Fish consumption as a source of human exposure to perfluorinated alkyl substances in Italy: Analysis of two edible fish from Lake Maggiore

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ABSTRACT

Extensive screening analyses of perfluorooctane sulfonate and related perfluorinated compounds (PFCs) in biota samples from all over the world have identified PFCs as global pollutants and have shown their bioaccumulation into higher trophic levels in the food chain.

Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) are environmental contaminants belonging to a chemical group known as perfluorinated compounds. PFOS and PFOA are very persistent in the environment and bioaccumulate in humans. They are potential reproductive and developmental toxicants and are considered to be emerging endocrine disrupters.

The United States Environmental Protection Agency (US EPA) considers both compounds to be carcinogenic and the European Food Safety Authority (EFSA) recently pointed out that they are associated with adverse health effects. Diet is considered the main source of exposure to PFCs, which have been found more frequently in fish and other seafood, compared to other food groups. In fact, aquatic ecosystems represent the final reservoir for PFCs due to their great affinity for sedimentary and living organic matter. In these systems, measured levels of persistent organic pollutants (POPs) could increase along the trophic web, ultimately affecting humans that consume aquatic species.

In this study, PFOS and PFOA was detected by LC-MS/MS in muscle samples of *Coregonus lavaretus (*European whitefish) and *Perca fluviatilis* (European perch) collected from Lake Maggiore, a large lake located on the south side of the Italian Alps. PFOA was not found in any of the investigated samples above the limit of quantitation of 0.50 ng g^{-1} fresh weight (fw), whereas PFOS was detected in all 90 samples with concentrations of up to 46.0 ng g^{-1} fw. Mean concentrations were 22.2 ng g^{-1} fw in *Perca fluviatilis* and 20.0 ng g^{-1} fw in *Coregonus lavaretus.*

Comparison of our results with literature data on PFOS intake suggested that fish from Lake Maggiore may be a significant source of dietary PFOS exposure, even if the reported values were lower than the Total Daily Intake (TDI) proposed by EFSA.

Keywords: PFOS; fish, Lake Maggiore, Liquid chromatography–tandem mass spectrometry (LC–MS/MS)

1. Introduction

Perfluorinated (fully fluorinated) organic compounds, such as perfluorooctane sulfonate (PFOS), represent a class of compounds showing high thermal, chemical and biological inertness. They can be widely found in the environment, primarily resulting from anthropogenic sources.

PFOS and a number of related perfluorinated organic sulfonates have been found to be present in fish, birds and mammals. In contrast to other fluorinated compounds that are partially degradable, PFCs are very persistent both to abiotic and biotic degradation processes. PFCs tend to bioaccumulate, and PFOS is also a suspected carcinogen and a candidate POP under the Stockholm Convention (EFSA, 2008; Paul *et al.*, 2009).

PFOS meets the criteria for being characterized as a persistent organic pollutant (POP), and restrictions for PFOS and other perfluoroalkylsulfonate (PFAS) commercialization were therefore fixed from 2001 to 2004 by the US EPA (2003), and in 2006 by the 2006/122/CE Directive.

In addition, in 2008, the EFSA Scientific Panel on Contaminants in the Food Chain (CONTAM Panel) acknowledged the limitations of the dietary exposure assessment, and recommended that more occurrence data of PFASs in different foodstuffs, along with biomonitoring data from humans should be collected, particularly with respect to monitoring trends in exposure.

PFOS has been shown to bioaccumulate in fish and in experimental animals, with exposure to PFOS resulting in hepatotoxicity and increased mortality.

Toxicology studies show that PFOS and PFOA are readily absorbed after oral exposure and accumulate primarily in the serum, kidney, and liver. No further metabolism is expected (EFSA 2008). PFOS and PFOA have a long half-life of about 4 years in humans. This continued exposure could increase body burdens to levels that would result in adverse outcomes (ATSDR 2009).

Acute- and intermediate- duration oral studies in rodents have raised concerns about potential developmental, reproductive, and other systemic effects of PFOS and PFOA (ATSDR 2009) while the ingestion of PFOA-contaminated water was found to cause adverse effects on mammary gland development in mice (Post et al. 2012). Both compounds are suspected endocrine disrupter, affecting fertility and development and are likely to have an analogous mode of actions (EFSA, 2008). PFOS and PFOA have a high affinity for binding to B-lipoproteins and liver fatty acid-binding protein. Several studies have shown that these compounds can interfere with fatty acid metabolism and may deregulate metabolism of lipids and lipoproteins (EFSA 2008). Some toxicological and epidemiological evidence indicate a link between PFOS/PFOA exposure and reduced fertility (Governini et al., 2011).

