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# Allocation of essential lipids in *Daphnia magna* during exposure to poor food quality

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**Summary**

**1.** In nature, food conditions change temporally and force consumers into trade-offs during resource allocation. In particular, under poor food conditions, for example during cyanobacterial blooms, herbivores have to optimize their resource allocation to maximize fitness, and face two decisions: (i) an individual might attempt to allocate acquired essential resources to reproductive tissues or use them for its own maintenance; and (ii) an individual might decide to optimize the chemical quality of its eggs.

**2.** As cyanobacteria feature a deficiency in some essential lipids that leads to a decline in the growth and fecundity of *Daphnia*, an important freshwater herbivore, we investigated *Daphnia magna*'s Strauss allocation of lipids such as polyunsaturated fatty acids (PUFAs) and cholesterol during an experimental non-toxic cyanobacterial bloom.

**3.** Generally, we found a substantial maternal investment of the particularly important omega-3 (n-3) PUFAs, in particular eicosapentaenoic acid (EPA), into the eggs. The concentration of EPA was 2·4-fold higher in eggs than in somatic tissue, a cumulative effect, which was not significantly changed under cyanobacterial food conditions.

**4.** Under poor conditions, *D. magna* not only decreased the number of eggs produced but, in principle, reduced the previously high concentrations of EPA in both eggs and somatic tissues to a similar degree. In contrast to EPA, the concentrations of α-linolenic acid and cholesterol, although lower than EPA, were more homeostatic in eggs than in somatic tissues, in which concentrations decreased.

**5.** When food quality was improved, *D. magna* were able to recover completely the fatty acid concentrations in their somatic tissues and eggs.

**6.** This study shows that the content of particular lipids in its food clearly affects resource allocation in *D. magna*, and suggests that cholesterol is important for somatic growth, while PUFAs are primarily needed for reproduction. As a decreasing investment of essential lipids into eggs implies a reduced fitness of the animals' progeny under poor food conditions, this could have a strong impact on population dynamics, which might also be valid for other species.

*Key-words*: cyanobacteria, fatty acids, food quality, fresh water, sterols, zooplankton

## Introduction

Strategies of resource allocation are fundamental factors for the understanding of animal life-history traits (Stearns 1992, 2000). In the past, investigations of resource allocation between somatic tissues and reproduction in *Daphnia* were restricted to body mass and the number or mass of eggs produced (Urabe & Sterner 2001). Some *Daphnia* species are known to produce larger eggs when food quantity is low, and such changes in egg size are believed to be an adaptive response, because neonates from larger eggs may survive longer without food (Gliwicz & Guisande 1992; Guisande & Gliwicz 1992). However, most studies have neglected the importance of nutrients other than carbon (which is often used as a surrogate for energy) as influences on egg quality.

In general, the elemental and biochemical composition of food (e.g. algae, detritus) is among the major factors that determine the nutritional value of algal and

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detrital particles for suspension feeders; other factors are morphology, cell-wall digestibility and toxicity (Ferrao, Azevedo & DeMott 2000). Aquatic animals are often discussed as having a fixed nutrient-to-carbon ratio – being, to some extent, homeostatic with respect to their mineral content (Hessen & Lyche 1991). However, the genus *Daphnia* can also change the relative chemical/mineral composition of its body tissues when fed with food sources deficient in minerals (DeMott, Gulati & Siewertsen 1998; DeMott 2003). In addition to the elements, there are numerous biochemicals that cannot be synthesized at all, or only at rates too slow to meet physiological requirements, and so these determine the quality of the units of C within the food (food quality; Cook 1996). As a consequence, the effective utilization of food is often limited (Sommer 1992; Sterner 1993; Müller-Navarra 1995a; Urabe, Clasen & Sterner 1997).

In particular, the availability of certain polyunsaturated fatty acids (PUFAs) is crucial. The significance of specific fatty acids as dietary compounds for animals is at least partly due to the inability of almost all animal species to introduce a second or a third double bond into fatty acids (Beenakkers, Van der Horst & Van Marrewijk 1985). Fatty acids that bear two or more double bonds play important roles in animal physiology and are therefore a dietary requirement for most animals. In general, linoleic acid (LIN, C18 : 2n-6) and α-linolenic acid (ALA, C18 : 3n-3; Cook 1996) are considered to be essential dietary compounds. Daphnids show a limited capacity to convert these fatty acids into longer, more unsaturated fatty acids such as eicosapentaenoic acid (EPA, C20 : 5n-3) (Farkas, Kariko & Csengeri 1981; Von Elert 2002). Moreover, *de novo* rates of synthesis in *Daphnia* are <2% (Goulden & Place 1990), and might be too low to meet metabolic demands. The absence of such essential (no synthesis) or nearly essential (too-low synthesis) dietary compounds strongly affects the somatic growth and fecundity of *Daphnia* (Müller-Navarra 1995b; Müller-Navarra *et al.* 2000; Wacker & Von Elert 2001; Von Elert 2002; Ferrao & Arcifa 2006). Additionally, daphnids may alter their growth and reproduction patterns if the requirements for certain elements or biochemicals differ between somatic and reproductive tissues (Urabe & Sterner 2001; Becker & Boersma 2003).

