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### ► To cite this version:

Matthias Pilecky, Margaux Mathieu-Resuge, Libor Závorka, Lena Fehlinger, Katharina Winter, et al.. Common carp (*Cyprinus carpio*) obtain omega-3 long-chain polyunsaturated fatty acids via dietary supply and endogenous bioconversion in semi-intensive aquaculture ponds. *Aquaculture*, 2022, 561, pp.738731. 10.1016/j.aquaculture.2022.738731 . hal-03838016

**HAL Id: hal-03838016**

**<https://hal.inrae.fr/hal-03838016>**

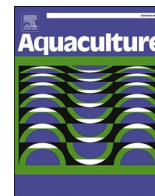
Submitted on 4 Nov 2022

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# Common carp (*Cyprinus carpio*) obtain omega-3 long-chain polyunsaturated fatty acids via dietary supply and endogenous bioconversion in semi-intensive aquaculture ponds

Matthias Pilecky<sup>a,b,1,\*</sup>, Margaux Mathieu-Resuge<sup>a,c,d,1</sup>, Libor Závorka<sup>a</sup>, Lena Fehlinger<sup>a,f</sup>, Katharina Winter<sup>a</sup>, Dominik Martin-Creuzburg<sup>e</sup>, Martin J. Kainz<sup>a,b</sup>

<sup>a</sup> WasserCluster – Biologische Station Lunz, Inter-University Center for Aquatic Ecosystem Research, Dr. Carl-Kupelwieser Promenade 5, 3293 Lunz/See, Austria

<sup>b</sup> Donau-Universität Krems, Department for Biomedical Research, Dr. Karl-Dorrek-Straße 30, 3500 Krems, Austria

<sup>c</sup> University of Brest, CNRS, IRD, Ifremer, LEMAR, F-29280 Plouzané, France

<sup>d</sup> UMR DECOD (Ecosystem Dynamics and Sustainability), Ifremer, INRAE, Institut Agro, 29280 Plouzané, France

<sup>e</sup> BTU Cottbus-Senftenberg, Department of Aquatic Ecology, Research Station Bad Saarow, Seestraße 45, 15526 Bad Saarow, Germany

<sup>f</sup> University of Vic - Central University of Catalonia, Aquatic Ecology Group, Carrer de la Sagrada Família, 7, 08500 Vic, Barcelona, Spain

## ARTICLE INFO

### Keywords:

Aquaculture  
Fatty acid bioconversion  
Compound-specific stable isotope analysis  
Deuterium  
DHA  
Fish ecophysiology

## ABSTRACT

Omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA) are essential micronutrients for aquatic consumers. Synthesized by aquatic primary producers, n-3 LC-PUFA are transferred across trophic levels and may eventually end up accumulating in fish. However, if short in dietary supply, fish may also biosynthesize n-3 LC-PUFA from dietary precursors (i.e., n-3 C18-PUFA). We applied compound-specific hydrogen stable isotope analysis (CSIA) of fatty acids to investigate sources and metabolic processes of n-3 LC-PUFA, and in particular of docosahexaenoic acid (22:6n-3, DHA), in common carp (*Cyprinus carpio*) raised in semi-intensive aquaculture ponds. Carp were feeding on natural pond zooplankton and benthic macroinvertebrates rich in n-3 LC-PUFA and cereal-based pellet feeds rich in C18-PUFA. Results provide isotopic evidence that carp obtained a significant amount of dietary lipids and nitrogen from added cereal-based feeds, while n-3 LC-PUFA were generally acquired by feeding on benthic macroinvertebrates and zooplankton. However, DHA retained in carp was also generated endogenously via bioconversion from dietary PUFA precursors, such as EPA. DHA was isotopically lighter than EPA and likely not supplied in sufficient quantities to meet the physiological requirements for DHA in carp. Our data show that depending on the natural abundance of dietary DHA in these eutrophic ponds, farmed carp can obtain DHA by two different pathways; i.e., directly via dietary uptake and indirectly via bioconversion. This field study highlights the importance of dietary LC-PUFA supply in eutrophic aquatic ecosystems and the ability of carp to biosynthesize highly valuable LC-PUFA, eventually also benefiting human health.

## 1. Introduction

Omega-3 long-chain ( $\geq 20C$ ) polyunsaturated fatty acids (n-3 LC-PUFA), in particular eicosapentaenoic acid (20:5n-3; EPA) and docosahexaenoic acid (22:6n-3; DHA), are essential structural components of cell membranes. Long-chain PUFA support somatic growth as well as cognitive traits and potentially behaviour in vertebrates, including fish (Salin et al., 2021; Tocher, 2010; Závorka et al., 2021) and humans

(Pilecky et al., 2021b). Long-chain PUFA are mainly produced by aquatic primary producers from which they are transferred and accumulated across various trophic levels (Arts et al., 2001). However, eutrophication as well as global warming are expected to favour cyanobacteria, which do not contain n-3 LC-PUFA (Galloway and Winder, 2015; Hixson and Arts, 2016), thus decreasing the availability of dietary n-3 LC-PUFA in primary producers and subsequent trophic transfer of n-3 LC-PUFA to higher trophic levels (Müller-Navarra et al., 2004).

\* Corresponding author at: WasserCluster – Biologische Station Lunz, Inter-University Center for Aquatic Ecosystem Research, Dr. Carl-Kupelwieser Promenade 5, 3293 Lunz/See, Austria.

E-mail addresses: [matthias.pilecky@donau-uni.ac.at](mailto:matthias.pilecky@donau-uni.ac.at) (M. Pilecky), [LiborZavorka@wcl.ac.at](mailto:LiborZavorka@wcl.ac.at) (L. Závorka), [katharina.winter@wcl.ac.at](mailto:katharina.winter@wcl.ac.at) (K. Winter), [Dominik.Martin-Creuzburg@b-tu.de](mailto:Dominik.Martin-Creuzburg@b-tu.de) (D. Martin-Creuzburg), [martin.kainz@donau-uni.ac.at](mailto:martin.kainz@donau-uni.ac.at) (M.J. Kainz).

<sup>1</sup> Both authors contributed equally to the manuscript.

<https://doi.org/10.1016/j.aquaculture.2022.738731>

Received 22 April 2022; Received in revised form 9 August 2022; Accepted 10 August 2022

Available online 13 August 2022

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Cyprinids, such as Common carp (*Cyprinus carpio*), belong to the most consumed fish in the world (FAO, 2018) and are typically raised in warm, eutrophic aquaculture ponds (Roy et al., 2019). Common carp raised in eutrophic aquaculture ponds is a relatively lean fish with total lipid content of ~5–10% dry weight<sup>-1</sup> in muscle tissue (Böhm et al., 2014) and a diet-dependent total n-3 PUFA content of ~5–20 mg g dry weight<sup>-1</sup> and a DHA content of ~3–10 mg g dry weight<sup>-1</sup> (Schultz et al., 2015). Experimental evidence suggests that increasing dietary DHA supply results in increasing DHA accumulation in carp muscle tissues (Schultz et al., 2015). Experimental evidence suggests that, depending on their diet, carp can adjust their *Fad6-a* and *Elovl5-a* gene expression levels (Ren et al., 2012; Monroig et al., 2022), and that they can potentially convert DHA from its precursors, i.e., EPA and potentially even  $\alpha$ -linolenic acid (18:3n-3; ALA) (Tocher, 2003; Zheng et al., 2004). However, it remains uncertain how carp raised in semi-intensive aquaculture ponds obtain and maintain their n-3 PUFA and particularly DHA contents. For example, it is unclear which dietary source is the main supply of DHA and if carp raised and exposed to a variety of diet sources is able to endogenously convert dietary PUFA to DHA, and by which proportion these processes contribute to the DHA content in the muscles.

The potentially large dichotomy between dietary DHA supply and endogenous DHA production is highly relevant for semi-intensive carp aquaculture in eutrophic ponds. Carp obtain their dietary energy and nutrients by consumption of zooplankton and benthic macro-invertebrates naturally occurring in ponds and by consumption of human-supplied cereal-based feeds (Schultz et al., 2015). Copepods are a major dietary source of DHA for carp in aquaculture ponds (Antón-Pardo and Adámek, 2015), while cladocerans and benthic invertebrates or cereal-based feeds contain only traces of DHA (Kainz et al., 2004; Schultz et al., 2015; Fehlinger et al., 2022). The addition of plant ingredients (i.e., cereals), containing less n-3 LC-PUFA, reduces the use of fish products in aquaculture (Adarme-Vega et al., 2014; Tocher et al., 2019) and is more relevant for aquaculture of non-piscivorous fish species, such as carp, which may accumulate DHA from natural food sources and/or are able to convert DHA from precursors obtained directly from the environment (Pauly and Christensen, 1995; Rodrigues et al., 2017). Thus, to maintain sufficient DHA carp must selectively retain DHA from consumed natural dietary sources (e.g., copepods and/or *Chaoborus* larvae), or convert dietary precursor PUFA (e.g., ALA or EPA) to DHA as suggested by Mráz et al. (2012).