Preliminary information indicate increasing levels of these substances in the environment within the food chain as well as in the general human population, there is therefore a crucial need to improve the database to assess the potential risks associated with human exposure to PFCs (EFSA 2008).

Most biota monitoring studies have so far focused either on liver, as a target organ for PFC accumulation, or on the blood or whole body homogenates (Houde *et al.*, 2006).

Fish are environmental bioindicators as they are essential components of various ecosystems (i.e. sea, rivers, lakes, etc.) as well as representing an important food source for humans.

Reports on PFC levels in edible fish muscle (Kannan *et al.*, 2005; Gulkowska *et al.*, 2006; Gruber *et al.*, 2007; Ericson *et al.*, 2008) have shown that perfluorooctane sulfonate (PFOS) is usually the prevailing PFAS, with concentrations in the range of <1 ng g^{-1} fresh weight (fw) to >100 ng g^{-1} fw, depending on fish species and sampling location (Berger *et al.*, 2009). Very little is known about PFAS levels in commercial fish species in Italy, and in particular on freshwater fish.

Lakes play a vital role in the landscape and ecosystems, as well as being central to recreational activities such as swimming and fishing. Given the high population density in areas surrounding lakes, and their year-round accessibility,

these water bodies are generally more vulnerable to contamination, especially those with the nearby presence of intensive industrial and commercial activities. Although natural processes often provide an effective means of removing many micro-contaminants, some effluent-derived contaminants seem to be resistant to biodegradation. Discharge of wastewater effluents into surface waters and drinking water aquifers, and subsequent re-use of the water, is becoming more common throughout the world, because the continuing growth of the human population creates a corresponding increase in the demand for fresh water supply.

Investigations on the PFOS/PFOA levels in fish muscle in Lake Maggiore, a North West Italian Lake, have not previously been carried out.

This lake, the second largest in Italy, has suffered from mercury and DDT contamination in the past and the quality of its water decreased substantially from the 1950s to the 1970s because of the effects of domestic and industrial contamination. Although nutrients and some non-polar contaminants have attracted widespread attention, polar substances have been less investigated. The region of Lake Maggiore can be characterized as a mountainous and tourist-affected area with little agricultural land use; there are many developed smaller urban centers, and some industrial impact on the west side (in Piedmont). Lake Maggiore receives municipal, agricultural, and industrial discharges, both directly and via its tributary rivers. The lake water is used in some areas for production of drinking water. It is, therefore, necessary to identify and quantify the chemicals likely to be present in recycled water, evaluate their potential effects on humans and aquatic ecosystems, and assess approaches for minimizing their release (Loos 2007).

The objective of this study was to investigate the occurrence of emerging polar anthropogenic contaminants (PFOS, PFOA) in two species of commercial fish, *Coregonus lavaretus* and *Perca fluviatilis,* from Lake Maggiore, in order to establish the contamination levels and to assess human dietary exposure through fish consumption.

2. Materials and methods

2.1 Study species

The European whitefish (*Coregonus lavaretus*), widely distributed in the freshwaters of Northern Europe and introduced into the major lakes of Northern Italy, are also present in the lakes of North and Central Italy, mainly as a result of stock enhancement practices (Orban *et al.* 2006). *C. lavaretus*, a stenothermic fish feeding mainly on zooplankton and benthos. This species lives in clear, cold and well-oxygenated waters, and is characterized by a rapid growth reaching sexual maturity in the second year of life, with a size of 30 cm circa. As a food source, whitefish is an appreciated and valuable freshwater fish species.

The European perch (*Perca fluviatilis* L.), a predatory freshwater fish species feeding on invertebrates and fish, was originally confined to the temperate waters of the northern hemisphere, mainly Europe and North America. In Italy, in the North-East sector of the Po basin, the presence of perch has been documented for centuries, while in Central Italy, this species is mainly present in the lakes of the Umbria and Latium regions (Orban *et al.* 2007). Within 2–3 years,

when the body length is 15–25 cm, the perch reaches sexual maturity. The average perch length is 20–25 cm, but it may grow up to 45 cm. On the other hand may be subjected to dwarfism when living in high density populations.

2.2 Fish collection

Fish were captured by gillnetting in Autumn 2012. The study included 40 specimens of European perch (*Perca fluviatilis*) and 50 specimens of European whitefish (*Coregonus lavaretus*).

