Chapter 6
Crustacean Zooplankton Fatty Acid Composition

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6.1 Introduction

Fatty acids (FA) are among the most important molecules transferred across the plant–animal interface in aquatic food webs. Particular classes of FA, such as the n-3 highly unsaturated fatty acids (HUFA), are important somatic growth limiting compounds for herbivorous zooplankton (Müller-Navarra 1995a; Müller-Navarra et al. 2000; Ravet et al. 2003). These molecules are also critical for the growth, disease resistance, and general well being of juvenile fish (Adams 1999; Olsen 1999; Sargent et al. 1999). Thus, knowing how nutritionally important FA are conveyed through food webs has important implications for understanding economically important fisheries. A very substantial literature shows these same molecules have a wide range of positive impacts on human health (Simopoulos 1999; Arts et al. 2001). Specific FA may also help interpret trophic relations in aquatic systems (Dalsgaard et al. 2003), as the group specific FA composition of primary producers varies greatly (Volkman et al. 1989; Ahlgren et al. 1992). Therefore, it is important to understand how much the FA composition of zooplankton is determined by taxonomic affiliation, changed by diet, and modified by starvation or temperature. It is also essential to know whether zooplankton maintain a semiconstant FA profile relative to their diets or, alternatively, bioconvert some FA into other FA molecules. This review will summarize the published information on how these factors regulate the FA composition of freshwater and marine zooplankton.

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6.1.1 Historical Context

The analysis of zooplankton FA started with Lovern (1935), who compared FA in the marine calanoid copepod *Calanus finmarchicus* and the freshwater zooplankters *Cyclops strenuous*, *Daphnia galeata*, and *Diaptomus gracilis* with the FA of fish caught from the same environments. Lovern observed that the FA composition of these zooplankton was quite similar to the lipids contained in "typical fish-oil" and concluded this indicated fish deposit dietary lipids into their tissue largely unchanged. Subsequently, Ackman and Eaton (1966) showed the most prevalent FA in the euphausiid *Meganyctiphanes norvegica* affected the FA composition of the fin whale in the North Atlantic. Variation in zooplankton FA composition on a seasonal basis was first explored by the pioneering research of Tibor Farkas (Csengeri and Halver 2006). When examining zooplankton samples collected from Lake Balaton, Hungary, in 1958, Farkas observed that zooplankton lipids always had lower melting points than the ambient water temperatures (Csengeri and Halver 2006). He also noted the proportions of eicosapentaenoic acid (20:5n-3; EPA) and especially docosahexaenoic acid (22:6n-3; DHA) in zooplankton lipids increased with decreasing temperatures (Farkas and Herodek 1964). He was the first to note that cladocerans nearly exclusively accumulate EPA whereas copepods predominately accumulate DHA (Farkas 1979). In several laboratory studies (Farkas 1979; Farkas et al. 1984), Farkas suggested copepods could readily adjust their n-3 HUFA and especially DHA content in response to cold stress, whereas the results he obtained for *Daphnia magna* suggested *Daphnia* only had a minimal capacity to adjust HUFA composition in response to temperature. Farkas explained these results within a homeoviscous[1] adaptation context and suggested that these differences were due to varying over-wintering strategies. He suggested that cladocerans as a group were primarily active when water temperatures exceeded 10°C, and over-wintered as inactive resting eggs. Farkas concluded that because cladocerans did not modify their DHA content in response to cold stress, they were unable to maintain lipid melting points below ambient winter water temperatures and therefore could not over-winter in an active life stage. In contrast, copepods readily increased their DHA content when exposed to lower temperatures and many species over-wintered in an active stage.

The first published studies of environmental impacts on the FA of marine zooplankton (Lewis 1969; Jeffries 1970) followed an approach similar to Farkas and focused on seasonal affects (changing water temperatures and phytoplankton community composition) on *Acartia* spp. FA. During the winter and spring, the phytoplankton at Jeffries' field site (Narragansett Bay, Rhode Island) was dominated by diatoms, and during the summer and fall, it was dominated by flagellates. Jeffries noted that during winter and spring, *Acartia* had higher monounsaturated fatty acid (MUFA) contents, and during summer and fall, they had higher saturated fatty acid (SAFA)

[1] Homeoviscous response refers to the modification of membrane lipid composition to maintain similar physical properties across a range of water temperatures.

contents. Paradoxically, he reported that *Acartia* accumulated more DHA during the warmer summer/fall months. This latter result could be because dinoflagellates (which often have high DHA content) were prevalent in the summer during his study.

One of the most pivotal studies of marine zooplankton FA was Lee et al.'s (1971) study of dietary influences on the accumulation and composition of wax esters. Wax esters are neutral storage lipids that are the dominant lipid class in polar/north temperate and deep-living calanoid copepods. These storage lipids play a critical role in the life history of copepods in these regions because they are dependent on brief, but intense, vernal phytoplankton blooms. On the basis of *Calanus helgolandicus* feeding experiments with three diatoms and one dinoflagellate, Lee et al. (1971) observed the wax ester and triacylglyceride (TAG) FA composition of this copepod closely matched the FA composition of their diets. They also noted the correspondence between diet and copepod FA increased when food concentrations were higher. In contrast to the results observed for TAG and wax esters, Lee and colleagues reported the FA composition of the structural phospholipids (PL) was not dependent on diet. Many studies have subsequently examined lipid accumulation in marine zooplankton; particularly for zooplankton from polar regions (Kattner and Hagen – Chap. 11).

6.1.2 Emphasis in the Marine and Freshwater Literature

The emphasis in the marine and freshwater dietary vs. zooplankton FA literature has been different for several reasons. Many early studies with both marine and freshwater zooplankton were focused on the nutritional needs of aquatic organisms as this affected their nutritional value as food for aquaculture fish (Provasoli and D'Agostino 1969). Also as previously noted, the marine literature was also quite focused on storage lipids and marine researchers were the first to realize the potential utility of FA as trophic markers (Graeve et al. 1994; reviewed by Dalsgaard et al. 2003).

The earliest freshwater field studies, e.g. Farkas (1964), focused on temperature impacts on zooplankton FA composition as this related to "cold adaptation". Subsequently, the importance of essential FA for zooplankton nutrition in nature was investigated (Ahlgren et al. 1990; Müller-Navarra 1995a; Jónasdóttir et al. 1995). Most recent freshwater studies looking at zooplankton FA composition (e.g., Persson and Vrede 2006; Brett et al. 2006; Müller-Navarra 2006) have focused on the somatic growth regulating properties of HUFA for zooplankton and fish and have therefore emphasized the trophic transfer of polyunsaturated fatty acids (PUFA) and HUFA[2] with an eye toward the availability of these molecules for upper trophic levels.

[2]In this chapter, we will use PUFA to refer to 16 and 18 carbon chain (C_{16} and C_{18}) FA with two or more double bonds and HUFA to represent the subset of C_{20} and C_{22} PUFA.

6.2 Zooplankton Taxonomic Differences in Fatty Acid Composition

Much is known about the FA dynamics of copepods from north temperate and polar marine systems (Dalsgaard et al. 2003, Kattner and Hagen – Chap. 11). Marine copepods are particularly rich in lipids (i.e., 37 ± 19% of dry mass), and these are strongly dominated by wax esters (56 ± 32% of lipids) and TAG (13 ± 18% of lipids), which serve as storage lipids (reviewed by Lee et al. 2006). Wax esters comprise a particularly important class of lipids, especially for polar, temperate, upwelling or deep water copepods, which are exposed to short but intense phytoplankton blooms and have adapted by developing an ability to accumulate pronounced seasonal lipid stores (Lee et al. 2006). Wax esters may also be important to seasonally diapausing copepods because the thermal expansion and compressibility of these molecules allows copepods to remain neutrally buoyant at great depth (Lee et al. 2006). Tropical epipelagic zooplankton species do not deposit storage lipids because they encounter much weaker seasonal food pulses and have higher metabolic rates. Instead, marine copepods in tropical regions rapidly utilize available food for growth and reproduction (Kattner and Hagen – Chap. 11).

Wax ester synthesis has been particularly well studied for marine *Calanus* spp. copepods. The following pattern can be concluded from the literature (e.g., Dalsgaard et al. 2003): In contrast to the FA moiety, the fatty alcohol component (mostly 20:1n-9, 22:1n-11, and 22:1n-9; Hagen et al. 1993) of wax esters is synthesized by copepods from the related FA, which can then be used as markers for fish copepod consumption (e.g. Sargent and Henderson 1986). Fatty alcohols can also be synthesized de novo from dietary carbohydrates and proteins (Lee et al. 2006). A recent ^{13}C labeling experiment (Graeve et al. 2005) concluded that the abundant MUFA 20:1n-9 and 22:1n-11 and corresponding fatty alcohols in three species of Arctic *Calanus* were most likely synthesized de novo from nonlipid dietary sources. In contrast, structural FA such as EPA and DHA were taken up directly from the diet and highly retained in the body (Graeve et al. 2005). Kattner and Hagen (Chap. 11) compared the wax esters and phospholipids (PL) of four calanoid copepods and found the FA of the wax ester fraction was composed of 61 ± 24% MUFA, especially 16:1n-7, 18:1n-9, 20:1n-9, and 22:1n-11. Fatty alcohols of the 20:1n-9 and 22:1n-11 moieties are also important components of wax esters. In contrast, the FA of the PL of these copepods only had 10 ± 4% MUFA, and was instead dominated by DHA (36 ± 6%), EPA (18 ± 2%) and the SAFA 16:0 (25 ± 3%). Scott et al. (2002) reported nearly identical results for the same copepod species.

Persson and Vrede (2006) found that freshwater zooplankton could be separated into groups based on their PUFA and HUFA composition. These authors found copepods contained a large fraction of DHA while cladocerans were rich in EPA and arachidonic acid (20:4n-6; ARA). Persson and Vrede (2006) also found that herbivorous zooplankton contained more HUFA than did seston, and that carnivores contained more HUFA than herbivores. Similar differences between *Daphnia* spp. and various copepod species have been noted previously (e.g. Farkas 1970; Ballantyne et al. 2003). The compilation of ARA, EPA, and DHA content in wild

caught zooplankton in Table 6.1 shows that these conclusions also hold for a wider dataset. The proportion of ARA was similar in cladocerans and copepods, but cladocerans have relatively high proportions of EPA compared with the copepods. Copepods have high proportions of DHA, while DHA is nearly absent in cladocerans. The greater relative content of EPA and ARA in cladocerans compared with copepods may be related to the cladocerans' higher potential for reproduction (Persson and Vrede 2006; Smyntek et al. 2008). The relationship between EPA and growth and reproductive capacity is speculative, however, and the physiological functions of EPA and ARA in crustaceans remain to be clarified.

