Fall composition of storage lipids is associated to the overwintering strategy of *Daphnia*

Heather L. Mariash[1,2], Mathieu Cusson[2] and Milla Rautio[1,2,3]

[1] Department of Environmental & Biological Science, University of Jyväskylä, P.O. Box 35
40014 Jyväskylä, Finland
[2] Département des sciences fondamentales, Université du Québec à Chicoutimi, Québec
G7H 2B1, Canada
[3] Centre for Northern Studies (CEN), Laval University, Quebec City, Quebec, Canada and
Group for Interuniversity Research in Limnology and aquatic environment (GRIL),
University of Montreal, Montreal, Quebec, Canada

Corresponding author: Heather L. Mariash
National Wildlife Research Centre
Science and Technology Division, Environment and Climate Change Canada
Carleton University, 1125 Colonel By Dr.,
Ottawa, ON, K1A 0H3 Canada

Email: heather.mariash@gmail.com

Abstract

Diapause, using dormant eggs, is a strategy some zooplankton use to avoid winter months of persistent low temperatures and low food availability. However, reports of active zooplankton under the ice indicate other strategies also exist. This study aimed at evaluating whether fall composition of storage lipids is different between diapausing and actively overwintering *Daphnia*. We assessed the quantity of storage lipids and fatty acid composition of *Daphnia* species along with fatty acid content of seston in six boreal, alpine and subarctic lakes at the onset of winter, and evaluated the association of storage lipids to *Daphnia* overwintering strategy. We found that active overwintering *Daphnia* had >55% of body fat and the highest fatty acid concentrations. Polyunsaturated fatty acids, especially stearidonic acid (18:4n3; SDA) and high ratios of n3:n6 were preferentially retained in active overwintering *Daphnia* compared to those that entered diapause. The *Daphnia* fatty acid compositions were independent of that of the seston diet, indicating *Daphnia* adjusted their storage lipids according to the physiological requirements of a given overwintering strategy. The occurrence of the active overwintering strategy has consequences to zooplankton community structure, and can have important implications for the transfer of high quality energy in 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, 20:5n3
MUFA	Monounsaturated fatty acid(s)
PUFA	Polyunsaturated fatty acid(s)
SDA	Stearidonic acid, 18:4n3
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, ephippia, in *Daphnia* (Cladocera) [4, 5]. Increases in population density and decreases in temperature, light and food supply are some of the most common triggers that induce resting egg production, and characterize lakes in late summer – fall. It is therefore commonly accepted 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 there exist also other adaptation mechanisms to overwinter. While most of the historic research has focused on environmental conditions initiating overwintering diapause, especially ephippia production in *Daphnia* [9], much less is known about physiological adaptations of cladocerans that overwinter actively.

An effective physiological overwintering mechanism known for fish and marine zooplankton is to accumulate 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 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 copepods that overwinter actively suggesting that there could be differences in lipid accumulation depending on whether cladocerans enter diapause or overwinter actively. The link between storage fats and FA, and zooplankton life history strategies has however not been explored in detail, especially in freshwater systems.

Typically the function of particular fatty acids is determined by the degree of saturation, and fatty acids can be grouped accordingly. Saturated and monounsaturated fatty acids (SFA and MUFA) tend to be used for 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 composition and thus in their requirement for specific FA [19]. The growth and reproduction of cladocerans has e.g. been strongly affected by the EPA (eicosapentaenoic, 20:5n3) content [20] but whether EPA or any other FA is linked or required to the active overwintering *Daphnia* is not known. Little is also known about how composition or relative differences between certain FAs 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 between omega-3 (n3) and omega-6 (n6) PUFA are favorable for all organisms, however the optimum dietary ratio of n3:n6 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 to survive the winter months under the ice. Rather, a selective accumulation of FAs 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 winter life history strategy of *Daphnia* and the quantity of their lipids and FA composition. *Daphnia* are well adapted to unstable habitats due to short life cycle, and have a wide phenotypic plasticity [6, 26]. These characteristics make *Daphnia* an ideal genus to compare the physiology and overwintering strategies among different populations in different parts of their environmental range. Our primary goal of 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 compositions 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 discussions 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 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 dataset 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 to three categories based on the occurrence of *Daphnia* in winter. The lakes Malla South and Saanajärvi with no record of ephippia-carrying *Daphnia* in earlier zooplankton samples or paleolimnological studies (e.g. [27]) were considered lakes with actively overwintering *Daphnia*. The lakes where all *Daphnia* entered diapause as ephippia due to freezing solid (Big Rock) or winter anoxia (Mekkojärvi) were called diapause lakes. The lakes Lunzersee and Simoncouche, 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 three months, in boreal Simoncouche (2.46±5.5 ind L^{-1}, n=12, December-April) with six 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 nine months of ice. The winter abundance was up to two fold lower than in summer, however, parthenogenetically reproducing individuals were found in both lakes in winter.

