double bonds in the side chain or in the sterol nucleus (Piironen et al. 2000; Moreau et al. 2002). Herbivorous arthropods can either use the sterols present in their diet directly, or they have to metabolize them to cholesterol to meet the requirements for growth and development (Svoboda and Thompson 1985). Other than a few exceptions, herbivorous insects and also the crustaceans examined to date use dietary sterols to synthesize cholesterol. Therefore, most species studied are capable of dealkylating and reducing common C-24-alkyl phytosterols, such as sitosterol or stigmasterol, to cholesterol (Grieneisen 1994; Behmer and Nes 2003). However, more than 200 different types of phytosterols have been reported in plant material (Moreau et al. 2002), and it is not surprising that not all of them are suitable as cholesterol precursors. Numerous studies have focused on dietary needs for sterols in insects and revealed that the pattern of sterol metabolism is by no means ubiquitous (Behmer and Nes 2003). In general, Δ^5 and $\Delta^{5,7}$ sterols (numbers indicate the position of the double bonds; see Fig. 3.2) meet the nutritional requirements of insects, while Δ^7 and Δ^{22} sterols are unsuitable, in at least some insect species. The ability to dealkylate C-24 methyl or ethyl sterols is widespread among insects, even though dealkylation capacities have been lost in some insect groups (Behmer and Nes 2003). It has been demonstrated that the ratio of suitable to unsuitable dietary sterols may constrain insect survival (Behmer and Elias 2000).

In crustaceans, comparable studies are scarce and mostly restricted to marine decapod crustaceans, which have received attention due to their relevance for aquaculture. As in insects, Δ^5 and $\Delta^{5,7}$ sterols are readily used by decapod crustaceans, and methyl or ethyl groups are effectively removed from the C-24 position of the side chain to form cholesterol (e.g. Teshima 1971, 1991; Grieneisen 1994). Only recently has been attention drawn to the nutritional sterol requirements of crustacean zooplankton; mostly to cladocerans of the genus *Daphnia* and to marine copepods.

In *Daphnia*, the absence of dietary sterols (accomplished by feeding a cyanobacterial diet) had serious consequences for a variety of life history traits (Martin-Creuzburg et al. 2005a). Somatic and population growth rates, the number of viable offspring, and the probability of survival were significantly reduced with the diminishing availability of sterols. Moreover, an insufficient sterol supply adversely affected the performance of the offspring, which points to strong maternal effects under sterol limitation.

In addition to low dietary sterol levels, the quality of dietary sterols may affect the assimilation of dietary carbon and thus growth and/or reproduction. In a first attempt, we have investigated to what extent structural features of sterols affect the

growth and reproduction of *Daphnia galeata* by supplementing a sterol-free diet with different sterols (Martin-Creuzburg and Von Elert 2004). The results indicated that sterols containing a double bond at position Δ^5 (e.g., sitosterol, stigmasterol, ergosterol) meet the nutritional requirements of the daphnids, regardless of additional double bonds in the nucleus or in the side chain. In contrast, the Δ^7 sterol lathosterol (5α-cholest-7-en-3β-ol, Fig. 3.4) supported growth and reproduction to a significantly lower extent than cholesterol. No effect on growth of *D. galeata* was observed by supplementation with dihydrocholesterol (5α-cholestan-3β-ol), a completely saturated molecule (Δ^0) (Martin-Creuzburg and Von Elert 2004). Likewise, lanosterol, a $\Delta^{8(9),24(25)}$ sterol with additional C-4-dimethyl and C-14-methyl substituents, did not affect the growth of *D. galeata*. Growth was adversely affected by the Δ^4 sterol allocholesterol (cholest-4-en-3β-ol). These data also indicated the presence of an efficient C-24 dealkylating system in *D. galeata*.

