Perfluorinated compounds (PFCs) in fish from Wisconsin's major rivers and Great Lakes

Meghan C.W. Williams, Candy S. Schrank
Wisconsin Department of Natural Resources
101 S Webster Street
Madison WI 53707

Abstract — The Wisconsin Department of Natural Resources (WDNR) has been tracking bioaccumulating pollutants in fish that are consumed by wildlife, anglers, and anglers' families since the 1970s. Beginning in 2006, this effort has included quantifying levels of perfluorinated compounds (PFCs) in Wisconsin fish from the Great Lakes and major river systems. The WDNR also has access to PFC data from fish collected as part of the United States Environmental Protection Agency's 2010 National Coastal Condition Assessment/Great Lakes Human Health Fish Tissue Study. This report summarizes the concentrations of PFCs found in 28 fish species from 7 river systems and Lakes Michigan and Superior, and explores the factors affecting PFC concentrations in fish fillets. PFC contamination was found to be spatially heterogeneous, with perfluorooctane sulfonate (PFOS) present in highest concentrations and present in the highest number of samples compared to other PFCs. PFCs in fish sampled from the Great Lakes were generally lower than those sampled from riverine locations, particularly the Mississippi River, suggesting that proximity to a PFC source is an important factor affecting concentrations. Advisory concentration ranges formulated by the Minnesota Department of Health were used evaluate PFOS concentrations in Wisconsin fish. PFOS levels in most fish from most locations did not supersede Wisconsin's general statewide advisories or advice already in place due to polychlorinated biphenyl (PCB) concentrations, although there are species from some Mississippi River locations where exceptions to general statewide advice are currently provided due to PFOS. We suggest continued monitoring of PFCs in Wisconsin fish, particularly in areas of known contamination or use.

Perfluorinated compounds (PFCs) are a group of man-made chemicals which are used for a variety of consumer and industrial purposes. In use since the 1950s (Lindstrom et al. 2011), PFCs function as stain–, oil–, and water–repellants for fabrics, carpet, cookware, and paper products, and are a component of industrial fire-fighting foams (Renner 2001; Prevedouros et al. 2006). Because of their widespread production and use (Lau et al. 2007), and because PFCs are formulated to be extremely-heat stable (Bhavsar et al. 2014), measurable concentrations of PFCs have been found in in nearly every corner of the world (Giesy & Kannan 2001; Ahrens 2011).

In the United States, commercial formulations of PFCs were produced by many companies, including Arkema, Asahi, Ciba, Clariant, Daikin, DuPont, 3M/Dyneon, and Solvay Solexis (Betts 2007). Commercial formulations contained many different PFC types having varying carbon chain lengths (Prevedouros et al. 2006) and functional groups. PFCs' ability to bioaccumulate is greater if the carbon chain length is greater than 7, and those containing a sulfonate (perfluoroalkyl sulfonates or PFSAs) tend to be more bioaccumulative than those with a carboxylate functional group (PFCAs) even when they have the same chain length (Murphy et al. 2012). Unlike other persistent organic pollutants such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs), PFCs are not lipophilic. They instead bind to protein, particularly in the liver and in blood (Jones et al. 2003; Consoer et al. 2014), meaning that PFC accumulation patterns and factors are dissimilar to PCBs and PBDEs.

The widespread nature of PFC contamination is particularly troubling given the wide array of negative health effects that are associated with their exposure. Between 2005 and 2013, an epidemiological study called the C8 Health Project was conducted to determine whether exposure to perfluorooctanoate (PFOA) was associated with adverse health outcomes in a highly exposed population (Frisbee et al. 2009). The C8 study found that cancers (kidney, testicular, and thyroid), pregnancy-induced hypertension, and ulcerative colitis were linked to high levels of PFOA or perfluorooctanesulfonate (PFOS) in the blood of study participants (Barry et al. 2013; Darrow et al. 2013; Steenland et al. 2013; Watkins et al. 2013; Stahl et al. 2014; Watkins et al. 2014). PFOA has been additionally linked to cardiac problems (Shankar et al. 2012) and cancer (Christensen et al. 2016) in adults. Exposure to PFOS is tentatively associated with immune problems (Grandjean et al. 2012) and low birth weight (Apelberg et al. 2007) in children. Although other adverse effects have been

documented using animal models (Murphy et al. 2012), it is difficult to determine whether these effects will also occur in humans.

As a result of the previously discussed evidence pointing towards PFCs' harmful health effects and widespread global presence, manufacturers began working with the United States Environmental Protection Agency (USEPA) to phase out PFC production. 3M was the primary manufacturer of PFOS and voluntarily phased out its use in 2002 (Lindstrom et al. 2011). Other companies agreed to reduce PFOA in their products by 95% by 2010 and completely eliminate their use by 2015 (Betts 2007).

Although phasing out PFC manufacture may eventually result in decreasing fish tissue concentrations (similar to what has been documented with PCBs; Rasmussen et al. 2014), fish presently represent a major PFC exposure route for humans (Jain 2014). Potential exposure due to fish consumption is a threat to Wisconsin anglers and their families, as several studies have demonstrated that PFCs can accumulate in freshwater fish to concentrations that pose a threat to human health (Martin et al. 2004; Ye et al. 2008; Delinsky et al. 2009; Xiao et al. 2013; Stahl et al. 2014).

In order to asses the threat of PFC exposure to those who consume fish caught in Wisconsin waters, we present here PFC concentrations measured in fillets of freshwater fish collected by the Wisconsin Department of Natural Resources (WDNR) and the USEPA from Wisconsin's major river systems and Great Lakes between 2006 and 2012. Similar to previous research on PFCs in freshwater fish (Ahrens & Bundschuh 2014), we expected to find PFOS most frequently and in highest concentrations in fillet samples. Further, we expected that concentrations of both PFOS and total PFCs (ΣPFCs) would be spatially heterogeneous, reflecting possible local usage or inputs to Wisconsin waters.

Sampling and analysis

This dataset includes fish samples collected by both the WDNR and the USEPA. WDNR fish were collected as part of regular population assessments and fisheries surveys using methods appropriate for each fish species and waterbody type (i.e. seining, electroshocking, gill netting, etc.). Collection locations were chosen in order to capture PFC variability in

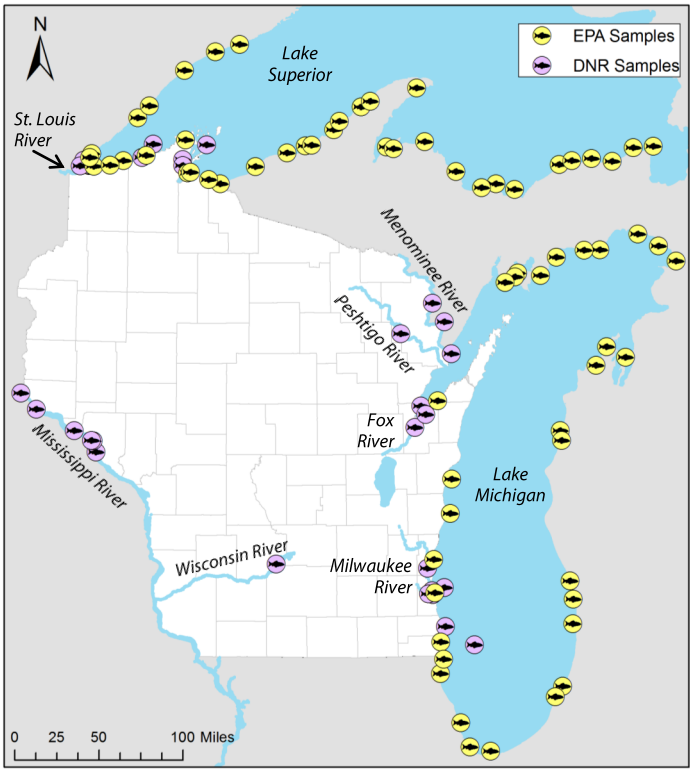

Figure 1. Locations from which samples were collected by the WDNR (purple icons) and USEPA (yellow icons) for PFC analysis.

fish species from waterbodies near industrial centers and/or Great Lakes Areas of Concern (Fig. 1). This dataset includes 28 fish species collected from Lake Michigan, Lake Superior, and the Fox, Menominee, Milwaukee, Mississippi (Pools 3, 4, and 5, 5A, and 6), Peshtigo, St. Louis, and Wisconsin Rivers.