Apart from PUFAs, sterols are also essential dietary compounds for all arthropods that cannot be synthesized *de novo*. Cholesterol, the principal sterol in crustaceans, is an indispensable structural component of cell membranes and serves as a precursor of steroid hormones, such as ecdysteroids, which are involved in the process of moulting (Goad 1981). The lack of dietary sterols has serious consequences for various life-history traits in *Daphnia* and accounts for the poor food quality of cyanobacteria (Von Elert, Martin-Creuzburg & Le Coz 2003; Martin-Creuzburg & Von Elert 2004; Martin-Creuzburg *et al.* 2005b).

The impact of food quality might be most severe on neonates, which have higher rates of specific metabolism and lower rates of resource acquisition than adults (Lynch, Weider & Lampert 1986). When essential compounds are scarce, adults may allocate extra provisions to each egg. Gliwicz & Guisande (1992) have demonstrated that female daphnids are capable of assessing food levels (food quantity) and, accordingly, adjust their fractional per-offspring allocation of reproductive resources. They have reported that high food levels lead to the production of large clutches with smaller eggs, and low food levels lead to the production of small clutches with larger eggs that are more resistant to starvation, due to a higher content of protein, lipid and C (Guisande & Gliwicz 1992). When feeding on food sufficient in energy but deficient in particular biochemical compounds, *Daphnia* also produce larger progeny (Martin-Creuzburg *et al.* 2005b), which raises the question as to whether daphnids are also capable of assessing nutrient levels in their food (food quality), and accordingly adjust their allocation of essential dietary compounds into reproductive tissues. Although the latter study has shown that larger neonates are produced from females with a poor-quality diet, it also revealed that these neonates do not necessarily have a higher growth capacity, suggesting that they were provided with higher nutrient stores in general, but not with sufficient amounts of essential nutrients. To understand this puzzling question, it is necessary to gain more insights into the chemical composition of the females and their eggs. Lipids, in particular, should be considered as they play highly important roles in aquatic environments (Arts & Wainman 1999), and are highly retained in zooplankton (Kainz, Arts & Mazumder 2004).

In general, animals might face two decisions. (i) From the viewpoint of a single animal, the individual might attempt to allocate offered resources to reproductive tissues or to retain essential resources in order to maintain important bodily functions. (ii) As it is also important that each progeny receives a sufficient amount of essential chemicals to survive in a given environment, an individual might adjust the chemical quality of the eggs. Therefore it is important to consider both *Daphnia*'s partition of available dietary lipid compounds between somatic and reproductive tissues, and the chemical quality of eggs. The problem of the allocation strategy of *Daphnia* may become more complicated if we consider that *Daphnia* inhabit environments that often exhibit substantial fluctuations in the availability of specific resources. Are the animals capable of enough storage to endure periods of poor food quality, such as a cyanobacterial bloom? For the first time we considered the allocation of several lipids in this context. The aim of the present study was to explain: (i) how *Daphnia magna* partition the dietary lipid compounds between somatic and reproductive tissues, including the question as to whether daphnids optimize the chemical composition of their eggs; and (ii) how *D. magna* can recover essential lipids in their somatic and reproductive tissues after a period of poor food quality.

## Methods

### CULTIVATION AND PREPARATION OF FOOD

Algae were obtained from the culture collection of the University of Göttingen (SAG, Germany). The cyanobacterium *Synechococcus elongatus* (SAG 89.79) and the heterokont chromophyte alga *Nannochloropsis limnetica* (SAG 18.99) were cultured semi-continuously in modified WC medium with vitamins (Guillard 1975) at 20 °C and an illumination of 120 μmol $m^{-2}$ $s^{-1}$ (16 : 8 h light : dark cycle) in aerated 2-l vessels. The C concentrations of the food suspensions were estimated from photometric light extinction (at 800 nm) using carbon-extinction equations determined previously (Wacker & Von Elert 2003; Martin-Creuzburg *et al*. 2005b). Aliquots of algal or cyanobacterial suspensions were added to filtered lake water (0·45 μm pore-sized membrane filter), and the resulting suspensions of 2 mg C $l^{-1}$ were used as food for daphnids. Food organisms were chosen because of their different lipid composition, while contents of amino acids were comparable (Ahlgren & Hyenstrand 2003). *Synechococcus elongatus* lacks essential PUFAs (Table 1) and sterols (Martin-Creuzburg *et al*. 2005a) that cannot be synthesized by *Daphnia*, and has been shown previously to be of poor quality for *Daphnia* (Martin-Creuzburg *et al*. 2005b). *Nannochloropsis limnetica* is rich in long-chain PUFAs and sterols, and is thought to be a superior food for *Daphnia*. *Synechococcus elongatus* contained 43·2% saturated fatty acids (SFAs), 55·6% monounsaturated fatty acids (MUFAs), and 0·8% (n-6) polyunsaturated fatty acids [(n-6) PUFAs], but no (n-6) PUFAs with more than 18 C atoms, and did not contain any (n-3) PUFAs (Table 1). In contrast, *N. limnetica* was rich in (n-3) and (n-6) PUFAs (30 and 5·9%, respectively), and contained smaller proportions of SFAs and MUFAs (27·3 and 36·8%, respectively). Major (n-6) PUFAs were C18 : 2n-6 (2·5%) and C20 : 4n-6 (2·4%), and major (n-3) PUFAs were C18 : 3n-3 (0·6%) and C20 : 5n-3 (29%) (Table 1). In addition, carbon : nitrogen : phosphorus molar ratios of 80 : 13 : 1 in *S. elongatus* and 100 : 11 : 1 in *N. limnetica* in the present study meant that mineral nutrients were sufficiently available in the diet.

