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ORIGINAL ARTICLE

# Fall Composition of Storage Lipids is Associated with the Overwintering Strategy of *Daphnia*

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**Abstract** Diapause, which occurs through the production of dormant eggs, is a strategy used by some zooplankton to avoid winter months of persistent low temperatures and low food availability. However, reports of active zooplankton under the ice indicate that other strategies also exist. This study was aimed at evaluating whether the composition of storage lipids in the fall differs between diapausing and active overwintering *Daphnia*. We assessed the quantity of storage lipids and fatty acid (FA) composition of *Daphnia* species, along with FA content of seston, in six boreal, alpine and subarctic lakes at the onset of winter, and evaluated the association between storage lipids and *Daphnia* overwintering strategy. We found that active overwintering *Daphnia* had >55% body fat and the highest FA concentrations. Polyunsaturated FA, especially stearidonic acid (18:4n-3; SDA) and high ratios of n-3:n-6, were preferentially retained to a greater extent in active overwintering *Daphnia* than in those that entered diapause. *Daphnia* FA composition was independent of that of the seston diet, indicating that *Daphnia* adjusted their storage lipids according to the physiological requirements of a given overwintering strategy. The occurrence of an active overwintering strategy has consequences for zooplankton community structure, and can have important implications for the transfer of high-quality energy at higher trophic levels.

**Keywords** Freshwater · Winter · Fatty acids · Ice · Life history strategies · Zooplankton · SDA

## Abbreviations

| | |
|---|---|
| FA | Fatty acid(s) |
| FAME | Fatty acid methyl esters |
| EPA | Eicosapentaenoic acid, 20:5n-3 |
| MUFA | Monounsaturated fatty acid(s) |
| PUFA | Polyunsaturated fatty acid(s) |
| SDA | Stearidonic acid, 18:4n-3 |
| SFA | Saturated fatty acid(s) |

## Introduction

Life history strategies of organisms are often determined by environmental cues, such as temperature and food availability [1–3]. This means that organisms can adjust their physiology and reproductive cycles to changes in their environment. One of the best documented life history adaptations to adverse changes in the environment is the production of resting eggs, or ephippia, in *Daphnia* (Cladocera) [4, 5]. Increases in population density and decreases in temperature, light and food supply are some

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of the most common triggers that induce resting egg production, and characterize lakes in late summer and fall. It is commonly accepted, therefore, that cladocerans prepare for winter by producing ephippia and are absent from the water column during the winter months with persistent low temperatures and limited food. However, there is increasing awareness of the presence of active zooplankton throughout the ice cover period [6–8], indicating that, at least for certain species, other adaptation mechanisms for overwintering exist as well. While most historical research has focused on the environmental conditions that trigger overwintering diapause, especially ephippia production in *Daphnia* [9], much less is known about physiological adaptations in cladocerans that overwinter actively.

An effective physiological overwintering mechanism known for fish and marine zooplankton is the accumulation of storage fat [5, 10, 11]. Storage fats and their fatty acids (FA) provide high-density energy reserves used to maintain metabolic function and reproduction during periods of low food supply [12–14]. Studies on freshwater copepods indicate that both the quantity of lipid reserves [15] and the high overall percentage of polyunsaturated fatty acids (PUFA) [16] are common characteristics in the fall for active overwintering copepods, suggesting that there could be differences in lipid accumulation depending on whether cladocerans enter diapause or overwinter actively. However, the link between storage fats and FA, and zooplankton life history strategies has not been explored in detail, especially in freshwater systems.

Typically, the function of particular FA is determined by the degree of saturation, and FA can be grouped accordingly. Saturated and monounsaturated fatty acids (SFA and MUFA) tend to be used as storage fats [17]. PUFA are particularly important for reproduction and early development [17], and are used to compensate for the loss of cell membrane flexibility in cold temperatures [18]. Further, consumers differ in their FA composition, and thus in their requirement for specific FA [19]. The growth and reproduction of cladocerans, for example, has been strongly affected by the content of eicosapentaenoic acid (EPA, 20:5n-3) [20], but whether EPA or any other FA is linked to or required by the active overwintering *Daphnia* is not known. Little is also known about how composition or relative differences between certain FA account for any species specific or individual metabolic requirement, such as an organism's reproduction, thermal compensation or life history stage. Generally, a higher quantity of PUFA and a higher ratio of omega-3 (n-3) to omega-6 (n-6) PUFA are favorable for all organisms; however, the optimal dietary ratio of n-3/n-6 seems to be highly species-specific [21], and very little is known about changes in seasonal requirements for these FA ratios.