From these six sites we compared the percent body fat and FA composition of adult *Daphnia* at the onset of winter. Considering the wide latitudinal variation in our study sites this period was defined as maximum the two months before ice cover for each water body (hereafter referred to as fall). Temperature in 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 site, 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 triplicates were made. 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

Fatty acids 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 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. Fatty acids (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 (TRACE GC-FID THERMO™). 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}). Fatty acid 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 DELTAplus 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 results into general lipid classes of saturated- (SFA), monounsaturated- (MUFA), polyunsaturated- (PUFA) fatty acids or as ratios of the sum of omega- 3 to omega-6 FAME (n3:n6).

Statistical analysis

Differences in seston and *Daphnia* fatty acid composition (data normalized, Euclidean distances), and *Daphnia* fall FAME characteristics (ΣFAME, %PUFA, %SFA, %SDA, SFA:PUFA and Σn3:n6) and body fat levels among strategies (active, diapause, both strategies) and lakes (Malla South, Saanajärvi, Simoncouche, Lunzersee, Big Rock, Mekkojärvi) were tested with a 2 factor (lake nested in strategy, strategy fixed and lake random) Permutational (Multivariate for fatty acid compositions) Analysis of Variance (PERMANOVA with 999 permutations). While comparing FAME characteristic values can be done with classical ANOVA, permutational ANOVA 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 restricted number of permutations were possible, Monte Carlo *p*-values were used. Non-metric multi-dimensional 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, Σn3:n6) to test for potential diet influence on *Daphnia* FAME composition. Pair-wise comparison tests among strategies were carried out for *Daphnia* FAME composition, total FAME, PUFA, SDA and n3:n6 in PERMANOVA with adjusted significance level method from Holm [30]. All analyses were conducted using PRIMER+PERMANOVA version 7.0.9. A significance level $\alpha = 0.05$ was used for all statistical tests.

Results

The *Daphnia* total lipid amount and FA profiles were different for *Daphnia* with different strategies (Table 2, Table 3, Fig. 1). In the lakes with actively overwintering *Daphnia*, the percent 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 (Table 2b, Table 3b, Fig. 2). The *Daphnia* FA compositions among the strategies were marginally different ($p = 0.068$) from each other (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 among differently overwintering *Daphnia* FA compositions was largely due to differences in their total PUFA, stearidonic acid (18:4n3; SDA) and the n3:n6 ratio (Fig. 2). The *Daphnia* PUFA were marginally different between strategies ($p = 0.061$; Table 3d) with higher values in actively overwintering *Daphnia* (Table 2c). SDA was significantly different among strategies (Table 3f, Fig. 2) and more than double in active winter *Daphnia* (26% of total FAME) compared to *Daphnia* with mixed strategies (12%) and 9-fold higher compared to the diapausing *Daphnia* (4%) (Table 2c). The SDA results were further reflected in the n3:n6 differences among strategies with significantly higher n3:n6 ratios for the active strategy (Table 2d, Table 3h). In summary, the main lipid parameters that were most different between strategies were total FAME concentration, SDA, PUFA, and n3:n6 FA ratio, as illustrated in Figure 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 to 89% were SFA. Of the PUFA, seston generally contained very small amounts of 18:3n6 (GLA), 20:5n3 (EPA), 22:6n3 (DHA), and notably 18:4n3 (SDA) was less than 2%. Seston FA composition was not different among strategies (Table 3i). Regression analyses revealed that variation in seston FA composition (ΣFAME, %PUFA, %SDA, Σn3:n6) 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 FAs 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 fatty acid concentrations in the zooplankton appeared more closely related to zooplankton overwintering strategy than seston fatty acid 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 fatty acids 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 % body fat and FAME concentration, than the other two strategies. Specifically PUFA, SDA (18:4n3), and high ratios of n3:n6 were preferentially retained in active overwintering *Daphnia* than in *Daphnia* using the other two strategies. While cladocerans should have fairly constant n3:n6 FA ratios, with an average of 3 [19, 31, 32], our results show that there were contrasting n3:n6 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 n3:n6 average value of three. High proportions of SDA mostly drive the n3:n6 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 indicated a preferential retention or accumulation of specific FA by *Daphnia*, rather than a direct reflection of 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 for the region's or ice cover's role on the strategy, our data shows 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 it is known that different species differ in their fatty acid 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 fatty acid 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 open water period is warm and long enough to allow for the emergence from ephippium and completing a life cycle before environmental conditions turn adverse again. Subsequently, species not capable for lipid accumulation should be absent from perennially cold arctic lakes, which could contribute to explaining the low species number of zooplankton in arctic lakes [40]. However, there exists evidence that lipid allocation is not only a species-specific trait but 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 less lipid droplets from individuals that accumulated large amounts of lipids to survive longer. Nevertheless, regardless of whether overwintering actively 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 which overwinter actively accumulate lipids for the subsequent use during the months when diet is scarce [8].