**Table 1.** Concentrations (μg $mg^{-1}$) of fatty acid groups and of individual PUFAs ±1 SE in *Nannochloropsis limnetica* and *Synechococcus elongatus*, used as diets for *Daphnia magna*

| Fatty acid | *N. limnetica* | *S. elongatus* |
|---|---|---|
| SFA | 42·9 ± 5·32 | 37·5 ± 0·88 |
| MUFA | 57·9 ± 3·21 | 48·2 ± 0·91 |
| (n-3) PUFAs | 47·1 ± 7·69 | nd |
| (n-6) PUFAs | 9·3 ± 0·86 | 0·7 ± 0·14 |
| C18 : 2n-6 | 3·9 ± 0·37 | 0·7 ± 0·14 |
| C20 : 4n-6 | 3·7 ± 1·88 | nd |
| C18 : 3n-3 | 0·8 ± 0·24 | nd |
| C18 : 4n-3 | nd | nd |
| C20 : 5n-3 | 46·3 ± 7·46 | nd |

nd = not detected; C22 : 6n-3 was not detected.
SFA contain all saturated fatty acids ≥ 14 carbons; MUFA contain all fatty acids with a single double bond ≥ 16 carbons; PUFAs contain all fatty acids with at least two double bonds and ≥ 18 carbons.

### ALLOCATION EXPERIMENT

The experiment was conducted with a clone of *D. magna* originally isolated from Großer Binnensee (Lampert 1991). The allocation experiment was carried out with juvenile daphnids (born <6 h), which were subjected to three different periods of feeding: a preconditioning phase, an experimental phase and a recovery phase. In the preconditioning phase, 10 newborn daphnids were kept at 20 °C in glass beakers filled with 200 ml filtered lake water (<0·45 μm), and fed with *N. limnetica* in a light : dark cycle of 16 : 8 h. As daphnids grew, they were distributed to larger, 400-ml beakers. After 7 days, daphnids released their first clutch and the adults were transferred to two sets of different experimental food regimes (the experimental phase). In one treatment they were fed with the alga *N. limnetica* (2 mg C $l^{-1}$) throughout the experiment, whereas in the other treatment they were fed with the cyanobacterium *S. elongatus* (2 mg C $l^{-1}$, 6 days). In the subsequent recovery phase (6 days), it was tested how animals recover from poor food quality (*S. elongatus*) when fed with *N. limnetica*. In both treatments, food suspensions were renewed at regular intervals to keep the food concentrations above the incipient limiting level, although concentrations may have fluctuated in both treatments throughout the experiment.

When animals released their first clutch at the end of the preconditioning phase, they had already been investing resources into the development of their second clutch (Tessier & Goulden 1982). As this investment originates from good-quality food from the preconditioning phase, we excluded the second clutch from the analysis. The production of new eggs was investigated visually under a stereomicroscope. As soon as animals produced third- and fourth-clutch eggs, daphnids and eggs were harvested from randomly chosen glass beakers of each experimental food treatment. Daphnids were dissected under a stereomicroscope and the eggs were sampled by gently blowing them out of the daphnids' brood chamber with a lengthened glass Pasteur pipette. All eggs sampled were in the first egg stage, and did not show any morphological differentiation (Threlkeld 1979). For determination of dry weight, at least 12 eggs and three adults were dried for 48 h at 50 °C and weighed on an electronic balance (±1 μg, CP2P, Sartorius, Goettingen, Germany).

### CHEMICAL ANALYSES

Aliquots of algal or cyanobacterial food suspensions were filtered through precombusted glass-fibre filters (Whatman GF/F, 25 mm diameter), dried, and

analysed for particulate organic carbon (POC) in the carbon analyser (HighTOC + N, Elementar). For lipid analyses, aliquots of algal or cyanobacterial food suspensions corresponding to ≈0·5 mg POC were filtered through a precombusted Whatman GF/F filter (25 mm diameter). At least 35 eggs and eight adults (with eggs removed) were prepared for lipid analyses, and were directly transferred into glass test tubes. The loaded filters, daphnids or eggs were disrupted in dichloromethane/methanol (2 : 1 v/v) using sonic waves, and were either extracted immediately for analysis of lipids or stored under nitrogen in dichloromethane/methanol at –80 °C for later analysis. Prior to extraction, 20 µg heptadecanoic acid methyl ester, 20 µg tricosanoic acid methyl ester and 10 µg 5-alpha-cholestan (all chemicals from Sigma-Aldrich) were added as internal standards. For extraction of lipids, algae, daphnids or eggs were extracted twice with 7 ml dichloromethane/methanol (2 : 1 v/v). Particles were removed by centrifugation (3500 ***g***, 5 min) and the supernatant evaporated to dryness under N. For fatty acid analysis, the dried sample was resuspended in 4 ml 3 mol $l^{-1}$ methanolic HCl (Sigma-Aldrich Chemie) and subsequently incubated for 20 min at 60 °C in a sealed vial to transesterify fatty acids into methyl esters. After the sample had cooled down, fatty acid methyl esters (FAMEs) were extracted three times with 3 ml isohexane. The fraction of isohexane was evaporated to dryness under N and resuspended in a volume of 50–100 µl isohexane. FAMEs were analysed by gas chromatography using a 6890 N gas chromatograph (Agilent Technologies, Waldbronn, Germany) with the following configuration: column, DB 225 (30 m × 0·25 mm inner diameter × 0·25 µm film; J & W Scientific, Cologne, Germany); oven, 60 °C (1 min) to 150 °C at 30 °C $min^{-1}$, then to 170 °C at 3 °C $min^{-1}$, then to 220 °C at 2 °C $min^{-1}$, and held for 6 min; carrier, helium, purity 5·0, 35 cm $s^{-1}$; flame-ionization detector (FID), 250 °C; injector, 250 °C; total run time, 42 min per sample. The sample (1 µl) was injected splitlessly. FAMEs were identified by comparison of retention times with those of reference compounds (Sigma-Aldrich). The fatty acids were quantified by comparison with internal standards and by using a multipoint standard calibration curve determined for each FAME, from mixtures of known composition (Sigma-Aldrich). It was not possible to distinguish between petroselinic acid (C18 : 1n-12) and oleic acid (C18 : 1n-9). The absolute amount of each FAME was normalized to the independently determined POC content or dry weight of the sample.

