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Original article

Effects of temperature and life stage on the fatty acid composition of Collembola

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ARTICLE INFO

Article history:
Received 14 June 2007
Accepted 27 September 2007
Published online 18 December 2007

Keywords:
Fatty acids
Temperature
Neutral lipids
Trophic biomarker
Fungi
Collembola

ABSTRACT

Fatty acid (FA) analysis is used as a promising tool to investigate trophic interactions in soil food webs. The FA profile of neutral lipids in consumers is affected by the diet, and the occurrence and amount of certain FAs can reflect feeding strategies. We investigated the lipid composition of the Collembola *Folsomia candida*, *Heteromurus nitidus* and *Protaphorura fimata* with the fungus *Chaetomium globosum* as food source. The impact of environmental temperature and life stage was assessed, with special respect to linoleic acid (18:2ω6,9) as a marker FA for fungal feeding. In all Collembola species the ratio of C16/C18 in neutral lipid fatty acids (NLFAs) increased with decreasing temperature. In the NLFAs of *F. candida* and *H. nitidus* the Unsaturation Index and the amount of 18:2ω6,9 decreased with temperature, whereas in *P. fimata* effects were the opposite. The composition of phospholipid fatty acids (PLFAs) differed between species, but was little affected by temperature. The degree of unsaturation in NLFAs increased with the age of Collembola, mainly due to higher amounts of 18:2ω6,9 and a lower proportion of 18:1ω9. The biomarker linoleic acid represented over 20% of FAs in all fungal feeding Collembola. Despite considerable influence of temperature and life stage on its proportion, the amount was always higher than in individuals reared on other diets. This suggests that linoleic acid can serve as marker for fungal feeding independent of such physiological variations in Collembola.

1. Introduction

Cellular fatty acid composition has been frequently used to investigate food webs in aquatic systems [6,23,24]. Only recently this approach was applied in soil food webs (e.g. [5,3,29,30]). Several studies investigated the feeding strategies of Collembola, a widespread and abundant group of soil animals, which play an important role in decomposition processes [38]. Laboratory experiments showed that specific FAs are typically found in the neutral lipid fatty acids (NLFAs) of consumers, when reared on a certain diet [13,30]. For bacterial feeding, specific marker FAs indicate consumption of either Gram-positive (i14:0, i15:0, a15:0, i17:0) or Gram-negative (cy17:0) bacteria [13]. The FA 20:1ω9 was only present in Collembola feeding on nematodes, whereas on fungal diet they contained a higher proportion of 18:2ω6,9 and on leaf diet a higher proportion of 18:1ω9 [31].

However, not only diet can influence the FA composition of an animal [8,16]. The relative abundance of FAs in hexapods is also determined by the specific type of biosynthetic pathway of the given species [10,12], life stage [25,33], and environmental conditions [18]. Especially thermal adaptation is a known

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doi:10.1016/j.ejsobi.2007.09.003

factor in alterations of fatty acid composition [14], but most studies deal with influence on phospholipid fatty acids (PLFAs), the main components of cell membranes, whereas changes in NLFAs are not well documented.

The changes during cold acclimation to maintain membrane viscosity and subsequently membrane transport and cell functionality are termed homeoviscous adaptation [14]. Two major metabolic compensation mechanisms exist. First, short-chain PLFAs result in higher membrane fluidity than long-chain PLFAs, leading to temperature induced changes in the ratio of C16 to C18. Second, unsaturated PLFAs result in more fluidity than saturated PLFAs, and the Unsaturation Index (UI) therefore increases with lower temperature [15].

In contrast, NLFAs have an important role as energy reserves and are closely related to nutritional requirements and metabolism [34]. The composition of NLFAs in the fat body results from different processes including the storage of dietary lipids, de novo synthesis, degradation and subsequent release for mobilisation to sites where they are metabolised [2]. Due to their use to investigate trophic relationships [13,30,31], it is important to assess if effects known to alter PLFA composition may also influence the NLFA pattern.