As observed also 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 separating between individuals that will overwinter actively versus those that will make ephippia. Even at a population level, it is difficult to discern the 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 water column under the ice and the high number of ephippia in fall. The more similar fatty acid composition between diapausing and mixed strategies in fall further suggests that most *Daphnia* in the mixed strategy lakes entered diapause, possibly because they did not possess physiological means to accumulate storage fats. FA composition between active, diapausing, and both strategies from different lakes and different species, in this study suggests that lipid accumulation is an important factor for *Daphnia* winter strategies, however, further experimental approach and tests would need to be used to explicitly determine this.

The ecology of the subarctic and arctic region may provide a framework to understand 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 n3 found in the active *Daphnia* can help to combat the low spring temperatures [43]. Active winter *Daphnia* and their parthenogenetic young are able to exploit the early spring phytoplankton, and are typically larger in body size [44] giving 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 are, however, 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 add to the ephippia bank for insurance [1]. We found the two winter life histories across the regions studied although a larger dataset 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 to 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 to 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* remaining 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 zooplankton use 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 change the phytoplankton community and production, and could lead to changes of storage fat accumulation for zooplankton.

Acknowledgments

We are grateful to Kilpisjärvi and Simoncouche biological stations for logistical support. We thank Martin Kainz and Jorge Watzke at the Wasserkluster Lunz for technical support during fatty acid analysis and two anonymous reviewers for their constructive comments that improved the paper. Core funding was provided by Academy of Finland with grants 19205 and 140775 to MR.

References

1. Thackeray SJ, Henrys P a., Jones ID, Feuchtmayr H (2012) Eight decades of phenological change for a freshwater cladoceran: what are the consequences of our definition of seasonal timing? Freshw Biol 57:345–359. doi: 10.1111/j.1365-2427.2011.02614.x

2. Chen C, Folt C. (1996) Consequences of fall warming for zooplankton overwintering success. Limnol Oceanogr 41:1077–1086.

3. Hagen W, Auel H (2001) Seasonal adaptations and the role of lipids in oceanic zooplankton. Zoology (Jena) 104:313–26. doi: 10.1078/0944-2006-00037

4. Lampert W, Lampert KP, Larsson P (2010) Coexisting overwintering strategies in *Daphnia pulex*: A test of genetic differences and growth responses. Limnol Oceanogr 55:1893–1900. doi: 10.4319/lo.2010.55.5.1893

5. Larsson P, Wathne I (2006) Swim or rest during the winter – what is best for an alpine daphnid? Arch für Hydrobiol 167:265–280. doi: 10.1127/0003-9136/2006/0167-0265

6. Slusarczyk M (2009) Extended lifespan traded for diapause in *Daphnia*. Freshw Biol 54:2252–2262. doi: 10.1111/j.1365-2427.2009.02256.x

7. Rautio M, Mariash H, Forsström L (2011) Seasonal shifts between autochthonous and allochthonous carbon contributions to zooplankton diets in a subarctic lake. Limnol Oceanogr 56:1513–1524. doi: 10.4319/lo.2011.56.4.1513

8. Schneider T, Grosbois G, Vincent WF, Rautio M (2016) Carotenoid accumulation in copepods is related to lipid metabolism and reproduction rather than to UV-protection. Limnol Ocean. doi: 10.1002/lno.10283

9. Carvalho GR, Hughes RN, Animal S (1983) The effect of food availability , female culture-density and photoperiod on ephippia production in *Daphnia magna* Straus (Crustacea: Cladocera). Freshw Biol 13:37–46. doi: 10.1111/j.1365-2427.1983.tb00655.x

10. Griffiths D, Kirkwood RC (1995) Seasonal variation in growth , mortality and fat stores of roach and perch in Lough Neagh , Northern Ireland. J Fish Biol 47:537–554.

