Effects of seasonal seston and temperature changes on lake zooplankton fatty acids

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Abstract

We investigated how seston fatty acids (FA) and water temperature explained seasonal variation in cladoceran and copepod FA over three years in pre-alpine, oligotrophic Lake Lunz, Austria. Using the mostly algal-derived polyunsaturated FA (PUFA: arachidonic, ARA; eicosapentaenoic, EPA; docosahexaenoic acid, DHA), terrestrial FA (TFA, 22 : 0, 24 : 0), and bacterial FA (BAFA, 15 : 0, 17 : 0 and their branched homologues) as source-specific biomarkers, we show that cladocerans consistently contained more ARA and EPA and copepods more DHA than the available food (seston). None of these physiologically important PUFA were significantly related between zooplankton and seston across the entire study period but copepod DHA increased with seston DHA during the coldest months (< 8°C, based on a significant seston FA*temperature interaction). EPA, conversely, increased with decreasing water temperature in both zooplankton groups. For the nonessential FA, TFA were lower in zooplankton than in seston and not related to dietary supply or water temperature. However, cladoceran and copepod BAFA increased significantly with increasing seston BAFA and decreasing water temperature. These findings suggest that physiological regulation in response to changing water temperature had a significant impact on cladoceran and copepod EPA and the extent of dietary tracking for copepod DHA. TFA available in the seston may not have been consumed or were poorly incorporated by zooplankton, but BAFA were good indicators of available resources throughout multiple seasonal cycles. Based on our study, both FA type and water temperature impact the extent that dietary vs. nondietary processes govern cladoceran and copepod FA in oligotrophic lakes.

Source-specific fatty acids (FA) are a promising tool for identifying trophic linkages in lakes (Taipale et al. 2009; Galloway et al. 2014). Herbivorous zooplankton, in particular, form crucial links between plants and upper trophic levels and identifying changes in zooplankton dietary sources through space and time is important for understanding pelagic food web structure (Arts et al. 2001; Brett et al. 2009). Ideally, FA biomarkers differ among available food sources and are incorporated conservatively by zooplankton from their diet. For example, long-chain polyunsaturated FA (LC-PUFA), particularly arachidonic acid (ARA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), are, theoretically, good dietary biomarkers in zooplankton because they can be highly conserved from the diet, differ among algae and cannot be biosynthesized de novo or efficiently

bioconverted from C18 precursors to entirely meet nutritional requirements (Taipale et al. 2011). Diet seems to be the major factor driving the LC-PUFA profile of laboratory zooplankton (e.g., Brett et al. 2006; Burns et al. 2011). Lake zooplankton, however, have been reported to track dietary LC-PUFA changes in some instances (e.g., Ravet et al. 2010) but not others (e.g., Persson and Vrede 2006). Physiological regulation, including some bioconversion from C18 precursors, of the nutritionally important LC-PUFA is a likely explanation for the observed differences between zooplankton and available dietary sources (Masclaux et al. 2012). However, the conditions under which dietary or nondietary processes govern natural zooplankton FA, and the reasons driving discrepancies among field studies, remain unknown.

FA serve a variety of functions in zooplankton (e.g., storage molecules and components of cell membranes) and, as a result, their proportions are regulated to meet the needs for survival, growth, and reproduction. For example, zooplankton retain (i.e., preferentially withhold from mobilization) some dietary FA in different proportions from those obtained in their diet and selectively allocate these FA to either the

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neutral (storage) or polar (membrane) lipid pools (Kainz et al. 2004; Brett et al. 2009). Increased retention or allocation of FA to the polar lipid pool (which is more tightly regulated than the neutral lipids; Olsen 1999) could obscure changes in the dietary FA signal through time. There is laboratory evidence that reduced dietary PUFA availability drives increased retention of certain FA in zooplankton (Koussoroplis et al. 2012), and temperature, through its effect on membrane fluidity and dietary FA requirements (Sperfeld and Wacker 2012), can increase both LC-PUFA retention (Masclaux et al. 2012) and allocation to the polar lipid pool during colder periods (Olsen 1999). Longer-term field data that extend past one year would help better understand how changes in natural zooplankton FA are explained by shifts in diet (e.g., phytoplankton community succession) or physiological regulation in response to temperature fluctuations.

In addition to LC-PUFA, it is as yet poorly understood how zooplankton track or regulate FA from other potential diet sources, such as bacterial or terrestrial sources, through time. Currently, terrestrial and bacterial FA (BAFA) serve unknown functions in zooplankton, and might, therefore, be subject to minimal physiological regulation and expected to conservatively track the diet. BAFA appear to be increasingly retained by larger zooplankton (Kainz and Mazumder 2005) but also to reflect seasonal dietary source changes (Taipale et al. 2009). Both bacteria and terrestrial carbon are currently receiving increased ecological interest as dietary sources for lake zooplankton (Taipale et al. 2013), but additional studies on their possible retention or accuracy as dietary biomarkers in zooplankton are still required.

Here, we conducted plankton sampling in pre-alpine Lake Lunz, Austria, over three consecutive years to evaluate the respective importance and interactions of diet FA composition and temperature as predictors of zooplankton biomarker FA. The following FA were explored as dietary, source-specific biomarkers in two separate zooplankton groups (cladocerans and copepods): (a) mostly algal-derived, LC-PUFA (ARA, EPA, DHA; Arts et al. 2001), (b) terrestrial FA (TFA: $22:0 + 24:0$; Samuels et al. 2008), and (c) BAFA ($15:0 + 17:0 + i15:0 + ai15:0 + i16:0 + i17:0$; Taipale et al. 2009; Taipale et al. 2012) to explore trends in presumably physiologically required (LC-PUFA) vs. "non-essential" FA (BAFA and TFA). Because identifying the dietary sources and functions of individual TFA and BAFA was not the objective of this study, we pooled all quantified TFA and BAFA to assess how these different dietary source markers were incorporated and represented in zooplankton through time. We also reviewed the literature to identify how the extent of dietary tracking by zooplankton might depend on physicochemical characteristics of study lakes that are expected to influence zooplankton physiology and, hence, the strength of the relationship between zooplankton and seston FA composition.