Collembola are known to feed on a large variety of food sources [32], but fungi are generally regarded as the most important diet [4,22]. The biomarker FA linoleic acid (18:2ω6,9) is found in high amounts in Collembola feeding on fungi, but is also found in Collembola feeding on other diets, although in lower amounts [3,13,31]. It is therefore considered as a relative biomarker for fungivory in Collembola. Physiological changes in Collembola metabolism may affect the abundance of linoleic acid, as well as related precursors and products. Linoleic acid is the precursor for eicosanoids, which play an important role in insect physiology. They influence reproduction, mediate cellular immune response and are involved in temperature regulation [35,37]. In addition, the amount of linoleic acid may be altered to maintain membrane fluidity at different environmental temperatures. We have analysed the influence of temperature and life stage on the lipid pattern in three Collembola species (*Protaphorura fimata*, *Heteromurus nitidus* and *Folsomia candida*) reared on fungal diet focusing on linoleic acid, and its reliability as trophic biomarker.

2. Materials and methods

2.1. *Fungi*

The soil decomposer fungus *Chaetomium globosum* Kunze was cultivated at 10 °C on Potato Dextrose Agar (PDA, Merck, Darmstadt). As food source for Collembola round pieces of fungal mats (diameter 10 mm) were cut out of the agar cultures under sterile conditions and offered to animals. For analysis of fungal FA composition *C. globosum* was grown on PDA covered by a membrane filter (Millipore, 0.8 μm). Cultures were kept at 5, 10 or 15 °C for 3 days before harvest to simulate the incubation conditions for fungi in the feeding experiment. To gain fungal biomass membrane filters were stripped of the agar with the adhering fungal mats. Hyphae were scrapped from the filter with a sterile scalpel and frozen at −20 °C until analysis.

2.2. *Collembola*

The Collembola species *Protaphorura fimata* (Gisin, 1952), *Heteromurus nitidus* (Templeton, 1835) and *Folsomia candida* (Willem, 1902) were taken from laboratory cultures fed with bakers yeast. Specimens were kept in plastic vessels (diameter 7 cm, height 4.5 cm) with a layer of plaster mixed with activated charcoal (2:1) at the bottom. Each vessel contained ten individuals. This low density was chosen to avoid cannibalism due to crowding. Vessels were kept in darkness and under moist conditions with distilled water (pH 7). Eggs, pellets and exuvia were removed once a week. To investigate the effect of temperature, Collembola were incubated at 5, 10 and 15 °C. Animals were fed with fungal diet ad libitum, thereby the fungal food source was renewed three times a week. After 6 weeks Collembola were sampled destructively and frozen at −20 °C until analysis. Three replicates (with 30 individuals each) per temperature were performed.

To study the influence of different life stages, eggs of *P. fimata* were kept in plastic vessels until hatching. Collembola hatched within one week were joined into the same life stage group. Eggs and newly hatched Collembola were removed once a week to insure cohorts of comparable age. Specimens were fed with *C. globosum* for 4, 8 and 12 weeks after hatching. Three replicates (with 30 individuals each) per developmental stage were performed and harvested destructively. Collembola were frozen at −20 °C until analysis.

2.3. *Analysis of fatty acid patterns*

Whole cellular lipids of fungi were extracted, whereas lipids of Collembola were divided into NLFA and PLFA fractions. Collembolan lipids were extracted by shaking in 5 ml single phase extraction solvent (chloroform/methanol/0.05 M phosphate buffer (pH 7.4) 1:2:0.8) overnight. The solvent was then transferred to new tubes and samples were re-extracted by shaking for 2–3 h with additional 2.5 ml. Extraction solvents of both steps were combined, 0.8 ml distilled water and 0.8 ml $CHCl_3$ were added and samples centrifuged at 1500 rpm for 5 min. Samples were allowed to stand and separate. Then the top two phases were removed and the chloroform fraction of each sample was transferred to a silica acid column (0.5 g silicic acid, mesh size 100–200 μm). Lipids were eluted with 5 ml chloroform (neutral lipids), 8 ml acetone (glycolipids) and 5 ml methanol (phospholipids). Neutral lipids and phospholipids were analysed further. The chloroform and methanol fraction was reduced by evaporation (50 °C, vacuum 200 hPa) in a Labconco RapidVap (Labconco Corp., Kansas City).