Like daphnids, copepods lack the capacity for de novo synthesis of cholesterol, and thus require a dietary source of sterols for somatic growth and reproduction. Supplementation of a diatom diet (*Thalassiosira weissflogii*) with cholesterol has been shown to positively affect egg production rates of the marine copepods *Acartia hudsonica*, *Acartia tonsa*, and *Calanus finmarchicus* (Hassett 2004). In contrast, egg production of the copepod *Centropages hamatus* feeding on *T. weissflogii* was unaffected by cholesterol supplementation, which suggests species-specific differences in the sterol requirements or sterol storage capacities among copepod species (Hassett 2004). Interestingly, egg production rates of *A. hudsonica* were unaffected by sterol supplementation when *Thalassiosira rotula* was used as food. The sterol composition of the two *Thalassiosira* species (*T. weissflogii* and *T. rotula*)

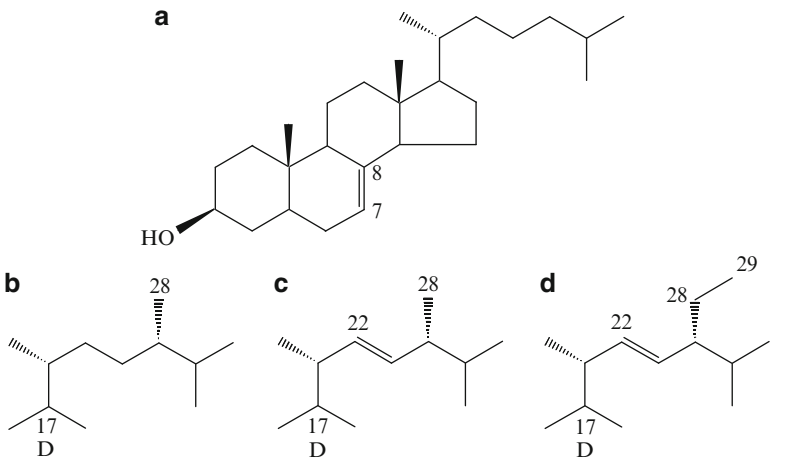

Fig. 3.4 Examples of structures of Δ^7-sterols: lathosterol (5α-cholest-7-en-3β-ol; **a**), fungisterol (5α-ergost-7-en-3β-ol; **b**), 5-dihydroergosterol ((22E)-5α-ergosta-7,22-dien-3β-ol; **c**), chondrillasterol ((24E)-5α-poriferasta-7,22-dien-3β-ol; **d**)

is very similar (Barrett et al. 1995), which makes it unlikely that the sterol composition accounted for the observed enhancement in copepod egg production. Instead, Hassett (2004) suggested that copepod egg production might have been constrained by a low concentration of dietary sterols, as the total sterol content varies considerably among and even within algal species.

Nevertheless, as described in various terrestrial insect species (Behmer and Nes 2003) and in the cladoceran *D. galeata* (Martin-Creuzburg and Von Elert 2004), structural features of dietary sterols may also affect the life history of copepods. Prahl et al. (1984) reported that in *Calanus helgolandicus* C_{28} and C_{29} sterols containing a Δ^5 or $\Delta^{5,7}$ double bond in the sterol nucleus are selectively removed from the diet (*Dunaniella primolecta*) relative to Δ^7 components, which were released unchanged as fecal lipids. The authors 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* lacks the ability to convert Δ^7 sterols to cholesterol, and that the Δ^7 components are therefore poorly assimilated. In agreement, Klein Breteler et al. (1999) found that the ontogenetic development of the marine copepods *Temora longicornis* and *Pseudocalanus elongatus* from larvae to the adult stage was impaired by the unsuitable sterol composition of the green alga *Dunaliella* sp., which contained only traces of Δ^7 sterols and no Δ^5 or $\Delta^{5,7}$ components.

Like Δ^7 sterols, ring-saturated sterols were found to pass through the gut of copepods quantitatively, i.e., the amounts of ring-saturated sterols found in the diet and in the fecal pellets did not differ, indicating their nutritional inadequacy (Harvey et al. 1987, 1989). The ring-saturated dihydrocholesterol also did not improve the growth of *D. galeata*, when supplemented to a sterol-free diet (Martin-Creuzburg and Von Elert 2004).

Harvey et al. (1987, 1989) reported that 4-methyl and 4-desmethyl sterols (i.e., sterols with and without a methyl substituent at C-4) having a $\Delta^{8(14)}$ and $\Delta^{17(20)}$ unsaturation were readily, and, to similar degrees, removed from the diet by *Calanus helgolandicus* feeding on the dinoflagellate *Scrippsiella trochoidea*, which may indicate that a methyl group at C-4 does not prevent sterols from being assimilated and converted into cholesterol. In contrast, the presence or absence of alkyl substituents at the sterol side chain may interfere with copepod sterol nutrition. Giner et al. (2003) suggested that sterols containing an alkyl group at C-23 are refractory to the C-24-dealkylation process, with which dietary phytosterols are converted to cholesterol. Likewise, they suggested that sterols without the C-27-methyl group (27-norsterols) cannot be converted to cholesterol.