Materials and methods

Sampling

Seston (1.2-30 μm) and larger zooplankton (cladocerans and copepods, > 500 μm) were sampled from oligotrophic ($<$ 10 μg total phosphorus L^{-1}), subalpine Lake Lunz, Austria (47°N, 15°E). Large zooplankton were targeted as the most likely prey size for planktivorous fish (Brooks and Dodson 1965), which has implications for trophic transfer of FA to higher trophic levels. The lake (608 m above sea level, 34 m maximum depth) is typically ice-covered for three months annually (December–February). Samples were collected monthly during August 2009 to December 2011. For logistical reasons, no samples were collected during January to April of any year or during October and November 2011. Still, 3 early winter (December) and 19 total sampling events were performed across the three-year period. All samples were collected from a stationary platform situated above the lake's deepest point. Seston was collected from integrated water column samples (0-25 m; 20 L) or from the chlorophyll a (Chl a) maximum. Because proportional FA data were reported (see below), differences in seston biomass did not affect our results. Lake water and particles were passed through a filter (30 μm) into a prerinsed plastic container (20 L). Seston (1.2–30 μm), defined as the most edible particle size fraction for cladocerans (Burns 1968) and copepods (Vanderploeg 1990), was retained on precombusted (2 h at 550°C) GF/C filters and immediately stored in the dark at -80°C.

Zooplankton were collected by vertically hauling a zooplankton net (100 μm mesh size, 36 cm diameter) from 25 m to the surface. Bulk zooplankton were retained on a 500 μm filter cup, transferred into a falcon tube (50 mL) and put on ice for transport to the laboratory. Within a few hours of sampling, cladocerans and copepods were separated under a light microscope and subsequently stored in cryovials at -80°C.

Water temperature was measured every meter from the lake surface to 25 m depth using a YSI multisonde (Yellow Springs Instruments 6920V2-2-O, Yellow Springs, Ohio) from the same stationary platform from which zooplankton were sampled. Temperature data were collected during all 19 plankton sampling events, except May 2011 and September 2011, resulting in 17 total sampling events for which both temperature and plankton FA data were obtained.

Particulate carbon and phosphorus analysis

Samples for particulate organic carbon (POC) and phosphorus (POP) analysis were filtrated onto precombusted and acid-washed glass-fiber filters (Whatman GF/C). POC was measured by combustion and infrared spectrometry (C-Mat 5500, Ströhlein, Korschenbroich, Germany), and POP by molybdate reaction after sulfuric acid digestion (Wetzel and Likens 2003). Seston C : P ratios were calculated as molar ratios. To determine phytoplankton community composition, integrated water column samples (unscreened) were

preserved with Lugol's solution and counted under an inverted microscope.

Fatty acid analysis

The detailed method of lipid extraction and fatty acid methyl ester (FAME) generation is provided in Heissenberger et al. (2010). Briefly, lipids were extracted from freeze-dried, homogenized samples in chloroform and FAME were generated using H_2SO_4-methanol (incubated at 50°C for 16 h). FAME were dried under N_2 before being redissolved in hexane and run on a gas chromatograph (TRACE GC THERMO coupled to flame ionization detection) with a Supelco[TM] SP-2560 column used for separation of FAME. FAME were identified using known standards. All data are reported as relative proportions (FA • Σ total FA^{-1} * 100), which is the conventional method of data reporting in biomarker studies (Müller-Navarra 2006).

Data analysis

Zooplankton FA values (%) were logit transformed prior to statistical analyses (Warton and Hui 2011) to meet assumptions of normality and equality of variances (assessed via Shapiro-Wilk's and Levene's tests, respectively). Water temperature, measured every meter from 0 m to 25 m, was expressed in two ways: (1) as the mean temperature across the epilimnion + metalimnion, referred to as "MetTemp" and (2) as the mean of the entire 0-25 m water column, referred to as "MeanTemp." The depth of the metalimnion was defined as the approximate depth of the thermocline (Fig. 1). Mean-Temp was highly correlated with MetTemp (Pearson's $r = 0.92$, $p < 0.05$; Fig. 1), yet both variables were included because the exact depth range utilized by seston and zooplankton in the water column was unknown. To first explore how the variation of each of the five source-specific FA biomarker proportions (ARA, EPA, DHA, BAFA, TFA) differed among seston and the two zooplankton groups, coefficients of variation were calculated from the mean and standard deviation taken across the study period. The ability of seston FA, water temperature and the seston FA*temperature interaction to explain individual FA for both cladocerans and copepods was determined using two sets of multiple linear regressions (one set each for MetTemp and MeanTemp) of the following form: cladoceran or copepod FA = $\beta_0 + \beta_1$(seston FA) + β_2(temperature) + β_3(seston FA*temperature). The interaction term was included to allow the effect of seston FA on zooplankton FA to vary with different MetTemp or Mean-Temp values. Predictor variables were centered prior to their inclusion in the regression to decrease correlations between each predictor and the interaction term and because values of zero were not informative for either predictor variable (whereas partial regression slopes for centered predictor variables reflect the slope of zooplankton FA on seston FA for the mean value of MetTemp or MeanTemp). In instances of a significant interaction term, the impact of seston FA on zooplankton FA for values of MetTemp ranging from 4.0°C to 16.0°C (in 1°C increments) and MeanTemp ranging from
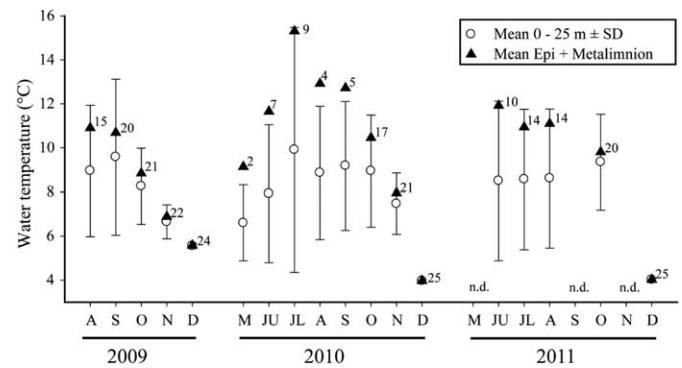

Fig. 1. Temperature of Lake Lunz during the study period, sampled monthly and recorded every m from 0 m to 25 m, and shown as: (1) mean and standard deviation of the entire 0-25 m water column (open symbols) and (2) mean of only the epi + metalimnion (filled symbols, numbers represent the approximate depth of the thermocline in m). Symbols along x axis indicate the month of sampling (M: May, JU: June, JL: July, A: August, S: September, O: October, N: November, D: December). No data were available for January through April of any year or for May, September or November of 2011 ("n.d.").