Sampling locations (Figure 1) were selected according to the ecological needs and the seasonal distribution of the two species. In fact, the European perch specimens were caught in the mouth of the River Tresa and close to the ground at a water depth of approximately 25 m (46.00006, 8.72228), while the individuals of European whitefish were captured in the town of Luino (Province of Varese) in open water, at a water depth of approximately 30 m (46.01419, 8.74468).

Fish were captured in agreement with the animal welfare legislation procedure. Perch specimens (10 males and 30 females) were collected at sizes of 16.5 –29.5 ± 0.5 cm, with weights ranging from 53 – 371 ± 1 g. European whitefish specimens (20 males and 30 females) were collected at sizes of 26.0 – 32.0 ± 0.5 cm, with weights ranging from 157 to 307 ± 1 g. Individual identification numbers were assigned to each sample .

Captured fish were preserved on ice and transported to the laboratory. Samples were sexed and dissected to obtain muscle tissues, which were immediately frozen and stored at −20 °C.

2.3 Reagents and Analytical methods

Certified standards of PFOS (96%) and PFOA (98%) were purchased from Sigma Aldrich (Milan, Iyaly). The internal standard (PFOA 13C8, 99%) was purchased from Cambridge Isotope Laboratories (Highwood Drive Tewksbury, MA, USA).

Ammonium acetate, 37% hydrochloric acid, and sodium acetate were purchased from Sigma Aldrich. Sodium hydroxide was supplied by Carlo Erba (Milan, Italy),while 33% ammonium hydroxide was from RDH (Seelze, Germany). Methanol, with a degree of purity suitable for HPLC-MS/MS analysis, was purchased from Sigma Aldrich, andultrapure water was produced by Maxima UltraPure Water (Thermo Scientific, MA, USA).

Purification of samples was performed by Oasis Wax SPE columns 6 cc cartridge 150 mg, 30 µg of Waters (MIlfrod, MA, USA).

Stock solutions of each analyte were prepared by dissolving the powder in methanol.

Diluted solutions for determining the matrix calibration line were prepared in methanol. The internal standard was also diluted in methanol.

Fish muscle (2.5 g) was homogenized with 2.5 ml of sodium hydroxide using Ultraturrax homogenizer (IKA, Staufen, Germany) , after addition of IS (13C8PFOA). Methanol (10 mL) was added to each homogenate. The shacked extract was centrifuged and purified using Oasis Wax SPE columns, followed by conditioning with 4 mL of methanol and 4 mL of water. The cartridge-purified extract, was then washed with 4 ml of 25 mM acetate buffer (pH 4-5), followed by

8 mL of methanol. Analytes were eluted with 1 mL of 2% ammonium hydroxide in methanol. The solvent was dried by evaporation by means of a nitrogen stream. The residue was reconstituted in the mobile phase and subjected to LC-MS/MS analysis.

LC-MS/MS analysis was performed by an Agilent HPLC 1100 procedure (Agilent Technologies, Palo Alto, CA, USA). Chromatographic separation was obtained by a reversed-phase Phenomenex Synergi Fusion column (150 x 2 mm, 4 μm) coupled to a guard column (Phenomenex Security Guard 4.0 x 2.0 mm). Eluents used were 20 mM aqueous ammonium acetate (eluent A) and 2 mM ammonium acetate in methanol (eluent B), at a flow-rate of 350 μl/min. The elution gradient consisted of the following steps: from 0 to 5 min, A at 50%; from 5 to 7 min, A at 100%; at 8 min, A% at 50; from 8 to 11 min, A% at 50%. The total run time for each injection was 11 min, with a 20 μl injection volume. Mass spectral analyses were performed using an Applied Biosystems API 4000 triple quadruple mass spectrometer (Applied Biosystems Sciex, Ontario, Canada) operating in electrospray ionization (ESI)negatiove ion mode. Detection and quantification of the two molecules were performed by selected reaction monitoring (SRM), as shown in Table 1.

The method limit of quantification (LOQ) was 0.50 ng g^{-1} for PFOA and 0.70 ng g^{-1} for PFOS, with a recovery rate of 99-102% and 96-108%, respectively. Commission Recommendation 2010/161 recommends to the Member States to apply methods of analysis with recovery rates ideally in the range of 70-120 %.

All analyses were carried out in duplicate. To check the purity of the reagents and any contamination, "blanks" were analyzed for each calibration run, using the same procedure.

2.3 Statistical analysis

Statistical analyses were performed in SPSS v. 20 (SPSS Inc., Chicago, IL, U.S.A.) for Macintosh. The analytical data (PFOS levels) were normally distributed in both species, either considering sexes together or separately (Kolmogorov–Smirnov test, $p > 0.05$).Initially, a linear regression analysis was performed to study the associations between body weight and PFOS concentrations in both species. Subsequently, differences in mean concentrations of PFOS between species and sexes were tested using a Student's t-test (all p-values were two-tailed)..