As the source of zooplankton lipids is diet, any seasonal accumulation of lipids in zooplankton is linked to food sources. Seasonal phytoplankton food availability has been reported to be the main driver for organisms to store FA [3, 17], and there is evidence that freshwater copepods use fall phytoplankton to build up the fats that allow for reproduction under the ice [8, 16]. Changes in phytoplankton community and FA composition are to some extent mirrored in the zooplankton FA composition [22, 23]. However, in fall, a simple reflection of the bulk phytoplankton community, where many taxa may be senescing, would not be a winning strategy for surviving the winter months under the ice. Rather, a selective accumulation of FA could be used as an energy source during winter. Given the predominance of ice cover in northern latitudes, it is important to understand the environmental and physiological mechanisms determining life history strategies. Ultimately, the presence or absence of an active zooplankton in a lake affects the lake's community structure and ecological functioning, such as winter food web dynamics [24, 25].

In this study, we investigate the link between the winter life history strategy of *Daphnia* and the quantity of their lipids and FA composition. *Daphnia* are well adapted to unstable habitats due to their short life cycle, and have wide phenotypic plasticity [6, 26]. These characteristics make *Daphnia* an ideal genus in which to compare the physiology and overwintering strategies among different populations in different parts of their environmental range. Our primary goal in this study was to compare the FA composition of *Daphnia* from boreal, alpine and subarctic lakes at the onset of winter. In this wide geographical range, different species composition of *Daphnia* and different overwintering strategies emerged, including *Daphnia* that entered diapause for winter, *Daphnia* that remained active under the ice, and lakes where both overwintering strategies were present. We hypothesized that in lakes where *Daphnia* overwinter actively, they will store lipids during the fall, particularly PUFA, in order to compensate for low temperature and limited food availability under the ice. Since the dormant strategy simply avoids the harsh winter conditions, we assumed that *Daphnia* which enter dormancy will not invest in storing lipids. We include further discussion on the evolutionary implications of winter strategies and the composition and physiology of storage lipids.

## Materials and Methods

### Study Sites and Sample Collection

We sampled *Daphnia* from six lakes across three geographically distinct regions in 2009–2011 (Table 1). The *Daphnia* were the dominant zooplankton species in three

**Table 1** Lake locations and physical characteristics separated by *Daphnia* winter strategy (active, both strategies, diapause)

| | Country | Region | Latitude, N | Longitude, E | Altitude, m | Area, m$^2$ | Depth, m | Ice cover, months | *Daphnia* species |
|---|---|---|---|---|---|---|---|---|---|
| Active | | | | | | | | | |
| Malla South | Finland | Subarctic | 69°03 | 20°43 | 599 | 7850 | 3.0 | 9 | *D. umbra* |
| Saanajärvi | Finland | Subarctic | 69°05 | 20°97 | 679 | 7.00E+05 | 24 | 8 | *D. umbra* |
| Both strategies | | | | | | | | | |
| Simoncouche | Canada | Boreal | 48°25 | −71°94 | 110 | 8.70E+05 | 6.0 | 6 | *D. galeata,*<br>*D. dubia,*<br>*D. longiremis* |
| Lunzersee | Austria | Alpine | 47°51 | 15°04 | 608 | 6.80E+05 | 34 | 3 | *D. longispina* |
| Diapause | | | | | | | | | |
| Big Rock | Finland | Subarctic | 69°10 | 20°43 | 527 | 47 | 0.5 | 9 | *D. longispina* |
| Mekkojärvi | Finland | Boreal | 61°13 | 25°08 | 130 | 3800 | 4 | 6 | *D. longispina* |

of the sites (Big Rock, Malla South and Mekkojärvi), while in the other sites they contributed to a zooplankton community dominated by copepods. Due to the wide geographical range, the data set included five different *Daphnia* species, and many lakes had different *Daphnia* assemblages (Table 1). In the text, they are collectively referred to as *Daphnia*. The lakes were divided into three categories based on the occurrence of *Daphnia* in winter. The Malla South and Saanajärvi lakes, with no record of ephippia-carrying *Daphnia* in earlier zooplankton samples or paleolimnological studies (e.g. [27]), were considered lakes with active overwintering *Daphnia*. The lakes where all *Daphnia* entered diapause as ephippia due to freezing solid (Big Rock) or winter anoxia (Mekkojärvi) were categorized as diapause lakes. The Lunzersee and Simoncouche lakes, where both ephippia and active individuals were present, were considered lakes with both overwintering strategies. *Daphnia* were present in winter in the alpine Lunzersee (0.0005 ind L$^{-1}$, $n = 1$, December), with the shortest ice cover period of 3 months; in boreal Simoncouche (2.46 ± 5.5 ind L$^{-1}$, $n = 12$, December–April), with 6 months of ice; and the two subarctic lakes Malla South and Saanajärvi (0.02 ± 0.01 ind L$^{-1}$, $n = 8$, November–May), with up to 9 months of ice. The winter abundance was up to twofold lower than that in summer; however, parthenogenetically reproducing individuals were found in both lakes in winter.

