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Effect of nutrition on fatty acid profiles of riverine, lacustrine, and aquaculture-raised salmonids of pre-alpine habitats

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Abstract We examined trophic positions and fatty acid concentrations of riverine, lacustrine, and aquaculture diet and fish in Austrian pre-alpine aquatic ecosystems. It was hypothesized that dietary fatty acid (FA) profiles largely influence the FA composition of the salmonids *Salvelinus alpinus*, *Salmo trutta*, and *Oncorhynchus mykiss*. We analyzed trophic positions using stable isotopes (δ^{15}N) and tested for correlations with polyunsaturated fatty acid (PUFA) concentrations. Gut content analysis revealed benthos (rivers), pellets (aquaculture), and zooplankton (lakes) as the predominant diet source. Results of dorsal muscle tissues analysis showed that the omega-3 PUFA, docosahexaenoic acid (DHA; 22:6$n-3$), was the mostly retained PUFA in all fish of all ecosystems, yet with the highest concentrations in *S. alpinus* from aquaculture (mean: 20 mg DHA/g dry weight). Moreover, we found that eicosapentaenoic acid (EPA; 20:5$n-3$) in fish of natural habitats (rivers, lakes) was the second most abundant PUFA (3–5 mg/g DW), whereas aquaculture-raised fish had higher concentrations of the omega-6 linoleic acid (18:2$n-6$; 9–11 mg/g DW) than EPA. In addition, PUFA patterns showed that higher omega-3/-6 ratios in aquacultures than in both riverine and lacustrine fish. Data of this pilot field study suggest that salmonids did not seem to directly adjust their PUFA to dietary PUFA profiles in either natural habitats or aquaculture and that some alterations of PUFA are plausible. Finally, we suggest that trophic positions of these freshwater salmonids do not predict PUFA concentrations in their dorsal muscle tissues.

Keywords Aquatic food webs · Dietary fatty acids · Stable isotopes · Aquatic habitats · Fish

Introduction

Organisms of aquatic food webs transfer dietary energy to consumers at different trophic levels. During the past decades of aquatic food web research, a number of ecological concepts have been developed to investigate how dietary energy gets conveyed from one trophic level to the next. Such concepts include, (a) gut content analysis of freshwater copepods (Fryer, 1957), amphipods (Quigley & Vanderploeg, 1991), and fish (Grey et al., 2002), (b) pigment analysis (Thys et al., 2003), and (c) stable isotope analysis (e.g., Cabana & Rasmussen, 1996; Post, 2002; Post et al., 2007).

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In addition to using these concepts for investigating dietary energy transfer, recent studies underlined the importance of the biochemical quality of food for aquatic consumers. Dietary lipids and their fatty acids (FA) have been identified to play a crucial role for somatic growth, reproduction, and consequently survival of aquatic organisms. In particular, polyunsaturated fatty acids (PUFA) are integral constituents of cell membranes and enhance membrane permeability (Jump, 2002). These PUFA-related membrane properties are linked with the ability of organisms to synthesize them de novo and/or to retain dietary PUFA selectively to satisfy their physiological requirements. Omega-3 ($n-3$) PUFA are mostly synthesized by primary producers and must thus be transferred to consumers at higher trophic levels as animals are generally not able to synthesize these $n-3$ PUFA themselves. Therefore, it is necessary to understand to what extent aquatic organisms retain PUFA along different trophic levels and at different aquatic habitats.

Stable isotopes are extensively used to investigate trophic positioning (δ^{15}N) as well as to assess bulk carbon sources (δ^{13}C). Minagawa & Wada (1984) and Post (2002) proposed an enrichment of 3.4 ± 1.1‰ of the δ^{15}N signature per trophic level due to respective fractionation processes. Signatures of δ^{13}C allow us to assess carbon sources and thus to determine which path an endconsumer's diet came from. So far, focus was mostly put on lacustrine systems (Lindeman, 1942; Vander Zanden & Rasmussen, 1996; Kainz et al., 2002, 2004; Vander Zanden & Vadebonceur, 2002), while fewer studies dealt with riverine food webs (Woodward & Hildrew, 2002; Townsend & Hildrew, 2006).

In this study, we used aquaculture as a reference model because its food chain is generally simple and short: fish feed—fish, i.e., 2-trophic level food web. In contrast, riverine food webs can be highly structured (Woodward & Hildrew, 2002) and their consumers can depend, in part, on terrestrial diet sources (e.g., invertebrates; Nakano & Kuhara, 1999). Riverine and lacustrine food webs are highly exposed to seasonal changes, which eventually affect the supply of diet. In lakes, there are seasonal shifts in plankton populations (Mazumder et al., 1990; Grey et al., 2001; Gladyshev et al., 2007) as well as in the preferred diet of fish. On a seasonal level, parameters, such as light, nutrients, oxygen, temperature, and water depth greatly affect organisms of lacustrine and riverine systems and thus their food webs. To better understand and finally protect dietary energy flow in lacustrine, riverine, and aquaculture systems, we designed this field study to concurrently investigate stable isotopes (δ^{13}C and δ^{15}N) and FA in fish and their potential prey.

Polyunsaturated fatty acids (PUFA) play an important role in the physiology of all species along aquatic food webs. PUFA cannot be produced in sufficient amounts in animals and must therefore be largely obtained from the diet. This is because vertebrates lack Δ12 and Δ15 desaturases to produce $n-6$ and $n-3$ FA, respectively (Tocher, 2003). Therefore, the shorter C-chain PUFA linoleic acid (LIN; 18:2$n-6$) and α-linolenic acid (ALA; 18:3$n-3$) are essential precursors for animals of longer chain PUFA, including arachidonic acid (ARA; 20:4$n-6$), as well as eicosapentaenoic acid (EPA; 20:5$n-3$) and docosahexaenoic acid (DHA; 22:6$n-3$). Using desaturases and elongases, LIN and ALA can be converted to EPA, ARA, and DHA (Tocher, 2003).

It has been shown that these FA contribute to proper development of the nervous system or sensory organs in fish larvae (Navarro et al., 1995; Benitez-Santana et al., 2006). From an overall food web perspective, it is thus important to understand how highly valuable dietary constituents, such as PUFA, are conveyed and retained in aquatic food webs and how their retention in fish is affected by dietary supply of different aquatic ecosystems.