Fish samples collected by the WDNR were wrapped in aluminum foil and frozen in the field before transport to the Wisconsin State Laboratory of Hygiene (WSLH) in Madison, WI. At the WSLH, thawed whole fish or fillets (depending on species) were homogenized and subsamples were refrozen until processing.

Partially thawed fish homogenates were quantified for up to 17 PFCs according to methods developed by Ye et al. (2008). Due to changes in instrument sensitivity (Table 1), different numbers of PFC types were quantified in different years. Furthermore, all fish samples analyzed within the same timeframe may not have been analyzed for the same number of PFCs.

A brief description of analytical techniques used by WSLH is given here: 0.5 g of partially thawed homogenized sub-sample and 0.5 mL of 18 Mohm·cm water were further

Table 1. Analyte information.

Abbreviation	Name	Formula	Carbon chain length	CAS number	Years analyzed	Detection limit (ng/g) NCCA/GL	Detection limit (ng/g) WSLH
PFBA	Perfluorobutanoate	C_3F_7COOH	3	375-22-4	2006-2012	0.07	0.12 – 3.7
PFPeA	Perfluoropentanoate	C_4F_9COOH	4	2706-90-3	2006-2012	0.13	0.12 – 2.5
PFBS	Perfluorobutane sulfonate	$C_4F_9SO_3$	4	375-73-5	2006-2012	0.10	0.12 – 5.0
PFHxA	Perfluorohexanoate	$C_5F_{11}COOH$	5	307-24-4	2006-2012	0.07	0.12 – 2.5
PFHpA	Perfluoroheptanoate	$C_6F_{13}COOH$	6	375-85-9	2006-2012	0.09	0.12 – 2.5
PFHxS	Perfluorohexane sulfonate	$C_6F_{13}SO_3$	6	355-46-4	2006-2012	0.12	0.50 – 5.0
PFOA	Perfluorooctanoate	$C_7F_{15}COOH$	7	335-67-1	2006-2012	0.10	0.12 – 2.5
PFHpS*	Perfluoro-1-heptanesulfonate	$C_7HF_{15}SO_3$	7	375-92-8	2009-2012	n/a	0.12 – 0.50
PFNA	Perfluorononanoate	$C_8F_{17}COOH$	8	375-95-1	2006-2012	0.08	0.12 – 2.5
PFOSA	Perfluorooctane sulfonamide	$C_8F_{17}SO_2NH_2$	8	754-91-6	2006-2010	0.08	2.26 – 2.5
PFOS	Perfluorooctanesulfonate	$C_8F_{17}SO_3$	8	1763-23-1	2006-2012	0.13	0.12 – 5.0
PFDA	Perfluorodecanoate	$C_9F_{19}COOH$	9	335-76-2	2006-2012	0.06	0.12 – 2.5
PFUnA	Perfluoroundecanoate	$C_{10}F_{21}COOH$	10	2058-94-8	2006-2012	0.11	0.12 – 2.5
PFDS*	Perfluoro-1-decanesulfonate	$C_{10}F_{21}SO_3$	10	335-77-3	2009-2012	n/a	0.12 – 0.50
PFDoA	Perfluorododecanoate	$C_{11}F_{23}COOH$	11	307-55-1	2006-2012	0.12	0.12 – 2.5
PFTrDA*	Perfluoro-n-tridecanoic acid	$C_{13}HF_{25}O_2$	13	72629-94-8	2010-2012	n/a	0.12 – 0.50
PFTeDA*	Perfluoro-n-tetradecanoic acid	$C_{14}HF_{27}O_2$	14	376-06-7	2010-2012	n/a	0.12 – 0.50

* PFC analyzed only by WI State Laboratory of Hygiene

homogenized in 50 mL polypropylene tubes using a probe mixer. Nine mL of 10 mM sodium hydroxide in methanol was used to rinse residual homogenate from the mixer probe while being added to the tube. This solution was spiked with a known amount of mixed mass labeled PFC internal standard solution and placed on an orbital shaker at room temperature for 12 to 16 hours.

After shaking, samples were centrifuged at 2,000 rpm for 5 minutes. One mL of the supernatant was combined with 9 mL of 18 Mohm·cm water in a 15 mL polypropylene tube and vortexed prior to solid phase extraction (SPE) cleanup using weak anion exchange (WAX) cartridges (60 mg, 3 cc; Waters, Milford, Ma). Samples were loaded onto solvent and water pre-conditioned WAX cartridges followed by 4 mL washes with 25 mM sodium acetate and methanol. PFCs were collected by elution of the WAX cartridges into 15 mL polypropylene tubes with 4 mL aliquots of 0.1% ammonium hydroxide in methanol.

The elution solvent was evaporated to <0.5 mL using 10 PSI nitrogen at 40°C. The final volume was brought to 0.5 mL with methanol and 0.5 mL of 2 mM aqueous ammonium acetate buffer was added. After brief vortexing, the final extract was syringe filtered using 13 mm, 0.2 µm Millex-GN® nylon discs into autosampler vials and capped with aluminum crimp caps containing polypropylene septa. Between 2006 and 2010, PFC analysis was performed using a high performance liquid chromatograph (Agilent 1100 HPLC; Santa Clara, CA) coupled to a quadrupole ion trap mass spectrometer (SCIEX 4000 MS/MS, Framingham, MA). After 2010, analysis was performed using an ultra-high performance liquid chromatograph (Waters Acquity UPLC, Milford, MA) coupled to an quadrupole-linear ion trap mass spectrometer (AB SCIEX QTRAP 5500, Framingham, MA).

Samples that were collected as part of the USEPA National Coastal Condition Assessment Great Lakes Human Health Fish Tissue Study (NCCA/GL) were analyzed for 14 PFCs by TestAmerica Labs in West Sacramento, CA (Table 1). NCCA/GL sample collection and analysis methods can be found in Stahl et al. (2014).

Detection limits for WSLH and NCCA/GL methods are presented in Table 1. (Ranges of detection limits reflect differing instrument sensitivity through time.) When an analyte was not detected, no data point was recorded for