Tracing methods, such as bulk stable isotope ratios (e.g., carbon, nitrogen, hydrogen) and fatty acid composition, are commonly used in trophic studies and food web reconstructions (Arts et al., 2009; Fry, 2006; Perga et al., 2006). However, these methods face inherent methodological limitations, such as low resolution or food resources having overlapping isotopic values (Burian et al., 2020; Twining et al., 2020). Compound-specific stable isotope analysis (CSIA) of carbon and hydrogen of fatty acids are newly developed methods in trophic studies and can help disentangle aquatic and terrestrial diet sources (Twining et al., 2020; Pilecky et al., 2021a; Mathieu-Resuge et al., 2021b). It has been shown that the essential fatty acids ALA and linoleic acid (LIN; 18:2n-6) show only little isotopic fractionation in consumers, due to the lack of de novo biosynthesis, and thus reflect the isotopic values of their diet (Twining et al., 2020; Pilecky et al., 2022). On the other hand, hydrogen stable isotopes ( $\delta^2\text{H}$ ) are susceptible to alteration if fatty acids (FA) are bioconverted, such as from ALA to EPA and further to DHA (Pilecky et al., 2022). Thus, it is expected that  $\delta^2\text{H}$  values from dietary DHA retention differ from  $\delta^2\text{H}$  obtained by metabolic conversion. Such differentiation assumes that if the isotopic hydrogen values of DHA remain unaltered between dietary and retained DHA, carp do not convert dietary PUFA to DHA. Alternatively, if DHA in carp is synthesized from dietary precursors,  $\delta^2\text{H}$  values of DHA in carp are expected to be isotopically lighter than the diet, or, if dietary DHA is absent, lighter than dietary EPA (Mathieu-Resuge et al., 2021b; Pilecky et al., 2022).

The aim of this study was to investigate how farmed carp, raised in semi-intensive aquaculture ponds and fed cereal-based feeds (terrestrial

diet), emergent insects, and zooplankton in natural abundances, obtain LC-PUFA, particularly EPA and DHA. We applied bulk stable isotope and FA analyses as well as CSIA of carbon and hydrogen to assess diet sources and the origin of n-3 LC-PUFA in carp muscle tissue.

## 2. Methods

### 2.1. Field sampling

Samples of potential food sources were taken from 8 fishponds (each ~1 m deep) around Waidhofen/Thaya, Austria (48° 49' N, 15° 17' E, 510 m), from June to September 2020. We collected cereal-based carp feeds, small plankton ( $\leq 30 \mu\text{m}$ ; particle size fraction edible for zooplankton, Burns, 1968), larger-sized zooplankton ( $\geq 500 \mu\text{m}$ ; zooplankton size fraction, Anton-Pardo and Adámek, 2015), emergent insect, and fish samples used for commercial fish farming.

For seston, three replicates were taken from each pond at different spots using a Schindler trap. Pond water was first prefiltered through a 30  $\mu\text{m}$  sieve and subsequently filtered through pre-weighed, muffled GF/C filters (1.2  $\mu\text{m}$ ) until they clogged. Triplicates of zooplankton samples were taken with a zooplankton net (500  $\mu\text{m}$  mesh size). The net was submerged to ~1 m below the pond surface to sample a representative volume of the water column and slowly towed for a few minutes. For each replicate, zooplankton were size separated ( $\geq 500 \mu\text{m}$ , 100–250  $\mu\text{m}$ , and  $< 100 \mu\text{m}$ ), put in sterile falcon tubes (50 mL), and subsequently frozen until further analyses. In 6 of the 8 ponds, zooplankton also contained Chaoboridae (*Chaoborus* spp.) larvae. Chironomidae, which were by far the most abundant group, were caught in emergence traps placed one week before the sampling, and immediately frozen after collecting. Three samples of both artificial foods, pellets and cereals have been taken during the sampling period. All samples were frozen at  $-80 \text{ }^\circ\text{C}$ , freeze dried (Genesis Freeze dryer, Virtis, NYC) and stored at  $-20 \text{ }^\circ\text{C}$  until further processing. Zooplankton was homogenized before analysis to obtain a representative sample as potential fish diet.

### 2.2. Carp in semi-intensive aquaculture ponds

During the first couple of months after hatching, young carp are fed with pellets, enriched with fish oil rich in n-3 LC-PUFA. Once they are released into the ponds, they are fed with cereals (mixture of triticale, rye, peas, maize, and barley), which contain only traces of n-3 LC-PUFA, until harvesting. In addition to pellets, carp also have access to pond zooplankton and benthic invertebrates. Five adult fish (from 1 to 2 yo) from each pond were provided together with fish feed (i.e., cereals) for analysis at the beginning of the harvest period in October. Fish were measured and weighed, and a sample of the dorsal muscle tissue was taken for further analyses. All fish samples were frozen at  $-80 \text{ }^\circ\text{C}$ , freeze dried, and stored at  $-20 \text{ }^\circ\text{C}$  until further processing.

### 2.3. Elemental analysis and bulk stable isotope

Freeze-dried and homogenized samples of emergent insects (~ 0.4 mg;  $n = 3$  per pond/month;  $n_{\text{total}} = 96$ ), zooplankton (~ 0.4 mg;  $n = 3$  of the 500  $\mu\text{m}$  size fraction per pond/month;  $n_{\text{total}} = 96$ ), seston (half of the filter  $>0.5 \text{ mg}$ ;  $n = 3$  per pond/month;  $n_{\text{total}} = 96$ ), fish (~ 0.4 mg;  $n = 5$  per pond in October;  $n_{\text{total}} = 24$ ) and artificial food (i.e., cereals, ~ 0.4 mg;  $n = 3$  of each one;  $n_{\text{total}} = 6$ ) were put into tin capsules (IVA Analysetechnik, Meerbusch, Germany). Their bulk stable isotope ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) values were quantified using an A flash HT Plus CNSOH elemental analyzer interfaced with a Conflo IV device (Thermo Co., Bremen, Germany) to a continuous flow stable isotope ratio mass spectrometer (Delta V Advantage IRMS, Thermo Co.). Values were normalized against reference gas injections of  $\text{N}_2$  and  $\text{CO}_2$  (Messer, Krefeld, Germany) and standardized using international standards IAEA-N-1, and IAEA-N-2 for nitrogen, and USGS24, and IAEA-CH-7 (IAEA, Vienna, Austria) for

carbon.

#### 2.4. Gas chromatography (GC) and stable isotope ratio mass spectrometry (IRMS)

Lipids were extracted according to Heissenberger et al. (2010). In brief, freeze-dried samples (~5 mg of each sample: seston, zooplankton, emergent insects, fish and food) were homogenized and mixed with chloroform:methanol (2:1 vol/vol) following sonication, vortexing and centrifuging 3 times to remove nonlipid materials. Extracted lipids were evaporated to a final volume of 1.5 mL under N<sub>2</sub>. For FAME formation, samples were incubated with sulfuric acid:methanol (1:100 vol/vol) for 16 h at 50 °C, following addition of KHCO<sub>3</sub> and hexane. Samples were shaken, vortexed and centrifuged and the upper organic layers collected, pooled and concentrated under N<sub>2</sub>.

Fatty acid methyl esters (FAME) were analysed using a gas chromatograph (TRACE GC, Thermo Co., Detector: FID 260 °C, Carrier gas: He: 1 mL/min, Detector gases: H<sub>2</sub>: 40 mL/min, N<sub>2</sub>: 45 mL/min, air: 450 mL/min, temperature ramp: 140 °C (5 min) – 4 °C min<sup>-1</sup>–240 °C (20 min) = 50 min) equipped with a temperature-programmable injector and an autosampler. A Supelco SP-2560 column (100 m, 25 mm i.d., 0.2 µm film thickness) was used for FAME separation. Chromeleon 7 was used for calculation and, if necessary, manual resetting of the chromatograms. FAME were identified and quantified by comparison of their retention times and signal areas to the concentrations of known standards (37-component FAME mix, 7-point standard curves, 47,885-U, Supelco; Sigma-Aldrich, Bellefonte, Pennsylvania). FAME concentrations are reported as mg g<sup>-1</sup> dry weight.