Data on sterol requirements of other zooplankton taxa, such as rotifers, are still scarce. The sterol content of the rotifer *Brachionus plicatilis* is positively correlated with the sterol content of its diet (Frolov et al. 1991). In a recent study, Boëchat and Adrian (2006) found that egg production, but not population growth, of the rotifer *Keratella quadrata* was correlated with the dietary sterol content. These findings may suggest that a dietary source of sterols is required for rotifer reproduction. To date, however, it is not known whether rotifers have a limited capacity, or even lack the ability, to synthesize sterols de novo.

3.5.3 Vertebrates

In aquatic vertebrates, sterol requirements are met by de novo synthesis and by dietary uptake in ratios depending on the amounts of sterols provided in the diet. Therefore, a dietary source of sterols is presumably not essential. In contrast, dietary phytosterols may act as endocrine and metabolic disrupters when present in high concentrations. The phytosterol sitosterol, for instance, has been shown to reduce sex steroid levels and the reproductive fitness of certain fish species in a concentration-dependent manner (e.g., MacLatchy and Van der Kraak 1995). To our knowledge, positive effects of dietary phytosterols on the performance of fish have as yet not been reported.

3.6 Ecological Implications: Competition and Succession in Zooplankton

From controlled experiments, it has been concluded that sterol limitation of somatic growth in *Daphnia magna* and *D. galeata* occurs at a sterol content of <5.4 μg/mg dietary carbon (Martin-Creuzburg et al. 2005a). Currently, the interpretation of these values in terms of the ecological relevance appears to be difficult due to the lack of data on the sterol content of eukaryotic phytoplankton. However, values as low as 5 μg/mg of carbon have been published for laboratory grown algae (Bec et al. 2006). In the field, the sterol content of algae might be even lower, as it varies significantly with environmental conditions (e.g., light and nutrient supply). It is worth emphasizing that the weight-specific sterol content of algae in general covers a wide range even within taxa (Tsitsa-Tzardis et al. 1993; Patterson et al. 1994). Thus, a limitation of *Daphnia* by a low availability of dietary sterols seems possible. Moreover, the size range of food particles ingested by the rather unselective filter feeder *Daphnia* includes bacteria, cyanobacteria, and ciliates, which all contain little or no sterols. These prey items may at least seasonally constitute a significant share of ingested carbon for *Daphnia* and thereby reduce the carbon-specific sterol content of the diet. Consequently, the dietary sterol content may strongly affect population dynamics of the herbivorous grazer *Daphnia* in nature.

In laboratory growth experiments with *D. magna* and *D. galeata*, Martin-Creuzburg et al. (2005a) showed that growth of offspring on a sterol-free diet was significantly affected by the sterol content of the mother's diet; i.e., somatic growth rates of offspring decreased with decreasing amounts of sterols in the maternal food. These findings strongly suggest that mothers allocate sterols, presumably cholesterol, to the eggs, and that this sterol content enables the offspring to buffer insufficient dietary sterol supply to some degree. The same study provides strong evidence that daphnids do not allocate a constant amount of sterol to an egg, but that less sterol is allocated under maternal sterol limitation. As a consequence, the offspring will become more sensitive to a nonsaturating sterol supply with increasing

maternal sterol limitation. Thus, the negative effects of limiting dietary sterol content on population growth of *Daphnia* will increase with the persistence of a dietary sterol deficiency. Therefore, the detrimental effects of low-sterol supply on *Daphnia* in the F1 generation may be underestimated in laboratory growth assays if the maternal diet is rich in sterols. In copepods, it has been found that the dietary cholesterol content does not only affect egg production rates, but also egg viability (Crockett and Hassett 2005), which suggests that negative effects of a low sterol availability may become most pronounced at the population level.