Recently, Scott et al. (2002), Persson and Vrede (2006) suggested that the high DHA content of copepods might be due to a more highly developed nervous system compared with other zooplankton. Copepods have highly developed prey attack and predator avoidance strategies, which allow them to respond to stimuli within milliseconds (Lenz et al. 2000). They also have abundant chemoreceptors on their antennae and mouth-parts, which allow them to taste food and track mates, see references in Persson and Vrede (2006). Lenz et al. (2000) noted that some calanoid copepods have thick myelin sheaths covering axons in their nervous system, which allow them to achieve exceptionally quick nerve impulse response times. Similar to other nervous tissues, DHA may be critical for the proper functioning of myelin and associated neural tissues. As Scott et al. (2002) concluded "the possibility that [DHA] has special properties in copepods relating to their mobility and migrations rather than to adaptation to low temperatures is worthy of future research". In contrast, Smyntek et al. (2008) suggested the high DHA content of copepods was an adaptation for over-wintering in an active life stage, as previously hypothesized by Farkas (1979).

The carnivorous cladoceran *Bythotrephes longimanus* and the carnivorous calanoid copepods *Epischura nevadensis* and *Heterocope* spp. have considerably higher proportions of PUFA than do herbivorous zooplankton. *B. longimanus* contains 22% EPA while the mean for the filter feeding cladocerans is 14% and *E. nevadensis* and *Heterocope* spp. contain 18% and 21% DHA, respectively, while omnivorous calanoids average 13% DHA. In a study analyzing zooplankton from Lake Tahoe, Müller-Navarra (unpublished data) found that *E. nevadensis* had a higher absolute n-3 PUFA content, and especially DHA, than the herbivore *Diaptomus tyrelli*, but lower than what was found in *Mysis relicta*. The higher relative PUFA proportions in carnivorous zooplankton might be a direct result of the fact that the food they consume (i.e., rotifers and crustacean zooplankton) is richer in PUFA than the seston diets of filter feeding cladocerans and the seston/micro-zooplankton diets of omnivorous calanoids. Since these differences in food have been present on an evolutionary time scale, it can be hypothesized that they have adapted to the high PUFA intake and that they may now be completely dependent on direct dietary sources of ARA, EPA, and DHA to meet their physiological demands. In this regard, it is worth noting several strictly carnivorous fish species such as northern pike (*Esox lucius*) have very limited abilities to convert LIN to ARA, and ALA to EPA and DHA (Henderson et al. 1995). Similarly, we speculate that carnivorous zooplankton may be dependent on a high intake of ARA, EPA, and DHA to meet their physiological demands.

The total FA composition of the major zooplankton groups for which substantial FA data exist (i.e., freshwater cladocerans and copepods, marine calanoid copepods

Table 6.1 Mean zooplankton fatty acid composition (as a percent of total FA) by FA functional groups

Group Trophic mode System	Cladoceran Herbivorous Freshwater	Cladoceran Carnivorous Freshwater	Calanoid copepod Omnivorous Freshwater	Calanoid copepod Carnivorous Freshwater	Cyclopoid copepods Omni.-Carni. Freshwater	Calanoid copepod Omnivorous Marine	Mysids Carnivorous FW & Marine	Euphausia superba Omnivorous Marine
n	13	6	9	3	4	11	4	8
SAFA	34.1 ± 7.2	34.6 ± 6.2	33.6 ± 5.9	34.6 ± 4.2	28.4 ± 10.6	25.5 ± 14.3	27.6 ± 3.5	32.9 ± 4.9
MUFA	23.5 ± 5.5	18.7 ± 5.1	13.2 ± 4.8	11.6 ± 1.4	17.6 ± 10.4	34.2 ± 18.4	33.1 ± 7.4	27.8 ± 4.0
LIN	6.2 ± 1.6	5.3 ± 0.8	4.7 ± 1.5	4.8 ± 0.9	5.2 ± 1.5	2.5 ± 2.2	3.4 ± 1.9	2.6 ± 0.2
ALA + SDA	14.4 ± 5.8	8.1 ± 1.3	13.1 ± 5.3	11.8 ± 1.0	13.1 ± 3.7	8.3 ± 5.6	4.6 ± 4.3	5.5 ± 3.8
ARA	5.2 ± 2.1	8.9 ± 1.0	3.8 ± 2.2	4.4 ± 0.6	3.8 ± 1.0	0.3 ± 0.6	2.2 ± 1.8	0.8 ± 0.4
EPA	14.7 ± 3.9	22.1 ± 2.3	13.0 ± 6.0	11.0 ± 1.7	10.9 ± 3.4	14.4 ± 4.2	16.5 ± 2.2	19.4 ± 5.9
22:2n-6	0.2 ± 0.3	0.3 ± 0.4	1.2 ± 1.3	1.8 ± 1.9	0.8 ± 0.7	0.0 ± 0.0	0.2 ± 0.4	0.0 ± 0.0
DHA	1.7 ± 1.5	2.0 ± 0.9	17.6 ± 9.1	20.1 ± 1.8	20.2 ± 7.3	14.8 ± 7.6	12.3 ± 3.4	11.0 ± 5.0
n-3:n-6 ratio	3.0 ± 0.7	2.3 ± 0.2	5.2 ± 2.8	4.2 ± 0.3	4.7 ± 1.8	18.1 ± 9.6	6.7 ± 4.6	9.8 ± 2.6

The values presented are average percent of total FA ± 1 SD. The freshwater zooplankton fatty acids data were obtained from Hessen and Leu (2006), Persson and Vrede (2006), Smyntek et al. (2008), M.T. Arts (unpublished data), M.T. Brett (unpublished data), C.W. Burns (unpublished data), and D.C. Müller-Navarra (unpublished data). The marine copepod data was taken from Peters et al. (2006), Veloza et al. (2006) and Kattner and Hagen (Chap. 11). The euphausiid FA data was obtained from Cripps et al. (1999), Hagen et al. (2001), Stübing et al. (2003) and Schmidt et al. (2006). Freshwater mysid FA data were obtained from D.C. Müller-Navarra (unpublished data) and M.T. Arts (unpublished data), and marine mysid data were obtained from Richoux et al. (2005)

and euphausiids) indicates considerable differences amongst these groups (Table 6.1). Freshwater cladocerans were notable for having much lower DHA (at ≈2%) than the other zooplankton groups. Cladocerans also had the lowest n-3:n-6 ratios (i.e., 2.4–3.0). Carnivorous freshwater cladocerans could be distinguished from herbivorous cladocerans by a higher ARA and EPA content, and lower ALA + SDA content and n3:n6 ratios (Table 6.1). Compared with other zooplankton, carnivorous cladocerans had particularly high proportions of ARA, i.e., 8.9 ± 1.0% (±1 SD) and low n-3:n-6 ratios, 2.3 ± 0.2. Freshwater calanoid and cyclopoid copepods had the highest proportion DHA (≈20%) and intermediate n-3:n-6 ratios (i.e. 4–6). In general, freshwater cladocerans and copepods had twice as much n-6 and n-3 PUFA, and 10× as much ARA, as did marine copepods and euphausiids. In contrast, marine zooplankton averaged twice as much MUFA and had much higher n-3:n-6 ratios (i.e. 10–20). The FA composition of marine omnivorous copepods differed from that of freshwater omnivorous copepods specifically, and from all freshwater copepods more generally, in their much higher MUFA content and n-3:n-6 ratios, and their much lower ARA and lower LIN and ALA + SDA content.

The zooplankton FA composition data summarized above ($n = 58$) was analyzed using discriminant function analysis (DFA; see Fig. 6.1). This DFA correctly classified 66% of the samples according to their major group (i.e., herbivorous

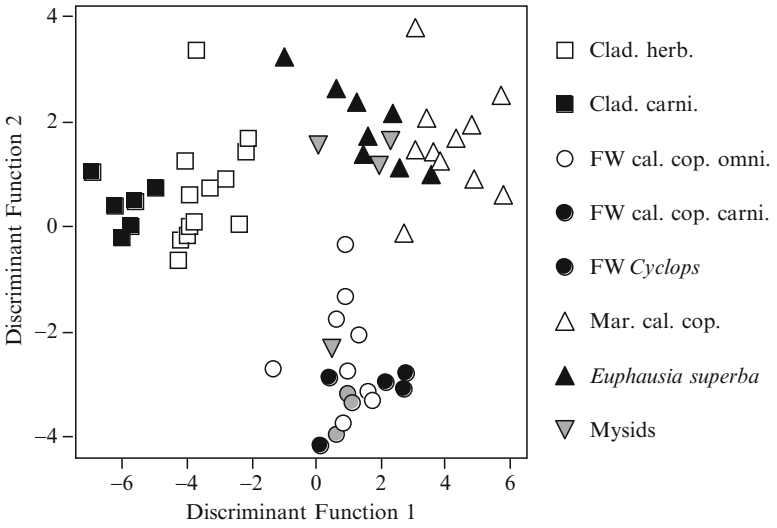

Fig. 6.1 A bivariate plot of the results of a discriminant function analysis of zooplankton fatty acid composition data presented in Table 6.1 ($n = 58$). The first axis explained 67.5% of the variability. This axis was positively correlated with the log(n-3:n-6) ratio and DHA and negatively correlated with ARA and LIN. The second axis explained 22.6% of the variability, and was positively associated with MUFA and EPA. *Clad. herb.* herbivorous cladocerans, *Clad. carni.* carnivorous cladocerans, *FW cal. cop. omni* freshwater omnivorous calanoid copepods, *FW Cyclops* freshwater cyclopoid copepods, *Mar. cal. cop.* marine calanoid copepods, and *Mysids* marine and freshwater mysids

cladocerans, carnivorous cladocerans, omnivorous freshwater calanoid copepods, etc.) using a "leave-one-out" algorithm. The large majority of the misclassification errors were within the freshwater copepod and marine and freshwater mysid groups. Overall, this DFA explained 98% of the variability using 3 axes, with the first axis explaining 67.5% of the variability. This axis was strongly positively correlated with the log(n-3:n-6) ratio and moderately positively correlated with DHA. The first axis was also strongly negatively correlated with ARA and moderately negatively correlated with LIN. The second axis explained 22.6% of the variability, and was positively associated with MUFA and EPA. This plot shows freshwater cladocerans and copepods formed distinct clusters, and the marine zooplankton formed a third distinct cluster. Within these groups, carnivorous and herbivorous cladocerans could be readily distinguished and marine copepods and euphausiids were mostly separated. Freshwater and marine mysids were poorly classified and tended to be confused with *Euphausia superba*. The results of this DFA strongly support Persson and Vrede's (2006) hypothesis that carnivorous cladocerans can be distinguished from herbivorous cladocerans. However, this DFA does not support their hypothesis that freshwater carnivorous copepods can be distinguished from freshwater omnivorous copepods.