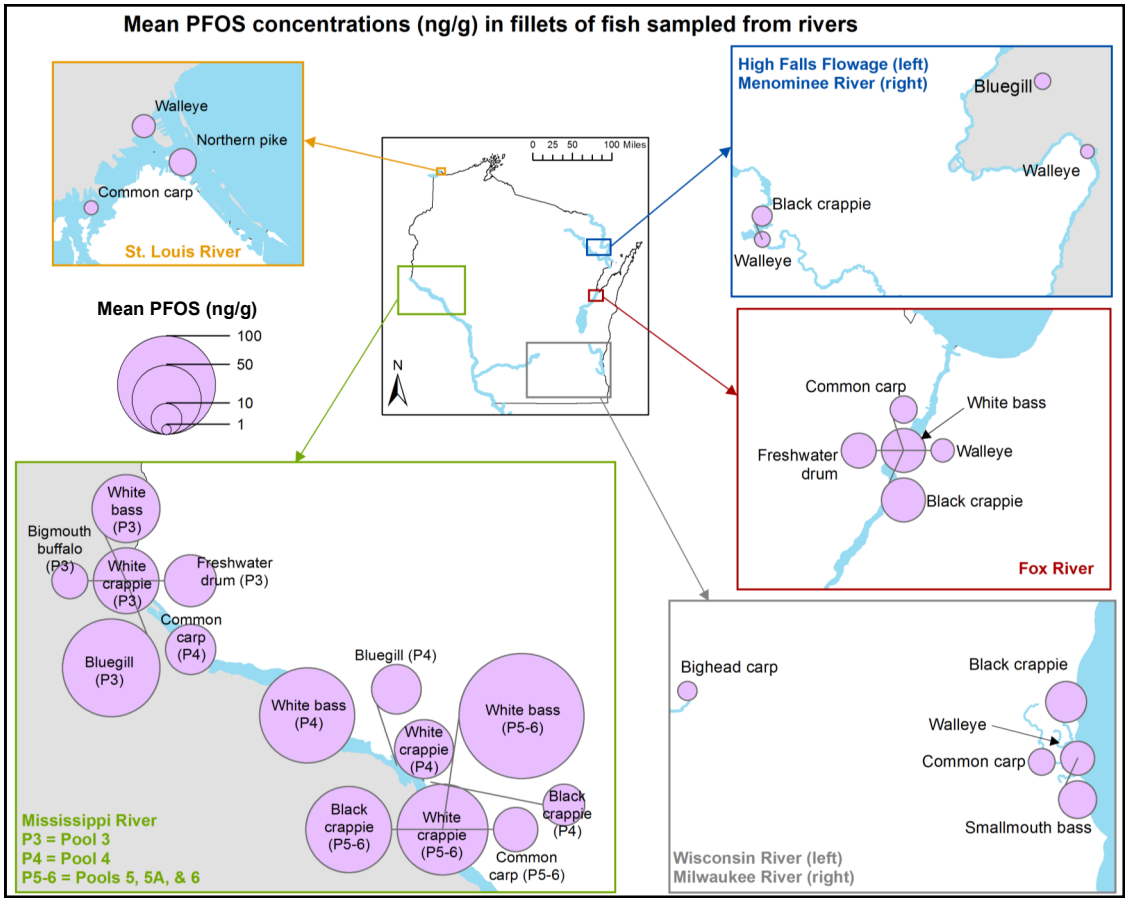

Figure 2. Mean concentrations (ng/g) of PFOS detected in fillets of fish sampled from river systems.

Sampling locations clockwise from top right: blue box - Peshtigo River at High Falls Flowage and Menominee River; red box - Fox River; gray box - Wisconsin and Milwaukee rivers; green box - Mississippi River; orange box - St. Louis River.

that sample; when an analyte was detected but was below the reporting limit, a data point of "0" was recorded for that sample.

PFCs in river fish fillets

PFC concentrations (hereafter [PFCs]) measured in fillets of fish sampled from rivers are detailed in Table 2. Eight PFC types were detected in >30% of fillets from river fish samples analyzed for that PFC (all samples were not necessarily analyzed for the same PFC types; Appendix Fig. A1). These included PFBA, PFHxS, PFOS, PFDA, PFUnA, PFDS, PFDoA, and PFTeDA (Appendix Fig. A1). PFOS was detected in >99% of fillets and was also measured in the highest concentrations in most fillets. It is also the PFC for which fish consumption advisories have been developed. As such, the remainder of this document focuses primarily on PFOS concentrations (hereafter [PFOS]).

Consistent significant relationships were not observed between fillet [PFOS] and fish length within the entire river dataset (all species, all locations). We did not have large enough sample sizes to investigate this relationship for every species/river location combination. Additionally, previous research has not observed a relationship between [PFCs] and fish size (Guo et al. 2012; Xiao et al. 2013).

We did find that [PFOS] was spatially heterogeneous (Fig 2.): highest concentrations were measured in white bass from Pools 5, 5A, and 6 of the Mississippi River (163.0 ng/g), downstream from 3M's Cottage Grove Facility, which formerly manufactured PFCs (MPCA 2009). The lowest concentration (2.0 ng/g) was measured in both walleye from the Menominee River and common carp from the St. Louis River/Superior Harbor (Table 2; Fig. 2).

Furthermore, [PFOS] varied among species. Regardless of sample location, white bass and centrarchids (white and black crappie, bluegill, smallmouth bass) generally contained higher [PFOS] than bigmouth buffalo, common carp, freshwater drum, or walleye (Fig. 2). This finding is supported by previous research suggesting that different fish species have differing bioaccumulation factors (Delinsky et al. 2010), although more research is needed before these pathways are fully understood (Martin et al. 2013).

While PFOS was measured in the highest concentrations in all river samples, there was variation in the mixture of other (non-PFOS) PFC types present by sample location and

Table 2. Concentrations of PFCs (ng/g) in fillets of fish from Wisconsin's major river systems. Neither PFBS nor PFOSA were detected in any river sample and are not presented here. ND = non-detect.