The finding that daphnids depend on dietary sterols means that sterols are a nonsubstitutable biochemical resource. Assuming that sterols constitute a seasonally limiting resource for daphnids raises the question of the role of sterols in zooplankton competition and succession. Martin-Creuzburg et al. (2005a) looked for interspecific differences of sterol limitation and compared reaction norms of *D. magna* and *D. galeata* on a range of food with decreasing sterol content. When the individual dry mass over time was taken as a proxy for fitness (Lampert and Trubetskova 1996) both species clearly ran into increasing degrees of sterol limitation when the resource concentration (sterol) was reduced. However, the reaction norms of the two species did not cross, neither when the two species were grown on nonlimiting sterol levels (the green alga *Scenedesmus obliquus*) and in the absence of sterols (the cyanobacterium *Synechococcus elongatus*) (Fig. 3.5a) nor when growth on the cyanobacterium with and without supplementation of cholesterol was compared (Fig. 3.5b): The reaction norms obtained for *D. magna* were steeper than those for *D. galeata*; hence a lower incremental increase in dietary sterol concentration is required for

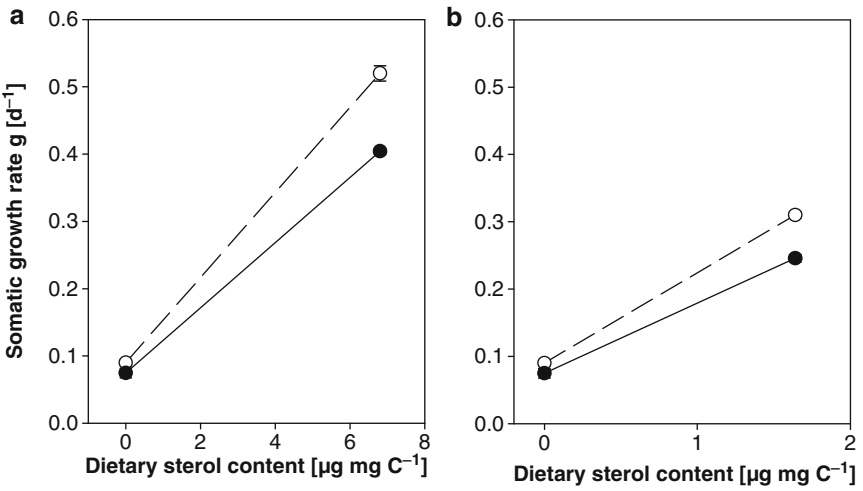

Fig. 3.5 Juvenile somatic growth rates of *Daphnia magna* and *D. galeata* fed with or without dietary sterols. Animals were grown either on (**a**) *Scenedesmus obliquus* or *Synechococcus elongatus* or (**b**) on *S. elongatus* with or without cholesterol supplementation. *Lines* represent reaction norms; *circles* are mean values ±SD. Data from Martin-Creuzburg et al. (2005a, b)

the same increase in growth in *D. magna*, which suggests a lower sterol requirement of the latter. Under all levels of sterol concentrations *D. magna* showed equal or higher juvenile growth rates than *D. galeata*, which corresponded to higher population growth rates (Martin-Creuzburg et al. 2005a) so that no effect of sterol limitation on competition could be demonstrated. Hence, as long as the extent of sterol limitation of herbivorous zooplankton in general and of different *Daphnia* species in particular in nature remains to be demonstrated, it cannot be estimated if the nonsubstitutable resource sterol affects zooplankton composition and seasonal succession.

3.7 Sterols and Carbon Transfer in Aquatic Food Webs

In aquatic food webs, the carbon transfer efficiency across the autotroph–herbivore interface is highly variable, which has far-reaching consequences for various ecosystem processes. One clear example is the low assimilation of cyanobacterial carbon by herbivorous zooplankton, which leads to a decoupling of primary and secondary production and therewith to the accumulation of cyanobacterial biomass (cyanobacterial blooms) especially in eutrophic systems. The food quality of cyanobacteria for herbivorous grazers such as *Daphnia* is determined by numerous factors (e.g., morphological and chemical properties, toxicity). However, the absence of sterols and essential fatty acids in cyanobacteria has been identified as a major food quality constraint for *Daphnia* (Müller-Navarra et al. 2000; Von Elert et al. 2003). In growth experiments with the sterol-free cyanobacterium *Synechococcus elongatus* and the sterol-containing green alga *Scenedesmus obliquus*, it was estimated that daphnids require 20–50% of the green alga in their diet to compensate for the low sterol content of a cyanobacterial food (Von Elert et al. 2003; Martin-Creuzburg et al. 2005a). Assuming that the eukaryotic phytosterols are fully available for the synthesis of cholesterol in *Daphnia*, this suggests that the food quality of natural seston is constrained by sterols if 50–80% of the biomass is prokaryotic, as is often the case with bloom-forming cyanobacteria (Oliver and Ganf 2000). It should be noted that this estimation is only valid if the phytosterol concentration of eukaryotic algae present in natural seston is similar to that of *S. obliquus*.

