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ORIGINAL ARTICLE

Fatty Acid Composition of Freshwater Wild Fish in Subalpine Lakes: A Comparative Study

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Abstract In this study, the proximate and fatty acid compositions of the muscle tissue of 186 samples of fish belonging to fifteen species of freshwater fish harvested in subalpine lakes (bleak, shad, crucian carp, whitefish, common carp, pike, black bullhead, burbot, perch, Italian roach, roach, rudd, wels catfish, chub and tench) were investigated. Most of the fish demonstrated a lipid content in the fillet lower than 2.0 g 100 g^{-1} wet weight (range 0.6–9.7). A strong relationship between feeding behavior and fatty acid composition of the muscle lipids was observed. Planktivorous fish showed the lowest amounts of n-3 fatty acids ($p < 0.05$), but the highest monounsaturated fatty acid (MUFA) contents, in particular 18:1n-9. Conversely, carnivorous fish showed the highest amounts of saturated fatty acids and n-3 fatty acids ($p < 0.05$), but the lowest MUFA contents. Omnivorous fish showed substantial proportions of n-3 fatty acids and the highest contents of n-6 fatty acids. Principal component analysis showed a distinct separation between fish species according to their feeding habits and demonstrated that the most contributing trophic markers were 18:1n-9, 18:3n-3, 22:6n-3 and 20:4n-6. The quantitative amounts n-3 polyunsaturated fatty acid in muscle tissues varied depending on the fish species, the lipid content and the feeding habits. Some species were very lean, and therefore would be poor choices for human consumption to meet dietary n-3 fatty acid requirements. Nevertheless, the more frequently consumed and appreciated fish, shad and whitefish, had EPA and DHA contents in the range 900–1,000 mg 100 g^{-1} fresh fillet.

Keywords Subalpine lakes · Freshwater fish · Fatty acids · Trophic markers · Fish nutrition

Abbreviations

DHA	Docosahexaenoic acid (22:6n-3)
EPA	Eicosapentaenoic acid (20:5n-3)
FAME	Fatty acid methyl esters
LC-PUFA	Long chain polyunsaturated fatty acid(s)
MUFA	Monounsaturated fatty acid(s)
PUFA	Polyunsaturated fatty acid(s)
SFA	Saturated fatty acid(s)

Dedication This work is dedicated to the memory of Prof. Maria Antonietta Paleari, a rare woman, an outstanding scientist and a precious friend, who passed away on 20th December 2013.

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Introduction

The subalpine lake district includes the large lakes of glacial origin (Como, Garda, Maggiore, Lugano and Iseo) and several small medium Insubrian lakes. It represents more than 80 % of the total Italian lacustrine volume. The estimated total amount of fish caught in these lakes is more than 1,800 tons per year [1]. The main fish species of interest are perch (*Perca fluviatilis*), whitefish (*Coregonus* sp.), shad (*Alosa fallax lacustris*), eel (*Anguilla anguilla*), brown trout (*Salmo trutta*), bleak (*Alburnus alburnus alborella*), pikeperch (*Sander lucioperca*), pike (*Esox* *lucius*), tench (*Tinca tinca*), burbot (*Lota lota*), roach (*Rutilus pigus*) and carp (*Cyprinus carpio*). The predominant fish harvested yearly are whitefish (200 tons), landlocked shad (80 tons) and perch (40 tons). Most fish are sold fresh; however, some are dried, salted and smoked in order to increase market value and improve shelf life, such as shad, tench and roach [2].

The small-scale traditional fishing activity in these lakes has changed in recent years. Although traditional fishing continues, the compositions of the catch have changed from valued pelagic species to less valued littoral species. These changes have primarily been attributed to eutrophication of the waters and to the introduction in inland water of alien species, such as rudd or wels catfish, that have contributed to the loss of biodiversity [3].

The proximate and fatty acid compositions of muscle tissue of freshwater fish vary from species to species and are influenced by many intrinsic and extrinsic factors. The innate factors are related to the biology of fish species, with particular reference to their food habits and spawning season. The food habits are strictly dependent on the physiology and morphology of the digestive systems, which are highly differentiated among species. In terms of reproduction, fish demonstrate a number of array of reproductive strategies that are the results of the interaction between their genetic heritage and environment circumstances. During the spawning season fish utilize the energy derived from lipid storage for gonad production and dramatically reduce the level of ingested food, with the consequence of changing body composition [4].

The external factors influencing fatty acid composition of fish are mainly related to water and environmental conditions and consequently, to the quality and availability of food items.

The fatty acid composition of freshwater fish has been extensively studied, from the earlier studies of Gruger [5], Kinsella [6] and Ackman [7] to more recent articles [8–11]. It is well established that freshwater fish contain lower amounts of C20, C22 and n-3 fatty acids and higher amounts of C18 and n-6 fatty acids, in particular 18:2n-6, than their wild marine counterparts [4, 6]. Generally, these differences reflect the fatty acid compositions of organisms in the marine food web compared to the freshwater food web [12]. Indeed, phytoplankton and zooplankton of the marine food chain contain lipids in which 18:3n-3, EPA and DHA predominate and which have lower levels of n-6 PUFA.

Protein content and amino acid profile are considered to be rather stable components among the principal constituents of fish muscle. They are influenced mainly by genetic factors and seem to be unaffected by temperature, feeding rate and fish size [13]. Conversely, the amount of tissue lipid is highly variable and correlated to the feed intake and spawning time, showing a specific seasonal trend.

At present, data on lipid compositions of freshwater species harvested in European subalpine lakes are partial and fragmentary. Luzzana et al. [14] studied the seasonal variations of fat content and fatty acid composition of landlocked shad from Lake Como. Orban et al. [15] analyzed the nutritional quality of European perch and whitefish [16] from central Italian lakes. Serrini et al. [17] reported the fatty acid compositions of some coregonids caught in European lakes.

Considering also that some of these species are marketed for human consumption, this study has been designed to determine proximate and fatty acid compositions of muscle tissue of major freshwater fish species harvested in the subalpine lakes. Such data could be useful either to food scientists to develop accurate processing and preservation technologies or to nutritionists to study the nutritional properties of these fish. From an ecological point of view, an accurate evaluation of the relationship between fish species, feeding habits and environment can help us to derive more accurate conclusions concerning fatty acids as trophic markers of freshwater ecosystems.

Materials and Methods

Standards and Reagents

All chemicals and reagents were of analytical grade and were purchased from Sigma-Aldrich (Milan, Italy). Demineralized water was obtained from an Elga purification system (Veolia Water Solutions and Technologies, Italy). Standards of individual fatty acids were purchased from Sigma-Aldrich (Milan, Italy). Stock solutions of standard compounds were prepared in hexane at a concentration of 10 mg mL^{-1} and stored at −20 °C. Further standard dilutions were prepared in hexane individually or in mixtures to reduce the concentration to 0.2–1 mg mL^{-1} before GC analysis. A 37-FAME mixture in dichloromethane was obtained from Supelco (Supelco, Bellafonte, PA, USA) and was used as a reference standard.

Aquatic Environment

Morphometric characteristics and productivity of the lakes and their representative phytoplankton and zooplankton communities were obtained from the Regional Agency for Environmental Protection [18]. These data were collected within the annual monitoring program of inland waters enforced by Italian and European legislation (Legislative Decree 152/99 and Water Framework Directive [19]). The following physico-chemical parameters were used to classify the ecological status of the lakes: transparency, hypolimnetic oxygen saturation, chlorophyll *a* and total

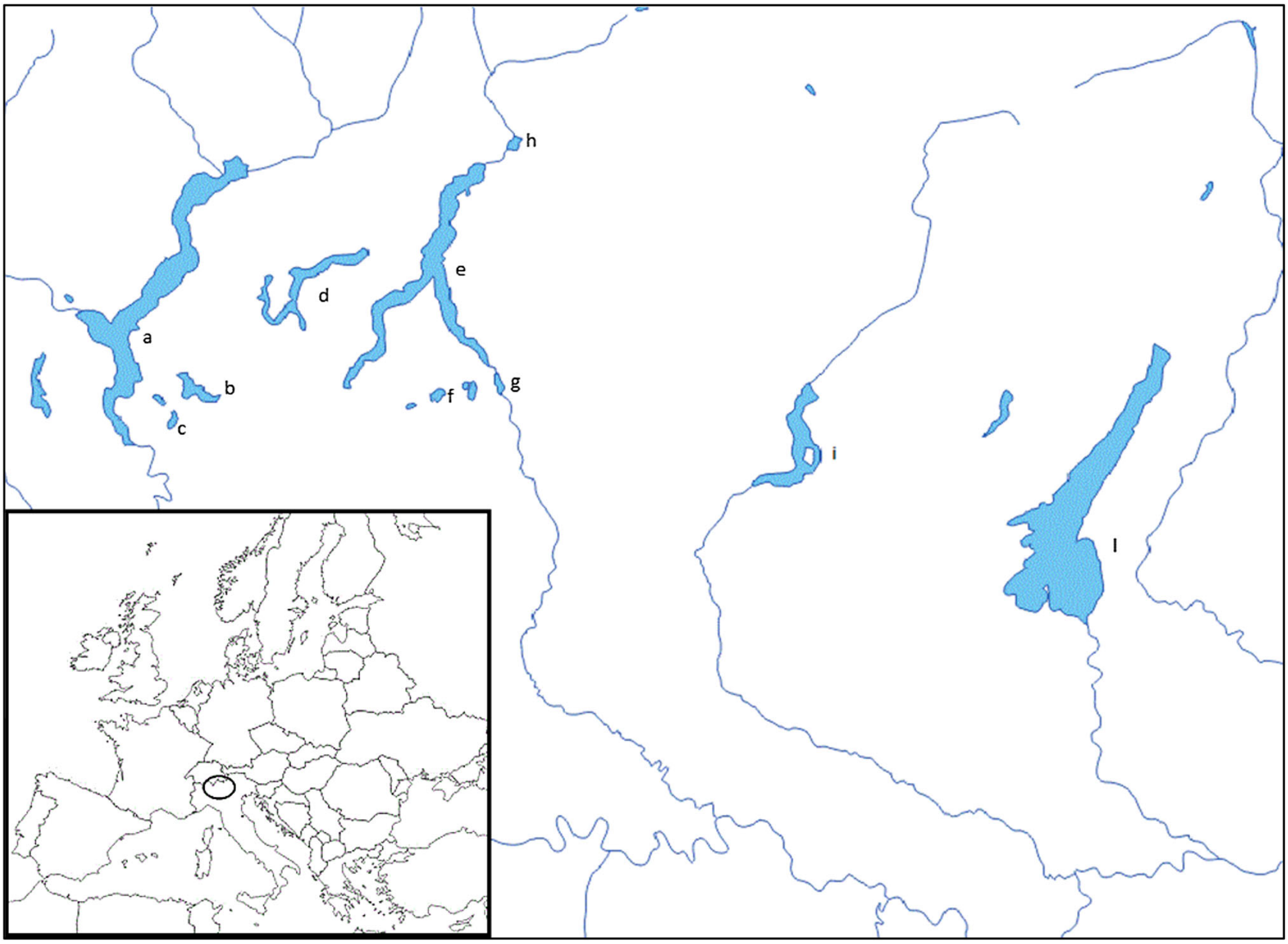

Fig. 1 Map of the subalpine lakes of this study. *a* L. Maggiore; *b* L. Varese; *c* L. Comabbio; *d* L. Lugano; *e* L. Como; *f* L. Alserio; *g* L. Garlate; *h* L. Mezzola; *i* L. Iseo; *l* L. Garda

phosphorus concentration. A map of the sampling locations is provided in Fig. 1.