6.3 Phytoplankton Fatty Acid Composition as Food for Zooplankton

The dominant phytoplankton available to herbivorous zooplankton in freshwater and marine planktonic systems differ greatly in their FA composition (Volkman et al. 1989; Ahlgren et al. 1992) (Table 6.2). When comparing the FA composition of freshwater and marine phytoplankton, a few differences are quite apparent. These dissimilarities may be adaptations to the respective environment of the algae. However, differences in experimental focus and/or methods cannot be excluded. For example, the marine literature reports considerably more EPA and DHA in chlorophytes than does the freshwater literature. On average these two FA comprise 4.8% and 1.0% of marine chlorophyte FA, respectively, but these FA are often not detected in freshwater chlorophytes (Table 6.2). The higher n-3 HUFA content of marine chlorophytes may be real or it may be due to the fact that most surveys of marine phytoplankton FA composition are geared toward identifying taxa with potential value as mariculture food stocks, and therefore HUFA-rich chlorophytes may be overly represented. This "aquaculture bias" does not exist in the freshwater phytoplankton literature. Probably because of this mariculture emphasis, and because of the fact that diatoms are quite important in marine systems, there are also far more observations of diatom FA composition for marine than for freshwater taxa. Conversely, there are far more observations of cyanophyte FA composition for freshwater than for marine taxa. This is probably because cyanobacteria are more prevalent in freshwater systems. In addition, they have a low n-3 FA content and are therefore of less interest to mariculturalists. There is also a very substantial

Table 6.2 Mean phytoplankton fatty acid composition (as a percent of total FA) by FA functional groups

Group system	Chlorophytes freshwater	Cryptophytes freshwater	Diatoms freshwater	Cyanophytes freshwater	Chlorophytes marine	Cryptophytes marine	Diatoms marine	*Isochrysis galbana* marine
n	11	9	6	9	10	11	14	8
SAFA	32.5 ± 9.5	28.4 ± 9.8	23.8 ± 11.0	58.6 ± 18.5	29.1 ± 10.4	23.3 ± 9.5	25.8 ± 6.3	31.2 ± 12.3
MUFA	27.3 ± 12.5	9.9 ± 5.1	40.3 ± 12.8	24.8 ± 16.6	15.3 ± 4.3	9.4 ± 3.6	24.4 ± 5.6	22.7 ± 5.5
C_{16} PUFA	0.0 ± 0.0	0.1 ± 0.4	9.1 ± 5.8	0.0 ± 0.0	17.8 ± 4.9	0.8 ± 1.5	18.5 ± 7.7	1.3 ± 1.3
LIN	14.4 ± 5.6	3.3 ± 2.4	2.0 ± 1.8	7.2 ± 6.5	7.7 ± 4.9	5.8 ± 6.4	1.8 ± 1.1	6.4 ± 2.0
ALA + SDA	25.5 ± 9.7	39.7 ± 10.4	2.9 ± 3.1	7.0 ± 10.2	24.3 ± 10.2	43.5 ± 12.7	2.4 ± 1.8	22.4 ± 7.9
ARA	0.2 ± 0.3	0.1 ± 0.2	2.3 ± 1.7	1.0 ± 2.4	0.9 ± 0.8	0.8 ± 1.0	1.9 ± 2.1	0.1 ± 0.1
EPA	0.1 ± 0.2	15.1 ± 6.1	16.9 ± 8.2	0.6 ± 1.2	4.0 ± 2.4	9.5 ± 3.1	22.0 ± 5.5	1.4 ± 0.8
22:2n-6	0.0 ± 0.0	0.6 ± 1.1	0.1 ± 0.2	0.1 ± 0.1	0.0 ± 0.0	0.4 ± 0.7	0.3 ± 0.4	1.2 ± 1.9
DHA	0.0 ± 0.0	2.9 ± 1.8	2.5 ± 3.0	0.7 ± 2.1	0.9 ± 1.4	6.5 ± 2.2	2.9 ± 1.7	13.3 ± 8.3
n-3:n-6	1.9 ± 0.9	16.8 ± 8.2	7.6 ± 4.8	1.0 ± 1.0	4.5 ± 2.4	22.4 ± 20.8	11.6 ± 8.9	5.1 ± 1.7

The values presented are average percent ±1 SD. The freshwater chlorophyte, cryptophyte and cyanobacteria FA data summarized in this table was taken from Ahlgren et al. (1992), Brett et al. (2006) and C.W. Burns (unpublished data). The freshwater diatom data was taken from Müller-Navarra (1995b), Desvilettes et al. (1997), Gatenby et al. (2003), Müller-Navarra (2006) and Caramujo et al. (2008). The marine chlorophyte data was taken from Volkman et al. (1989), Renaud et al. (1999), and Lourenco et al. (2002). The marine cryptophyte data was taken from Renaud et al. (2002), Broglio et al. (2003), Dunstan et al. (2005), Veloza et al. (2006), and Tremblay et al. (2007). The marine diatom FA data was taken from Dunstan et al. (1994). The *Isochyrsis galbana* FA data were taken from Volkman et al. (1989), Reitan et al. (1994), Nanton and Castell (1998), Renaud et al. (1999), Lourenco et al. (2002), Renaud et al. (2002), Wacker et al. (2002), and Patil et al. (2007)

literature reporting variation in the FA composition of the marine prymnesiophyte *Isochrysis galbana* because this species is the most important phytoplankton food source for aquaculture. Another striking difference between the marine and freshwater literature is that most marine studies report results for a wide range of 16 carbon chain (C_{16}) PUFA and many freshwater studies do not report these FA. In fact, some of the common FA standards used in freshwater studies (e.g. the Supelco® 37-FAME Standard [47885U]) do not contain these FA, making their identification in actual samples problematic. From the marine literature, it is clear C_{16} PUFA are very prevalent in diatoms and chlorophytes, but they do not appear to be common in cryptophytes (but see Müller-Navarra 2006), cyanophytes or the prymnesiophyte *I. galbana*.

Amongst freshwater phytoplankton, chlorophytes are notable for having a high proportion of C_{18} n-6 FA, in particular linoleic acid (18:2n-6; LIN). Freshwater chlorophytes also tend to have very little C_{20} and C_{22} n-3 and n-6 FA. Marine chlorophytes have similar FA composition, except they have, on average, about half as much MUFA and C_{18} n-6 FA, more EPA (4.8% vs. 0.1%), more DHA (1.0% vs. 0%) and a clearly higher n-3:n-6 ratio than freshwater chlorophytes (Table 6.2). Much of the difference between marine and freshwater chlorophyte FA composition may be due to the fact that FA composition data have been reported for a wide variety of freshwater chlorophytes, whereas the marine literature seems to be focused on species with potential aquaculture value (and hence tend to have a high n-3 HUFA content).

Freshwater and marine cryptophytes have a low MUFA content, very high and roughly equal proportions of the C_{18} n-3 FA α-linolenic acid (18:3n-3; ALA) and stearidonic acid (18:4n-3; SDA), high EPA and moderately high DHA content, and a very high n-3:n-6 ratio (i.e., ≈17:1 and 22:1, respectively). Marine cryptophytes have about one third less EPA and twice as much DHA as do freshwater cryptophytes. In general, diatoms have the highest MUFA content, low proportions of both n-3 and n-6 C_{18} FA, high EPA and ARA content and moderately high DHA. Diatoms also have considerable amounts of C_{16} MUFA and PUFA, which are a characteristic of this group. Few studies have reported the FA composition of marine cyanobacteria, but freshwater cyanobacteria are characterized by having a very high SAFA content, very little n-3 FA in general, and a particularly low n-3:n-6 ratio. The marine flagellate *I. galbana* has nearly the global average FA composition for phytoplankton, except it has little EPA and exceptionally high DHA content (Table 6.2). Its high DHA content and the ease with which it can be grown are the reasons this species is very widely used in aquaculture.

The FA composition of the freshwater and marine phytoplankton summarized above (n = 74) was analyzed using DFA (Fig. 6.2). This DFA correctly classified 91% of the samples according to their major group (i.e., diatom, chlorophyte, cryptophyte, cyanophyte, and *Isochrysis*) using a "leave-one-out" algorithm. The DFA correctly classified 100% of the diatom and *Isochrysis* monocultures and 95% of the cryptophyte monocultures. Two marine chlorophytes were misclassified

