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Per- and Polyfluoroalkyl Substances (PFAS) in Fish from European Lakes: Current Contamination Status, Sources, and Perspectives for Monitoring

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Abstract: Concentrations in fish of per- and polyfluoroalkyl substances (PFAS) were reported for 7 deep lakes in the European subalpine area: Lakes Geneva, Lugano, Maggiore, Iseo, Como, Garda, and Mergozzo; one shallow lowland lake (Varese); and 2 high-altitude alpine lakes (>2000 m a.s.l). Fillets and, in selected cases, other body fractions (viscera, liver, and residual carcass) from 8 fish species were analyzed. The possibility of harmonizing the monitoring protocols was tested. Results suggest that the sampling season is not critical for PFASs and the total protein content cannot be used for normalization of tissue concentrations because PFASs bind to specific proteins. Moreover, the polar lipid content could be used to reduce the variability of PFAS concentrations in phospholipid rich fractions of fish such as viscera and carcass. The data comparison and analysis show that the PFAS contamination in lake fish is generally correlated with the degree of urbanization of the lake catchment; however, it is sometimes difficult to compare absolute concentrations in lake fish because the lake hydromorphological characteristics play a substantial role in determining the chemical concentrations of persistent and mobile contaminants. *Environ Toxicol Chem* 2021;40:658–676. © 2020 SETAC

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INTRODUCTION

Within continental waters, large lakes present special features because of their physical characteristics, especially a long residence time, and the services they can provide to human populations. The southwestern part of the Alps in Europe holds several of these large lakes, among the largest in Europe. These lakes are the main source of drinking waters for residential population; they also sustain recreational as well as economic activities such as professional fishing, tourism, and shipyards. Nevertheless, they suffer from significant anthropic pressures because they are surrounded by densely populated areas, industries, and extensive agriculture.

Monitoring persistent contaminants in fish is therefore an essential component of environmental and health risk assessment in such large lakes (Mazzoni et al. 2019). Institutional monitoring programs of legacy contaminants have been running for many years, especially in the transboundary lake basins in this region such as Lake Geneva (Commission Internationale pour la Protection des eaux du Léman 2019) and Lake Maggiore with Lake Lugano (Commissione Internazionale per la Protezione delle Acque Italo-Svizzere).

Poly- and perfluoroalkyl substances (PFAS) are a wide class of persistent chemicals that has attracted attention in the last 2 decades because of their unique properties, widespread uses in consumer products, and presence in various environmental compartments (Houde et al. 2006; Ahrens 2011; Houde et al. 2011; Gewurtz et al. 2013). Among PFAS, perfluoroalkyl sulfonates (PFSA) such as perfluorooctane sulfonate (PFOS) and many perfluoroalkyl carboxylic acids (PFCAs) have been shown

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to be bioaccumulative (Kannan et al. 2005; Houde et al. 2006; Houde et al. 2011) or toxic to humans and other species (Beach et al. 2006; Lau et al. 2007). Perfluorooctane sulfonate was listed on Annex B of the Stockholm Convention in 2009 (United Nations Environment Programme–Persistent Organic Pollutants 2009). Consequently, the states that signed the Stockholm Convention must monitor PFOS in their environment; therefore, the European Commission added PFOS to the list of priority pollutants to be monitored in continental waters in 2013 (European Commission 2013). Perfluorooctanoate (PFOA) was listed on Annex A of the Stockholm Convention in 2019 (United Nations Environment Programme–Persistent Organic Pollutants 2019) and perfluorohexane sulfonate (PFHxS) has been proposed for listing under the Stockholm Convention and is currently under review (United Nations Environment Programme–Persistent Organic Pollutants 2017).

The aim of the present study is to review the status of PFAS contamination in fish of lakes from the Alpine area, comparing large deep lakes and smaller shallow lakes belonging to the same catchments that also include 2 high-altitude reference lakes. Data gathered from different monitoring programs, carried out by local authorities for each lake, allowed a wide assessment of PFAS contamination in fish across the southwestern subalpine area for the last 5 yr (2015–2019). The collected dataset gave us the opportunity to highlight PFAS sources and transport mechanism in this area as well as to discuss some technical aspects of PFAS monitoring in fish, with a specific focus on the European Commission regulation for biota monitoring in European freshwaters.

MATERIALS AND METHODS

Study area

Ten European glacial lakes in pre-alpine and alpine areas were investigated in the present study (Figure 1). These lakes, from remote to densely populated and industrialized areas, were chosen to cover similar aquatic environments with a gradient of anthropogenic pressures.

Lakes Geneva, Lugano, and Maggiore are transboundary subalpine lakes between France and Switzerland or between Italy and Switzerland. Five lakes (Lakes Como, Iseo, Garda, Varese, and Mergozzo) are entirely on Italian territory on the southern side of the Alps. The 2 high-altitude alpine lakes (Lake Sassolo upper and Lake Sassolo lower) are interconnected and located in Switzerland.

Lake Geneva in the Rhône river basin is a deep lake on the western side of the Alps and one of the largest lakes in Western Europe. Lakes Maggiore, Como, Iseo, and Garda are deep

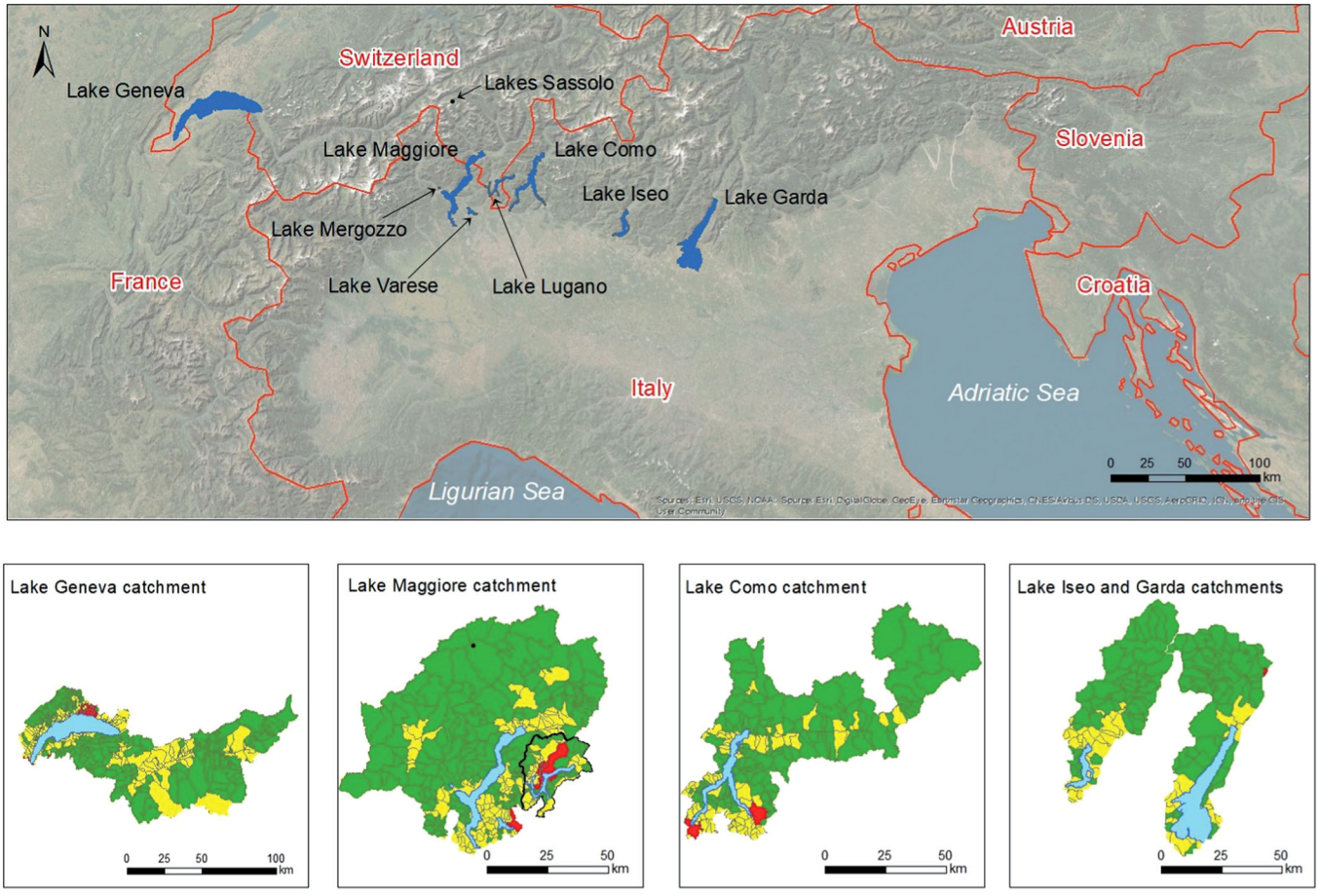

FIGURE 1: (**Top**) Map of the studied lakes and (**Bottom**) their respective catchments. Catchment areas are colored according to the DEGURBA (Degree of Urbanisation; European Union Statistical Office 2014). Red: class 1, densely populated areas; yellow: class 2, intermediate density areas; and green: class 3, thinly populated areas.

glacial lakes that form the subalpine Italian lacustrine district belonging to the Po river basin and constitute approximately 70% of all Italian freshwater resources. Lakes Lugano, Varese, and Mergozzo belong to the Lake Maggiore hydrological catchment; their outlet rivers (Tresa and Bardello, and an artificial canal, respectively) directly flow into Lake Maggiore. Lake Mergozzo is a small and deep lake located in a less urbanized and protected area (Mazzoni et al. 2020), whereas Lake Varese is a shallow, medium-size lake situated in a densely populated and industrialized territory.

Lake Sassolo upper and Lake Sassolo lower are located in the Maggia Valley (Canton of Ticino, CH), which is a tributary of Lake Maggiore. They have been chosen as monitoring sites of the International Cooperative Programme on Assessment and Monitoring Effects of Air Pollution on Rivers and Lakes (ICP Waters) because they are located at an altitude of more than 2000 m a.s.l but in a region highly affected by long-range transport of atmospheric pollutants (Steingruber 2018).

The main geographical, chemical, and physical characteristics of the lakes are reported in Supplemental Data, Table S1.

Study species

Eight fish species were collected: shad (*Alosa agone*), European whitefish (*Coregonus lavaretus*), burbot (*Lota lota*), rainbow trout (*Oncorhynchus mykiss*), European perch (*Perca fluviatilis*), roach (*Rutilus rutilus*), brown trout (*Salmo trutta*), and Arctic char (*Salvelinus alpinus*).

These species have different habitats, feeding behaviors, and spawning times. For example, shad is a pelagic nonmigratory species, mainly zooplanktivorous, and its spawning period ranges from June to August (Kottelat and Freyhof 2007), whereas burbot usually lives in deep waters, feeds on benthic invertebrates, and reproduces between November and March. Roach feeds on zooplankton, algae or plants, and detritus (Horppila and Peltonen 1997; Kamjunke et al. 2002), whereas perch is considered an opportunistic diurnal predator, living in the littoral zone. In Supplemental Data, Table S2, the main biological and ecological characteristics of all sampled species are reported.

