Dietary polyunsaturated fatty acid supply improves *Daphnia* performance during vertical migration

Jana Isanta Navarro^{1*}, Matthias Fromherz¹, Michelle Dietz¹, Bettina Zeis², Anke Schwarzenberger¹, and Dominik Martin-Creuzburg¹

¹Limnological Institute, University of Konstanz, Konstanz, Germany

²Institute of Zoophysiology, University of Muenster, Muenster, Germany

*Correspondence:

Jana Isanta Navarro

jana.isanta-navarro@uni-konstanz.de

Email addresses of co-authors:

matthias.fromherz@uni-konstanz.de

michelle.dietz@uni-konstanz.de

zeis@uni-muenster.de

anke.Schwarzenberger@uni-konstanz.de

dominik.martin-creuzburg@uni-konstanz.de

Abstract

1. Diel vertical migration (DVM) is a common predator avoidance strategy of zooplankton. Migration to deeper water layers during the day to escape visually hunting predators most likely requires physiological adaptations to periodically changing temperatures. Polyunsaturated fatty acids (PUFA) are essential nutrients that play crucial roles in membrane temperature acclimation. Exposure to cold temperatures typically results in an increase in the relative abundance of PUFA in cell membranes and PUFA requirements of *Daphnia* have been shown to increase with decreasing temperatures.
2. To assess the significance of dietary PUFA for coping with temperature fluctuations experienced during DVM, we reared *Daphnia magna* at either constantly warm or fluctuating temperatures simulating DVM both with and without dietary PUFA supplementation.
3. We show that the well-known positive effect of dietary eicosapentaenoic acid (EPA) supplementation on offspring production and population growth rates of *Daphnia* is more pronounced at alternating temperatures than at constantly warm temperatures. Exposure to alternating temperatures caused modification in body PUFA concentrations and, consequently, increased lipid peroxidation. However, detrimental effects of lipid peroxidation were not evident.
4. Our data demonstrate that the capacity to cope with the distinct temperature fluctuations experienced during DVM increases with dietary EPA supplementation, suggesting that an adequate dietary PUFA supply is crucial especially for migrating *Daphnia* populations. A dietary deficiency in long-chain PUFA may thus severely constrain predator evasion, potentially resulting in increased mortality and cascading effects on lower trophic levels.

Key Words – Diurnal vertical migration, eicosapentaenoic acid, food quality, lipid peroxidation, predator avoidance

Introduction

Diel vertical migration (DVM) has been recognized as a very efficient predator avoidance strategy of aquatic organisms, which is induced by chemical cues released from visually hunting predators and triggered by light availability (Siebeck 1960; Hays, 2003; Lampert, 2011). Species of the genus *Daphnia* are important model organisms for studying DVM (Rinke & Petzoldt, 2008; Lampert, 2011). Many *Daphnia* population are migrating to deeper water layers during the day to escape fish predation and thus are confronted with diurnal changes in temperature and food supply (Loose & Dawidowicz, 1994; Lampert, McCauley, & Manly, 2003). The amplitude of DVM can vary from a few up to 40 and more meters, depending on a variety of different factors, such as lake depth, light conditions and predation risk (Stich & Lampert, 1981; Dodson, 1990; von Elert & Pohnert 2000; Lampert, 2011). During DVM, *Daphnia* populations can experience temperature changes exceeding 15 °C (Stich & Lampert, 1981). Metabolic disadvantages associated with the exposure to cold temperatures have been identified as the primary cost of DVM, resulting in reduced growth and reproduction (Stich & Lampert, 1984; Lampert, 2011).

Exposure to cold temperature requires physiological adaptations, which have been poorly studied in regard to DVM. Restructuring the lipid composition of cell membranes is one important mechanism by which ectotherms adapt to changing temperatures. The homeoviscous adaptation hypothesis predicts that the proportion of unsaturated fatty acids in cell membranes increases with decreasing temperature to maintain vital membrane properties (Hazel & Williams, 1990; Hazel, 1995). Polyunsaturated fatty acids (PUFA) are essential nutrients for most animals; they are indispensable as structural components of cell membranes and as precursors for eicosanoids, signaling molecules acting primarily on reproduction and immunity (Stanley, 2000; Leonard, Pereira, Sprecher *et al.*, 2004; Valentine & Valentine, 2004). The dietary supply with long-chain (>C18) PUFA, such as eicosapentaenoic acid (EPA), strongly affects growth and reproduction of *Daphnia* (von Elert, 2002; Becker &