3. Results and discussion

3.1. PFC concentrations in fish and PFOS levels in relation to the data from the literature

Several studies have indicated that PFC concentrations are higher in fish caught from fresh water compared to marine water and in particular the PFC contamination levels of fish caught in waters affected by anthropogenic pollution is generally higher than concentrations in fish from open oceans (Berger et al., 2009; Schuetze et al., 2010). In the last EFSA Opinion concerning PFCs (2012), a constantly higher mean concentration in fish from fresh water was demonstrated, while diadromous fish had slightly higher mean concentrations, tending to be more similar to the marine fish. The highest contributors to dietary PFOS exposure across all age classes examined were 'Fish and other

174 seafood'(50 to 80 %). Among PFCs, PFOS had the highest mean concentrations in all three fish categories and in the
175 food group 'Fish and other seafood', the concentrations found in fish meat ranged from 0.04 to 211 ng g^{-1}. In the
176 present study, PFOS mean values were 22.20 ng g^{-1} fw in *Perca fluviatilis* and 19.98 ng g^{-1} fw in *Coregonus lavaretus*,
177 while PFOA values were less than the limit of quantitation in all samples. All 90 samples had detectable levels of PFOS
178 (> LOQ) and concentrations ranged from 5.0 to 45.8 ng g^{-1} fw (Table 2).
179 The presence of PFCs in Lake Maggiore, and in drinking water produced from the lake, was previously observed by
180 Loos (2007), who showed that these compounds were relatively equally distributed in the lake water. In fact, PFOS was
181 detected at a concentration of 7.8 ng g^{-1} fw (range 7.2-8.6 ng g^{-1} fw) while PFOA was reported at a lower concentration
182 2.4 ng g^{-1} fw (range (1.8-2.9 ng g^{-1} fw), while no biota data were reported. Even if the fate properties of PFOA are
183 similar to those of PFOS, and once in the environment they both are extremely persistent and not known to undergo
184 significant further abiotic or biotic degradation, the bio accumulation pattern in fish seems to differ. In fact, PFOS
185 exhibits a higher tendency to bind to organic matter and bioaccumulate compared to PFOA, due to its longer
186 perfluoroalkyl chain length (Conder et al. 2010). Moreover, PFOS has been shown to bioaccumulate and biomagnify in
187 wildlife species such as fish and piscivorous birds and is the only PFC that has been shown to accumulate to levels of
188 concern in fish tissue (EFSA 2008). Because PFOS is both hydrophobic and lipophobic it does not follow the typical
189 pattern of partitioning into fatty tissues followed by accumulation, the typical pattern of many persistent organic
190 pollutants. Instead, it binds to proteins in the plasma and, as a result, is present in highly perfused tissues such as the
191 liver and kidneys rather than lipid tissue. Therefore, the mechanism of bioaccumulation likely differs from most other
192 bioaccumulative chemicals (UNEP, 2006). However, monitoring data from top predators at various locations show
193 highly elevated levels of PFOS and demonstrate the substantial bioaccumulation and biomagnification (BMF)
194 properties of PFOS. Monitoring studies and field studies indicate that biomagnification in various terrestrial and marine
195 mammals occur (Bossi et al., 2005a,b).
196 Most international studies on PFOS levels in fish have been focused on liver, blood, or whole body homogenates as
197 analytical matrices, and only a few reports on levels in fish muscle are available for comparison with our data (Hoff *et*
198 *al.*, 2003; Järnberg and Holmström, 2003; Kannan *et al.*, 2005; Gulkowska *et al.*, 2006; Tittlemier *et al.*, 2007; Ericson
199 *et al.*, 2008).
200 A Swedish study of PFOS and PFOA in perch from waters without local pollution reported low PFOS levels in fish
201 muscle tissue (1–2 ng g^{-1} fw), (Järnberg and Holmström, 2003). Higher levels were found in perch caught in Lake
202 Mälaren (20–44 ng g^{-1} fw), an important lake for both commercial and recreational fishing, situated in one of the most
203 densely populated areas in Sweden. PFOA levels were, however, below the method detection limit (MDL) of 0.5 ng g^{-1}
204 fw in all samples (Järnberg and Holmström, 2003). These data are comparable to ours that were obtained in Lake
205 Maggiore, where PFOS levels in perch ranged from 11 to 46. No data are available in the literature about European