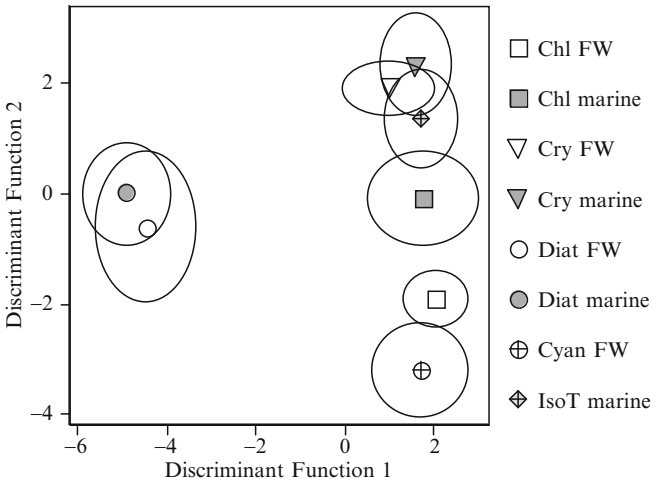

Fig. 6.2 A bivariate plot of the results of a discriminant function analysis of phytoplankton fatty acid composition for the phytoplankton data presented in Table 6.2 ($n = 78$). The ovoids around the phytoplankton group centroids represent the area delineated by ±1 SD on the *X* and *Y* axes. The first axis of this DFA explained 61.4% of the overall variation in these data and was correlated positively with C_{18} n-3 and C_{18} n-6 FA and negatively with EPA, arachidonic acid (20:4n-6; ARA) and MUFA. The second axis explained 22.0% of the variation and was positively associated with the n-3:n-6 ratio, DHA and C_{18} n-3 FA and negatively associated with SAFA. The third axis (not shown) explained an additional 10.3% of the overall variation and was positively associated with DHA. *Chl* chlorophytes, *Cry* cryptophytes, *Diat* diatoms, *Cyan* cyanophytes, *IsoT Isochrysis galbana*, and *FW* freshwater taxa. This analysis is based on data obtained from the sources described in Table 6.2

as cryptophytes and two freshwater cyanophytes (both *Oscillatoria*) were misclassified as chlorophytes. The first axis of this DFA explained 61.4% of the overall variation and was correlated positively with C_{18} n-3 and C_{18} n-6 FA and negatively with EPA, ARA, and MUFA. This axis distinguished freshwater and marine diatoms from all other phytoplankton. The second axis explained 22.0% of the variation and was positively associated with the n-3:n-6 ratio, DHA and C_{18} n-3 FA and negatively associated with SAFA. This axis clustered freshwater and marine cryptophytes with *Isochrysis*, and diatoms with marine chlorophytes. These two clusters where easily distinguished from each other, as well as from freshwater chlorophytes and cyanophytes, which were distinct from each other and all other groups. The third axis (not shown) explained an additional 10.3% of the overall variation and was positively associated with DHA. This DF strongly distinguished *Isochrysis* from all other taxonomic groups. The marine and freshwater diatom and cryptophytes clusters were nearly indistinguishable. In contrast, the marine chlorophyte cluster was most similar to the cryptophyte cluster and the freshwater chlorophyte cluster was most similar to the cyanobacteria cluster, consistent with an over-representation of n-3 HUFA rich taxa in the marine literature.

6.4 Dietary Impacts on Zooplankton Fatty Acid Composition

6.4.1 Freshwater Zooplankton: Laboratory Studies

Several studies have examined the impact of algal diets on crustacean FA composition and found a great similarity between the consumer's FA pattern and that of their diet (see e.g. Lewis 1969 for marine amphipods; Bourdier and Amblard 1989 for *Acanthodiaptomus denticornis*; Elendt 1990 for *Daphnia magna*). The FA composition of the neutral lipid fraction (mainly TAGs) is especially affected by dietary FA (Langdon and Waldock 1981; Parrish et al. 1995). Some FA could even be traced across several trophic levels, from phytoplankton via zooplankton to fish larvae (Fraser et al. 1989).

Elendt (1990) was one of the first to study dietary impacts on daphnid FA composition using artificial supplements. D'Abramo and Sheen (1993) found that the FA composition in the freshwater prawn (*Macrobrachium rosenbergii*) whole body tissue reflects that of purified artificial diets. However, concentrations of SAFA and MUFA seemed to change in relation to additions of PUFA. ALA, EPA, and ARA were conserved in the polar lipid fraction of the tissue even when these FA were not provided with the diet. In contrast, n-3 PUFA decreased in the neutral lipids when not provided in the diet whereas n-6 PUFA remained unchanged or increased. They suggest further that n-6 and n-3 PUFA have different metabolic and nutritional functions (*see* Ahlgren et al. – Chap. 7). Using HUFA enriched supplements, Weers et al. (1997) showed that when *Daphnia galeata* were fed combinations of the alga *Chlamydomonas reinhardtii* and emulsions with varying DHA to EPA ratios, the DHA content of *D. galeata* increased with the emulsion DHA:EPA ratio, but even at the highest DHA/EPA ratio tested (≈4:1) *D. galeata* still contained 4× more EPA. These authors suggested this indicated *D. galeata* were retro-converting much of the DHA to EPA. Weers et al. (1997) also showed that *D. galeata* that consumed *Cryptomonas* spp. contained 3× more SDA and 25× more EPA than *D. galeata* that consumed *Scenedesmus* spp. These findings are supported by recent research which has also shown that phytoplankton FA composition has pronounced impacts on the FA profiles of *Daphnia* spp. (Brett et al. 2006; Müller-Navarra 2006). Most FA groups (i.e., SAFA, MUFA, C_{18} n-6, etc.) show moderate correlations (r^2 = 0.40–0.68) between the diet and *Daphnia* FA. However, EPA and, even more so, the sum of EPA and DHA show a particularly strong correlation between diet and *Daphnia* FA composition ($r^2 \approx 0.85$). Differences between these studies might suggest some differences in the FA accumulation patterns for different *Daphnia* species. For example, Brett et al. (2006) studied dietary impacts on the FA composition of a clone of *D. pulex* isolated from a lake in California and found diet and somatic FA were most strongly correlated for ARA and EPA. In contrast, Burns et al. (unpublished data) studied a clone of *D. carinata* isolated in New Zealand and observed the best correlations for MUFA, C_{18} n-3s, EPA + DHA, and the n-3:n-6 ratio.

Despite the strong dietary impacts on *Daphnia* FA, they tend to accumulate less SAFA, more MUFA, and especially more ARA than what is found in their diets.

Also, when consuming diets that contain DHA, *Daphnia* tend to accumulate far less of this FA than what is present in their food. However, the differences in *Daphnia* FA composition when consuming different phytoplankton monoculture diets is pronounced. For example, *Daphnia* that consumed cryptophytes had on average 16 ± 4% (±1 SD) EPA in their FA pool, whereas *Daphnia* that consumed chlorophytes averaged only 1 ± 1% EPA.

As previously noted, the major phytoplankton groups have distinct FA profiles by which they can be readily separated using discriminant function analysis (Fig. 6.2). We used the freshwater phytoplankton FA data depicted in Fig. 6.2 and the FA composition of *Daphnia* fed monoculture phytoplankton diets (Brett et al. 2006; Müller-Navarra 2006; Müller-Navarra et al. unpublished data, Burns et al. unpublished data) to graphically demonstrate the strong impact of dietary FA on *Daphnia* FA composition (Fig. 6.3). In this DFA, the phytoplankton and *Daphnia* samples were treated as a single class, and 94.6% of these samples were correctly classified to phytoplankton group (e.g., chlorophyte), or to *Daphnia* eating phytoplankton from that group, using the "leave-one-out" algorithm. The first axis of this DFA explained 54.7% of the overall variation in these data and was strongly negatively

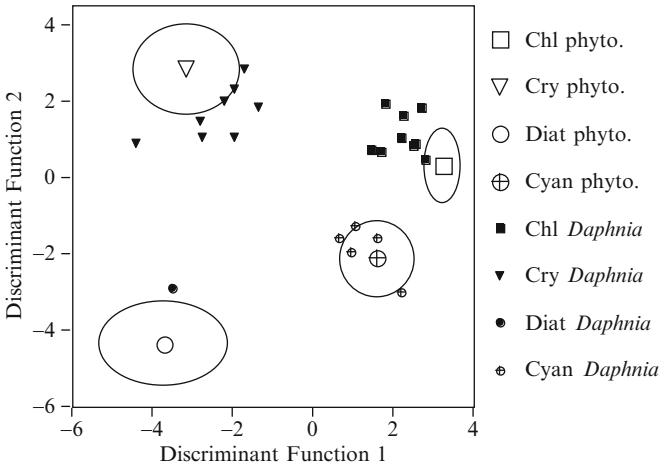

Fig. 6.3 A bivariate plot of the results of a discriminant function analysis of phytoplankton fatty acid composition and the FA composition of *Daphnia* consuming these phytoplankton. The ovoids around the phytoplankton group centroids represent the area delineated by ±1 SD on the *X* and *Y* axes. The large open symbols represent the phytoplankton group centroids. The small filled symbols represent the individual *Daphnia*-phytoplankton monoculture treatments. The first axis of this DFA explained 54.7% of the overall variation in these data and was very strongly negatively correlated with EPA, moderately negative correlated with DHA and the n-3:n-6 ratio and moderately positively correlated with C_{18} n-6 FA. The second axis explained 36.1% of the variation and was strongly positively associated with C_{18} n-3 FA, and moderately negatively associated with MUFA. A third axis (not shown) explained 9.1% of the variability and was moderately correlated with SAFA. *Chl* chlorophytes, *Cry* cryptophytes, *Diat* diatoms, *Cyan* cyanophytes, and *phyto.* phytoplankton. This figure is based on data from Brett et al. (2006), Müller-Navarra (2006), C.W. Burns (unpublished data) and D.C. Müller-Navarra (unpublished data)

correlated with EPA, moderately negatively correlated with DHA, and the n-3:n-6 ratio and moderately positively correlated with LIN. The second axis explained 36.1% of the variation and was strongly positively associated with C_{18} n-3 FA, and moderately negatively associated with MUFA. A third axis (not shown) explained 9.1% of the variability and was moderately correlated with SAFA. The results in Fig. 6.3 show diet FA composition had a distinct impact on *Daphnia* FA composition. However, this figure also shows that in 22 out of 23 cases the FA of the *Daphnia* was slightly more inclined toward a general central tendency relative to their diets. These data demonstrate that diet has a dominating impact on *Daphnia* FA composition but that, irrespective of diet, *Daphnia* retain some internally consistent features of their FA profiles.

The unpublished data of Burns et al. showed the cladoceran *Ceriodaphnia dubia* had similar responses to dietary FA as those noted above for *Daphnia* spp. *C. dubia* accumulated more ARA relative to their diets, and even when their diets were rich in DHA they accumulated very little of this FA. The SAFA, MUFA, and EPA content and n-3:n-6 ratio of *C. dubia* was moderately correlated with their monoculture diets, whereas the C_{18} n-3 and LIN where strongly correlated ($r^2 \approx 0.85$).