11. Hagen W, Van Vleet E, Kattner G (1996) Seasonal lipid storage as overwintering strategy of Antarctic krill. Mar Ecol Prog Ser 134:85–89. doi: 10.3354/meps134085

12. Tessier AJ, Goulden CE (1982) Estimating Food Limitation in Cladoceran Populations. Limnol Oceanogr 27:707–717.

13. Reznick DN, Braun B (1987) Fat cycling in the mosquitofish (*Gambusia affinis*): fat storage as a reproductive adaptation. Oecologia 73:401–413. doi: 10.1007/BF00385257

14. Lee RF, Hagen W, Kattner G (2006) Lipid storage in marine zooplankton. Mar Ecol Prog Ser 307:273–306.

15. van der Meeren T, Olsen RE, Hamre K, Fyhn HJ (2008) Biochemical composition of copepods for evaluation of feed quality in production of juvenile marine fish. Aquaculture 274:375–397. doi: 10.1016/j.aquaculture.2007.11.041

16. Hiltunen M, Strandberg U, Keinänen M, et al (2014) Distinctive Lipid Composition of the Copepod Limnocalanus macrurus with a High Abundance of Polyunsaturated Fatty Acids. Lipids 919–932. doi: 10.1007/s11745-014-3933-4

17. Kattner G, Hagen W, Lee RF, et al (2007) Perspectives on marine zooplankton lipids. Can J Fish Aquat Sci 64:1628–1639. doi: 10.1139/F07-122

18. Guschina IA, Harwood JL (2009) Algal lipids and effect of the environment on their biochemistry. In: Arts MT, Brett MT, Kainz MJ (eds) Lipids Aquat. Ecosyst. Springer, New York, pp 1–24

19. Persson J, Vrede T (2006) Polyunsaturated fatty acids in zooplankton: variation due to taxonomy and trophic position. Freshw Biol 51:887–900. doi: 10.1111/j.1365-2427.2006.01540.x

20. Müller-Navarra DC, Brett MT, Liston a M, Goldman CR (2000) A highly unsaturated fatty acid predicts carbon transfer between primary producers and consumers. Nature 403:74–7. doi: 10.1038/47469

21. Yang XW, Dick TA (1994) Arctic Char (*Salvelinus Alpinus*) and Rainbow Trout (*Oncorhynchus Mykiss*) Differ in Their Growth and Lipid Metabolism in Response to Dietary Polyunsaturated Fatty Acids. Can J Fish Aquat Sci 51:1391–1400.

22. Burns CW, Brett MT, Schallenberg M (2011) A comparison of the trophic transfer of fatty acids in freshwater plankton by cladocerans and calanoid copepods. Freshw Biol 56:889–903. doi: 10.1111/j.1365-2427.2010.02534.x

23. Taipale SJ, Kainz MJ, Brett MT (2011) Diet-switching experiments show rapid accumulation and preferential retention of highly unsaturated fatty acids in Daphnia. Oikos 120:1674–1682. doi: 10.1111/j.1600-0706.2011.19415.x

24. Eloranta AP, Mariash HL, Rautio M, Power M (2013) Lipid-rich zooplankton subsidise the winter diet of benthivorous Arctic charr (*Salvelinus alpinus*) in a subarctic lake. Freshw Biol 58:2541–2554. doi: 10.1111/fwb.12231

25. Hampton SE, Moore M V, Ozersky T, et al (2015) Heating up a cold subject : prospects for under-ice plankton research in lakes. J Plankton Res 0:1–8. doi: 10.1093/plankt/fbv002

26. Dufresne F, Hebert PDN (1995) Polyploidy and clonal diversity in an arctic cladoceran. Heredity (Edinb) 75:45–53.

27. Heissernberger M, Watzke J, Kainz MJ (2010) Effect of nutrition on fatty acid profiles of riverine, lacustine, and aquaculture-raised salmonids of pre-alpine habitats. Hydrobiology 650:234–254.

28. Rautio M, Sorvari S, Korhola A (2000) Diatom and crustacean zooplankton communities , their seasonal variability and representation in the sediments of subarctic Lake Saanajärvi. Methods 59:81–96.

29. Fairclough D V., Clarke KR, Valesini FJ, Potter IC (2008) Habitat partitioning by five congeneric and abundant Choerodon species (Labridae) in a large subtropical marine embayment. Estuar Coast Shelf Sci 77:446–456. doi: 10.1016/j.ecss.2007.10.004

30. Holm S (1979) A Simple Sequentially Rejective Multiple Test Procedure. Scand J Stat 6:65–70.

31. Ahlgren G, Vrede T, Geodkoop W (2009) Fatty acid ratios in freshwaer fish, zooplanknton and zoobenthos. In: Arts MT, Brett MT, Kainz MJ (eds) Lipids Aquat. Ecosyst. Springer, New York, pp 147–178

32. Hessen DO, Leu E (2006) Trophic transfer and trophic modification of fatty acids in high Arctic lakes. Freshw Biol 51:1987–1998. doi: 10.1111/j.1365-2427.2006.01619.x

33. Brett MT, Mu C, Ballantyne AP, et al (2006) Daphnia fatty acid composition reflects that of their diet. Limnol Oceanogr 51:2428–2437.