Boersma, 2003; Martin-Creuzburg, Wacker, & Basen, 2010). Dietary PUFA requirements of *Daphnia* have been shown to increase with decreasing temperature, suggesting that the dietary PUFA supply is crucial for coping with low temperatures (Sperfeld & Wacker, 2011; Martin-Creuzburg, Wacker, Ziese *et al.*, 2012). Behavioral experiments suggest that *Daphnia* with access to dietary EPA are migrating to deeper water layers, presumably because of a higher PUFA-mediated capacity to cope with colder temperatures (Brzezinski & von Elert, 2015). The influence of fluctuating temperatures on PUFA requirements has not been studied yet. Despite the significance of PUFA for physiological processes, PUFA accumulation may also come along with costs because PUFA are particularly sensitive to lipid peroxidation, resulting in radical formation and oxidative stress (Yin, Xu, & Porter, 2011). Effects of temperature on lipid peroxidation and oxidative stress are controversial but most studies on ectotherms have found increasing oxidative stress with increasing temperature (Lushchak, 2011). To assess the significance of dietary PUFA for coping with temperature fluctuations experienced during DVM, we reared *Daphnia magna* at either constantly warm or fluctuating temperatures simulating DVM both with and without dietary EPA supply, measured various life history traits, and analyzed the fatty acid composition of the animals. The thiobarbituric acid (TBA) assay was used to assess the effects of temperature and dietary PUFA supply on lipid peroxidation. We used *D. magna* as a model because this species is known to perform pronounced DVM if conditions are appropriate (Lampert, 2011) and is extensively used to study essential lipid requirements.

Methods

Cultivation of organisms

The experiment was performed with a *Daphnia magna* clone originally isolated from Grosser Binnensee, Germany (Lampert, 1991), which has been used previously to study DVM under laboratory conditions (von Elert & Pohnert, 2000; Brzezinski & von Elert, 2015). *Daphnia*

were pre-raised at 20 °C in 1-L jars (10 individuals per jar) containing filtered lake water (<0.2 µm) and 2 mg C L⁻¹ of the green alga *Acutodesmus obliquus* (formerly referred to as *Scenedesmus obliquus*; SAG 276-3a). *A. obliquus* was used as food also in the growth experiment because it lacks long-chain PUFA (i.e. >C18) and thus is of moderate food quality for *Daphnia* (von Elert, 2002, Martin-Creuzburg *et al.*, 2012). *A. obliquus* was grown at 20 °C in 5-L batch cultures in Cyano medium (Jüttner, Leonhardt, & Möhren, 1983) and harvested in the late-exponential growth phase (illumination at 100 µmol quanta m⁻² s⁻¹). Food suspensions were prepared by centrifugation and resuspension in fresh medium. Carbon concentrations of the food suspensions were estimated from photometric light extinctions using previously established carbon-extinction regressions.

114

115 **Growth experiment**

The experiment was conducted with third-clutch offspring born within 12 h. Neonates were placed individually in jars containing 80 ml of filtered (<0.2 µm) lake water and exposed to two different temperature regimes and two different food qualities in a full-factorial design. A subsample of 30 animals was taken from the isolated cohort of neonates for initial dry mass determination after freeze-drying. The jars were placed in a flow-through system in which they were constantly surrounded by temperature-controlled water of either constantly 20 °C or alternating 20 °C and 6 °C. The alternating temperature regime was set up to simulate the temperature changes that *Daphnia* potentially experience during DVM in deep, temperate lakes, i.e. eight hours at 20 °C, two hours for cooling down to 6 °C, 12 hours at 6 °C, and two hours for warming up to 20 °C again. All jars were provided with 2 mg C L⁻¹ of *A. obliquus*. Food quality was manipulated by adding EPA-containing liposomes to the jars; liposomes prepared without adding EPA were used as a control supplement. Liposomes were prepared as described previously (Martin-Creuzburg, Sperfeld, & Wacker, 2009). Initially, each treatment consisted of 40 jars each containing one animal. Daphnids were transferred daily

130 into new jars with freshly prepared food suspensions. At day six, 12 daphnids (jars) were
 131 subsampled, rinsed with ultra-pure water, transferred into pre-weighed aluminum boats, and
 132 stored at -80°C until they were freeze-dried and weighed ($\pm 0.1 \mu\text{g}$) for dry mass
 133 determination and subsequent fatty acid and lipid peroxidation analysis. Fatty acids were
 134 analyzed as described in Martin-Creuzburg *et al.* (2010). Mass-specific juvenile somatic
 135 growth rates (g) were determined as the increase in dry mass from the beginning (M_0) until
 136 day six of the experiment (M_t) with time (t) expressed as age in days:

$$g = \frac{\ln(M_t) - \ln(M_0)}{t}$$

137 The remaining daphnids (28 replicates) were kept in their respective treatments until they
 138 released their third-clutch offspring. Population growth rates (r) were estimated iteratively
 139 using the Euler-Lotka equation:

$$1 = \sum_{x=0}^1 l_x m_x e^{-rx}$$

140 where l_x is the age-specific survivorship, m_x is the number of offspring at each reproduction
 141 cycle, and x is the age at reproduction in days (offspring production was recorded once a day,
 142 when the animals were transferred to new jars).