Sample collection and preparation

The choice of fish species for the present study depended on their abundance in the study area as well as their catching in as many lakes as possible. Fish specimens were caught by professional fishermen. Most of the fish had reached their sexual maturity. Lake Geneva fish were collected throughout the lake during the summer of 2018, outside the reproductive period. Fish from the other lakes were obtained from 2015 to 2019 in different seasons. Fish from both Lakes Sassolo were sampled during the 2018 monitoring campaign in the framework of ICP Waters activities (Steingruber 2018). Fish from Lake Lugano were sampled in the framework of the monitoring programmes of the International Commission for the Protection of Italian–Swiss Waters (Solcà 2016, 2019). Generally, 2 species of fish were collected per lake with the exception of Lake Como and Lake Garda, where only shad was caught. In Lake Maggiore, 3 species were sampled (shad, European whitefish, and roach), whereas in Lake Mergozzo 6 fish species were collected (shad, European whitefish, burbot, European perch, roach, and Arctic char). Shad is the fish species caught in most lakes (Lakes Mergozzo, Maggiore, Lugano, Como, Iseo, and Garda). Fish were measured and weighed. Supplemental Data, Table S3, summarizes sampling information and fish characteristics.

Fish from Lake Geneva were refrigerated (~4 °C) and stored until they could be frozen (–20 °C), and then sent to the French Reference Laboratory for Halogenated Pollutants in Food of LABERCA for further treatment and analysis. Fish specimens from the other lakes arrived at the Water Research Institute laboratory for further treatments and analysis within a few hours after collection.

Sample treatment

The fish dorsal muscle (i.e., the fillet) from all specimens was separated from the skin (European Commission 2006). Some fish specimens were dissected into 3 or 4 fractions: muscle (separated from the skin), whole viscera (including the liver), or entrails and liver separately, and carcass (consisting of all the rest of the fish, i.e., head, fishbone, skin, and fins). The weights of each fraction (i.e., muscle [F], viscera [V], or entrails [E] and liver [L], and carcass [C]) in each sample are reported in Supplemental Data, Table S3.

Muscle and viscera samples from Italian lakes, consisting of single or pooled samples of up to 21 specimens (Supplemental Data, Table S3), were homogenized in 15-mL polyethylene vials from Ultra-Turrax T25 (Janke & Kunkel, IKA®-Labortechnik), whereas the carcass samples were frozen at –21 °C and crumbled with an ice crusher before the extraction. Dry weight was determined after drying an aliquot (from 2–3 g wet wt) of fish fractions at 105 °C overnight. Lipid content (f_{Lip}) was measured by the cyclohexane/isopropanol extraction standard method developed by Smedes (1999) for marine biota monitoring programs. Protein content evaluation (f_{Pr}), polar lipid (f_{LP}), and neutral lipid (f_{LN}) determinations were conducted on selected fish, according to the methods described by Bradford (1976) and Palacios and Wang (2005) and detailed in the Supplemental Data, Section III.

Fish specimens from Lake Geneva were defrosted and dissected into 4 fractions: fillets, liver, entrails (viscera without liver), and carcass. However, in some cases liver and entrails were pooled, in order to obtain a sufficient mass for analysis. Fractions were further freeze-dried and finely ground to acquire a homogenous powder. Dry weight was determined on whole samples subjected to freeze drying.

PFAS chemical analysis

In the case of Italian samples, fish tissues were analyzed for the determination of 9 perfluorocarboxylic acids: perfluorohexanoate (PFHxA), perfluoroheptanoate (PFHpA), PFOA, perfluorononanoate (PFNA), perfluorodecanoate (PFDA), perfluoroundecanoate (PFUnDA), perfluorododecanoate

(PFDoDA), perfluorotridecanoate (PFTrDA), perfluorotetradecanoate (PFTeDA), and 2 perfluoroalkyl sulfonates (PFHxS and PFOS). A full list of chemicals, solvents, and standards is provided in Supplemental Data, Table S4. The extraction and the analysis by liquid chromatography tandem mass spectrometry were carried out according to Mazzoni et al. (2016) and described in Supplemental Data, Section III.

For Lake Geneva fish, the analytical method was developed to determine the concentration of 5 perfluoroalkyl sulfonates (perfluorobutane sulfonate [PFBS], PFHxS, perfluoroheptane sulfonate [PFHpS], PFOS, and perfluorodecane sulfonate [PFDS]) and 9 perfluorocarboxylic acids (perfluorobutanoate [PFBA], perfluoropentanoate [PFPeA], PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, and PFDoDA; Riviere et al. 2014). Details are provided in Supplemental Data, Section III.

Quality assurance/quality control. In the Water Research Institute laboratory, limits of detection (LODs) and limits of quantification (LOQs) in fish tissue were estimated according to International Organisation for Standardisation Standard 6107-2:2006 as, respectively, 3-fold and 10-fold the standard deviation of an extract of biological tissue fortified at 1 µg/L. Limit of detection and LOQ values ranged from 0.01 to 0.13 and from 0.02 to 0.33 ng/g wet weight, respectively (Supplemental Data, Table S5). A procedural blank was run for every extraction batch; PFAS concentrations were always below respective LODs. Method trueness was assessed by the analysis of IRMM-427, a reference fish fillet certified for the mass fraction of PFASs (Dabrio Ramos et al. 2015).

In LABERCA, quality assurance/quality control procedures included the use of appropriate internal standards in each sample, whereas labeled external standards were systematically added at the end of each analytical batch to determine recoveries. Furthermore, a continuous monitoring of the analytical procedure was implemented through procedural blanks, to check for the absence of external contamination. Reproducibility was assessed using a quality control sample regularly characterized over several years. Limits of detection and LOQs were determined similar to the Water Research Institute process and were in the range of 0.01 to 0.10 ng/g wet weight, except for short-chain perfluorinated alkyl acids (PFBA and PFPA) for which the sensitivity was lower.

Both laboratories participated in the IMEP-42 round-robin study, which used the above-mentioned IRMM-427 certified sample. The performance of participating laboratories was assessed by calculating *Z*-scores according to International Organisation for Standardisation IEC17043 (Dehouck et al. 2015). The *Z*-scores of both methods were satisfactory because their absolute values were close to 1, ranging from –0.52 to +0.82 for the Water Research Institute and from –0.77 to +1.15 for LABERCA for all the 6 compounds (PFHxS, PFOS, PFNA, PFDA, PFUnDA, and PFDoDA) reported by the CRM provider (Dabrio Ramos et al. 2015).

Data processing

Viscera concentrations. In cases where entrails and liver were analyzed separately, PFAS concentrations in whole viscera (*V*) were determined as the weighted mean of concentrations in fractions according to the following equation:

$$Conc_V = \frac{(Conc_E \times weight_E) + (Conc_L \times weight_L)}{weight_E + weight_L} \quad (1)$$

where the subscript *E* means entrails and the subscript *L* corresponds to liver.

Whole-body concentrations. Whole-body (*WB*) PFAS concentrations were determined as the weighted means of concentrations in fractions according to the following equation:

$$Conc_{WB} = \frac{(Conc_F \times weight_F) + (Conc_V \times weight_V) + (Conc_C \times weight_C)}{weight_F + weight_V + weight_C} \quad (2)$$

where *F* means fillet, *C* corresponds to carcass, and *V* to viscera, including liver. In the same way, the whole-body dry weight, fresh weight, lipid content, polar lipids, and protein contents were calculated. Dry weight fraction ($f_{dry\,wt} = g_{dry\,wt}/g_{wet\,wt}$) was determined in most fillet samples and also in viscera and carcass of the dissected fish.

Degree of Urbanisation Index. For the catchments of the largest lakes, we applied the Degree of Urbanisation (DEGURBA) classification developed by the statistical office of the European Union as a proxy for the anthropic pressure (European Union Statistical Office 2020a). Based on the share of local population living in urban clusters and centers, this index classifies local administrative units into 3 categories: 1) class 1, urban centers (densely populated areas), 2) class 2, urban clusters (intermediate density areas), and 3) class 3, rural areas (thinly populated areas). Because urban clusters (class 2) are defined as a population density of at least 300 inhabitants per km² whereas urban centers (class 1) are identified as a population density of at least 1500 inhabitants per km² (i.e., 5 times the class 2 density), we described a Degree of Urbanization Index in the following equation:

$$\text{Degree of Urbanization Index} = 5 * (\%Class\ 1) + (\%Class\ 2) \quad (3)$$

The DEGURBA data for the catchments of the largest lakes and their calculated Degree of Urbanization Indexes are shown in Supplemental Data, Table S6.

Statistics. Distributions of concentrations in fish accounting for nondetects were obtained with software ProUCL Ver 5.1.00 (US Environmental Protection Agency 2016). For datasets with more than 50% of censored data (i.e., less than 50% of data above the detection limits), only median and concentration ranges were reported.

Shapiro–Wilk tests were conducted to check for normality within groups. An unpaired two-sample *t* test, following an *F* test for variance homogeneity, was used to evaluate

significant differences between 2 normal distributed and homogeneous sets of data. A Wilcoxon rank-sum test was applied to evaluate significant differences between 2 non-normal distributed sets of data. One-way analysis of variance (for normally distributed data) or Kruskal–Wallis (in the case of non-normal distributed data) tests were used for variance analysis. The Kruskal–Wallis test was followed by Dunn's post hoc test in the case of significant differences.

The correlations between fillet and whole-body concentrations were assessed by the Theil–Sen regression to include censored data in the datasets, after having carried out a trend analysis by the Mann–Kendall test. We also applied the analysis of covariance (ANCOVA) to compare the slopes of regressions between fillet and whole-body concentrations when the detection rates equaled 100%.

Significance was set at $\alpha = 0.05$ in all tests.

RESULTS

Characterization of fish

The biometric data of fish and their fractions are reported in Supplemental Data, Table S7. The weight percentage of the 3 fractions (fillet, viscera, and carcass) was determined in the 4 dissected species (shad, burbot, roach, and brown trout; Supplemental Data, Table S7). Viscera were the smallest fraction and constituted from 9 to 14% of the fresh whole-body weight. Carcass was the largest fraction (47–55%), whereas fillet represented from 32 to 41% of the fresh whole-body weight. The dry weight fraction of whole-body weight ($f_{dry\,wt}$) was calculated according to Equation 2 and ranged from 0.22 to 0.32 ($g_{dry\,wt}/g_{wet\,wt}$). Lipid content (f_{Lip}) was determined in most fillets and also in the dissected viscera and carcass samples. Shad had the highest lipid content (f_{Lip}) in fillets. Analysis of protein (f_{Pr}), polar lipid (f_{LP}), and neutral lipid (f_{LN}) content was carried out for only 3 different fractions of trout and shad. Protein content ranging from 0.07 ± 0.02 to 0.08 ± 0.02 was the same in the 3 fractions. The highest content of polar lipids was observed in viscera (0.41 ± 0.13), whereas the highest content of neutral lipids was detected in the carcass fractions (0.11 ± 0.02).

PFAS levels in fish

The dataset presents PFAS contamination in fish in the subalpine area during the last 5-yr period. Aggregated data are summarized in Tables 1–3, which are divided according to lakes and matrices, whereas the complete dataset is available in Supplemental Data, Tables S8–S11.