<sup>13</sup>C- and <sup>2</sup>H-CSIA was performed using a Thermo Trace 1310 GC (ThermoFisher Scientific, Waltham, MA), which was connected via a ConFlo IV (ThermoFisher Scientific) to an Isotope Ratio Mass Spectrometer (IRMS, DELTA V Advantage, ThermoFisher Scientific). FAMES were separated using either a VF-WAXms 60m column, 0.25 mm ID, film thickness 0.25 µm; or a VF-WAXms 30m column, 0.32mm ID, film thickness 1 µm (both Agilent, Santa Clara, CA) and then for <sup>13</sup>C analysis oxidized to CO<sub>2</sub> in a combustion reactor, filled with Ni, Pt and Cu wires, at a temperature of 1000 °C, or for <sup>2</sup>H analysis reduced to H<sub>2</sub> by passing through a high thermal conversion reactor kept at 1200 °C. The reactors were oxidized, respectively conditioned using 2 × 1 µL hexane before each sequence (40–60 samples) following He-flushing for 1 h and measurement of 6 standards before running samples. After 15 samples, standards were measured again to ensure system stability.

The injector temperature was kept at 250 °C. Up to a maximum volume of 3.5 µL of sample were injected in spitless mode, following activation of purge flow after 1 min. The injection volumes were adjusted in order to obtain amplitudes between 300 and 8000 mV for all peaks of interest. The temperature program for the 60 m GC column started at 80 °C, which was kept for 2 min, after which the temperature was raised by 30 °C min<sup>-1</sup> to 175 °C, by 5 °C min<sup>-1</sup> to 200 °C and finally by 2.4 °C min<sup>-1</sup> to 250 °C, which was maintained for 30 min. The total run time was 62 min. The temperature program for the 30 m GC column started at 80 °C, which was kept for 2 min, after which the temperature was raised by 30 °C min<sup>-1</sup> to 175 °C, and then by 5 °C min<sup>-1</sup> to 240 °C, which was held for 35 min. The total run time was 52 min. For <sup>2</sup>H measurements, H<sub>3</sub><sup>-</sup>-factor determination has been performed before and after each measurement sequence using a dilution series of reference gas. The factor was stable in course of the whole study, which was additionally validated using dilution series of samples.

Samples were run against certified Me-C20:0 standards (USGS70: <sup>13</sup>C = -30.53‰, <sup>2</sup>H = -183.9‰, USGS71: <sup>13</sup>C = -10.5‰, <sup>2</sup>H = -4.9‰ and USGS72: <sup>13</sup>C = -1.54‰, <sup>2</sup>H = +348.3‰), which were used for drift and linear correction. The <sup>13</sup>C and <sup>2</sup>H value of individual FAME were determined by automated integration, defining 0.5 mV/s as start and end point of a peak and using individual background values. All peaks were validated and corrected manually if necessary. FA <sup>13</sup>C/<sup>2</sup>H values ( $\delta I_{FA}$ ) were corrected for the methyl group addition during

methylation according to the formula

$$\delta I_{FA} = \frac{(n+1) * (\delta I_{FAME} - \delta I_{MeOH})}{n}$$

where  $\delta I_{FAME}$  are the <sup>2</sup>H or <sup>13</sup>C values of the measured FAME and  $\delta I_{MeOH}$  the <sup>2</sup>H or <sup>13</sup>C values of the methanol used during methylation and  $n$  equals the total number of H-/C-atoms of the FAME molecule. Values for <sup>13</sup>C are referenced to Vienna PeeDee Belemite (<sup>13</sup>C:<sup>12</sup>C = 0.01118)

$$\delta^{13}C_{FA} = \left( \frac{^{13}C/^{12}C_{Sample}}{^{13}C/^{12}C_{VPDB}} - 1 \right) \times 1000$$

Values for <sup>2</sup>H are standardized against Vienna Standard Mean Ocean Water (<sup>2</sup>H:<sup>1</sup>H = 155.76 ppm)

$$\delta^2H_{FA} = \left( \frac{^2H/^1H_{Sample}}{^2H/^1H_{VSMOW}} - 1 \right) \times 1000$$

16:1n-7 and 16:1n-9, as well as 18:1n-7 and 18:1n-9 coeluted on the column, therefore their isotopic values were simultaneously analysed and reported as  $\Sigma 16:1$  and  $\Sigma 18:1$ .

#### 2.5. Data analysis

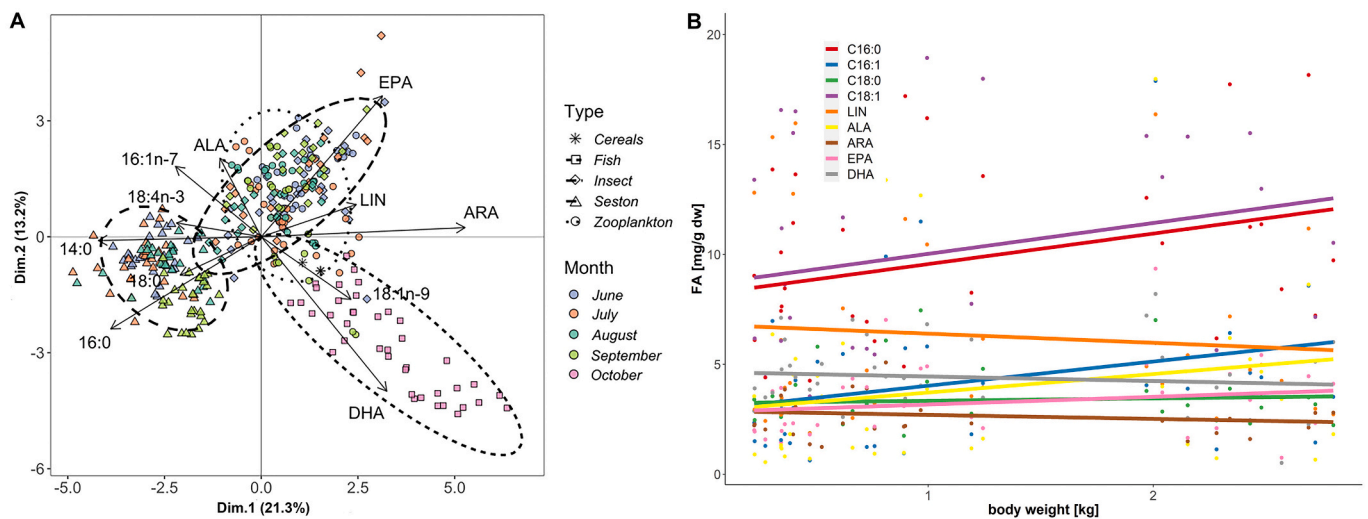
Data analysis and graphics design were performed in R (Version 4.1.0) using the packages rstatix, ggplot2, ggpubr, multcomp, rcompanion, RVAideMemoir, FactoMineR and lme4. Values were presented as means ± standard deviation. Normality was tested using Shapiro-Wilks test. Principal component analyses (PCA) were performed to investigate the variation in FA composition (%) among fish and their potential food sources. To prevent excessive weight of rare species, Euclidean distances were applied on FA contents (Legendre and Gallagher, 2001), and a test of similarity percentages analysis (SIMPER) was carried out to assess the most discriminant FA responsible for the difference (>80%) among the groups (i.e., fish, seston, zooplankton, emergent insects, and cereals). Ellipses, showing 95% CI for each of these groups were added for visualization. Paired *t*-test has been used for direct comparison of dietary and consumer compound specific values. Multiple group comparison, using individual sample time points and ponds as factors, was performed using Scheirer-Ray-Hare (H) test following Dunn's multiple comparison. Pearson method was used for linear correlation analysis. Differences in dual isotopic compositions were tested by MANOVA following pairwise comparison by permutation and Pillai post-hoc test. Differences in single isotopic compositions were tested by ANOVA following Tukey post-hoc test, while accounting for pond differences.

### 3. Results

#### 3.1. Fatty acids composition of fish and its potential diets

Principal component analysis (PCA) clearly separated carp from zooplankton and emergent insects as well as from seston (mostly phytoplankton) based on their fatty acid composition (Fig. 1). Fish were richer in ARA, DHA, LIN and 22:5n-3, while seston had high levels of saturated fatty acids (SFA) and 18:4n-3 (SDA). Bulk zooplankton and emergent insects had similar FA compositions and seemed more enriched in EPA and 16:1n-7 compared to seston and fish.