From these six sites we compared the percentage of body fat and the FA composition of adult *Daphnia* at the onset of winter. Considering the wide latitudinal variation in our study sites, this period was defined as a maximum of 2 months before ice cover for each water body (hereafter referred to as "fall"). Temperature at all sites was close to 10 °C during the sampling and decreasing from the maximum summer values. *Daphnia* were collected by vertical tows of a 50-μm mesh net at the deepest point of the lake, or by horizontal pulls in the shallow pond Big Rock. The *Daphnia* were hand-sorted, aiming for at least 0.5–1.5 mg dry weight (DW), when possible in triplicate. Seston, representing the dietary source of FA available for *Daphnia*, was taken from a depth-integrated water sample that was pre-filtered through a 50-μm net to remove larger organisms, then filtered onto a GF/F filter using 1–3 L per triplicate. All samples were freeze-dried and stored in −80 °C until lipid and FA analysis.

## Fatty Acid Analyses

FA were analysed using a three-step extraction–methylation protocol [27]. Freeze-dried and weighed *Daphnia* and seston samples were extracted using a chloroform–methanol wash cycle, and the concentration of extracted lipids determined by gravimetry. The body fat percentage was calculated as a function of extracted lipid weight and *Daphnia* DW. The extracted lipids were methylated using toluene and sulphuric acid–methanol and then solubilized in hexane. FA (C14–C24) were identified as fatty acid methyl esters (FAME) using a gas chromatograph equipped with a Supelco® SP-2560 column (100 m, 25 mm i.d., 0.2 μm film thickness) and a flame ionization detector (Thermo Scientific™ TRACE™ GC-FID). Total fatty acid concentrations (ΣFAME) were calculated using calibration curves based on known external standard concentrations, and are reported as μg FAME per mg carbon weight (μg FA mgC$^{-1}$). FA concentrations were calculated using calibration curves of known standard concentrations. *Daphnia* dry weights were converted to carbon (C) weight using a *Daphnia* summer average of 42% C and 15% for seston that were available from mass spectrometry (Thermo Finnigan DELTA$^{plus}$ Advantage) and stable isotope analyses

(Mariash unpubl). Only the FAME greater than 0.1% were used in the analysis. All FAME results are presented as a proportion of the total FAME concentration in moles. Individual FA are summarized in the results according to general lipid classes of SFA, MUFA or PUFA, or as ratios of the sum of omega-3 to omega-6 FAME (n-3/n-6).

### Statistical Analysis

Differences in seston and *Daphnia* FA composition (data normalized, Euclidean distances), and *Daphnia* fall FAME characteristics (ΣFAME, %PUFA, %SFA, %SDA, SFA/PUFA and Σn-3/n-6) and body fat levels among strategies (active, diapause, both strategies) and lakes (Malla South, Saanajärvi, Simoncouche, Lunzersee, Big Rock, Mekkojärvi) were tested with a two-factor (lake nested in strategy, strategy fixed and lake random) permutational (multivariate for FA composition) analysis of variance (PERMANOVA, with 999 permutations). Although comparisons of FAME characteristic values can be done with classical ANOVA, PERMANOVA was preferred, as it did not require observations fitting the normality assumptions (see [29]). The nested design was used to control for variation among lakes within strategy. When only a limited number of permutations were possible, Monte Carlo $p$ values were used. Non-metric multidimensional scaling (nMDS) was used to visualize *Daphnia* FA composition among factors. Linear regressions were carried out between *Daphnia* and seston FAME variables that were significantly different in *Daphnia* in different overwintering strategies (ΣFAME, %PUFA, %SDA, Σn-3/n-6) to test for potential influence of diet on *Daphnia* FAME composition. Pair-wise comparison tests among strategies were carried out for *Daphnia* FAME composition, total FAME, PUFA, SDA and n-3:n-6 in PERMANOVA, with an adjusted significance level method from Holm [30]. All analyses were conducted using PRIMER software version 7.0.9 with PERMANOVA+. A significance level $\alpha = 0.05$ was used for all statistical tests.