4.0°C to 9.5°C (in 0.5°C increments) was explored by calculating simple slopes ($\beta_1 + \beta_3$*temperature; Quinn and Keough 2002). The *deltamethod* command in the R package msm (Jackson 2011) was used to determine which simple slopes significantly differed from zero based on 95% confidence intervals. Pearson's correlation coefficients were calculated between each set of predictor variables (i.e., each of the five seston FA and water temperature) prior to performing multiple linear regressions to explore possible instances of colinearity. Because this correlation was significant between seston BAFA and water temperature (but not for the other FA and water temperature, see results), the effect of each predictor on zooplankton BAFA was also determined separately using ordinary least squares simple linear regressions. The extent of FA similarity among seston, cladocerans, and copepods was finally explored using principal component analysis (PCA) performed on the five FA biomarker proportions from all 19 sampling events. PCA was used in addition to multiple linear regression to visually explore relationships among seston and zooplankton FA throughout the study in multivariate space. FA were scaled to unit variance prior to their inclusion in the PCA. Loadings for each FA variable on each principal component (PC) axis were obtained by calculating the correlation coefficient between the original FA proportions and the scores extracted from each PC axis. All analyses were performed in the statistical program R (R Development Core Team 2010). Package vegan was used for the PCA (Oksanen et al. 2010). The significance level was set at 0.05 and all values are provided as mean ± SD.

Literature synthesis

We synthesized previously published reports of linear relationships between lake seston and zooplankton LC-PUFA

Table 1. Mean, standard deviation (SD) and coefficient of variation (CV, %) of seston, cladoceran and copepod fatty acids across n = 19 sampling events.

Fatty acid	Sample type	Mean	SD	CV
ARA	Seston	0.7	0.3	42.9
	Cladocerans	4.0	0.8	20.0
	Copepods	1.7	0.4	23.5
EPA	Seston	6.2	2.0	32.8
	Cladocerans	14.3	2.8	19.6
	Copepods	8.6	1.2	14.0
DHA	Seston	4.4	1.5	34.1
	Cladocerans	1.7	1.2	70.6
	Copepods	12.6	2.3	18.3
Terrestrial	Seston	1.0	0.6	60.0
	Cladocerans	0.2	0.1	50.0
	Copepods	0.3	0.1	33.3
Bacterial	Seston	3.8	1.3	34.2
	Cladocerans	4.2	0.9	21.4
	Copepods	3.9	0.8	20.5

(ARA, EPA, DHA), as well as physiochemical variables of the study lakes, to explore if lake trophic status might explain the observed discrepancies in the extent of dietary FA tracking by natural zooplankton. Study findings were only included if analyses were performed on proportional FA data. Studies reporting seston vs. zooplankton relationships derived from μg mg^{-1} data (Smyntek et al. 2008; Hartwich et al. 2013) were not included. Seston quality data (PUFA values) were reported as proportions (%) except for Müller-Navarra (2006) who provided these data only on an absolute μg mg^{-1} basis (although correlation coefficients were still provided from proportional FA data). As an indication of seston FA variability through time or across the lakes sampled in each study, seston coefficient of variation (CV) was calculated from provided seston mean and SD values for each study. When mean values were only provided for individually sampled lakes or seasons (Bychek and Guschina 2001; Hessen and Leu 2006; Taipale et al. 2009), we calculated an overall mean and SD from these values.

Results

Lake physicochemical characteristics

POC of seston (< 30 μm) in Lake Lunz ranged between 0.09 mg L^{-1} and 0.16 mg L^{-1} (0.12 ± 0.02 mg C L^{-1}), particulate POP (< 30 μm) from 0.71 μg L^{-1} to 2.8 μg L^{-1} (1.35 ± 0.58 μg L^{-1}) and the molar C:P ratio from 120 to 463 throughout the seasons. There were no obvious seasonal declines from spring to winter in either POC or POP and the lowest POC (0.09 mg L^{-1}) was observed during the summer. Phytoplankton community composition data were not collected throughout the study period. However, based on data gathered during 2012, which agree with more historical data

(Malicky 1985), the typical phytoplankton community of Lake Lunz includes: (1) Cryptophyta (*Rhodomonas* and *Cryptomonas*) that constitute around half of the biomass, (2) diatoms (including *Nitzschia*, *Cyclotella*, and *Asterionella*) which become particularly abundant in spring (up to 35% of biomass) but decreased dramatically later in the season, and (3) Chlorophyta (*Micractinium* and *Chlamydomonas*), Euglenophyta (*Trachelomonas* and *Euglena*), and Dinophyta (*Gymnodinuim* and *Peridinium*) that are present throughout the seasons (Malicky 1985). Cladocerans consisted of *Daphnia longispina* and *Bosmina longirostris*, and copepods of calanoids (*Eudiaptomus gracilis*) and cyclopoids (*Cyclops tatricus*).