Seston FA mass fractions fluctuated over the course of the field study. EPA (H<sub>3,64</sub> = 21.709,  $p < 0.0001$ ) and DHA (H<sub>3,64</sub> = 28.249,  $P < 0.0001$ ) levels of seston were significantly higher in September (Dunn post-hoc) than in other months. Mass fractions of FA of Chironomidae did not change significantly during the course of the sampling, except for EPA (H<sub>3,48</sub> = 16.519,  $P = 0.0008$ ), which was higher in June compared to the other months (Dunn post-hoc). In zooplankton, SDA (H<sub>3,54</sub> = 16.878,  $P$



**Fig. 1.** (A) Principal Component Analyses (PCA) of fatty acid (FA) compositions (mass %) of seston, emergent insects, zooplankton, carp, and fish feed (i.e., cereals). Only FA that account for >80% of the contribution to dissimilarity between taxa are shown. (B) Mass fractions of FA, except 16:0 and 18:1 in carp muscle tissue were not significantly correlating with weight and levels of DHA remained stable. A slight positive trend was observed for ALA and EPA.

= 0.0007), EPA ( $H_{3,54} = 18.946$ ,  $P = 0.0003$ ) and docosapentaenoic acid (DPA, 22:5n-3;  $H_{3,54} = 8.059$ ,  $P = 0.045$ ) were higher in September compared to the other months (Dunn post-hoc). While the FA mass fractions of emergent insects did not significantly differ among ponds, FA profiles of zooplankton varied from pond to pond, according to its taxonomic composition (cladocerans: Chaoboridae larvae; copepods; relative biomass; data not shown), which was particularly evident in ALA ( $H_{7,54} = 26.480$ ,  $P = 0.0004$ ), LIN ( $H_{7,54} = 19.551$ ,  $P = 0.00662$ ), SDA ( $H_{7,54} = 19.291$ ,  $P = 0.0073$ ), and DHA ( $H_{7,54} = 19.187$ ,  $P = 0.0076$ ). Cereal-based carp feed contained mainly C18 FA, such as 18:1n-9 (~ 1.4 mg g<sup>-1</sup>), LIN (~ 7.5 mg g<sup>-1</sup>) and ALA (~ 1.2 mg g<sup>-1</sup>). Fish muscle tissues did not significantly vary in any PUFA mass fractions among ponds (Table 1).

Regarding fish muscle tissue, a significant increase in FA mass fractions of  $\sum 16:1$  and  $\sum 18:1$  in proportion to the fish body weight could be observed (Pearson,  $r(38) = 0.27$ ,  $P = 0.004$  and  $r(38) = 0.21$ ,  $P = 0.008$ ; respectively), while no significant changes could be observed for other FA including EPA and DHA.

### 3.2. Isotopic composition of fish, potential diet sources and their fatty acids

Bulk carbon and nitrogen stable isotopes showed a clear separation of fish feed and seston from other sample types (Fig. 2A, MANOVA,  $F_{5,282} = 46.3$ ,  $P < 0.001$ , Pillai post-hoc). However, the separation among fish, insects, and zooplankton was less clear and only significant between fish and zooplankton (Pillai,  $P = 0.0415$ ). Overall, the bulk stable isotope values of zooplankton, insects and fish were similar in both  $\delta^{13}\text{C}$  ( $-29.77 \text{‰} \pm 3.41$  vs.  $-28.06 \text{‰} \pm 3.73$  vs.  $-27.79 \text{‰} \pm 1.22$  respectively; Tukey,  $P > 0.05$ ) and  $\delta^{15}\text{N}$  ( $8.74 \text{‰} \pm 1.46$  vs.  $9.30 \text{‰} \pm 2.67$  vs.  $9.21 \text{‰} \pm 1.52$ , respectively; Tukey,  $P > 0.05$ ) (Fig. 2A). The  $\delta^{15}\text{N}$  values of the cereal-based feed were significantly lower than those of all other sample types (Tukey,  $P < 0.001$ ), including seston, whose  $\delta^{15}\text{N}$  value spanned between 1.98 ‰ and 9.64 ‰ (Fig. 2A). Fish did not present any differences in  $\delta^{13}\text{C}$  values among ponds (ANOVA,  $F_{7,16} = 2.42$ ,  $P = 0.068$ ), showing values ranging from  $-30.6 \text{‰} \pm 1.5$  to  $-25.8 \text{‰} \pm 0.7$ . However,  $\delta^{15}\text{N}$  values of carp were significantly different among ponds (ANOVA,  $F_{7,16} = 23.64$ ,  $P < 0.001$ ), ranging from  $6.41 \text{‰} \pm 0.10$  in pond Kiebitzteich to  $11.15 \text{‰} \pm 0.42$  in pond Herrenteich.

MANOVA revealed a similar isotopic composition between  $\delta^{13}\text{C}$  and  $\delta^2\text{H}$  of FA in fish and insects, which only differed in 18:0 (MANOVA,  $F_{4,154} = 81.0$ ,  $p < 0.001$ ; Pillai,  $P < 0.001$ ), 18:1 (MANOVA,  $F_{5,218} =$

$135.0$ ,  $P < 0.001$ ; Pillai,  $P = 0.013$ ) and EPA (MANOVA,  $F_{4,161} = 4.2$ ,  $P = 0.047$ , Pillai,  $P = 0.008$ ). Fish and zooplankton had similar  $\delta^{13}\text{C}$  and  $\delta^2\text{H}$  values in ALA, while EPA (Pillai:  $P = 0.012$ ) and DHA (MANOVA,  $F_{3,134} = 153.8$ ,  $P < 0.001$ ; Pillai:  $P = 0.058$ ) differed significantly (Fig. 2B).

Isotopic composition of PUFA showed taxa specific differences of  $\delta^2\text{H}_{\text{LIN}}$  (ANOVA,  $F_{5,375} = 136.5$ ,  $P < 0.001$ ),  $\delta^2\text{H}_{\text{ALA}}$  (ANOVA,  $F_{5,375} = 10.0$ ,  $P < 0.001$ ),  $\delta^2\text{H}_{\text{EPA}}$  (ANOVA,  $F_{4,367} = 67.6$ ,  $P < 0.001$ ) and  $\delta^2\text{H}_{\text{DHA}}$  (ANOVA,  $F_{4,253} = 17.8$ ,  $P < 0.001$ ), as well as in  $\delta^{13}\text{C}_{\text{LIN}}$  (ANOVA,  $F_{5,109} = 21.0$ ,  $P < 0.001$ ),  $\delta^{13}\text{C}_{\text{ALA}}$  (ANOVA,  $F_{5,109} = 5.3$ ,  $P < 0.001$ ),  $\delta^{13}\text{C}_{\text{EPA}}$  (ANOVA,  $F_{4,109} = 14.1$ ,  $P < 0.001$ ) and  $\delta^{13}\text{C}_{\text{DHA}}$  (ANOVA,  $F_{4,79} = 10.3$ ,  $P < 0.001$ ). The isotopic composition of LIN of fish muscle ( $\delta^2\text{H}$ :  $-196.4 \text{‰} \pm 21.1$ ;  $\delta^{13}\text{C}$ :  $-32.9 \text{‰} \pm 4.4$ ) resembled that of cereal feed ( $\delta^2\text{H}$ :  $-240.4 \text{‰} \pm 2.5$ ;  $\delta^{13}\text{C}$ :  $-31.7 \text{‰} \pm 0.2$ , Tukey,  $P = 0.11$  and  $P = 0.93$ ) and chironomids ( $\delta^2\text{H}$ :  $-194.8 \text{‰} \pm 41.6$ ;  $\delta^{13}\text{C}$ :  $-32.7 \text{‰} \pm 3.0$ , Tukey,  $P = 0.16$  and  $P = 0.99$ ), while diverging from zooplankton ( $\delta^2\text{H}$ :  $-80.6 \text{‰} \pm 35.7$ ;  $\delta^{13}\text{C}$ :  $-36.4 \text{‰} \pm 3.0$ , Tukey, both  $P < 0.001$ ).  $\delta^2\text{H}_{\text{EPA}}$  of fish ( $-258.6 \text{‰} \pm 19.3$ ) resembled both chironomids ( $-258.3 \text{‰} \pm 23.9$ , Tukey,  $P = 0.99$ ) and zooplankton ( $-268.6 \text{‰} \pm 28.3$ , Tukey,  $P = 0.77$ ), while their  $\delta^{13}\text{C}_{\text{EPA}}$  values ( $-33.4 \text{‰} \pm 1.4$ ) were different, lying between insects ( $-31.3 \text{‰} \pm 3.3$ , Tukey,  $P = 0.005$ ) and zooplankton ( $-36.9 \text{‰} \pm 3.6$ , Tukey,  $P < 0.001$ ). Chironomids did not contain DHA.  $\delta^2\text{H}_{\text{DHA}}$  of fish were slightly but not significantly lower than of zooplankton ( $-239.1 \text{‰} \pm 18.8$  vs.  $-206.9 \text{‰} \pm 25.5$ , Tukey,  $P = 0.11$ ), while  $\delta^{13}\text{C}_{\text{DHA}}$  values were significantly higher ( $-34.6 \text{‰} \pm 1.4$  vs.  $-36.5 \text{‰} \pm 3.2$ , Tukey,  $P < 0.001$ ). Additionally, the difference of  $\delta^2\text{H}_{\text{DHA}}$  values between fish muscle tissue and zooplankton correlated significantly with the mass fraction of DHA in bulk zooplankton samples (Pearson,  $r(6) = 0.72$ ,  $P = 0.04$ ; Fig. 2C).