			PFC type Mean (Median)														
	Species	**N**	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoA	PFHxS	PFOS	PFDS	PFTeDA	PFTrDA	PFHpS
Fox River: DePere to Green Bay	Black crappie	2	8.6 (8.6)	3.5 (3.5)	4.1 (4.1)	ND	ND	ND	ND	ND	ND	ND	20.0 (20.0)	1.2 (1.2)	ND	ND	ND
	Common carp	4	ND	ND	ND	ND	ND	ND	1.9 (1.9)	ND	ND	ND	7.7 (7.5)	ND	ND	ND	ND
	Freshwater drum	3	3.5 (2.9)	3.7 (2.8)	2.6 (2.4)	ND	ND	ND	ND	ND	ND	ND	12.6 (13.0)	0.70 (0.70)	ND	ND	ND
	Walleye	4	ND	ND	ND	ND	ND	ND	0.94 (1.0)	ND	ND	ND	5.7 (5.0)	ND	ND	ND	ND
	White bass	2	5.6 (5.6)	3.4 (3.4)	2.8 (2.8)	ND	ND	ND	ND	ND	ND	ND	20.0 (20.0)	1.0 (1.0)	ND	ND	ND
Peshtigo River at High Falls Flowage	Black crappie	3	ND	ND	ND	ND	ND	0.53 (0.53)	0.37 (0.40)	0.28 (0.28)	ND	0.56 (0.56)	4.2 (3.2)	ND	ND	ND	0.18 (0.18)
	Walleye	4	ND	ND	ND	1.0 (1.0)	5.3 (5.3)	ND	0.31 (0.35)	ND	ND	ND	2.6 (2.2)	ND	ND	ND	ND
Menominee River: Piers Gorge to Lower Scott Flowage	Bluegill	6	ND	ND	ND	ND	1.3 (1.3)	ND	0.69 (0.71)	ND	ND	ND	2.7 (3.1)	ND	ND	ND	ND
	Walleye	3	ND	ND	0	0	ND	0	0.43 (0.57)	ND	ND	0	2.0 (2.0)	ND	ND	ND	ND
Milwaukee River Estuary: Estabrook Falls to mouth	Common carp	4	ND	ND	0	0	1.7 (1.7)	0	0.55 (0.60)	ND	ND	0	7.3 (8.1)	ND	ND	ND	ND
	Smallmouth bass	3	ND	ND	ND	ND	ND	ND	1.9 (1.0)	1.2 (1.4)	1.9 (2.2)	0.61 (0.62)	14.9 (12.0)	1.3 (1.5)	1.3 (1.5)	1.0 (1.0)	ND
	Walleye	6	ND	ND	ND	ND	ND	0.15 (0.15)	0.90 (0.76)	0.83 (0.83)	0.91 (0.66)	0.67 (0.64)	12.0 (11.0)	0.50 (0.54)	0.89 (0.91)	ND	0.23 (0.22)
Milwaukee River: Grafton to Estabrook Falls	Black crappie	3	ND	ND	ND	ND	ND	0.82 (0.52)	2.0 (2.3)	1.3 (1.3)	ND	0.74 (0.74)	17.3 (16.0)	ND	ND	ND	ND
Mississippi River: Pool 3	Bigmouth buffalo	3	1.9 (2.1)	0.75 (0.78)	1.6 (1.7)	ND	ND	ND	ND	ND	ND	ND	28.3 (30.0)	ND	ND	ND	ND
	Bluegill	8	1.3 (1.3)	1.0 (1.0)	1.9 (1.6)	ND	ND	ND	1.3 (1.2)	0.42 (0.36)	1.3 (1.2)	ND	99.5 (71.5)	47.0 (51.0)	0.31 (0.31)	ND	0.21 (0.16)
	Freshwater drum	3	1.5 (1.5)	1.3 (1.3)	1.4 (1.40	ND	ND	ND	ND	ND	ND	ND	13.5 (9.3)	0.64 (0.64)	ND	ND	ND
	White bass	8	2.4 (2.4)	0.94 (1.1)	1.1 (1.1)	ND	ND	0.39 (0.39)	1.5 (0.99)	0.54 (0.69)	0.66 (0.65)	ND	48.0 (50.0)	11.7 (6.5)	0.37 (0.36)	ND	0.14 (0.14)
	White crappie	5	1.7 (1.7)	0.63 (0.63)	0.69 (0.71)	ND	ND	ND	ND	ND	ND	ND	44.8 (42.0)	0.62 (0.62)	ND	ND	ND
Mississippi River: Pool 4	Black crappie	3	0.76 (0.79)	ND	ND	ND	ND	ND	ND	ND	ND	ND	18.0 (16.0)	0.53 (0.53)	ND	ND	ND
	Bluegill	8	1.1 (0.68)	0.89 (0.89)	0.68 (0.53)	0.25 (0.13)	0.44 (0.38)	0.30 (0.30)	0.78 (0.56)	0.41 (0.35)	0.62 (0.62)	1.2 (1.1)	25.7 (20.0)	6.8 (3.2)	0.25 (0.25)	ND	0.32 (0.31)
	Common carp	3	0	0	0	0	0	0	0	0	0	0	26.3 (12.9)	ND	ND	ND	ND
	White bass	10	0	0	0.04 (0.0)	0.03 (0.0)	0	0.27 (0.0)	1.5 (1.9)	0.46 (0.0)	0.21 (0.0)	0.65 (0.32)	94.3 (93.5)	20.8 (20.0)	0.36 (0.32)	ND	0.32 (0.17)
	White crappie	4	ND	ND	ND	0.17 (0.17)	0.36 (0.39)	1.8 (1.8)	1.1 (0.75)	1.1 (1.1)	0.42 (0.42)	1.3 (1.4)	36.2 (19.5)	8.8 (2.2)	0.37 (0.37)	ND	0.19 (0.19)
Mississippi River: Pools 5, 5A, & 6	Black crappie	2	0	0	0	0	0	0	0	0	0	0	76.9 (76.9)	ND	ND	ND	ND
	Common carp	4	0	0	0	0	0	0	0	0	0	0	20.3 (20.0)	ND	ND	ND	ND
	White bass	3	0	0	0	0	0	0	2.0 (2.6)	0	2.1 (0.0)	0	163.0 (163.0)	ND	ND	ND	ND
	White crappie	2	0	0	0	0	0	0	0	0	0	0	86.2 (86.2)	ND	ND	ND	ND
St. Louis River & Superior Harbor	Common carp	2	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.71 (0.71)	2.0 (2.0)	ND	ND	ND	ND
	Northern pike	4	ND	ND	ND	ND	ND	0.54 (0.50)	0.80 (0.79)	0.89 (0.89)	1.0 (1.0)	0.74 (0.74)	7.7 (7.5)	0.33 (0.32)	ND	ND	0.19 (0.19)
	Walleye	4	ND	ND	ND	ND	ND	0.61 (0.60)	0.73 (0.73)	ND	ND	0.63 (0.57)	5.5 (4.0)	0.33 (0.33)	ND	ND	ND
Wisconsin River: Prairie du Sac to Mississippi River	Bighead carp	1	ND	ND	ND	0.12	ND	ND	0.35	ND	ND	ND	3.8	0.13 (0.13)	ND	ND	0.12 (0.12)

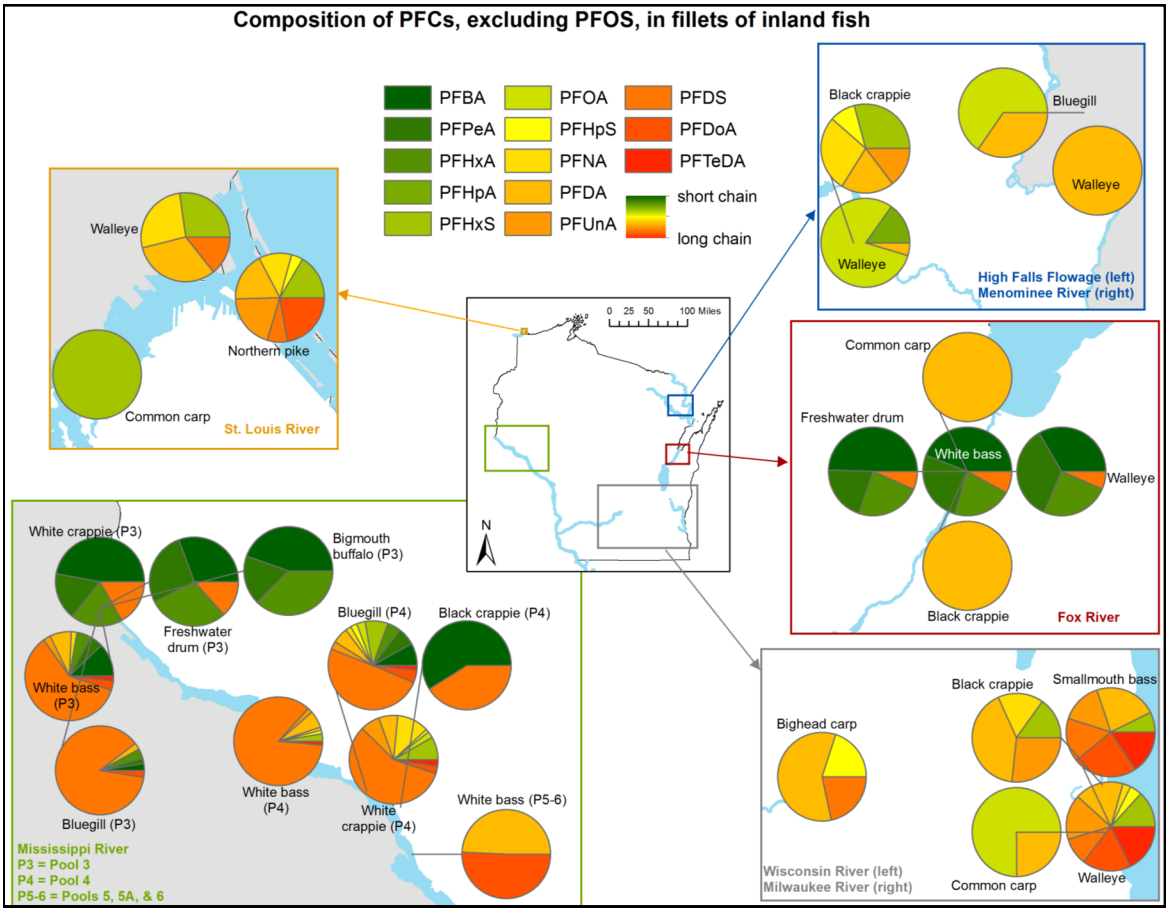

Figure 3. Proportions of PFCs, *excluding* PFOS, that were detected in fillets of fish sampled from rivers. Color ramp indicates carbon chain length: green = short chain (less bioaccumulative), red = long chain (more bioaccumulative).

Sampling locations clockwise from top right: blue box - Peshtigo River at High Falls Flowage and Menominee River; red box - Fox River; gray box - Wisconsin and Milwaukee rivers; green box - Mississippi River; orange box - St. Louis River.

species (Fig. 3). Shorter chain PFCs (≤6 carbons) predominated in fillets of common carp sampled from the St. Louis River (orange box), white crappie, freshwater drum, bigmouth buffalo, and black crappie sampled from Mississippi River Pools 3 and 4 (green box) and freshwater drum, white bass, and black crappie from the Fox River (red box). Fillets containing a range of PFCs having up to 9 carbons were present in all fish sampled from the Peshtigo River at High Falls Flowage and the Menominee River (blue box), common carp and walleye from the Fox River (red box), and common carp from the Milwaukee River (gray box). The longest chain PFCs (≥10 carbons) were found in many species from the Mississippi and Milwaukee rivers, bighead carp from the Wisconsin River (gray box), and walleye and northern pike from the St. Louis River (orange box).