143 The concentration of malonic dialdehyde (MDA), an indicator of lipid peroxidation, was
 144 estimated using the thiobarbituric acid (TBA) assay (Ohkawa, Ohishi, & Yagi, 1979) and the
 145 data were expressed as TBA-reactive substances (TBARS). The concentration of TBARS was
 146 used as an approximation for the amount of damage caused by lipid peroxidation.

147

148 **Data analyses**

149 Somatic growth rates, population growth rates, C18 body PUFA concentrations, and TMOP-
 150 equivalents were analysed using two-factorial ANOVAs followed by Tukey's HSD post hoc

151 tests (STATISTICA, StatSoft, Inc., 2011, Version 10.0.). Population growth rate data were
 152 log-transformed to meet ANOVA assumptions, i.e. homogeneity of variances. Age at first
 153 reproduction and cumulative counts of offspring were analysed using a generalized linear
 154 model (GLM) with log as the link function for quasi-Poisson errors to compensate for
 155 overdispersion (R Development Core Team, 2017). To compare the increase in somatic
 156 growth rates (g ; d^{-1}), offspring production (off ; No.), and population growth rates (r ; d^{-1}) upon
 157 EPA supplementation between the two temperature regimes (Fig. 1, inserted bar graphs), the
 158 data were standardized as improvement (I) upon EPA supplementation relative to the values
 159 obtained on the unsupplemented food using the equation:

$$I = \frac{(g, off, r_{suppl} - g, off, r_{unsuppl})}{g, off, r_{unsuppl}}$$

160

161 **Results**

162 Juvenile somatic growth rates were significantly affected by food and temperature (Fig. 1A,
 163 Table 1), i.e. they increased upon EPA supplementation in both temperature regimes and were
 164 significantly lower in the alternating temperature regime than in the constantly warm
 165 temperature regime. The increase in somatic growth rates upon EPA supplementation (Fig.
 166 1A, inserted bar graph) and the significant interaction between food and temperature reflect
 167 that the positive effect of EPA supplementation was higher in the constantly warm
 168 temperature regime than in the alternating temperature regime.

169 The age at first reproduction was significantly affected by food and temperature (Fig. 1B,
 170 Table 1). Although EPA supplementation did not affect the age at first reproduction in the
 171 constantly warm temperature regime (on average 7.3 days in both food treatments) it resulted
 172 in a significantly younger age at first reproduction in the alternating temperature regime (on
 173 average 18.1 days without EPA, 15.7 days with EPA; Fig. 1B, Table 1).

174 The cumulative numbers of offspring were also significantly affected by food and temperature
 175 (Fig. 1C, Table 1). Without EPA supplementation, offspring numbers were significantly lower
 176 in the alternating temperature regime than in the constantly warm temperature regime. With
 177 EPA supplementation, offspring numbers did not differ significantly between temperature
 178 regimes. The increase in offspring production upon EPA supplementation (Fig. 1C, inserted
 179 bar graph) and the significant interaction between food and temperature reflect that the
 180 positive effect of EPA supplementation on offspring production was higher in the alternating
 181 temperature regime.

182 Population growth rates were also significantly affected by food and temperature (Fig. 1D,
 183 Table 1), i.e. they increased upon EPA supplementation in both temperature regimes and were
 184 significantly lower in the alternating temperature regime than in the constantly warm
 185 temperature regime. The increase in population growth rates upon EPA supplementation (Fig.
 186 1D, inserted bar graph) and the significant interaction between food and temperature indicate
 187 that the positive effect of EPA supplementation was higher in the alternating temperature
 188 regime than in the constantly warm temperature regime. Mortality was negligible in all
 189 treatments.