34. Galloway AWE, Taipale SJ, Hiltunen M, et al (2014) Diet-specific biomarkers show that high-quality phytoplankton fuels herbivorous zooplankton in large boreal lakes. Freshw Biol 59:1902–1915. doi: 10.1111/fwb.12394

35. Sarma SSS, Nandini S, Gulati RD (2005) Life history strategies of cladocerans: Comparisons of tropical and temperate taxa. Hydrobiologia 542:315–333. doi: 10.1007/s10750-004-3247-2

36. Cáceres CE, Tessier AJ (2004) To sink or swim: Variable diapause strategies among *Daphnia* species. Limnol Oceanogr 49:1333–1340. doi: 10.4319/lo.2004.49.4_part_2.1333

37. de Senerpont Domis LN, Mooij WM, Hülsmann S, et al (2007) Can overwintering versus diapausing strategy in *Daphnia* determine match-mismatch events in zooplankton-algae interactions? Oecologia 150:682–98. doi: 10.1007/s00442-006-0549-2

38. Demott W, Müller-Navarra D (1997) The importance of highly unsaturated fatty acids in zooplankton nutrition: evidence from experiments with *Daphnia* , a cyanobacterium and lipid emulsions. Freshw Biol 38:649–664. doi: 10.1046/j.1365-2427.1997.00222.x

39. Elert E Von (2004) Food Quality Constraints in Daphnia: Interspecific Differences in the Response to the Absence of a Long Chain Polyunsaturated Fatty Acid in the Food Source. Hydrobiologia 526:187–196. doi: 10.1023/B:HYDR.0000041604.01529.00

40. Patalas K (1990) Diversity of zooplankton communities in Canadian lakes as a function of climate. Verhanlungen des Int verein für Limnol 24:360–368.

41. Forsström L, Sorvari S, Rautio M, et al (2007) Changes in Physical and Chemical Limnology and Plankton during the Spring Melt Period in a Subarctic Lake. Int Rev Hydrobiol 92:301–325. doi: 10.1002/iroh.200610928

42. Roiha T, Laurion I, Rautio M (2015) Carbon dynamics in highly heterotrophic subarctic thaw ponds. Biogeosciences 12:7223–7237. doi: 10.5194/bg-12-7223-2015

43. Farkas T, Herodek S (1964) The effect of environmental temperature on the fatty acid composition of crustacean plankton. J Lipid Res 5:369–373.

44. Dufresne F, Hebert PDN (1998) Temperature-related differences in life-history characteristics between diploid and polyploid clones of the *Daphnia pulex* complex 1. Ecoscience 5:433–437.

45. Gliwicz M, Slusarczyk A, Slusarczyk M (2001) Life history synchronization in a

long-lifespan single-cohort *Daphnia* population in a fishless alpine lake. Oecologia 128:368–378. doi: 10.1007/s004420100673

46. Tessier AJ, Henry L, Goulden CE, Durand MW (1983) Starvation in *Daphnia*: Energy Reserves and Reproductive Allocation. Limnol Oceanogr 28:667–676.

47. Hartwich M, Martin-Creuzburg D, Wacker A (2013) Seasonal changes in the accumulation of polyunsaturated fatty acids in zooplankton. J Plankton Res 35:121–134. doi: 10.1093/plankt/fbs078

48. Lampert W, Fleckner W, Rai H, Taylor BE (1986) A study zooplankton : on the Phytoplankton control by grazing spring phase '. Limnol Oceanogr 31:478–490.

49. Dupuis AP, Hann BJ (2009) Climate change, diapause termination and zooplankton population dynamics: an experimental and modelling approach. Freshw Biol 54:221–235. doi: 10.1111/j.1365-2427.2008.02103.x

Tables and Figures

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,* *D. dubia,* *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*

Table 2. Average (±SD) values of *Daphnia* a) body fat (% of dry weight), b) total FAME concentration (μgFA mgC^{-1}), c) fatty acid composition (mole % of total fatty lipids) for saturated (ΣSFA), monounsaturated (ΣMUFA), and polyunsaturated (ΣPUFA) fatty acids, d) ratios of ΣSFA:PUFA and Σn3:n6 per overwintering strategy. n = number of replicates, nd = no data. Bold values are sums of the individual FAME.