Integrating stable isotopes and PUFA in aquatic food webs, we expect to advance our understanding of PUFA retention in organisms at various food web levels and eventually provide more detailed insights into the functioning of aquatic food webs. The following PUFA are known to be crucial dietary components of aquatic consumers: (a) ARA, which is important for cortisol formation (Koven, 2001) and signal transduction (Smith & Fitzpatrick, 1996); (b) EPA, which yields higher reproduction success in zooplankton (Müller-Navarra et al., 2000; Brett et al., 2009); and (c) DHA, which is a crucial molecule of the cell membrane structure and thus for cell processes (Spector, 1999), and it improves somatic growth of daphnids (Müller-Navarra, 1995) and fish (Izquiero et al., 2000; Copeman et al., 2002; Ballantyne et al., 2003).

The objectives of this study were to examine: (a) the trophic position of salmonids in the three different

aquatic ecosystems, i.e., rivers, lakes, and aquaculture of pre-alpine Austria, and (b) how PUFA retention of these salmonids differs with respect to their trophic position and potential diets of these ecosystems.

Therefore, we tested the hypothesis that (a) existing concepts of trophic position by which $\delta^{15}N$ signatures as trophic markers may increase by ~3.4‰ (Post, 2002) and bulk diet sources by which $\delta^{13}C$ signatures generally shift <1‰ per trophic level, applies to all of these aquatic systems, and (b) the FA composition of the different consumer species resembles that of their diet. Our overall assumption was that fish from rivers feed mainly on benthic organisms, lacustrine fish primarily on zooplankton, while farmed fish are fed fish feed.

Methods

This study was performed during summer (July–September 2008) in pre-alpine Lower Austria. The study area is located ca. 100 km southwest of Vienna, at the foothills of the Austrian limestone Alps. Forest soils (mostly rendzina) are mostly composed of spruce (*Picea abies)* and larch (*Larix decidua*).

We investigated fish and their potential diet of (a) rivers (River Erlauf, Ois, and Ybbs; see Table 1), (b) lakes (Upper Lake Lunz, LOS; and Lower Lake Lunz, LUS), and (c) freshwater aquaculture (i.e., AC-1, AC-2, AC-3). Fish were caught by hand nets (fish farms) and fishing rods (lakes and rivers, and electrical fishing at the river Ois) and their body size and weight were immediately measured before subsequent analyses.

At LOS, *Salvelinus alpinus* (the abundant fish species of LOS) was caught at oligotrophic (1.6 μg Chl *α*/l) LOS, and *S. alpinus* and *Salmo trutta* were collected at oligotrophic (0.4 μg Chl *α*/l) LUS (Table 1). In order to investigate their potential planktonic food we analyzed large zooplankton (>500 μm body size) that were collected using vertical net hauls (64 μm mesh size). This large zooplankton size class is generally the preferred zooplankton prey size of planktivorous fish (Brooks & Dodson, 1965) and gut content analysis of fish showed that the primary food source was zooplankton and macrozoobenthos. Two brown trouts also had small fish in their stomachs (*Phoxinus phoxinus* and *Cottus gobio*). Zooplankton consisted mainly of copepodes (*Eudiaptomus* sp.) in LOS and of cladocerans (*Daphnia* sp., and *Bosmina* sp.) in LUS.

Due to fishery restrictions of these rivers, we were only able to collect rainbow trout (*Oncorhynchus mykiss*) in these study rivers. Gut content analysis of these *O. mykiss* revealed that their recent feeding success consisted predominantly of insect larvae (Table 2).

We analyzed three salmonid farms as our reference model systems since fish meal pellets were the only known food source, supplied via standardized pulse feeding. Due to this simple food web, we were able to examine the generality of the trophic concept that, independent of the aquatic ecosystem, consumers are enriched in $\delta^{15}N$ values (by ~3.4‰; Post, 2002) with respect to the values of their diet.

Gut contents of fish were identified and pure white dorsal muscle tissues were used for lipid and stable isotope analyses. Muscle tissue was stored at cryogenic temperatures (−80°C) and freeze dried before lipid extraction and formation of FA methyl esters (FAME). Riverine macrozoobenthos was determined taxonomically (Table 2). All benthic and plankton

Table 1 Fish species analyzed per habitat (Lakes: Upper Lake Lunz, LOS; Lower Lake Lunz, LUS; Rivers: Erlauf, Ybbs, Ois; Aquacultures: AC 1–3)

Gut contents: Benthos (B), Zooplankton (Z), Insects (I), Fish (F), Pellets (P). Mean length (cm)/mean weight (g)

Habitats	*Salmo trutta*	*Oncorhynchus mykiss*	*Salvelinus alpinus*
LOS			Z; 29.5/291.5
LUS	B, Z, F; 28.8/236		Z; 28.6/218.8
Erlauf		B; 21.1/96.6	
Ybbs		B; 25.9/254.8	
Ois		B; 24.8/173.3	
AC-1		P; 29.5/302.2	P; 20.7/112.5
AC-2	P; 29.5/376.7	P; 26.5/295.1	P; 28.1/274.5
AC-3	P, I; 35.5/472.7	P, I; 32.3/375.9	

Table 2 Potential diet composition of study lakes, rivers, and aquaculture systems (Lakes: Upper Lake Lunz, LOS; Lower Lake Lunz, LUS; Rivers: Erlauf, Ybbs, Ois; Aquacultures: AC 1–3)

Aquatic systems	Analyzed potential diet
LOS	Zooplankton, dominated by copepods*: Eudiaptomus* sp.*, Cyclops* sp.
LUS	Zooplankton dominated by cladocerans: *Daphnia* sp. *Bosmina* sp.
Erlauf	Caddisflies (*Lepidostomatidae, Limnephilidae, Glossosomatidae), Cottus gobio*, Stoneflies (*Perla* sp.)
Ybbs	*Gammaridae, Simulidae, Chironomidae, Stoneflies (Perla* sp.*, Baetis* sp.*)*
Ois	*Limnephilidae* (caddisfly), *Ephemerelidae* (Mayflies), *Hydrachnellae* (Milbes), *Simulidae*
AC-1	Pellets-Alpenlachs^{TM} (pigmented)
AC-2	Pellets, Danex^{TM} (pigmented)
AC-3	Pellets, Danex^{TM} (unpigmented)

samples were stored at −80°C, freeze dried to limit lipolytic degradation processes.