Fish Sampling and Biometric Measurements

This study was carried out in two successive years, from September 2010 to June 2011. Fifteen fish species were sampled during catches in 10 different subalpine lakes by different commercial inland fishermen, using gill nets (Table 3). Bleak ($n = 100$), burbot ($n = 11$) and northern pike ($n = 3$) were caught in Iseo lake; landlocked shad in Como ($n = 17$) and Iseo ($n = 16$) lakes; European whitefish ($n = 10$) and Italian roach ($n = 14$) in Como lake; crucian carp in Alserio ($n = 7$), Comabbio ($n = 10$), Mezzola ($n = 10$) and Varese ($n = 10$) lakes; common carp ($n = 5$) in Varese lake; chub in Como ($n = 30$), Garlate ($n = 9$), Iseo ($n = 3$), Maggiore ($n = 5$) lakes; roach in Lugano ($n = 10$) and Maggiore ($n = 10$) lakes; black bullhead ($n = 10$) in Alserio lake, rudd in Alserio ($n = 10$), Comabbio ($n = 10$) and Garlate ($n = 10$) lakes; tench in Comabbio ($n = 7$), Como ($n = 5$), Iseo ($n = 5$), Mezzola ($n = 7$) and Varese ($n = 3$) lakes; European perch in Garda ($n = 19$) and Iseo ($n = 11$) lakes and wels catfish ($n = 8$) in Comabbio lake. A total of 385 fish specimens were collected.

After catching the fish were immediately stored under ice in polystyrene boxes until their arrival in the laboratory, where fish were measured for total length (L_T) and weighed for total mass (M_T), eviscerated mass (M_E), gonad mass (M_G), perivisceral fat mass (M_F) and liver mass (M_L). Fulton's condition index was calculated using the formula $K = 100\ M_T\ L_T^{-3}$. This morphometric index assumes that, within the same species, heavier fish for the same length are in better nutritional condition. When possible, macroscopic differentiation of gender was done, according to the shape, color and texture of the gonads. All fish were then manually filleted, with the exception of bleaks that, due to their small size, were eviscerated and analyzed as whole fish. Gonado-somatic index ($I_G = 100\ M_G\ M_T^{-1}$), hepatosomatic index ($I_H = 100\ M_L\ M_T^{-1}$) and coefficient of fatness ($C_F = 100\ M_F\ M_T^{-1}$) were calculated.

Dorsal fillet samples were skinned, vacuum packed and stored at −20 °C until analysis. Biometric measurements of wels catfish were not recorded because fish fillets were directly purchased from a fishmonger.

Proximate Composition and Fatty Acid Analysis

All assays for proximate composition analysis were performed using AOAC methods [20]. Moisture content of fillets was determined by drying samples in an oven at 100 °C for 16–18 h (Method 950.46). Total protein was determined by the Kjeldahl method (Method 940.25). For the analysis an automated distillation unit (Büchi 339, Switzerland) was used. Ash was determined by incineration of samples in a muffle furnace at 550 °C for 18 h (Method 938.08).

The extraction and determination of total lipids was performed according to the Folch method [21]. The preparation of fatty acid methyl esters was performed according to Christie [22]. Briefly, the lipid sample (20 mg) was dissolved in 10 % methanolic hydrogen chloride (2 mL). A 1 mL solution of tricosanoic acid (1 mg mL^{-1}) in toluene was added as an internal standard. The sample was sealed and heated at 50 °C overnight; then, 2 mL of a 1 M potassium carbonate solution were added to each sample. The FAME were extracted with 2 × 2 mL of hexane and the mixture was evaporated to dryness under a stream of nitrogen. The sample was dissolved in 1 mL hexane and 1 μL was injected into the gas chromatograph, in split mode (split ratio 1:100). Fatty acid analysis was carried out on an Agilent gas chromatograph (Model 6890 Series GC) fitted with an automatic sampler (Model 7683) and FID detector. The carrier gas was helium with a flow rate of 1.0 mL min^{-1} and an inlet pressure of 16.9 psi. An HP-Innowax fused silica capillary column (30 m × 0.25 mm I.D., 0.25 μm film thickness; Agilent Technologies) was used to separate fatty acid methyl esters. The oven temperature program for separation was from 100 to 180 °C at 3 °C min^{-1}, then from 180 to 250 °C at 2.5 °C min^{-1} and held for 10 min. Fatty acids were identified relative to known external standards and the resulting peak areas were then corrected by theoretical relative FID response factors [23] and quantified relative to the internal standard. All analyses were done in duplicate. Data were expressed as the mol% of fatty acids.

Statistical Analysis

Firstly, data were checked for normal distribution using a Shapiro–Wilk's test ($p > 0.05$) and an evaluation of the skewness and kurtosis measures. A Levene's test was used to verify the homogeneity of variance in the samples. Normal distribution and homogeneity of variance was confirmed and comparison between means was performed by analysis of variance. The Student–Newman–Keuls was used as a post hoc test for comparison of the means among different fish species. Significance was accepted at probabilities of 0.05 or less.

Principal component analysis was performed using fatty acid data in order to compare different samples and to detect the most important fatty acids affecting the distribution of fish samples. Since the measured variables differed significantly in their relative magnitudes, prior to performing PCA the dataset was submitted to centering and scaling. Centering consists of subtracting the mean from each variable and ensures that all results will be interpretable in terms of variation around the mean. In the scaling procedure each variable is scaled by dividing the centered value by the corresponding standard deviation, ensuring that each scaled variable gets the same variance. In this way, the correlation matrix was obtained and investigated by PCA, using the Non-linear Iterative Partial Least Squares algorithm. NIPALS algorithm calculates one principal component at a time and handles missing values. All the statistical analyses were performed by SPSS version 22.0 (SPSS Inc. Chicago, Illinois) and The Unscrambler version 10.0 (Camo, Norway). Data in the tables are reported as mean values ± standard deviations (SD).

Results

Aquatic Environment

Morphometric characteristics and productivity of the lakes of this study and their representative phytoplankton and zooplankton communities are presented in Tables 1 and 2. In all lakes, mostly meso-eutrophic, a common pattern in the sequence of seasonal changes can be identifiable. During the cold season Bacillariophyceae (diatoms) are dominant, with the exception of Lake Lugano in which Cyanobacteria are the predominant species all over the year. During summer, Cyanobacteria become prevalent in almost all lakes, with the exception of Iseo and Garda, where conjugatophytes are the algae more adapted to the mesotrophic conditions [24]. As concerns the zooplankton populations, copepods are present in high numbers during winter, especially in Lake Garda, while cladocerans became the dominant population during the summer, even if they are present with a consistent population all over the year. The rotifer population is more irregular and unpredictable, with increases in abundance that are not linked with the change of season but with algal blooms [25].

Table 1 Morphometric characteristics and productivity of the lakes in which the fish were caught for this study

Lake	Latitude (N)	Longitude E (G)[a]	Area (km^2)	Maximum depth (m)	Mean depth (m)	Trophic status	Ecological status[b]
Alserio	45°47′	09°12′	1.44	8	5.4	Eutrophic	4
Comabbio	45°45′	08°41′	3.59	8	4.3	Eutrophic	3
Como	46°10′	09°16′	145	410	155	Meso-eutrophic	3
Garda	45°40′	10°41′	368	350	133	Mesotrophic	2
Garlate	45°49′	09°24′	4.64	35	15	Mesotrophic	3
Iseo	45°44′	10°04′	61	251	125	Eutrophic	3
Lugano	45°59′	08°58′	48.9	288	85	Eutrophic	4
Maggiore	45°47′	08°40′	213	370	176	Mesotrophic	3
Mezzola	46°11′	09°26′	5.85	69	26	Oligo-mesotrophic	2
Varese	45°48′	08°45′	14.8	26	11	Eutrophic	4

[a] G: Greenwich

[b] Classification scheme for water quality includes five status classes: 1 high. 2 good. 3 moderate. 4 poor. 5 bad [18]

Fish Biometric Measurements

The biometric measurements taken on each sample and the biological characteristics of fish species of this study are listed in Tables 3 and 4. A total of 385 fish was caught in ten different lakes. All harvested fish were specimens of commercial size, according to the regulations enforced for the access to the lakes' resources. Each species occupies a particular habitat, designated as pelagic, littoral or benthonic, which for some species could change during the reproductive season. On the basis of a macroscopic and microscopic examination of the content of digestive system of some representative specimens and according to published information [26] a generalized feeding pattern of the analyzed fish is presented (Table 4). Bleak, shad and whitefish are planktivorous fish and feed on algae, cladocerans and copepods. Crucian carp, common carp, chub, roach, black bullhead, Italian roach, rudd and tench may be defined as omnivorous, and their diets depend on lacustral and seasonal availability of food. Some of them, such as tench, carp, black bullhead and Italian roach have benthic habits.

Burbot, pike, perch and wels catfish are carnivorous and ichthyophagous species and are placed at the top of the food chain. The trophic level of fish ranged from 2.8 of rudd to 4.4 of pike [27]. The length and weight of sampled fish varied in different lakes according to the occurrence and abundance of species. Shad were caught in the Como and Iseo lakes during autumn, their average weight ranged from 89 to 111 g. Crucian carp were caught in Alserio, Comabbio, Mezzola and Varese lakes during the spring season, with a mean weight ranged from 956 to 1,437 g. It is worth noting that crucian carp of such a size were all female specimens, due to their gynogenetic reproduction [28]. Chub were sampled in Como, Garlate, Iseo and Maggiore lakes. The weight of chub coming from Iseo was higher when compared to the others. Roach were caught in the Lugano and Maggiore lakes and the fish sizes were similar in both lakes. Rudd were harvested in Alserio, Comabbio and Garlate lakes during spring. Tench were from five different lakes and their weight varied from 874 to 2,702 g.

The Fulton index (K) varied among fish species according to their shape and morphology. Within the same species, K varied from 0.75 to 0.77 for shad, from 2.11 to 3.05 for crucian carp, from 1.21 to 1.43 for chub, from 1.52 to 1.63 for roach, from 1.21 to 1.25 for perch, from 1.73 to 2.28 for rudd and from 1.57 to 2.12 for tench. Significant statistical differences were detected among crucian carp caught from different lakes, as well as among rudd and tench.

Proximate and Fatty Acid Compositions

The proximate and fatty acid compositions of muscle tissues of freshwater fish collected in this study are presented in Table 5. The lipid content varied from 0.57 g 100 g^{-1} in perch to 9.69 g 100 g^{-1} in shad, with most fish having a lipid content lower than 2.0 g 100 g^{-1} wet weight. Protein ranged from 18.07 g 100 g^{-1} in burbot to 21.38 g 100 g^{-1} in whitefish. The ash levels of muscle tissue were similar among species and were approximately 1–1.5 g 100 g^{-1}. Bleak samples, due to their small size (adult maximum length around 10 cm), were analyzed as whole eviscerated fish and consequently presented an ash amount higher than fillets from the other species. The moisture content varied from 69.44 g 100 g^{-1} in shad to 80.71 g 100 g^{-1} in burbot.

A total of 19 major fatty acids, including 3 saturated fatty acids (SFA), 5 monounsaturated fatty acids (MUFA) and 11 polyunsaturated fatty acids (PUFA) were identified and quantified. All fatty acids varied significantly among fish species.