Like prokaryotes, ciliates presumably lack the ability to synthesize sterols de novo (see above). Recently, it was hypothesized that the comparatively poor food quality of ciliates for crustacean grazers is caused by the lack of sterols in ciliates (Martin-Creuzburg et al. 2006). This hypothesis is complicated by the finding that ciliates are able to incorporate dietary 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 dietary 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, 2006). Hence, the occurrence of sterols in ciliates, and thereby the food quality of ciliates for higher trophic levels, might depend on the food source preyed upon by the ciliates; i.e., ciliates feeding

on a sterol-free prokaryotic food are expected to be nutritionally inadequate for crustacean grazers due to the lack of sterols, whereas ciliates feeding on a sterol-containing eukaryotic food are expected to be upgraded in food quality due to the incorporation of dietary sterols. This was corroborated experimentally by using the ciliate *Colpidium campylum* (grown on bacteria) as a food for *D. magna* (Martin-Creuzburg et al. 2006). As expected, the food quality of the ciliate for *D. magna* was significantly improved by feeding the ciliate with sterol-supplemented albumin beads, which clearly indicates that the deficiency in sterols in the bacteria-fed ciliates has limited the somatic growth of the crustacean grazer. Ciliates are abundant protists in freshwater and marine ecosystems, and their significance in transferring mass and energy from the picoplankton via the microbial loop to higher trophic levels has often been recognized (Stoecker and Capuzzo 1990; Martin-Creuzburg et al. 2005b, and references therein). However, the efficiency of carbon transfer via the microbial loop might be constrained by a deficiency in sterols as long as ciliates are the predominant heterotrophic protozoa.

The carbon transfer efficiency in aquatic food webs might not only be restricted by the absence of dietary sterols, but also by the predominance of sterols which cannot be used as cholesterol precursors by the herbivorous zooplankton. For instance, the marine "red tide" dinoflagellate *Karenia brevis* is characterized by an unusual sterol profile ($\Delta^{8(14)}$-sterols methylated at C-4 or C-23, or demethylated at C-27), which is unlikely to satisfy the sterol nutritional requirements of marine invertebrates (Giner et al. 2003). The same authors proposed that the predominance of these unsuitable sterols in *Karenia brevis* may reduce predation losses, and thereby enhance the ability of the dinoflagellate to form massive blooms. Conceivably, such sterol-mediated metabolic constraints may temporarily also exist in freshwater habitats.

It should also be noted that the assimilation efficiencies of dietary phytosterols may differ significantly depending on structural features of phytosterols (see above) but also depending on food levels. Harvey et al. (1987) reported that the copepod *Calanus helgolandicus* has low assimilation efficiencies for sterols (compared with fatty acids) of the dinoflagellate *Scrippsiella trochoidea*, and that the assimilation efficiencies increase with decreasing food levels.

Detrimental effects of unsuitable dietary sterols on the performance of the consumer might not only be mediated by the inability of these sterols to serve as cholesterol precursors or by low assimilation efficiencies, but these sterols may also have adverse effects on other physiological processes. In grasshoppers, for example, the accumulation of unsuitable sterols in the body leads to high mortality rates, even if suitable sterols are present in ample concentrations (Behmer and Elias 1999). It has been suggested that the incorporation of unsuitable sterols (e.g., alkylated phytosterols) into phospholipid bilayers may result in leaky membranes because alkyl groups prevent phospholipids from packing tightly around the sterol molecules (Behmer et al. 1999b; Haines 2001). In addition, some sterols may inhibit or destroy enzymes involved in sterol dealkylation or may interfere with ecdysteroid synthesis (Giner et al. 2003). Hence, the accumulation of unsuitable dietary sterols may have severe effects on the performance of herbivores, an aspect that should be considered when analyzing carbon transfer efficiencies in aquatic food webs. Another intriguing

area that has not been considered in aquatic systems is the topic of food selection. In grasshoppers, it has been demonstrated that dietary phytosterols can affect the feeding duration on a particular diet, and that unsuitable dietary phytosterols can mediate a learned aversion to the food (Champagne and Bernays 1991; Behmer et al. 1999a).