Stable isotope analysis

The freeze dried samples of fish, zooplankton, benthos, and fish pellets were homogenized and put into tin capsules (~0.7 mg each). Stable isotopes (δ^{15}N and δ^{13}C) were analyzed using an elemental analyzer (EA 1110, CE Instruments, Milan, Italy) interfaced via a ConFlo II device (Finnigan MAT, Bremen, Germany) to a continuous flow stable isotope ratio mass spectrometer (Delta Plus, Finnigan MAT, Bremen, Germany). Our reference gas was N_2 (air liquide) and CO_2. It was calibrated to the at air international standard using IAEA-N-1, IAEA-N-2, and IAEA-NO-3 (IAEA, Vienna, Austria) for nitrogen and IAEA-CH-6, IAEA-CH-7 (International Atomic Energy Agency) for carbon. All stable isotope values were reported in the δ notation where $\delta^{13}C$ or $\delta^{15}N = ([R_{sample}/R_{standard}] - 1) \times 1000$, where R is $^{13}C{:}^{12}C$ or $^{15}N{:}^{14}N$. We used 0.15% for δ^{15}N and 0.10% for δ^{13}C standard deviation of measurement.

Lipid analysis

Freeze dried samples (5–30 mg) were homogenized and stored in chloroform (2 ml) under N_2 atmosphere over night at −80°C. The overnight storage in chloroform at −80°C proved to enhance lipid extraction efficiency as sample tissues were further broken up during this process. Subsequently, methanol (1 ml), 2:1 chloroform–methanol (1 ml), and NaCl (0.8 ml; salt wash) was added and topped with N_2. Sonication and vortexing were employed to further break up sample tissue. After vortexing and centrifugation, the lipid layer (bottom organic layer) was collected using double pipetting technique, which involves placing a long Pasteur pipette inside a short one. The removed layer was transferred into a pre-cleaned vial and stored on ice under N_2. The mixture was washed thrice with ice-cold chloroform (3 ml) with sonication, vortexing, centrifugation, and removal of the organic layer being repeated each time. The amount of total lipids was determined gravimetrically by weighing aliquots of lipid extracts (duplicates) on a microscale (±1 μg; Gibertini^{TM}) as a measure for the required quantity for subsequent formation of FAME.

For esterification, the total lipid extracts (parent solution) were evaporated under N_2 and toluene (1 ml) and H_2SO_4–methanol (2 ml; 1% v/v) were added, vortexed, and stored for 16 h at 50°C. Schlechtriem et al. (2008) suggested that this sulphuric acid esterification method is more suitable for the formation of FAME than others (e.g., boron-trifluoride/methanol). Subsequently, $KHCO_3$ (2 ml; 2% v/v) and BHT (5 ml; 0.01%) were added, shaken, and CO_2 released. After centrifugation, the top layer was removed and again BHT (5 ml) added. CO_2 was released and after centrifugation the top layer was removed once again. The formed FAME were dried under N_2 and re-dissolved in hexane.

Fatty acid methyl esters (FAME) were analyzed using a gas chromatograph (TRACE GC THERMO, Detector: FID 260°C, Carrier gas: He: 1 ml/min, Detector gases: H_2: 40 ml/min, N_2: 45 ml/min, air: 450 ml/min, temperature ramp: 140°C (5 min)–4°C/min–240°C (20 min) = 50 min) equipped with a temperature-programmable injector and an autosampler. A Supelco^{TM} SP-2560 column (100 m, 25 mm i.d.,

0.2 μm film thickness) was used for FAME separation. Excalibur 1.4^{TM} was used for calculation and, if necessary, manual resetting of the chromatograms. Fatty acid concentrations were calculated using calibration curves based on known standard concentrations.

We used paired *t*-test analysis to compare PUFA concentrations between two groups (e.g., diets and fish or between two habitats). Analysis of variance (ANOVA) was applied to examine differences of FA concentrations among more than two groups (e.g., among the salmonid species and among the three different habitats). Linear regression models were used to examine relationships between lipid concentrations (including total FA and PUFA) and fish weight and between trophic dependence (δ^{15}N values between fish and potential diets) and concentrations PUFA compounds (dependent variables); however, due to the limited sample sizes (*n*), we could not test for the effect of habitat.

Results

Epilimnetic lake temperature was 6.8°C in LOS and 13.8°C in LUS. Both were oligotrophic with a Secchi depth of 6.5 and 11.5 m, respectively. River temperatures ranged between 9.1°C (River Ois) and 16.8°C (River Ybbs). Samples of riverine fish were taken within the trout region (low temperatures, highly oxygenated, high current, and gravel/rock underground) of these rivers. Water temperatures of farms ranged between 8.9 and 13.1°C (Table 3).

Charr were collected at LOS ($n = 7$), LUS ($n = 5$), AC-1 ($n = 5$), and AC-2 ($n = 5$), rainbow trouts were taken from every aquaculture system ($n = 5$) and every river ($n = 5$), while brown trout was available in LUS ($n = 5$), AC-2 ($n = 5$), and AC-3 ($n = 3$; Table 1). LOS zooplankton (>500 μm) consisted mainly of *Eudiaptomus* sp. (85%) and occasionally of *Cyclops* sp., while LUS was largely populated with *Bosmina* sp. and *Daphnia* sp. River benthos consisted of insect larvae of the order Trichoptera, Ephemeroptera, Plecoptera, Diptera, and also *Gammaridae* (Table 2). AC-1 and AC-2 provided pigmented fish feed (pellets), while AC-3 provided unpigmented trout feed.

Total fatty acids and PUFA concentrations

Total FA concentrations did not differ significantly among the three salmonid species ($P = 0.4$): charr (80 ± 30 mg/g dw), brown trout (80 ± 40 mg/g dw), rainbow trout (70 ± 30 mg/g dw) as well as among the habitats ($P = 0.2$): lake (70 ± 30 mg/g dw), river (70 ± 30 mg/g dw), and aquaculture (90 ± 30 mg/g dw). Furthermore, no significant difference of total FA was seen between river and aquaculture rainbow trout ($P = 0.98$), between lake and aquaculture brown trout ($P = 0.2$), and lake and aquaculture charr ($P = 0.06$).