For analysis of cholesterol, the extracted and evaporated sample was saponified with 4 ml 0·2 mol $l^{-1}$ methanolic KOH (60 min at 70 °C). After addition of 1 ml ultra-pure water, the neutral lipids (including cholesterol) were partitioned into isohexane : diethyl ether (9 : 1 v/v). This fraction was evaporated to dryness under N and resuspended in a volume of 50–100 µl.

Free cholesterol was quantified with a gas chromatograph (6890 N, Agilent Technologies) with the following configuration: DB-5 capillary column (30 m × 0·25 mm inner diameter × 0·25 µm film; J & W Scientific); oven, 150 °C (1 min) to 280 °C at 15 °C $min^{-1}$, then to 320 °C at 2 °C $min^{-1}$, and held for 3 min; carrier, helium, purity 5·0, 39 cm $s^{-1}$; FID, 350 °C; injector, 350 °C; total run time, 33 min per sample. The sample (1 µl) was injected splitlessly. Cholesterol was quantified using 5-α-cholestan as an internal standard and was identified by comparison of the retention times with authentic cholesterol and by using a gas chromatograph–mass spectrometer (Finnigan MAT GCQ, Egelsbach, Germany), equipped with a fused silica capillary column (DB-5 ms); the instrumental settings are described elsewhere (Martin-Creuzburg & von Elert 2004).

### DATA ANALYSIS

For direct measurements of clutch size and egg mass, data were analysed using a two-way ANOVA with the factors designated as food regime and time interval. To meet assumptions, data were square-root transformed. For data analysis of lipids, we divided the interval during which the animals were fed with poor-quality food into two steps: the first production of a clutch on poor-quality food, which is the visible production of the third clutch; and the successive production of a clutch on poor-quality food, which is the fourth clutch. Changes of lipid concentration in somatic tissues and eggs were compared between the two different food regimes using a two-way ANOVA for each clutch separately. The factors were food regime and the allocation target, the latter representing concentration differences between somatic tissues and eggs. To meet assumptions, data were $\log(x + 1)$-transformed when necessary. A reduced number of degrees of freedom for the data analysis of fatty acids are due to the fact that some samples were used exclusively for the identification of cholesterol. To test if the fatty acid concentration in the recovery phase reached previous control values, an ANOVA was performed, which should not be significant if recovery was successful.

## Results

### CLUTCH SIZE AND EGG SIZE

The clutch size of the daphnids decreased significantly over time when the animals (producing the third and fourth clutch) were fed with poor-quality food during the experimental time interval (significant interaction; Fig. 1; Table 2). In contrast, the egg mass of their produced eggs was constant, although there might have been a marginal interaction, suggesting a slight increase of egg mass with poor food quality (Fig. 1; Table 2). When animals were allowed to recover from the poor food source *S. elongatus* by feeding on *N. limnetica*, they produced a clutch size similar to the previous control after 6 days (ANOVA, $P = 0{\cdot}93$), although they did produce slightly smaller eggs (Fig. 1, ANOVA, $P < 0{\cdot}001$).

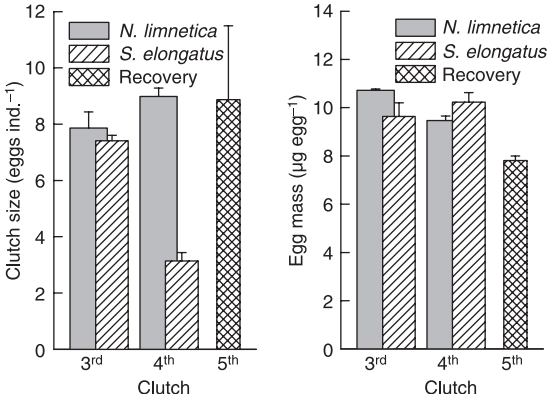


**Fig. 1.** Mean clutch size (left) and mean egg mass (right) (±1 SE) during simulation of an experimental cyanobacteria bloom (*Synechococcus elongatus*) lasting for the development of two subsequent clutches of *Daphnia magna* (third and fourth clutch, respectively), compared with *D. magna* fed continuously with good-quality food (*Nannochloropsis limnetica*). Cross-hatched bars show what happens when animals previously fed with poor-quality food are given the chance to recover with good-quality food.