The PFTrDA and PFTeDA were not determined in Lakes Geneva, Mergozzo, and Lugano in 2015. The PFBA, PFPeA, PFBS, PFHpS, and PFDS were measured only in Lake Geneva but were always below the LODs (respectively, 0.2, 0.2, 0.02, 0.02, and 0.02 ng/g wet wt); they were not further discussed or included in the tables.

Taking into consideration the whole fillet dataset, which is the most comparable, the most frequently found compounds were PFOS and PFDoDA (100% of detection) followed by PFDA and PFUnDA (92–98% of detection). The highest concentrations were measured for PFOS (from 0.2–50.5 ng/g wet wt, median 6.0 ng/g wet wt) followed by PFDA (<LOD–12.0 ng/g wet wt, median 0.5 ng/g wet wt), PFUnDA (<LOD–8.9 ng/g wet wt, median 0.3 ng/g wet wt), and PFDoDA (0.01–4.81 ng/g wet wt, median 0.3 ng/g wet wt).

Similar concentration results were obtained for carcass and viscera, where long-chain PFCAs (from C10–C14) and PFOS were detected in more than 95% of the samples. The highest concentrations were measured for PFOS (viscera from 3.6–77.0 ng/g wet wt, median 25.9 ng/g wet wt, and carcass from 2.1–55.2 ng/g wet wt, median 14.8 ng/g wet wt) and PFDA (viscera from 0.6–7.0 ng/g wet wt, median 2.1 ng/g wet wt, and carcass from <LOD–3.5 ng/g wet wt, median 1.2 ng/g wet wt).

From PFNA (9 carbon atoms) to PFHxA (6 C), the frequency of detection significantly decreased from 48 to 9% in fillet samples and from approximately 80 to 10–20% for the other examined matrices (Tables 1–3), following the well-known decrease of the bioaccumulation potential as a function of the decrease in perfluorinated chain length (Martin et al. 2003a, 2003b; Zhao et al. 2013).

For the investigation of the possible influence of seasonality on PFAS concentrations in fish, data from 4 lakes (Lakes Como, Garda, Lugano, and Maggiore) that were sampled in the four seasons of 2018 were pooled according to the season. Shads were sampled in all the lakes in every season, whereas European perches were seasonally caught only in Lake Lugano. No statistical differences were found among the seasons for all the compounds (Kruskal–Wallis $p > 0.5$) regardless of whether 2 species were considered (shad and European perch) or only one species was considered (shad; Supplemental Data, Figure S1).

The differences among species could be detected only in Lakes Lugano, Varese, and Geneva where at least 3 specimens for each different species were available (roach and burbot in Lake Geneva, shad and European perch in Lake Lugano, and European perch and roach in Lake Varese). Statistical analysis carried out between the couples of species showed no significant differences ($p > 0.05$) for PFOS and long-chain PFCAs in fillets (Supplemental Data, Figure S2). It was not possible to perform the same comparison for viscera samples because of the paucity of data.

PFAS pattern of contamination in lakes

One of the aims of the present study was to assess the status of fish contamination by PFASs in the European subalpine lakes in the Alpine area. In this section we focus mainly on fillet data because the largest dataset is available for this matrix, allowing us to compare concentrations in fish among lakes.

The dataset covers a wide concentration range from high altitude and remote lakes (e.g., PFOS from 0.2–0.8 ng/g wet wt, median 0.3 in Lake Sassolo upper and Lake Sassolo lower) to low altitude and highly populated lakes (e.g., PFOS from 3.7–50.5 ng/g wet wt, median 15.7 in Lake Lugano).

TABLE 1: Summary of the concentrations of per- and polyfluoroalkyl substances in fish fillets[a,b,c,d,e]

		PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	PFTeDA	PFHxS	PFOS	∑PFAS
Lake Como	% detect	15	15	31	77	100	92	100	100	92	8	100	—
A. agone	Mean ± SD	—	—	—	0.04 ± 0.03	0.25 ± 0.17	0.15 ± 0.12	0.23 ± 0.29	0.30 ± 0.26	0.13 ± 0.13	—	3.7 ± 1.3	4.9 ± 1.8
(No. = 13)	Median	<LOD	<LOD	<LOD	0.03	0.22	0.10	0.15	0.17	0.08	<LOD	4.1	5.4
	Min-Max	<LOD–0.58	<LOD–0.04	<LOD–0.14	<LOD–10	0.05–0.49	<LOD–0.42	0.03–1.01	0.09–1.03	<LOD–0.41	<LOD–0.10	1.6–6.0	2.1–7.5
Lake Garda	% detect	17	17	17	67	100	67	100	100	100	17	100	—
A. agone	Mean ± SD	—	—	—	0.04 ± 0.03	0.12 ± 0.10	0.06 ± 0.04	0.08 ± 0.06	0.11 ± 0.06	0.07 ± 0.05	—	1.8 ± 1.5	2.5 ± 1.7
(No. = 6)	Median	<LOD	<LOD	<LOD	0.03	0.12	0.06	0.08	0.12	0.06	<LOD	1.4	1.8
	Min-Max	<LOD–0.77	<LOD–0.05	<LOD–0.02	<LOD–0.09	0.01–0.27	<LOD–0.12	0.02–0.16	0.01–0.19	0.01–0.13	<LOD–0.11	0.7–4.8	0.85–5.6
Lake Iseo	% detect	22	22	33	67	89	78	100	89	89	11	100	—
A. agone	Mean ± SD	—	—	—	0.04 ± 0.04	0.13 ± 0.10	0.11 ± 0.09	0.15 ± 0.10	0.11 ±0.07	0.09 ± 0.06	—	1.1 ± 0.6	1.8 ± 0.8
S. trutta	Median	<LOD	<LOD	<LOD	0.02	0.09	0.07	0.14	0.09	0.06	<LOD	0.9	1.9
(No. = 9)	Min-Max	<LOD–0.09	<LOD–0.05	<LOD–0.35	<LOD–0.11	<LOD–0.36	<LOD–0.29	0.06–0.37	<LOD–0.23	<LOD–0.19	<LOD–0.12	0.4–2.5	0.73–3.5
Lake Maggiore	% detect	18	27	18	64	100	100	100	73	73	0	100	—
A. agone	Mean ± SD	—	—	—	0.10 ± 0.20	0.32 ± 0.21	0.20 ± 0.12	0.17 ± 0.10	0.09 ± 0.09	0.04 ± 0.06	—	9.54 ± 5.0	10.7 ± 5.5
C. lavaretus	Median	<LOD	<LOD	<LOD	0.03	0.28	0.15	0.13	0.08	0.01	<LOD	8.6	10.2
R. rutilus	Min-Max	<LOD–0.96	<LOD–0.16	<LOD–1.19	<LOD–0.69	0.02–0.76	0.03–0.39	0.04–0.29	<LOD–0.30	<LOD–0.17	<LOD	3.7–19.9	4.5–21.8
(No. = 11)													
Lake Mergozzo	% detect	11	11	0	44	89	100	100	—	—	0	100	—
A. agone	Mean ± SD	—	—	—	—	1.73 ± 2.31	1.89 ± 2.75	1.15 ± 1.45	—	—	—	8.9 ± 11.7	13.8 ± 18.2
C. lavaretus	Median	<LOD	<LOD	<LOD	<LOD	0.78	0.95	0.61	—	—	<LOD	5.4	8.5
S. alpinus	Min-Max	<LOD–0.10	<LOD–0.10	<LOD	<LOD–0.21	<LOD–7.92	0.05–8.93	0.04–4.81	—	—	<LOD	0.27–38.4	0.36–60.2
L. lota													
P. fluviatlis													
R. rutilus													
(No. = 9)													
Lakes Sassolo	% detect	0	0	25	25	100	75	100	100	75	25	100	—
O. mykiss	Mean ± SD	—	—	—	—	0.14 ± 0.18	0.29 ± 0.27	0.27 ± 0.32	0.74 ± 1.10	0.19 ± 0.29	—	0.42 ± 0.30	2.1 ± 2.6
S. alpinus	Median	<LOD	<LOD	<LOD	<LOD	0.08	0.21	0.16	0.24	0.03	<LOD	0.32	1.0
(No. = 4)	Min-Max	<LOD	<LOD	<LOD–0.05	<LOD–0.07	0.01–0.41	<LOD–0.73	0.01–0.74	0.11–2.38	<LOD–0.70	<LOD–0.03	0.20–0.83	0.35–6.0
(Steingruber 2018)													
Lake Varese	% detect	0	0	0	67	100	100	100	0	50	0	100	—
R. rutilus	Mean ± SD	—	—	—	0.18 ± 0.19	2.85 ± 2.09	0.70 ± 0.51	0.14 ± 0.13	—	0.02 ± 0.01	—	7.1 ± 4.4	11.0 ± 7.3
P. fluviatlis	Median	<LOD	<LOD	<LOD	0.12	2.40	0.59	0.13	<LOD	0.02	<LOD	7.0	10.24
(No. = 6)	Min-Max	<LOD	<LOD	<LOD	<LOD–0.51	0.66–5.58	0.16–1.37	0.01–0.32	<LOD	<LOD–0.04	<LOD	2.07–12.5	2.9–19.6
Lake Geneva	% detect	0	0	36	50	100	100	100	—	—	71	100	—
L. lota	Mean ± SD	—	—	—	0.08 ± 0.08	0.81 ± 0.30	0.37 ± 0.15	0.98 ± 0.57	—	—	0.26 ± 0.29	9.8 ± 4.2	12.4 ± 4.6
R. rutilus	Median	<LOD	<LOD	<LOD	0.09	0.81	0.33	0.87	—	—	0.10	9.1	11.7
(No. = 14)	Min-Max	<LOD	<LOD	<LOD–0.14	<LOD–0.23	0.12–1.34	0.03–0.58	0.09–2.32	—	—	<LOD–0.95	2.4–19.3	2.7–22.6

(*Continued*)

TABLE 1: (*Continued*)

		PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	PFTeDA	PFHxS	PFOS	∑PFAS
Lake Lugano	% detect	9	0	0	13	100	91	100	75[f]	75[f]	0	100	—
A. agone	Mean ± SD	—	—	—	—	4.02 ± 3.71	1.11 ± 0.83	1.28 ± 0.81	0.47 ± 0.37	0.22 ± 0.16	—	21.4 ± 15.3	28.1 ± 19.7
P. fluviatlis	Median	<LOD	<LOD	<LOD	<LOD	2.16	0.96	1.11	0.41	0.21	<LOD	15.7	18.6
(No. = 23) (Solcà 2016 and present study)	Min-Max	<LOD–0.43	<LOD	<LOD	<LOD–0.27	0.40–11.99	<LOD–2.86	0.18–2.72	<LOD–1.23	<LOD–0.45	<LOD	3.7–50.5	4.5–60.4
		—	—	—	—	—	—	—	—	—	—	—	—
All fillet	% detect	11	9	17	48	98	92	100	79[g]	81[g]	15	100	—
samples	Mean ± SD	—	—	—	—	1.53 ± 2.53	0.61 ± 1.07	0.65 ± 0.81	0.24 ± 0.38	0.11 ± 0.14	—	9.8 ± 11.3	13.0 ± 14.8
(No. = 95)	25th percentile	—	—	—	<LOD	0.20	0.10	0.12	0.05	<LOD	—	2.4	3.5
	Median	<LOD	<LOD	<LOD	0.02	0.54	0.28	0.28	0.11	0.05	<LOD	6.0	8.5
	75th percentile	—	—	—	0.08	1.24	0.67	1.01	0.30	0.14	—	11.6	14.2
	Min-Max	<LOD–0.96	<LOD–0.16	<LOD–1.19	<LOD–0.69	<LOD–11.99	<LOD–8.93	0.01–4.81	<LOD–2.38	<LOD–0.70	<LOD–0.95	0.2–50.5	0.35–60.4