Less is known about dietary impacts on freshwater copepods, but Bourdier and Amblard (1989) explored this subject for the calanoid *Acanthodiaptommus denticornis*, Burns and colleagues (unpublished data) studied the calanoid *Boeckella* spp., and Caramujo et al. (2008) studied the harpacticoid *Attheyella trispinosa*. After feeding with chlorophyte, cyanophyte, and diatom monocultures, Bourdier and Amblard (1989) noted the neutral lipid composition of *A. denticornis* was closely linked to that of their diets. These authors also indicated that diet did not affect the FA composition of the structural polar lipids. These different dietary responses for neutral and polar lipids are well characterized for marine copepods (e.g., Lee et al. 1971). Burns et al. (unpublished data) noted that when fed chlorophyte, cyanophyte, and cryptophyte monocultures, the calanoid *Boeckella* spp. accumulated significantly more ARA, EPA, and DHA than its diet. The MUFA and LIN content and n-3:n-6 ratio of *Boeckella* was moderately correlated ($r^2 = 0.52–0.62$) with their diets, while SAFA and LIN were strongly correlated ($r^2 \approx 0.90$). Caramujo et al. (2008) fed diatom and cyanobacteria monocultures to *A. trispinosa* and found the neutral lipid 16:1n-7, LIN and EPA content of this harpacticoid was clearly influenced by diet. These authors also observed the FA of polar lipids was not influenced by diet.

6.4.2 Freshwater Zooplankton: Field Studies

Several recent studies have examined the FA composition of freshwater zooplankton collected from the field. Ballantyne et al. (2003) noted that cladocerans collected from Lake Washington tend to accumulate EPA, whereas copepods accumulated both EPA and especially DHA. Kainz et al. (2004) examined the accumulation of essential fatty acids (EFA) in different zooplankton size classes for a series of lakes on Vancouver Island, British Columbia. These authors found that all zooplankton

size classes accumulated 2–4× more EFA than the seston, and that copepod-dominated "meso-zooplankton" (200–500 μm) tended to accumulate DHA, while cladoceran-dominated "macro-zooplankton" (>500 μm) tended to accumulate EPA. As suggested in their study, and more clearly shown in subsequent studies, size per se is not a rational basis for examining differences in zooplankton FA composition because very large differences exist in the EPA and DHA accumulation patterns between cladocerans and copepods, which are unrelated to size. That is, small herbivorous cladocerans have much more similar FA profiles to large herbivorous cladocerans than they do to small-sized copepods (Persson and Vrede 2006).

Persson and Vrede (2006) noted that zooplankton from oligotrophic alpine lakes in Sweden were greatly enriched with PUFA and HUFA relative to seston. Persson and Vrede (2006) also found that the FA composition of zooplankton was unrelated to that of the seston, but was related to zooplankton taxonomic affiliation and trophic mode. Similary, Smyntek et al. (2008) noted the FA profiles of the major freshwater zooplankton groups (i.e., cladocerans and copepods) differed systematically in the large lake systems they sampled, but within individual zooplankton taxa FA profiles appeared to be independent of the seston's FA composition. These results were similar to those of Müller-Navarra (2006), who found pronounced food dependency of *Daphnia's* FA composition when fed cultured algae but much weaker patterns for natural diets. In the field, significant relationships between the FA composition of seston and zooplankton were recorded for 18:4n-3 and LIN for gravid daphnids and in *Eudiaptomus* spp. for ARA, DHA (gravid animals), and ALA (animals without eggs) (Müller-Navarra 2006). As Müller-Navarra (2006) noted, the weaker relationships between seston and zooplankton FA composition in the field may be because there was less variation in the FA composition of the seston than there is for the phytoplankton monocultures utilized as food in laboratory studies. Persson and Vrede (2006) also noted that the seston FA composition varied little in the suite of relatively similar lakes they sampled making it more difficult to detect dietary impacts.

In contrast to the field studies mentioned earlier, Ravet et al. (2009) observed strong relations between seston and zooplankton FA composition in the mesotrophic Lake Washington. Lake Washington has dramatic shifts in phytoplankton biomass and community composition (Arhonditsis et al. 2003) that make it particularly amendable to studies of natural seston impacts on zooplankton FA composition. Overall, Ravet et al. (2009) found quite similar results for *Leptodiaptomus ashlandi* feeding on natural seston in Lake Washington compared with Burns et al.'s results for *Boeckella* spp. feeding on phytoplankton monocultures in the lab. Lake Washington *L. ashlandi* had a significantly lower proportion MUFA and LIN and significantly more C_{18} n-3, ARA and DHA and a higher n-3:n-6 ratio than did the seston collected on the same dates. This pattern was particularly pronounced for DHA, which was, on average, 4× more prevalent in *L. ashlandi* than in the seston. The seston's SAFA and n-3 HUFA content was moderately correlated with that of *L. ashlandi* ($r^2 \approx 0.77$). Although the sample sizes were much smaller for *Cyclops bicuspidatus thomasi* ($n = 5$), *Epischura nevadensis* ($n = 5$), and *Daphnia* spp. ($n = 4$), than for *L. ashlandi* ($n = 15$), these zooplankton also showed evidence of seston impacts on their FA composition. Similar to *L. ashlandi*, *Cyclops* had significantly

less LIN and significantly more C_{18} n-3 and DHA and a higher n-3:n-6 ratio than the seston. SAFA, DHA, and the n-3:n-6 ratio had the strongest relations with diet for *Cyclops* ($r^2 \approx 0.90$). Probably because this copepod is predominantly predaceous, the FA composition of *E. nevadensis* was not correlated with any of the main FA functional groups in seston. However, *E. nevadensis* had 38% less SAFA, 2× more C_{18} n-3 and 21× as much DHA as the seston from the same dates. Lake Washington *Daphnia* had less SAFA, and more C_{18} n-3 FA, ARA, and EPA than their diets and the MUFA, C_{18} n-3 FA and EPA content of *Daphnia* was correlated with that of the seston.

6.4.3 Marine Calanoid Copepods

It is well established that the FA composition of the storage lipid fraction in marine copepods is influenced by their diet, and it is generally believed that dietary FA are incorporated, unmodified, into these lipids (Lee et al. 1971). These authors also noted the total lipid content of copepods was correlated with phytoplankton concentrations, as was the strength of the association between the FA composition of the diet and storage lipids. Lee et al. (1971) also reported that the FA composition of the structural phospholipids was not affected by diet. In another classic study, Graeve et al. (1994) showed that the FA profile of the boreal herbivorous copepod *Calanus finmarchicus* could be changed from a presumptive dinoflagellate dominated (as indicated by a high 18:4n-3 content) to a diatom dominated profile (i.e., high 16:1n-7 content) by feeding wild collected *C. finmarchicus* a diatom monoculture diet for 42 days. Similarly, these authors were able to switch the FA composition of *C. hyperboreus* from diatom-like to dinoflagellate-like by feeding this copepod a dinoflagellate diet for 47 days. Since these studies, many marine copepod field studies have assumed the FA 16:1n-7 and EPA represent diatom consumption and 18:4n-3 and DHA represent dinoflagellate consumption (Kattner et al. 1994; Scott et al. 2002). It has also been suggested that C_{14} and C_{16} SAFA and the MUFA 18:1n-9 are trophic markers for omnivorous feeding on ciliates and 18:1n-7 indicates bacterial consumption (Stevens et al. 2004; Peters et al. 2006). Recently, Peters et al. (2006) used a FA trophic marker approach to infer that the glacial relict copepod *Pseudocalanus acuspes* exhibited an opportunistic feeding strategy in the Baltic Sea. The FA profiles of *P. acuspes* indicated that their diet was dominated by ciliates, diatoms, dinoflagellates, and cyanobacteria depending on the time of year. In contrast, the FA patterns of carnivorous and omnivorous copepods cannot be as easily linked to diet as is the case for herbivorous copepods from temperate to polar regions. It should be noted that there is considerable overlap in the FA composition of the major phytoplankton groups, so caution should be exercised when attributing consumer FA to particular dietary sources based on individual FA. For example, LIN is prevalent in both cyanobacteria and chlorophytes, whereas cryptophytes share a high EPA and DHA content with diatoms and a high ALA and 18:4n-3 content with chlorophytes.

6.4.4 Harpacticoid Copepods

When looking at dietary impacts on the FA composition of the marine harpacticoid *Tisbe holothuriae,* Norsker and Støttrup (1994) reported this copepod accumulated between 27 and 50% n-3 HUFA when consuming diets containing 1–13% HUFA. These authors concluded that *T. holothuriae* was able to synthesize n-3 HUFA from ALA at high rates. However, despite this bioconversion capacity *T. holothuriae* achieved considerably higher nauplii production when consuming HUFA-rich diets. Similarly, Nanton and Castell (1998) found *Tisbe* sp. had a high n-3 HUFA content (i.e., 19–41% of total FA) regardless of the HUFA content of baker's yeast and phytoplankton diets (which had n-3 HUFA content varying between 1 and 36%). Furthermore, these authors found the DHA/EPA ratio (a larval fish nutritional index) of these copepods varied between 2.6:1 and 3.3:1 despite the fact that this ratio in their diets ranged between 0.1:1 and 12:1. Nanton and Castell (1998) concluded *Tisbe* had a high capacity to convert ALA to EPA and DHA, which they suggested was an adaptation to the fact that *Tisbe* occupies detritus-rich benthic habitats where n-3 HUFA might be scarce.

6.4.5 Artemia spp

Considerable research effort has been devoted to understanding how the FA composition of aquaculture food organisms like *Artemia* spp. is affected by diet and supplements. The vast majority of the research on *Artemia* FA concerns short-term supplementation (i.e. <24 h) designed to boost the EPA and DHA content in these naturally HUFA deficient crustaceans (Palmtag et al. 2006). *Artemia* are, in some regards, ideal food sources for aquaculture because their nauplii are of suitable size for a wide range of first feeding larval fish and these nauplii do not themselves require food. Unfortunately, *Artemia* normally have very low EFA content, particularly DHA. Furthermore, when starved *Artemia* readily catabolize EFA(Coutteau and Mourente 1997). One of the few studies that extended *Artemia* diet studies beyond 24 h found that when fed algae containing ARA and EPA, *Artemia* readily accumulated these FA; however, *Artemia* accumulated very little DHA irrespective of diet (Vismara et al. 2003).