Concentrations of PUFA of potential diets and fish are listed in Tables 4 and 5, respectively. Total PUFA concentrations were significantly ($P = 0.01$) higher in aquaculture charr (30.6 ± 11.9 mg/g dw) than lake charr (17.5 ± 4.8 mg/g dw). Comparing the three aquatic systems, the highest PUFA concentrations were found in aquaculture salmonids (26.7 ± 9.1 mg/g dw), but were not significantly ($P = 0.06$)

Table 3 Physico-chemical parameters of lakes (LOS: Upper Lake Lunz; LUS: Lower Lake Lunz), rivers (Ybbs, Erlauf, and Ois), and aquacultures AC 1–3

	Coordinates	Max. depth (m)	Secchi depth (m)	Temperature (°C)	O_2 mg/l	Chl *a* μg/l
LOS	47°48′N, 15°04′E	15	6.5	6.8	9.2	1.6
LUS	47°51′N, 15°03′E	32	11.5	13.8	11.4	0.4
YBBS[1]	47°48′N, 14°46′E	<0.5	n.d.	16.8	10.8	0.6
ERLAUF[1]	47°55′N, 15°09′E	<0.5	n.d.	13.3	10.9	0.7
OIS[1]	47°51′N, 15°02′E	<0.5	n.d.	9.1	12	0.4
AC-1	47°44′N, 14°54′E	1	n.d.	13.2	10.5	0.2
AC-2	47°51′N, 15°01′E	1	n.d.	10.7	10.2	2.5
AC-3	47°51′N, 14°59′E	1	n.d.	8.9	19.9	0.9

n.d. not detected (all rivers and aquaculture tanks were clear to the bottom)

Table 4 Polyunsaturated fatty acids concentrations (PUFA; mg/g dw) of potential salmonid diet from different habitats

	18:2*n* − 6	18:3*n* − 3	20:4*n* − 6	20:5*n* − 3	22:6*n* − 3	Total PUFA
mzb-Y	5.5 ± 0.1	9.9 ± 2.4	1.5 ± 0.0	20.9 ± 2.4	0.2 ± 0.1	39.1 ± 4.8
mzb-E	3.6 ± 1.2	7.8 ± 2.3	1.0 ± 0.5	11.8 ± 3.6	0.1 ± 0.1	24.8 ± 7.5
mzb-O	4.1 ± 1.8	10.1 ± 5.7	0.8 ± 0.3	7.6 ± 2.2	0.2 ± 0.1	23.3 ± 10.0
LUS-P	4.6 ± 0.7	8.1 ± 1.2	2.9 ± 0.4	9.8 ± 1.5	1.9 ± 0.3	28.8 ± 4.3
LOS-P	7.0 ± 1.1	21.1 ± 3.2	1.8 ± 0.3	23.6 ± 3.5	20.0 ± 3.0	77.7 ± 11.6
AC-1	10.5 ± 1.6	2.5 ± 0.4	0.5 ± 0.1	5.2 ± 0.8	7.3 ± 1.1	27.8 ± 4.2
AC-2	12.8 ± 1.9	3.7 ± 0.6	0.8 ± 0.1	9.3 ± 1.4	10.3 ± 1.5	39.3 ± 5.9
AC-3	15.7 ± 2.4	3.2 ± 0.5	0.6 ± 0.1	9.6 ± 1.4	5.3 ± 0.8	35.2 ± 5.3

Y River Ybbs, *E* River Erlauf, *O River Ois*, *AC* Aquaculture, *LUS* Lower Lake Lunz, *LOS* Upper Lake Lunz, *mzb* riverine macrozoobenthos, *P* lacustrine zooplankton. See text for taxonomy of macrozoobenthos and zooplankton

Table 5 Polyunsaturated fatty acids concentrations (PUFA; mg/g dw) of riverine, lacustrine, and aquaculture-raised salmonids

	18:2*n* − 6	18:3*n* − 3	20:4*n* − 6	20:5*n* − 3	22:6*n* − 3	Total PUFA
RT-Y	0.9 ± 0.7	0.5 ± 0.2	0.4 ± 0.1	3.0 ± 1.1	8.9 ± 4.2	14.2 ± 3.3
RT-E	2.2 ± 0.9	2.3 ± 1.1	0.8 ± 0.3	4.2 ± 1.4	10.8 ± 4.7	21.4 ± 8.5
RT-O	2.3 ± 1.6	5.0 ± 3.5	0.8 ± 0.5	5.2 ± 1.8	9.3 ± 3.7	24.8 ± 11.8
RT-AC1	4.2 ± 2.0	0.8 ± 0.3	0.4 ± 0.1	2.3 ± 0.4	12.4 ± 2.4	21.0 ± 5.6
RT-AC2	5.1 ± 1.8	0.9 ± 0.3	0.4 ± 0.1	2.7 ± 0.5	12.1 ± 2.0	22.3 ± 4.5
RT-AC3	5.0 ± 5.9	0.9 ± 0.7	0.5 ± 0.2	2.8 ± 1.2	11.6 ± 3.4	21.6 ± 11.3
CH-LUS	1.1 ± 0.4	1.3 ± 0.5	1.7 ± 0.2	5.0 ± 0.9	5.0 ± 0.7	15.6 ± 3.0
CH-LOS	1.5 ± 0.5	2.2 ± 0.9	1.5 ± 0.4	5.0 ± 1.5	6.6 ± 1.6	18.2 ± 5.2
CH-AC1	5.0 ± 1.3	0.8 ± 0.2	0.6 ± 0.1	3.3 ± 0.5	11.9 ± 1.8	22.7 ± 4.2
CH-AC2	9.3 ± 3.4	1.5 ± 0.6	0.8 ± 0.2	5.7 ± 1.8	18.0 ± 5.9	37.0 ± 12.2
BT-LUS	1.2 ± 0.3	0.9 ± 0.3	1.4 ± 0.6	2.8 ± 0.7	9.1 ± 2.3	16.2 ± 3.8
BT-AC2	12.9 ± 12.5	1.8 ± 1.4	0.7 ± 0.3	3.6 ± 2.1	13.8 ± 6.1	34.4 ± 23.4
BT-AC3	6.4 ± 5.5	1.2 ± 1.0	0.5 ± 0.2	3.2 ± 1.5	15.3 ± 5.8	28.1 ± 14.7

AC Aquaculture, *CH* Charr, *RT* Rainbow trout, *BT* Brown trout, *E* Erlauf, *Y* Ybbs, *O* Ois, *LUS* Lower Lake Lunz, *LOS* Upper Lake Lunz

higher than salmonids of rivers (20.1 ± 5.4 mg/g dw) and lakes (16.7 ± 1.4 mg/g dw). No significant difference of total PUFA concentrations ($P = 0.4$) was observed among charr (23.7 ± 11 mg/g dw), brown trout (25 ± 15.1 mg/g dw), and rainbow trout (20.8 ± 8.2 mg/g dw).