**Table 2.** Statistical results for changes in clutch size and egg mass during an experimental cyanobacteria bloom (factor = food regime, which describes the change in food from *Nannochloropsis limnetica* to *Synechococcus elongatus*), lasting for the development of two subsequent clutches (factor = time interval) of *Daphnia magna*

| Measurement | Factor | df | *F* | *P* |
|---|---|---|---|---|
| Clutch size | Food regime (F) | 1,12 | 48·98 | <0·0001*** |
| | Time interval (T) | 1,12 | 7·76 | 0·0165* |
| | F × T | 1,12 | 17·64 | 0·0012** |
| Egg mass | Food regime (F) | 1,12 | 0·12 | 0·7389 |
| | Time interval (T) | 1,12 | 0·06 | 0·8182 |
| | F × T | 1,12 | 4·00 | 0·0687 |

## CHEMICAL QUALITY OF MATERNAL TISSUES AND EGGS

In all cases, the concentrations of SFAs, MUFAs, (n-6) PUFAs and (n-3) PUFAs per dry weight decreased under poor food quality (factor, food regime; Table 3; Fig. 2), and particular fatty acid concentrations were significantly higher in eggs than in somatic tissues (shown by the significant factor, allocation target). Eggs of the third clutch of daphnids that were fed with good-quality food had 1·4-fold higher concentrations of SFAs, MUFAs and (n-6) PUFAs compared with the fatty acid concentrations of the somatic tissues (Fig. 2; Table 3). This cumulative effect was more pronounced in (n-3) PUFAs, which were 2·4-fold more concentrated in eggs than in somatic tissue. This effect was also observed when daphnids were fed with the poor-quality diet. Then daphnids still showed higher concentrations of SFAs, MUFAs, (n-6) PUFAs and (n-3) PUFAs (1·8-fold, 1·4-fold, 1·3-fold and 2·1-fold, respectively; Table 3) in eggs than in somatic tissues, although concentrations were generally lower (Fig. 2). Since, for all groups of fatty acids, no significant interactions were found between the two factors (allocation target and food regime), under poor food-quality conditions the animals reduced fatty acid concentrations in both eggs and somatic tissues to a similar degree (significant effects of food regime in all fatty acid groups in subsequent clutches; Table 3; Fig. 2).

As PUFAs are complex mixtures, with some believed to have high physiological and ecological importance, individual fatty acids within the groups of PUFAs were tested separately. The major (n-6) PUFAs were linoleic acid (LIN, C18 : 2n-6) and arachidonic acid (ARA, C20 : 4n-6). In contrast to ARA, which was significantly more concentrated in eggs, the concentration of LIN in eggs and somatic tissue was not

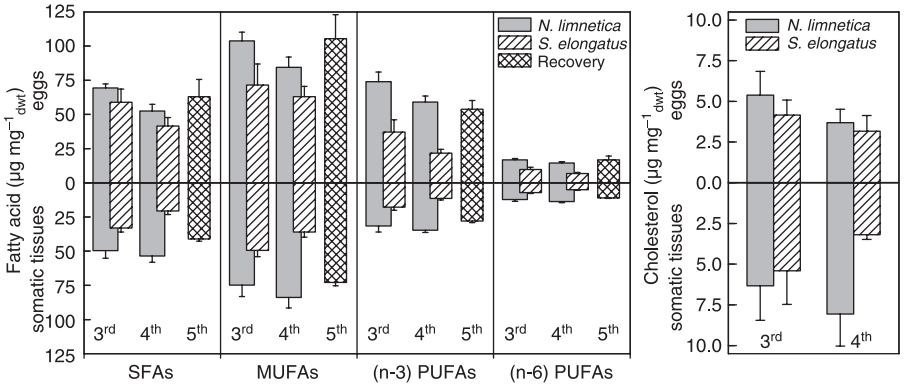


**Fig. 2.** Chemical quality of egg tissues (positive direction of bars) and of maternal tissues (negative direction of bars). After a preconditioning phase, during which animals were fed with identical food, animals were transferred to two different experimental food regimes until they produced their third and fourth clutch. The experimental food regimes represented a good-quality diet (*Nannochloropsis limnetica*) and a poor-quality diet (*Synechococcus elongatus*). After production of the fourth clutch, animals were allowed to recover from poor food quality by feeding again on *N. limnetica*, and produced their fifth clutch. Concentrations of lipids ±1 SE are given for saturated, monounsaturated, n-3 polyunsaturated, n-6 polyunsaturated fatty acids [SFAs, MUFAs, (n-3) PUFAs and (n-6) PUFAs, respectively; left], and cholesterol (right) per dry weight of egg or tissue mass. Groups of fatty acids are summarized as in Table 1.