**Table 2** Average (±SD) values of *Daphnia* (a) body fat (% of dry weight), (b) total FAME concentration (μg FA $\text{mgC}^{-1}$), (c) FA composition (mole% of total fatty lipids) for saturated (ΣSFA), monounsaturated (ΣMUFA) and polyunsaturated (ΣPUFA) FA, and (d) ratios of ΣSFA/PUFA and Σn-3/n-6 per overwintering strategy

| | Active $n = 7$ | Both strategies $n = 9$ | Diapause $n = 7$ |
|---|---|---|---|
| (a) Body fat | 58.6 ± 11.0 | 30.9 ± 3.3[a] | 32.6 ± 4.1 |
| (b) ΣFAME | **742.6 ± 132.0** | **189.7 ± 57.3** | **214.7 ± 10.6** |
| (c) ΣSFA | **27.0 ± 0.1** | **32.1 ± 6.1** | **26.5 ± 5.5** |
| 14:0 | 9.7 ± 0.3 | 11.6 ± 7.2 | 3.7 ± 2.03 |
| 15:0 | 0.9 ± 0.1 | 0.7 ± 0.9 | 1.1 ± 0.1 |
| 16:0 | 10.7 ± 0.3 | 15.6 ± 0.5 | 14.9 ± 5.9 |
| 17:0 | 0.2 ± 0 | 0.8 ± 0.2 | 0.8 ± 0.1 |
| 18:0 | 5.6 ± 0.4 | 3.4 ± 0.2 | 6.1 ± 1.9 |
| ΣMUFA | **15.4 ± 0.6** | **26.4 ± 2.3** | **43.0 ± 16.3** |
| 16:1n-7c | 5.9 ± 0.1 | 8.9 ± 0.4 | 25.3 ± 19.8 |
| 18:1n-9 | 5.6 ± 0.4 | 8.5 ± 0.9 | 8.2 ± 4.8 |
| 16:1n-9 | 1.4 ± 0.1 | 4.0 ± 3.6 | 0.9 ± 4.7 |
| 18:1n-7 | 2.6 ± 0.2 | 5.0 ± 2.5 | 8.7 ± 1.1 |
| ΣPUFA | **57.6 ± 0.8** | **41.5 ± 8.3** | **30.5 ± 10.8** |
| 18:2n-6 | 3.5 ± 0.3 | 4.5 ± 0.3 | 5.0 ± 2.3 |
| 18:3n-3 | 5.2 ± 0.2 | 6.9 ± 2.6 | 6.8 ± 4.8 |
| 18:4n-3 | 25.7 ± 0.4 | 11.6 ± 1.6 | 3.5 ± 1.1 |
| 18:3n-6 | 1.3 ± 0.1 | 0.8 ± 0.2 | 0.6 ± 0 |
| 20:4n-3 | 9.0 ± 1.8 | 0.6 ± 0.1 | 0.6 ± 0.1 |
| 20:4n-6 | 1.6 ± 0.2 | 3.8 ± 0.1 | 4.7 ± 1.1 |
| 20:5n-3 | 10.6 ± 0.5 | 12.8 ± 3.6 | 9.0 ± 4.4 |
| 22:6n-3 | 0.8 ± 0.2 | 0.8 ± 0.1 | 0.4 ± 0.2 |
| (d) ΣSFA/PUFA | **0.5 ± 0.4** | **0.8 ± 0.3** | **0.9 ± 0.1** |
| Σn-3/n-6 | **7.9 ± 0.2** | **3.6 ± 0.8** | **2.0 ± 0.1** |

Bold values are sums of the individual FAME

*n* number of replicates

[a] This value is only from Lake Simoncouche; Lunzersee did not have a sample for fall body fat

## Results

The *Daphnia* total lipid amount and FA profiles were different for *Daphnia* with different strategies (Tables 2, 3; Fig. 1). In the lakes with active overwintering *Daphnia*, the percentage of body fat was highest (58%), although not statistically different from the other strategies (Table 3a). In the lakes with diapause or mixed overwintering strategy, *Daphnia* had lower body fat (~30%; Table 2a). Total FAME concentrations (mean ± SD) were statistically higher for active *Daphnia* than for the diapause strategy (Tables 2b, 3b; Fig. 2). The *Daphnia* FA composition differed marginally among the strategies ($p = 0.068$; Table 3c; Fig. 1). When the lake factor was not considered, all strategies were highly statistically different from each other ($p < 0.003$ for all multiple pair-wise comparisons), and sample scores from the active and diapausing populations were furthest away from each other (Fig. 1). The difference in FA composition among the differently overwintering *Daphnia* was largely due to differences in their total PUFA, stearidonic acid (18:4n-3; SDA) and the n-3/n-6 ratio (Fig. 2). The *Daphnia* PUFA were marginally different between strategies ($p = 0.061$; Table 3d), with higher values in active overwintering *Daphnia* (Table 2c). SDA was significantly different among strategies (Table 3f; Fig. 2): more than double in active wintering *Daphnia* (26% of total FAME) compared to *Daphnia* with mixed strategies (12%), and ninefold higher compared to the diapausing *Daphnia* (4%;