It is not unexpected to find a range of medium and long-chain PFC types in these fish, as they have been documented previously (Ye et al. 2008, Delinsky et al. 2010, Stahl et al. 2014). However, the presence of short-chain PFCs in fish from the Mississippi and Fox rivers possibly may reflect changes in the formulation of products that previously contained long chain PFCs (Ahrens & Bundschuh 2014; Chu et al. 2016).

PFCs in Lakes Michigan & Superior fish fillets

[PFCs] detected in fillets of fish sampled from the Great Lakes are detailed in Table 3. Eight PFC types were detected in >30% of Great Lakes samples analyzed for that PFC (all samples were not necessarily analyzed for the same PFC types; Appendix Fig. A1). These included PFHxS, PFHpS, PFNA, PFOSA, PFOS, PFDA, PFUnA, and PFDoA (Appendix Fig. A1). Similar to trends observed in riverine fish, PFOS was detected in >99% of Great Lakes fillets and was also detected in the highest concentrations in most fillets (Table 3).

As in riverine samples, consistent relationships between [PFOS] and length were not detected within the entire Great Lakes dataset (all fish species, all locations), nor in any individual fish species sampled from the Great Lakes.

In Lake Michigan, the highest [PFOS] was detected in Michigan's Upper Peninsula offshore of the eastern portion of the Garden Peninsula (Fig. 4). Elevated [PFOS] was also

Table 3. Concentrations of PFCs (ng/g) in fillets of fish from Lake Michigan (including Green Bay), Lake Superior, and their tributaries up to the 1st impassible barrier (i.e. dam or falls). ND = non-detect.

		Sample N	PFC type Mean (Median)																
	Species	(composite N)	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoA	PFBS	PFHxS	PFOS	PFOSA	PFDS	PFTeDA	PFTrDA	PFHpS
Lake Michigan, including Green Bay & tributaries	Alewife	3 (15)	ND	ND	ND	ND	ND	ND	0.44 (0.44)	ND	ND	ND	ND	7.4 (7.4)	ND	ND	ND	ND	ND
	Bloater chub	2 (60)	2.8 (2.8)	3.1 (3.1)	0.83 (0.83)	ND	ND	ND	ND	ND	ND	ND	ND	13.5 (13.5)	ND	3.5 (3.5)	ND	ND	ND
	Brown trout	4	ND	0.31 (0.31)	0	0	ND	0.04 (0)	0.22 (0.19)	0.69 (0.69)	0.23 (0.23)	0	0.30 (0)	13.7 (13.5)	0.65 (0.65)	ND	ND	ND	ND
	Channel catfish	2	ND	0.28 (0.28)	ND	ND	ND	ND	0.71 (0.71)	0.80 (0.80)	0.54 (0.54)	ND	1.7 (1.7)	2.9 (2.9)	0.40 (0.40)	ND	0.19 (0.19)	ND	ND
	Chinook salmon	18 (20)	0.21 (0.21)	0.37 (0.31)	0.16 (0.12)	0.24 (0)	1.0 (1.0)	0.32 (0.32)	0.46 (0.48)	0.70 (0.69)	0.30 (0.29)	ND	0.92 (0.92)	21.9 (21.5)	0.17 (0.17)	0.18 (0.18)	0.35 (0.35)	0.76 (0.76)	0.19 (0.15)
	Coho salmon	5	ND	ND	ND	ND	0.43 (0.43)	0.12 (0.12)	0.39 (0.37)	0.18 (0.18)	0.13 (0.13)	ND	1.1 (1.2)	13.5 (16.0)	ND	0.12 (0.12)	ND	ND	0.20 (0.20)
	Freshwater drum	1	ND	ND	ND	ND	ND	ND	0.36	0.68	ND	ND	ND	33.0	ND	ND	ND	ND	ND
	Lake whitefish	3	ND	ND	0	0	ND	0.93 (0.90)	1.9 (1.5)	ND	ND	0	0	16.7 (18.0)	ND	ND	ND	ND	ND
	Lean lake trout	7 (13)	ND	0.28 (0.28)	ND	ND	0.43 (0.28)	2.37 (2.37)	1.0 (0.47)	1.9 (0.96)	0.48 (0.19)	ND	0.56 (0.56)	12.7 (12.0)	0.38 (0.38)	ND	ND	ND	0.21 (0.21)
	Northern pike	1	ND	ND	ND	ND	ND	ND	0.44	0.86	ND	ND	ND	5.8	0.40	ND	ND	ND	ND
	Rainbow smelt	2 (10)	1.3 (1.3)	1.0 (1.0)	1.3 (1.3)	ND	ND	ND	ND	ND	ND	ND	ND	42.5 (42.5)	ND	1.1 (1.1)	ND	ND	ND
	Rainbow trout	9	ND	ND	0	0	ND	0.35 (0.29)	0.57 (0.57)	0.42 (0.42)	0.07 (0.07)	0	0.53 (0)	12.8 (8.1)	ND	0.15 (0.15)	ND	ND	0.17 (0.15)
	Round whitefish	1	ND	ND	ND	ND	ND	0.26	0.65	1.1	0.45	ND	ND	5.8	1.2	ND	ND	ND	ND
	Small-mouth bass	1 (5)	0.36	ND	ND	ND	ND	ND	3.0	6.8	1.6	ND	ND	24.0	4.2	ND	ND	ND	ND
	Walleye	5 (11)	ND	ND	0.07 (0.07)	ND	0.19 (0.19)	0.27 (0.25)	0.61 (0.60)	0.99 (0.86)	0.50 (0.48)	ND	0.24 (0.24)	10.7 (12.0)	2.4 (2.2)	ND	0.13 (0.13)	ND	ND
	White sucker	1 (2)	0.10	ND	ND	ND	0.32	ND	0.85	0.87	0.32	ND	0.30	15.0	0.13	ND	ND	ND	ND
	Yellow perch	6	ND	ND	ND	ND	ND	0.26 (0.26)	1.3 (1.1)	1.1 (1.2)	0.47 (0.43)	0.20 (0.20)	ND	16.4 (15.0)	ND	0.22 (0.22)	0.58 (0.58)	1.5 (1.5)	0.15 (0.14)
Lake Superior & tributaries	Bloater chub	2 (10)	0.51 (0.51)	0.84 (0.84)	ND	ND	ND	ND	ND	ND	ND	ND	ND	4.7 (4.7)	ND	0.56 (0.56)	ND	ND	ND
	Brown trout	1	0.47	0.68	0.25	ND	ND	1.0	0.79	2.4	0.51	ND	ND	35.0	0.26	ND	ND	ND	ND
	Chinook salmon	3	ND	ND	ND	ND	ND	ND	0.24 (0.24)	ND	ND	ND	ND	1.9 (1.9)	ND	ND	ND	ND	0.21 (0.21)
	Cisco (lake herring)	2 (20)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	4.6 (4.6)	ND	ND	ND	ND	ND
	Coho salmon	3	ND	ND	ND	ND	ND	0.36 (0.36)	ND	ND	ND	ND	0.97 (0.81)	2.2 (1.6)	ND	ND	ND	ND	ND
	Lake whitefish	3	ND	ND	ND	1.1 (0.96)	17.2 (15.0)	ND	0.28 (0.28)	ND	ND	ND	ND	0.57 (0.58)	ND	ND	ND	ND	ND
	Lean lake trout	27 (91)	0.35 (0.33)	0.90 (0.57)	0.29 (0.26)	1.0 (0.94)	10.4 (11.1)	0.88 (0.78)	0.68 (0.58)	1.5 (1.7)	0.40 (0.37)	ND	1.8 (1.6)	9.8 (9.0)	0.16 (0.16)	ND	ND	ND	ND
	Longnose sucker	5 (15)	0.83 (0.83)	0.50 (0.39)	0.23 (0.23)	ND	0.61 (0.61)	4.8 (4.5)	3.0 (2.8)	6.4 (6.9)	2.0 (2.3)	ND	0.29 (0.23)	8.1 (7.2)	0.25 (0.19)	ND	ND	ND	ND
	Round whitefish	6 (24)	0.45 (0.45)	0.43 (0.41)	0.19 (0.23)	ND	0.19 (0.19)	1.8 (1.2)	0.70 (0.23)	1.1 (0.38)	0.50 (0.28)	ND	0.47 (0.38)	9.4 (9.3)	0.33 (0.32)	ND	ND	ND	ND
	Siscowet lake trout	3	ND	ND	ND	ND	ND	0.39 (0.38)	0.48 (0.51)	0.75 (0.75)	0.26 (0.26)	ND	ND	2.7 (2.3)	ND	0.15 (0.15)	ND	ND	0.16 (0.16)
	Splake	1	ND	ND	ND	ND	ND	2.2	0.88	2.1	0.40	ND	ND	8.7	ND	ND	ND	ND	ND
	White sucker	1 (2)	1.1	0.67	ND	ND	0.45	0.38	0.31	0.76	0.21	ND	0.19	2.7	0.81	ND	ND	ND	ND