whitefish PFC levels. The concentrations of PFOS in the present study were higher than those reported in fish from other areas in different wild fish species from the Mediterranean Sea (Brambilla et al., 2009, Nania et al., 2009). Brambilla and coworkers analyzed wild and farmed fish from different areas in the Mediterranean and reported concentrations in wild fish ranging from 0.09 to 5.96 ng g^{-1} w.w. Nania and coworkers analysed fish samples included pelagic fish and benthonic fish caught in the Mediterranean Sea, reporting PFOS mean values of 4 and 13 ng g^{-1} w.w respectively. Benthonic fish showed PCFs levels, on average, higher than pelagic fish and this could be explained by the fact that benthonic fish can absorb contaminants both from water and from sandy and muddy sediments (Berger et al., 2004). From Nania results emerged the presence of few fish that showed an extremely high contamination by PFOS, maybe linked to ‘‘dot-like” pollutants release. In our findings, analytical data showed a normal distribution for PFOS in both species, either considering sexes together or separately.

3.2 Intraspecies and between-species comparisons

Gender related length, weight and PFOS levels in fish, are shown in Table 2. In European whitefish length and weight were not affected by gender, while highest PFOS levels were found in female specimens (42.3 ng g^{-1} fw), although mean and median PFOS values were comparable in both sexes.

In perch, highest PFOS levels (45.8 ng g^{-1} fw) were still found in females with an average length and weight of 29.5 cm and 371 g, respectively. However, mean and median values were comparable in both sexes. In our sample, there was no correlation (R = 0.016) between fish weight and PFOS concentration in the muscle (Figure 2).

The Student’s t-test showed that there was no significant difference in the mean concentration of POFS, neither between the two species (*Coregonus lavaretus* = 50, *Perca fluviatilis* = 40, t = 1.430, df = 88, p = 0.154), nor between sexes (*Coregonus lavaretus*: males = 30, females = 20, t = - 0.735, df = 48, p = 0.466; *Perca fluviatilis*: males = 30, females = 10, t = 0.79, df = 38, p = 0.938). Our data confirmed previous findings (Giesy and Kannan, 2005), underlining that the variations in concentrations of perfluorochemicals found between sexes and among different age groups are not statistically significant.

3.3. Fish as a source of human exposure to PFOS

Diet, particularly consumption of fish and fishery products, is known to be a source of exposure to PFOS, PFOA, and other PFCs (Ericson et al., 2008; Guruge et al., 2008; Nania et al., 2009; Clarke et al., 2010). Public health concern regarding PFASs was raised after several studies indicated that PFOS and PFOA are present in the environment, including within the human body (EFSA 2012). Several adverse health effects, e.g. hepatotoxicity, developmental toxicity, neurobehavioral toxicity, immunotoxicity, reproductive toxicity, lung toxicity, hormonal effects, as well as a weak genotoxic and carcinogenic potential have been demonstrated in experimental studies in animals (Lau et al., 2006; Zhang et al., 2009; Pinkas et al., 2010). Recently, an epidemiological study performed on a cohort of children in the Faroe Islands indicated that high exposure to PFASs was associated with reduced humoral immune response to

238 immunizations in children (Grandjean et al., 2012). Moreover, the Italian project PREVIENI, funded by the Italian
239 Environment Ministry, aims to link environment and human health through the investigation of exposure to selected
240 endocrine disrupters (EDs) and associated biomarkers related to human infertility conditions. Preliminary results related
241 to the metropolitan area indicate that subjects affected by infertility factors tend to have both higher PFOS levels and
242 higher gene expression of specific nuclear receptors (La Rocca et al., 2012).
243 The Scientific Panel on Contaminants in the Food Chain (CONTAM) identified 30 ng g^{-1} body weight (b.w.) per day as
244 the lowest no-observed-adverse-effect level (NOAEL) (effect: changes in lipids and thyroid hormones) to derive a
245 provisional TDI of 150 ng kg^{-1} b.w. per day by applying an overall uncertainty factor of 200 to the NOAEL (EFSA,
246 2008, Schuetze et al., 2010).
247 In an adult consumer with a body weight of 60 kg, this value is reached when consuming fish that contains 30 ng g^{-1} of
248 PFOS, considering a consumption of 300 g fish per day. We estimated human exposure from fish consumption by
249 calculating the Estimated Human Daily Intake (EHDI), as follows:
250 EHDI = (C X DC)/BW
251 where C is the contaminant mean concentration, DC indicates the daily fish consumption for the Italian population, as
252 reported by the National Research Institute for Food and Nutrition (Leclercq et al., 2009), and BW is the human body
253 weight (60 kg).
254 The consumption figures used were the 50th and 95th percentile intakes for the total population in consumers of all
255 ages; we obtained a value of 11.9 ng kg^{-1} bw day and 54.39 ng kg^{-1} bw day respectively. Comparing our EHDI with
256 the Tolerable Daily Intake (TDI) established for PFOS (150 ng kg) we can conclude that the intake related to fish
257 consumption from Lake Maggiore is below the tolerable daily intake (TDI).
258 However, other food and sources other than food may contribute to the total human exposure; than particularly for high
259 fish consumers the intake from fish consumption may constitute a considerable contribute to the total daily intake of
260 PFOS.