[a]Data are expressed in ng/g wet weight.
[b]The reported values take nondetects into account and they were calculated by ProUCL Ver 5.1.00 (US Environmental Protection Agency 2016).
[c]For datasets with less than 50% results above the detection limits, only median and concentration ranges were reported.
[d]In the calculation of ∑PFAS, values <LOD have been set at zero.
[e]LODs were 0.01 ng/L for PFHxA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, and PFTeDA; 0.02 ng/L for PFHpA, PFOA, and PFHxS; and 0.04 ng/L for PFOS.
[f]PFTrDA and PFTeDA were determined only in 2018 samples (No. = 8).
[g]PFTrDA and PFTeDA were not determined in Lake Geneva, Lake Mergozzo, and Lake Lugano in 2015 (No. = 57).
PFAS = perfluoroalkyl substances; PFHxA = perfluorohexanoate; PFNA = perfluorononanoate; PFDA = perfluorodecanoate; PFUnDA = perfluoroundecanoate; PFDoDA = perfluorododecanoate; PFTrDA = perfluorotridecanoate; PFTeDA = perfluorotetradecanoate; PFHpA = perfluoroheptanoate; PFOA = perfluorooctanoate; PFHxS = perfluorohexane sulfonate; PFOS = perfluorooctane sulfonate; SD = standard deviation; LOD = limit of detection.
A. agone = shad; *S. trutta* = brown trout; *C. lavaretus* = European whitefish; *R. rutilus* = roach; *S. alpinus* = Arctic char; *L. lota* = burbot; *P. fluviatilis* = European perch; *O. mykiss* = rainbow trout.

TABLE 2: Summary of the concentrations of per- and polyfluoroalkyl substances in fish liver and viscera[a,b,c,d,e]

		PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	PFTeDA	PFHxS	PFOS	∑PFAS
Liver													
Lake Geneva	% detect	0	0	22	33	100	78	100	—	—	56	100	—
L. lota	Mean ± SD	—	—	—	—	2.11 ± 1.80	0.99 ± 0.92	2.49 ± 1.95	—	—	0.44 ± 0.31	28.5 ± 19.7	36.9 ± 23.6
R. rutilus	Median	<LOD	<LOD	<LOD	<LOD	1.15	0.54	1.76	—	—	0.53	20.2	27.8
(No. = 10)	Min-Max	<LOD	<LOD	<LOD–0.23	<LOD–0.26	0.60–4.84	<LOD–2.86	0.82–6.89	—	—	<LOD–0.85	9.4–57.8	13.5–67.8
Viscera													
Lake Como	% detect	20	0	0	90	100	100	100	100	100	20	100	—
A. agone	Mean ± SD	—	—	—	0.61 ± 0.44	2.88 ± 1.99	1.39 ± 0.79	1.16 ± 0.61	2.14 ± 1.31	0.93 ± 0.54	—	38.0 ± 20.1	48.1 ± 20.4
(No. = 10)	Median	<LOD	<LOD	<LOD	0.51	2.26	1.18	0.95	2.15	1.08	<LOD	30.0	44.8
	Min-Max	<LOD–7.68	<LOD	<LOD	<LOD–1.37	0.89–6.96	0.42–2.98	0.32–2.40	0.23–3.88	0.09–1.47	<LOD–2.11	14.5–77.0	21.6–88.6
Lake Garda	% detect	20	20	40	100	100	100	100	80	100	0	100	—
A. agone	Mean ± SD	—	—	—	0.45 ± 0.12	1.62 ± 1.41	0.50 ± 0.22	1.01 ± 0.53	0.84 ± 0.77	0.65 ± 0.46	—	16.2 ± 9.7	21.8 ± 9.8
(No. = 5)	Median	<LOD	<LOD	<LOD	0.48	1.07	0.49	0.93	0.43	0.77	<LOD	15.2	23.7
	Min-Max	<LOD–2.30	<LOD–0.30	<LOD–0.06	0.27–0.57	0.78–4.14	0.18–0.74	0.33–1.77	<LOD–1.87	0.13–1.14	<LOD	5.8–31.5	11.5–35.1
Lake Iseo	% detect	20	20	40	80	100	80	80	100	100	20	100	—
A. agone	Mean ± SD	—	—	—	0.66 ± 0.52	1.97 ± 1.06	1.61 ± 1.24	1.72 ± 0.93	1.77 ± 1.17	0.84 ± 0.50	—	10.70 ± 4.7	20.2 ± 4.9
S. trutta	Median	<LOD	<LOD	<LOD	0.54	1.72	1.25	2.08	2.28	0.88	<LOD	10.4	21.4
(No. = 5)	Min-Max	<LOD–3.38	<LOD–0.08	<LOD–0.45	<LOD–1.59	0.69–3.48	<LOD–3.75	<LOD–2.70	0.17–3.03	0.12–1.42	<LOD	3.6–15.1	12.1–25.3
Lake Maggiore	% detect	25	25	25	100	100	100	100	100	100	0	100	—
A. agone	Mean ± SD	—	—	—	0.46 ± 0.14	2.96 ± 1.50	1.19 ± 0.32	0.95 ± 0.16	1.09 ± 0.22	0.70 ± 0.41	—	49.4 ± 10.4	59.0 ± 13.0
(No. = 4)	Median	<LOD	<LOD	0.07	0.52	3.31	1.28	0.97	1.15	0.51	<LOD	52.5	59.6
	Min-Max	<LOD–8.00	<LOD–0.48	<LOD–0.27	0.25–0.56	0.86–4.34	0.75–1.47	0.75–1.13	0.77–1.29	0.47–1.31	<LOD	34.8–57.8	42.8–74.0
Lake Geneva	% detect	0	0	21	57	100	100	100	—	—	64	100	—
L. lota	Mean ± SD	—	—	—	0.17 ± 0.19	2.26 ± 1.02	1.04 ± 0.51	2.68 ± 1.77	—	—	0.64 ± 0.64	28.0 ± 10.9	32.2 ± 11.1
R. rutilus	Median	<LOD	<LOD	<LOD	0.07	2.19	1.02	1.83	—	—	0.37	28.3	32.0
(No. = 14)	Min-Max	<LOD	<LOD	<LOD–0.20	<LOD-0.54	0.50–4.76	0.14–2.33	0.30–4.67	—	—	<LOD–2.18	8.7–55.2	12.8–63.1
All viscera	% detect	13	8	21	79	100	97	97	96	100	32	100	—
samples	Mean ± SD	—	—	—	0.42 ± 0.38	2.27 ± 1.38	1.10 ± 0.73	1.61 ± 1.04	1.62 ± 1.14	0.82 ± 0.48	—	28.4 ± 17.2	36.3 ± 18.3
(No. = 38)	25th percentile	—	—	—	0.07	1.31	0.60	0.89	0.72	0.45	—	15.3	24.0
	Median	<LOD	<LOD	<LOD	0.41	2.10	1.10	1.49	1.49	0.86	<LOD	26.9	32.8
	75th percentile	—	—	—	0.54	3.02	1.31	2.05	2.32	1.21	—	32.7	41.8
	Min-Max	<LOD–8.00	<LOD–0.48	<LOD–0.45	<LOD–1.59	0.64–6.96	<LOD–3.75	<LOD–4.67	<LOD–3.88	0.09–1.47	<LOD–2.18	3.6–77.0	11.5–88.6

[a]Data are expressed in ng/g wet weight.
[b]Reported values take nondetects into account and were calculated by ProUCL Ver 5.1.00 (US Environmental Protection Agency 2016).
[c]For datasets with less than 50% results above the detection limits, only median and concentration ranges were reported.
[d]In the calculation of ∑PFAS, values <LOD have been set as zero.
[e]LODs were 0.04 ng/L for PFHxA; 0.07 ng/L for PFHpA, PFOA, and PFHxS; 0.05 ng/L for PFNA, PFDA, and PFTrDA; 0.03 ng/L for PFUnDA and PFTeDA; 0.02 ng/L for PFDoDA; and 0.13 ng/L for PFOS.
PFAS = perfluoroalkyl substances; PFHxA = perfluorohexanoate; PFHpA = perfluoroheptanoate; PFOA = perfluorooctanoate; PFHxS = perfluorohexane sulfonate; PFNA = perfluorononanoate; PFDA = perfluorodecanoate; PFTrDA = perfluorotridecanoate; PFUnDA = perfluoroundecanoate; PFTeDA = perfluorotetradecanoate; PFDoDA = perfluorododecanoate; PFOS = perfluorooctane sulfonate; SD = standard deviation; LOD = limit of detection.
L. lota = burbot; *R. rutilus* = roach; *A. agone* = shad; *S. trutta* = brown trout.

TABLE 3: Summary of the concentrations of per- and polyfluoroalkyl substances in fish carcasses[a,b,c,d,e]

		PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	PFTeDA	PFHxS	PFOS	∑PFAS
							Carcass						
Lake Como	% detect	20	20	10	90	90	90	90	100	100	10	100	—
A. agone	Mean ± SD	—	—	—	0.15 ± 0.12	1.17 ± 0.74	0.59 ± 0.36	0.43 ± 0.22	0.95 ± 0.50	0.38 ± 0.27	—	13.5 ± 7.0	17.4 ± 7.6
(No. = 9)	Median	<LOD	<LOD	<LOD	0.10	0.89	0.48	0.44	0.96	0.32	<LOD	12.0	16.4
	Min-Max	<LOD–1.25	<LOD–0.09	<LOD–0.08	<LOD–0.35	<LOD–2.46	<LOD–1.23	<LOD–0.88	0.29–1.62	0.09–1.03	<LOD–0.25	6.9–30.2	9.6–36.1
Lake Garda	% detect	40	20	40	80	100	100	100	100	100	20	100	—
A. agone	Mean ± SD	—	—	—	0.19 ± 0.14	0.69 ± 0.35	0.27 ± 0.22	0.33 ± 0.20	0.65 ± 0.61	0.32 ± 0.41	—	4.7 ± 1.2	7.72 ± 3.8
(No. = 5)	Median	<LOD	<LOD	<LOD	0.13	0.49	0.21	0.28	0.41	0.20	<LOD	4.3	6.3
	Min-Max	<LOD–2.03	<LOD–0.21	<LOD–0.06	<LOD–0.44	0.40–1.11	0.07–0.62	0.15–0.65	0.11–1.69	0.02–1.03	<LOD–0.22	3.6–6.7	5.3–14.4
Lake Iseo	% detect	20	0	0	80	100	100	100	100	100	20	100	—
A. agone	Mean ± SD	—	—	—	0.18 ± 0.14	0.68 ± 0.44	0.64 ± 0.59	0.59 ± 0.45	0.77 ± 0.62	0.48 ± 0.39	—	2.7 ± 0.4	6.4 ± 3.0
S. trutta	Median	<LOD	<LOD	<LOD	0.15	0.71	0.40	0.52	0.59	0.33	<LOD	2.7	5.2
(No. = 5)	Min-Max	<LOD–1.88	<LOD	<LOD	<LOD–0.41	0.16–1.33	0.07–1.60	0.14–1.30	0.14–1.43	0.09–1.00	<LOD–0.21	2.1–3.2	3.8–11.3
Lake Maggiore	% detect	50	25	0	100	100	100	100	100	100	0	100	—
A. agone	Mean ± SD	0.05 ± 0.03	—	—	0.19 ± 0.10	1.22 ± 0.69	0.61 ± 0.31	0.45 ± 0.15	0.52 ± 0.18	0.16 ± 0.15	—	19.6 ± 1.8	22.8 ± 2.3
(No. = 4)	Median	0.02	<LOD	<LOD	0.19	1.32	0.72	0.47	0.52	0.09	<LOD	18.9	23.1
	Min-Max	<LOD–0.07	<LOD–0.06	<LOD	0.10–0.28	0.38–1.86	0.17–0.83	0.27–0.58	0.37–0.68	0.07–0.38	<LOD	18.4–22.3	19.8–25.2
Lake Geneva	% detect	0	0	36	71	100	100	100	—	—	79	100	—
L. lota	Mean ± SD	—	—	—	0.22 ± 0.18	2.13 ± 0.88	1.02 ± 0.51	2.70 ± 1.87	—	—	0.50 ± 0.54	24.3 ± 11.8	31.0 ± 13.1
R. rutilus	Median	<LOD	<LOD	<LOD	0.21	2.14	0.89	2.33	—	—	0.20	22.6	31.5
(No. = 14)	Min-Max	<LOD	<LOD	<LOD–0.30	<LOD–0.56	0.49–3.54	0.15–1.77	0.29–7.32	—	—	<LOD–1.64	6.7–55.2	9.1–64.5
All carcass	% detect	18	11	21	82	97	97	97	100	100	37	100	—
samples	Mean ± SD	—	—	—	0.19 ± 0.15	1.40 ± 0.91	0.72 ± 0.49	1.27 ± 1.56	0.75 ± 0.51	0.34 ± 0.31	—	15.6 ± 11.4	20.3 ± 13.1
(No. = 37)	25th percentile	—	—	—	0.06	0.72	0.37	0.32	0.37	0.10	—	6.7	—
	Median	<LOD	<LOD	<LOD	0.13	1.22	0.63	0.57	0.62	0.26	<LOD	14.8	19.2
	75th percentile	—	—	—	0.28	1.85	0.93	1.44	1.23	0.39	0.19	21.8	—
	Min-Max	<LOD–2.03	<LOD–0.21	<LOD–0.30	<LOD–0.56	<LOD–3.54	<LOD–1.77	<LOD–7.32	0.11–1.69	0.02–1.03	<LOD–1.64	2.1–55.2	3.8–64.5

[a]Data are expressed in ng/g wet weight.
[b]Reported values take nondetects into account and were calculated by ProUCL Ver 5.1.00 (US Environmental Protection Agency 2016).
[c]For datasets with less than 50% results above the detection limits, only median and concentration ranges were reported.
[d]In the calculation of ∑PFAS, values <LOD have been set as zero.
[e]LODs were 0.02 ng/L for PFHxA, PFUnDA, and PFTeDA; 0.04 ng/L for PFHpA, PFOA, and PFHxS; 0.03 ng/L for PFNA, PFDA, and PFTrDA; 0.01 ng/L for PFDoDA; and 0.08 ng/L for PFOS.
PFAS = perfluoroalkyl substances; PFHxA = perfluorohexanoate; PFUnDA = perfluoroundecanoate; PFTeDA = perfluorotetradecanoate; PFHpA = perfluoroheptanoate; PFOA = perfluorooctanoate; PFHxS = perfluorohexane sulfonate; PFNA = perfluorononanoate; PFDA = perfluorodecanoate; PFTrDA = perfluorotridecanoate; PFDoDA = perfluorododecanoate; PFOS = perfluorooctane sulfonate; SD = standard deviation; LOD = limit of detection.
A. agone = shad; *S. trutta* = brown trout; *L. lota* = burbot; *R. rutilus* = roach.

Even if monitoring programs were not designed for the compliance checking with the European Union Environmental Quality Standards for biota (EQS_{biota}) derived from the Water Framework Directive regulation, we could get a rough assessment of each lake status by comparing geometric means of the whole dataset—without distinction of fish species and years—with the European Union EQS_{biota} for PFOS (9.1 ng/g wet wt; European Commission 2014). Geometric means of PFOS concentrations in Lakes Iseo, Garda, Como, Mergozzo, and Varese (1.0, 1.4, 3.5, 4.5, and 5.7 ng/g wet wt, respectively) were lower than the European Union EQS_{biota} for PFOS. On the contrary, geometric means of Lakes Maggiore and Geneva (8.4 and 8.9 ng/g wet wt, respectively) were close to this standard, which was widely exceeded in Lake Lugano (PFOS geometric mean 16.0 ng/g wet wt; Figure 2).

With regard to the sum of long-chain PFCAs (Supplemental Data, Table S12), Lake Lugano showed the highest values (median 4.2 ng/g wet wt but with a wide variability from 0.7 to 16.8 ng/g wet wt), followed by Lakes Varese, Mergozzo, and Geneva (medians 3.3, 3.1, and 2.2 ng/g wet wt, respectively). On the contrary, long-chain PFCA concentrations in Lakes Maggiore, Como, Iseo, and Garda (medians from 0.36–0.64 ng/g wet wt) were of the same order of magnitude as those determined in the high-altitude Sassolo Lakes (median 0.44 ng/g wet wt).

Of the PFASs measured in all low-altitude lakes, PFOS represented more than 50%, ranging from 62% in Lake Iseo to 88% in Lake Maggiore (Figure 3). In the high-altitude lakes, C10 to C14 PFCAs represented approximately 65% of the total PFASs. The PFDA made up 25% of ∑PFASs in Lake Varese, far above the proportion observed in all other investigated lakes. Similarly, PFUnDA was present in a significant proportion (13%) of ∑PFASs in Lake Mergozzo, whereas PFHxS (2.5%) was present in Lake Geneva—both higher than in all other lakes. These observations suggest specific, but as yet not identified, PFAS sources in these lakes. It is interesting to note that PFOA has been detected only in 6 lakes but with a very limited percentage contribution; the maximum PFOA percentage (1.5%) was measured in Lake Iseo, confirming that PFOA has a limited accumulation in biota.

The morphology for Lakes Como and Lugano permits the discernment of 2 distinct areas. Lake Como is divided into 2 branches named Como and Lecco, whereas the Melide Dam divides the northern and southern parts of Lake Lugano. In both cases, the differences in concentrations and patterns were not significant, showing that the site of catching was not critical regarding the collected fish species that included both pelagic and demersal.

PFAS distribution in fish fractions

Distribution of perfluoroalkyl acids (PFAAs) among fish fractions was assessed for the most detected compounds: PFOS, PFNA, PFDA, PFUnDA, and PFDoDA ($n = 38$ fish). Whatever the compound, the fraction that displayed the highest concentrations was the viscera, thus including the liver and some blood, followed by the carcass, whereas the dorsal muscle had the lowest concentrations (Figure 4).

Nevertheless, the respective fraction loads differed among species (Supplemental Data, Figure S3). The muscle (fillet) represented approximately 10% of the total body burden in

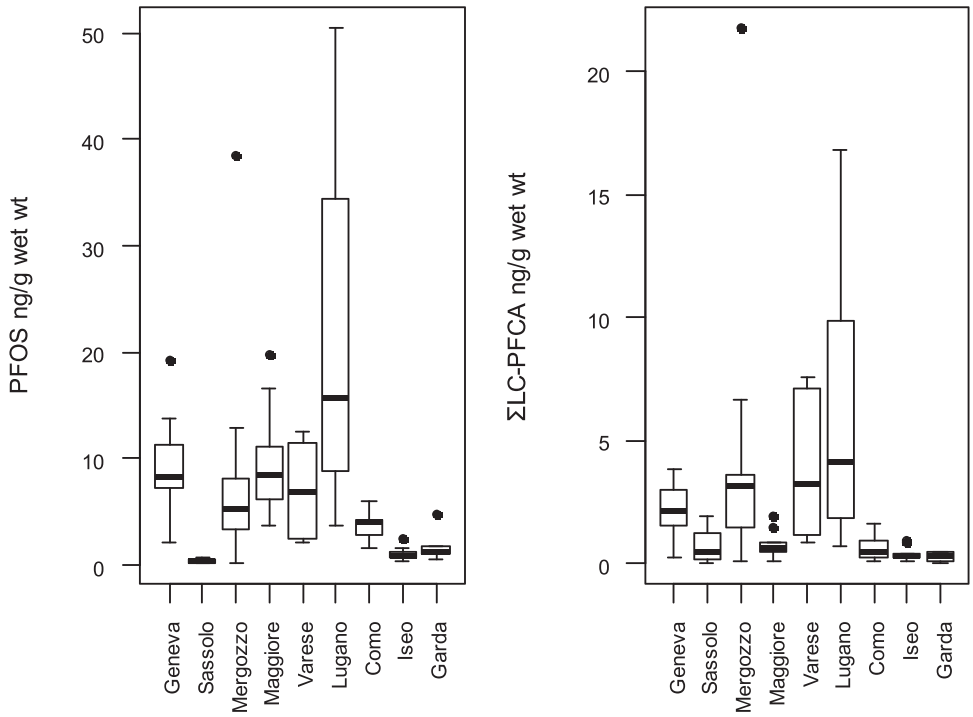

FIGURE 2: Box-whisker plot of the fillet per- and polyfluoroalkyl substance concentrations in the different lakes. PFOS = perfluorooctane sulfonate; ∑LC-PFCA = total long-chain perfluoroalkyl carboxylic acids.