Surface temperature (upper 5 m) of Lake Lunz ranged from a low of 4°C in December of 2009, 2010, and 2011 to a maximum of 22°C during July 2010 (data not shown). The time of warmest temperatures and most stratified water column based on mean ± SD of the 0-25 m temperature data occurred in July through September (summer), with temperatures and water column stratification declining in October through November (fall) to December (winter; Fig. 1). Monthly temperature data were not consistent across all
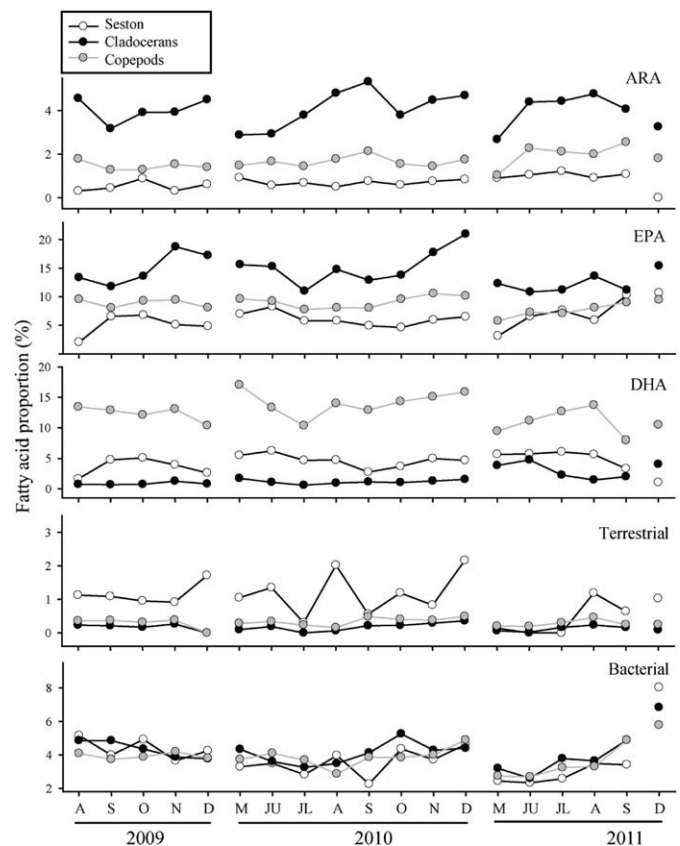

Fig. 2. Fatty acid proportions of seston (open symbols), cladocerans (black symbols), and copepods (grey symbols) from Lake Lunz. Note, on the *x* axis, that no data were available for January through April of any year or for October 2011 and November 2011.

Table 2. Relationships between zooplankton biomarker fatty acids (dependent variable) and seston FA, water temperature and the seston FA*temperature interaction (centered predictor variables) based on multiple linear regressions. Temperature was included in two sets of regressions as either: 1) MetTemp: mean epi + metalimnion temperature, and 2) MeanTemp: mean 0 – 25 m temperature. Intercepts and partial regression slopes are provided (significance at $\alpha = 0.05$ indicated by '*' and test statistic provided in footnote). All FA were logit transformed prior to analysis.

| Temperature | FA | Intercept | Cladocerans Partial slopes | | | | Copepods Partial slopes | | | |
			Seston FA	Temp.	Seston FA*Temp.	r^2	Intercept	Seston FA	Temp.	Seston FA*Temp.	r^2
MetTemp	ARA	−3.156	0.482	−0.003	−0.064	0.094	−4.092	0.768	0.016	0.262	0.257
	EPA	−1.771	−0.087	−0.063*	0.052	0.645	−2.349	−0.089	−0.023[†]	−0.020	0.392
	DHA	−4.450	0.401	−0.042	0.174	0.323	−1.871	0.066	−0.021	−0.052[‡]	0.448
	TFA	−1.622	0.837	−0.100	0.539	0.210	−0.777	−0.187	0.031	0.181	0.043
	BAFA	−3.176	0.502[§]	−0.001	−0.020	0.621	−3.266	0.222	−0.018	−0.048	0.573
Mean Temp	ARA	−3.165	0.506	0.004	−0.045	0.088	−4.092	0.739	0.010	0.332	0.185
	EPA	−1.763	−0.204	−0.124[¶]	0.070	0.728	−2.353	−0.103	−0.038	−0.033	0.361
	DHA	−4.422	0.368	−0.109	0.223	0.351	−1.876	0.061	−0.041	−0.086[‖]	0.459
	TFA	−1.670	1.705	−0.025	0.423	0.110	−0.831	0.196	0.129	−0.072	0.060
	BAFA	−3.166	0.541[#]	0.019	−0.057	0.639	−3.247	0.256**	−0.030	−0.059	0.641

*t = −4.839, P < 0.001;
[†]t = −2.342, P < 0.05;
[‡]t = −2.447, P < 0.05 ;
[§]t = 3.285, P < 0.01;
[¶]t = −5.868, P < 0.01 ;
[‖]t = −2.612, P < 0.05;
[#]t = 3.997, P < 0.01;
**t = 2.27, P < 0.05

three years, however, because mean temperatures were lower in June, July, and August of 2011 than 2010 (Fig. 1).

Fatty acids in seston, cladocerans, and copepods

Based on CV, cladocerans and copepods had less variable ARA, EPA, TFA, and BAFA proportions compared to seston across the study period (Table 1). DHA was less variable in copepods but more variable in cladocerans than seston (Table 1). Cladocerans had higher ARA and EPA proportions than copepods and seston, and copepods had higher DHA than cladocerans and seston for the entire study period (Fig. 2). Cladocerans and copepods had lower TFA than in seston except for May, June, and July 2011 (Fig. 2). Unlike the other FA, BAFA proportions overlapped among these three groups during the three years sampling period (Fig. 2).

Effect of seston and temperature on zooplankton fatty acids

Seston FA and water temperature were not significantly correlated for any FA ($p > 0.05$) except for BAFA (MetTemp: Pearson's $r = −0.64$, $p < 0.05$; MeanTemp: Pearson's $r = −0.54$, $p < 0.05$). Based on multiple linear regressions, neither seston FA, water temperature nor their interaction explained a significant amount of the variation in ARA or TFA for either cladocerans or copepods (all $p > 0.05$; Table 2). Seston EPA was also unrelated to EPA for both cladocerans and copepods (Table 2),