261 **4. Conclusions**

262 Fish caught in polluted freshwater systems can be a significant source of dietary human PFOS exposure, and may
263 continue to be for many years or decades to come; however the occurrence of suspected carcinogens, such as PFOS, in
264 food for human consumption should not be tolerated, even at low levels. This study has contributed to the knowledge of
265 PFOS levels in fish from an important Italian lake, and whilst our biomonitoring results did not show a particularly
266 alarming level of pollution by PFCs, the PFOS contamination levels and the resulting consumer's intake were mostly
267 higher than those found in similar European monitoring. Then, measures should therefore be taken to reduce the
268 consumption of these damaging substances by humans.
269

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Table 1.
MS experimental condition of PFOA and PFOS and internal standard

Analyte	Parent ion	Product Ion	Decluring Potential (V)	Collision Energy (V)
PFOA	412.7	368.9	-61	-14
		169.0		-26
		219.0		-22
PFOS	498.5	80.1	-50	-94
		99.0		-72
		169.0		-51
Internal standard	420.7	376.0	-32	-14

Table 2
Concentrations of PFOS in fish muscle from Lake Maggiore

Coregonis lavaretus

	PFOS (ng g-1)		**length** (cm)		**weight** (g)	
	male	female	male	female	male	female
min	9.4	5.0	26.0	27.5	157.0	195.0
max	29.5	42.3	31.5	32.0	307.0	306.0
mean	19.1	20.6	29.1	30.0	2258	243.5
median	19.0	19.0	29.3	30.0	229.0	238.5

Perca fluviatilis

	PFOS (ng g-1)		**length** (cm)		**weight** (g)	
	male	female	male	female	male	female
min	11.0	11.3	16.5	18.0	53.0	65.0
max	31.8	45.8	22.0	29.5	126.0	371.0
mean	22.4	22.1	20.6	21.8	101.4	120.3
median	20.9	20.5	21.0	21.5	108.0	109.5

Table 3

Length and weight versus gender in *Coregonis lavaretus*

	Length (cm)		Weight (g)	
	male	female	male	female
Min.	26.0	27.5	157.0	195.0
Max.	31.5	32.0	307.0	306.0
Mean	29.1	30.0	225.8	243.5
Median	29.2	30.0	229.0	238.5

Table 4

Length and weight versus gender in *Perca fluviatilis*

	Length (cm)		Weight (g)	
	male	female	male	female
Min.	16.5	18.0	53.0	65.0
Max.	22.0	29.5	126.0	371.0
Mean	20.5	21.8	101.4	120.3
Median	21.0	21.5	108.0	109.5