190 The body fatty acid composition of all animals was characterized by a number of saturated
 191 (mostly C16:0) and monounsaturated (mostly C18:1) fatty acids, which were not significantly
 192 affected by temperature and dietary EPA supply (data not shown). Polyunsaturated fatty acids
 193 (PUFA) were represented mostly by C18:3n-3 and C18:2n-6. PUFA with more than 18
 194 carbon atoms were not detected in any of the animals, except in those that were reared on the
 195 EPA supplemented diet, which contained EPA. C18 PUFA concentrations were significantly
 196 affected by temperature but not by food (Fig. 2A, Table 1). Without EPA supplementation,
 197 C18 PUFA concentrations were significantly higher in animals exposed to alternating
 198 temperatures than in those exposed to the constantly warm temperature. This effect was not
 199 significant with EPA supplementation. EPA body concentrations were significantly higher in

animals exposed to alternating temperatures than in animals exposed to the constantly warm temperature (ANOVA, $F_{1,4} = 20.8$, $p = 0.01$; Fig. 2B). The ratio of saturated to unsaturated fatty acids did not differ significantly among treatments (data not shown).

The concentration of TBARS in whole body extracts was significantly affected by temperature but not by food and did not reveal a significant interaction between temperature and food (Fig. 2C, Table 1). With EPA supplementation, the concentration of TBARS was significantly higher in animals exposed to the alternating temperatures than in those exposed to the constantly warm temperature. This effect was not significant without EPA supplementation.

Discussion

We show here that the ability of *Daphnia* to cope with alternating temperatures is significantly affected by lipid-mediated food quality. While the effect of EPA supplementation on juvenile somatic growth rates was higher in the constantly warm temperature regime, the effect of EPA supplementation on offspring production and population growth rates was higher in the alternating temperature regime. The more pronounced effect of EPA supplementation on juvenile somatic growth rates in the constantly warm temperature regime presumably reflects that the overall growth potential was higher at constantly warm temperatures and thus also the potential of EPA to increase somatic growth rates. Despite a higher growth potential at constantly warm temperatures, the positive effect of EPA supplementation on offspring production was higher at alternating temperatures. Without dietary EPA supply, offspring production was significantly lower in the alternating temperature regime than in the constantly warm temperature regime. With dietary EPA supplementation, however, offspring production was similar in both temperature regimes, i.e. the negative effect of alternating temperatures on offspring production was equalized by dietary EPA supplementation. This supports previous findings showing that the dietary EPA

supply gains in importance at lower temperatures (Sperfeld & Wacker, 2011; Martin-Creuzburg *et al.*, 2012). The more pronounced effect of EPA supplementation on reproduction in the alternating temperature regime resulted in higher population growth rates. These findings suggest that vertically migrating *Daphnia*, which in deep temperate lakes are often confronted with similar temperature changes during vertical migration as applied in our study (Stich & Lampert, 1981; Lampert, 2011), rely more on an adequate dietary PUFA supply than non-migrating *Daphnia*. Experiments on the vertical migration behavior of *Daphnia* demonstrated that animals with access to dietary EPA are migrating to deeper water layers, implying that the migration amplitude and thus the capacity to seek shelter from fish predation is affected by the dietary PUFA supply (Brzezinski & von Elert, 2015). Together with our findings, indicating that the ability of *Daphnia* to cope with alternating temperatures increases with dietary EPA supplementation, this suggests that the vertical migration behavior is strongly affected by PUFA-mediated food quality.

Daphnia are commonly considered as unselective filter-feeders, which do not discriminate between food particles differing in nutritional quality (DeMott, 1986). The predominance of long-chain PUFA-deficient phytoplankton taxa, such as green algae and cyanobacteria, in the food-rich epilimnion of lakes may constrain the performance of both migrating and non-migrating *Daphnia* populations. However, migrating *Daphnia* populations may be even more susceptible to dietary PUFA deficiencies because the shortage in dietary PUFA may exacerbate the trade-off between light-dependent predation losses and reduced growth and reproduction and thus increase the demographic costs associated with DVM. Our data imply that the vertical migration behavior of *Daphnia* is more pronounced under natural conditions when the seston is dominated by long-chain PUFA-rich algae, such as diatoms or cryptophytes. However, more empirical research is required to assess whether the dietary PUFA supply needs to be considered as another proximate cue triggering DVM (Fig. 3). Moreover, further studies are required to assess the physiological significance of dietary

252 PUFA for DVM in a more natural context, considering that migrating *Daphnia* are typically
 253 confronted with rather low food concentrations during the day (Winder, Boersma, & Spaak
 254 2003). Food quality effects often decrease with decreasing food quantity because of changes
 255 in nutrient allocation (Sterner & Schulz 1998; Schällicke, Sobisch, Martin-Creuzburg *et al.*,
 256 2019). At high food quantity, PUFA can be used to modulate membrane properties or to fuel
 257 distinct physiological processes (e.g. the eicosanoid pathway). At low food quantity, however,
 258 PUFA might be also catabolized to gain energy. It remains to be tested whether migrating
 259 *Daphnia* retain higher amounts of EPA and other PUFA than non-migrating *Daphnia* under
 260 natural conditions and whether the positive effect of EPA supplementation observed here is
 261 less pronounced when the animals are temporarily exposed to low food quantity.