**Table 3.** Statistical results for differences in chemical quality of *Daphnia*'s produced biomass, measured as concentration per dry weight

| | | third clutch | | | fourth clutch | | |
|---|---|---|---|---|---|---|---|
| Sample | Factor | df | *F* | *P* | df | *F* | *P* |
| Cholesterol | Food regime (F) | 1,12 | 0·39 | 0·5461 | 1,10 | 6·33 | 0·0306* |
| | Allocation target (A) | 1,12 | 0·41 | 0·5323 | 1,10 | 3·15 | 0·1062 |
| | F × A | 1,12 | 0·01 | 0·9316 | 1,10 | 4·13 | 0·0695 |
| SFA | Food regime | 1,10 | 6·29 | 0·0311* | 1,10 | 13·03 | 0·0048** |
| | Allocation target | 1,10 | 16·55 | 0·0023** | 1,10 | 4·00 | 0·0733 |
| | F × A | 1,10 | 0·30 | 0·5987 | 1,10 | 1·91 | 0.1973 |
| MUFA | Food regime | 1,10 | 10·17 | 0·0097** | 1,10 | 14·33 | 0·0036** |
| | Allocation target | 1,10 | 8·09 | 0·0174* | 1,10 | 3·84 | 0·0785 |
| | F × A | 1,10 | 0·15 | 0·7076 | 1,10 | 0·98 | 0·3454 |
| (n-3) PUFAs | Food regime | 1,10 | 14·75 | 0·0032** | 1,10 | 103·67 | <0·0001*** |
| | Allocation target | 1,10 | 22·93 | 0·0007*** | 1,10 | 42·92 | <0·0001*** |
| | F × A | 1,10 | 0·30 | 0·5970 | 1,10 | 0·08 | 0·7807 |
| (n-6) PUFAs | Food regime | 1,10 | 26·48 | 0·0004*** | 1,10 | 62·88 | <0·0001*** |
| | Allocation target | 1,10 | 9·88 | 0·0104* | 1,10 | 3·76 | 0·0811 |
| | F × A | 1,10 | 0·95 | 0·3523 | 1,10 | 0·02 | 0·8899 |
| C18 : 2n-6 | Food regime | 1,10 | 26·02 | 0·0005*** | 1,10 | 36·18 | <0·001*** |
| | Allocation target | 1,10 | 2·51 | 0·1445 | 1,10 | 0·47 | 0·5093 |
| | F × A | 1,10 | 0·04 | 0·8402 | 1,10 | 0·99 | 0·3424 |
| C20 : 4n-6 | Food regime | 1,10 | 24·76 | 0·0006*** | 1,10 | 112·27 | <0·0001*** |
| | Allocation target | 1,10 | 15·13 | 0·0030** | 1,10 | 11·82 | 0·0064** |
| | F × A | 1,10 | 1·85 | 0·2039 | 1,10 | 3·01 | 0·1132 |
| C18 : 3n-3 | Food regime | 1,10 | 1·08 | 0·3241 | 1,10 | 3·76 | 0·0812 |
| | Allocation target | 1,10 | 0·10 | 0·7544 | 1,10 | 1·17 | 0·3047 |
| | F × A | 1,10 | 0·03 | 0·8739 | 1,10 | 5·01 | 0·0491* |
| C18 : 4n-3 | Food regime | 1,10 | 7·86 | 0·0187* | 1,10 | 2·57 | 0·1397 |
| | Allocation target | 1,10 | 1·12 | 0·3146 | 1,10 | 1·57 | 0·2387 |
| | F × A | 1,10 | 0·24 | 0·6363 | 1,10 | 0·08 | 0·7805 |
| C20 : 5n-3 | Food regime | 1,10 | 15·75 | 0·0026** | 1,10 | 67·64 | <0·0001*** |
| | Allocation target | 1,10 | 26·59 | <0·001*** | 1,10 | 35·91 | <0·001*** |
| | F × A | 1,10 | 0·32 | 0·5863 | 1,10 | 0·50 | 0·4970 |

The factor 'allocation target' represents possible differences between maternal tissues and egg tissue. The factor 'food regime' describes differences in chemical quality dependent on the food source, i.e. if animals were reared on good or poor-quality food during the two time steps. Results for each clutch are given separately. Groups of fatty acids are summarized as given in Table 1.

significantly different (Table 3). During poor food quality, the concentrations of these two fatty acids were similarly reduced in eggs and tissues (Tables 3 and 4). However, in the group of (n-3) PUFAs, such a proportional decrease during poor quality could be observed only in EPA (C20 : 5n-3), where the interaction between both statistical factors was not significant (Tables 3 and 4). Additionally, EPA was preferentially allocated into eggs up to a concentration of ≈70 μg $mg^{-1}$ DW, 2·4-fold higher than in somatic tissues. In contrast, the concentrations of ALA (C18 : 3n-3) were low, but similar in somatic tissues and eggs (≈1 μg $mg^{-1}$ DW) until the animals were exposed to poor food quality. Under poor-quality conditions, daphnids were able to keep the ALA in their eggs constant (≈1 μg $mg^{-1}$ DW), whereas simultaneously, ALA in their somatic tissues was significantly reduced to 0·6 μg $mg^{-1}$ DW (significant interaction; Table 3). After the experimental phase, food quality was improved, and after 6 days all concentrations were not significantly different from previous values for the good food-quality treatment (ANOVA, $P > 0·05$). Daphnids were therefore able to recover completely the concentrations of fatty acids in their somatic tissues and eggs (Fig. 2).

In addition to these changes in fatty acid concentration, cholesterol concentration was also affected under poor food-quality conditions. ANOVA showed that, although the concentration of cholesterol in the fourth clutch appears to be comparable in tissue and eggs (Fig. 2; Table 3, factor = allocation target), it is significantly influenced by food quality (factor = food regime). While the cholesterol concentration in the somatic tissue of animals fed with the cyanobacterium decreased during production of the fourth clutch (Fig. 2), the concentration in eggs appeared to be homeostatic (marginally significant interaction, Table 3).