## 4. Discussion

This study highlights that farmed carp obtain PUFA from macro-invertebrates and zooplankton naturally abundant in carp ponds. Bulk  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values indicated that human-supplied cereal feed are an important source of dietary energy and nitrogen for carp. Additionally, both  $\delta^{13}\text{C}_{\text{LIN}}$  and  $\delta^2\text{H}_{\text{LIN}}$  values of carp were very similar to those of cereals, indicating that carp assimilated a substantial part of these essential n-6 PUFA from cereal-based fish feed. However, when looking at n-3 and n-6 LC-PUFA, the isotopic values of EPA and ARA suggested zooplankton and benthic macroinvertebrates as the main source of these essential micronutrients. Carp obtained DHA mainly by feeding on

Table 1

Fatty acid mass fractions [mean µg/mg dw ± standard deviation] of pooled seston, feed, zooplankton, insects, and fish in the study ponds.

|             | Pond                      | 14:0       | 16:0        | 16:1n-7    | 16:1n-9   | 18:0      | 18:1n-7   | 18:1n-9    | LIN        | GLA        | ALA        | SDA        | 20:1n-9   | 20:3n-6   | ARA       | 20:3n-3   | ETA       | EPA        | DPA       | DHA       |           |
|-------------|---------------------------|------------|-------------|------------|-----------|-----------|-----------|------------|------------|------------|------------|------------|-----------|-----------|-----------|-----------|-----------|------------|-----------|-----------|-----------|
| Seston      | Dach                      | 0.6 ± 0.2  | 2.4 ± 0.9   | 0.8 ± 0.3  | 0.3 ± 0.2 | 1.1 ± 0.5 | 0.2 ± 0.1 | 0.7 ± 0.3  | 0.3 ± 0.2  | 0.0 ± 0.0  | 0.8 ± 0.4  | 0.3 ± 0.3  | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.1 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.4 ± 0.2  | 0.0 ± 0.0 | 0.3 ± 0.2 |           |
|             | Eng                       | 0.5 ± 0.3  | 5.8 ± 2.1   | 0.6 ± 0.3  | 0.5 ± 0.4 | 1.9 ± 1.4 | 0.3 ± 0.1 | 3.5 ± 2.4  | 0.9 ± 0.5  | 0.1 ± 0.1  | 2.9 ± 0.5  | 1.8 ± 1.3  | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.0 ± 0.0 | 0.1 ± 0.0 | 0.9 ± 0.5  | 0.0 ± 0.1 | 0.3 ± 0.2 |           |
|             | Ger                       | 1.2 ± 0.7  | 6.0 ± 2.7   | 1.9 ± 1.2  | 0.5 ± 0.1 | 1.1 ± 0.5 | 0.4 ± 0.2 | 1.6 ± 0.6  | 1.0 ± 0.7  | 0.1 ± 0.0  | 3.8 ± 2.3  | 1.6 ± 1.9  | 0.0 ± 0.0 | 0.2 ± 0.2 | 0.1 ± 0.1 | 0.0 ± 0.0 | 0.1 ± 0.0 | 1.2 ± 1.0  | 0.0 ± 0.0 | 0.5 ± 0.4 |           |
|             | Herr                      | 0.9 ± 0.7  | 4.0 ± 1.8   | 1.8 ± 1.4  | 0.5 ± 0.1 | 1.0 ± 0.9 | 0.3 ± 0.1 | 1.4 ± 0.3  | 0.6 ± 0.3  | 0.1 ± 0.0  | 1.7 ± 0.5  | 0.6 ± 0.4  | 0.0 ± 0.0 | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.9 ± 0.5  | 0.0 ± 0.0 | 0.3 ± 0.2 |           |
|             | Jag                       | 0.5 ± 0.2  | 3.3 ± 2.3   | 0.8 ± 0.4  | 0.3 ± 0.2 | 1.2 ± 1.0 | 0.3 ± 0.2 | 0.9 ± 0.4  | 0.5 ± 0.3  | 0.0 ± 0.0  | 2.0 ± 2.0  | 1.3 ± 2.3  | 0.1 ± 0.2 | 0.1 ± 0.2 | 0.1 ± 0.0 | 0.0 ± 0.1 | 0.0 ± 0.0 | 0.8 ± 1.1  | 0.0 ± 0.0 | 0.3 ± 0.5 |           |
|             | Kieb                      | 0.4 ± 0.2  | 1.8 ± 1.1   | 0.5 ± 0.2  | 0.2 ± 0.1 | 0.9 ± 0.5 | 0.1 ± 0.1 | 0.3 ± 0.2  | 0.2 ± 0.2  | 0.0 ± 0.0  | 0.6 ± 0.6  | 0.3 ± 0.3  | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.2 ± 0.2  | 0.0 ± 0.0 | 0.1 ± 0.1 |           |
|             | Furt                      | 1.2 ± 0.7  | 3.0 ± 1.5   | 1.0 ± 0.4  | 0.3 ± 0.2 | 1.1 ± 0.4 | 0.3 ± 0.2 | 0.7 ± 0.6  | 0.6 ± 0.4  | 0.1 ± 0.0  | 1.7 ± 1.0  | 0.7 ± 0.8  | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.2 ± 0.2 | 0.0 ± 0.0 | 0.1 ± 0.0 | 0.8 ± 0.4  | 0.0 ± 0.0 | 0.6 ± 0.4 |           |
|             | Stadt                     | 0.8 ± 0.4  | 3.3 ± 1.3   | 1.9 ± 1.3  | 0.3 ± 0.1 | 1.2 ± 0.8 | 0.3 ± 0.1 | 0.9 ± 0.6  | 0.4 ± 0.2  | 0.0 ± 0.0  | 0.9 ± 0.4  | 0.3 ± 0.1  | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.1 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.3 ± 0.2  | 0.0 ± 0.0 | 0.1 ± 0.1 |           |
|             | cereals                   | 0.0 ± 0.0  | 2.1 ± 0.3   | 0.0 ± 0.0  | 0.0 ± 0.0 | 0.2 ± 0.0 | 0.1 ± 0.1 | 1.4 ± 1.1  | 7.5 ± 1.0  | 0.0 ± 0.0  | 1.2 ± 0.2  | 0.0 ± 0.0  | 0.1 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0  | 0.0 ± 0.0 | 0.0 ± 0.0 |           |
|             | Feed pellets <sup>a</sup> | 0.6 ± 0.0  | 9.9 ± 0.6   | 1.0 ± 0.1  | 0.1 ± 0.0 | 1.9 ± 0.1 | 1.6 ± 0.1 | 25.5 ± 1.3 | 23.8 ± 1.5 | 0.0 ± 0.0  | 2.6 ± 0.1  | 0.0 ± 0.0  | 0.1 ± 0.1 | 0.1 ± 0.0 | 0.1 ± 0.0 | 0.1 ± 0.0 | 0.1 ± 0.0 | 0.1 ± 0.0  | 0.5 ± 0.0 | 0.2 ± 0.0 | 0.7 ± 0.0 |