262 Alternating temperatures presumably impose additional challenges on *Daphnia* in terms of
 263 physiological temperature acclimation. Restructuring the lipid composition of membranes is a
 264 fundamental mechanism by which ectothermic organisms adapt to changing environmental
 265 temperatures. Exposure to low temperatures typically results in an increase in the relative
 266 abundance of unsaturated fatty acids in cell membranes, suggesting that these temperature-
 267 mediated modifications in membrane fatty acids are crucial to maintain vital membrane
 268 properties (Hazel & Williams, 1990; Hazel, 1995). *Daphnia* have limited capacities to modify
 269 the incorporation of dietary PUFA and their body fatty acid profile largely reflects that of
 270 their food (Brett, Müller-Navarra, Ballantyne *et al.*, 2006). There is evidence, however, that
 271 *Daphnia* accumulate higher amounts of PUFA within their body when exposed to colder
 272 temperatures (Schlechtriem, Arts, & Zellmer, 2006); whether this also involves a higher
 273 degree of fatty acid unsaturation within membrane phospholipids remains unclear. In our
 274 experiment, *Daphnia* exposed to alternating temperatures contained higher amounts of C18
 275 PUFA than animals exposed to constantly warm temperatures. However, this effect was
 276 significant only without EPA supplementation. In animals exposed to the alternating
 277 temperatures, EPA supplementation resulted in significantly lower body C18 PUFA

278 concentrations. Instead, animals exposed to the alternating temperatures contained
279 significantly higher amounts of EPA, suggesting that the supplemented EPA was used as a
280 substitute for C18 PUFA. The concentration of EPA within the body was generally much
281 lower than that of the predominant C18 PUFA, 18:3n-3 and 18:2n-6, which reflects dietary
282 availability. Assuming that EPA was used as a substitute for C18 PUFA implies that EPA is
283 more efficient in modulating membrane-related temperature acclimation than C18 PUFA,
284 which has been suggested previously based on experiments showing that the positive effects
285 of EPA supplementation on a C18 PUFA-rich diet is higher at colder temperatures (Martin-
286 Creuzburg *et al.*, 2012).

287 Higher body PUFA concentrations in animals exposed to the alternating temperatures are in
288 accordance with the homeoviscous adaptation hypothesis, considering that the animals in the
289 alternating temperature regime were on average exposed to colder temperatures. However,
290 exposure to alternating temperatures may also hamper lipid-mediated temperature
291 acclimation. Despite the significance of PUFA for growth and reproduction, high body PUFA
292 concentrations may also become detrimental because PUFA are sensitive to lipid
293 peroxidation, resulting in radical formation and oxidative stress (Esterbauer, Schaur, &
294 Zollner, 1991; Yin *et al.*, 2011). The influence of temperature on oxidative stress is
295 controversial, albeit most studies on ectothermic animals revealed increasing oxidative stress
296 with increasing temperature (Lushchak, 2011). In *Daphnia*, lipid peroxidation has been found
297 to increase in response to heat exposure, but did not appear to be a predictive measure of
298 susceptibility to thermal damage (Coggins, Collins, Holbrook *et al.*, 2017). To the best of our
299 knowledge, lipid peroxidation in response to fluctuating temperatures has not been studied
300 yet. We show here that the concentration of thiobarbituric acid (TBA)-reactive substances
301 (TBARS), as a measure of the major lipid peroxidation end product malonic dialdehyde
302 (MDA), is higher in animals exposed to fluctuating temperatures than in animals exposed to
303 constantly warm temperatures, suggesting that exposure to fluctuating temperatures resulted

in increased lipid peroxidation. Although this effect was observed with and without EPA supplementation, it was significant only for the EPA supplemented diets, suggesting enhanced temperature-mediated lipid peroxidation in the presence of long-chain PUFA. It has been proposed that lipid peroxidation in *Daphnia* may increase in response to heat exposure as a by-product of changes in phospholipid fatty acid unsaturation (Coggins *et al.* 2017). In our study, higher EPA concentrations in *Daphnia* exposed to fluctuating temperatures were accompanied by higher lipid peroxidation, implying a trade-off between long-chain PUFA accumulation and oxidative stress during DVM. The accumulation of long-chain PUFA as a mechanism to cope with low temperatures experienced during the day may thus come at the expense of increased oxidative stress associated with high temperature exposure during the night. Our data suggest, however, that the positive effects of EPA supplementation outweigh potential negative effects associated with lipid peroxidation during DVM.