3.8 Sterol Mediated Trophic Upgrading

In the field, a sterol-depleted food source might be biochemically "upgraded" by heterotrophic protists because of their ability to produce sterols from these substrates (and other essential nutrients, see Chap. 2) thereby making them available for assimilation by higher trophic levels. For instance, Klein Breteler et al. (1999) suggested that the poor food quality of the green alga *Dunaliella* sp. for marine copepods is due to a sterol deficiency in the green alga, which contained only small amounts of Δ^7-sterols that are unsuitable to support the ontogenetic development of copepods. The authors demonstrated that the Chlorophycean food was biochemically upgraded by the heterotrophic dinoflagellate *Oxyrrhis marina* to high-quality copepod food and attributed this trophic upgrading by an intermediary protozoan to the production of Δ^5 sterols in the dinoflagellate. Likewise, sterol-mediated trophic upgrading has been documented in freshwater organisms (Bec et al. 2006). In a simplified food chain consisting of the sterol-free picocyanobacterium *Synechococcus* spp., the heterotrophic nanoflagellate *Paraphysomonas* sp., and the crustacean grazer *D. magna*, the poor food quality of the picocyanobacterial carbon was improved by the addition of sterols (and fatty acids; see Chap. 2) produced by the intermediary flagellate (Bec et al. 2006). Thus, the production of sterols by intermediary protozoans might improve carbon transfer efficiency via the microbial loop from nutritionally inadequate primary producers to metazoan grazers.

3.9 Sterols from Aquatic Sources and Their Potential Role in Human Nutrition

Humans are capable of de novo synthesis of cholesterol, which is negatively regulated by dietary cholesterol. Elevated serum cholesterol levels have been shown to increase the risk of coronary heart disease in humans, which is a leading cause of mortality in many western countries. Thus, the main target in the prevention of coronary heart disease is lowering serum cholesterol levels, either by dietary restrictions or food additives, which limit cholesterol intake (Piironen et al. 2000; Moreau et al. 2002). Phytosterols and phytostanols (and their fatty acid esters) have been shown to reduce plasma cholesterol levels by interfering with transport-mediated processes of cholesterol uptake; i.e., in presence of dietary phytosterols intestinal cholesterol absorption is reduced and more cholesterol is excreted in the feces (Trautwein et al. 2003;

Normén et al. 2004). With the growing interest in functional food, phytosterols are increasingly used as food additives, e.g., in margarine-like spreads (Moreau et al. 2002). The exploitation of sterols from aquatic microorganisms for biotechnological or nutritional purposes is largely in its infancy (Volkman 2003). However, the large variety of sterols in microalgae and the ability to mass culture these organisms may offer new biotechnological applications, e.g., for the production of steroids, nutraceuticals or food additives for human health benefits (Volkman 2003).

3.10 Perspectives

The sterol composition of numerous phytoplankton species has been investigated, mostly to assess the use of sterols as biomarkers for certain phytoplankton taxa. However, due to the large variety of naturally occurring phytosterols in eukaryotic algae, the use of sterols as biomarkers appears difficult. The sterol profile of eukaryotic algae is often dominated by Δ5 sterols alkylated at C-24 with no methyl groups at C-4 (4-desmethyl-sterols); the distributions range from the predominance of a single sterol to a mixture of ten or more sterols (Volkman 2003). Diatoms, for instance, show a great variety of sterol compositions and, although 24-methylcholesta-5,22E-dien-3β-ol (diatomsterol), and 24-methylcholesta-5,24(28)-dien-3β-ol are widely distributed, no sterol appears to be either unique or representative (Volkman et al. 1998). The sterol composition of dinoflagellates is often, but not always, dominated by 4α-methyl sterols, such as dinosterol (4α,23,24-trimethyl-5α-cholest-22E-en-3β-ol), and by sterols with a fully saturated ring system (stanols). Green algae from the Chlorophyta are often characterized by a high content of Δ7 sterols, which are not common in other eukaryotic algae (Volkman 2003). Although some general patterns have been identified, our knowledge of the sterol and composition of common phytoplankton taxa is far from being complete, in particular in freshwater habitats. Another topic that deserves further investigation is the use of sterols as trophic biomarkers in aquatic food webs. Suitable sterols, however, might be difficult to identify, as dietary sterols are often considerably metabolized at each trophic level (e.g., to cholesterol in the crustacean zooplankton).