Individual PUFA concentrations

Eicosapentaenoic acid

No significant difference of EPA concentrations was found within the charr ($P = 0.5$) and brown trout ($P = 0.5$) populations, while a significant ($P = 0.001$) difference between rainbow trout populations of riverine habitats (4.1 ± 1.6 mg/g dw) and farms (2.6 ± 0.8 mg/g dw) was determined. Rainbow trout of the river Ois had the highest EPA concentrations (5.2 ± 1.8 mg/g dw), while rainbow trout of AC-1 the lowest (2.3 ± 0.7 mg/g dw). Comparing all salmonids, no significant difference ($P = 0.1$) of EPA concentrations was observed among habitats, while EPA of charr (4.8 ± 1.5 mg/g dw) was significantly higher ($P = 0.001$) than of brown trout (3.2 ± 1.3 mg/g dw) and rainbow trout (3.4 ± 1.5 mg/g dw).

Docosahexaenoic acid

Rainbow trout ($P = 0.08$) and brown trout ($P = 0.05$) populations did not differ significantly in their DHA concentrations, while charr from aquaculture (14.9 ± 5.2 mg/g dw) contained significantly higher ($P = 0.001$) DHA concentrations than lake charr (6 ± 1.5 mg/g dw). No significant difference of DHA concentrations among species could be detected, but DHA concentrations of aquaculture-raised salmonids (13.6 ± 4.5 mg/g dw) were significantly higher ($P = 0.001$) than of river (9.6 ± 3.4 mg/g dw) and lake (6.9 ± 2.2 mg/g dw) salmonids.

Arachidonic acid

No significant difference of ARA concentrations ($P = 0.5$) was found within the rainbow trout populations, while charr ($P = 0.001$; lake: 1.6 ± 0.3 mg/g dw; aquaculture: 0.7 ± 0.2 mg/g dw) and brown trout ($P = 0.01$; lake: 1.6 ± 0.3 mg/g dw; aquaculture: 0.7 ± 0.2 mg/g dw) were significantly higher in lakes than from aquaculture systems. When comparing species, the highest ARA concentrations were found in charr (1.2 ± 0.5 mg/g dw) and brown trout (0.9 ± 0.6 mg/g dw), while ARA concentrations of rainbow trout (0.5 ± 0.3 mg/g dw) were significantly lower ($P = 0.01$). Lake salmonids had significantly higher ($P = 0.001$) ARA concentrations (1.5 ± 0.4 mg/g dw) than those from rivers (0.6 ± 0.4 mg/g dw) and aquaculture systems (0.6 ± 0.2 mg/g dw).

Alpha-linolenic acid

ALA concentrations of rainbow trout of the river Ybbs (0.5 ± 0.2 mg/g dw) were significantly ($P = 0.02$) lower than that of the rivers Erlauf (2.3 ± 1.1 mg/g dw) and Ois (5.0 ± 3.5 mg/g dw). ALA concentrations were significantly ($P = 0.04$) higher in rainbow trout of rivers (2.6 ± 2.8 mg/g dw) than from farmed rainbow trout (0.8 ± 0.5 mg/g dw). There was no significant difference of ALA concentrations of brown trout ($P = 0.3$) or between charr from lakes (1.8 ± 0.9 mg/g dw) and aquaculture systems (1.2 ± 0.7 mg/g dw; $P = 0.06$). No significant differences ($P = 0.6$) of ALA concentrations were detected among the three salmonid species (rainbow trout: 1.8 ± 2.3 mg/g dw; brown trout: 1.2 ± 0.9 mg/g dw; charr: 1.5 ± 0.8 mg/g dw). However, fish from riverine habitats had significantly ($P = 0.04$) higher ALA concentrations (2.6 ± 2.8 mg/g dw) than fish from lakes (1.5 ± 0.8 mg/g dw) and farmed fish (1.1 ± 0.7 mg/g dw).

Linoleic acid

There was no significant difference ($P = 0.3$) of LIN concentrations among the brown trout populations of the different habitats. Similarly, LIN concentrations of rainbow trout were not significantly different between aquaculture and river habitats ($P = 0.4$), and farmed charr (1.2 ± 0.3 mg/g dw) and charr of lakes (1.8 ± 0.7 mg/g dw) were also not significantly different ($P = 0.06$). No significant ($P = 0.6$) difference was observed among the three species, whereas salmonids of riverine habitats (2.6 ± 0.7 mg/g dw) had significantly ($P = 0.01$) higher LIN concentrations than in lake (1.5 ± 0.8 mg/g dw) and aquaculture systems (1.1 ± 0.7 mg/g dw).

Total fatty acids and PUFA in potential diets

Total FA concentrations did not differ significantly ($P = 0.8$) between benthos (116 ± 37 mg/g dw) and pellets (114 ± 36 mg/g dw), while zooplankton had significantly higher total FA concentrations (210 ± 98 mg/g dw; $P = 0.03$).