**Table 3** Summary of the permutational ANOVA comparing *Daphnia* (a) body fat, (b) total FAME, (c) FA composition, (d) PUFA, (e) SFA, (f) SDA, (g) SFA/PUFA, (h) n-3/n-6 and (i) seston FA composition between the overwintering strategies (active, diapause, both) at the onset of winter

| Source | *df* | MS | Pseudo-*F* | *p* value |
|---|---|---|---|---|
| (a) Body fat | | | | |
| Strategy | 2 | 1318.9 | 4.27 | 0.182 |
| Lake (strategy) | 2 | 256.29 | 3.17 | 0.069 |
| Residual | 13 | 80.9 | | |
| Total | 17 | | | |
| (b) Total FAME | | | | |
| Strategy | 2 | $7.08 \times 10^5$ | 17.4 | 0.023 |
| Lake (strategy) | 3 | 40,852 | 4.69 | 0.008 |
| Residual | 17 | 8717 | | |
| Total | 22 | | | |
| (c) FA composition | | | | |
| Strategy | 2 | 82.67 | 2.67 | 0.068 |
| Lake (strategy) | 3 | 31.22 | 5.71 | 0.001 |
| Residual | 17 | 5.47 | | |
| Total | 22 | | | |
| (d) PUFA | | | | |
| Strategy | 2 | 0.16 | 7.53 | 0.061 |
| Lake (strategy) | 3 | 0.02 | 12.2 | 0.001 |
| Residual | 17 | 0.002 | | |
| Total | 22 | | | |
| (e) SFA | | | | |
| Strategy | 2 | 0.01 | 1.46 | 0.344 |
| Lake (strategy) | 3 | 0.009 | 3.17 | 0.058 |
| Residual | 17 | 0.003 | | |
| Total | 22 | | | |
| (f) SDA | | | | |
| Strategy | 2 | 0.10 | 249.3 | 0.001 |
| Lake (strategy) | 3 | 0.0004 | 0.283 | 0.279 |
| Residual | 17 | 0.0003 | | |
| Total | 22 | | | |
| (g) SFA/PUFA | | | | |
| Strategy | 2 | 0.411 | 2.68 | 0.217 |
| Lake (strategy) | 3 | 0.154 | 2.31 | 0.107 |
| Residual | 17 | 0.067 | | |
| Total | 22 | | | |
| (h) n-3/n-6 | | | | |
| Strategy | 2 | 75.46 | 52.9 | 0.004 |
| Lake (strategy) | 3 | 1.43 | 1.21 | 0.308 |
| Residual | 17 | 1.18 | | |
| Total | 22 | | | |
| (i) FA composition seston | | | | |
| Strategy | 2 | 29.52 | 1.01 | 0.522 |
| Lake (strategy) | 3 | 26.82 | 5.02 | 0.001 |
| Residual | 9 | 5.34 | | |
| Total | 14 | | | |

Except for seston FA composition, all *p* values were obtained with the Monte-Carlo routine (see "Materials and Methods")

Table 2c). The SDA results were further reflected in the n-3/n-6 differences among strategies, with significantly higher n-3:n-6 ratios for the active strategy (Tables 2d, 3h). In summary, the main lipid parameters that were most different between strategies were total FAME concentration, SDA, PUFA and n-3/n-6 FA ratio, as illustrated in Fig. 2. Further, the active and diapausing populations differed in species. Lakes with active strategy had only *D. umbra*, and lakes with diapause had only *D. longispina*, which differed in their FA composition (SI Table 1).