but MetTemp explained a significant amount of the variation in EPA for both zooplankton groups (Table 2). The simple linear relationships, after removing nonsignificant terms, were explained by: (1) cladocerans: logit(EPA) = −1.261 + (−0.054)*MetTemp, $r^2 = 0.575$, $p < 0.001$ (Fig. 3a) and (2) copepods: logit(EPA) = −2.120 + (−0.023)*MetTemp, $r^2 = 0.316$, $p < 0.05$ (Fig. 3d). MeanTemp was only significantly related to cladoceran EPA: logit(EPA) = −1.047 + (−0.095)*MeanTemp, $r^2 = 0.584$, $p < 0.001$. EPA ranged from a low of 10.8% in cladocerans (June 2011) and 5.8% in copepods (May 2011) to a high of 21.0% (December 2010) and 10.6% (November 2010) in cladocerans and copepods, respectively (Fig. 2), supporting the observed negative relationship between zooplankton EPA and water temperatures (Fig. 3a,d). Neither seston DHA (Fig. 3b,e) nor water temperature were related to DHA in either zooplankter across the entire study period, but the interaction between these two predictor variables was significant for copepods for both temperature variables (Table 2). Simple slopes of copepod vs. seston DHA were positive and significantly different from zero at values of MetTemp ≤ 8.0°C and at values of Mean-Temp ≤ 6.5°C (when 95% confidence intervals did not bound zero, Table 3). November and December (winter) had MetTemp < 8.0°C and December had Mean-Temp ≤ 6.5°C. A positive relationship, therefore, existed
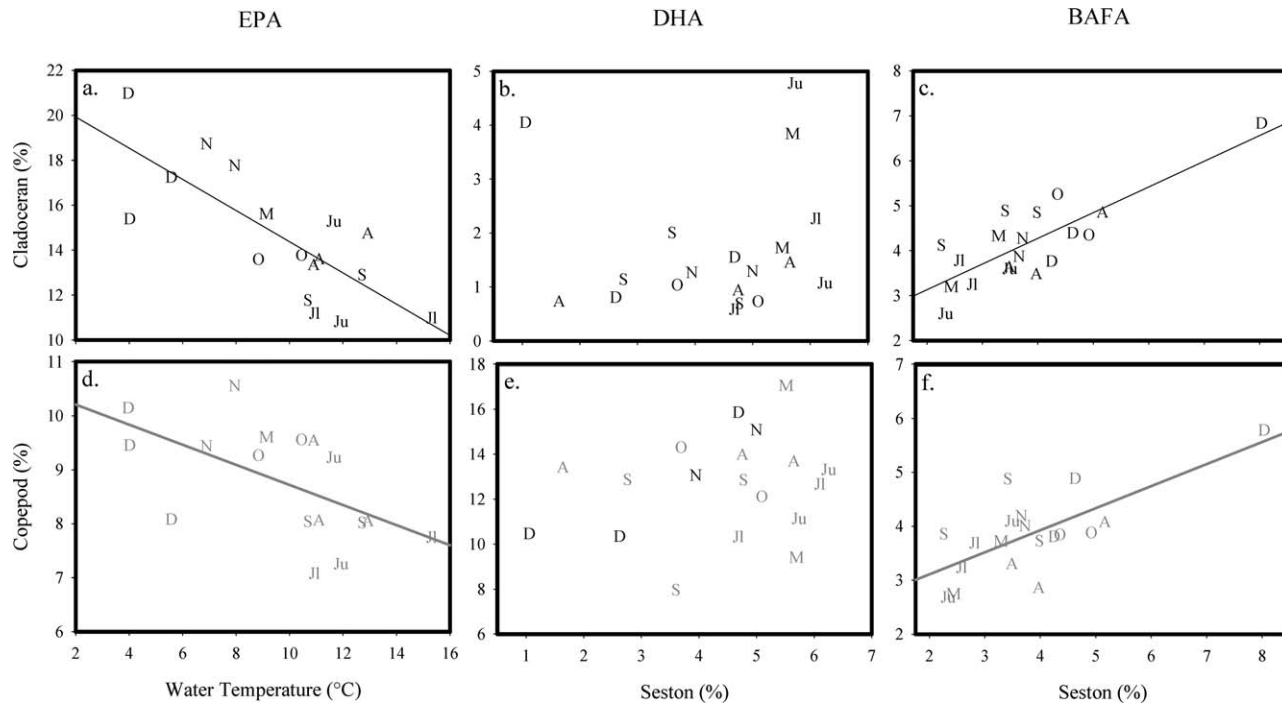
Fig. 3. Relationships between zooplankton (cladoceran or copepod) FA and two predictor variables: (1) water temperature (MetTemp: a, d), and (2) seston FA (b, c, e, f). Significant simple linear regression lines ($p < 0.05$) are shown (see text for parameter estimates). Seston DHA was not significantly related to either cladoceran (b) or copepod DHA (e) across the entire study period, but based on the seston DHA*MetTemp interaction, simple slopes were significant between copepod and seston DHA for months with MetTemp $\leq 8.0°$C (black letters). See Table 2 for full model parameters and Table 3 for simple slopes.

between DHA in seston and copepods during these months (Fig. 3e; Table 3).

Due to the significant, negative correlation between seston BAFA and water temperature, the effect of these predictor variables on zooplankton BAFA was assessed with both multiple and separate simple linear regressions. Seston BAFA significantly explained the variation in both cladoceran and copepod BAFA in the full multiple regression model when MeanTemp was included as a predictor but neither temperature variable was significant in any full model (Table 2). Based on simple linear regressions seston BAFA (via a positive relationship) was a significant predictor of BAFA in cladocerans and copepods (Fig. 3) via the following relationships:

1. cladocerans: logit(BAFA) = $-1.433 + 0.526$*logit(sestonBAFA), $r^2 = 0.593$, $p < 0.001$
2. copepods : logit(BAFA) = $-1.855 + 0.422$*logit(sestonBAFA), $r^2 = 0.455$, $p < 0.01$

MetTemp also became a significant predictor of zooplankton BAFA when included separately in simple regressions for both zooplankton groups (suggesting some increased variance of regression coefficients due to colinearity in the full model) as follows:

3. cladocerans : logit(BAFA) = $-2.790 + (-0.037)$*MetTemp, $r^2 = 0.276$, $p < 0.05$

4. copepods : logit(BAFA) = $-2.842 + (-0.040)$*MetTemp, $r^2 = 0.460$, $p < 0.01$

MeanTemp was only significantly related to copepod BAFA as follows:

logit(BAFA) = $-2.842 + (-0.040)$*MeanTemp, $r^2 = 0.460$, $p < 0.01$

Thus, zooplankton BAFA increased with seston BAFA and was higher during times with lower water temperature. Removal of one high value from December did not change the significance of the seston vs. zooplankton FA relationship for either zooplankton group ($p < 0.05$) but temperature remained significant only for copepods.