detected in fish sampled north of Manistee, MI, and in the southern portion of the lake offshore of Gary, IN. Among species where $n > 1$, rainbow smelt contained the highest mean [PFOS] (42.5 ng/g) and channel catfish contained the lowest mean concentration (2.9 ng/g; Table 3).

The spatial distribution observed in Lake Michigan [PFOS] (Fig. 4) is similar to that documented in previous research which found highest [PFOS] in sediments in the northern basin of Lake Michigan (Codling et al. 2014) compared to southern basin sediments. Codling et al. (2014) put forth two (possibly co-occurring) explanations to describe PFOS distribution: first, river inputs—unnamed but potentially the Manistique River—initially contributed PFOS to the northern basin; and second, the presence of a "northern gyre" which prevented mixing between northern and southern basins. It is possible that the trends affecting sediment [PFOS] also affect fish [PFOS], although to date we have not found

any other research investigating intra-lake spatial variability and/or associations between sediment and fish [PFOS] concentrations, so it is difficult to be certain.

Concentrations of PFOS in species sampled from Lake Superior were lower (Fig. 4) than those sampled from Lake Michigan: among species where $n > 1$, Lake Superior lean lake trout contained the highest [PFOS] (9.8 ng/g) and lake whitefish contained the lowest [PFOS] (0.57 ng/g; Table 3). Spatially, the highest [PFOS] was found offshore of the Keweenaw Peninsula, MI and in eastern Lake Superior near Luce County, MI (Fig. 4).

Scott et al. (2010) posits that atmospheric deposition in the form of precipitation and tributary inputs are the predominant sources of PFCs to Lake Superior. As such, locations where we measured slightly elevated [PFOS] in Lake Superior fish fillets could potentially reflect site-specific inputs from Michigan's Upper Peninsula. However, as Lake Superior fish fillet [PFOS] is so low, we cannot definitively point to one source.

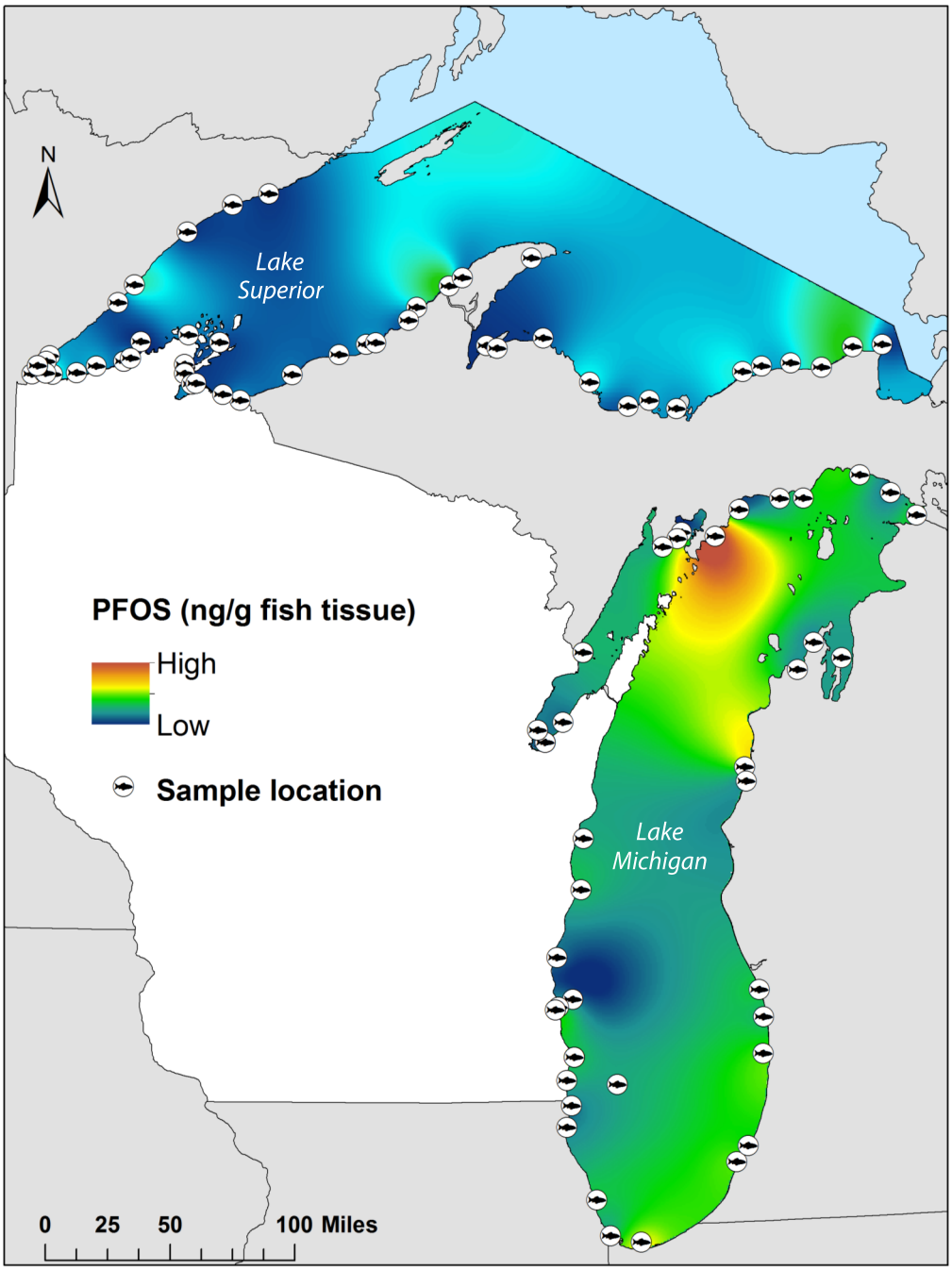

Figure 4. Spatial distribution of PFOS measured in fillets of fish sampled from Lake Michigan and the U.S. waters of Lake Superior. Gradient was interpolated from point measurements in ArcMap 10.1 (ESRI) using the Spline with Barriers tool (Spatial Analyst).