Chloroform and methanol fractions of the Collembola samples and total fungal biomass were saponified and methylated following the procedures given for the Sherlock Microbial Identification System (MIDI Inc., Newark, DE). Saponification of lipids was conducted in a sodium hydroxide/methanol solution (45 g sodium hydroxide, 150 ml methanol, 150 ml distilled water) at 100 °C for 30 min, followed by acid methanolysis in HCl/methanol (325 ml 6.0 N hydrochloric

acid, 275 ml methyl alcohol) at 80 °C for 10 min. The fatty acid methyl esters were extracted into hexane/methyl tertiary butyl ether (1:1) and washed with aqueous NaOH (10.8 g sodium hydroxide, 900 ml distilled water). The lipid-containing phase was then transferred to test tubes and stored at −20°C until analysis.

Fatty acid methyl esters were analysed by gas chromatography (GC) using the Sherlock Microbial Identification System (MIDI Inc.) consisting of a Hewlett Packard 5890 Series II gas chromatograph and flame ionisation detector equipped with an HP Ultra 2 phenyl methyl silicone fused capillary column (25 m × 0.2 mm i.d., film thickness 0.33 μm), an automated sampler and computer with associated software (Sherlock Pattern Recognition Software, MIDI). The fatty acid methyl esters were identified on the basis of their retention times and quantified. To verify correct identification of fatty acids methyl esters (chain length and saturation) all samples were analysed by GC-mass spectrometry using an Agilent Series 6890 GC System and 5973 Mass Selective Detector, equipped with a HP5MS capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm). Due to insufficient biomass, only major FAs were detected.

The fluidity of lipids is expressed as the fatty acid unsaturation index (UI). This was calculated as follows:

$$UI = (C{:}1 * 1) + (C{:}2 * 2) + (C{:}3 * 3) + (C{:}4 * 4)/100$$

where C:1, C:2, C:3 and C:4 represent the proportion (%) of fatty acids with 1, 2, 3, and 4 double bonds, respectively.

2.4. *Statistical analysis*

Differences in fatty acid profiles of fungi and Collembola were arcsin-radical-transformed and analysed using ANOVA. If significant effects were suggested, means were compared with Tukey's honestly significant difference test. Statistical analyses were performed using SAS (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. *Effects of temperature*

3.1.1. *Fungi*

Major cellular lipids of *C. globosum* were palmitic (16:0; 18–20%), oleic (18:1ω9; 29–32%) and linoleic (18:2ω6,9; 44–46%) acid. Additionally, 14:0, 16:1ω7, 17:0 and 18:0 were present in proportions between 0.5% and 4.7%. The FA profile of *C. globosum* was not significantly affected by temperature within 3 days of incubation at 5, 10 or 15 °C (MANOVA: $F_{8,2} = 0.834$, $P = 0.6496$) (Table 1). Turkey's HSD detected no differences between temperature treatments for any FA.

3.1.2. *Collembola*

Ambient temperature significantly affected body weight of Collembola ($F_{2,18} = 20.66$, $P < 0.0001$, Fig. 1). Irrespective of species individuals kept at 5 °C had a lower fresh weight (340–437 μg) than Collembola maintained at 10 or 15 °C (485–557 μg); however this was only significant for *P. fimata* and *F. candida*.