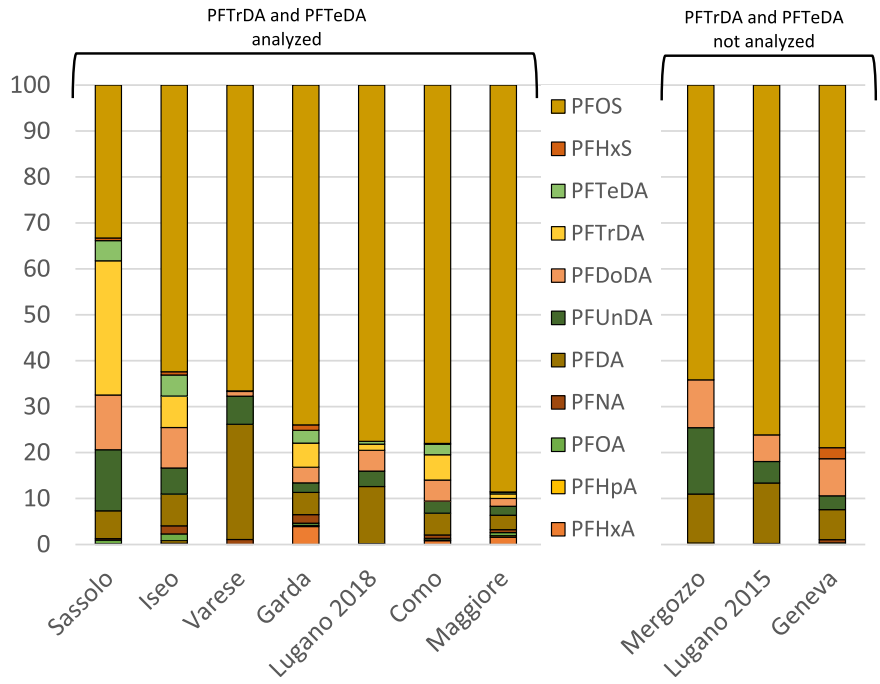

FIGURE 3: Mean percentage composition of per- and polyfluoroalkyl substance concentrations in the fish fillets from different lakes. (**Left**) Lakes whose dataset includes PFTrDA and PFTeDA concentrations. (**Right**) Lakes whose dataset does not include PFTrDA and PFTeDA concentrations. PFAS = perfluoroalkyl substances; PFOS = perfluorooctane sulfonate; PFHxS = perfluorohexane sulfonate; PFTeDA = perfluorotetradecanoate; PFTrDA = perfluorotridecanoate; PFDoDA = perfluorododecanoate; PFUnDA = perfluoroundecanoate; PFDA = perfluorodecanoate; PFNA = perfluorononanoate; PFOA = perfluorooctanoate; PFHpA = perfluoroheptanoate; PFHxA = perfluorohexanoate.

shad for PFOS, PFDA, PFUnDA, and PFDoDA, whereas it totaled approximately 20% in burbot and roach; these differences were significant ($p = 0.0003$). Conversely, the loads in viscera (i.e., liver + entrails) were significantly higher ($p < 0.0001$) in shad (~35%) than in burbot and roach (15–20%) because of anatomical and physiological differences among these species.

Whole-body concentrations generally increased with concentrations in fillet, as illustrated in Figure 5 for the most detected compounds. However, because roach and burbot

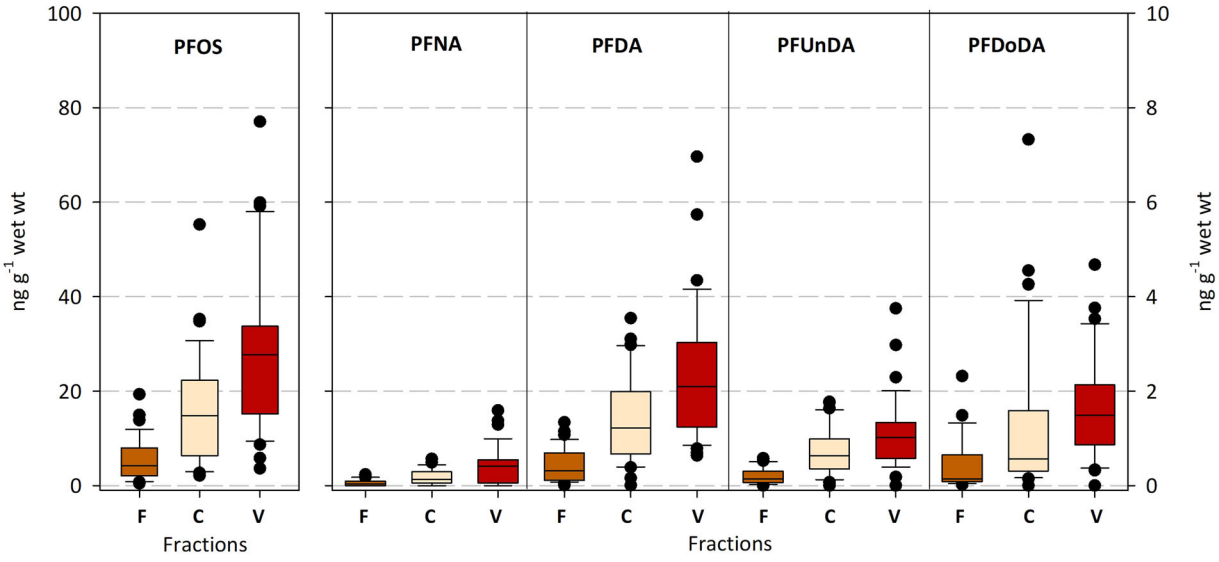

FIGURE 4: Box-whisker plot of the per- and polyfluoroalkyl substance concentrations in the different fish fractions. All species and lakes together. F = fillet; C = carcass; V = viscera, including liver; PFOS = perfluorooctane sulfonate; PFNA = perfluorononanoate; PFDA = perfluorodecanoate; PFUnDA = perfluoroundecanoate; PFDoDA = perfluorododecanoate.

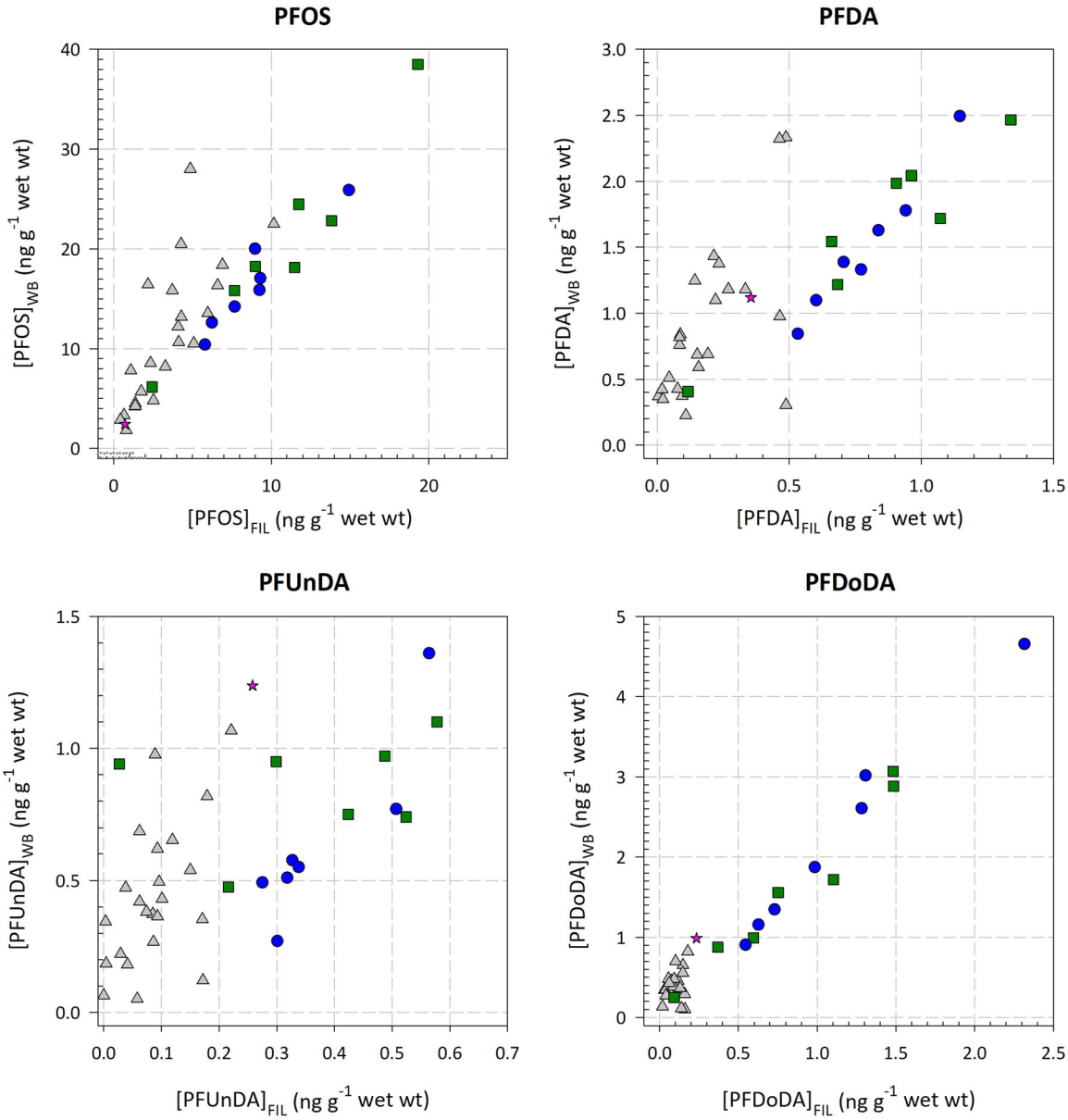

FIGURE 5: Relationship between fillet (FIL) and whole-body (WB) concentrations for perfluorooctane sulfonate (PFOS), perfluorodecanoate (PFDA), perfluoroundecanoate (PFUnDA), and perfluorododecanoate (PFDoDA). Gray triangles represent shad; pink stars are trout; green squares denote roach; and blue dots identify burbot.

were analyzed only in Lake Geneva and shad was analyzed only in the Italian lakes, we chose to test the correlations separately (Mann–Kendall test followed by the Theil–Sen regression). Results are reported in Table 4. Except for PFUnDA in roach and PFDoDA in shad, all regressions were significant, with mean slopes ranging from 1.59 to 3.54. The lack of significance for both PFDoDA in shad and PFUnDA in roach was probably caused by the limited concentration gradient for these compounds in our dataset (Figure 5).

To test whether the slopes of the regressions were different in the case of PFOS, we applied an ANCOVA to the dataset composed of fillet and whole-body concentrations for the shad, roach, and burbot species ($n = 34$). The effect of species (qualitative variable) was significant ($p = 0.043$), meaning that the slopes of the respective regressions were different.

DISCUSSION

Technical aspects for monitoring compliance or for data comparison

Monitoring of chemical pollutants in fish is an important way to assess the contamination status of water bodies and identify the pollution sources. This is especially true in the case of lipophilic contaminants that are difficult to determine in water, whereas they tend to accumulate in biota. Starting from these

considerations, the European Union derived EQS_{biota} for 11 substances and substance groups including the perfluoroalkyl compound PFOS (European Commission 2013).

It is necessary to consider many aspects (species selection, sampling period, selection of suitable matrices, etc.) when a fish-sampling campaign is designed and implemented. The sampling strategies should be designed according to the purposes of the studies; however, sometimes research studies must fit into existing monitoring programs for practical or logistical reasons. Guidance Document No. 32 on Biota Monitoring under the Water Framework Directive (hereafter termed GD-Biota; European Commission 2014) addresses many of the controversial issues in biota sampling. Nevertheless, it does not provide specific recommendations on all aspects because these are largely influenced by site-specific characteristics (e.g., availability of fish species and their exposure ways to contaminants) that imply a wide variability in accumulation behavior. A recent article (Fliedner et al. 2018) analyzed the available fish monitoring data of the German Danube for some bioaccumulative compounds including PFOS. Some open questions such as the relationship between contaminant concentrations in fillet and whole fish and the use of normalization to overcome tissue and species-specific differences in accumulation were discussed.