Conclusions

We show here that the well-known positive effect of dietary EPA supplementation on offspring production and thus population growth rates of *Daphnia* is more pronounced at alternating temperatures than at constantly warm temperatures, suggesting that an adequate dietary PUFA supply is important especially for migrating *Daphnia*. Exposure to alternating temperatures caused modification in body PUFA concentrations and, consequently, increased lipid peroxidation. Detrimental effects of lipid peroxidation on *Daphnia* performance were not evident, suggesting that the positive effects of PUFA accumulation at alternating temperatures mask potential negative effects of PUFA-mediated oxidative stress. Our data imply that a dietary deficiency in long-chain PUFA has strong implications for predator evasion and thus *Daphnia* population dynamics.

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333

334 **Author Contributions**

335 DMC designed the experiment. MF conducted the experiment and performed the lipid
336 analyses. BZ performed the lipid peroxidation assay. All authors analysed the data. JIN and
337 DMC wrote the first draft of the manuscript. All authors contributed to subsequent versions of
338 the manuscript.

339

340 **Conflict of Interest**

341 The authors declare that they have no conflict of interest.

342

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346

347 **Data accessibility**

348 The datasets used and/or analysed during the current study are available from the
349 corresponding author on reasonable request.

350

References

- Becker, C. & Boersma, M. (2003). Resource quality effects on life histories of *Daphnia*.
Limnology and Oceanography, 48, 700-706.
- Brett, M.T., Müller-Navarra, D.C., Ballantyne, A.P., Ravet, J.L. & Goldman, C.R. (2006).
Daphnia fatty acid composition reflects that of their diet. *Limnology and Oceanography*,
51, 2428-2437.
- Brzeziński, T. & Von Elert, E. (2015). Predator evasion in zooplankton is suppressed by
polyunsaturated fatty acid limitation. *Oecologia*, 179, 687-697.
- Coggins, B.L., Collins, J.W., Holbrook, K.J. & Yampolsky, L.Y. (2017). Antioxidant
capacity, lipid peroxidation, and lipid composition changes during long-term and short-
term thermal acclimation in *Daphnia*. *Journal Of Comparative Physiology B-*
Biochemical Systemic And Environmental Physiology, 187, 1091-1106.
- Demott, W.R. (1986). The role of taste in food selection by freshwater zooplankton.
Oecologia, 69, 334-340.
- Dini, M.L., O'Donnell, J., Carpenter, S.R., Elser, M.M., Elser, J.J. & Bergquist, A.M. (1987).
Daphnia size structure, vertical migration, and phosphorus redistribution.
Hydrobiologia, 150, 185-191.
- Dodson, S. (1990). Predicting diel vertical migration of zooplankton. *Limnology and*
Oceanography, 35, 1195-1200.
- Esterbauer, H., Schaur, R.J. & Zollner, H. (1991). Chemistry and biochemistry of 4-
hydroxynonenal, malonaldehyde and related aldehydes. *Free Radical Biology and*
Medicine, 11, 81-128.
- Hays, G.C. (2003). A review of the adaptive significance and ecosystem consequences of
zooplankton diel vertical migrations. *Hydrobiologia*, 503, 163-170.
- Hazel, J.R. (1995). Thermal adaptation in biological membranes: Is homeoviscous adaptation
the explanation? *Annual Review of Physiology*, 57, 19-42.

377 Hazel, J.R. & Williams, E.E. (1990). The role of alterations in membrane lipid composition in
378 enabling physiological adaptation of organisms to their physical environment. *Progress*
379 *in Lipid Research*, 29, 167-227.

380 Jüttner, F., Leonhardt, J. & Möhren, S. (1983). Environmental factors affecting the formation
381 of mesityloxy, dimethylallylic alcohol and other volatile compounds excreted by
382 *Anabaena cylindrica*. *Journal of General Microbiology*, 129, 407-412.

383 Lampert, W. (1991). The dynamics of *Daphnia* in a shallow lake. *Verh. Int. Ver. Limnol.*, 24,
384 795-798.

385 Lampert, W. (2011). *Daphnia: Development of a model organism in ecology and evolution*,
386 International Ecology Institute, Oldendorf/Luhe, Germany.

387 Lampert, W., Mccauley, E. & Manly, B.F.J. (2003). Trade-offs in the vertical distribution of
388 zooplankton: ideal free distribution with costs? *Proc. R. Soc. B*, 270, 765-773.

389 Leonard, A.E., Pereira, S.L., Sprecher, H. & Huang, Y.S. (2004). Elongation of long-chain
390 fatty acids. *Progress in Lipid Research*, 43, 36-54.