Table 1 – Effects of incubation temperature on the proportion of fatty acids (in % ± S.D.) in cellular lipids of the fungus *Chaetomium globosum* after 3 days at 5, 10 or 15 °C

	5 °C	10 °C	15 °C
14:0	0.5 ± 0.2	0.6 ± 0.1	0.5 ± 0.1
16:0	18.9 ± 0.7	20.3 ± 4.2	18.2 ± 0.4
16:1ω7	1.0 ± 0.2	1.1 ± 0.4	1.0 ± 0.1
17:0	0.8 ± 0.1	1.0 ± 0.3	0.9 ± 0.2
18:0	4.7 ± 1.2	1.8 ± 1.7	3.8 ± 0.1
18:1ω9	30.4 ± 1.4	29.0 ± 2.9	31.8 ± 2.0
18:2ω6,9	43.8 ± 1.2	46.2 ± 5.5	43.8 ± 2.2

Dominant NLFAs of Collembola were similar in *P. fimata*, *H. nitidus* and *F. candida*, but differed in their relative amounts (Table 2). Major FAs were 16:0 (17.3–25.7%), 16:1ω7 (2.0–7.5%), 18:0 (2.0–7.2%), 18:1ω9 (45.9–51.3%) and 18:2ω6,9 (12.6–24.8%). Temperature had a significant influence on the proportion of palmitic and palmitoleic acid (16:1ω7) ($F_{2,18} = 5.03$, $P = 0.0184$; $F_{2,18} = 14.72$, $P = 0.0002$, respectively), which decreased with elevated temperature in all three species. The proportion of linoleic acid in *H. nitidus* kept at 5 °C was about half than incubation at 10 and 15 °C and significantly affected by temperature ($F_{2,6} = 8{,}41$, $P = 0.0182$). Stearic acid (18:0) had a high frequency in *P. fimata* at 15 °C with 4.4%, but made up only 2.0% and 2.4% at 5 and 10 °C, respectively ($F_{2,6} = 21{,}89$, $P = 0.0018$).

The ratio C16/C18 in neutral lipids was affected significantly and decreased with increasing temperature in all Collembola ($F_{2,18} = 11.93$, $P = 0.0005$). The Unsaturation Index (UI) of NLFAs differed between Collembolan species ($F_{2,18} = 19.9$, $P < 0.0001$) with *H. nitidus* always having the lowest index. The UI in *P. fimata* increased from 0.97 to 1.00 with declining temperature ($F_{6,2} = 3.36$, $P = 0.1048$), whereas in *H. nitidus* and *F. candida* the UI was lower in animals maintained at 5 °C than in animals at 15 °C (0.92 to 0.83, 1.00 to 0.96, respectively; $F_{6,2} = 10.79$, $P = 0.0103$; $F_{6,2} = 1.86$, $P = 0.2358$; respectively).

Phospholipids of *H. nitidus* could not be analysed due to insufficient biomass and a high mortality at 5 °C. Major PLFAs of

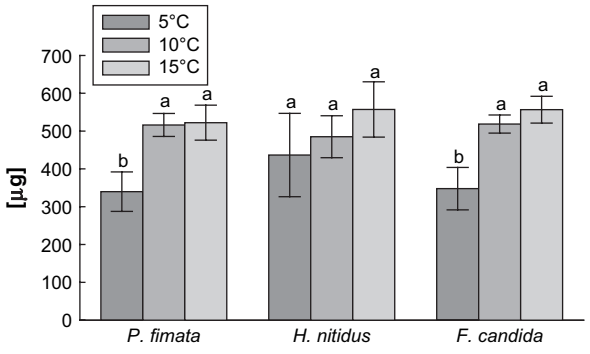

Fig. 1 – Individual fresh weight (μg ± S.D.) of the Collembola species *Protaphorura fimata*, *Heteromurus nitidus* and *Folsomia candida* fed with the fungus *Chaetomium globosum* under different temperature conditions (5, 10, 15 °C). Bars sharing the same letters within a Collembolan species are not significantly different (Tukey's HSD, $P < 0.05$).