PUFA concentrations of zooplankton at LOS (77.7 ± 11.6 mg/g dw) were significantly higher ($P = 0.001$) than of any other diet (ranging from 23 to 39 mg/g dw). LIN was significantly higher ($P = 0.001$) in aquaculture pellets (13.0 ± 2.6 mg/g dw) than in dietary organisms of rivers (4.3 ± 1.4 mg/g dw) and lakes (5.8 ± 1.7 mg/g dw), while dietary ALA in aquacultures (3.1 ± 0.6 mg/g dw) was significantly lower ($P = 0.03$) than in rivers (9.2 ± 3.6mg/g dw) and lakes (14.6 ± 9.2 mg/g dw). Dietary ARA was significantly ($P = 0.01$) higher in lakes (2.4 ± 0.4 mg/g dw) compared to the other systems, and dietary DHA was significantly ($P = 0.01$) lower in rivers (0.2 ± 0.1 mg/g dw) compared to the other systems. No significant differences ($P = 0.3$) were found for dietary EPA concentrations, which were highest in lake zooplankton (6.1 ± 2.5 mg/g dw). Finally, results from linear regression analysis revealed that fish weight was not significantly related to total lipid concentrations, total FAME, or PUFA concentrations of fish dorsal muscle tissues from any of these habitats ($P > 0.3$).

Stable isotopes

Stable nitrogen values (δ^{15}N) were highest in aquaculture fish (9.3 ± 0.4‰), while the δ^{15}N values of their food were consistently lower (i.e., 6.6 ± 0.9‰; Fig. 1). This isotopic difference between diet and consumer (2.7‰) is in line with the suggested range of 3.4 ± 1.1‰ per trophic level (Minagawa & Wada, 1984; Post, 2002). The highest δ^{13}C values were detected in aquaculture fish (−21.4 ± 0.7‰) and, on average, 3.6‰ lighter in their diet (−24 ± 0.8‰).

Fish from the riverine habitats, Ybbs and Ois had similar δ^{15}N values (3.2‰), while fish of the river Erlauf had higher values (3.8 ± 0.2‰). The corresponding δ^{13}C values were −29.3 ± 0.5‰ for Erlauf, −30.7 ± 0.3‰ for Ois, and −28.2 ± 1.6‰ for Ybbs. Fish and benthos of the rivers Erlauf and Ois differed by 1.2–1.3‰ in δ^{13}C, whereas fish and benthos of the river Ybbs differed by 3.6‰ in their δ^{13}C values. Benthic organisms of the three rivers showed that the following differences of δ^{15}N values: Erlauf (0.8 ± 1‰) had higher δ^{15}N values than benthos of the rivers Ybbs (0.2 ± 0.6‰) and Ois (−1.7 ± 0.8‰). Benthos of the river Ois had the lowest δ^{13}C values (−33.0 ± 3.4 ‰) compared to those of the rivers Ybbs (−31.8 ± 1.1‰) and Erlauf (−30.5 ± 1.0‰). Organisms of these river food webs had the lowest δ^{15}N values when compared with those of lakes and aquacultures. Comparing δ^{15}N values between fish and diet a difference of 4.9‰ for the river Ois was detected, while fish from the rivers Erlauf and Ybbs had a 3‰ higher δ^{15}N value than their diet.

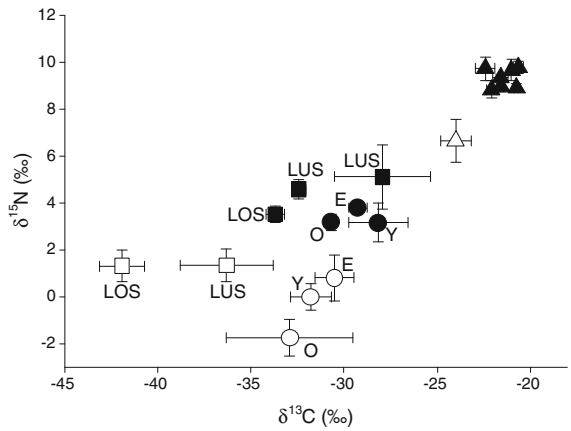

Fig. 1 Stable isotope signatures (δ^{15}N and δ^{13}C) of fish (aquaculture ponds: *filled triangle*; lakes: *filled square*; rivers: *filled circle*) and their respective diet (aquaculture ponds: *open triangle*; lakes: *open square*; rivers: *open circle*) in pre-alpine Austria. *LUS* Lower Lake Lunz, *LOS* Upper Lake Lunz, *E* Erlauf, *O* Ois, *Y* Ybbs

Brown trout from the lacustrine habitat LUS had the highest δ^{15}N values (5.1 ± 1.4‰) followed by charr of LUS (4.6 ± 0.4‰) and LOS (3.5 ± 0.3‰). For LUS, comparing δ^{13}C values of charr (−32.4 ± 0.4‰) and brown trout (−27.9 ± 2.6‰), different diet sources were present which was consistent with gut content analysis where predominantly zooplankton was found in charr stomachs, while brown trout contained mostly benthos and occasionally small fish (*Cottus gobio* and *Phoxinus phoxinus*). LOS charr had the lowest δ^{13}C values (−33.7 ± 0.5‰) of all fish as well as the LOS zooplankton which was 6.4‰ lower in δ^{13}C than at LUS.

Similar trophic positions (δ^{15}N values: 1.6 ± 0.3‰) were determined for large zooplankton (>500 μm) of both LOS and LUS (Fig. 1). Charr at LOS charr were 2.7‰ higher δ^{15}N values than those of their potential diet, whereas δ^{15}N values of charr and brown trout at LUS had 3.3 and 3.8‰ higher δ^{15}N values than those of their potential diet, respectively.

We calculated the PUFA retention ratios between fish and potential diets by dividing PUFA concentrations of fish by PUFA concentrations of their potential diet (i.e., $[PUFA]_{fish}/[PUFA]_{diet}$) to assess the trophic relationship between dietary PUFA supply and PUFA retention in the consumer (Fig. 2). Linear regression analyses were performed to test how concentrations of individual PUFA compounds in fish varied with differences of δ^{15}N signatures between fish and potential diets (Fig. 3A–E). This analysis linking trophic enrichment values and individual PUFA compounds of the salmonids resulted in no significant correlations for LIN ($P = 0.5$), ALA ($P = 0.01$, but $P = 0.9$ when not including the outlier value; see Fig. 3B), ARA ($P = 0.4$), EPA ($P = 0.2$), or DHA ($P = 0.6$).