Swiss
Confederation

2

Luino

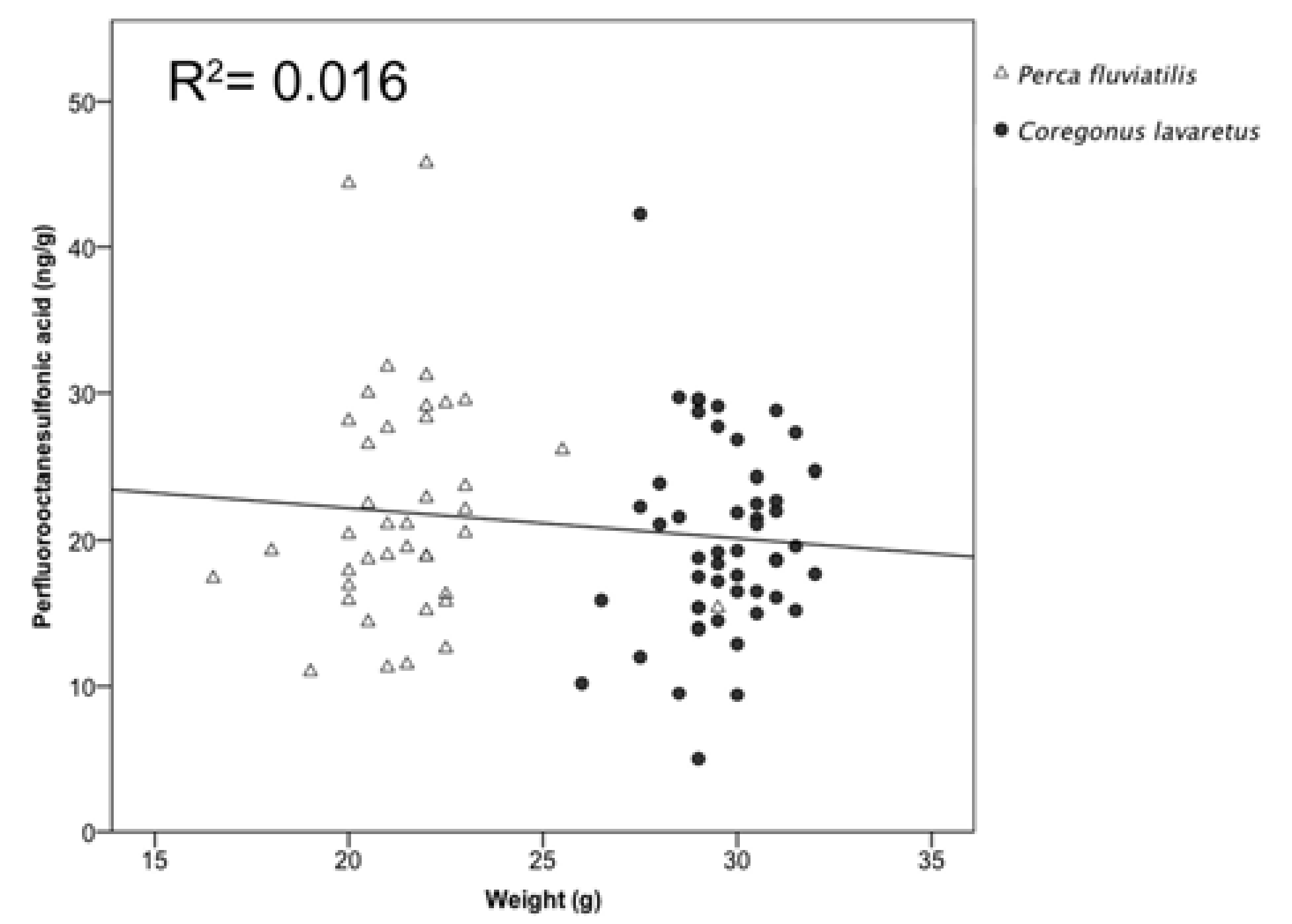
Tresa

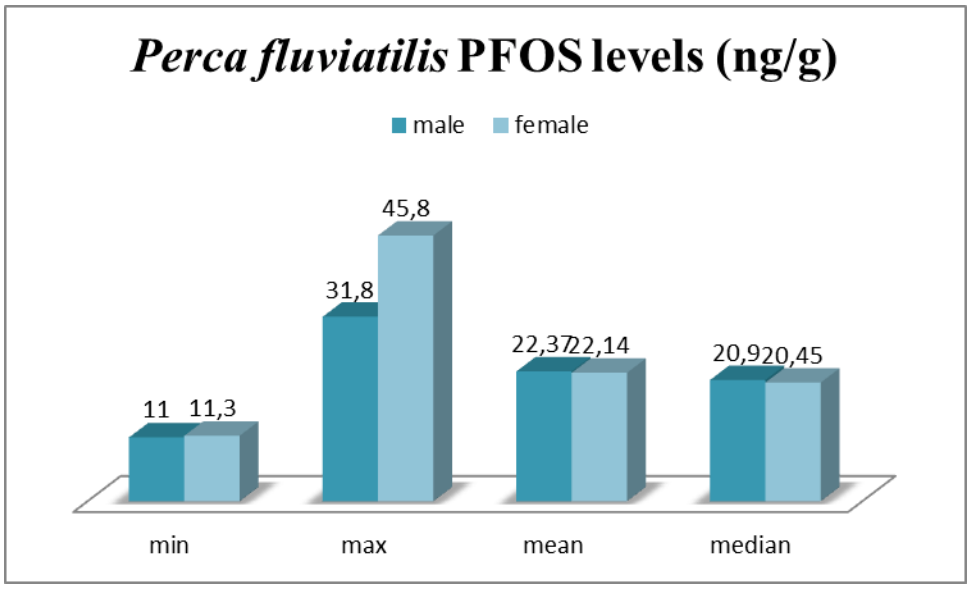
1

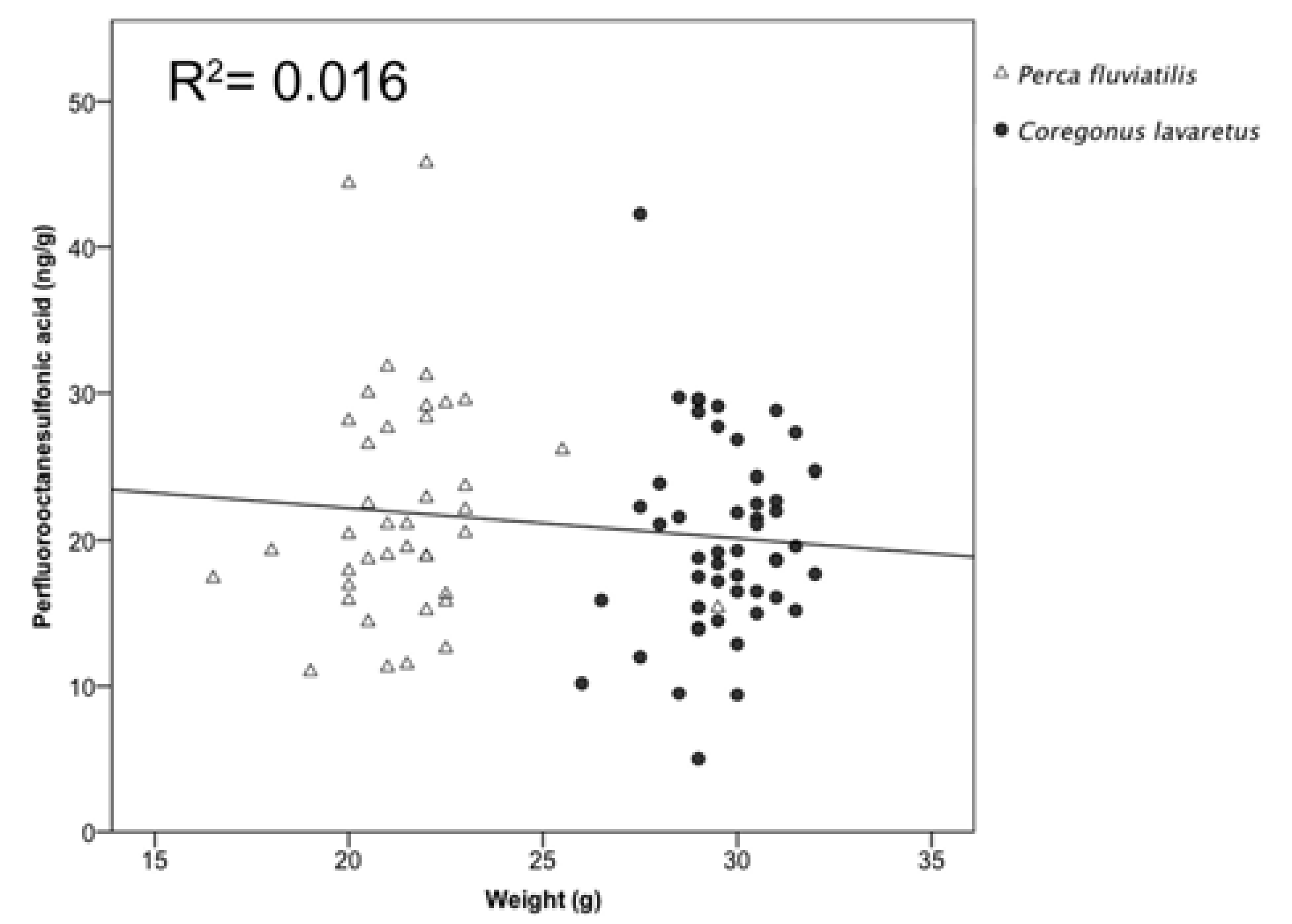
Italy

- 372 m

Lake Maggiore


R^2= 0.016
Perca fluviatilis
Coregonus lavaretus
Perfluorooctanesulfonic acid (ng/g)
0
10
20
30
40
50
15
20
25
30
35
Weight (g)


Perca fluviatilis PFOS levels (ng/g)
male
female
11
11,3
31,8
45,8
22,37
22,14
20,9
20,45
min
max
mean
median


R^2= 0.016
Perca fluviatilis
Coregonus lavaretus
Perfluorooctanesulfonic acid (ng/g)
0
10
20
30
40
50
15
20
25
30
35
Weight (g)