Table 2 – Major neutral lipid fatty acids (in % ± S.D.) of the Collembola *Protaphorura fimata*, *Heteromurus nitidus* and *Folsomia candida* maintained at three different temperatures (5, 10, 15 °C)

Collembola	*P. fimata*			*H. nitidus*			*F. candida*			ANOVA		
Temperature	5 °C	10 °C	15 °C	5 °C	10 °C	15 °C	5 °C	10 °C	15 °C	C	T	C × T
16:0	22.3 ± 0.6	22.4 ± 0.6	20.5 ± 0.8	25.7 ± 1.9a	22.0 ± 0.6b	24.9 ± 1.2ab	20.2 ± 1.3ab	21.2 ± 2.3a	17.3 ± 0.7b	***	*	**
16:1 ω7	4.5 ± 1.7	3.5 ± 1.3	2.0 ± 0.5	7.4 ± 2.2	5.0 ± 1.2	3.4 ± 2.0	7.5 ± 1.1a	4.7 ± 1.1ab	3.1 ± 1.1b	*	***	
18:0	2.0 ± 0.4b	2.4 ± 0.2b	4.4 ± 0.7a	4.2 ± 0.7	3.2 ± 0.2	3.7 ± 0.2	5.2 ± 1.9	4.9 ± 0.2	7.2 ± 1.7	***	**	*
18:1 ω 9	47.0 ± 2.4	49.3 ± 3.3	51.3 ± 0.1	50.2 ± 4.1	48.0 ± 3.3	47.1 ± 1.2	46.0 ± 2.9	45.9 ± 2.2	47.5 ± 0.5			
18:2 ω 6,9	24.2 ± 0.3	22.4 ± 2.3	21.8 ± 0.9	12.6 ± 3.0b	21.8 ± 4.0a	20.9 ± 2.1a	21.2 ± 1.0b	23.4 ± 1.2ab	24.8 ± 1.6a	***	**	**
C16:C18	0.37 ± 0.04	0.35 ± 0.03	0.29 ± 0.02	0.50 ± 0.09	0.37 ± 0.01	0.39 ± 0.03	0.38 ± 0.05	0.35 ± 0.06	0.26 ± 0.01	***	***	
UI	1.00 ± 0.01	0.98 ± 0.02	0.97 ± 0.01	0.83 ± 0.04	0.97 ± 0.04	0.92 ± 0.03	0.96 ± 0.01	0.97 ± 0.03	1.00 ± 0.04	***	**	**

ANOVA indicates significant effects of Collembola (C), temperature (T) and interactions (C × T), with *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Means within lines and per species with different letters are significantly different from each other (Tukey's HSD, $P < 0.05$). UI, Unsaturation Index.

F. candida, and *P. fimata* were 16:0 (24.4–31.2%), 18:0 (7.5–23.9%), 18:1ω9 (17.9–34.8%) and 18:2ω6,9 (28.8–34.9%) (Table 3). No significant influence of temperature on the PLFAs was observed, whereas the composition of PLFAs varied with species. 18:0 and 18:2ω6,9 were significantly different between species ($F_{1,6} = 20.0$, $P = 0.0011$; $F_{1,6} = 18.86$, $P = 0.0013$; respectively) with 18:0 being less and 18:2ω6,9 more abundant in *P. fimata* than in *F. candida*. This corresponded to the UI, which was also influenced by temperature ($F_{1,6} = 70.5$; $P = 0.0002$) and was higher in *P. fimata* (0.97–1.03) than in *F. candida* (0.69–0.75). The ratio C16/C18 decreased with increasing temperature in both species, more pronounced in *F. candida* (0.45 to 0.33) than in *P. fimata* (0.32 to 0.28).

3.2. *Influence of life stage*

The life stage of Collembola affected the body mass of specimens. *P. fimata* at an age of 4 weeks had a lower fresh weight (159 ± 36 μg) than 8- and 12-week-old individuals (297 ± 63 and 262 ± 129 μg, respectively, data not presented). Additionally, the proportion of dominant NLFAs changed with developmental stage. The amount of 18:2ω6,9 increased ($F_{5,2} = 9.17$, $P = 0.0213$; respectively), whereas the frequency of 18:1ω9 decreased with increasing age ($F_{5,2} = 5.77$, $P = 0.0503$, Fig. 2). The C16/C18 ratio ranged between 0.31 and 0.33 (data not presented) and was not influenced by life stage. The UI tended to increase from 0.94 to 1.08 in older Collembola ($F_{5,2} = 4.94$, $P = 0.0655$, data not presented).