The PCA performed on the five biomarker FA in seston and zooplankton revealed that 91% of the variation in the data was explained by the first three PC (Table 4). PC1 separated cladocerans, copepods, and seston and indicated that cladocerans differed the most from seston (i.e., had the most positive PC1 scores, associated with higher EPA and ARA; Table 4) during December (Fig. 4), supporting the findings from the multiple linear regressions that water temperature, but not seston EPA, was related to cladoceran EPA. BAFA and TFA loaded positively and DHA negatively on PC2 (Table 4). In December, seston and copepods, and one cladoceran sample, had more positive PC2 scores than generally observed for other months (Fig. 4), which supports the finding that

Table 3. Probing the significant seston DHA*Temperature interaction for copepods at different values of MetTemp (mean metalimnion + epilimnion temperature) and MeanTemp (mean across entire 0 – 25 m water column) via simple slopes ($\beta_1 + \beta_3$*MeanTemp, see methods) and 95% confidence intervals. Predictor variables were centered prior to analysis.

Temperature	Actual °C	Centered °C	Simple slope	95% CI	
MetTemp	4.0	−5.7	0.36	0.58	0.14
	5.0	−4.7	0.31	0.500	0.12
	6.0	−3.7	0.26	0.42	0.09
	7.0	−2.7	0.21	0.36	0.05
	8.0	−1.7	0.15	0.30	0.01
	9.0	−0.7	0.10	0.26	−0.05
	10.0	0.3	0.05	0.22	−0.12
	11.0	1.3	−0.01	0.19	−0.19
	12.0	2.3	−0.05	0.17	−0.28
	13.0	3.3	−0.10	0.15	−0.36
	14.0	4.3	−0.16	0.13	−0.45
	15.0	5.3	−0.21	0.12	−0.53
	16.0	6.3	−0.26	0.10	−0.62
MeanTemp	4.0	−3.7	0.38	0.60	0.15
	4.5	−3.2	0.34	0.54	0.14
	5.0	−2.7	0.29	0.47	0.11
	5.5	−2.2	0.25	0.41	0.09
	6.0	−1.7	0.21	0.36	0.06
	6.5	−1.2	0.16	0.31	0.02
	7.0	−0.7	0.12	0.27	−0.02
	7.5	−0.2	0.08	0.23	−0.07
	8.0	0.3	0.04	0.20	−0.13
	8.5	0.8	−0.01	0.18	−0.19
	9.0	1.3	−0.05	0.16	−0.26
	9.5	1.8	−0.09	0.14	−0.32

DHA was more similar between seston and copepods during winter, and that BAFA were positively related between seston and zooplankton, and higher during times of colder water temperatures. DHA loaded negatively on PC3, which separated copepods (negative PC3 scores) from seston and cladocerans (positive PC3 scores) due to high DHA proportions in copepods (Table 4).

Literature Synthesis

Only those studies on mesotrophic lakes ($n = 3$; Table 5) reported significant relationships between seston and zooplankton ARA, EPA, or DHA. Studies performed in oligotrophic and eutrophic lakes ($n = 3$ and 1, respectively) failed to identify significant tracking of dietary PUFA by copepod or cladoceran populations (Table 5).

Discussion

Results of this multiannual study in oligotrohpic Lake Lunz indicate that: (1) water temperature was a better predic-

tor of seasonal cladoceran and copepod EPA changes than dietary EPA supply, (2) copepod DHA was related to seston DHA changes, but only during the coldest months, (3) ARA and TFA were not related to either diet or temperature changes in zooplankton, but (4) seston BAFA predicted BAFA in cladocerans and copepods throughout the entire sampling period. Therefore, BAFA appear to be suitable biomarkers of available dietary bacterial energy in cladocerans and copepods inhabiting this oligotrophic lake. Patterns of mostly algae-derived and physiologically required LC-PUFA in zooplankton, however, could not be predicted by dietary FA supply throughout all seasons and may instead be more closely related to environmental conditions, such as temperature.

Explanatory power of seston fatty acids

The observation that ARA and EPA in cladocerans and DHA in copepods were higher and less variable (based on CV) than corresponding values available in the seston agrees with the widely recognized ability of zooplankton to actively retain and maintain PUFA close to taxa-specific, physiologically required proportions (Persson and Vrede 2006; Kainz et al. 2009). The lack of significant relationships between zooplankton and seston PUFA throughout the entire study period further suggests that zooplankton PUFA are not always reflective of changing dietary PUFA levels. Based on our findings, however, the extent of dietary PUFA tracking can be influenced by abiotic factors such as temperature, because copepod DHA tracked changes in seston DHA when water temperatures were at their coldest for the year (based on the significant seston FA and water temperature interaction). Increased DHA demand for copepods to maintain cell membrane fluidity during colder temperatures could explain

Table 4. Eigenvalues, proportion explained and loadings of each FA variable on the first three PC axes extracted from a PCA performed on seston, cladoceran and copepod FA proportions. Loadings are the correlation coefficient between the PC axis and the actual FA variable. Mean ± SD of sample scores for seston and each zooplankter are also provided.

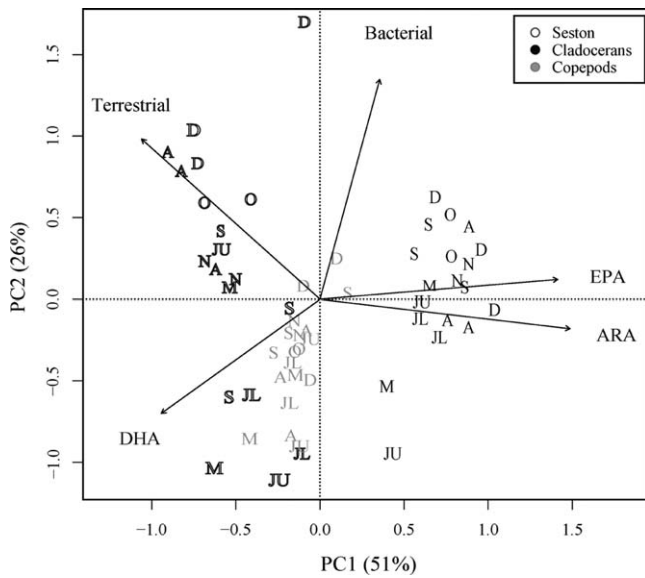|	PC1	PC2	PC3
Eigenvalue	2.54	1.31	0.69
Proportion explained	0.51	0.26	0.14
Variable loadings			
ARA	0.94	−0.11	0.03
EPA	0.89	0.08	−0.24
DHA	−0.60	−0.44	−0.65
Terrestrial	−0.67	0.62	0.15
Bacterial	0.22	0.85	−0.43
Sample scores			
Seston	−0.13 ± 0.06	0.05 ± 0.18	0.09 ± 0.08
Cladocerans	0.17 ± 0.04	0.02 ± 0.09	0.05 ± 0.1
Copepods	−0.04 ± 0.03	−0.07 ± 0.07	−0.14 ± 0.08