|             | Zooplankton               | Dach       | 2.6 ± 1.7   | 10.9 ± 3.4 | 3.1 ± 1.5 | 0.7 ± 0.3 | 2.6 ± 1.0 | 1.5 ± 0.6  | 5.6 ± 3.0  | 2.5 ± 0.8  | 0.3 ± 0.1  | 6.4 ± 1.9  | 2.2 ± 1.4 | 0.0 ± 0.1 | 0.3 ± 0.1 | 2.4 ± 1.0 | 0.1 ± 0.1 | 0.4 ± 0.2  | 7.4 ± 2.3 | 0.3 ± 0.2 | 4.6 ± 3.3 |
|             |                           | Eng        | 1.4 ± 0.5   | 14.5 ± 4.6 | 2.3 ± 1.1 | 2.2 ± 1.2 | 3.3 ± 1.1 | 2.0 ± 0.7  | 12.9 ± 5.1 | 4.9 ± 2.0  | 0.5 ± 0.2  | 16.4 ± 4.6 | 7.8 ± 4.4 | 0.3 ± 0.4 | 0.2 ± 0.1 | 1.9 ± 0.6 | 0.2 ± 0.2 | 0.8 ± 0.4  | 8.7 ± 4.0 | 0.2 ± 0.2 | 2.6 ± 2.2 |
| Ger         |                           | 0.9 ± 0.2  | 7.7 ± 3.0   | 2.2 ± 0.6  | 0.9 ± 0.7 | 2.0 ± 0.5 | 1.4 ± 0.9 | 5.2 ± 3.9  | 2.4 ± 1.2  | 0.2 ± 0.1  | 6.3 ± 4.3  | 1.9 ± 1.6  | 0.0 ± 0.0 | 0.1 ± 0.0 | 1.3 ± 0.5 | 0.1 ± 0.2 | 0.5 ± 0.2 | 6.1 ± 2.6  | 0.4 ± 0.2 | 2.8 ± 1.6 |           |
| Herr        |                           | 2.2 ± 1.3  | 13.3 ± 4.0  | 8.1 ± 5.4  | 2.2 ± 1.3 | 3.1 ± 1.2 | 2.6 ± 0.9 | 9.7 ± 5.3  | 3.8 ± 1.6  | 0.5 ± 0.2  | 11.9 ± 6.3 | 3.3 ± 1.2  | 0.1 ± 0.1 | 0.2 ± 0.1 | 2.0 ± 0.6 | 0.2 ± 0.2 | 0.5 ± 0.2 | 10.7 ± 3.9 | 0.2 ± 0.1 | 0.8 ± 0.9 |           |
| Jag         |                           | 1.1 ± 0.7  | 12.3 ± 7.1  | 3.0 ± 3.2  | 1.8 ± 0.9 | 2.8 ± 1.6 | 2.1 ± 1.1 | 9.1 ± 5.3  | 3.6 ± 1.8  | 0.4 ± 0.3  | 10.5 ± 8.3 | 4.4 ± 3.7  | 0.1 ± 0.1 | 0.2 ± 0.1 | 1.8 ± 0.6 | 0.2 ± 0.2 | 0.7 ± 0.5 | 8.1 ± 5.2  | 0.1 ± 0.1 | 1.0 ± 0.8 |           |
| Kieb        |                           | 1.7 ± 0.7  | 11.6 ± 4.5  | 4.8 ± 1.8  | 1.0 ± 0.5 | 2.7 ± 1.2 | 2.1 ± 0.6 | 4.9 ± 2.1  | 3.1 ± 1.3  | 0.4 ± 0.1  | 8.6 ± 4.0  | 3.3 ± 3.2  | 0.0 ± 0.0 | 0.2 ± 0.2 | 2.1 ± 0.7 | 0.1 ± 0.1 | 0.5 ± 0.2 | 8.1 ± 2.9  | 0.2 ± 0.2 | 2.3 ± 1.8 |           |
| Furt        |                           | 2.1 ± 1.1  | 7.1 ± 4.7   | 3.2 ± 3.8  | 0.5 ± 0.5 | 1.9 ± 1.0 | 1.8 ± 1.5 | 2.9 ± 2.2  | 1.6 ± 1.1  | 0.3 ± 0.3  | 5.3 ± 3.9  | 1.9 ± 2.4  | 0.0 ± 0.0 | 0.2 ± 0.1 | 1.8 ± 1.3 | 0.0 ± 0.1 | 0.4 ± 0.2 | 4.8 ± 2.8  | 0.2 ± 0.1 | 2.0 ± 1.7 |           |
| Stadt       |                           | 2.5 ± 1.3  | 12.2 ± 5.5  | 8.4 ± 4.4  | 1.4 ± 0.9 | 3.1 ± 1.4 | 3.0 ± 1.8 | 6.5 ± 3.2  | 4.0 ± 1.9  | 0.6 ± 0.3  | 8.3 ± 4.1  | 2.3 ± 1.2  | 0.0 ± 0.0 | 0.2 ± 0.1 | 2.8 ± 1.5 | 0.1 ± 0.1 | 0.4 ± 0.2 | 7.5 ± 3.0  | 0.2 ± 0.1 | 1.4 ± 1.4 |           |
| Dach        |                           | 1.4 ± 1.1  | 9.8 ± 5.0   | 2.4 ± 1.1  | 0.2 ± 0.1 | 3.9 ± 1.2 | 1.6 ± 0.7 | 7.0 ± 3.4  | 6.3 ± 4.1  | 0.2 ± 0.2  | 4.1 ± 1.8  | 0.7 ± 0.8  | 0.0 ± 0.0 | 0.1 ± 0.0 | 1.4 ± 0.6 | 0.0 ± 0.0 | 0.1 ± 0.1 | 5.2 ± 2.3  | 0.1 ± 0.1 | 0.3 ± 0.4 |           |
| Eng         |                           | 1.2 ± 0.8  | 11.9 ± 10.2 | 3.2 ± 2.3  | 0.4 ± 0.4 | 5.0 ± 3.3 | 2.1 ± 1.4 | 6.2 ± 4.2  | 6.8 ± 1.8  | 0.4 ± 0.4  | 7.3 ± 7.6  | 1.2 ± 2.2  | 0.0 ± 0.0 | 0.0 ± 0.0 | 1.2 ± 0.4 | 0.0 ± 0.0 | 0.2 ± 0.1 | 5.7 ± 3.4  | 0.0 ± 0.0 | 0.0 ± 0.0 |           |
| Ger         |                           | 2.3 ± 2.0  | 14.8 ± 6.2  | 6.0 ± 5.7  | 0.5 ± 0.3 | 5.3 ± 2.0 | 2.7 ± 2.1 | 6.6 ± 2.1  | 7.4 ± 2.4  | 0.3 ± 0.2  | 5.4 ± 2.6  | 0.6 ± 0.2  | 0.0 ± 0.0 | 0.1 ± 0.1 | 1.2 ± 0.4 | 0.1 ± 0.1 | 0.2 ± 0.1 | 4.8 ± 1.6  | 0.0 ± 0.0 | 0.1 ± 0.2 |           |
| Herr        |                           | 1.2 ± 0.6  | 10.0 ± 4.9  | 3.6 ± 2.6  | 0.4 ± 0.3 | 4.4 ± 2.4 | 3.0 ± 1.9 | 5.7 ± 3.3  | 5.6 ± 2.1  | 0.3 ± 0.2  | 5.5 ± 3.0  | 0.7 ± 0.5  | 0.0 ± 0.0 | 0.0 ± 0.0 | 1.3 ± 0.9 | 0.1 ± 0.1 | 0.1 ± 0.1 | 6.0 ± 3.0  | 0.0 ± 0.1 | 0.1 ± 0.1 |           |
| Jag         | 1.2 ± 0.7                 | 10.5 ± 6.8 | 3.5 ± 2.3   | 0.3 ± 0.2  | 4.2 ± 2.3 | 2.7 ± 1.7 | 5.6 ± 3.4 | 5.9 ± 2.0  | 0.3 ± 0.1  | 5.6 ± 3.1  | 0.7 ± 0.4  | 0.0 ± 0.0  | 0.1 ± 0.0 | 1.0 ± 0.6 | 0.0 ± 0.1 | 0.1 ± 0.0 | 5.0 ± 2.2 | 0.0 ± 0.0  | 0.1 ± 0.2 |           |           |
| Kieb        | 1.4 ± 0.9                 | 11.9 ± 6.0 | 2.9 ± 1.5   | 0.3 ± 0.2  | 5.2 ± 3.1 | 1.8 ± 0.8 | 5.8 ± 3.0 | 7.7 ± 3.3  | 0.2 ± 0.1  | 11.4 ± 7.3 | 0.4 ± 0.2  | 0.0 ± 0.0  | 0.1 ± 0.1 | 1.4 ± 0.7 | 0.1 ± 0.1 | 0.2 ± 0.1 | 6.0 ± 4.0 | 0.0 ± 0.1  | 0.3 ± 1.0 |           |           |
| Furt        | 2.0 ± 1.4                 | 14.7 ± 6.6 | 3.5 ± 1.8   | 0.4 ± 0.3  | 5.8 ± 2.7 | 3.3 ± 1.2 | 6.4 ± 3.4 | 6.7 ± 2.3  | 0.3 ± 0.2  | 7.4 ± 4.9  | 0.6 ± 0.3  | 0.0 ± 0.0  | 0.1 ± 0.0 | 1.5 ± 0.9 | 0.1 ± 0.1 | 0.2 ± 0.1 | 4.7 ± 3.1 | 0.0 ± 0.0  | 0.1 ± 0.0 |           |           |
| Insect Fish | 1.0 ± 0.5                 | 9.1 ± 4.0  | 2.7 ± 1.7   | 0.3 ± 0.2  | 3.8 ± 1.7 | 2.4 ± 1.6 | 4.9 ± 2.2 | 5.3 ± 2.6  | 0.2 ± 0.2  | 3.9 ± 2.2  | 0.5 ± 0.7  | 0.0 ± 0.0  | 0.1 ± 0.0 | 1.5 ± 0.8 | 0.0 ± 0.0 | 0.1 ± 0.1 | 4.9 ± 3.7 | 0.0 ± 0.0  | 0.1 ± 0.1 |           |           |