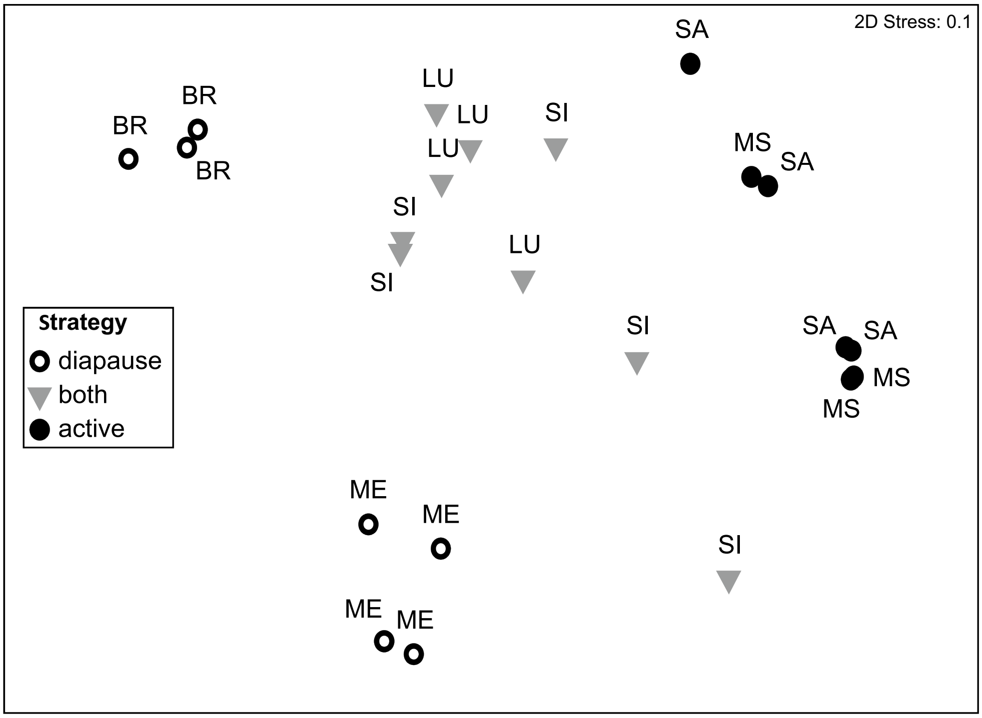
FAME concentrations in seston were an order of magnitude lower than *Daphnia* FAME concentrations (SI Table 2). Seston had high SFA/PUFA ratios, indicating that SFA dominated the seston. Of all FAME in seston, between 41 and 89% were SFA. Of the PUFA, seston generally contained very small amounts of 18:3n-6 (GLA), 20:5n-3 (EPA) and 22:6n-3 (DHA), and notably, 18:4n-3 (SDA) was less than 2%. Seston FA composition did not differ among strategies (Table 3i). Regression analyses revealed that variation in seston FA composition (ΣFAME, %PUFA, %SDA, Σn-3/n-6) across the six lakes did not explain a significant amount of corresponding variation in the FA composition of any *Daphnia* community ($R^2 < 0.20$ and $p > 0.10$ for all comparisons). While average concentrations of individual FA in the *Daphnia* varied substantially among overwintering strategies, FA concentrations in seston were more uniform and did not reflect these differences. The patterns in FA composition and total FA concentrations in the zooplankton appeared more closely related to zooplankton overwintering strategy than seston FA composition.

## Discussion

We compared the quantity of storage lipids and FA composition of *Daphnia* at the onset of winter from lakes where there is an active winter community, lakes where the *Daphnia* enter diapause for winter and from lakes where the *Daphnia* use both strategies but are dominated by the diapausing strategy. Our results suggest that FA may have an important role for *Daphnia* that prepare to overwinter actively and are in accordance with earlier observations from cladocerans and calanoid copepods that have demonstrated a high accumulation of lipids and PUFA in fall [5, 15, 16]. Storing fat could be a physiological mechanism zooplankton use to adapt to life under the ice, and therefore can potentially be used as a physiological indicator for zooplankton's winter survival.

The active winter *Daphnia* stored more FA, both in percentage of body fat and FAME concentration, than the other two strategies. Specifically PUFA, SDA (18:4n-3), and high ratios of n-3:n-6 were preferentially retained in

**Fig. 1** Non-metric multidimensional scaling (nMDS) plot of fatty acid (FA) methyl ester concentrations from lakes with *Daphnia*, showing **a** active (*black symbols*), **b** diapause (*white symbols*) or **c** both (*grey symbols*) overwintering strategies. *Data points* represent samples collected in the fall from the six lakes (*MS* Malla South, *SA* Saanajärvi, *SI* Simoncouche, *LU* Lunzersee, *BR* Big Rock, *ME* Mekkojärvi)