Fig. 4. PCA biplot of seston (bold black symbols), cladoceran (black symbols), and copepod (grey symbols) fatty acids. The amount of variance explained by each PC axis is shown in parentheses. Sample score symbols indicate the month of sampling (M: May, JU: June, JL: July, A: August, S: September, O: October, N: November, D: December). Fatty acid variables and sample scores are scaled relative to eigenvalues for graphing purposes, with scaling = 2 in the rda function of package vegan in the statistical program R.

this result and might have increased copepod dependency and tracking of the available supply of DHA in the seston.

Weak relationships between individual PUFA in seston and zooplankton throughout the study period are in agreement with findings from previous field studies (Bychek and Guschina 2001; Hessen and Leu 2006; Persson and Vrede 2006) but in contrast to laboratory and other field observations that reported significant relationships (Müller-Navarra 2006; Taipale et al. 2009; Ravet et al. 2010). Although we cannot exclude the possibility that sampling zooplankton with a time lag from seston would have improved the fit of seston and zooplankton PUFA relationships, Hartwich et al. (2013) also reported no significant zooplankton vs. seston PUFA correlations (similar to our results) even when zooplankton were sampled with a one-week time lag of seston sampling. Moreover, if such a lag was the main reason for the weak seston-zooplankton PUFA relationships reported here, it should be the same for all FA including BAFA. This suggests that certain yet unidentified factors, which differ between lab conditions and the field or even among ecosystems, might affect the strength of dietary PUFA tracking by zooplankton. For example, lab studies performed under conditions of sufficient, high quality, and monoalgal food sources may limit the need for zooplankton to physiologically retain dietary FA (Taipale et al. 2011). Additionally, field studies often differ in taxonomic resolution and sampling design. For example, Ravet et al. (2010) analyzed a larger size

range of seston (0.7-50 μm) and zooplankton (> 120 μm) than in this study. Freshwater zooplankton also have some capacity for converting C18 to C20 and C22 PUFA (Masclaux et al. 2012). In this study, however, seston $\Sigma n-6$ and $\Sigma n-3$ PUFA also exhibited weak relationships with cladoceran and copepod ARA, EPA, and DHA (simple linear regressions, all $p > 0.05$), similar to findings for individual seston LC-PUFA.

Based on findings currently available in the literature, an additional hypothesis is that differences in lake trophic status might impact the extent of dietary tracking in zooplankton because studies that have reported significant seston-zooplankton PUFA correlations were conducted in mesotrophic rather than eutrophic or oligotrophic lakes (Table 5). Drawing from current knowledge of zooplankton FA physiology (Arts et al. 2001; Brett et al. 2009), we propose several nonmutually exclusive mechanisms that could lead to such a result. First, due to the relatively unselective accumulation of dietary FA in storage lipids, greater seston quantity (e.g., Chl a or POC) or quality (e.g., % EPA) in mesotrophic than eu- or oligotrohpic lakes (Table 5) could support more lipid-rich zooplankton with a higher storage to membrane lipid ratio that more readily reflect dietary changes (Olsen 1999; Müller-Navarra 2006). Lipids of leaner zooplankton, conversely, mostly consist of membrane lipids, which are more strictly regulated and, thus, less responsive to dietary FA composition. Additionally, after a dietary shift, tissues of faster growing zooplankton could increasingly reflect the FA of the new diet by following a simple growth dilution model, a phenomenon shown for fish (Jobling 2004). Burns et al. (2011) reported no correlation between Chl a and seston PUFA (i.e., food quality) among a variety of different lakes, based on a literature review, but higher food quantity in mesotrophic lakes might still support higher zooplankton growth, faster lipid turnover and tighter coupling between seston and zooplankton FA compared to oligotrophic lakes. Finally, mesotrophic lakes seem to exhibit more pronounced seasonal fluctuations in seston EPA and DHA than oligotrophic lakes based on CV. For example, CV for EPA across season was 61.9% in the mesotrophic lake of Ravet et al. 2010 and 32.8% in the oligotrophic Lake Lunz sampled in this study (Table 5). The larger magnitude of seston FA variation through time in mesotrophic lakes might overpower physiological FA regulation capacities and increase responsiveness of zooplankton PUFA to dietary changes, whereas more strict FA profiles might be maintained in oligotrophic lakes that experience smaller seasonal changes in seston FA. Additional studies, including those that combine lipid patterns with somatic growth rates of zooplankton (e.g., RNA/DNA ratios), are clearly needed to establish a mechanistic understanding of how PUFA transfer at the phytoplankton–zooplankton interface varies across lake ecosystems of different trophic status.

Zooplankton TFA were lower than and not related to TFA found in the seston, suggesting that zooplankton in this study did not efficiently retain dietary long-chain saturated

Table 5. Previously published correlations between seston and zooplankton LC-PUFA proportions in lakes of different trophic classes (Eu: eutrophic, Meso: mesotrophic, Oligo: oligotrophic). For each study, seston quantity (POC or Chl *a*, both μg L^{-1}), seston coefficient of variation (%) and seston quality (mean LC-PUFA values reported as % or μg mg^{-1}) are provided. Instances of significant correlations between seston and zooplankton LC-PUFA are noted (no significant correlations indicated by 'None') and n.d. = no data reported. All studies reported FA data from the total lipid fraction except for Taipale et al. 2009 (polar lipids).