Because contaminant levels in fish are known to be influenced by a range of biological and environmental factors (European Commission 2014), natural variability within tissues and among samples should be minimized as much as possible to strengthen the comparisons among different monitoring programs. One of the factors that most impacts fish biology is seasonality. We did not find any statistical difference in the different seasons regarding both the analyzed PFASs and the considered fish species (shad and European perch). We do not have enough data to confirm the results for other species; nonetheless, we can assume that the sampling season is less critical for PFASs than for legacy lipophilic substances (Supplemental Data, Figure S1), as already demonstrated for zooplankton accumulation in the same subalpine lakes (Pascariello et al. 2019).

It is necessary to be flexible in the choice of the fish species when monitoring programs cover many water bodies because we can monitor only species that are actually present in the sampling sites. It is also necessary to evaluate the comparability of the concentration data of different species. In the present study, comparison of fish species was possible only between roach and burbot in Lake Geneva, shad and European perch in Lake Lugano, and European perch and roach in Lake Varese. The differences between the couples of species for PFOS and long-chain PFCA in fillet were not significant (Supplemental Data, Figure S2). This result might be caused by the limited sample size or because of the lack of distinction between feeding behaviors. Examining the PFAS concentrations of fish from the Rhône River, it was shown that the differences in concentrations among 3 species (*Barbus barbus*, *Gobio gobio*, and *R. rutilus*) could be explained by their diet, based on stomach contents and the analysis of food sources (Babut et al. 2017). We cannot exclude the fact that food webs are different in lotic and deep lentic environments because a food web based on a benthic or detritus source (mainly allochthonous) could prevail in the former and a phytoplankton-based pelagic food web could exist in the latter. However, we do not have sufficient data to utilize the dataset in a more detailed manner from this perspective.

There are other possible sources of variability such as: 1) the within-body contaminant distribution in the different fish tissues, 2) the differences in accumulation among fish of different sizes of the same species, and 3) among different species from the same water body. One possible solution to overcome these intrinsic variabilities is the normalization of the concentrations in fish against any biological components such as lipids, dry matter, or protein, as suggested by the GD-Biota.

It is known that chemical contaminants are not evenly distributed in fish. For example, the concentrations of hydrophobic substances tend to be higher in the liver than in other fractions of fish but the difference widely disappears when the results are lipid-normalized (Jurgens et al. 2013). The GD-Biota (European Commission 2014) already points out that lipid normalization is not appropriate for PFOS but suggests normalization against another parameter, such as dry weight, as a proxy for the total protein content.

According to our dataset, concentrations of PFASs in viscera, which include liver and some blood, were higher than in the remaining fractions (carcass or fillet; Figure 4). If normalization succeeds in reducing the concentration differences among the fractions, the ratio among the normalized concentrations in the different fish fractions should approach the unit value. The comparison between the ratios of carcass-to-viscera and fillet-to-viscera concentrations, based both on fresh and dry weight, are reported in Supplemental Data, Table S13. The dry weight normalization was ineffective in reducing both the concentration differences among the fish fractions for any PFASs (i.e., the median values of the ratios did not change if based on fresh or dry weight) and their variability (expressed as relative standard deviation [RSD]; Supplemental Data, Table S13). These results agree with the Fliedner et al. (2018) study that showed normalizing to 26% dry mass as suggested by GD-Biota had a very partial effect in adjusting fillet and whole-fish data for nonlipophilic substances such as PFOS.

Normalization based on proteins could be an effective alternative because it is known that PFAS preferentially bind to proteins (Kelly et al. 2009; Houde et al. 2011). However, total protein contents did not vary much among fish fractions in our dataset (Supplemental Data, Table S7). The PFAS have high affinities only for specific proteins (Ng and Hungerbuhler 2013; Cheng and Ng 2018; Zhong et al. 2019); thus normalization to the total protein content is not likely to improve data variability.

Finally, some studies also suggested that phospholipid binding could play a significant role in tissue distribution of PFAS (Armitage et al. 2012; Droge 2019); for this reason, polar lipid content was determined in fractions of some dissected fish. The highest polar lipid content was measured in viscera (Supplemental Data, Table S7) that also presented the highest PFAS content (Tables 1–3 and Figure 4). The comparisons between the carcass-to-viscera and fillet-to-viscera ratios of

TABLE 4: Correlation between fillet data and whole-body data

		Detection frequency (%)					
Chemical	Species	F %	WB %	*p* value (MK)	Slope	95% CI of slope	Intercept
PFNA	*A. agone*	83	96	0.004	2.28	1.039–3.676	0.07
	L. lota	100	100	0.018	2.29	0.377–3	–0.12
	R. rutilus	0	100	—	—	—	—
PFDA	*A. agone*	100	100	0.0002	2.78	1.602–3.599	0.25
	L. lota	100	100	0.003	2.53	1.691–3.053	–0.56
	R. rutilus	100	100	0.011	1.59	0.751–2.085	0.39
PFUnDA	*A. agone*	96	100	0.0007	3.54	1.781–4.296	0.10
	L. lota	100	100	0.008	2.38	0.987–7	–0.24
	R. rutilus	100	100	0.184	NS	—	—
PFDoDA	*A. agone*	100	100	0.06	NS	—	—
	L. lota	100	100	0.001	2.23	1.972–2.779	–0.34
	R. rutilus	100	100	0.001	1.82	1.444–2.233	0.20
PFOS	*A. agone*	100	100	0.00001	2.19	1.74–2.931	2.93
	L. lota	100	100	0.008	1.61	1.06–2.71	1.44
	R. rutilus	100	100	0.003	1.85	0.94–2.134	–3.04

PFNA = perfluorononanoate; PFDA = perfluorodecanoate; PFUnDA = perfluoroundecanoate; PFDoDA = perfluorododecanoate; PFOS = perfluorooctane sulfonate; MK = Mann-Kendall test; NS = not significant.
A. agone = shad; *L. lota* = burbot; *R. rutilus* = roach.

concentrations, based on fresh weight, and the same ratios normalized to polar lipids are reported in Supplemental Data, Table S14, for PFAS congeners with data above the detection limits. The dataset is rather poor and no clear conclusion can be deduced. In the case of fillet-to-viscera ratio, the ratios for polar lipid-normalized concentrations were similar to those expressed as fresh weight for all the substances (median values ranged from 0.21–0.35) and even an increase of the ratio variability (expressed as RSD) was detected. On the contrary, in carcass-to-viscera ratios the polar lipid normalization improved the comparability between fractions (median values ranged from 0.59–0.79) without a substantial increase in variability (RSD). This suggests that polar lipids might be used as a surrogate for normalization of the viscera and carcass concentrations, at least for PFOS and long-chain PFCAs. As a result of the size limitation of our dataset, further studies are needed to strengthen this conclusion. Furthermore, it is important to underline that neither carcass nor viscera can be considered the ideal matrix to be monitored because they cannot be strictly defined.

Normalization of contaminant concentrations is also used to minimize the natural variability of collected fish at a sampling location. Again, it is suggested that normalization with respect to lipid content and dry weight could be useful to account for this major influence on bioaccumulation in monitoring programs (European Commission 2014).

We have already demonstrated that in the case of PFAS these 2 variables are not appropriate to account for variability among fish fractions; nevertheless, we would like to assess whether this conclusion can also be extended to the normalization of different fish specimens in the same lake. The dataset for the most detected PFAS (PFOS, PFNA, PFDA, PFUnDA, and PFDoDA) was analyzed as a whole, without distinction between species and lakes. The RSD values of fresh weight concentrations in fish fillet (the most populated dataset) ranged from 103 to 167%. Any normalization procedure, if effective, should reduce the total variability of the dataset. Both lipid and dry weight normalization of the concentrations led to an increase in variability, with RSD values ranging from 184 to 227% and from 111 to 244% for lipid or dry-weight normalized concentrations, respectively. The results confirm that neither lipid nor dry weight normalizations of PFAS concentrations have a positive effect on reducing the total variability. This finding is supported by the lack of correlation between the concentrations of PFASs and the fish lipid content or dry weights. Supplemental Data, Figure S4, plots for fillet samples of Lake Lugano are provided as an example.

Currently the only perfluorinated chemical regulated for water quality in the European Union is PFOS. The PFOS EQS_{biota} was derived to protect fish consumers; thus it applies to concentrations in fish meat (fillets). The fillet is more easily analyzed because it is more homogenous, and it yielded generally lower LODs and LOQs than other fish fractions in the present study (Supplemental Data, Table S5). Nevertheless, the fillet was generally the fraction displaying the lowest detection rates, especially for short-chain compounds (Tables 1–3). Carcasses and viscera presented higher concentrations than fillets for all long-chain PFASs in the present study (Figure 4), consistent with previous studies that showed similar distribution patterns (Martin et al. 2003a, 2003b; Peng et al. 2010). Measurements in the fillet would therefore be more appropriate and provided to have a fit for the purpose of LOQ. Nevertheless, it would be more relevant to use whole-body concentrations when the assessment of the risk of secondary poisoning for piscivorous fauna is needed. In this perspective, knowing the relationships between fillet and whole-body concentrations should be very useful. Fliedner et al. recently proposed a simple linear model for extrapolating whole-body concentrations based on measurements in fillets (Fliedner et al. 2018). Their model was based on pool samples of several species

(bream, chub, and perch) from one location in the Danube River. They obtained 2 different slopes: 1.93 when concentrations were normalized according to dry weight fraction and 2.85 when they were not normalized. These are higher than those derived in the present study but included in their confidence intervals, except for burbot (Table 4). Predicted whole-body concentrations of PFOS based on this model (wet wt slope) were correlated to measured whole-body concentrations ($R^2 = 0.75$); however, the slope of the regression between predicted and measured concentrations (0.56 ± 0.05) strongly deviated from one. This model tended to underestimate whole-body PFOS concentrations in more contaminated shad samples, and systematically overestimated whole-body PFOS concentrations in burbot and roach up to 78%. In less contaminated shad samples whole-body concentrations were also overestimated by more than 25%. We therefore do not recommend using this generic equation for predicting PFOS whole-body concentrations for species not considered in Fliedner et al. (2018). Consistent with the above-mentioned ANCOVA results, a global model based on our data did not perform better than that of Fliedner et al. (2018), suggesting that species- and ecosystem-specific models would probably be more relevant.