The sterol profile of eukaryotic algae varies considerably among strains, but also within a single strain depending on the growth conditions (Ballantine et al. 1979; Wright et al. 1980). Klein Breteler et al. (2005) reported that the lipid composition (PUFA and sterols) of the diatom *Thalassiosira weissflogii* was strongly affected by nitrogen and phosphorus limitation, which, in turn, explained the observed differences in copepod growth. This example illustrates that algal growth conditions (nutrient availability, light intensity, temperature, etc.) are crucial for the interpretation of food quality experiments, and it suggests that food quality constraints caused by a stoichiometric or biochemical imbalance might be difficult to separate.

Dietary sterol requirements may vary throughout the life cycle, e.g., the impact of sterol limitation might be most severe on juvenile crustaceans, as they have higher specific growth rates than older animals, and therefore might require larger

amounts of sterols to: (a) build-up and maintain cell membranes and (b) to synthesize ecdysteroids required for molting. With age, sterol requirements may gradually be reduced, as the animals increase their relative investment into reproduction (Lynch et al. 1986). Most crustaceans, however, continue to grow throughout their lifetime and hence a requirement for dietary sterols persists. It is also likely that sterols are allocated into the eggs to provide the developing embryo with sufficient amounts of sterols, which suggests that sterols are also needed for egg production (Wacker and Martin-Creuzburg 2007). Thus, sterol availability in the diet, inherent sterol biosynthetic capacity, and sterol requirements of the various zooplankton life stages have all to be determined to more fully assess the ecological role of sterols in structuring zooplankton communities.

Excess free sterols are harmful for the proper function of membranes (Leppimäki et al. 2000), therefore the dietary intake of sterols must be tightly regulated to prevent toxic effects due to over-accumulation and abnormal deposition within the body. In copepods, the cholesterol content of membranes, which contain the majority of total body cholesterol, is constant despite varying levels of dietary sterols (Crockett and Hassett 2005), suggesting sterol homeostasis. In contrast, the cholesterol content of whole-body extracts of *D. galeata* was found to decrease rapidly when they were fed a sterol-deficient diet and to increase with sterol supplementation (Martin-Creuzburg and Von Elert 2004). Sterols can be, to some extent, stored by esterifying them to long chain fatty acids (sterylesters). Therefore, this decrease in whole-body cholesterol might be attributed to the exhaustion of stored cholesterol and not to a decrease in membrane cholesterol levels. Alternatively, the observed decrease in body cholesterol levels under poor food conditions might be due to the catabolism of membranes, and it remains to be tested if low food quality or low food quantity (energy limitation) have similar effects on body cholesterol levels. In addition, more studies are needed to elucidate the ability of zooplankton to cope with varying levels of dietary sterols, i.e., to identify incipient limiting sterol levels at which somatic growth is limited by the low availability of sterols to better predict the fate of natural zooplankton populations.

The need to maintain a more or less constant level of sterols in the body implies that the absorption of sterols in the gut is strictly regulated. In vertebrates, sterol absorption is presumably a protein-mediated process, i.e. specific receptors and transporters are involved (Trautwein et al. 2003). In general, the absorption of phytosterols in the gut of vertebrates is much lower than that of cholesterol, which suggests discrimination between cholesterol and phytosterols by highly specific mechanisms (Trautwein et al. 2003). In invertebrates, sterol absorption has not been investigated in such detail. However, a selective absorption or excretion of certain sterols has also been reported (Prahl et al. 1984; Harvey et al. 1987). Further studies will have to reveal assimilation efficiencies of dietary sterols to understand sterol mediated food quality constraints on zooplankton performance.