(continued on next page)

Table 1 (continued)

| Pond      | 14:0      | 16:0       | 16:1n-7   | 16:1n-9   | 18:0      | 18:1n-7   | 18:1n-9     | LIN        | GLA       | ALA       | SDA       | 20:1n-9   | 20:3n-6   | ARA       | 20:3n-3   | ETA       | EPA       | DPA       | DHA       |
|-----------|-----------|------------|-----------|-----------|-----------|-----------|-------------|------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| Dach (1)  | 0.7 ± 0.5 | 10.4 ± 4.3 | 2.7 ± 1.8 | 0.3 ± 0.1 | 3.2 ± 1.2 | 1.6 ± 1.0 | 10.1 ± 5.4  | 5.8 ± 2.9  | 0.1 ± 0.0 | 1.9 ± 1.2 | 0.0 ± 0.0 | 0.7 ± 0.4 | 0.5 ± 0.3 | 2.7 ± 0.9 | 0.2 ± 0.1 | 0.3 ± 0.1 | 2.8 ± 1.2 | 1.2 ± 0.3 | 5.2 ± 1.3 |
| Eng (1)   | 0.2 ± 1.1 | 2.8 ± 14.3 | 0.7 ± 4.3 | 0.4 ± 0.4 | 1.4 ± 3.2 | 0.5 ± 2.7 | 4.6 ± 15.5  | 5.4 ± 8.8  | 0.1 ± 0.1 | 1.7 ± 4.4 | 0.0 ± 0.0 | 0.4 ± 1.1 | 0.1 ± 0.5 | 0.6 ± 2.3 | 0.1 ± 0.3 | 0.1 ± 0.4 | 0.4 ± 2.8 | 0.2 ± 1.2 | 0.8 ± 3.8 |
| Ger (3)   | 1.2 ± 1.1 | 9.7 ± 15.5 | 3.8 ± 5.3 | 0.5 ± 1.6 | 2.7 ± 3.8 | 2.1 ± 3.2 | 11.6 ± 22.6 | 7.9 ± 7.3  | 0.1 ± 0.2 | 5.2 ± 7.6 | 0.0 ± 0.0 | 0.7 ± 1.8 | 0.2 ± 0.4 | 0.6 ± 2.8 | 0.3 ± 0.5 | 0.3 ± 0.8 | 1.3 ± 4.3 | 0.2 ± 1.7 | 0.9 ± 4.6 |
| Herr (2)  | 0.9 ± 0.8 | 8.8 ± 13.7 | 5.1 ± 4.8 | 1.6 ± 0.7 | 1.9 ± 3.0 | 2.6 ± 1.9 | 14.0 ± 16.7 | 5.2 ± 6.2  | 0.1 ± 0.2 | 6.1 ± 5.3 | 0.0 ± 0.2 | 1.5 ± 1.2 | 0.3 ± 0.3 | 2.1 ± 2.5 | 0.5 ± 0.2 | 0.8 ± 0.7 | 3.2 ± 4.4 | 1.3 ± 1.5 | 3.3 ± 4.2 |
| Jag (2)   | 0.4 ± 1.1 | 4.0 ± 11.0 | 1.8 ± 2.9 | 0.4 ± 0.7 | 0.7 ± 3.2 | 1.2 ± 2.4 | 7.2 ± 8.8   | 3.2 ± 7.4  | 0.1 ± 0.2 | 2.5 ± 4.7 | 0.3 ± 0.0 | 0.4 ± 0.6 | 0.2 ± 0.5 | 0.7 ± 3.5 | 0.2 ± 0.4 | 0.2 ± 0.7 | 0.4 ± 4.5 | 0.4 ± 1.6 | 1.8 ± 5.0 |
| Kieib (1) | 1.4 ± 0.5 | 8.1 ± 10.0 | 3.4 ± 2.8 | 0.7 ± 0.4 | 1.7 ± 3.5 | 2.3 ± 1.4 | 8.4 ± 13.1  | 8.6 ± 6.2  | 0.2 ± 0.1 | 5.2 ± 1.5 | 0.0 ± 0.1 | 0.7 ± 0.8 | 0.3 ± 0.4 | 1.2 ± 2.0 | 0.3 ± 0.1 | 0.4 ± 0.2 | 1.5 ± 2.1 | 0.4 ± 1.0 | 1.8 ± 4.4 |
| Furt (1)  | 0.5 ± 1.1 | 5.7 ± 13.6 | 1.9 ± 3.7 | 0.2 ± 0.5 | 1.9 ± 4.0 | 0.6 ± 3.0 | 9.9 ± 14.6  | 5.1 ± 13.6 | 0.1 ± 0.2 | 1.2 ± 4.2 | 0.3 ± 0.0 | 0.5 ± 1.1 | 0.2 ± 0.6 | 1.5 ± 3.3 | 0.0 ± 0.3 | 0.0 ± 0.4 | 0.8 ± 3.2 | 0.5 ± 1.2 | 1.3 ± 4.7 |
| Stadt (1) | 0.6 ± 0.6 | 4.7 ± 1.2  | 1.8 ± 1.2 | 0.2 ± 0.2 | 1.2 ± 1.2 | 1.1 ± 1.1 | 7.5 ± 6.6   | 6.6 ± 6.6  | 0.1 ± 0.1 | 1.9 ± 1.9 | 0.0 ± 0.0 | 0.6 ± 0.6 | 0.2 ± 0.2 | 0.3 ± 0.3 | 0.1 ± 0.1 | 0.2 ± 0.2 | 0.7 ± 0.7 | 0.2 ± 0.2 | 1.4 ± 1.4 |

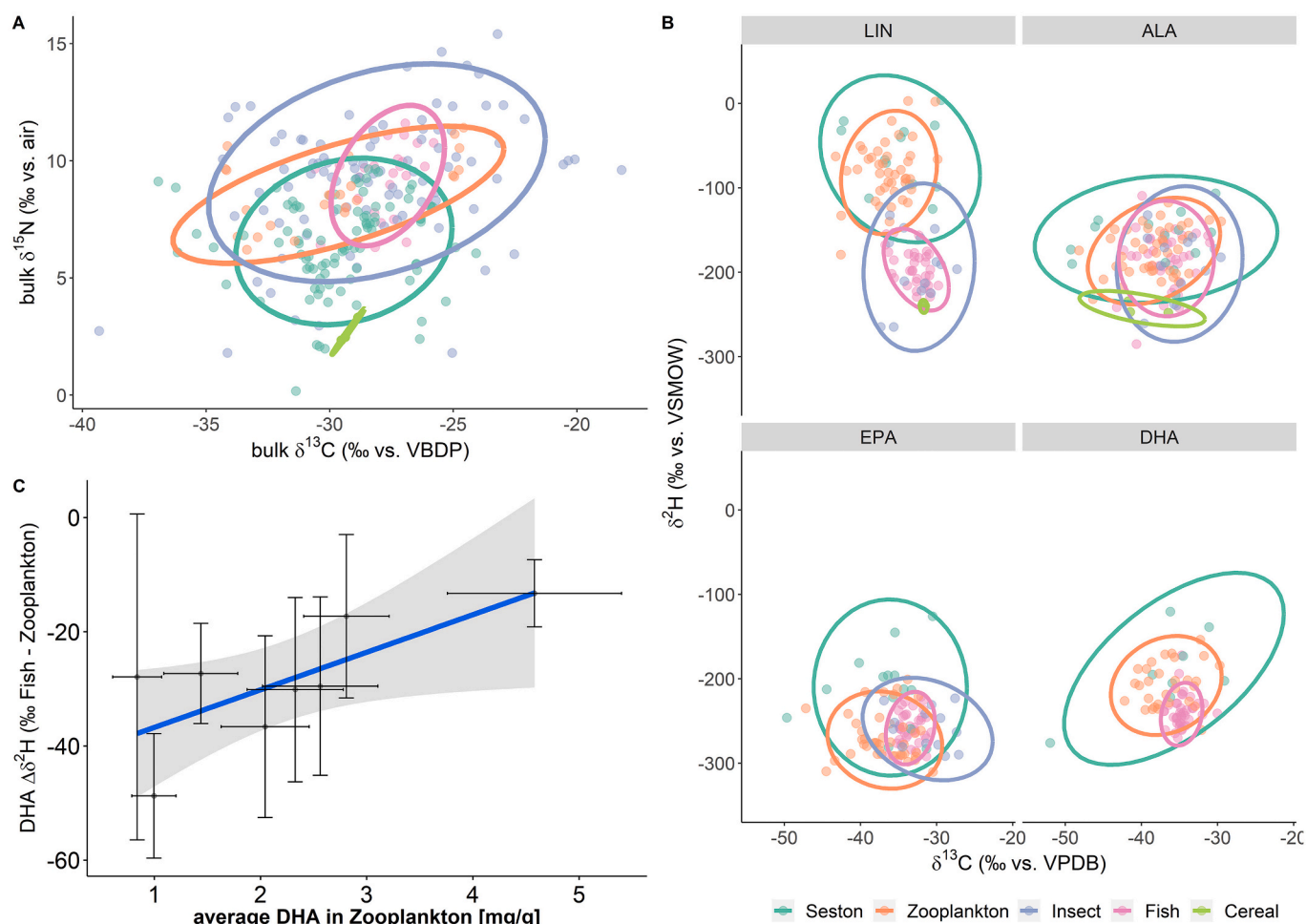
<sup>a</sup> Pellets were only fed to fish within the first months after hatching and are not used for feeding fish in the ponds; Number in parenthesis after ponds represents mean age of fish in years.