6.4.6 Euphausiids (krill)

Krill are one of the most important zooplankton groups in terms of understanding the global trophic transfer of EFA from primary producers to upper trophic levels. Because of this, considerable effort has been directed at untangling the environmental factors that exert the greatest influence on euphausiid FA composition, particularly

for the Antarctic species *Euphausia superba*. The FA of krill larval stages are dominated by EPA, DHA and 16:0. In contrast, in the adult stages with larger TAG stores, the FA 14:0, 16:0, and 18:1n-9 are dominant (Hagen et al. 2001). Lipid concentrations are the highest in gravid females. However, during the spawning season females may lose half of their total lipids because they typically spawn multiple times (e.g., Hagen et al. 2001). Because of these ontogenetic variations accompanied with the accumulation of very large lipid stores, it is particularly challenging to assess dietary impacts on euphausiid FA composition (Stübing et al. 2003). For example, *E. superba* store up sufficient lipid reserves to last through 6 months of near starvation conditions. Because of these reserves and experimental constraints (i.e., most feeding experiments by necessity last <2 months), it is difficult to impart strong dietary signals in the lipid composition of adult or juvenile euphausiids. Furthermore, some researchers have observed declining adult body mass during experiments indicating the krill were not feeding efficiently (Stübing et al. 2003), and it is generally thought that dietary impacts on zooplankton FA composition will be most evident when they are actively accumulating lipids.

In contrast to the studies above, which suggest krill maintain FA profiles that are somewhat independent of their diets, Falk-Petersen et al. (2000) used FA trophic markers to deduce the trophic levels of various polar euphasiids. These authors concluded that high 16:1n-7, 18:1n-7, SDA and EPA composition indicated herbivory. Specifically 16:1n-7 and EPA are indicators of diatom consumption and SDA and DHA are indicators of dinoflagellate consumption. High 18:1n-9 content and/or a high 18:1n-9/18:1n-7 ratio were suggested to be indicators of carnivory. Furthermore, a high 20:1n-9 and 22:1n-11 content was suggested as indicating carnivory on calanoid copepods specifically. On the basis of these assumptions, Falk-Petersen et al. (2000) inferred the euphausiids *Thysanoessa inermis* and *Euphausia crystallorophias* are herbivores, *T. rashii*, *T. macrura*, and *E. superba* are omnivores (with switching between phytoplankton and zooplankton diets during the year), and *T. longicaudata* and *Meganyctiphanes norvegia* are carnivores that primarily feed on the copepod *Calanus*. These inferences depend on an assumption that these euphausiids metabolize and bioconvert FA in similar ways. However, it is well established that many marine copepods can bioconvert C_{18} MUFA to 20:1n-9 and 22:1n-11 (Lee et al. 2006), and if any euphausiids had this capacity (but clearly some, such as *E. superba*, do not) they could be misclassified as copepod predators according to Falk-Petersen et al.'s scheme.

Stübing et al. (2003) showed that the FA composition of larval krill is very clearly modified by their diet. Larval *E. superba*, which start out with far less lipids than adults, also gained weight during these experiments and this weight gain was primarily due to lipids. In contrast to the sometimes ambiguous results obtained in dietary studies with adult krill FA, Stübing observed very clear trends when comparing the FA composition of krill diets to those of "fecal strings." These comparisons showed larval *E. superba* preferentially assimilated EPA, 16:1n-7, 16:4n-1, and DHA, with the pattern being particularly strong for EPA. Because of this, the residual FA in the fecal strings were relatively enriched with 16:0, 18:0 and 18:1n-9.

However, these results do not prove the preferentially assimilated FA were also accumulated, as the HUFA could have been catabolized. This type of question could be more easily resolved if ^{13}C-labeled FA were employed in feeding experiments as Graeve et al. (2005) did for marine copepods. More recently, Pond et al. (2005) showed that short-term inter-moult growth responses in krill were positively correlated with the concentration of diatom lipid biomarkers (i.e., 16:4n-1 and EPA) and the flagellate biomarker SDA. Furthermore, when feeding adult krill an exclusive diet of copepods, Cripps and Atkinson (2000) demonstrated clear changes in *E. superba* FA in 16 day feeding trails. In these experiments, the krill's EPA + DHA content increased from 20 to 45% and the sum of SAFA and MUFA declined from 41 to 27%.

6.4.7 The FATM Approach Applied to Zooplankton

As previously noted, there is great interest in using FA as trophic markers (Iverson – Chap. 12), particularly for studies of zooplankton feeding ecology (Dalsgaard et al. 2003). The FATM approach is a semiquantitative approach to reveal consumption of items with distinctive FA signatures. For more quantitative information (e.g., food web, carbon mass-balance calculations) information is needed on how the various FA are selectively metabolized as they are conveyed through aquatic food webs. Recently, a quantitative experimental approach was advanced that used ^{13}C-labeled phytoplankton to examine FA turnover times in marine copepods (e.g., Graeve et al. 2005). Knowing FA turnover times is particularly important (especially during short term experiments) as the FATM concept assumes equilibrium conditions in the system. Recent marine studies have tried to improve applications of the FATM approach. Instead of single FA and FA ratios, the whole FA pattern is analyzed by means of multivariate statistical methods (e.g., Quantitative FA Signature Approach, QFASA; see also Iverson – Chap. 12).

It is also possible to combine the FATM or QFASA approach with other food web tracer methods such as analyses of stable isotopes, sterols, gut pigments, gut content (including genetic markers), and lipophilic or even non-lipophilic anthropogenic substances (e.g., new and legacy contaminants) (Kainz and Fisk – Chap. 5). Studies have shown that lipid production in the zooplankton and seasonal variability for zooplankton dietary sources increases uncertainty within diet-zooplankton FA patterns. The ecological relevance of zooplankton species, their feeding strategies, and life history traits (incl. biosynthetic pathways and specific lipid composition requirements, differential catabolism of specific lipids, etc.) still needs to be better understood to allow us to more accurately interpret patterns observed in field studies before the full potential of the QFASA approach is realized (Iverson – Chap. 12). However, despite these caveats this approach has the potential to greatly improve our understanding of food web mass transfer, especially for the nutritionally critical lipids.

6.4.8 *Inconsistencies Between Taxa and Laboratory and Field Studies*

The published research on dietary impacts on zooplankton FA composition shows a wide range of responses from laboratory studies of *Daphnia* fed phytoplankton monocultures, where strong dietary impacts are observed, to field studies of adult euphausiids, where dietary responses are much more muted. Several field studies of freshwater zooplankton have failed to observe dietary impacts even for those taxa which show clear responses in laboratory studies. Some of these differences might be due to the fact that some zooplankton (e.g., *Daphnia*) are very fast growing and relatively lean so it only takes a short period of time to replace their lipid reserves with new dietary lipids (see Sect. 6.6). At the other extreme, marine copepods and especially euphausiids grow much more slowly and build up much larger lipid reserves over a period of months. Thus, recently acquired lipids are diluted into a much larger pool of previously stored lipids. In this case, it may only be possible to discern clear dietary signals in experiments lasting several months or more, which can be problematic for marine zooplankton that might be difficult to rear in the laboratory. It is noteworthy that studies looking at FA accumulation in larval euphausiids, which grow faster and have much smaller lipid reserves, show clearer evidence of dietary impacts on FA composition (Stübing et al. 2003).

It should also be noted that in oligotrophic freshwater systems zooplankton may often be food quantity limited (Persson et al. 2007) and for that reason zooplankton collected from these systems may have accumulated less storage lipids – which are more readily influenced by diet. In contrast, many laboratory studies utilize relatively high food concentrations >1 mg C (l^{-1}), which makes it more likely that the zooplankton will acquire new lipids. In addition, because most laboratory studies employ phytoplankton monocultures, and natural phytoplankton assemblages are rarely dominated by one group, the differences in the dietary FA profiles are much greater in laboratory than field studies. Finally, zooplankton probably also vary in their tendency to modify the FA profiles of stored lipids relative to their diets. Knowing how the major zooplankton groups differ in this regard is an important and as of yet unresolved research question.

6.5 Homeostatic Fatty Acid Composition Responses

Even amongst the zooplankton taxa that have FA profiles strongly influenced by diet, clear evidence of quasi-homeostatic[3] responses to dietary FA availability is evident. A quasi-homeostatic EFA content could be observed for freshwater

[3] A homeostatic response refers to a generally fixed elemental or biochemical composition in a consumer despite considerable variation in their diet. A "quasi-homeostatic" response indicates some (but much less) variation in the elemental or biochemical composition of a consumer compared to their diet.

zooplankton, although variability for individual FA was considerably higher than for phosphorus in daphnids (Müller-Navarra 2006). For example, when feeding *Daphnia galeata*, three algal cultures (*Scenedesmus obliquus*, *Cryptomonas erosa*, *Nitzschia palea*), for which EPA concentrations varied by a factor of 20×, EPA only varied by a factor of 2 in *D. galeata* (Müller-Navarra 2006). In Schöhsee, *Daphnia* spp. and *Eudiaptomus* spp. had higher and much less variable PUFA composition than the seston, especially for LIN and n-3 HUFA. (Müller-Navarra 2006). In Lake Washington, Ravet et al. (2009) observed the SAFA content of the freshwater calanoid copepod *Leptodiaptomus ashlandi* was clearly correlated with that of the seston over the course of a yearly sampling cycle ($r^2 = 0.75$) (see Fig. 6.4a). However, *L. ashlandi* had more SAFA than the seston during the spring diatom bloom in Lake Washington, i.e., 35 ± 1 (±1 SD) vs. 27 ± 1, respectively, and considerably less SAFA than the seston during the summer stratified period, i.e., 49 ± 6 vs. 66 ± 8, respectively. Since these samples were collected from the field, seasonal fluctuations in water temperatures as they affect the homeoviscous response should have also influenced the FA composition of *L. ashlandi*. However, in this case the trends observed (i.e., more SAFA than the seston when the water temperatures were low and less SAFA than the seston during the warm summer period) were more consistent with a quasi-homeostatic than a homeoviscous response.

In a laboratory study, Burns et al. (unpublished data) showed that the proportion of ALA plus SDA in the diet correlated strongly with the proportion of these FA in *Ceriodaphnia dubia* (see Fig. 6.4b). However, when *C. dubia* were fed cyanobacteria that had very little C_{18} n-3 FA, they still had ≈15% of these FA in their FA pool.