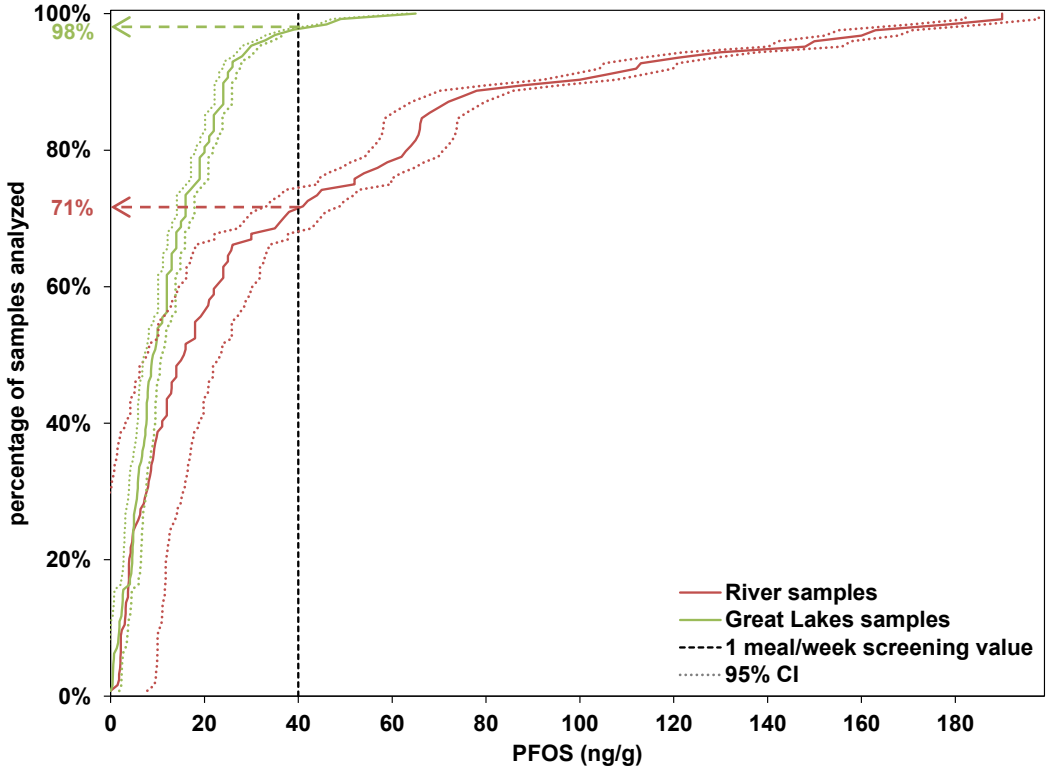

Figure 5. Cumulative distribution functions of PFOS in fish fillet samples (± 95% confidence interval) from rivers (red) and the Great Lakes (green). Dotted line indicates the Minnesota Department of Health 1 meal/week screening value of 40 ng/g. Two percent of the Great Lakes fillet samples and 29% of river fillet samples contained PFOS in excess of the screening value. All river samples exceeding 40 ng/g came from Mississippi River fish fillets.

Implications for Wisconsin fish consumption advisories

In Wisconsin, advice for people who want to consume fish is provided based upon the concentration of several contaminants: mercury, PCBs, dioxins/furans, and PFCs. PFCs are assessed by comparing the amount of PFOS in fish fillets to meal frequency ranges developed by the Minnesota Department of Health (0.08 µg/kg-day; MDH 2008). Fish consumption advisories due to PFOS are warranted because PFOS accumulates in fish (Stahl et al. 2014) and there is reliable evidence showing human health risks are associated with elevated levels of PFOS (Darrow et al. 2013).

Wisconsin DNR and Department of Health Services first issued PFOS-based consumption advice in 2007 for some Mississippi River species at 1 meal/week. Currently, there are 3 locations in the Mississippi River where PFOS is measured at concentrations high enough to warrant advice more stringent than Wisconsin's general statewide advice. In Pool 3 and Pools 5-6 advice is provided for bluegill and crappie, and in Pool 4 advice is provided for bluegill.

Cumulative distribution functions of [PFOS] in fillets of fish sampled were used to determine the percentage of samples exceeding 40 ng/g, (Minnesota Department of Health's 1 meal/week lower meal range; Stahl et al. 2014). In this dataset, 2% of the Great Lakes fillet samples and 29% of river fish fillet samples (all from the Mississippi River) contained PFOS in excess of 40 ng/g (Fig. 5). It is important to note that although some amount of PFOS was measured in most fillets, in most Wisconsin locations where higher concentrations of contaminants are found, PCBs and mercury remain the contaminants of concern in terms of consumption advice.

Recommendations for future work

Monitoring fish from Wisconsin's rivers and Great Lakes for PFCs should continue. In particular, future work should target analysis of fish collected near possible sources or uses of PFCs. Extremely high levels of PFOS (up to 9580.0 ng/g) have been measured in fish near locations in Michigan that use aqueous film-forming foams for fire fighting (MDCEQ 2015).

Fish consumption is thought to be a major pathway for human exposure to PFCs, and although products containing PFOS and PFOA

have been phased out of production by the 8 major manufacturers, these pollutants are expected to remain in the environment for a long time. In addition, product formulations have evolved in response to regulations, and replacement chemicals have recently been detected in the environment (Strynar et al. 2015; Chu et al. 2016). Continued monitoring is needed to assess evolving trends in PFCs in the environment and risk to people who consume fish.

Acknowledgments

This work was supported in part by a Wisconsin Department of Health Services grant from the Great Lakes Restoration Initiative (EPA-R5-GL2010-1). Special thanks to Pat McCann, Agnes Mukasa, Wayne Richter, and Sarah Yang for feedback on this report. Thanks also to Ryan Alger for assistance with figures and to Curtis Hedman for review of Wisconsin State Laboratory of Hygiene methods. Thank you to the WSLH staff for fish processing and analysis.

Literature cited

Ahrens, L. 2011. Polyfluoroalkyl compounds in the aquatic environment: a review of their occurrence and fate. Journal of Environmental Monitoring 13(1):20-31.

Ahrens, L., and M. Bundschuh. 2014. Fate and effects of poly- and perfluoroalkyl substances in the aquatic environment: a review. Environmental Toxicology and Chemistry 33(9):1921–1929.

Apelberg, B.J., F.R. Witter, J.B. Herbstman, A.M. Calafat, R.U. Halden, L.L. Needham, and L.R. Goldman. 2007. Cord serum concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in relation to weight and size at birth. Environmental Health Perspectives 115(11):1670–1676.

Barry, V., A. Winquist, and K. Steenland. 2013. Perfluorooctanoic acid (PFOA) exposures and incident cancers among adults living near a chemical plant. Environmental Health Perspectives 121(11-12):1313–1318.

Betts, K.S. 2007. Perfluoroalkyl acids: what is the evidence telling us? Environmental Health Perspectives 115(5):A250–6.

Bhavsar, S.P., X. Zhang, R. Guo, E. Braekevelt, S. Petro, N. Gandhi, E.J. Reiner, H. Lee, R. Bronson, and S.A. Tittlemier. 2014. Cooking fish is not effective in reducing exposure to perfluoroalkyl and polyfluoroalkyl substances. Environment International 66:107–114.

Christensen, K.Y., M. Raymond, B.A. Thompson, and H.A. Anderson. 2016. Perfluoroalkyl substances in older male anglers in Wisconsin. Environment International 91: 312-318.

Chu, S., R.J. Letcher, D.J. McGoldrick, and S.M. Backus. 2016. A new fluorinated surfactant contaminant in biota: perfluorobutane sulfonamide in several fish species. Environmental Science and Technology 50(2): 669-675.

Codling, G., A. Vogt, P.D. Jones, T. Wang, P. Wang, Y.-L. Yu, M. Corcoran, S. Bonina, N.C. Sturchio, K.J. Rockne, K. Ji, J.-S. Khim, J.E. Naile, J.P. Giesy. 2014. Historical trends of inorganic and organic fluorine in sediments of Lake Michigan. Chemosphere 114:203-209.

Consoer, D.M., A.D. Hoffman, P.N. Fitzsimmons, P.A. Kosian, and J.W. Nichols. 2014. Toxicokinetics of perfluorooctanoate (PFOA) in rainbow trout (*Oncorhynchus mykiss*). Aquatic Toxicology 156:65–73.

Darrow, L.A., C.R. Stein, and K. Steenland. 2013. Serum perfluorooctanoic acid and perfluorooctane sulfonate concentrations in relation to birth outcomes in the Mid-Ohio Valley, 2005-2010. Environmental Health Perspectives 121(10):1207–1213.