Comparison with European lakes and North American Great Lakes

The present study provides the first survey of PFAS contamination in lake fish in a wide area covering the northern and southern slopes of the Alps where the largest and deepest European lakes are situated. Lakes are located in densely urbanized subalpine regions characterized by dynamic economic activities including tourism and industries (European Union Statistical Office 2020b). As a large reservoir of freshwater for some of the most important European river basins, subalpine lake ecosystems must be protected from chemical pollution from industrial sources as well as everyday domestic uses. Because of their persistence and bioaccumulation potential, perfluoroalkyl acids are good tracers of the anthropic pressures on the chemical status of these precious freshwater ecosystems. The PFAS concentration data on European lake fish are available mainly for smaller lakes in Northern Europe (Norway and Sweden) with sporadic data from impacted lakes in Germany and The Netherlands (Supplemental Data, Table S15). Compared with other European lakes, our data show that the subalpine lakes are generally in the lowest contamination range for PFOS and the ∑PFAS, in the same order of magnitude as the least-impacted Swedish and Norwegian lakes (Berger et al. 2009; Hansen et al. 2016). The highest PFOS concentrations in European lake fish (hundreds of ng/g wet wt) were measured in the lakes that are impacted by specific sources such as the drainage from neighboring airports (Ahrens et al. 2015; Filipovic et al. 2015; Hansen et al. 2016), the run-off from PFAS-amended soils (Holzer et al. 2011), or wastewater discharges (Schuetze et al. 2010).

The most polluted lakes (Lakes Maggiore, Varese, Geneva, and Lugano) in our study showed average concentrations in fish (∑PFAS from 10.7–28.1 ng/g wet wt) comparable to the least-contaminated Laurentian Great Lakes, Lake Superior and Lake Michigan, located upstream in the West (Stahl et al. 2014; Remucal 2019). The ∑PFASs in lake trout varied widely across the Great Lakes with a consistent spatial gradient that increases from west to east and ranging from 11 ng/g in Lake Superior to 24 ng/g in Lake Michigan, and 46 ng/g in Lake Huron. The highest ∑PFAS concentrations were measured in the farther eastern lakes, Lake Ontario (92 ng/g) and Lake Erie (136 ng/g). On a mass basis, PFOS percentage on the ∑PFAS ranged from 35% in Lake Superior to 64% of PFASs in Lake Huron to 80 to 82% of PFASs in Lakes Erie and Ontario (Remucal 2019). In another study, PFOS was detected in 100% of 157 Great Lakes fish samples from 18 species, with a median of 15 ng/g and a maximum concentration of 80 ng/g in fillets (Stahl et al. 2014), 2 to 3 times higher than our data (median 6.0 ng/g and a maximum of 50.5 ng/g; Table 1).

Sources of PFASs in lakes

The availability of a dataset of 10 lakes from Lake Geneva to Lake Garda, which span about 400 km from northwest to southeast in the Alps, gave us the possibility to study the sources and the transport mechanisms of PFAS in this area.

Together with subalpine deep and shallow lakes, we collected data also from 2 small natural Alpine lakes, Lakes Sassolo Lower and Upper, located in an uninhabited mountainous territory at more than 2000 m of altitude in the Lake Maggiore catchment. The absence of direct sources allowed to estimate the contribution of atmospheric transport to PFAS contamination and to compare data with those collected in remote lakes in the French Alps (Ahrens et al. 2010), in Sweden (Åkerblom et al. 2017), and in the Faroe Islands and Greenland (Bossi et al. 2015; Supplemental Data, Table S15). The PFOS concentrations in fish from the Sassolo Lakes (mean 0.4 ± 0.3; median 0.3; range 0.2–0.8 ng/g wet wt) were close to the mean of pristine Swedish lakes (mean 0.2; range <0.025–0.93 ng/g wet wt; Åkerblom et al. 2017). The median of long-chain PFCAs (C9–C12) in the Sassolo Lakes was approximately 0.7 ng/g wet weight, which is the same as in all examined Swedish lakes (Åkerblom et al. 2017). This value can be considered a continental background level in fish caused by the atmospheric contribution because in remote areas the long-chain PFCAs necessarily originate from oxidative transformation of airborne long-chain fluorotelomer precursors (Schenker et al. 2008; Benskin et al. 2011).

It is interesting to note that in the remote lakes in Sweden, ∑PFAS content decreases with the latitude but the relative ∑LC-PFCA content increases (Åkerblom et al. 2017). A similar trend (i.e., the decrease of total PFASs and the corresponding increase of long-chain PFCAs with respect to PFOS) was also observed in the Great Lakes, moving from east to west as a function of the decrease in industrialization and urbanization (Remucal 2019). Consequently, we studied the use of the ratio between PFOS and long-chain PFCA concentrations in fish (ratio PFOS/∑LC-PFCA) as a proxy of the impact of human activities (Supplemental Data, Table S12). In our study, we added only C9 to C12 PFCA in

the ∑LC-PFCA because longer PFCAs (C13–C14) have not been analyzed in all lakes.

First of all, it should be noted that these ratios were very similar among carcass, liver, and viscera (Supplemental Data, Table S12) and in some lakes also in fillets (e.g., in Lake Geneva 4.9 in fillet, 4.4 in carcass, 5.5 in liver, and 5.5 in viscera but in Lake Maggiore 15.1 in fillet, 8.3 in carcass, and 8.0 in viscera). In the case of remote areas, PFOS/∑LC-PFCA ratios measured in fish fillets from the Sassolo Lakes ranged from 0.4 to 0.8 (with an anomalous value of 9.5). These ratios were ≤0.3 in the liver of fish in the French Alpine lakes (Åkerblom et al. 2017), and most of them were ≤0.5 in the liver of fish caught at the Faroe Islands and Southern Greenland (Bossi et al. 2015; Supplemental Data, Table S15). These results suggest that a ratio PFOS/∑LC-PFCA < 1 in every monitoring tissue could be an indicator of the absence of direct water emission sources.

In general, the ratio PFOS/∑LC-PFCA could be used as a tracer of the distance from the emission sources in remote areas; however, in the urbanized and industrialized areas this ratio is more influenced by the presence of direct sources of PFOS or long-chain PFCAs that are generally different from each other. In the subalpine lakes the median values of the ratio PFOS/∑LC-PFCA for fillets (matrix with the amplest dataset) ranged from 1.9 in Lake Mergozzo to 15.1 in Lake Maggiore.

The main problem in interpreting these data is that the long-chain PFCA releasing points into the environment have not yet been recognized together with the timing of release, given their high persistence and potential transformation from precursors. The investigation—recently carried out by the German Environmental Agency (Wirth et al. 2019) and the European Chemical Agency (2018) as support for preparing the restriction proposal under the European registration, evaluation, authorisation and restriction of chemicals regulation—found no indication that these chemicals are used intentionally in any industrial sector within the European Union. Applications containing PFCAs as impurities were seen to be of low relevance, whereas no direct or indirect uses of these substances could be identified. No manufacturers or users of C9 to C14 PFCAs and only one importer have been identified in the European Union (Wirth et al. 2019). Potential environmental sources of long-chain PFCAs include the breakdown of their fluorotelomer alcohol precursors during wastewater and sewage treatment processes, the oxidation of their precursors in the atmosphere, and the degradation of commercial products containing their precursors (Ellis et al. 2004; Ahrens et al. 2011). These precursors have been detected in various consumer articles and mixtures such as textiles, carpets, upholstery, paper, leather, toner, cleaning agents and carpet care solutions, sealants, floor waxes, paints, and impregnating agents that might be imported into the European Union (European Chemical Agency 2018).

Because long-chain PFCA are ubiquitarian chemical compounds that are present in widespread products and industrial formulations, we tested the possibility to correlate ∑LC-PFCA concentrations in fish with the extent of anthropic pressure in lake catchments, to assess the hypothesis that the main source for these compounds is the release from products used in everyday life.

As an index of the anthropic pressure in the catchment we propose the DEGURBA Index (Degree of Urbanization Index) calculated according to Equation 3 and Supplemental Data, Table S6. The regression between Degree of Urbanization Index and medians of ∑LC-PFCA for the largest lakes was highly significant ($R^2 = 0.941$; $p = 0.0013$; Supplemental Data, Figure S5), suggesting that the emissions are strongly linked to the degree of urbanization of the lake catchment. Even if the regression between Degree of Urbanization Index and PFOS median concentrations was still significant ($R^2 = 0.798$; $p = 0.016$), PFOS experimental data were more scattered and presented higher residuals than the modeled PFOS data. In particular, the highest residual was found for the PFOS median of Lake Maggiore, suggesting that in this catchment there is an additional source other than those derived from the life cycle of consumer products. The ratio of the regression slopes of PFOS and ∑LC-PFCA is 3.4 (±1.3) and this range could be considered an indication of the typical ratio PFOS/∑LC-PFCA of urbanized areas, when no specific industrial sources are present.

CONCLUSIONS

Monitoring the accumulation of persistent substances such as long-chain perfluoroalkyl acids in aquatic biota should be the method of choice in large and deep lakes to assess their quality status. Fish monitoring allows overcoming the problems of the high water dilution of the contaminants in these environments and gives a spatially and temporally integrated picture of the contamination. On the other hand, this tool raises some controversial issues in terms of procedure harmonization and data evaluation. For these reasons, monitoring protocols must take into account variabilities in water bodies' characteristics as well as in the accumulation mechanisms of contaminants.

Based on these considerations, we gathered data from different monitoring programs that made it possible to discuss some technical aspects of biota monitoring and, at the same time, to obtain the first large survey of PFASs in European lakes of the subalpine region.

In particular, our approach tested the possibility to harmonize the monitoring protocols, especially in terms of fish species, seasonality, and fish matrix to be analyzed. The natural variability of fish should be minimized when designing and implementing a fish-sampling campaign, as far as possible, by selecting the sampling period by applying data normalization. Our results suggest that the sampling season is not critical for PFAS and that neither lipid nor dry weight normalizations of PFAS concentrations has a positive effect on reducing the total variability both for PFOS and long-chain PFCAs.

The data comparison and analysis showed that the PFAS contamination in lake fish is generally correlated with the degree of the urbanization of the lake catchment; nevertheless, it is sometimes difficult to compare absolute concentrations in lake fish because the lake hydro-morphological characteristics such as volume and residence time have a substantial role in

determining the chemical concentrations of persistent and mobile contaminants. In fact, we can find that some lowland lakes (Garda, Iseo, Como, and Maggiore) have the same concentrations in fish of long-chain PFCAs as in high-altitude lakes. Long-chain PFCA do not have any dominant and specific industrial or human activity source; however, they are more related to everyday use of products/urbanization and are most probably transported to remote areas as airborne precursors. On the contrary, PFOS might have specific sources, such as fire-training activity, never linked to the catchment urbanization. The use of ratio PFOS/∑LC-PFCA can help to identify remote areas where the only source is atmospheric (ratio is usually <1), whereas it can give an idea of the relative weights of sources of PFOS and long-chain PFCAs in lakes sited in urbanized areas.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at https://doi.org/10.1002/etc.4815.

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Author Contributions statement—S. Valsecchi, M. Babut, M. Mazzoni and S. Polesello conceived the study design. M. Mazzoni, S. Pascariello, B. De Felice, and B. Veyrand carried out the investigation (sampling design, sample treatments and methodologies and chemical analysis). C. Ferrario, S. Pascariello, S. Valsecchi, M. Babut, M. Mazzoni and S. Polesello performed data curation and statistical analysis. S. Valsecchi, M. Babut and S. Polesello wrote manuscript. P. Marchand and R. Bettinetti reviewed and edited the manuscript.

Data Availability Statement—Data obtained during this study are accessible from the corresponding author (valsecchi@irsa.cnr.it and marc.babut@inrae.fr).

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