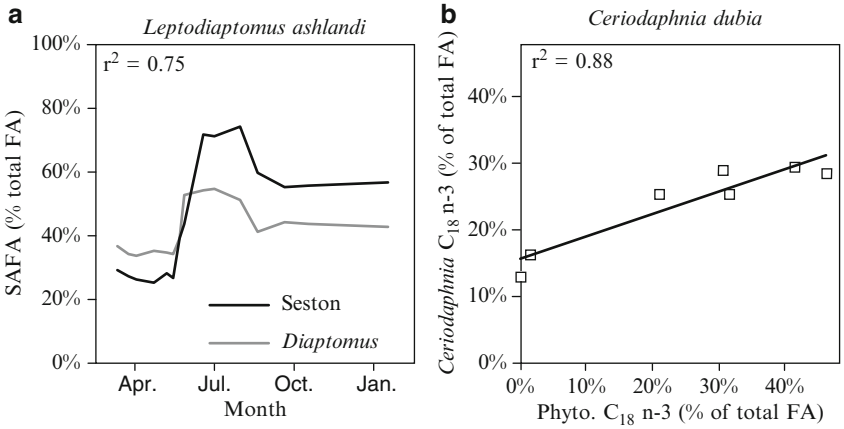

Fig. 6.4 Quasi-homeostatic responses in zooplankton-dietary FA composition. (**a**) This shows the relationship between the FA composition of natural seston and the calanoid copepod *Leptodiaptomus ashlandi* collected over an annual cycle in Lake Washington, USA (Ravet et al. 2009). (**b**) This shows the relationship between the FA composition of phytoplankton monocultures and the cladoceran *Ceriodaphnia dubia* obtained in a laboratory experiment (Burns et al. unpublished data)

Conversely, when *C. dubia* consumed cryptophytes that had ≈46% C_{18} n-3s, they contained ≈30% of these FA. Burns et al. (unpublished data) obtained similar results for *Daphnia carinata* and the copepod *Boeckella* spp. In both cases, the quasi-homeostatic responses primarily manifested themselves at the low range of dietary ALA and SDA availability where *D. carinata* and *Boeckella* contained substantially more C_{18} n-3 FA than their diets.

Daphnia spp. that consume SAFA-rich cyanobacteria tend to have only about half as much of these FA as their diet. In contrast, when *Daphnia* consume MUFA poor cryptophytes, they accumulate about twice the proportion of these FA as in their diets (Brett et al. 2006; Müller-Navarra 2006). This quasi-homeostatic response was particularly clear for *Daphnia* n-3:n-6 ratios, which averaged 16 ± 8 (±1 SD) in cryptophytes and 7 ± 5 in *Daphnia* that consume cryptophytes. Cyanobacteria had an average n-3:n-6 ratio of 0.3 and *Daphnia* that consumed cyanobacteria had an average n-3:n-6 ratio of 1.0. Similarly, Nanton and Castell (1998) observed the DHA/EPA ratio of the marine harpacticoid copepod *Tisbe* spp. only varied between 2.6 and 3.3 despite the fact that this same ratio in their phytoplankton and baker's yeast diets ranged between 0.1 and 12. It should also be noted that zooplankton that have systematically different FA composition than their diets also represent a form of homeostatsis.

6.6 Fatty Acid Turnover Times in Freshwater and Marine Zooplankton

When applying the FATM approach to zooplankton studies or merely trying to infer the impact of diet on zooplankton FA composition, it is important to know how long it takes for zooplankton to turn over their FA. That is, does the observed FA profile in a zooplankter reflect the food they consumed in the last 24 h or the last 2 months? In fact, much of the differences in zooplankton FA composition responses to dietary FA (see Sect. 6.4) may be due to widely varying FA turnover times (and experimental conditions employed) for the major zooplankton groups summarized. In its simplest sense, the FA turnover question can be conceptualized as a simple dilution model (see Jobling 2004, for an example with a fish context). According to a dilution model, the turnover time for particular FA should be most strongly influenced by the initial pool of a particular FA in the zooplankton tissues, normalized according to accrual of new FA. Even when lipid reserves are not being actively accumulated, FA can be replaced as older molecules of a particular FA are replaced by newer ones. The dilution of preexisting FA with new dietary FA should be simpler to conceptualize and easier to measure for neutral storage FA than for polar FA which have important structural roles and should therefore vary less with diet (Jobling 2004).

In experimental systems, where very fast growing zooplankton such as *Daphnia* are used and the experiments are initiated with <24 h old neonates, this is easy to address because new biomass is accrued so rapidly that nearly all of the maternal lipids initially present will be rapidly diluted by new growth. For example, a *Daphnia*

growing at a rate of 0.4 $(d)^{-1}$ will increase its mass by a factor of 10 in 6 d. In this case, one could assume that nearly all of the observed FA were accrued during the experiment. However, in natural systems, even fast growing zooplankton like *Daphnia* often grow well below their optima. In these cases, the FA accumulated in the daphnids may have accrued over one or more weeks. This could make it more difficult to detect unequivocal signals of dietary impacts on the FA composition of wild collected zooplankton. This question is even more challenging for zooplankton such as marine copepods and especially euphausiids, which accumulate very large lipid reserves over long periods. In this case, accumulation of new FA on a daily basis is minor relative to the lipids previously stored. This and the fact that many marine zooplankton are difficult to rear in a laboratory setting can make it more challenging to detect dietary signals.

To date only one study has addressed the zooplankton FA turnover question in a systematic fashion. Graeve et al. (2005) used ^{13}C labeled diets to determine how long it took three different species of Arctic *Calanus* to turn over their FA and fatty alcohol pools. These authors concluded the marine copepod *C. hyperboreus* exchanged nearly all of its original lipid pool after 11 d. In contrast, *C. finmarchicus* and *C. glacialis* exchanged 22% and 45% of their lipids, respectively, after 14 days, even though they were not actually growing. Since lipid turnover rates will depend on growth rates, FA turnover should also vary with zooplankter life stage, food availability, and water temperature.

6.7 Zooplankton Reproductive Investment in Fatty Acids

Müller-Navarra (2006) compared the FA composition of *Daphnia* spp. somatic tissues and subitaneous eggs to that of monoculture chlorophyte, cryptophyte, and diatom diets. She demonstrated that the LIN, ALA, SDA, and EPA composition and n-3:n-6 ratios of *Daphnia* somatic tissues were strongly correlated with those of their diets. Müller-Navarra's results also showed that *D. galeata* tended to have a higher absolute and relative content of ARA and less DHA than their diets, both in somatic tissue and eggs. Compared with the respective somatic tissues, subitaneous eggs had substantially higher total FA, SAFA, n-3 PUFA and n-6 PUFA content and higher n-3:n-6 ratios. Overall the FA composition of both somatic tissues and subitaneous eggs was very strongly influenced by diet when fed monocultures. In a similar study, Wacker and Martin-Creuzburg (2007) compared the FA composition of *Daphnia magna* somatic tissues and subitaneous eggs to those of high and low food quality diets. They found *D. magna* eggs contained significantly more SAFA, MUFA, and n-6 and n-3 PUFA than somatic tissues irrespective of diet quality. Among the n-6 PUFA, ARA was significantly enriched in eggs but LIN was not. Müller-Navarra (2006) found that ALA, SDA, and EPA were 2–3× enriched in eggs over somatic tissues. Wacker and Martin-Creuzburg (2007) found that EPA was the most strongly enriched, being 2.4× higher in subitaneous eggs than in somatic tissues. These authors suggested that *Daphnia* enrich their subitaneous eggs with EPA as a buffer against low food quality cyanobacteria blooms.

Abrusán et al. (2007) compared the FA composition of *Daphnia pulicaria* subitaneous and resting eggs when they consumed *Scenedesmus obliquus*. [Unfortunately, these authors did not present FA composition data for the somatic tissues of the *Daphnia* they used]. These authors noted that *D. pulicaria* hatched from resting eggs had higher growth and egg production rates than *D. pulicaria* from subitaneous eggs, so it is likely *D. pulicaria* invest more essential biochemicals in resting eggs. Consistent with this expectation, Abrusán et al. (2007) found that when *D. pulicaria* consumed EPA deficient *S. obliquus*, their starvation induced resting eggs contained 3.5× higher amounts of total FA and these resting eggs contained a substantially lower proportion of the SAFA 16:0 and the MUFA 16:1n-9. These resting eggs also had higher proportions of the C_{18} PUFA LIN, and in particular ALA and SDA, and a higher n-3:n-6 ratio. Furthermore, resting eggs contained approximately 0.7 ± 0.5% EPA, but this FA was barely detected in the subitaneous eggs of *D. pulicaria* fed *S. obliquus*. Overall, both n-3 and n-6 PUFA and HUFA accounted for 52% of total FA in the resting eggs and only 27% in the subitaneous eggs. Therefore, these studies show *Daphnia* invest heavily in the total FA content of both subitaneous and resting eggs and that these eggs were preferentially enriched with LIN and ARA and especially n-3 FA, although the specific EFA enriched (i.e., LIN or ARA, ALA or EPA) seems to vary from one case to the other.

The results obtained by Ederington et al. (1995) for the marine copepod *Acartia tonsa* contrast notably in several regards from the *Daphnia* results presented above. These authors' data showed that when *Acartia* consumed diatoms both their somatic tissues and their eggs were clearly enriched with EPA, whereas the somatic tissues and eggs of *A. tonsa* consuming the bacterivorous ciliate *Pleuronema* (which had 18:1n-11 as its dominant FA) were enriched with 18:1n-11. However, irrespective of diet both the somatic tissues and eggs of *A. tonsa* were dramatically enriched with the SAFA 16:0 and 18:0. Remarkably, Ederington et al.'s (1995) results suggest that the eggs of *A. tonsa* fed ciliates were almost completely devoid of n-3 and n-6 FA, and were almost entirely composed of 18:0 (50%), 16:0 (30%), and 18:1n-11 (12%) with other SAFA and MUFA making up the balance of FA in these eggs. While these authors did report that ciliate consumption was associated with dramatically reduced egg production, they also reported that 95% of the eggs from copepods fed ciliates were viable, which they suggested meant EFA were not essential for egg hatching. This latter result is paradoxical and should be validated in future studies.