## Discussion

Under poor food-quality conditions, *D. magna* may optimize the allocation of their essential lipid resources between somatic and reproductive tissues, to maximize fitness. Animals may be able to bridge periods of poor diet quality if food quality switches quickly between

**Table 4.** Concentrations (μg $mg^{-1}$ DW) of individual PUFAs ± 1 SE in eggs and somatic tissue of daphnids fed with *Nannochloropsis limnetica* or *Synechococcus elongatus* during the experimental period

| | | | *N. limnetica* | | *S. elongatus* | |
|---|---|---|---|---|---|---|
| Sample | | | Third clutch | Fourth clutch | Third clutch | Fourth clutch |
| (n-6) | C18 : 2n-6 | Eggs | 6·8 ± 0·38 | 5·5 ± 0·42 | 4·2 ± 0·76 | 3·2 ± 0·46 |
| | | Soma | 5·9 ± 0·53 | 6·4 ± 0·49 | 3·5 ± 0·24 | 2·3 ± 0·26 |
| | C20 : 4n-6 | Eggs | 10·0 ± 0·66 | 8·9 ± 0·62 | 5·5 ± 1·06 | 3·5 ± 0·44 |
| | | Soma | 6·3 ± 0·71 | 7·3 ± 0·46 | 3·7 ± 0·35 | 2·7 ± 0·26 |
| (n-3)* | C18 : 3n-3 | Eggs | 1·2 ± 0·10 | 0·9 ± 0·06 | 0·9 ± 0·64 | 1·0 ± 0·26 |
| | | Soma | 1·1 ± 0·14 | 1·1 ± 0·09 | 0·8 ± 0·10 | 0·6 ± 0·06 |
| | C18 : 4n-3 | Eggs | 1·3 ± 0·10 | 1·1 ± 0·12 | 0·6 ± 0·35 | 0·7 ± 0·07 |
| | | Soma | 1·0 ± 0·14 | 1·1 ± 0·11 | 0·5 ± 0·16 | 0·5 ± 0·05 |
| | C20 : 5n-3 | Eggs | 71·6 ± 6·76 | 57·2 ± 4·29 | 35·6 ± 8·05 | 19·9 ± 2·63 |
| | | Soma | 29·4 ± 4·35 | 32·4 ± 1·44 | 16·6 ± 2·02 | 10·3 ± 1·00 |

A detailed description of the experimental period is given in the legend to Fig. 2.
*C22 : 6n-3 was not detected.

good and poor (Becker & Boersma 2005). In nature, however, cyanobacterial blooms of poor lipid quality usually persist for 1–2 weeks (Arts, Robarts & Evans 1997). Consequently, if animals have to face longer periods of poor quality (as in the present study), they reduce the fatty acid concentrations over time both in eggs and in their soma, consistent with the observations of Becker & Boersma (2005). Nevertheless, *D. magna* allocated high amounts of fatty acids into eggs. The concentration of (n-6) PUFAs such as ARA was 1·3× higher in eggs than in somatic tissue, and this was in the same order of magnitude for SFAs and MUFAs. This indicates that a general mechanism exists for concentrating fatty acids into eggs, which provides eggs with sufficient biochemical compounds for development. It is obvious that *D. magna* fed a diet deficient in ARA is still able to allocate ARA into eggs, which can also be observed in zebra mussels (Wacker & von Elert 2004). Since LIN, which is a precursor of ARA, was available in *S. elongatus*, *D. magna* possibly used elongation and desaturation of LIN to provide eggs with additional amounts of ARA. The importance of ARA can be attributed mainly to its function as a precursor of prostaglandins (Cook 1996), which regulate the reproduction of invertebrates (Martinez, Olivares & Mettifogo 2000). However, there is no indication about the potential influence of (n-6) PUFAs, as well as of SFAs and MUFAs, in the determination of fitness of daphnids in nature, while (n-3) PUFAs are suggested to play significant roles (Müller-Navarra *et al*. 2000; Wacker & von Elert 2001).

In the present study, *D. magna* invested huge amounts of EPA into eggs, which might underline the importance of EPA; other (n-3) PUFAs may not be equally important, as some serve as EPA precursors. This adds to recent findings that EPA is of high ecological and physiological significance (Von Elert 2002; Becker & Boersma 2003; Kainz *et al*. 2004; Wacker & Von Elert 2004; Hessen & Leu 2006; Ravet & Brett 2006). Additionally, we found that *D. magna* invested differently into the biochemical quality, as identified by lipid profiles, of their offspring and into somatic tissues when food quality changed. The concentrations of PUFAs decreased proportionally in eggs and in somatic tissue, except for the initially similar concentrations of ALA (C18 : 3n-3), which were homeostatic in eggs but not in somatic tissues. This shows that the females followed a strategy that invests ALA into eggs even under poor conditions, when ALA is not sufficiently available, and this also suggests that ALA is of high importance (Wacker & von Elert 2001). The concentration of stearidonic acid (C18 : 4n-3), which is desaturated from ALA and used as precursor for EPA, did not show the same clear behaviour as ALA. During production of the third clutch it was less abundant in animals and eggs fed with the cyanobacterium, and did not show any homeostasis throughout the whole experimental phase (Tables 3 and 4).