Discussion

Total lipids and PUFA

Dietary FA composition differed among rivers, lakes, and aquaculture systems. While EPA and ALA were the main FA in these riverine zoobenthos, pellets consisted to a much larger extent of LIN, and these

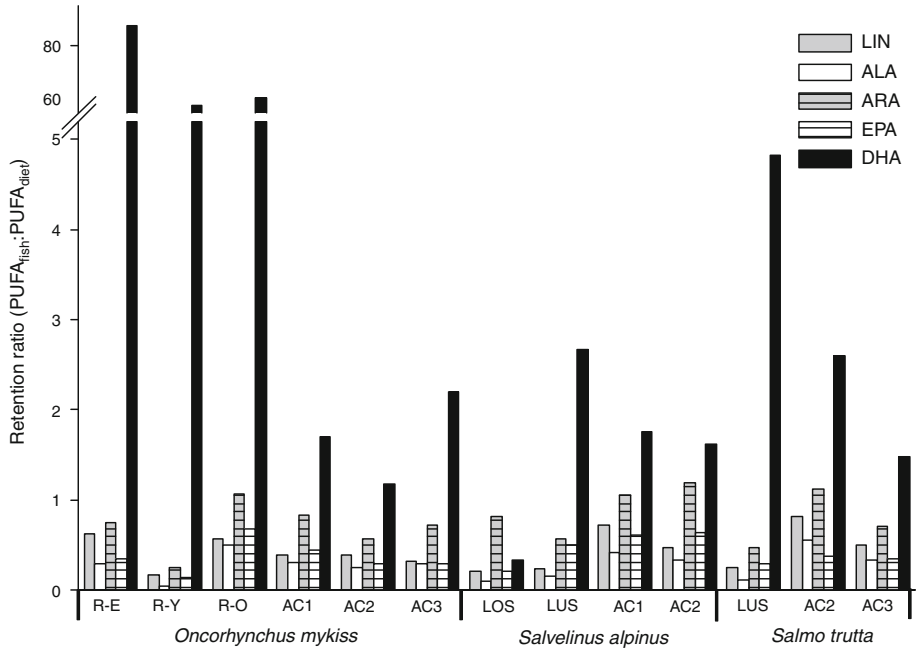

Fig. 2 Retention ratios of polyunsaturated fatty acid concentrations between fish and potential diets (concentration of $PUFA_{fish}/PUFA_{diet}$). The higher these ratios, the more the particular PUFA compounds are retained. *R-Y* River Ybbs, *R-E* River Erlauf, *R-O* River Ois, *LOS* Upper Lake Lunz, *LUS* Lower Lake Lunz, *AC 1–3* Aquaculture 1–3, *LIN* linoleic acid, *ALA* α-linolenic acid, *ARA* arachidonic acid, *EPA* eicosapentaenoic acid, *DHA* docosahexaenoic acid

zooplankton taxa had high concentrations of ALA and EPA. This is in contrast to what we found in fish with DHA as the most abundant FA in all examined species and habitats. These results show that these freshwater salmonids preferentially retained DHA. Dietary supply of DHA was highly variable and very low in riverine macrozoobenthos, which suggests highly efficient DHA retention and/or bioconversion of precursor PUFA to DHA. Such enzymatic conversion of PUFA has been demonstrated for freshwater salmonids, such as rainbow trout (Buzzi et al., 1996), brown trout, and Artic charr (Tocher et al., 2001).

When compared with DHA concentrations in fish, all other reported PUFA (LIN, ALA, ARA, and EPA) show lower concentrations, per unit biomass, than their potential diet supply. From a habitat point of view, it is evident that salmonids in these rivers retain and/or convert DHA more efficiently than salmonids of lake or aquaculture habitats. The presented PUFA retention ratios indicate that DHA was highly required in these salmonids and, from a diet supply perspective, possibly more in riverine habitats. In addition to such observational data, detailed comparative research is required to reveal whether river salmonids are more efficient in converting dietary PUFA than salmonids of other habitats. For lakes, data from a recent study on seasonal PUFA dynamics of lake zooplankton (Lower Lake Lunz) indicate that their potential dietary supply of $n-3$ PUFA, DHA, in particular, correlates with the abundance of copepods, known to be rich in DHA (e.g., Persson & Vrede, 2006; Kainz et al., 2009), whereas $n-3$ PUFA and DHA concentrations were lower when cladocerans prevailed during late spring and summer (unpubl. data). This present field study suggests that PUFA concentrations and profiles of these salmonids did not linearly reflect direct dietary supply.

Stable isotopes

Results on stable nitrogen isotopes ($\delta^{15}N$) show that fish examined from rivers, lakes, and aquacultures were different in their trophic positions. Fish of the habitat aquaculture had about two trophic levels higher $\delta^{15}N$ values than those of the rivers. The source of these high $\delta^{15}N$ values resulted from the