Total SFA ranged from 24.4 % in Italian roach to 38.0 % in perch, MUFA ranged from 12.2 % in pike to 36.9 % in shad and PUFA from 32.7 % in shad to 61.2 % in roach. The most abundant SFA in all fish studied was

Table 2 Phytoplankton and zooplankton communities in studied lakes

Lake	Phytoplankton community		Zooplankton community	
	Summer	Winter	Summer	Winter
Alserio	*Tetraedron muticum* (Clorophyceae)	*Cyclotella comensis* (Bacillariophycae)	*Daphnia hyalina* (Cladocera)	*Bosmina longirostris* (Cladocera)
	Tetraedron minimum (Clorophyceae)	*Rhodomonas minuta* (Cryptophycae)	*Thermocyclops crassus* (Copepoda)	*Asplanchna priodonta* (Rotifera)
Comabbio	*Merismopedia glauca* (Cyanobacteria)	*Synedra ulna* (Bacillariophycae)	*Cyclops abyssorum* (Copepoda)	*Keratella quadrata* (Rotifera)
	Microcystis incerta (Cyanobacteria)	*Synedra acus* (Bacillariophycae)	*Daphnia hyalina* (Cladocera)	*Synchaeta pectinata* (Rotifera)
Como	*Planktothrix rubescens* (Cyanobacteria)	*Asterionella formosa* (Bacillariophycae)	*Conochilus unicornis* (Rotifera)	*Conochilus unicornis* (Rotifera)
	Ceratium hirundinella (Dynophceae)	*Fragilaria crotonensis* (Bacillariophycae)	*Daphnia hyalina* (Cladocera)	*Keratella cochlearis* (Rotifera)
Garda	*Mougeotia spp* (Conjugatophyceae)	*Fragilaria crotonensis* (Bacillariophycae)	*Keratella quadrata* (Rotifera)	*Mesocyclops leuckartii* (Copepoda)
	Closterium aciculare (Conjugatophyceae)	*Planktothrix rubescens* (Cyanobacteria)	*Kellicottia longispina* (Rotifera)	*Copipodiaptomus steueri* (Copepoda)
Garlate	–	*Asterionella formosa* (Bacillariophycae)	–	*Eudiaptomus padanus padanus* (Cladocera)
	–	*Planktothrix rubescens* (Cyanobacteria)	–	*Eubosmina longispina* (Cladocera)
Iseo	*Mougeotia spp* (Conjugatophyceae)	*Aulacoseira islandica* (Bacillariophycae)	*Daphnia hyalina* (Cladocera)	*Asplanchna priodonta* (Rotifera)
	Planktothrix rubescens (Cyanobacteria)	*Fragilaria crotonensis* (Bacillariophycae)	*Keratella cochlearis* (Rotifera)	*Bosmina longirostris* (Cladocera)
Lugano	*Pseudanabaena limnetica* (Cyanobacteria)	*Limnothrix redeckei* (Cyanobacteria)	*Daphnia hyalina* (Cladocera)	*Eubosmina coregoni* (Cladocera)
	Planktothrix rubescens (Cyanobacteria)	*Stephanodiscus parvus* (Bacillariophycae)	*Cyclops abyssorum* (Copepoda)	*Mesocyclops leuckartii* (Copepoda)
Maggiore	*Aphanocapsa/Aphanothece sp.* (Cyanobacteria)	*Diatoma tenuis* (Bacillariophycae)	*Eudiaptomus padanus padanus* (Cladocera)	*Eudiaptomus padanus padanus* (Cladocera)
	Oscillatoria limnetica (Cyanobacteria)	*Fragilaria crotonensis* (Bacillariophycae)	*Daphnia hyalina* (Cladocera)	*Conochilus unicornis* (Rotifera)
Mezzola	*Synedra ulna* (Bacillariophycae)	*Cyclotella comensis* (Bacillariophycae)	*Keratella cochlearis* (Rotifera)	–
	Tabellaria fenestrata (Bacillariophycae)	*Gymnodinium helveticum* (Dynophceae)	*Cyclops abyssorum* (Copepoda)	–
Varese	*Planktothrix rubescens* (Cyanobacteria)	*Asterionella formosa* (Bacillariophycae)	*Daphnia cucullata* (Cladocera)	*Keratella quadrata* (Rotifera)
	Oscillatoria limnetica (Cyanobacteria)	*Cyclotella comensis* (Bacillariophycae)	*Bosmina longirostris* (Cladocera)	*Synchaeta pectinata* (Rotifera)

palmitic acid (16:0), which varied from 16.6 % in crucian carp to 21.5 % in shad, followed by stearic acid (18:0), which varied from 4.5 % in shad to 16.1 % in perch.

The most represented MUFA in freshwater fish were oleic acid (18:1n-9) (6.3 % in roach to 26.6 % in shad), 16:1n-7 (1.9 % in pike to 8.9 % in tench) and 18:1n-7 (2.4 % in pike to 5.9 % in wels catfish). Other minor MUFA detected were 20:1n-9 (0.3 % in perch to 1.7 % in shad) and 22:1. Among long chain polyunsaturated fatty acids, docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3) varied dramatically among fish species. DHA ranged from 4.7 % in bleak to 29.7 % in roach, whereas EPA ranged from 5.7 % in pike to 15.9 % in burbot. Linoleic (18:2n-6) and arachidonic (20:4n-6) acids were also present in moderate proportions and varied in fish fillets from 2.0 % in roach to 5.9 % in chub and from 4.6 % in shad to 11.2 % in common carp, respectively. Total n-3 fatty acids ranged from 21.5 % in shad to 47.3 in roach, whereas total n-6 fatty acids ranged from 11.0 % in whitefish to 20.0 % in common carp.

Table 3 Sampling and biometric measurement of fish species collected in subalpine lakes

Fish	Lake (abbr.)	Month of capture	No. fish	Length (cm)	Weight (g)	Fulton index
Landlocked shad	Como (CO)	September	17	22.5 ± 3.6	89.1 ± 39.4a	0.75 ± 0.06
	Iseo (IS)	November	16	24.4 ± 1.3	111.8 ± 12.7b	0.77 ± 0.06
Bleak	Iseo (IS)	November	100	9.3 ± 0.5	8.5 ± 1.3	1.05 ± 0.11
Burbot	Iseo (IS)	November	11	34.1 ± 3.0	318.5 ± 57.4	0.80 ± 0.08
Crucian carp	Alserio (AL)	March	7	35.1 ± 5.1	1,379.6 ± 561.3b	3.05 ± 0.38a
	Comabbio (CM)	April	10	38.7 ± 1.7	1,437.4 ± 194.1b	2.5 ± 0.42b
	Mezzola (ME)	May	10	35.5 ± 2.6	956.8 ± 260.0a	2.11 ± 0.16c
	Varese (VA)	May	10	37.0 ± 4.3	1,160.5 ± 408.4ab	2.2 ± 0.25c
Common carp	Varese (VA)	June	5	26.0 ± 3.3	406.1 ± 138.8	2.25 ± 0.17
Chub	Como (CO)	November	20	35.3 ± 4.8a	559.0 ± 175.7a	1.34 ± 0.25
	Garlate (GR)	April	9	32.2 ± 1.7a	483.3 ± 58.9a	1.43 ± 0.11
	Iseo (IS)	October	3	54.3 ± 1.9b	1,938.2 ± 170.9b	1.21 ± 0.07
	Maggiore (MA)	March	5	35.3 ± 3.8a	632.1 ± 230.2a	1.38 ± 0.09
	Como (CO)	April	10	32.9 ± 4.3a	497.3 ± 227.5a	1.31 ± 0.15
Roach	Lugano (LU)	April	10	22.7 ± 2.5	184.8 ± 68.8	1.52 ± 0.08
	Maggiore (MA)	March	10	26.5 ± 3.9	317.9 ± 167.4	1.63 ± 0.52
European whitefish	Como (CO)	September	10	27.6 ± 1.2	186.9 ± 10.9	0.89 ± 0.13
Pike	Iseo (IS)	November	3	50.7 ± 16.2	1,053.6 ± 1,006.8	0.65 ± 0.06
European perch	Garda (GA)	November	19	23.8 ± 3.7	181.5 ± 88.8	1.25 ± 0.13
	Iseo (IS)	November	11	24.0 ± 1.5	167.5 ± 24.4	1.21 ± 0.09
Black bullhead	Alserio (AL)	April	10	20.3 ± 1.3	140 ± 29.7	1.69 ± 0.39
Italian Roach	Como (CO)	April	14	33.1 ± 4.9	517.5 ± 243.7	1.34 ± 0.10
Rudd	Alserio (AL)	April	10	22.7 ± 1.9a	240.8 ± 41.5a	2.1 ± 0.48ab
	Comabbio (CM)	April	10	20.8 ± 5.9a	225.1 ± 319.9a	1.73±0.45a
	Garlate (GR)	April	10	30 ± 2.1b	594.6 ± 59.2b	2.28 ± 0.60b
Tench	Comabbio (CM)	June	7	51.2 ± 3.0a	2,513.9 ± 466.8a	1.86 ± 0.18ab
	Como (CO)	September	5	37.6 ± 6.0b	874.4 ± 363.8c	1.57 ± 0.10a
	Iseo (IS)	November	5	39.8 ± 4.0b	1,083.5 ± 282.7c	1.71 ± 0.12ab
	Mezzola (ME)	May	7	43.8 ± 3.8b	1,773.2 ± 444b	2.12 ± 0.46b
	Varese (VA)	May	3	53.7 ± 2.0a	2,702.9 ± 500.2a	1.73 ± 0.13ab
Wels Catfish		April	8	nd	nd	nd

Value within the species on the same column not sharing a common letter are significantly different ($p < 0.05$)

The fatty acid compositions of planktivorous fish (whitefish, shad and bleak) were significantly different among species (Table 5). Whitefish was richer in n-3 fatty acids, EPA and DHA, while bleak was richer in n-6 fatty acid, especially 18:2n-6, when compared to the other two species. The oleic acid percentage was higher in bleak and lower in shad and whitefish. The n-3/n-6 ratio was 0.94 in bleak, 1.97 in shad and 2.8 in whitefish. The predominant fatty acids in bleak were 18:1n-9, 16:0, 18:2n-6 and 16:1n-7; the predominant fatty acid in shad were 18:1n-9, 16:0, EPA and 18:3n-3, whereas the predominant fatty acids in whitefish were 16:0, 18:1n-9, EPA and DHA.

Interestingly, the fatty acid compositions of omnivorous fish showed high variability among species. Total SFA ranged from 24.4 to 27.9 %, MUFA ranged from 13.0 to 32.0 % and PUFA from 40.0 to 61.2 %. The most abundant SFA was 16:0, which ranged from 16.6 % in crucian carp to 19.7 % in rudd, followed by 18:0, which

Table 4 Biology of fish species collected in this study

Family	Species	Common name	Lacustrine habitat	Feeding habits	Food items found in digestive system	Trophic level[a]	Reproductive season
Clupeidae	*Alosa fallax lacustris*	Landlocked shad	Pelagic	Planktivorous	Cladocerans. copepods and small fish	3.6	Early summer
Cyprinidae	*Alburnus arborella*	Bleak	Pelagic	Planktivorous	Planktivorous: cladocerans and copepods. insects larvae and invertebrates	3.0	Summer
Salmonidae	*Coregonus macrophtalmus*	European whitefish	Pelagic	Planktivorous	Insects larvae and zooplankton	3.3	Winter
Cyprinidae	*Scardinius erythrophthalmus*	Rudd	Littoral	Opportunistic omnivorous	Plankton. terrestrial insects and plant material	2.9	Spring
Cyprinidae	*Squalius squalus*	Chub	Littoral	Opportunistic omnivorous	Aquatic and terrestrial animal. plant material and fish	3.0	Spring
Cyprinidae	*Carassius carassius*	Crucian carp	Littoral	Omnivorous/ benthophagous	Plankton. benthic invertebrates. plant materials and detritus	3.1	Late spring
Cyprinidae	*Cyprinus carpio*	Common carp	Littoral	Omnivorous/ benthophagous	Benthic organisms and plant material	3.0	Late spring
Cyprinidae	*Rutilus rutilus*	Roach	Littoral	Opportunistic omnivorous	Benthic invertebrates. zooplankton. plant material and detritus	2.8	Spring
Cyprinidae	*Rutilus pigus*	Italian Roach	Littoral	Omnivorous/ benthophagous	Benthic macroinvertebrates. macrophytes	3.1	Spring
Cyprinidae	*Tinca tinca*	Tench	Littoral	Omnivorous/ benthophagous	Detritus. mollusks. benthic animals and plant materials	3.5	Summer
Ictaluridae	*Ictalurus melas*	Black bullhead	Benthonic	Omnivorous	Insects. leeches and crustaceans. clams. snails. plant material and fish	3.7	Spring
Gadidae	*Lota lota*	Burbot	Benthonic	Ichthyophagous	Crayfish. mollusks. fish and other invertebrates	4.0	Winter
Percidae	*Perca fluviatilis*	European perch	Littoral	Ichthyophagous	Benthic prey and fish	4.4	Spring
Esocidae	*Esox lucius*	Pike	Littoral	Ichthyophagous	Invertebrates. terrestrial vertebrates and fish	4.4	Late winter
Siluridae	*Silurus glanis*	Wels Catfish	Benthonic	Ichthyophagous	Invertebrates. fish and other aquatic vertebrates	4.4	Early summer

[a] *Source*: Fishbase.org

varied from 5.5 % in Italian roach to 9.1 % in common carp. The most represented MUFA were 18:1n-9, 16:1n-7 and 18:1n-7.

The proportion of DHA and EPA ranged from 8.2 % in tench to 29.7 % in roach, and from 6.7 % in common carp to 12.5 % in burbot, respectively. Linoleic and arachidonic acids varied in muscle tissue from 2.1 % in roach to 6.2 % in chub and from 7.8 % in black bullhead to 11.2 % in common carp, respectively. Among omnivorous, the fish with the highest content of n-3 fatty acids was roach (47.3 %), whereas the species with the highest content of n-6 fatty acids was common carp (20.0 %).