Overall, little is known about the PUFA requirements for subitaneous and diapause eggs in both marine and freshwater copepods. In copepods, variability of FA independent of diets may be due to different requirements of the different developmental stages, with differences for somatic growth and egg production. During egg production, conversion of storage lipids and/or dietary lipids to PL takes place. These PL are then transported to the gonads where they become part of the egg yolk (lipovitelin production). In cladocerans, these fractions seem to be especially rich in PUFA as *Daphnia* eggs have several fold higher PUFA and especially HUFA contents than the somatic tissue (Müller-Navarra 2006). Thus, one can hypothesize that egg production in copepods may also be HUFA intensive. However, the FA composition of freshwater and marine copepod eggs warrants further research.

6.8 Temperature Impacts on Zooplankton Fatty Acids: The Homeoviscous Response

Although the first studies to examine the FA composition of zooplankton were specifically directed at examining temperature impacts on zooplankton FA (Farkas & Herodek 1964), few studies have focused on this topic since this pioneering research. Farkas and colleagues initially noted that those freshwater zooplankton that over-winter in an active life stage (i.e., copepods) were able to strongly modify their FA composition in response to temperature variation (Farkas 1979; Farkas et al. 1984). Copepods exposed to cold stress greatly increased the proportion of DHA and decreased the proportion of the SAFA 18:0 in their FA pools. It is now well known that nearly all poikilotherms adapt to cold stress, by increasing the proportions of PUFA, and in particular HUFA, in their membrane lipids (Hazel 1995). It was also suggested that zooplankton that do not over-winter in an active phase (i.e., cladocerans) do not have the capacity to modify their lipid composition in response to cold challenges and therefore over-winter as resting-eggs (Farkas 1979; Farkas et al. 1984). These authors also stressed the particular importance of DHA for zooplankton cold-water adaptation.

Several of Farkas assertions were challenged in a recent study by Schlechtriem et al. (2006), as well as by Arts et al.'s (1992) observation that some *Daphnia* are able to over-winter in ice-covered lakes. While supporting Farkas' fundamental observation that zooplankton adapt to cold stress by increasing the proportion of HUFA in their lipids, Schlechtriem et al. challenged the assertion that *Daphnia* cannot over-winter in an active phase and presented very clear evidence that *Daphnia* can increase their n-3 HUFA composition in response to cold stress. Schlechtriem et al. (2006) showed *Daphnia pulex* fed *Ankistrodesmus falcatus* at 11°C had 4× as much EPA (12.7% vs. 3.1%) compared with *Daphnia* grown at 22°C. Furthermore, cold-adapted *Daphnia* had 4× more 16:1n-7 (13.0% vs. 3.3%) and only half as much LIN (6.8% vs. 14.3%) and ALA (11.3% vs. 22.4%) as warm adapted *Daphnia* (see Fig. 6.5). Cold-adapted *Daphnia* also did not accumulate DHA, which is consistent with the broader observation that cladocerans as a group do not accumulate this FA. This taxa-specific observation also contradicts Farkas' assertion that DHA plays an essential role in membrane adaptation for zooplankton.

Kattner and Hagen (Chap. 11) also noted the high EPA and DHA content of tropical marine copepods calls into question the membrane fluidity hypothesis, and suggested these molecules may play important roles in membrane structure and function besides maintaining fluidity. Nanton and Castell (1999) reported the FA composition responses of the benthic marine harpacticoid copepods *Amonardia* and *Tisbe* to 6, 15, and 20°C temperature treatments. Unfortunately, this study produced rather equivocal results because the copepod FA profiles were generally more similar for the 6 and 20°C treatments than for the 15°C treatment. Specifically, both copepods had substantially lower DHA content when cultured at 15°C (Nanton and Castell 1999).

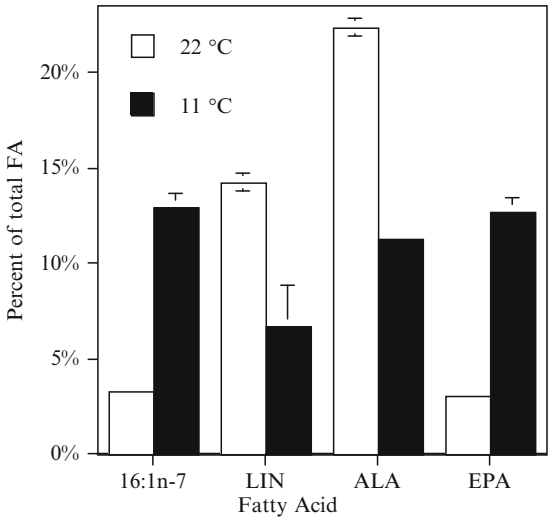

Fig. 6.5 The percent of total fatty acids for those FA in *Daphnia pulex* that showed a clear response (i.e. 16:1n-7, LIN, ALA and EPA) to high (22°C) and low (11°C) temperature treatments. All other FA showed much smaller responses to the temperature treatments. The results presented are treatment means ±1 SD. This figure is based on the data reported in Schlechtriem et al. (2006)

6.9 Starvation Impacts on Zooplankton Fatty Acids

Several studies have attempted to quantify starvation impacts on zooplankton FA composition, with Schlechtriem et al. (2006) presenting the clearest results. These authors cultured *Daphnia pulex* on the chlorophyte *Ankistrodesmus falcatus* and then starved individuals at 22 and 11°C. The daphnids starved at 22°C died within three days, during which time their FA composition did not change markedly. However, many of the daphnids starved at 11°C survived until day six during which time their FA composition changed dramatically. In particular, the mass of SAFA, MUFA, and ALA per individual declined markedly, whereas LIN, ARA, and EPA were conserved. This study could serve as a model for other studies attempting to assess starvation impacts on zooplankton FA composition.

6.10 Unanswered Questions

1. Why do freshwater zooplankton accumulate ARA? A number of studies have shown pronounced ARA accumulation by a variety of zooplankters, but so far no study has demonstrated a clear physiological justification (e.g., enhanced growth or reproduction) for ARA accumulation.

2. Are differences in the homeoviscous response the reason why cladocerans are less likely to over-winter in an active phase than copepods? Farkas and colleagues originally hypothesized that cladocerans were forced to over-winter as resting eggs because they were unable to modify the HUFA content of their lipids. In contrast, copepods were observed to dramatically increase their DHA content in response to cold stress. However, recent results challenge this hypothesis for daphnids and suggest *Daphnia* are able to exist in an active phase even at very low temperatures. Do the storage lipids and greater starvation tolerance of copepods also play an important role in over-wintering as suggested by research on marine copepods and krill?
3. Is the FA composition of polar lipids unaffected by diet for all zooplankton? Many studies have mentioned structural lipids are relatively unaffected by diet, whereas the composition of neutral lipids should be highly responsive to dietary lipid accumulation. Is this generally the case or are there exceptions to this "rule"? [See Stübing et al. (2003) and Hagen et al. (1996) for contradictory results in certain euphausiids].
4. How much endogenous FA production occurs in zooplankton? Many studies have cited Goulden and Place's (1990) observation that only 2% of *Daphnia* FA are produced de novo when consuming *Ankistrodesmus falcatus*. However, the generality of this result has not been tested for different *Daphnia* species or different diets, much less for other freshwater zooplankton. In contrast to *Daphnia*, it is well known that polar copepods synthesize C_{20} and C_{22} MUFA from dietary carbohydrates and proteins (Lee et al. 2006). Are marine zooplankton better able to synthesize FA than freshwater zooplankton, or is this difference between cladocerans and copepods independent of habitat?
5. To what extent are zooplankton capable of converting one EFA molecule to another; for example, LIN to ARA or ALA, and SDA to EPA or DHA? If zooplankton can make these conversions, what are the energetic costs of these conversions and how do these costs vary from one group to another (e.g., between cladocerans and copepods in freshwater and copepods and euphausids in marine systems)? Are carnivorous zooplankton less able to bioconvert C_{18} PUFA to C_{20} and C_{22} HUFA than herbivorous zooplankton? Knowing how these conversion capacities vary among zooplankton taxa will allow us to better understand to what extent zooplankton are able to "upgrade" the FA content of the food they consume.
6. Which zooplankton groups have FA profiles that are the most strongly influenced by diet and which have FA profiles which are the most "fixed" and why? The research conducted to date indicates cladocerans such as *Daphnia* have quite plastic FA profiles that are strongly influenced by diet, whereas some zooplankton have less obvious responses to the FA composition of their diets. Are these differences due to the greater role (and slower turnover) of storage lipids in the latter group? Or alternatively: Are some zooplankton more inclined to modify the composition of the dietary lipids they accumulate?
7. Why do cladocerans tend to accumulate relatively more EPA whereas copepods accumulate DHA? Various hypotheses have been offered to explain these patterns.

For example, the hypothesis that DHA plays an important role in conveying nerve impulses. However, so far none of these hypotheses have been rigorously tested.

8. What role do the "congener" molecules of ARA, EPA, and DHA (e.g., 20:3n-6, 20:3n-3, and 22:5n-3, respectively) play in the nutritional ecology and FA metabolism of marine and freshwater zooplankton? While most studies only report results for these HUFA, it is clear that congeners of these molecules are commonly encountered and may in some cases actually be more prevalent than the classically considered forms of HUFA. At this time, virtually nothing is known about how these molecules are utilized by zooplankton.

6.11 Conclusions

The various crustacean zooplankton groups found in the ocean and freshwater lakes have markedly different FA composition. Marine copepods and euphausids in temperate and polar regions store large pools of lipids as wax esters and to a lesser extent TAG composed predominantly of MUFA. Freshwater copepods accumulate EPA and especially DHA, while freshwater cladocerans preferentially accumulate EPA. Both freshwater copepods and cladocerans accumulate ARA compared with their diets. In general, nearly all zooplankton accumulate less SAFA, and a higher portion n-3 PUFA and especially n-3 and n-6 HUFA relative to their available diets. Diet has a very strong impact on the FA composition of some zooplankton (in particular *Daphnia* spp.), a moderate impact on some copepods, and only a small impact on the FA composition of zooplankton such as detritivorous harpacticoid copepods and adult euphausids. However, even the zooplankton taxa with the most plastic FA composition show clear homeostatic FA responses to diets with widely varying FA composition. Most zooplankton exposed to cold-stress show a clear homeoviscous response, with greatly increased accumulation of n-3 HUFA. When starved, zooplankton preferentially retain n-3 and n-6 HUFA. It has been shown that *Daphnia* heavily invest both C_{18} n-3 PUFA and EPA in their eggs. As outlined in this review, there remain many challenging research questions pertaining to zooplankton FA composition to occupy aquatic ecologists during the next decade.

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