	Active	Both Strategies	Diapause
	n=7	n=9	n=7
a) Body fat	58.6 ±11.0	30.9 ±3.3*	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**
16:1n7c	5.9 ±0.1	8.9 ±0.4	25.3 ±19.8
18:1n9	5.6 ±0.4	8.5 ±0.9	8.2 ±4.8
16:1n9	1.4 ±0.1	4.0 ±3.6	0.9 ±4.7
18:1n7	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**
18:2n6	3.5 ±0.3	4.5 ±0.3	5.0 ±2.3
18:3n3	5.2±0.2	6.9 ±2.6	6.8 ±4.8
18:4n3	25.7 ±0.4	11.6 ±1.6	3.5 ±1.1
18:3n6	1.3 ±0.1	0.8 ±0.2	0.6 ±0
20:4n3	9.0 ±1.8	0.6 ±0.1	0.6 ±0.1
20:4n6	1.6 ±0.2	3.8 ±0.1	4.7 ±1.1
20:5n3	10.6 ±0.5	12.8 ±3.6	9.0 ±4.4
22:6n3	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**
Σn3:n6	**7.9 ±0.2**	**3.6 ±0.8**	**2.0 ±0.1**

*this value is only from Lake Simoncouche, Lunzersee did not have a sample for fall body fat.