Perch and pike presented a similar muscle fatty acid composition. When compared to burbot and wels catfish they showed a higher amount of DHA and SFA, in particular 18:0, and a lower amount of MUFA, in particular 18:1n-9. Total n-3 fatty acid accounted for 41.6 % of fatty acids in pike, 35.5 % in burbot, 35.3 % in perch and 32.2 % in wels catfish.

As shown in Table 6, aquatic environment affected lipid compositions of shad and perch. Interestingly, shad from Iseo in November contained a higher amount of lipid (11.1 vs 9.3 %), which were richer in DHA (9.5 vs 3.6 %). In contrast, lipid of shad from Como in September were richer in 18:1n-9 (28.0 vs 20.7 %). Perch caught in Garda and Iseo were of analogous size and presented similar proximate and fatty acid compositions. The main differences were related to the content of EPA and DHA. As regards whole bleak (Table 5), the fatty acid composition was characterized by high percentages of oleic and linoleic acid.

Proximate and fatty acid compositions of crucian carp caught in different lakes are presented in Table 7. All fish were caught during spring, which for cyprinids coincides

Table 5 Proximate (g 100 g^{-1} fresh tissue) and fatty acid composition (mol%) of muscle tissue of freshwater fish analysed in this study

Feeding habits	Planktivorous			Omnivorous			
Fish Fish no.	Bleak[3] 100	Shad 22	Whitefish 10	Crucian carp 21	Common carp 5	Rudd 14	Roach 10
Weight (g)	8.51 ± 1.32	94.53 ± 31.37	186.87 ± 10.87	1,175.2 ± 3,98.53	406.1 ± 1,38.88	430.1 ± 2,71.80	237.2 ± 1,42.04
Moisture	72.91 ± 1.12	69.44 ± 2.00a	71.38 ± 1.43b	78.24 ± 0.68d	78.77 ± 0.44de	78.77 ± 0.73de	78.47 ± 0.88d
Protein	19.83 ± 0.57	19.44 ± 1.49ab	21.38 ± 0.52c	19.57 ± 0.67ab	19.26 ± 0.57ab	19.07 ± 0.59ab	19.32 ± 0.74ab
Lipid	4.54 ± 1.54	9.69 ± 2.30b	5.99 ± 1.71c	0.84 ± 0.27a	0.63 ± 0.08a	0.87 ± 0.13a	0.94 ± 0.10a
Ash	2.72 ± 0.72	1.43 ± 0.10	1.26 ± 0.06	1.35 ± 0.18	1.35 ± 0.20	1.30 ± 0.18	1.26 ± 0.24
C14:0	2.35 ± 0.38	4.35 ± 0.42e	3.62 ± 0.48d	1.49 ± 0.38bc	0.76 ± 0.14ab	0.90 ± 0.21ab	0.51 ± 0.16a
C16:0	16.74 ± 1.17	21.47 ± 1.08c	19.65 ± 1.09bc	16.61 ± 1.94a	17.78 ± 1.47ab	19.69 ± 0.81bc	18.53 ± 2.43ab
C16:1n-7	11.87 ± 1.95	4.48 ± 0.54ab	7.25 ± 1.17cd	6.01 ± 1.24bc	4.43 ± 0.63ab	3.82 ± 0.70ab	2.63 ± 1.01a
C18:0	3.85 ± 0.84	4.52 ± 0.51a	4.27 ± 0.33a	7.10 ± 1.18bc	9.12 ± 0.56c	6.52 ± 0.65b	6.75 ± 0.34bc
C18:1n-9	30.74 ± 4.74	26.62 ± 4.52e	19.31 ± 2.31d	11.64 ± 4.29abc	10.27 ± 1.29abc	8.04 ± 3.54ab	6.29 ± 1.22a
C18:1n-7	3.63 ± 1.30	3.90 ± 0.39c	3.55 ± 0.24bc	5.34 ± 0.48de	4.93 ± 0.44d	2.71 ± 0.44ab	3.39 ± 0.41bc
C18:2n-6	10.14 ± 0.72	4.61 ± 0.87abc	4.19 ± 0.44abc	3.55 ± 0.95abc	4.98 ± 2.17bc	3.50 ± 2.49abc	2.01 ± 1.07a
C18:3n-6	0.44 ± 0.07	0.56 ± 0.10	0.29 ± 0.04	0.23 ± 0.06	0.17 ± 0.07	0.16 ± 0.07	0.10 ± 0.04
C18:3n-3	6.54 ± 0.59	5.27 ± 1.12c	5.43 ± 0.67c	2.90 ± 0.69ab	2.26 ± 1.73ab	4.97 ± 4.24c	1.16 ± 0.38a
C18:4n-3	0.30 ± 0.32	2.71 ± 0.78	3.29 ± 0.60	1.02 ± 0.30	0.23 ± 0.05	0.68 ± 0.23	0.37 ± 0.19
C20:1n-9	1.11 ± 0.27	1.69 ± 0.26	0.67 ± 0.15	1.09 ± 0.22	0.78 ± 0.17	0.30 ± 0.17	0.54 ± 0.16
C20:2n-6	0.57 ± 0.09	0.28 ± 0.14a	0.36 ± 0.04ab	0.42 ± 0.17ab	1.10 ± 0.10d	0.54 ± 0.19abc	0.80 ± 0.21cd
C20:3n-6	0.53 ± 0.05	0.33 ± 0.06	0.19 ± 0.02	0.55 ± 0.11	1.04 ± 0.17	0.49 ± 0.21	0.44 ± 0.16
C20:4n-6	3.36 ± 1.06	4.63 ± 0.89a	5.18 ± 0.63a	8.90 ± 1.75bcd	11.21 ± 1.66d	8.62 ± 2.50bcd	9.94 ± 1.17cd
C20:5n-3	3.66 ± 1.16	6.77 ± 1.45a	11.18 ± 1.16cd	9.40 ± 1.31bc	6.71 ± 1.04a	12.51 ± 3.37d	11.44 ± 3.42cd
C22:1	0.03 ± 0.01	0.25 ± 0.03	0.13 ± 0.01	0.14 ± 0.06	0.27 ± 0.06	0.08 ± 0.04	0.15 ± 0.06
C22:4n-6	0.24 ± 0.06	0.83 ± 0.20ab	0.75 ± 0.14ab	0.97 ± 0.24ab	1.51 ± 0.26bc	0.80 ± 0.46ab	0.73 ± 0.24ab
C22:5n-3	0.34 ± 0.50	2.22 ± 0.94ab	2.74 ± 0.32abc	4.01 ± 0.69cde	4.34 ± 0.63cde	3.78 ± 0.81bcde	4.60 ± 1.07de
C22:6n-3	3.58 ± 1.83	4.70 ± 2.81	8.00 ± 2.12a	18.64 ± 3.36def	18.13 ± 2.29def	21.93 ± 2.51efg	29.69 ± 3.16h
SFA	22.94 ± 1.62	30.34 ± 1.31b	27.54 ± 0.98ab	25.20 ± 1.72a	27.66 ± 1.30ab	27.11 ± 1.19ab	25.78 ± 2.50a
MUFA	47.37 ± 5.03	36.94 ± 4.29e	30.86 ± 3.54d	24.20 ± 5.44 cd	20.68 ± 1.18abc	14.92 ± 4.64ab	12.98 ± 2.16a
PUFA	29.69 ± 3.99	32.72 ± 4.22a	41.60 ± 2.75b	50.59 ± 5.26c	51.66 ± 1.78cd	57.98 ± 3.68de	61.25 ± 3.98e
n-3	14.41 ± 2.65	21.48 ± 4.30a	30.65 ± 2.57b	35.97 ± 4.01bc	31.66 ± 1.38b	43.87 ± 5.70de	47.25 ± 4.62e
n-6	15.28 ± 1.43	11.24 ± 1.70a	10.95 ± 0.58a	14.63 ± 2.53a	20.01 ± 2.82b	14.11 ± 2.36a	14.00 ± 1.71a
n-3 LC PUFA[1]	7.58 ± 2.74	13.60 ± 3.64a	21.93 ± 3.50b	32.05 ± 4.50cde	29.17 ± 3.05c	38.22 ± 5.43de	45.72 ± 4.24f
n-6 LC PUFA[2]	4.70 ± 1.23	6.07 ± 1.10a	6.47 ± 0.70a	10.85 ± 2.14bcd	14.86 ± 2.11e	10.46 ± 2.65bc	11.91 ± 1.43cde

Table 5 continued

Feeding habits	Planktivorous			Omnivorous			
Fish	Bleak[3]	Shad	Whitefish	Crucian carp	Common carp	Rudd	Roach
Fish no.	100	22	10	21	5	14	10
n3/n6	0.94 ± 0.09	1.97 ± 0.57abc	2.80 ± 0.25def	2.53 ± 0.48cde	1.61 ± 0.25a	3.24 ± 0.87efg	3.45 ± 0.71fg
FA mg/100 g tissue	3,405.93 ± 1,160.52	8,604.72 ± 1,725.56	5,429.95 ± 1,284.02	738.36 ± 204.95	546.84 ± 63.27	761.25 ± 105.56	815.92 ± 75.25