391 Loose, C.J. & Dawidowicz, P. (1994). Trade-offs in diel vertical migration by zooplankton:
392 the costs of predator avoidance. *Ecology*, 75, 2255-2263.

393 Lushchak, V.I. (2011). Environmentally induced oxidative stress in aquatic animals. *Aquatic*
394 *Toxicology*, 101, 13-30.

395 Martin-Creuzburg, D., Sperfeld, E. & Wacker, A. (2009). Colimitation of a freshwater
396 herbivore by sterols and polyunsaturated fatty acids. *Proceedings of the Royal Society B*
397 *- Biological Sciences*, 276, 1805-1814.

398 Martin-Creuzburg, D., Wacker, A. & Basen, T. (2010). Interactions between limiting
399 nutrients: Consequences for somatic and population growth of *Daphnia magna*.
400 *Limnology and Oceanography*, 55, 2597-2607.

401 Martin-Creuzburg, D., Wacker, A., Ziese, C. & Kainz, M.J. (2012). Dietary lipid quality
402 affects temperature-mediated reaction norms of a freshwater key herbivore. *Oecologia*,
403 168, 901-912.

404 Ohkawa, H., Ohishi, N. & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by
405 thiobarbituric acid reaction. *Analytical Biochemistry*, 95, 351-358.

406 Ringelberg, J. & Van Gool, E. (2003). On the combined analysis of proximate and ultimate
407 aspects in diel vertical migration (DVM) research. *Hydrobiologia*, 491, 85-90.

408 Sakwinska, O. & Dawidowicz, P. (2005). Life history strategy and depth selection behavior as
409 alternative antipredator defenses among natural *Daphnia hyalina* populations.
410 *Limnology and Oceanography*, 50, 1284-1289.

411 Schällicke, S., Sobisch, L.-Y., Martin-Creuzburg, D. & Wacker, A. (2019). Food quantity-
412 quality co-limitation: Interactive effects of dietary carbon and essential lipid supply on
413 population growth of a freshwater rotifer. *Freshwater Biology*, in press. DOI:
414 10.1111/fwb.13272.

415 Schlechtriem, C., Arts, M.T. & Zellmer, I.D. (2006). Effect of temperature on the fatty acid
416 composition and temporal trajectories of fatty acids in fasting *Daphnia pulex* (crustacea,
417 cladocera). *Lipids*, 41, 397-400.

418 Siebeck, O. (1960). Untersuchungen über die Vertikalwanderung planktischer Crustaceen
419 unter Berücksichtigung der Strahlungsverhältnisse. *Internationale Revue der gesamten*
420 *Hydrobiologie und Hydrographie*, 45, 381-454.

421 Sperfeld, E. & Wacker, A. (2011). Temperature- and cholesterol-induced changes in
422 eicosapentaenoic acid limitation of *Daphnia magna* determined by a promising method
423 to estimate growth saturation thresholds. *Limnology and Oceanography*, 56, 1273-1284.

424 Stanley, D.W. (2000). *Eicosanoids in invertebrate signal transduction systems*, Princeton
425 University Press, New Jersey.

- 426 Sterner, R.W. & Schulz, K.L. (1998). Zooplankton nutrition: Recent progress and a reality
427 check. *Aquatic Ecology*, 32, 261-279.
- 428 Stich, H.B. & Lampert, W. (1981). Predator evasion as an explanation of diurnal vertical
429 migration by zooplankton. *Nature*, 293, 396-398.
- 430 Stich, H.B. & Lampert, W. (1984). Growth and reproduction of migrating and non-migrating
431 *Daphnia* species under simulated food and temperature conditions of diurnal vertical
432 migration. *Oecologia*, 61, 192-196.
- 433 Valentine, R.C. & Valentine, D.L. (2004). Omega-3 fatty acids in cellular membranes: a
434 unified concept. *Progress in Lipid Research*, 43, 383-402.
- 435 Von Elert, E. (2002). Determination of limiting polyunsaturated fatty acids in *Daphnia*
436 *galeata* using a new method to enrich food algae with single fatty acids. *Limnology and*
437 *Oceanography*, 47, 1764-1773.
- 438 Von Elert, E. & Pohnert, G. (2000). Predator specificity of kairomones in diel vertical
439 migration of *Daphnia*: a chemical approach. *Oikos*, 88, 119-128.
- 440 Winder, M., Boersma, M. & Spaak, P. (2003). On the cost of vertical migration: are feeding
441 conditions really worse at greater depths? *Freshwater Biology*, 48, 383-393.
- 442 Yin, H., Xu, L. & Porter, N.A. (2011). Free radical lipid peroxidation: Mechanisms and
443 analysis. *Chemical Reviews*, 111, 5944-5972.
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Table 1: Results of the two-factorial ANOVA used to assess differences in juvenile somatic growth rates, population growth rates, C18 body PUFA concentrations, and TBARS-equivalents between food and temperature treatments (Fig. 1 and Fig. 2). For the age at first reproduction and the cumulative numbers of offspring produced in the first three reproduction cycles results of the generalized linear model are shown. Population growth rates were log-transformed to meet ANOVA assumptions. Asterisks (*) indicate significant factors and interactions.