Reference	Bychek & Guschina 2001	Ravet et al. 2010	Taipale et al. 2009	Müller-Navarra 2006	Persson & Vrede 2006	Hessen & Leu 2006	Current study
Seston versus zooplankton PUFA (%) correlations							
Cladocerans	None	EPA	ΣPUFA	None	None	None	None
Copepods	n.d.	ARA, DHA	n.d.	ARA, DHA	None	n.d.	None
Trophic status	Eu	Meso	Meso	Meso	Ultra-oligo to oligo	Oligo	Oligo
Study system	Russian reservoir	Lake Washington	Finnish lake	Schöhsee (Germany)	12 Swedish lakes	6 high arctic ponds	Lake Lunz
Seston quantity	n.d.	Chl *a* 2.8 - 11.9	Chl *a* 3.7 - 7.2	n.d.	POC 110 - 291	Chl *a* 0.90 - 2.63	POC < 200 Chl *a* < 3
Dates sampled	June - Aug.	Apr. – Jan.	May – Oct.	Apr.– Oct.	Jul.– Aug.	Jul.	May – Dec.
Seston coefficient of variation							
ARA	65.3	75.0	148	110	144	59.8	42.9
EPA	70.2	61.9	31.5	49	33.3	23.8	33.9
DHA	n.d.	74.3	n.d.	82	47.6	48.5	34.1
Seston quality							
ARA	4.7 %	3.2 %	0.33 %	0.18 μg mg^{-1}	1.6 %	1.1 %	0.7 %
EPA	1.7 %	8.4 %	1.10 %	0.71 μg mg^{-1}	3.0 %	6.1 %	6.2 %
DHA	n.d.	3.5 %	n.d.	0.50 μg mg^{-1}	2.1 %	2.8 %	4.4 %
Sample size (n) & zooplankton species sampled							
Seston	12	15	16	7	19	18	19
Cladocerans	9 *Daphnia galeata*	4 *Daphnia spp.*	12 *Daphnia longispina*	7 *Daphnia spp.*	5 *Daphnia spp.*	18 *Daphnia tenebrosa*	19 see text
Copepods	n.d.	15 *Diaptomus ashlandi*	n.d.	7 *Eudiaptomus*	8 *Arctodiaptomus laticeps*	n.d.	19 see text

FA (LC-SAFA) or feed on particles with which these FA were associated. Previous studies show that laboratory *Daphnia* can assimilate carbon and FA from dietary terrestrial POC but only when the contribution of this food source was high (> 60%, Taipale et al. 2013). Future work is needed to determine whether, being physiologically less required as cell membrane lipids, LC-SAFA are low in zooplankton because they are not assimilated, are rapidly oxidized for energy (Schulz 2002) or are readily converted into other FA.

The positive linear relationship between seston and zooplankton BAFA indicates that cladoceran and copepod BAFA are reliable biomarkers of dietary bacterial availability. *Daphnia magna* BAFA also increased with increasing bacterial contribution to the diet in controlled feeding trials (Taipale et al. 2013). Although seston BAFA were generally available at considerably lower levels than dietary algae-derived PUFA in this field study, significant seston vs. zooplankton BAFA relationships indicate little effect of the filter (cladocerans) or selective (copepods) feeding modes of zooplankton on BAFA acquisition and/or retention. Dietary BAFA serve a different yet largely unknown physiological purpose compared

to PUFA (Taipale et al. 2012) and the underlying reasons of the similarity to dietary BAFA in both cladocerans and copepods (e.g., perhaps BAFA are not efficiently catabolized and instead routed to storage molecules) need to be further elucidated. It would also be informative to increase the dietary resolution of TFA and BAFA by assigning individual FA to more specific terrestrial or bacterial sources. Furthermore, experimental work using compound-specific stable isotope tracing could help understanding how BAFA and TFA are routed and used in zooplankton.

Effect of water temperature on zooplankton fatty acids

Slight differences were apparent between the ability of the two highly correlated temperature variables to explain zooplankton FA. MetTemp was related to EPA and BAFA in both zooplankton groups, but MeanTemp was related only to EPA in cladocerans and BAFA in copepods. The reasons for this discrepancy are unclear because the actual location and movements of zooplankton and seston in the water column are unknown (samples reflect integration over 0-25 m). Further work is required to elucidate the precise link between

the lake's thermal profile and dietary FA acquisition or retention in zooplankton. Regardless, our findings suggest that natural temperature fluctuations in this oligotrophic lake were related to changes in certain zooplankton FA, which has important implications for the use of FA as dietary biomarkers in lake zooplankton.

Cladocerans and copepods had significantly higher EPA with decreasing water temperature, which was not related to changes in seston EPA throughout the seasons. Higher EPA in zooplankton in response to declining temperature likely reflect increased EPA demand and retention (Masclaux et al. 2012). Previous studies reported no evidence for increased EPA accumulation by *Daphnia* in certain seasons (Taipale et al. 2009; Hartwich et al. 2013), although these studies only included samples from spring through fall. Gladyshev et al. (2010), however, reported a significant increase in EPA of combined cladoceran, copepod, and rotifer samples with decreasing water temperature ($r = -0.69$). Based on our findings, EPA in cladocerans and copepods did not vary due to changes in seston EPA but instead increased and decreased during colder and warmer times of the year, respectively, likely due to physiological regulation.

In contrast to seston EPA, BAFA in both cladocerans and copepods significantly tracked seston BAFA throughout changes in water temperature, and BAFA in seston and both zooplankters increased with decreasing temperature. Based on this finding, bacteria (as indicated by BAFA) constituted a higher proportion in seston, that was consumed by zooplankton (either directly or through bacterivorous protists), during colder seasons. Although it may not explain why zooplankton steadily track bacterial biomarkers, bacteria can convey dietary nutrients (Taipale et al. 2013) and could, therefore, be an important source of energy for zooplankton during certain times of the year.

Based on our results, only BAFA and not mostly algal-derived PUFA in oligotrophic lake zooplankton can be expected to track monthly changes in the FA of available seston throughout annual seasons and water temperature changes. Future work is needed to establish whether seston TFA are not consumed, not assimilated or readily catabolized by natural zooplankton in oligotrophic lakes. Importantly, quantitative estimates of zooplankton diet remain feasible in light of our findings, as long as physiological modification of FA from the diet can be estimated (by laboratory feeding trials; Galloway et al. 2014). However, our study sheds new light on the importance of FA type, seasonal temperature changes and possibly lake trophic status for governing whether natural zooplankton FA profiles are most reflective of dietary supply or physiological regulation.

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