Fish	Italian roach	Chub	Tench	Black bullhead	Perch	Burbot	Wels catfish	Pike
Fish no.	9	25	23	5	10	10	5	3
Weight (g)	517.5 ± 24.76	783.1 ± 505.07	1,755.3 ± 801.25	150.0 ± 29.72	201.3 ± 61.73	333.5 ± 57.44	*nd*	1,053.6 ± 1,006.87
Moisture	78.73 ± 0.98de	78.56 ± 1.12d	77.70 ± 2.25a	78.15 ± 0.91d	79.03 ± 0.67de	80.71 ± 0.56e	78.24 ± 0.95d	79.23 ± 0.94de
Protein	19.85 ± 1.06b	19.16 ± 1.20ab	18.93 ± 1.37ab	19.29 ± 0.72ab	19.16 ± 0.57ab	18.07 ± 0.64a	19.16 ± 0.78ab	18.93 ± 0.85ab
Lipid	0.72 ± 0.49a	1.00 ± 0.58a	2.01 ± 2.14a	1.13 ± 0.20a	0.57 ± 0.47a	1.28 ± 0.11a	0.62 ± 0.18a	1.50 ± 0.55a
Ash	1.46 ± 0.19	1.27 ± 0.22	1.36 ± 0.18	1.43 ± 0.37	1.24 ± 0.22	0.96 ± 0.05	1.98 ± 0.17	1.34 ± 0.12
C14:0	0.77 ± 0.75ab	1.05 ± 0.57ab	1.95 ± 1.14c	1.20 ± 0.47ab	0.95 ± 0.20ab	1.04 ± 0.29ab	1.11 ± 0.47ab	1.29 ± 0.64ab
C16:0	18.12 ± 2.42ab	18.52 ± 1.70ab	18.66 ± 1.91ab	18.41 ± 1.07ab	20.92 ± 1.41c	18.03 ± 1.42ab	18.14 ± 1.15ab	18.05 ± 0.82ab
C16:1n-7	7.30 ± 3.46cd	3.36 ± 1.48ab	8.87 ± 4.64d	3.20 ± 0.91ab	2.78 ± 0.83a	3.70 ± 0.84ab	4.47 ± 1.34ab	1.94 ± 1.34a
C18:0	5.49 ± 1.14ab	7.85 ± 2.94bc	6.99 ± 2.80bc	7.23 ± 0.42bc	16.13 ± 1.57e	11.30 ± 1.82d	7.35 ± 0.73bc	15.73 ± 2.28e
C18:1n-9	15.99 ± 8.24cd	13.52 ± 4.83bc	14.34 ± 4.77c	13.67 ± 2.21bc	6.74 ± 1.78a	12.05 ± 0.96abc	10.83 ± 3.37abc	7.64 ± 2.54ab
C18:1n-7	4.07 ± 1.27c	2.86 ± 0.57ab	5.51 ± 1.02de	5.44 ± 0.57de	2.82 ± 0.50ab	5.30 ± 0.66de	5.90 ± 0.39e	2.37 ± 0.82a
C18:2n-6	2.75 ± 1.18ab	5.95 ± 2.58c	5.75 ± 2.45c	3.77 ± 0.45abc	2.88 ± 0.76ab	2.16 ± 0.35a	4.34 ± 1.13abc	3.37 ± 1.08abc
C18:3n-6	0.17 ± 0.05	0.31 ± 0.28	0.18 ± 0.14	0.08 ± 0.01	0.17 ± 0.02	0.25 ± 0.07	0.10 ± 0.02	0.44 ± 0.28
C18:3n-3	2.21 ± 1.36ab	1.61 ± 0.87ab	3.81 ± 1.93bc	3.38 ± 0.38abc	1.15 ± 0.36a	1.85 ± 0.45ab	2.28 ± 0.87ab	2.08 ± 1.10ab
C18:4n-3	0.31 ± 0.18	0.54 ± 0.39	0.84 ± 0.49	0.66 ± 0.15	0.66 ± 0.22	1.37 ± 1.19	0.43 ± 0.04	1.27 ± 0.82
C20:1n-9	0.56 ± 0.26	0.56 ± 0.24	0.60 ± 0.34	0.80 ± 0.08	0.26 ± 0.06	0.55 ± 0.19	1.27 ± 0.21	0.55 ± 0.18
C20:2n-6	0.84 ± 0.34 cd	0.86 ± 0.24cd	0.85 ± 0.38cd	0.66 ± 0.12bc	0.29 ± 0.05a	0.57 ± 0.07abc	1.05 ± 0.18d	0.52 ± 0.13abc
C20:3n-6	0.53 ± 0.26	0.86 ± 0.53	0.65 ± 0.20	0.55 ± 0.14	0.26 ± 0.06	0.28 ± 0.03	0.76 ± 0.08	0.54 ± 0.36
C20:4n-6	9.28 ± 2.62bcd	9.65 ± 2.22cd	8.70 ± 3.56bcd	7.79 ± 1.04bc	9.50 ± 0.61cd	9.10 ± 0.99bcd	11.06 ± 2.18d	6.44 ± 0.59ab
C20:5n-3	10.35 ± 2.91cd	6.93 ± 1.37a	8.20 ± 0.91ab	12.48 ± 0.38d	6.94 ± 1.58a	15.92 ± 1.24e	5.91 ± 0.66a	5.67 ± 1.17a
C22:1	0.12 ± 0.05	0.12 ± 0.09	0.18 ± 0.11	0.14 ± 0.01	0.15 ± 0.04	0.26 ± 0.08	0.11 ± 0.15	0.14 ± 0.12
C22:4n-6	0.76 ± 0.31ab	0.73 ± 0.15ab	1.68 ± 1.23c	0.86 ± 0.11ab	1.50 ± 0.62bc	0.46 ± 0.05a	1.46 ± 0.18bc	0.74 ± 0.27ab
C22:5n-3	4.02 ± 0.99cde	3.02 ± 0.52bcd	3.40 ± 1.51bcd	5.29 ± 0.39e	1.50 ± 2.33a	3.70 ± 0.52bcde	3.49 ± 0.24bcd	4.61 ± 0.36de
C22:6n-3	16.40 ± 6.55de	22.71 ± 5.72fg	10.75 ± 5.60ab	14.39 ± 2.73bc	26.68 ± 3.91gh	16.29 ± 1.71cd	20.25 ± 3.76def	30.31 ± 8.13h
SFA	24.39 ± 2.84a	27.41 ± 4.35ab	27.61 ± 3.71ab	26.85 ± 1.36ab	38.00 ± 2.67d	30.26 ± 2.70b	26.60 ± 1.49ab	35.06 ± 1.26c
MUFA	27.99 ± 12.52cd	20.39 ± 6.49abc	29.48 ± 10.09cd	23.24 ± 3.04bcd	12.49 ± 2.90a	21.77 ± 2.14bc	22.49 ± 5.06bcd	12.17 ± 5.06a
PUFA	47.63 ± 9.99bc	52.20 ± 5.09cd	42.91 ± 7.09b	49.91 ± 4.08c	49.51 ± 1.89c	47.97 ± 1.62bc	50.91 ± 4.25c	52.76 ± 3.95cd
n-3	33.29 ± 9.11b	33.90 ± 5.99b	25.14 ± 4.53a	36.21 ± 2.97bc	35.28 ± 2.39bc	35.53 ± 1.71bc	32.18 ± 3.12b	41.55 ± 5.21cd
n-6	14.33 ± 2.17a	18.30 ± 3.38b	17.77 ± 3.52b	13.70 ± 1.22a	14.24 ± 1.26a	12.44 ± 1.13a	18.74 ± 1.62b	11.22 ± 1.29a
n-3 LC PUFA[1]	30.78 ± 10.01cd	31.91 ± 6.45cde	20.68 ± 6.49b	32.17 ± 3.35cde	34.07 ± 2.66cde	33.32 ± 2.33cde	29.65 ± 4.08c	39.05 ± 7.01e

Table 5 continued

Fish Fish no.	Italian roach 9	Chub 25	Tench 23	Black bullhead 5	Perch 10	Burbot 10	Wels catfish 5	Pike 3
n-6 LC PUFA[2]	11.41 ± 2.81cde	12.07 ± 2.55cde	11.87 ± 4.88cde	9.85 ± 1.22bc	11.32 ± 1.12cde	10.27 ± 0.88bc	14.33 ± 2.40de	7.70 ± 0.12ab
n3/n6	2.34 ± 0.71bcd	1.94 ± 0.60abc	1.44 ± 0.25a	2.65 ± 0.11cde	2.50 ± 0.33cde	2.88 ± 0.35def	1.72 ± 0.15ab	3.77 ± 0.84g
FA mg/100 g tissue	627.12 ± 369.15	862.15 ± 436.50	1,724.58 ± 1,611.07	987.62 ± 156.21	502.74 ± 352.59	1,134.08 ± 83.05	552.42 ± 134.73	1,311.20 ± 413.81

Value within the same raw not sharing a common letter are significantly different ($p < 0.05$)

[1] Sum of 20:5n-3 + 22:5n-3 + 22:6n-3

[2] Sum of 20:2n-6 + 20:3n-6 + 20:4n-6 + 22:4n-6

[3] Whole body eviscerated fish (ten fish per pool). Bleak was not included in the statistical analysis

with the reproductive season. Fish weights were highly variable, from 824 to 1,372 g, with a fillet lipid content ranging from 0.61 g 100 g^{-1} to 1.09 g 100 g^{-1}. The most abundant fatty acids in muscle tissue were DHA (15.0–21.0 %), followed by 16:0 (14.4–19.0 %), 18:1n-9 (8.3–14.4 %), EPA (8.7–10.7 %) and 20:4n-6 (7.5–10.1 %). Almost all fatty acids showed significant differences among lakes.

Rudd were caught during April in Alserio, Comabbio and Garlate lakes (Table 8). In spite of different weights among groups, ranging from 248 to 584 g, their proximate compositions were rather similar. Fatty acid profiles showed significant differences in 18:3n-3, 20:4n-6 and EPA content.

Only a few differences in fatty acid compositions between two groups of roach were found (Table 8). Roach from Maggiore presented higher 16:0 and lower EPA compared to those from Lugano. The most abundant fatty acids in rudd muscle tissue were DHA (20.5–23.2 %), 16:0 (19.0–20.0 %), EPA (9.9–15.4 %) and 20:4n-6 (5.2–10.0 %). Lipid content varied from 1.26 g 100 g^{-1} to 1.38 g 100 g^{-1} wet muscle tissue.

A total of 25 chub caught in Como, Garlate, Iseo and Maggiore lakes were analyzed (Table 9). Mean weights were highly variable, ranging from 452 to 1,938 g, depending on the lake and month of capture. In spite of different sizes, fish did not show significant differences in proximate compositions. Lipid contents varied from 0.63 to 1.56 g 100 g^{-1}, with a mean content of 1.0 g 100 g^{-1}. Fatty acid compositions of chub from Iseo lake were significantly different from the fish of the other lakes. They presented a higher percentage of 18:0 and 20:4n-6 and a lower percentage of DHA. The most abundant fatty acids in chub muscle tissue were DHA (17.0–28.0 %), 16:0 (17.5–19.8 %), 18:1n-9 (11.6–15.1 %), 20:4n-6 (8.5–12.5 %) and EPA (5.7–7.5 %).

A comparison of fatty acid and proximate compositions of tench harvested in different lakes is presented in Table 10. Fish caught in different seasons showed different weights, starting from 874 g in early autumn to 2,702 g in early summer. Lipid contents varied from 0.58 to 3.57 g 100 g^{-1}. The fatty acid content was extremely affected by lakes and month of capture. Oleic acid ranged from 9.2 % (Como) to 22.2 % (Varese), EPA ranged from 3.3 % (Comabbio) to 14.7 % (Como), 20:4n-6 ranged from 4.2 % (Varese) to 13.2 % (Como). The most representative fatty acids were, in decreasing order, 18:0, 18:1n-9, DHA, 20:4n-6 and EPA.

Total fatty acid compositions of muscle lipid of fish species grouped according to their feeding habits are shown in Table 11. Planktivorous fish (bleak, whitefish and shad) had the lowest PUFA, n-3 and n-6 fatty acids, but had the highest MUFA content, in particular 18:1n-9. Carnivorous and ichthyophagous fish (pike, perch, burbot, wels

Table 6 Proximate (g 100 g^{-1} wet weight) and fatty acid composition (mol%) of the muscle tissue of shad and perch captured in different lakes (mean ± SD)

Fish	Shad		p^2	Perch		p^2
Lake	Iseo	Como		Garda	Iseo	
Fish n.	5	17		5	5	
Catch month	November	September		November	November	
Weight (g)	113.18 ± 13.88	89.05 ± 39.37	*ns*	227.9 ± 44.21	174.7 ± 23.67	*ns*
Moisture	69.89 ± 2.20	69.31 ± 1.99	*ns*	79.09 ± 0.59	78.98 ± 0.81	*ns*
Protein	17.75 ± 2.26	19.94 ± 0.70	*	19.04 ± 0.48	19.28 ± 0.68	*ns*
Lipid	11.06 ± 2.50	9.28 ± 2.15	*ns*	0.63 ± 0.69	0.52 ± 0.10	*ns*
Ash	1.31 ± 0.06	1.46 ± 0.08	*	1.24 ± 0.24	1.23 ± 0.22	*ns*
Fatty acid						
C14:0	4.51 ± 0.40	4.31 ± 0.42	*ns*	0.86 ± 0.12	1.04 ± 0.24	*ns*
C16:0	21.1 ± 0.71	21.56 ± 1.15	*ns*	21.26 ± 1.03	20.57 ± 1.75	*ns*
C16:1n-7	5.03 ± 0.56	4.35 ± 0.45	**	2.66 ± 0.49	2.89 ± 1.13	*ns*
C18:0	5.09 ± 0.47	4.38 ± 0.43	**	15.31 ± 0.78	16.96 ± 1.80	*ns*
C18:1n-9	20.7 ± 3.94	28.03 ± 3.41	**	6.86 ± 0.67	6.62 ± 2.57	*ns*
C18:1n-7	4.09 ± 0.22	3.85 ± 0.41	*ns*	2.57 ± 0.18	3.06 ± 0.62	*ns*
C18:2n-6	4.80 ± 0.59	4.57 ± 0.93	*ns*	2.38 ± 0.56	3.38 ± 0.61	*
C18:3n-6	0.47 ± 0.06	0.57 ± 0.10	*	0.17 ± 0.02	0.20 ± 0.00	*ns*
C18:3n-3	7.17 ± 0.83	4.82 ± 0.56	**	0.95 ± 0.14	1.34 ± 0.42	*ns*
C18:4n-3	3.35 ± 0.64	2.59 ± 0.75	*ns*	0.66 ± 0.01	0.66 ± 0.00	*nd*
C20:1n-9	1.47 ± 0.35	1.75 ± 0.22	*	0.28 ± 0.06	0.22 ± 0.02	*ns*
C20:2n-6	0.51 ± 0.06	0.22 ± 0.09	**	0.30 ± 0.04	0.27 ± 0.03	*ns*
C20:3n-6	0.35 ± 0.02	0.33 ± 0.06	*ns*	0.31 ± 0.03	0.21 ± 0.03	*
C20:4n-6	3.74 ± 0.42	4.85 ± 0.85	*	9.59 ± 0.62	9.40 ± 0.65	*ns*
C20:5n-3	7.42 ± 0.52	6.62 ± 1.57	*ns*	5.77 ± 0.89	8.10 ± 1.20	**
C22:1	0.25 ± 0.02	0.24 ± 0.03	*ns*	0.12 ± 0.01	0.18 ± 0.03	*ns*
C22:4n-6	0.57 ± 0.17	0.89 ± 0.15	**	1.65 ± 0.70	1.35 ± 0.57	*ns*
C22:5n-3	0.71 ± 1.09	2.51 ± 0.58	**	0.15 ± 0.74	4.19 ± 1.20	*ns*
C22:6n-3	9.48 ± 2.07	3.56 ± 1.40	**	29.39 ± 1.56	23.97 ± 3.69	*

p^2 Statistical significance between lakes within the same species

ns not statistically significant

* $p < 0.05$, ** $p < 0.01$

catfish) showed the highest SFA, n-3 LC-PUFA and n-3 fatty acids, but the lowest MUFA content, whereas omnivorous fish, which eat both vegetal and animal food, had a substantial proportion of n-3 fatty acids and the highest content of n-6 LC-PUFA and n-6 fatty acids, when compared to the other fish.