Delinsky, A.D., M.J. Strynar, P.J. McCann, J.L. Varns, L. Mcmillan, S.F. Nakayama, and A.B. Lindstrom. 2010. Geographical Distribution of Perfluorinated Compounds in Fish from Minnesota Lakes and Rivers. Environmental Science and Technology 44(7):2549–2554.

Delinsky, A.D., M.J. Strynar, S.F. Nakayama, J.L. Varns, X. Ye, P.J. McCann, and A.B. Lindstrom. 2009. Determination of ten perfluorinated compounds in bluegill sunfish (*Lepomis macroshirus*) fillets. Environmental Research 109(8): 975–984.

Frisbee, S. J., A.P. Brooks, A. Maher, P. Flensborg, S. Arnold, T. Fletcher, K. Steenland, A. Shankar, S.S. Knox, C. Pollard, J.A. Halverson, V.M. Vieira, C. Jin, K.M. Leyden, and A.M. Ducatman. 2009. The C8 health project: design, methods, and participants. Environmental Health Perspectives 117(12):1873–1882.

Giesy, J.P., and K. Kannan. 2001. Global distribution of perfluorooctane sulfonate in wildlife. Environmental Science and Technology 35(7):1339–1342.

Grandjean, P., E.W. Andersen, E. Budtz-Jørgensen, F. Nielsen, K. Mølbak, P. Weihe, and C. Heilmann. 2012. Serum vaccine antibody concentrations in children exposed to perfluorinated compounds. Jama 307(4):391–7.

Guo, R., E.J. Reiner, S.P. Bhavsar, P.A. Helm, S.A. Mabury, E. Braekevelt, and S.A. Tittlemier. 2012. Determination of polyfluoroalkyl phosphoric acid diesters, perfluoroalkyl phosphonic acids, perfluoroalkyl phosphinic acids, perfluoroalkyl carboxylic acids, and perfluoroalkane sulfonic acids in lake trout from the Great Lakes region. Analytical and Bioanalytical Chemistry 404(9):2699–2709.

Jain, R.B. 2014. Contribution of diet and other factors to the levels of selected polyfluorinated compounds: data from NHANES 2003–2008. International Journal of Hygiene and Environmental Health 217(1): 52–61.

Jones, P.D., W. Hu, W. De Coen, J.L. Newsted, and J.P. Giesy. 2003. Binding of perfluorinated fatty acids to serum proteins. Environmental Toxicology and Chemistry 22(11):2639–2649.

Lau, C., K. Anitole, C. Hodes, D. Lai, A. Pfahles-

Hutchens, and J. Seed. 2007. Perfluoroalkyl acids: a review of monitoring and toxicological findings. Toxicological Sciences 99(2):366–394.

Lindstrom, A.B., M.J. Strynar, and E.L. Libelo. 2011. Polyfluorinated compounds: past, present, and future. Environmental Science and Technology 45(19):7954–7961.

Martin, J.W., D.M. Whittle, D.C.G. Muir, and S.A. Mabury. 2004. Perfluoroalkyl contaminants in a food web from Lake Ontario. Environmental Science and Technology 38(20): 5379–5385.

Martin, J.W., S.A. Mabury, K.R. Solomon, and D.C.G. Muir. 2013. Progress toward understanding the bioaccumulation of perfluorinated alkyl acids. Environmental Toxicology and Chemistry 32(11):2421–2423.

Michigan Department of Environmental Quality (MDEQ) . 2015. Water Resources Division Staff Report MI/DEQ/WRD-15/019: Reconnaissance sampling of perfluorinated compounds in Michigan surface waters and fish, 2010-2014. Available: http://www.michigan.gov/documents/mdch/MDCH_GL-00E01122-0_Final_Report_493494_7.pdf

Minnesota Department of Health (MDH). 2008. Fish consumption advisory program meal advice categories. Available: http://www.health.state.mn.us/divs/eh/fish/eating/mealadvicetables.pdf

Minnesota Pollution Control Agency (MPCA). 2009. Fact Sheet: 3M Cottage Grove site proposed cleanup plan for PFCs. Report pfc3-04. Available: https://www.pca.state.mn.us/sites/default/files/pfc3-04.pdf

Murphy, M.B., E.I.H. Loi, K.Y. Kwok, and P.K.S. Lam. 2012. Ecotoxicology of Organofluorous Compounds. Page 24 *in* I. T. Horváth, editor. Fluorous Chemistry, 308th edition. Springer Berlin Heidelberg, Berlin.

Prevedouros, K., I.T. Cousins, R.C. Buck, and S.H. Korzeniowski. 2006. Sources, fate and transport of perfluorocarboxylates. Environmental Science and Technology 40(1):32–44.

Rasmussen, P.W., C. Schrank, and M.C.W. Williams. 2014. Trends of PCB concentrations in Lake Michigan coho and chinook salmon, 1975-2010. Journal of Great Lakes Research 40(3): 748-754.

Renner, R. 2001. Growing concern over perfluorinated chemicals. Environmental Science and Technology 35 (7):154A–160A.

Scott, B.F., A.O. de Silva, C. Spencer, E. Lopez, S.M. Backus, and D.C.G. Muir. 2010. Perfluoroalkyl acids in Lake Superior water: Trends and sources. Journal of Great Lakes Research 36(2):277–284.

Shankar, A., J. Xiao, and A. Ducatman. 2012. Perfluorooctanoic acid and cardiovascular disease in US adults. Archives of Internal Medicine 172(18):1397–1403.

Stahl, L.L., B.D. Snyder, A.R. Olsen, T.M. Kincaid, J.B. Wathen, and H. B. McCarty. 2014. Perfluorinated compounds in fish from U.S. urban rivers and the Great Lakes. The Science of the Total Environment 499:185–195.

Steenland, K., L. Zhao, A. Winquist, and C. Parks. 2013. Ulcerative colitis and perfluorooctanoic acid (PFOA) in a highly exposed population of community residents and workers in the mid-Ohio valley. Environmental Health Perspectives 121(8):900–905.

Strynar, M., S. Dagnino, R. McMahen, S. Liang, A. Lindstrom, E. Anderson, L. McMillan, M. Thurman, I. Ferrer, and C. Ball. 2015. Identification of novel perfluoroalkyl ether carboxylic acids (PFECAs) and sulfonic acids (PFESAs) in natural waters using accurate mass time-of-flight mass spectrometry (TOFMS). Environmental Science and Technology 49(19): 11622-11630.

Watkins, D.J., J. Josson, B. Elston, S.M. Bartell, H. Shin, V.M. Vieira, D.A. Savitz, T. Fletcher, and G.A. Wellenius. 2013. Exposure to perfluoroalkyl acids and markers of kidney function among children and adolescents living near a chemical plant. Environmental Health Perspectives 121(5):625–630.

Watkins, D.J., G.A. Wellenius, R.A. Butler, S.M. Bartell, T. Fletcher, and K.T. Kelsey. 2014. Associations between serum perfluoroalkyl acids and LINE-1 DNA methylation. Environment International 63:71–76.

Xiao, F., J.S. Gulliver, and M.F. Simcik. 2013. Perfluorooctane sulfonate (PFOS) contamination of fish in urban lakes: a prioritization methodology for lake management. Water Research 47(20):7264–72.

Ye, X., M.J. Strynar, S.F. Nakayama, J. Varns, L. Helfant, J. Lazorchak, and A.B. Lindstrom. 2008. Perfluorinated compounds in whole fish homogenates from the Ohio, Missouri, and Upper Mississippi Rivers, USA. Environmental Pollution 156(3):1227–1232.

Appendix, Figure A1. Detection information for each PFC type in river samples (top) and Great Lakes samples (bottom). Left panels display the numbers of samples analyzed compared to the number of detections of each PFC. Because analysis methods and analytes varied through time and between laboratories, the same number of PFCs were not necessarily measured in every sample. Right panels display percent detection for each PFC type. Asterisks in bottom panel indicate PFC types that were only analyzed for by the Wisconsin State Laboratory of Hygiene.