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) n3:n6, and i) seston FA composition between the overwintering strategies (active, diapause, both) at the onset of winter. Except for i), all *p*-values were obtained with Monte-Carlo routine (see methods).

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	7.08×10^5	17.4	0.023
Lake(strategy)	3	40852	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) n3: n6				
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			

Figure 1. Nonmetric 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 fall from the six lakes (MS = Malla South, SA = Saanajärvi, SI = Simoncouche, LU = Lunzersee, BR = Big Rock, ME = Mekkojärvi).

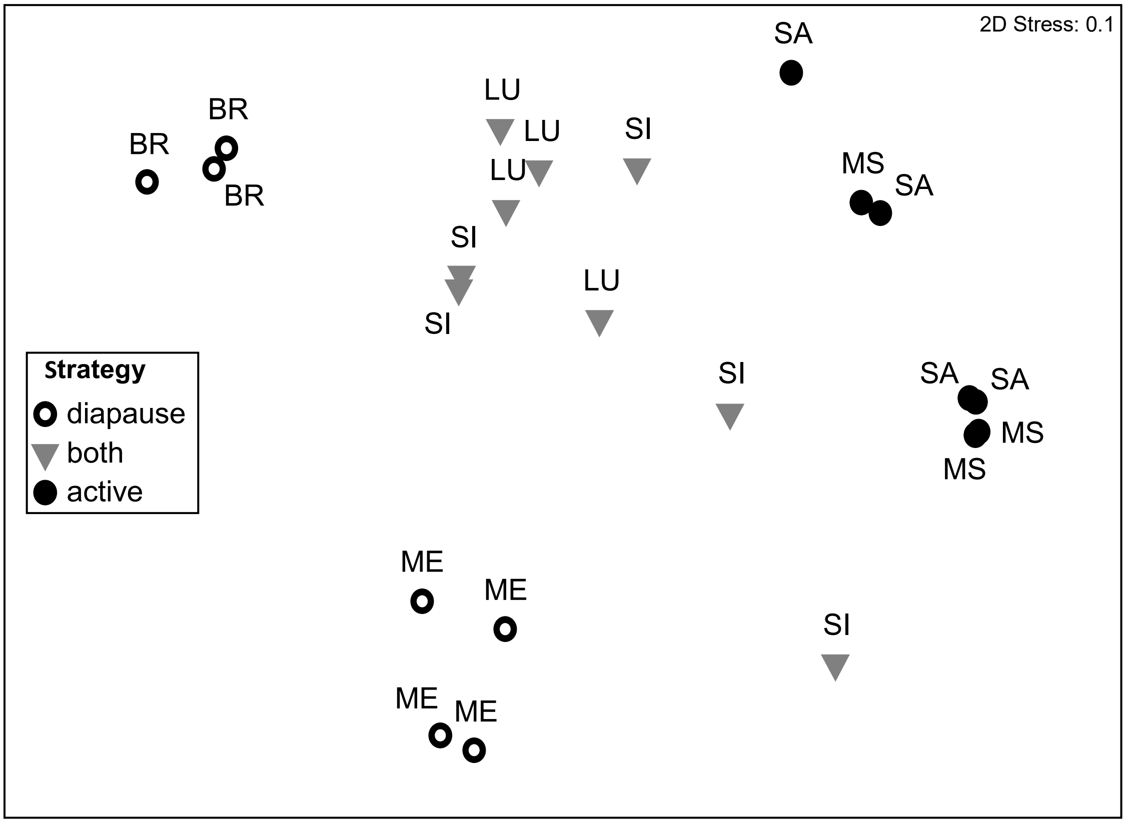
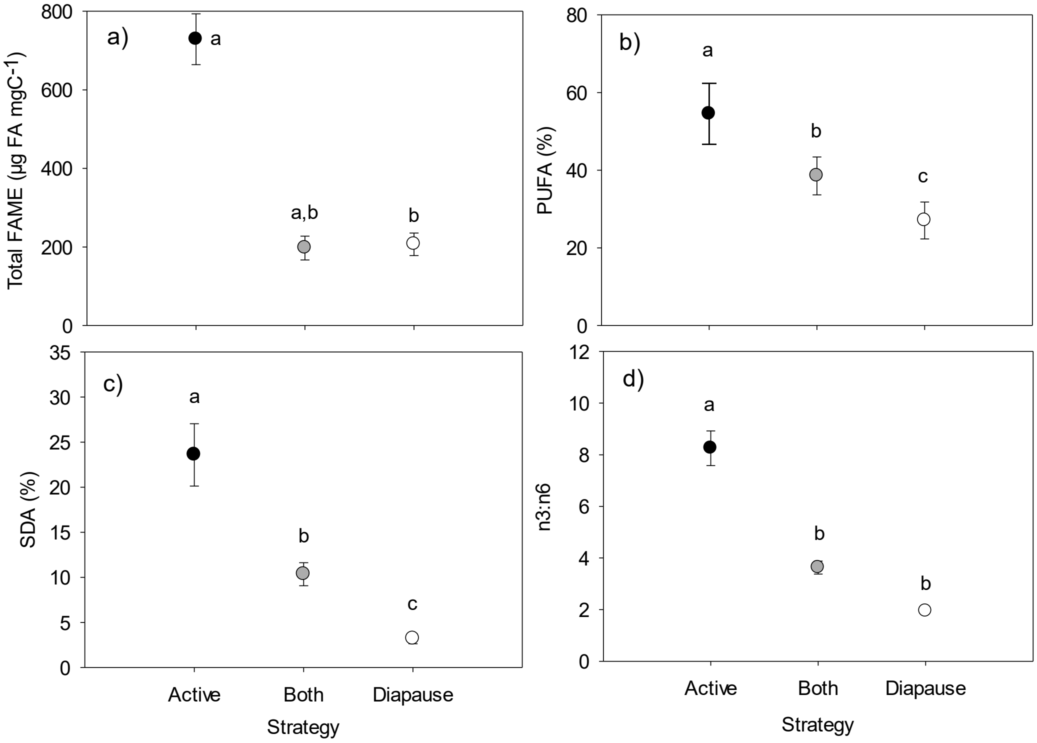