Principal component analysis (PCA) was performed using 19 fatty acid variables. The correlation loadings and the scores plots were obtained from the fatty acid data matrix (19 × 186). PCA explained the 54 % of the variance with PC1 accounting for 38 % and PC2 for 16 % of the total variance. The separation of species was predominantly based on differences in the proportion of 18:1n-9, 18:2n-6, 20:4n-6 and 22:6n-3. Two species, shad and whitefish, were clearly grouped and differentiated from the others, and were characterized by higher amount of 18:1n-9, 14:0, 16:0 and 18:4n-3. FA as 18:2n-6 and 16:1n-7 were higher in bleaks whilst 22:6n-3, 20:4n-6 and 18:0 were biomarkers of carnivorous species.

Discussion

The lipid compositions of freshwater fish are influenced by several interdependent factors, including genetics, physiological status, reproductive cycle, water temperature, aquatic environment and feeding habits. All these factors have been extensively reviewed by Henderson and Tocher [29] and in a recent book edited by Arts and Wainman [30]. In this study we have sampled 15 fish species from 10 subalpine lakes. For practical reasons related to fishing activities and to the occurrence and abundance of species in different aquatic environments, it was not possible to harvest all fish species in the same period from all lakes. As a consequence, the effects of aquatic environment and season on fatty acid composition was presented only in certain species. Most fish caught belonged to the family of cyprinids, which are abundant in freshwater environments, especially in warm and eutrophic conditions. Some of them were low value and invasive species, such as rudd, roach

Table 7 Proximate (g 100 g^{-1} wet weight) and fatty acid composition (mol%) of the muscle tissue of Crucian carp captured in different lakes (mean ± SD)

Lake	Alserio	Comabbio	Mezzola	Varese	*p*
Fish n.	6	5	5	5	
Catch month	March	April	May	May	
Weight (g)	1,316.4 ± 587.0	1,372.5 ± 156.5	894.2 ± 63.4	1,089.3 ± 455.3	*ns*
Moisture	77.61 ± 0.37	78.85 ± 0.65	78.16 ± 0.30	78.46 ± 0.72	**
Protein	20.00 ± 0.31	18.87 ± 0.68	19.98 ± 0.22	19.33 ± 0.70	**
Lipid	1.09 ± 0.31	0.76 ± 0.08	0.61 ± 0.08	0.86 ± 0.26	*
Ash	1.29 ± 0.15	1.52 ± 0.04	1.25 ± 0.21	1.35 ± 0.18	*ns*
Fatty acid					
C14:0	1.44 ± 0.52	1.44 ± 0.14	1.45 ± 0.34	1.65 ± 0.45	*ns*
C16:0	19.05 ± 0.95	16.85 ± 0.14	14.39 ± 0.63	15.65 ± 0.93	**
C16:1n-7	5.92 ± 1.54	5.4 ± 0.52	5.7 ± 0.64	7.02 ± 1.47	*ns*
C18:0	6.21 ± 1.08	8.08 ± 0.30	7.96 ± 0.27	6.34 ± 1.15	**
C18:1n-9	14.44 ± 5.05	8.32 ± 1.02	9.28 ± 0.64	13.97 ± 4.40	*
C18:1n-7	5.01 ± 0.60	5.21 ± 0.32	5.33 ± 0.26	5.86 ± 0.15	*
C18:2n-6	2.54 ± 0.22	3.34 ± 0.71	4.28 ± 0.71	4.25 ± 0.72	**
C18:3n-6	0.16 ± 0.03	0.27 ± 0.04	0.21 ± 0.03	0.27 ± 0.06	**
C18:3n-3	2.77 ± 0.70	2.24 ± 0.04	3.17 ± 0.47	3.43 ± 0.76	*
C18:4n-3	0.98 ± 0.36	0.88 ± 0.05	1.25 ± 0.33	0.97 ± 0.27	*ns*
C20:1n-9	1.06 ± 0.14	0.89 ± 0.01	1.23 ± 0.25	1.18 ± 0.24	*
C20:2n-6	0.32 ± 0.06	0.47 ± 0.03	0.37 ± 0.31	0.55 ± 0.10	*ns*
C20:3n-6	0.40 ± 0.05	0.63 ± 0.02	0.58 ± 0.07	0.63 ± 0.05	**
C20:4n-6	7.52 ± 1.96	10.10 ± 0.91	9.34 ± 1.36	8.93 ± 1.73	*ns*
C20:5n-3	8.75 ± 1.18	9.23 ± 0.35	10.74 ± 1.50	9.02 ± 1.15	*
C22:1	0.09 ± 0.02	0.1 ± 0.01	0.16 ± 0.02	0.2 ± 0.06	**
C22:4n-6	0.72 ± 0.19	1.2 ± 0.13	0.96 ± 0.15	1.05 ± 0.17	**
C22:5n-3	3.42 ± 0.63	4.31 ± 0.20	4.44 ± 0.71	4.01 ± 0.69	*
C22:6n-3	19.22 ± 3.61	21.05 ± 1.44	19.15 ± 1.63	15.01 ± 3.27	*

ns not statistically significant

* $p < 0.05$, ** $p < 0.01$

and crucian carps, whereas others were of commercial interest for human consumption, such as perch, whitefish and shad.

Most analyzed fish can be considered as lean fish [12], having a lipid content lower than 2.0 g 100 g^{-1} muscle tissue. An inverse relationship between the water and the lipid content of muscle tissue was present, as well described by other authors [4]. Protein content of fish tissue for most species was in the range of 18–20 g 100 g^{-1} on wet basis. Protein is considered to be a rather stable component of the fish body, depending on fish size and genetic factors and it is known that fish are a good protein source ranging from 16 to 21 g 100 g^{-1} [31].

The more representative fatty acids in muscle tissue of fish were 16:0 and 18:1n-9. Palmitic acid has been reported by many authors as the major SFA in freshwater fish [32]. Kinsella et al. [6] found that 16:0 was one of the major fatty acids of fillets in 19 species of freshwater fish. Guler et al. [33] reported that 16:0 was the major saturated fatty acid in zander fillets (*Sander lucioperca*), ranging from 14.2 to 17.9 %. Celik et al. [34] found similar results in the same species harvested from two different Turkey lakes, with a 16:0 content of 19.6 and 20.8 %. Ozogul et al. [10] reported a 16:0 content varying from 15.9 to 20.5 % in some freshwater fishes.

Stearic and oleic acid are also known to be widespread in freshwater aquatic habitats. They are present in all classes of microalgae [35] and are characteristics of bacterial and animal detrital FA profiles, because bacteria mainly synthesize 18:0, MUFA and branched chain odd-fatty acids to achieve the correct physical state of the membrane lipids [36].

Concerning other MUFA, 16:1n-7 and 18:1n-7 were abundant mainly in tench, Italian roach and crucian carp. These fatty acids are the primary products of Δ9-desaturation occurring during the synthesis of unsaturated fatty acids in algae. They have been associated to blue-green algae, *Oscillatoria* and *Microcystis* [37]. Interestingly, their occurrence is abundant in fish with benthic habits (tench, Italian roach, carps). Desvilettes et al. [35] showed that 18:1n-7 and 16:1n-7 should be considered, together with branched chain odd-fatty acids, characteristics of the

Table 8 Proximate (g 100 g^{-1} wet weight) and fatty acid composition (mol%) of the muscle tissue of rudd and roach captured in different lakes (mean ± SD)

Species	Rudd				Roach		
Lake	Alserio	Comabbio	Garlate	*p*[1]	Lugano	Maggiore	*p*[1]
Fish no.	5	5	4		5	5	
Catch month	April	April	April		April	March	
Weight (g)	248.0 ± 53.8	488.3 ± 537.0	584.9 ± 49.1	*ns*	215.8 ± 83.2	248.6 ± 58.4	*ns*
Moisture	79.16 ± 0.50	78.61 ± 1.02	78.48 ± 0.48	*ns*	78.63 ± 0.85	78.31 ± 0.98	*ns*
Protein	18.77 ± 0.43	19.27 ± 0.86	19.18 ± 0.20	*ns*	19.32 ± 0.79	19.33 ± 0.78	*ns*
Lipid	0.80 ± 0.04	0.86 ± 0.17	0.96 ± 0.17	*ns*	0.92 ± 0.08	0.97 ± 0.12	*ns*
Ash	1.28 ± 0.12	1.26 ± 0.27	1.38 ± 0.14	*ns*	1.13 ± 0.24	1.40 ± 0.17	*ns*
Fatty acid							
C14:0	0.73 ± 0.10	1.11 ± 0.13	0.84 ± 0.20	**	0.38 ± 0.05	0.63 ± 0.12	**
C16:0	20.04 ± 0.36	19.03 ± 1.01	20.08 ± 0.44	*ns*	16.46 ± 0.42	20.59 ± 1.57	**
C16:1n-7	3.39 ± 0.06	4.23 ± 1.00	3.84 ± 0.47	*ns*	1.83 ± 0.31	3.43 ± 0.76	**
C18:0	7.09 ± 0.42	6.38 ± 0.55	5.99 ± 0.50	*	6.81 ± 0.31	6.68 ± 0.40	*ns*
C18:1n-9	6.47 ± 0.99	10.66 ± 5.03	6.72 ± 1.14	*ns*	5.63 ± 0.80	6.94 ± 1.28	*ns*
C18:1n-7	2.6 ± 0.24	3.19 ± 0.18	2.25 ± 0.19	**	3.4 ± 0.39	3.37 ± 0.47	*ns*
C18:2n-6	1.69 ± 0.32	4.35 ± 3.53	4.69 ± 1.21	*ns*	2.22 ± 1.18	1.81 ± 1.04	*ns*
C18:3n-6	0.13 ± 0.05	0.18 ± 0.11	0.15 ± 0.03	*ns*	0.11 ± 0.04	0.08 ± 0.02	*ns*
C18:3n-3	2.53 ± 0.37	2.54 ± 0.21	11.06 ± 2.91	**	1.39 ± 0.20	0.93 ± 0.40	*
C18:4n-3	0.9 ± 0.14	0.56 ± 0.20	0.55 ± 0.14	*	0.52 ± 0.14	0.22 ± 0.05	**
C20:1n-9	0.21 ± 0.04	0.43 ± 0.24	0.25 ± 0.05	*ns*	0.45 ± 0.08	0.64 ± 0.17	*ns*
C20:2n-6	0.45 ± 0.09	0.44 ± 0.17	0.78 ± 0.06	**	0.87 ± 0.25	0.73 ± 0.16	*ns*
C20:3n-6	0.39 ± 0.13	0.5 ± 0.32	0.6 ± 0.06	*ns*	0.41 ± 0.11	0.47 ± 0.20	*ns*
C20:4n-6	10.01 ± 0.57	9.95 ± 1.85	5.23 ± 0.79	**	9.56 ± 1.21	10.31 ± 1.11	*ns*
C20:5n-3	15.38 ± 0.75	11.77 ± 4.02	9.84 ± 1.62	*	14.35 ± 1.10	8.53 ± 2.00	**
C22:1	0.07 ± 0.05	0.07 ± 0.01	0.15 ± 0.03	*ns*	0.11 ± 0.03	0.18 ± 0.07	*ns*
C22:4n-6	1.11 ± 0.38	0.87 ± 0.43	0.33 ± 0.14	*	0.63 ± 0.19	0.82 ± 0.26	*ns*
C22:5n-3	4.52 ± 0.49	3.22 ± 0.67	3.57 ± 0.62	*	5.33 ± 0.94	3.87 ± 0.59	*
C22:6n-3	22.29 ± 1.97	20.53 ± 2.43	23.22 ± 2.93	*ns*	29.58 ± 3.41	29.79 ± 3.30	*ns*

ns not statistically significant

[1] Statistical significance between lakes within the same species

* $p < 0.05$, ** $p < 0.01$

presence of aquatic bacteria, while 18:3n-3 is described as being associated with the organic matter of vegetal detritus.