However, an effect comparable to that of ALA was observed for concentrations of cholesterol. While animals invested considerable amounts of substances into their eggs, in particular EPA, the concentration of cholesterol was not higher in somatic tissues than in eggs. Under prolonged low diet quality, for example until production of the fourth clutch, animals invested lower amounts of cholesterol into somatic tissues, and kept the composition of eggs constant. A relative shift in comparison to EPA, for example, indicates that cholesterol might be more important for growth than for reproduction, in contrast to PUFAs, which might be more important for reproduction than for growth. This opinion is consistent with results from feeding experiments with *S. elongatus*, which also suggest that growth of daphnids is primarily constrained by the absence of sterols. In this case, PUFAs become limiting only when the shortage of sterols has been overcome by sterol supplementation and when the animals increase their investment into reproductive tissues (Von Elert *et al*. 2003). However, in the present study, animals tried to keep the concentration of cholesterol in eggs

constant (Fig. 2), indicating that a threshold cholesterol concentration in the somatic tissue may exist (<3·19 μg mg $C^{-1}$, approximated from the minimal value of somatic tissue), at which reproduction is limited by the low availability of sterols. If we suppose that a food source should contain amounts of essential compounds similar to those needed for production of a daphnid's body tissue, our estimation fits well with the calculations of Martin-Creuzburg *et al*. (2005b). They calculated that population growth rates of *D. magna* will decline at sterol contents of <3·4 μg $mg^{-1}$ dietary C.

We had expected that mothers would modify egg size and composition with changes in resource acquisition in a variable environment such as our experiment. Indeed, Guisande & Gliwicz (1992) documented that daphnids produce larger eggs with an increased content of protein, lipid and C as food concentration decreased. This might be adaptive, because larger eggs improved the ability of neonates to withstand starvation (Tessier & Consolatti 1989; Gliwicz & Guisande 1992). Bychek *et al*. (2005) recently showed that *D. magna* displayed only small changes in its lipid metabolism during short starvation periods (24 h). If the nutritional limitation lasts longer, and essential compounds such as particular fatty acids and sterols are not sufficiently available in the environment (e.g. during cyanobacterial blooms), internal reserves are exhausted and animals change their metabolism or their strategy. Nevertheless, the production of fewer, larger eggs might be adaptive for the parent's fitness only when the food conditions remain poor. Therefore it is also important to know if, and how quickly, females can recover from a bad food source and revert to their usual behaviour. We found that, although animals were able to recover from a period of poor food quality, this recovery period lasted 6 days. Consequently, the strategy to produce larger progeny might be highly adaptive in the case of cyanobacterial blooms. We have shown previously that the dry weight of neonates was enhanced slightly as maternal food quality (sterol content) decreased (Martin-Creuzburg *et al*. 2005b). This might be considered an adaptive response to lower food quality that gives progeny an opportunity to survive poor food-quality conditions. Accordingly, this suggests that a low-quality maternal diet (in terms of lipids) should lead to an increased allocation of PUFAs and cholesterol into eggs, to enhance the survival of offspring.

Unfortunately, this hypothesis is contradicted by data presented here, which suggest an allocation of lipids approximately proportional to their availability in the diet. As a consequence, the period of insufficient food quality results in a decrease of quality in both maternal and offspring tissue, and might explain the finding that neonates performed less well when maternal food quality was poor (Brett 1993; Martin-Creuzburg *et al*. 2005b). Hence increased eggs sizes might result from the allocation of energy-rich compounds into the eggs (cf. Gliwicz & Guisande 1992; Guisande & Gliwicz 1992); however, the low availability of cholesterol and PUFAs might limit growth of offspring.

Alternatively, the finding that energy-rich foods deficient in key biochemical compounds led to production of large progeny that did not necessarily have high growth capacity (present study; Martin-Creuzburg *et al*. 2005b) might result from a noxious effect of high nutrient intake. It has been shown recently that animals benefit from the increasing availability of limiting micronutrients until the costs of disposing excess micronutrients overcome these positive effects, or excesses become toxic. This approach can be extended to caloric macronutrients such as carbohydrates and proteins (Raubenheimer, Lee & Simpson 2005). While herbivorous insect species usually have sufficient ability to equilibrate a dietary mismatch by compensatory feeding or selection of complementary food sources (Raubenheimer & Jones 2006), such a mechanism is unlikely in the present study because daphnids are unselective filter feeders (DeMott 1986). However, daphnids might respond postingestively (animals may excrete excess dietary compounds, which are associated with metabolic costs; Elser & Urabe 1999; Anderson *et al*. 2005). Unfortunately, changes in postingestive food processing are not necessarily sufficient, so that too much surplus of specific nutrients compared with others may result in large or obese progeny with decreased fitness (Raubenheimer *et al*. 2005; Warbrick-Smith *et al*. 2006). Such a mechanism could lead to our *Daphnia*'s large offspring and decreased fitness, a fact recently suggested for *Daphnia*'s growth response to excess phosphorus (Boersma & Elser 2006). As, in the present study, mineral nutrients were sufficiently available in food organisms, increased costs might originate from ingesting excess cyanobacterial food (that contained surplus nutrients such as phosphorus and SFAs) to gain the limiting compounds (e.g. specific PUFAs, sterols). It is still not known why potentially surplus nutrients such as phosphorus, SFAs and MUFAs may be noxious compared with less-available limiting compounds such as PUFAs and sterols. Therefore future research is important, and is needed to clarify if surplus compounds might be either directly noxious or just correlated with another unhealthy mechanism.

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