4. Discussion

4.1. *Temperature*

The ambient temperature did not influence the fatty acid composition of the fungal diet *C. globosum* within 3 days, the maximum incubation period with Collembola before exchanged with fresh fungus. The altered FA composition of Collembola due to temperature regime in the experiment therefore originates from changes in animal metabolism and is not related to differences in the food source.

Fluidity in storage fat is maintained at lower temperatures to permit accessibility of enzymes to energy reserves [11,17]. In our experiments the C16/C18 ratio of NLFAs increased with decreasing temperature. Individuals kept at lower environmental temperatures contained higher proportions of palmitic acid (16:0). Similar observations are reported from *Drosophila* spp. [26]. In contrast to the C16/C18 ratio, the UI of NLFAs increased with temperature in *F. candida* and *H. nitidus*, but decreased in *P. fimata*, mainly due to corresponding proportions of the unsaturated FA linoleic acid. Oleic acid (18:1ω9) did not respond to temperature at all. These results are surprising, as adaptation to lower temperature by shifting from more saturated to more unsaturated FAs is a widespread phenomenon in poikilothermes. For instance in nematodes the UI of NLFA and PLFA increased with decreasing temperature [17]. However, Petersen and Holmstrup [28] found an increase in UI of whole cell fatty acids of *Lumbricus rubellus* from 1.4 to 1.6 when kept at 0 °C instead of 20 °C. In our experiments only *P. fimata* showed an increase in UI with cold

Table 3 – Major phospholipid fatty acids (in % ± S.D.) of the Collembola *Protaphorura fimata* and *Folsomia candida* maintained at 3 different temperatures (5, 10, 15 °C)

Collembola	*P. fimata*			*F. candida*			ANOVA
Temperature	5 °C	10 °C	15 °C	5 °C	10 °C	15 °C	C
16:0	24.4	24.5	21.6 ± 1.6	31.2 ± 2.3	26.3 ± 6.9	24.7 ± 1.2	
18:0	11.7	7.5	10.7 ± 3.7	18.5 ± 1.0	21.9 ± 3.6	23.9 ± 2.1	***
18:1 ω 9	30.9	34.8	32.9 ± 2.1	18.6 ± 1.5	23.0 ± 2.3	17.9 ± 4.4	
18:2 ω 6,9	33.0	33.2	34.9 ± 3.7	31.7 ± 1.9	28.8 ± 2.2	33.6 ± 1.0	***
C16:C18	0.32	0.32	0.28 ± 0.03	0.45 ± 0.05	0.37 ± 0.13	0.33 ± 0.02	
UI	0.97	1.01	1.03 ± 0.05	0.69 ± 0.05	0.75 ± 0.07	0.69 ± 0.02	***

UI, Unsaturation Index. ANOVA indicates significant effects of Collembola species (C) with ***$P < 0.001$.

acclimation, whereas all species displayed a distinct increase in the C16/C18 ratio. This indicates that Collembola change the fluidity in their NLFAs mainly by alterations in FA chain length and not by degree of saturation.

In contrast to other studies which found the degree of unsaturation in PLFAs to vary inversely with growth temperature in poikilothermic animals [1,9,19,27,28] the impact of ambient temperature on the PLFA pattern of Collembola in our experiment was minor. Declining temperature led to a shift towards short chain FAs, which increased the fluidity of membranes; however, these effects were not significant. The variation in the PLFA composition between Collembola species was much more pronounced than temperature effects. The pattern of PLFAs, which are structurally and functionally involved in biomembranes is individually arranged in the specific tissue and controlled at a cellular level [34].