When comparing the proportion of n-3 LC-PUFA and MUFA in analyzed fish, three distinctive patterns can be distinguished. The first is that of shad, whitefish and tench, which had the lower amount of EPA and DHA and higher MUFA (n-3 LC-PUFA/MUFA ratio: <1). The second profile is typical of chub, black bullet, Italian roach, carps, burbot and wels catfish characterized by an intermediate proportion of n-3 and MUFA (n-3 LC-PUFA/MUFA ratio: between 1 and 2). The third group was represented by rudd, roach, perch and pike, with elevated amounts of n-3 LC-MUFA with respect to the other fish (n-3 LC-PUFA/MUFA ratio: >2). Remarkably, to this last group belonged fish with extremely different habits: rudd and roach share the same pelagic environment and have omnivorous feeding habits, whilst perch and pike are icthyophagous fish.

Three analyzed species were planktivorous: landlocked shad, belonging to a family of essentially marine distribution (*Clupeidae*), which spawn from June to July; whitefish (*Salmonidae*) which spawn during winter and bleak, a small gregarious cyprinid fish, spawning in spring. The feeding habits of these species are nearly identical. Depending on the season, their diet is based on cladocerands in summer and copepods in winter (Table 4) [38]. In spite of this, the total fatty acid compositions of these species were quite different. The fatty acid pattern of bleak was characterized by a higher amount of 16:1n-7, 18:1n-9 and 18:2n-6 and, in general, by higher MUFA and lower n-3 fatty acids. In contrast, whitefish differed from shad and bleak for higher content of EPA and DHA. As expected in a cold-water fish, whitefish contained high amount of PUFA. Although they share the same lake environment, whitefish and shad have a different thermal *preferendum*: the first inhabits colder hypolimnetic waters, while shad prefers epilimnetic waters [14]. Early studies demonstrated that the degree of unsaturation of total lipids in fish is inversely related to the environmental

Table 9 Proximate (g 100 g^{-1} wet weight) and fatty acid composition (mol%) of the muscle tissue of chub captured in different lakes (mean ± SD)

Lake	Como	Garlate	Iseo	Maggiore	*p*
Fish no.	12	6	3	4	
Catch month	April/November	April	November	March	
Weight (g)	694.7 ± 202.3	452.9 ± 43.9	1,938.2 ± 171.0	632.1 ± 230.2	**
Moisture	78.54 ± 0.91	78.05 ± 1.53	79.40 ± 1.14	78.62 ± 1.21	*ns*
Protein	19.22 ± 0.96	18.99 ± 2.03	18.72 ± 0.54	19.47 ± 1.28	*ns*
Lipid	0.93 ± 0.45	1.56 ± 0.80	0.63 ± 0.75	0.83 ± 0.09	*ns*
Ash	1.30 ± 0.12	1.40 ± 0.16	1.25 ± 0.53	1.09 ± 0.12	*ns*
Fatty acid					
C14:0	1.27 ± 0.60	0.77 ± 0.40	1.44 ± 0.14	0.45 ± 0.12	*
C16:0	18.36 ± 1.97	17.51 ± 1.47	19.76 ± 0.86	19.30 ± 0.63	*ns*
C16:1n-7	3.82 ± 1.53	3.17 ± 1.58	3.44 ± 1.41	2.16 ± 0.80	*ns*
C18:0	8.23 ± 3.29	5.85 ± 1.34	11.36 ± 1.78	6.53 ± 0.22	*
C18:1n-9	13.88 ± 5.72	15.11 ± 4.71	11.60 ± 2.77	11.89 ± 3.43	*ns*
C18:1n-7	2.96 ± 0.68	2.79 ± 0.37	3.11 ± 0.58	2.46 ± 0.28	*ns*
C18:2n-6	5.78 ± 3.45	7.28 ± 0.95	5.67 ± 0.55	5.00 ± 1.22	*ns*
C18:3n-6	0.34 ± 0.38	0.30 ± 0.02	0.23 ± 0.14	0.21 ± 0.10	*ns*
C18:3n-3	1.85 ± 0.69	1.51 ± 1.10	2.31 ± 0.34	0.52 ± 0.07	*
C18:4n-3	0.69 ± 0.35	0.46 ± 0.49	0.69 ± 0.24	0.28 ± 0.07	*ns*
C20:1n-9	0.55 ± 0.31	0.60 ± 0.18	0.53 ± 0.14	0.54 ± 0.14	*ns*
C20:2n-6	0.73 ± 0.21	1.06 ± 0.26	0.97 ± 0.13	0.92 ± 0.16	*
C20:3n-6	0.57 ± 0.33	1.39 ± 0.66	0.61 ± 0.10	1.16 ± 0.34	**
C20:4n-6	8.52 ± 1.54	9.18 ± 2.07	12.50 ± 1.42	11.52 ± 1.92	**
C20:5n-3	7.30 ± 1.45	6.75 ± 1.50	7.46 ± 0.65	5.66 ± 0.65	*ns*
C22:1	0.16 ± 0.10	0.10 ± 0.01	0.15 ± 0.03	0.09 ± 0.02	*ns*
C22:4n-6	0.66 ± 0.13	0.68 ± 0.07	0.90 ± 0.02	0.85 ± 0.15	*
C22:5n-3	3.01 ± 0.53	3.19 ± 0.40	2.82 ± 0.04	2.64 ± 0.31	*ns*
C22:6n-3	22.58 ± 6.28	22.29 ± 4.14	16.96 ± 0.64	27.98 ± 3.55	*

ns not statistically significant

* $p < 0.05$, ** $p < 0.01$

temperatures. In addition, cold-water species, such as coregonids, are more efficient at converting the C18 PUFA to highly unsaturated C20 and C22 PUFA, due to more effective desaturation and elongation enzymatic activity in their metabolic pathways, and the conversion rates of freshwater fish are greater than those observed in marine fish [4], to which shad originally belonged [39].

Fatty acid compositions of whitefish of this study resemble those reported in the literature for coregonids by other authors [14, 17]. Linko et al. [40] and Wirth et al. [41], when studying the relationship between vendace (a related whitefish species, *Coregonus albula*) fatty acids and its available plankton feed, found that plankton fatty acids (mainly 16:0, 18:1n-9, 16:1n-7, 18:1n-7, 20:4n-6 and EPA) were transferred to vendace flesh lipids without major modifications.

Concerning shad, it is worth noting that fish caught in autumn contained a higher amount of lipids which were richer in DHA, compared to fish caught in summer, which showed lower lipid contents richer in 18:1n-9. These changes might be related to the fish reproductive cycle. Shad caught in November were in their post spawning phase, during which a marked increase in fillet lipid content and a reduction of ovary lipid content usually occurred [42].

When comparing shad and whitefish fatty acid profiles with those of other species, their lipid contents should be considered. As a consequence of the high lipid levels of these two fish, it is likely that their muscle tissues have triacylglycerols as their principal lipid class, whereas phospholipids generally predominate in fish tissues with low lipid contents [43]. Since phospholipids have a much higher proportion of PUFA and a lower proportion of MUFA, this factor might help to explain a higher PUFA percentage in fish with lower lipid contents.

The planktivorous fish contained higher proportions of 18:3n-3 and 18:4n-3 fatty acids ($p < 0.05$), lower proportions of EPA and DHA ($p < 0.05$) and higher amounts of 16:1n-7 and 18:1n-9 ($p < 0.05$) when compared to omnivorous and carnivorous fish. These differences may originate from the fatty acid composition of freshwater microalgae [37, 44], zooplankton feed [35, 45] and

Table 10 Proximate (g 100 g^{-1} wet weight) and fatty acid composition (mol%) of the muscle tissue of tench captured in different lakes (mean ± SD)

Lake	Comabbio	Como	Iseo	Mezzola	Varese	*p*
Fish no.	5	5	5	5	3	
Catch month	June	September	November	May	May	
Weight (g)	2,667.9 ± 431.1	874.4 ± 363.8	1,083.5 ± 282.8	1,826.6 ± 524.1	2,702.9 ± 500.7	**
Moisture	77.55 ± 1.00	79.14 ± 2.06	79.33 ± 1.13	76.00 ± 2.74	75.63 ± 1.51	*
Protein	19.04 ± 0.67	19.09 ± 2.28	18.49 ± 1.26	18.84 ± 1.52	19.34 ± 0.82	*ns*
Lipid	1.94 ± 1.09	0.58 ± 0.36	0.93 ± 0.85	3.66 ± 3.56	3.57 ± 1.67	*ns*
Ash	1.48 ± 0.06	1.19 ± 0.07	1.25 ± 0.23	1.58 ± 0.07	1.46 ± 0.14	**
Fatty acid						
C14:0	3.19 ± 0.71	1.08 ± 0.55	1.08 ± 0.56	2.19 ± 0.73	3.19 ± 0.16	**
C16:0	18.39 ± 0.71	20.57 ± 0.76	19.44 ± 1.57	16.71 ± 0.66	15.60 ± 0.34	**
C16:1n-7	12.59 ± 2.47	3.80 ± 1.18	6.49 ± 1.48	12.05 ± 4.50	14.71 ± 0.27	**
C18:0	5.23 ± 0.82	9.40 ± 0.84	9.33 ± 1.20	4.44 ± 1.66	3.04 ± 1.00	**
C18:1n-9	16.88 ± 2.91	9.22 ± 1.13	11.92 ± 1.47	17.05 ± 2.59	22.24 ± 0.77	**
C18:1n-7	6.63 ± 0.59	4.48 ± 0.61	5.13 ± 0.51	6.23 ± 1.24	5.85 ± 0.29	**
C18:2n-6	6.63 ± 1.08	2.88 ± 0.62	6.64 ± 2.82	5.64 ± 1.37	8.59 ± 1.05	*
C18:3n-6	0.24 ± 0.18	0.11 ± 0.03	0.21 ± 0.14	0.23 ± 0.15	0.13 ± 0.16	*ns*
C18:3n-3	5.55 ± 0.85	1.68 ± 0.21	4.12 ± 1.54	2.69 ± 1.10	6.02 ± 0.12	**
C18:4n-3	1.34 ± 0.29	0.42 ± 0.13	0.50 ± 0.04	0.74 ± 0.67	1.17 ± 0.01	**
C20:1n-9	0.46 ± 0.27	0.76 ± 0.46	0.55 ± 0.32	0.82 ± 0.07	0.46 ± 0.40	*ns*
C20:2n-6	0.37 ± 0.28	1.08 ± 0.45	0.87 ± 0.13	1.14 ± 0.31	0.62 ± 0.13	*
C20:3n-6	0.65 ± 0.03	0.44 ± 0.07	0.86 ± 0.23	0.69 ± 0.10	0.57 ± 0.11	**
C20:4n-6	6.02 ± 1.17	13.22 ± 0.89	9.58 ± 1.07	7.00 ± 2.80	3.72 ± 1.29	**
C20:5n-3	9.19 ± 0.70	7.69 ± 0.53	7.72 ± 1.10	8.06 ± 0.31	8.89 ± 0.49	*ns*
C22:1	0.09 ± 0.01	0.24 ± 0.06	0.24 ± 0.12	0.16 ± 0.08	0.08 ± 0.04	*ns*
C22:4n-6	0.67 ± 0.21	3.26 ± 0.59	1.92 ± 0.69	1.20 ± 0.33	0.18 ± 0.21	**
C22:5n-3	2.65 ± 0.42	5.01 ± 0.62	2.83 ± 1.53	3.64 ± 1.09	2.28 ± 0.67	*
C22:6n-3	7.26 ± 2.39	14.71 ± 3.38	13.22 ± 3.34	9.31 ± 4.41	2.66 ± 3.56	**

ns not statistically significant

* $p < 0.05$, ** $p < 0.01$

freshwater insects [46] that contribute to the population of the lake ecosystems.