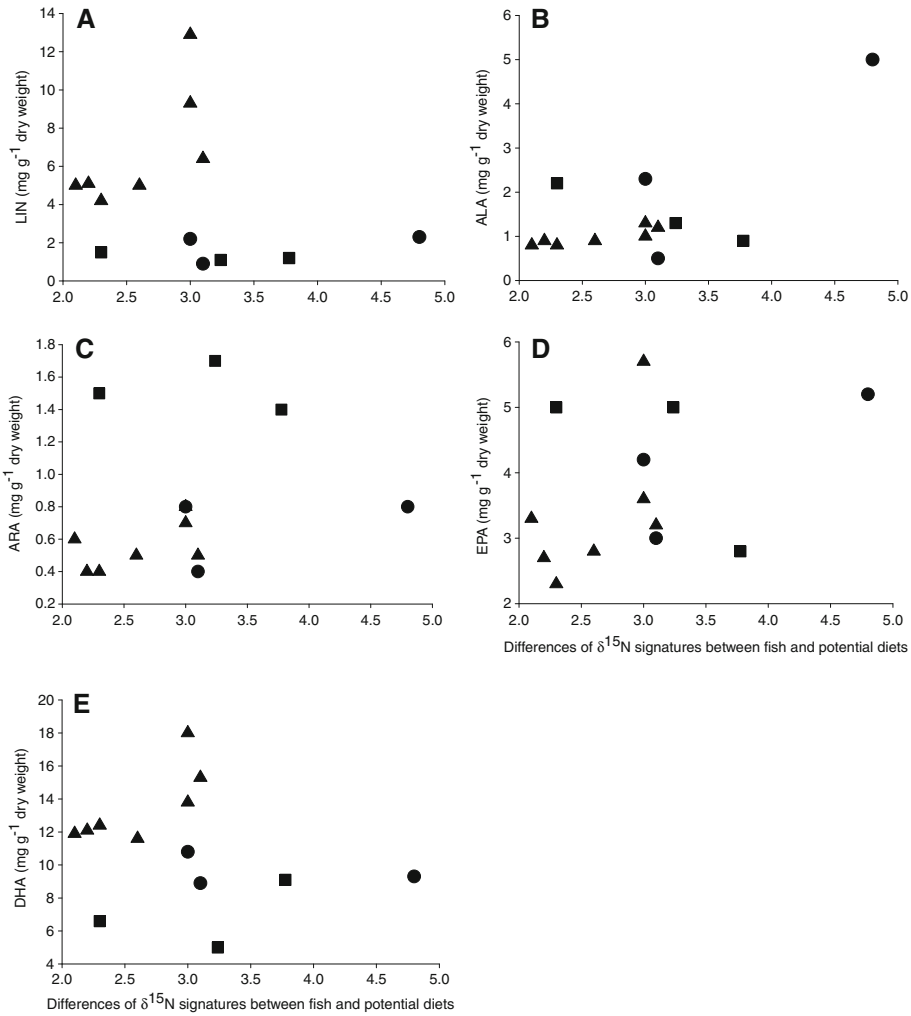

Fig. 3 **A**–**E** Trophic enrichment values (δ^{15}N; between fish and potential diet) versus retention of **A**: LIN, **B**: ALA, **C**: ARA, **D**: EPA, and **E**: DHA concentrations in fish of pre-alpine habitats (rivers: *filled circle*, lakes: *filled square*, and aquacultures: *filled triangle*)

diet, i.e., fish pellets that had higher δ^{15}N values than the measured lake zooplankton and riverine macrozoobenthos. Different δ^{13}C values of charr and brown trout (LUS) indicate different food sources. This argument is supported by gut content analysis where zooplankton was predominantly found in charr stomachs and benthos (mostly Plectoptera, partly digested), and occasionally small fish (*Cottus gobio* and *Phoxinus phoxinus*), in brown trouts. Charr at LOS contained marginally lower δ^{15}N values than charr at LUS, which is in line with the gut content analysis (visual observation) that charr at LUS did consume, occasionally, small fish. However, charr at LUS fed to a larger extent on zooplankton, which differed from the diet regime of brown trout at LUS. Due to mostly herbivorous feeding of these large zooplankters in both LUS and LOS, planktivorous fish had access to similar dietary δ^{15}N signatures.

In rivers, δ^{15}N values of fish were consistent. Fish in rivers were limited to benthos (benthivory) and/or other fish (omnivory), while fish in lakes were also exposed to zooplankton. Such different feeding strategies of riverine fish may have resulted in a higher variability of δ^{15}N (Vander Zanden & Vadebonceur, 2002). The low δ^{15}N values (even below zero) may have been due to nitrification/denitrification processes by methanotrophic bacteria which can be the diet of benthic organisms. Grey

et al. (2004) correlated such low $\delta^{15}N$ values of chironomid larvae to their excretion of ammonium that can be taken up by bacteria. Thus, N may be recycled and fractionated within the microhabitat resulting in low $\delta^{15}N$ values of benthic organisms.

The study systems aquaculture and river followed the proposed $\delta^{15}N$ enrichment of $\sim$3.4‰ per trophic level (Minagawa & Wada, 1984; Post, 2002), while lakes showed more complex diet flows. Fish as the top aquatic predator in lakes had more diverse diet sources (as indicated by large deviations of $\delta^{13}C$ signatures between fish and potential diet) and feeding grounds (e.g., pelagic and/or benthic feeding on both herbivores and carnivores), while riverine fish were, in this study, limited to mostly zoobenthic feeding, and to pellet feeding in aquacultures. The observed $\delta^{15}N$ enrichment of 2.7‰ between aquaculture fish and their diet was within the often proposed $\delta^{15}N$ range of 3.4 ± 1.1‰ per trophic level. Interestingly, we found a higher trophic enrichment at LUS (3.8‰ for brown trout, 3.2‰ for charr) than at LOS (2.7‰ for charr), suggesting that LOS charr mainly fed on plankton, while both fish species at LUS may have also fed on an additional trophic level, including prey fish.

The trophic enrichment between rainbow trout and macrozoobenthos of the rivers Erlauf and Ybbs was ca. 3‰, whereas it was much higher (4.9‰) in the river Ois. Such a clear trophic difference among these riverine habitats suggests that rainbow trout of the river Ois had access to other, trophically intermediate prey items. Alternatively, nitrification/denitrification processes by methanotrophic bacteria, yielding even negative $\delta^{15}N$ values (Grey et al., 2004), may also have caused such a large $\delta^{15}N$ difference between macrozoobenthos and rainbow trout. Although it could be shown that trophic enrichment occurred within all three examined aquatic habitats [ca. 3.4 ± 1.1‰; as proposed by Post (2002)], it is clear that further research is required to identify processes leading to such low $\delta^{15}N$ values of riverine benthos.

This study showed small differences in PUFA compositions of these freshwater salmonids. DHA was the dominant PUFA in salmonids, regardless of plankton, pellets, or benthos feeding. This was exemplified by PUFA of rainbow trout, which contained $\sim$10 mg DHA/g dw from both rivers and aquaculture systems. Interestingly, DHA concentrations of their diet were significantly different among the rivers, which contained hardly any DHA, while fish feed had higher DHA concentrations (mean: 7 mg DHA/g dw). Finally, no significant relationships between LIN, ALA, ARA, EPA, or DHA concentrations and $\delta^{15}N$ enrichment were found, indicating that the accumulation of these PUFA was not a simple function of trophic transfer. Data of this pilot field study suggest that freshwater salmonids seem to be able to regulate their PUFA retention, which is not a direct function of dietary PUFA supply as trophic positions of fish could not predict concentrations of PUFA compounds in their dorsal muscle tissues.

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