It has already been demonstrated that structural features of dietary sterols can have pronounced effects on somatic growth and development of herbivorous grazers, in particular with regard to their suitability to serve as cholesterol precursors. However, such sterol-mediated metabolic constraints might be highly specific (as in insects, Behmer and Nes 2003) and should not be representative of the situation

in invertebrate grazers in general. In addition to "free" sterols, significant amounts of sterol conjugates (steryl esters, steryl glycosides) might be present in algal food sources (Véron et al. 1998). Whether these compounds are suitable to satisfy the sterol requirements of the herbivorous grazers has not yet been investigated. Zooplankton is likely to ingest both suitable and unsuitable sterols when feeding on a mixture of eukaryotic algae. This implies that the relative proportion of suitable to unsuitable sterols may constrain proper growth and survival of the herbivorous zooplankton, as has been shown for a terrestrial insect (Behmer and Elias 2000). Thus, further studies are needed to improve our knowledge of possible metabolic constraints mediated by the multitude of free and conjugated sterols that occur in aquatic food webs.

The dietary sterol content itself or the predominance of unsuitable dietary sterols might also affect the synthesis of ecdysteroids in arthropods. In general, the prohormone ecdysone is synthesized from dietary sterols (i.e., specifically from cholesterol) and subsequently hydroxylated by target tissues to 20-hydroxyecdysone, the most active ecdysteroid in arthropods (Grieneisen 1994; Gilbert et al. 2002; Martin-Creuzburg et al. 2007; Fig. 3.3). In crustaceans, ecdysteroids are produced mainly in steroidogenic glands, the so-called Y-organs. Our knowledge of ecdysteroid biosynthesis in crustaceans others than decapods is scarce and needs to be extended to the major zooplankton taxa to assess possible effects of dietary sterols on developmental processes.

Sterols are often considered as important structural components of eukaryotic cell membranes. In ciliates, however, the triterpenoid alcohol tetrahymanol and the related hopanoids have been shown to serve as sterol surrogates, as long as a dietary source of sterols is not available; i.e. when ciliates prey on prokaryotic food sources (bacteria, picocyanobacteria; cp. Martin-Creuzburg et al. 2005b, 2006). The finding that ciliates, as intermediary grazers, improve the poor food quality of a picocyanobacterial (sterol-free) diet for subsequent use by *D. magna* implies that tetrahymanol and related compounds can be used as sterol surrogates also in crustacean tissues (Martin-Creuzburg et al. 2005b). Ederington et al. (1995) reported the assimilation of tetrahymanol in copepod tissues (mainly eggs) when ciliates were offered as food, and 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 serve as precursors for many bioactive molecules. 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.

3.11 Conclusions

Aquatic herbivores face a complex pattern of nutritional challenges in natural environments. Beside sterols, biochemical food quality depends on a multitude of other factors such as long chain polyunsaturated fatty acids (e.g., Von Elert 2002),

amino acids (Guisande et al. 2000), and possibly vitamins (Conklin and Provasoli 1977). However, sterol limitation might be one important factor with the potential to affect the structure of aquatic food webs, at least seasonally and in certain habitats. In general, several "compensatory" mechanisms within the prey community and within the herbivorous zooplankton may lead to a less pronounced sterol limitation than would be predicted from determination of the sterol content of edible phytoplankton alone. Within the prey community, trophic upgrading within the microbial loop and synthesis of functionally equivalent compounds like tetrahymanol may reduce the impact of sterol-deficient photoautotrophs on metazoan grazers. Within the metazoan grazer community, the capability to store excess sterols and to change food selectivity may provide a means to temporarily buffer low sterol provision. However, herbivorous zooplankton differs with regard to prey size and selectivity and, though coexisting, may be limited by different resources. The relevance of the different "compensatory" mechanisms may differ substantially for different taxa, e.g., a change in food selectivity may protect calanoid copepods against sterol limitation while nonselective grazers become sterol limited. This suggested example is meant to illustrate that, counterintuitive, compensatory mechanisms may lead to even more pronounced differences of low sterol provision on different zooplankton taxa, since a particular "compensatory" mechanism may not be available to all taxa. As a result, though sterols are essential to all crustacean taxa, a low sterol provision may greatly differ in its effects on coexisting zooplankton taxa and thus affect competition among herbivorous zooplankton. Predatory zooplankton will be protected from sterol limitation due to the presence of cholesterol in its prey and will only indirectly be affected by the sterol concentration in natural seston when it runs into quantitative food limitation if the abundance of herbivorous zooplankton declines as a consequence of sterol limitation. In conclusion, results from controlled laboratory experiments strongly suggest that sterol limitation is a likely scenario for freshwater communities; one that has probably led to a variety of compensatory responses within communities and thereby affected the structure of aquatic food webs.

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