Taking into account inter- and intra-species-specific variation, nutrient concentration and environmental conditions, a generalized profile of total fatty acids occurring in algae and cyanobacteria of freshwater ecosystems can be synthesized as follows [47, 48]: (a) 18:0 is the major fatty acid and occurs in all microalgae classes; (b) 18:1n-9 is the most representative monounsaturated fatty acid in all algae class; (c) Cyanobacteria are usually rich in 18:2n-6 and 18:3n-3; (d) polyunsaturated C16 fatty acids of the n-3 series and 20:4n-6 are very abundant in Chlorophyceae (green algae); (e) Bacillariophyceae (diatoms) are generally rich in 16:1n-7 and EPA, when compared to the others; (f) the presence of 18:4n-3 is indicative of Chrysophyceae, Cryptophyceae and Dinophyceae (diflagellates).

With regard to insects, Ghioni and others [46], in a work on the fatty acid compositions of neutral lipids and phospholipids of freshwater insects, reported that SFA and MUFA together represented up to 85 % of the fatty acids of total neutral lipids, whereas 16:0 is the most abundant SFA and 16:1n-7, 18:1n-9 and 18:1n-7 the most abundant MUFA. This might help to explain the MUFA composition of bleak, being particularly fond of midges and insects larvae.

Black bullhead is particularly rich in EPA. This fish is active mainly at night, feeding on immature insects, leeches and crustaceans when young, while adults also feed on clams, snails and also fish. In this case, due to their young age (150 g), is likely that their muscle EPA derived from benthic invertebrates [49].

Among carnivorous/icthyophagous fish, perch and pike showed muscle tissues significantly richer in DHA in comparison to burbot and wels catfish. Wels catfish (*Silurus glanis*) is a very aggressive and invasive large carnivore fish that takes advantage of incredibly different sources of food, such as fish, frogs or aquatic birds [50]. Muscle tissue from wels catfish was found very lean and containing 50.9 % of PUFA, 22.5 % MUFA and 26.6 % of SFA. The most abundant fatty acids were DHA, 16:0, 18:1n-9 and 20:4n-6. These results were rather different to those obtained by other authors, who reported fatty acid profiles of smoked catfish meat [50] or catfish reared under different conditions [51]. In the first work, fresh meat from a 28 kg catfish contained 29.9 % SFA, 41.6 % MUFA and 28.3 % PUFA. In the second work, a 1.3 kg catfish reared in earthen ponds contained 25.4 % SFA, 39.9 % MUFA and 34.7 % PUFA. The differences in our study consisted mainly of a higher percentage of MUFA and a lower percentage of PUFA, probably due to the differences in fish size, environmental conditions and availability of food items.

Among carnivorous fish, burbot showed the highest content of EPA and the lowest amount of DHA. This fish belongs to the *Gadidae* family with many phylogenetically close species (cod) that are of marine origin. This fish show the highest activity in cold water and is an efficient feeder of benthic invertebrates, that are rich in EPA [52, 53], especially in winter.

Principal component analysis was used to provide an overview of the capacity of the total fatty acid composition to discriminate fish groups, irrespective of lake of origin and month of capture. The percentages of variance explained by first and second PC were 38 and 16 %, respectively. According to the loading of the fatty acids in the first PC, the most contributing descriptors were 18:1n-9, 18:3n-3, 14:0, DHA and 20:4n-6, while the most contributing variables in the second PC were 16:0, 18:2n-6 and 16:1n-7. Furthermore, the correlation loadings showed a strong correlation between 18:1n-9 and 18:3n-3 and between 20:4n-6 and DHA. When representing the scores of the fish samples on the two dimensional space defined by the calculated PC, shad and whitefish appeared well distinguished from each other according to their fatty acid composition. Carnivorous species were grouped together, while other species showed more samples dispersion. In particular tench, the species with the highest variability of fatty acid composition, evidenced by high standard deviations in Table 5, presented the widest distribution on the two dimensional space. In the scores and loadings plots, shad and whitefish appeared correlated to 18:4n-3, 14:0, 20:1n-9 and 18:3n-3, whilst roach, pike and perch were correlated to 20:4n-6, DHA and 18:0.

Since a strong relationship between feeding behavior and fatty acid composition of fish muscle tissue was observed, fish were tentatively grouped according to their feeding habits (Table 11).

Planktivorous fish showed the lowest PUFA, n-6 and n-3 fatty acids, but presented the highest MUFA content, in particular 18:1n-9, when compared to the other groups. Ichthyophagous fish showed the highest amount of SFA and n-3 fatty acids, but the lowest MUFA content, whereas omnivorous fish had the highest content of n-6 fatty acids, in comparison to the other species.

Table 11 Fatty acid composition (mol%) of the muscle tissue of freshwater fish grouped according to their feeding habits (mean ± SD)

Fatty acid	Planktovorous[α] ($n = 46$)	Omnivorous[β] ($n = 112$)	Ichthyophagous[γ] ($n = 28$)
C14:0	3.75 ± 0.90a	1.30 ± 0.91b	1.01 ± 0.39b
C16:0	20.05 ± 2.19a	18.32 ± 2.02b	19.08 ± 1.87b
C16:1n-7	6.69 ± 3.16a	5.54 ± 3.84a	3.32 ± 1.21b
C18:0	4.32 ± 0.61a	7.00 ± 2.07b	12.80 ± 3.71c
C18:1n-9	25.93 ± 5.66a	12.08 ± 5.77b	9.46 ± 3.07c
C18:1n-7	3.76 ± 0.68	4.68 ± 3.34	4.20 ± 1.53
C18:2n-6	5.72 ± 2.47a	4.51 ± 2.65b	2.86 ± 1.19c
C18:3n-6	0.47 ± 0.13a	0.19 ± 0.16b	0.12 ± 0.13c
C18:3n-3	5.58 ± 1.05a	2.78 ± 2.25b	1.63 ± 0.78c
C18:4n-3	2.25 ± 1.31a	0.58 ± 0.43b	0.31 ± 0.66b
C20:1n-9	1.34 ± 0.49a	0.65 ± 0.34b	0.47 ± 0.46b
C20:2n-6	0.36 ± 0.16a	0.76 ± 0.41b	0.47 ± 0.36a
C20:3n-6	0.34 ± 0.12a	0.65 ± 0.35b	0.30 ± 0.26a
C20:4n-6	4.48 ± 1.07a	9.00 ± 2.57b	9.31 ± 1.64b
C20:5n-3	7.05 ± 2.84a	9.11 ± 3.09b	9.82 ± 4.80b
C22:1	0.16 ± 0.10a	0.12 ± 0.09b	0.10 ± 0.12b
C22:4n-6	0.68 ± 0.29a	0.96 ± 0.67b	1.02 ± 0.64b
C22:5n-3	1.88 ± 1.17a	3.46 ± 1.49b	1.51 ± 1.91a
C22:6n-3	5.17 ± 2.90a	18.30 ± 7.91b	22.21 ± 6.43c
SFA	28.12 ± 3.25a	26.62 ± 3.13b	32.89 ± 5.03c
MUFA	37.89 ± 7.02a	23.08 ± 9.92b	17.55 ± 5.81c
PUFA	33.99 ± 5.70a	50.30 ± 9.32b	49.56 ± 2.88b
n-3	21.94 ± 6.52a	34.24 ± 9.61b	35.49 ± 3.52b
n-6	12.05 ± 2.24a	16.06 ± 3.72b	14.07 ± 2.71c
n-3 LC-PUFA[1]	14.10 ± 5.87a	30.87 ± 9.91b	33.54 ± 4.06b
n-6 LC-PUFA[2]	5.86 ± 1.21a	11.36 ± 3.18b	11.10 ± 2.23b
n-3/n-6	1.82 ± 0.76	2.13 ± 1.43	2.52 ± 0.67

[1] Sum of 20:5n-3 + 22:5n-3 + 22:6n-3

[2] Sum of 20:2n-6 + 20:3n-6 + 20:4n-6 + 22:4n-6

Value within the same raw not sharing a common letter are significantly different ($p < 0.05$)

[α] Italian bleak (*Alburnus arborella*). Landlocked shad (*Alosa fallax lacustris*). European whitefish (*Coregonus macrophtalmus*)

[β] Crucian carp (*Carassius carassius*). Common carp (*Cyprinus carpio*). Chub (*Squalius squalus*). Roach (*Rutilus rutilus*). Black bullhead (*Ichtalurus melas*). Italian roach (*Rutilus pigus*). Rudd (*Scardinius erythrophtalmus*). Tench (*Tinca tinca*)

[γ] Burbot (*Lota lota*). Northern pike (*Esox lucius*). European perch (*Perca fluviatilis*). Wels catfish (*Silurus glanis*)

Concerning nutritional aspects, the importance of the n-6/n-3 fatty acids ratio in the diet has been seriously addressed in human nutrition [54]. During the past century, the imbalance of the n-6/n-3 ratio in the Western diet has been characterized by a lower intake of n-3 LC-PUFA and a concurrent higher intake of n-6 PUFA. It is estimated that the present human diet is deficient in n-3 fatty acids with a n-6/n-3 ratio of 15-20/1, while a ratio of around 5/1 is considered as optimal [55]. In order to better understand the nutritive values of these fish products and their possible contribution to the intake of n-3 fatty acids in humans, their total fatty acid content, expressed in mg fatty acids 100 g^{-1} fresh muscle, is presented in Table 5. All the analyzed fish showed a n-6/n-3 ratio ranging from 0.3 to 0.9, typical of similar freshwater fish [56]. Due to their high lipid content, shad and whitefish provided the higher amount of n-3 LC PUFA, that consist mainly of EPA and DHA, with an average of 1,170 and 1,190 mg 100 g^{-1} of fresh fillet, respectively. A serving portion of 100 g of whitefish could completely meet the dietary intake of EPA + DHA according to recommendations of many organizations and expert committees [57] which suggest a regular fish consumption (one to two servings per week; each serving should provide the equivalent of 250–500 mg of EPA + DHA). Other species such as roach, rudd, chub and black bullhead showed a EPA + DHA content between 200 and 300 mg 100 g^{-1}. A serving portion of 100 g of these lean species could ensure the minimum recommended daily intake of EPA and DHA. The other species, such as carp, pike, perch, wels catfish and tench contained a poor amount of n-3 LC-PUFA, with a EPA + DHA in the lowest range of 100–200 mg 100 g^{-1} fillet. The lowest amount of n-3 fatty acids was found in burbot fillet. It should be noted, however, that potential benefits derived from the intake of these fish may be counteracted by the risks related to their exposure to chemical pollutants [15, 16, 58, 59].

The present study contributed to the lipid characterization of muscle tissue from fifteen species of freshwater fish caught in subalpine lakes. To the authors' knowledge, it is the first time that an accurate assessment of the lipid composition of these specimens has been carried out. The results have mainly demonstrated that total fatty acid composition of lipid muscle of wild freshwater fish is a suitable tool to distinguish feeding behavior and to provide their position in the food web of the aquatic environment. Predatory fish showed a tendency to accumulate substantial amounts of n-3 LC-PUFA, especially DHA in their muscles, although the lipid content was low. Planktivorous fish were generally medium or high fat fish and their fatty acid profile could be considered to be of a lower quality. Omnivorous fish of our study were the leanest and their fatty acid profiles were in between.

Nutritional considerations highlight that the consumption of these fish do not necessarily result in a sufficient supply of EPA and DHA for humans. Some of them are very lean and contributed poorly to satisfying the requirements of n-3 LC-PUFA in the human diet. Nevertheless, the present work confirmed that two of the more frequently consumed and highly appreciated fish, whitefish and shad, are a good source of nutrients and could meet the dietary recommendations for EPA and DHA.

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Conflict of interest The authors declare that there are no conflicts of interest.

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