	factor	df	<i>F</i>	<i>P(>F)</i>
somatic growth rates	food	1/44	70.7	<0.001*
	temperature	1/44	1289.8	<0.001*
	food×temp	1/44	11.9	<0.001*
population growth rates	food	1/104	196.0	<0.001*
	temperature	1/104	4321.1	<0.001*
	food×temp	1/104	74.6	<0.001*
C18 PUFA	food	1/9	0.6	0.48
	temperature	1/9	11.7	0.008*
	food×temp	1/9	3.6	0.09
TBARS equivalents	food	1/11	1.2	0.30
	temperature	1/11	15.1	0.003*
	food×temp	1/11	2.5	0.14
	factor	df	<i>Deviance</i>	<i>P(>X)</i>
cumulative offspring	food	1/106	399.3	<0.001*
	temperature	1/105	10.36	0.03 *
	food×temp	1/104	70.04	<0.001*
age at first reproduction	food	1/106	2.33	<0.001*
	temperature	1/105	209.7	<0.001*
	food×temp	1/104	1.54	<0.001*

Figure legends

Fig. 1: Juvenile somatic growth rates, age at first reproduction, cumulative numbers of offspring produced within the first three reproduction cycles, and population growth rates of *Daphnia magna* exposed to two different temperature regimes, i.e. either constantly 20°C or alternating 6 and 20°C. Data represent means $\pm$ standard deviation (n = 12 for somatic growth rates; n = 26-28 for offspring and population growth rates). Different letters indicate significant differences among treatments (Tukey's HSD, $P < 0.05$). Inserted bar graphs depict the relative improvement in somatic growth rates, age at first reproduction, offspring production, and population growth rates upon EPA supplementation in the two temperature regimes.

Fig. 2: C18 PUFA (A) and EPA (B) concentrations and TBARS equivalents (C) in whole body extracts of *Daphnia magna* exposed to two different temperature regimes, i.e. either constantly 20°C or alternating 6 and 20°C. Data represent means $\pm$ standard deviation (n = 3-4). Different letters indicate significant differences among treatments (Tukey's HSD, $P < 0.05$). EPA was detected only in animals reared on the EPA supplemented diet.

Fig. 3: Conceptual model of *Daphnia* diel vertical migration (DVM) considering dietary PUFA supply as an additional proximate factor. In spring, when water temperatures rise and lakes become stratified in a warm and food-rich epilimnion (light green beam) and a cold and food-poor hypolimnion, *Daphnia* start to migrate when fish kairomones are present (Ringelberg & van Gool, 2003). DVM is controlled by the relative change in light intensity, which is greatest in the mornings and in the evenings (Siebeck, 1960). The amplitude of DVM is determined by a variety of different factors, such as water transparency (Dodson, 1990), the concentration of fish kairomones (von Elert & Pohnert, 2000), oxygen availability (Sakwinska & Dawidowicz, 2005), body size (Dini, O'Donnell, Carpenter *et al.*, 1987) and potentially the

478 availability of dietary PUFA (Brzeziński & von Elert, 2015). The scheme also shows the
 479 potential trade-off between predator evasion and food quality: While non-migrating *Daphnia*
 480 remain in the epilimnion during the day (red *Daphnia*), irrespective of dietary PUFA
 481 availability, migrating *Daphnia* seek shelter in the hypolimnion given that they are
 482 sufficiently supplied with dietary PUFA (green *Daphnia* (+)). The predominance of long-
 483 chain PUFA-deficient phytoplankton taxa (green algae, cyanobacteria) may either prevent
 484 DVM (green *Daphnia* (--)) or may reduce the migration amplitude (green *Daphnia* (-); see
 485 Brzeziński & von Elert, 2015), depending on dietary PUFA concentrations. *Daphnia* that are
 486 migrating at moderate dietary PUFA concentrations may be more susceptible to temperature-
 487 caused physiological constraints (present study). A shortage in dietary PUFA may thus
 488 exacerbate the trade-off between light-dependent predation losses and reduced growth and
 489 reproduction and thus increase the demographic costs associated with DVM.

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Temperature

Thermocline

Depth and light intensity

24 h