Surprisingly, the NLFA pattern was also affected by Collembola species, suggesting changes based on species specific lipid metabolism. Further temperature had a significant impact. Nevertheless, the proportion of 18:2ω6,9 in NLFAs, the marker FA for fungal feeding in Collembola [31], was over 20% in all species and at all incubation temperatures. This is well above the amount in Collembola feeding on bacteria (2.0–12.0%) [13], leaves (13.9%) and nematodes (13.6%) [31], or yeast (2.0–12.0%) [3]. Proportions below 20% occurred only in *H. nitidus* at 5 °C (12%), where this species had a high mortality. Likely, the NLFA pattern at 5 °C is not predominantly affected by the major diet ingested, but by altered metabolism due to temperatures below the tolerated threshold. Suboptimal growth temperatures are reported to result in qualitative and quantitative changes in lipid composition of nematodes [1]. *H. nitidus* had the lowest UI at all temperatures, suggesting poorer adaptation to temperature decline than in *P. fimata* and *F. candida*. This may be due to different temperature optima in enzymes that synthesise unsaturated FAs, a fact known for eukaryotic organisms [7]. A comparable inability to adapt to decreasing temperatures was also found by Jagdale and Gordon [17] in a nematode species inhabiting warmer regions, which showed a poor survival at 5 °C.

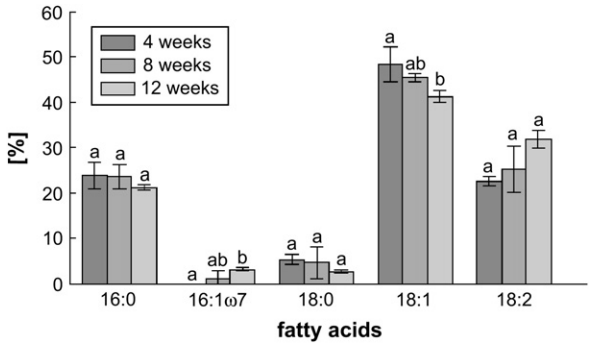

Fig. 2 – Major NLFAs (in % ± S.D.) of 4-, 8- and 12-week-old *Protaphorura fimata* fed with the fungus *Chaetomium globosum*. Bars sharing the same letters within individual fatty acids are not significantly different (Tukey's HSD, $P < 0.05$).

4.2. *Life stage*

Fatty acid profiles of many hexapods change over the course of development [34]. In gypsy moth both NLFA and PLFA pattern changed with life stage [36]. In our experiments NLFA composition of *P. fimata*, especially the UI, was influenced by the age of the Collembola. The UI increased from 0.94 to 1.08 in older animals, mainly due to the higher proportion of 18:2ω6,9 and 16:1ω7 and the lower amount in 18:1ω9. Also, Kamler et al. [20] observed in fish that the level of some unsaturated fatty acids increased with age and size. Kattner and Krause [21] found more long-chain unsaturated FAs in older stages of copepods.

The influence of age on C18 FAs may be due to their use as precursors for hormones, such as eicosanoids, which involved in reproduction, predominantly egg laying and moulting [35]. They also modulate lipid mobilisation in hexapods and are mediators of hemocytic immunity [35]. Due to the requirement of eicosanoids in reproduction processes, the life stage of the Collembola may affect the amount of FAs which are involved in the biosynthesis of C20 PUFAs such as 18:2ω6,9. In our experiments, we found only an insignificant increase in 18:2ω6,9 in neutral lipids with Collembola age. Due to the broad time span for building age groups this could not directly be related to distinct Collembolan life stages. However, within these cohorts changes in linoleic acid were minor, suggesting that developmental needs for precursor FAs of C20 PUFAs do not affect NLFA pattern and consequently the trophic marker linoleic acid.

5. Conclusion

Temperature and developmental stage affected the composition of NLFAs and PLFAs in Collembola. However, linoleic

acid, the marker FA for fungal feeding in Collembola, was present in high proportions (over 20%) at all temperatures and stages (except *H. nitidus* at 5 °C), and was more abundant than in Collembola feeding on other food sources. This indicates that high amounts of 18:2ω6,9 in NLFAs can serve as robust biomarker for fungal food in Collembola, and may also hold for other soil decomposer invertebrates.

Acknowledgements

D.H. was funded by the DFG, Graduiertenkolleg, TU Darmstadt, and FAZIT-*Stiftung* Gemeinnützige Verlagsgesellschaft mbH.

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