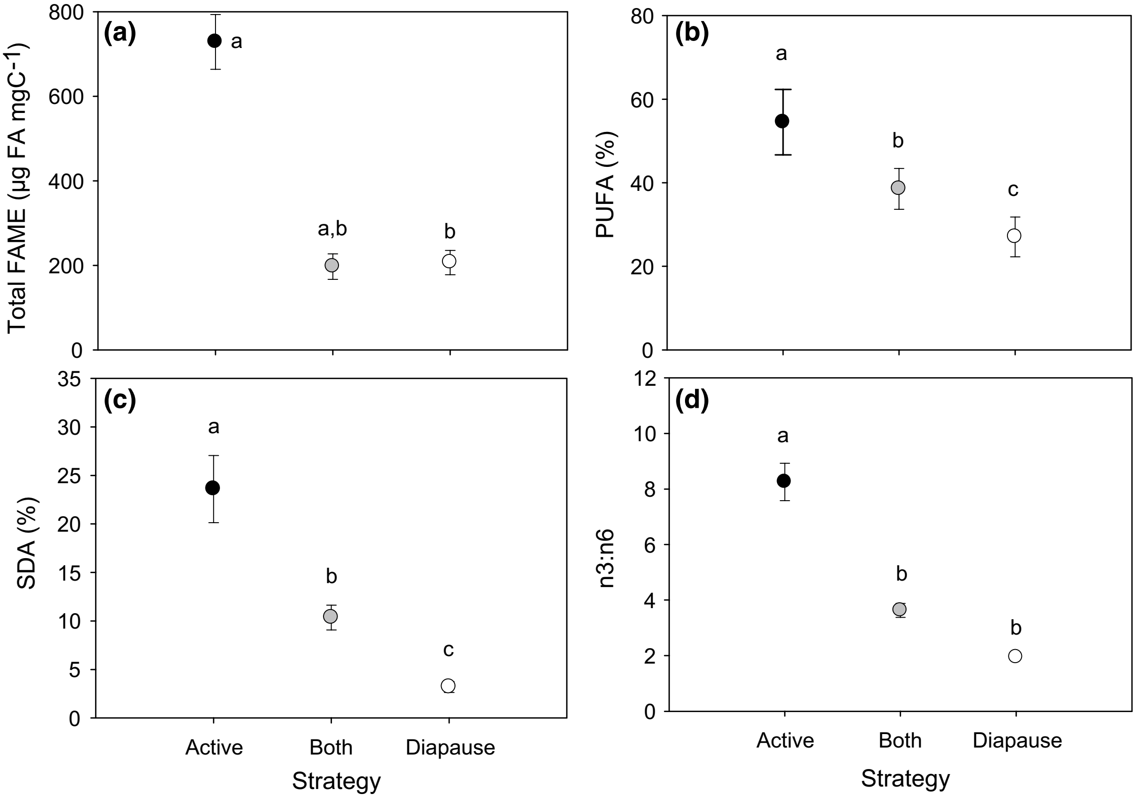


**Fig. 2** *Daphnia* **a** total FAME concentration (µg FA mgC$^{-1}$), **b** molar percentage of PUFA of total FA, **c** molar percentage of SDA (18:4n-3) of total FA, and **d** n-3/n-6 ratio in communities showing active, diapause or both overwintering strategies at the onset of winter. *Error bars* are SE. *Different letters* indicate statistically different values

active overwintering *Daphnia* than in *Daphnia* using the other two strategies. While cladocerans should have fairly constant n-3/n-6 FA ratios, with an average of 3 [19, 31, 32], our results show that there were contrasting n-3/n-6 ratios between winter life history strategies. The active *Daphnia*, especially in the subarctic, had more than double (8.25) the average, while the diapausing *Daphnia* were below (2.0) the n-3/n-6 average value of three. High proportions of SDA mostly drive the n-3/n-6 ratios in our results, highlighting that SDA could have an important role in physiological functions for *Daphnia* winter life history strategy.

We also considered the alternative explanation that the patterns in FA among *Daphnia* may stem from differences in basal resources [33] rather than the overwintering strategy. For example, changes in FA among *Daphnia* in boreal lakes were attributed to changes in resource assimilation between summer and fall diets [34]. However, our results show that the composition of the *Daphnia* FA indicate a preferential retention or accumulation of specific FA by *Daphnia*, rather than a direct reflection of the availability of specific FA in seston during the fall. Further, as winter seston diet is more scarce in subarctic than in boreal lakes, one would expect diapause to be the dominant strategy at high-latitude lakes, which was not the case here. The *Daphnia* entered diapause only when environmental constraints such as loss of habitat (drying, freezing solid, anoxia) prevented the active stage. While the limited number of lakes in this study does not allow for more accurate testing of the role of the region or ice cover on the strategy, our data show that *Daphnia* overwinter actively in subarctic lakes even when there is 9 months of ice cover.