Figure 2. *Daphnia* a) total FAME concentration (µg FA mgC^{-1}), b) percentage of PUFA of total FA, c) percentage of SDA (18:4n3) of total FA and d) n3:n6 ratio in communities showing active, diapause or both overwintering strategies at the onset of winter. Error bars are SE. Different letters indicate statistically different values.



Supplementary Information

SI Table 1. Seston a) total FAME concentration (µgFA mgC^{-1}), b) fatty acid composition (mole % of total fatty lipids) for saturated (ΣSFA), monounsaturated (ΣMUFA), and polyunsaturated (ΣPUFA) fatty acids, and c) ratios of ΣSFA:PUFA and Σn3:n6 during the fall. All values are mean ±SD, no value is shown when SD <0.1. n = number of replicates, nd = no data.

	Active		**Both strategies**		**Diapause**	
	Malla South	Saana-järvi	Simon-couche	Lunzersee	Big Rock	Mekkojärvi
	n=3	n=3	n=1	n=4	n=1	n=3
a) **ΣFAME**	**28.9 ±9,5**	**33.8 ±4,8**	**19.1**	**65.3 ±29**	**80.2**	**42.2 ±19**
b) **ΣSFA**	**67,2 ±0.3**	**48.4**	**43.8**	**41.3 ±0.05**	**47.9**	**88.6 ±0.1**
14:0	41±0.4	6.7	8.2	9.7	3.4	80.0
15:0	1,6	4.0	2.2	2.1	11.1	0.0
16:0	21.8 ±0.2	33.9	29.4	23.1	22.5	6.8
18:0	2.6	2.2	4.0	6.5	10.9	1.8
ΣMUFA	**24.5 ±0.2**	**44.0**	**31.9**	**27.1**	**39.2**	**7.5**
16:1n7	13.6	29.4	15.0	19.1	18.5	4.2
18:1n9	8,3	10.9	11.5	4.1	15.7	3.3
16:1n9	2.7	3.7	5.1	3.6	5.1	0.0
18:1n7	0.0	0.0	0.4	0.2	0.0	0.0
ΣPUFA	**8.3**	**7.6**	**24.3**	**31.5**	**12.9**	**3.9**
18:2n6	1.0	2.9	4.2	6.9	2.9	0.0
18:3n3	2.9	0.0	8.2	9.3	1.0	0.0
18:4n3	0.0	0.0	1.4	0.7	1.0	0.0
18:3n6	1.6	0.0	0.0	0.2	0.0	0.0
20:4n3	0.0	2.1	4.9	5.8	1.9	2.3
20:4n6	0.0	0.4	2.8	4.3	0.0	0.0
20:5n3	2.9	2.1	2.8	2.3	1.5	1.5
22:6n3	1.5	nd	nd	1.9	nd	0.0
c) **ΣSFA:PUFA**	**8.1 ±1.2**	**6.4 ±0.4**	**1.8**	**1.3 ±0.3**	**3.7**	**22.8±9.2**
Σn3:n6	**7.1 ±0.2**	**1.3 ±0.1**	**2.5**	**1.7**	**3.3**	**0.0**

SI Table 2. Site-specific average (±SD) values of *Daphnia* a) body fat (% of dry weight), b) total FAME concentration (μgFA mgC^{-1}), c) fatty acid composition (mole % of total fatty lipids) for saturated (ΣSFA), monounsaturated (ΣMUFA), and polyunsaturated (ΣPUFA) fatty acids, d) ratios of ΣSFA:PUFA and Σn3:n6 during the fall when *Daphnia* are either preparing to enter diapause or remain active during winter ice cover. Error values not shown when SD <0.1. n = number of replicates, nd = no data.

	Active		**Both strategies**		**Diapause**	
	Malla South	Saanajärvi	Simoncouche	Lunzersee	Big Rock	Mekkojärvi
	D. umbra	*D. umbra*	*D. galeata,* *D. dubia,* *D. longiremis*	*D. longispina*	*D. longispina*	*D. longispina*
	n=3	n=4	n=5	n=4	n=3	n=4
a) Body fat	59.5 ±9.2	57.7 ±12.8	30.9 ±3.3	nd	23.9 ±5.3	41.2 ±11.1
b) **ΣFAME**	**838.9±83.7**	**646.3±181**	**258.4 ±65.6**	**121±49**	**270.2±43**	**159.2 ±58**
c) **ΣSFA**	**26.9**	**27.0**	**36.4 ± 0.1**	**27.8**	**30.4**	**22.6**
14:0	9.9	9.5 ±0.1	16.7 ± 0.1	6.5	2.1	5.3
15:0	1.0	0.8	0.7	0.9	1.0	1.1
16:0	10.5	10.9	15.2	15.9	19.1	10.7
17:0	0.2	0.2	0.6	0.9	0.8	0.7
18:0	5.3	5.8	3.2	3.5	7.4	4.7
ΣMUFA	**15.0**	**15.8**	**28.0**	**24.8**	**31.5**	**54.5**
16:1n7	5.9	5.8	9.2	8.6	11.4	39.3
18:1n9	5.3	5.9	9.1	8.0	11.5	4.8
16:1n9	1.3	1.4	6.5 ±0.1	1.4	0.7	1.1
18:1n7	2.5	2.7	3.2	6.8	7.9	9.4
ΣPUFA	**58.1**	**57.0**	**35.6 ±0.1**	**47.4**	**38.1**	**22.9**
18:2n6	3.3	3.7	4.2	4.7	6.6	3.3
18:3n3	5.0	5.3	5.0	8.7	10.2	3.4
18:4n3	26.0	25.4	10.4	12.7	2.7	4.3
18:3n6	1.2	1.3	0.9	0.6	0.6	0.6
20:4n3	10.2 ±0.1	7.8 ±0.1	0.5	0.7	0.5	0.7
20:4n6	1.5	1.7	3.7	3.9	5.4	4.0
20:5n3	10.3	10.9	10.2	15.3	11.9	6.1
22:6n3	0.6	0.9	0.7	0.9	0.2	0.5
d) **ΣSFA:PUFA**	**0.5**	**0.5 ±0.1**	**1.0 ±0.5**	**0.6 ±0.1**	**0.8**	**1.0 ±0.1**
Σn3:n6	**8.7 ±1.5**	**7.5 ±2.1**	**3.0 ±0.6**	**4.2 ±0.4**	**2.0 ± 0.1**	**1.9 ±0.2**