zooplankton (i.e., copepods and *Chaoborus* larvae), but also bioconverted EPA from benthic macroinvertebrates (e.g., chironomids) to DHA, when DHA-rich zooplankton was not sufficiently abundant in the pond.

Bulk stable isotope analysis could not resolve the food web structure in these study ponds and no isotopic separation between zooplankton, emergent insects, and fish was apparent. Therefore, we used CSIA to disentangle and identify the importance of each diet source for carp. In semi-intensive aquaculture systems, cereals are one of the main sources of nutrients and energy required to improve fish growth and production (Abdelghany and Ahmad, 2002). This was substantiated by the  $\delta^{13}C_{LIN}$  and  $\delta^2H_{LIN}$  values of fish that showed close similarity with the ones of cereals, suggesting direct dietary retention of C18-PUFA from cereals (Twining et al., 2020). However, as cereal-based feeds do not contain any LC-PUFA, they cannot be the source of n-3 LC-PUFA for carp, which thus may be provided by other natural food sources, such as zooplankton (Anton-Pardo and Adámek, 2015).

Omega-3 LC-PUFA mass fractions of carp muscle tissues did not vary in these study ponds, irrespective of the variation of food sources. The levels of n-3 LC-PUFA in carp are sustained by dietary sources or internal bioconversion throughout their somatic growth. The isotopic evidence in our study points at carp to use both aquatic insects and zooplankton as main dietary sources of EPA and ARA, as suggested by fish isotopic values which were close to those of both invertebrate groups (Bec et al., 2011; Burian et al., 2020; Twining et al., 2020). It is well known that benthic invertebrates are important sources of EPA for aquatic (Scharnweber et al., 2019) and riparian consumers (Twining et al., 2016; Mathieu-Resuge et al., 2021b), and it is likely that carp feed on invertebrates at the water-sediment interface. Compared to zooplankton and benthic invertebrates, seston  $\delta^2H_{ARA}$  and  $\delta^2H_{EPA}$  values of fish were higher. This phenomenon has previously been described in zooplankton communities switching from strict dietary allocation to bioconversion, due to a decrease of n-3 LC-PUFA availability in dietary seston. The capacity to bioconvert dietary PUFA precursors to LC-PUFA may provide some resilience to higher trophic levels in regard to LC-PUFA fluctuations in primary producers (Pilecky et al., 2022). In carp ponds, eutrophication may be accelerated by the addition of cereal-based feeds (Rahman, 2015), favouring cyanobacteria-dominated phytoplankton communities that are nutritionally less suitable for zooplankton and higher trophic levels due to the lack of sterols and LC-PUFA (De Serpont Domis et al., 2014; Lürling et al., 2017; Martin-Creuzburg and von Elert, 2009). Under these conditions, the capacity of zooplankton to compensate for n-3 LC-PUFA-poor diets is required (Pilecky et al., 2022). This PUFA conversion ability highlights the pivotal role of zooplankton in eutrophic carp ponds to provide n-3 LC-PUFA to carp.

Carp obtained DHA from two distinct sources. The only natural source of DHA were copepods and *Chaoborus* larvae, as the majority of the emergent insects consisted of chironomids that lack DHA (Martin-Creuzburg et al., 2017; Mathieu-Resuge et al., 2021a). Importantly, the isotopic difference in  $\delta^2H_{DHA}$  values between zooplankton and fish significantly correlated with the average zooplankton DHA mass fraction in ponds, indicating that DHA in fish is obtained by different pathways. In cases of sufficient dietary DHA supply, the isotopic differences in  $\delta^2H_{DHA}$  values between fish and zooplankton are very low, suggesting direct dietary assimilation (Bec et al., 2011; Twining et al., 2020). However, when zooplankton is poor in DHA, the isotopic difference in  $\delta^2H_{DHA}$  values increases and carp are more  $^2H$ -depleted compared to zooplankton, indicating endogenous bioconversion from dietary precursors. Using natural variabilities of fatty acid-specific  $\delta^2H$  values had the advantage to resolve the food web structure while simultaneously tracking bioconversion activity without the requirement for any pre-emptive intervention (e.g., labelling). This study supports previous experimental findings in carp and perch, showing that dietary n-3 LC-PUFA depletion increases their capacity to bioconvert these nutrients from precursors (Henrotte et al., 2011; Scharnweber et al., 2021). To our knowledge, this is the first study to demonstrate, using



**Fig. 2.** (A) Stable isotopes profiles of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of fish and potential food sources. (B)  $\delta^2\text{H}$  and  $\delta^{13}\text{C}$  values of FA in fish and potential food sources (i.e., seston, zooplankton, emergent insects, and fish feed) (C) the relationship between the average contents of DHA in zooplankton and the  $\Delta\delta^2\text{H}$  of DHA in fish muscle tissue to zooplankton.

compound-specific stable isotopes, that carp of semi-intensive aquaculture exposed to different qualitative resources can maintain and bioconvert n-3 LC-PUFA at different life stages. The new method presented here is likely also applicable for other fishes and study systems.

## 5. Conclusion

This study shows that, in addition to sufficient energy delivered by cereal feeds (i.e., nitrogen and short chain FA), natural occurrence of zooplankton and emergent insects in carp ponds is necessary for the production of high quality, DHA-rich carp in semi-intensive pond aquaculture. In contrast to cladocerans and many other invertebrates, fish rely more on DHA than EPA as the key LC-PUFA potentially limiting their growth (Nehra et al., 2012; Tocher, 2010). By using natural variability in compound-specific deuterium values, we were able to show that in addition to dietary allocation of zooplankton DHA (from copepods and/or *Chaoborus* larvae), farmed carp likely convert dietary EPA to DHA, especially if the zooplankton consists primarily of cladocerans, which contain EPA, but lack DHA. Thus, food quantity and quality (e.g., n-3 LC-PUFA) is important for carp production in eutrophic aquaculture ponds. Therefore, preserving primary producer and consumer diversity in fish ponds can help promote the transfer of highly valuable nutrients in natural pond food webs, and eventually to humans.

## Author's contribution

MP and MMR conceived the ideas and designed methodology; MMR, LF, MJK, and MP collected the data; LF, MP and KW conducted laboratory analyses; MP, MMR and LZ analysed data; MP and MMR led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

## Funding

This work has been supported by the Austrian Science Fund (FWF I-3855) and the German Research Foundation (DFG; MA 5005/8-1) within the framework of the DACH collaboration (project 'AquaTerr' to DMC and MJK).

## CRediT authorship contribution statement

**Matthias Pilecky:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft. **Margaux Mathieu-Resuge:** Conceptualization, Formal analysis, Investigation, Project administration, Visualization, Writing – original draft. **Libor Závorka:** Data curation, Formal analysis, Software, Validation, Writing – review & editing. **Lena Fehlinger:** Investigation, Project administration. **Katharina Winter:** Data curation, Methodology. **Dominik Martin-Creuzburg:** Funding acquisition, Investigation, Project administration, Resources, Supervision, Writing – review &



editing. **Martin J. Kainz**: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

## Acknowledgements

The authors would like to thank Erik Wassenaar, Hannes Hager and Samuel Kämmer for assisting with sample collection and processing. The authors report no conflict of interest.

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