Other factors known to affect life history strategies are species type [35], environmental stressors like temperature [2], and/or lake size and depth [36, 37]. Our study included five different *Daphnia* species, and studies have shown that different species differ in their FA composition and allocation of lipids [38, 39]. Lakes with active winter *Daphnia* had only *D. umbra*, a species that is not known to occur in temperate lakes, and both lakes with diapause had only *D. longispina*, a species common in the boreal region. It is possible that lakes only support populations of species that are matched to the environment in terms of their physiology, including FA signatures. Hence, it could be that not all *Daphnia* species are able to regulate their storage fats and lipid composition to the same extent. The species that do not possess this ability could be geographically restricted to lakes where the open water period is warm and long enough to allow for emergence from ephippium and completion of a life cycle before the return of adverse environmental conditions. Subsequently, species not capable of lipid accumulation should be absent from perennially cold arctic lakes, which could in part explain the low number of zooplankton species in arctic lakes [40]. However, there exists evidence that lipid allocation is not only a species-specific trait, but is also determined by environmental conditions. Based on the number of lipid droplets in *D. umbra*, Larsson and Wathne [5] were able to separate the ephippia-producing individuals with fewer lipid droplets from individuals that accumulated large amounts of lipids to survive longer. Nevertheless, regardless of whether active overwintering is an intrinsic physiological adaptation of only certain species or a more common response of *Daphnia* genus to seasonally changing environmental conditions, the results here are in line with other studies that have shown that zooplankton with active overwintering strategies accumulate lipids for subsequent use during the months when food is scarce [8].

As has been observed in other studies, both strategies can coexist in the same lake [5, 36, 37]. Although it would be interesting to separate the different life history strategies within the same lake, there is no way of visually distinguishing between individuals that will overwinter actively and those that will make ephippia. Even at a population level, it is difficult to discern overwintering strategy. It is however, noteworthy, that in lakes where both strategies existed, the majority of *Daphnia* entered diapause, based on the low numbers of active *Daphnia* in the water column under the ice and the high number of ephippia in the fall. The more similar fall FA composition between individuals with diapausing and mixed strategies further suggests that most *Daphnia* in mixed-strategy lakes entered diapause, possibly because they did not possess the physiological means to accumulate storage fats. Patterns of FA composition among active, diapausing and both strategies from different lakes and different species in this study suggest that lipid accumulation is an important factor in *Daphnia* wintering strategies; however, further experimental approaches and testing would be needed to explicitly determine this.

The ecology of the subarctic and arctic region may provide a framework for understanding how the active winter strategy evolved. The intense light regime in late spring at higher latitudes promotes a thriving phytoplankton community, even under the ice [7, 41, 42], creating favorable growing conditions for *Daphnia*. At this time, ephippia would not yet be primed for emergence, as water temperatures are still too low (3–5 °C), and would not be able to benefit from the spring phytoplankton as the already active community would. Further, the high levels of the polyunsaturated SDA and n-3 found in the active *Daphnia* can help combat the low spring temperatures [43]. Active wintering *Daphnia* and their parthenogenetic young are able to exploit the early spring phytoplankton, and are typically larger in body size [44], affording them a decisive competitive advantage over the resting egg community, made possible by energy reserves stored as >55% body fat, as suggested by our study. In addition, the interaction between low temperature and starvation during winter months could effectively help to extend winter survival by lowering metabolism [45]. When the open water season is a major part of the year, *Daphnia* have time to feed and regenerate within the longer productive period, and ephippia have the flexibility to postpone emergence until spring conditions improve.

The evolutionary advantages of reproducing even in times of starvation, however, must be weighed against living longer but with increased probability of death from senescence [46]. Complete abandonment of ephippia would be risky, as a single season of low survival and failed reproduction could eliminate an entire population. Further, the diapause strategy maintains genetic diversity and ensures that a population may persist during potentially fatal conditions [4]. The most profitable strategy would be to produce many parthenogenetic offspring in spring, plus adding to the ephippia bank for insurance [1]. We found both winter life histories across the regions studied, although a larger data set would be needed to confirm the occurrence and potential dominance of this strategy at a global scale.

The occurrence of different overwintering strategies has consequences for the plankton community structure and food web dynamics. Not only is the seasonal accumulation of PUFA in cladocerans important to the accumulation of quality energy at higher trophic levels [47], but the actual presence of winter *Daphnia* is important to winter-feeding fish ([24] and ref therein) and can affect the composition of the spring phytoplankton community [48]. Our results show that *Daphnia* using the diapause strategy do not need to invest in storing a large reserve of lipids, having only half as much body fat in the fall compared to the *Daphnia* that remain active. Therefore, both population- and community-level dynamics are affected by the life history strategies of *Daphnia*. Further, the physiological adaptations and life history strategies used by zooplankton on an annual scale may provide insight into the degree of population plasticity in response to a changing climate. As temperatures continue to increase around the globe, earlier spring ice-off or later fall ice-on will begin to affect the environmental cues (temperature and photoperiod) for diapause stages [35, 49]. A longer ice-free period will result in changes to phytoplankton community structure and production, and could lead to changes in storage fat accumulation for zooplankton.

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