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Research Paper

Absorption and elimination of per and poly-fluoroalkyl substances substitutes in salmonid species after pre-fertilization exposure

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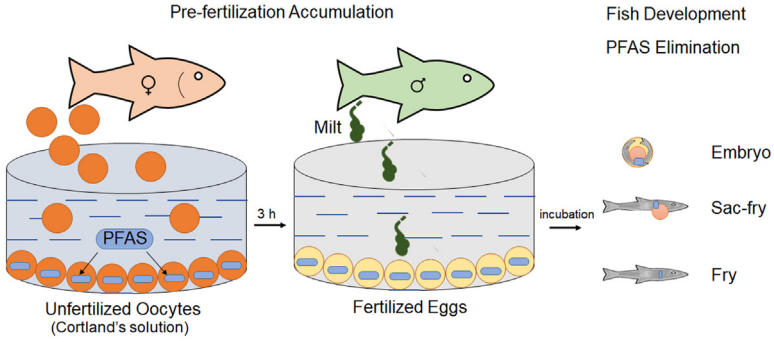
HIGHLIGHTS

- PFAS could enter the salmonid egg yolk through pre-fertilization exposure.
- Rapid elimination of PFAS during first three days after fertilization of eggs.
- Faster rates of elimination from eggs and larvae of newer substitute PFAS.

GRAPHICAL ABSTRACT



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ABSTRACT

Due to their relatively large production and few restrictions on uses, novel substitutes for historically used per and poly-fluoroalkyl substances (PFAS) are being used and accumulating in the environment. However, due to a lack of information on their toxicological properties their hazards and risks are hard to estimate. Before fertilization, oocytes of two salmonid species, Arctic Char *(Salvelinus alpinus)* and Rainbow Trout *(Oncorhynchus mykiss)*, were exposed to three PFAS substances used as substitutes for traditional PFAS, PFBA, PFBS or GenX or two archetypical, historically used, longer-chain PFAS, PFOA and PFOS. Exposed oocytes were subsequently fertilized, incubated and were sampled during several developmental stages, until swim-up. All five PFAS were accumulated into egg yolks with similar absorption rates, and their concentrations in egg yolks were less than respective concentrations in/on egg chorions. Rapid elimination of the five PFAS was observed during the first 3 days after fertilization. Thereafter, amounts of PFOS and PFOA were stable until swim-up, while PFBA, PFBS and GenX were further eliminated during development from one month after the fertilization to swim-up. In these two salmonid species, PFBA, PFBS and GenX were eliminated faster than were PFOS or PFOA.

1. Introduction

Per and poly-fluoroalkyl substances (PFAS) are a group of industrial chemicals that contain a hydrophobic alkyl chain and a hydrophilic functional group such as carboxylate, sulfonate, or phosphonate (Buck et al., 2011). The alkyl chain consists of one or more carbon atoms in

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which all the available valence electrons are bound to fluorine (F) atoms and can be straight-chain or branched (Buck et al., 2011). Therefore, PFAS are defined as chemicals with at least one perfluorocarbon moiety (C_nF_{2n+1}), although structurally, they can differ by the addition of more *per*-fluorinated (fully fluorinated) or poly-fluorinated (partially fluorinated) chains (Buck et al., 2011; Organization for Economic Co-operation and Development, 2020). Because of the presence of multiple strong carbon-carbon and carbon-fluorine bonds, PFAS are resistant to degradation, making them versatile synthetic chemicals used in a range of industrial processes and products since the 1950s (Giesy and Kannan, 2001, 2002; Paul et al., 2009). As excellent surfactants, PFAS have been and continue to be used widely in commercial and industrial products, like paints, textiles including carpet, food package, and in aqueous film forming foams (AFFF) (Buck et al., 2011). The widespread application of PFAS has resulted in some PFAS being ubiquitous in the environment, where their resistance to degradation has allowed them to accumulate in wildlife and humans (Giesy and Kannan, 2001, 2002; Nakayama et al., 2019). Of particular concern are effects PFAS might have on aquatic environments. The potential and known toxic effects and potencies of some PFAS have been determined to include reproductive toxicity, growth and developmental defects, neuro-behavioral defects, and other general disorders arising from immune system disruption and alterations in structure and function of membranes (Lee et al., 2017, 2020). Perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA), both containing eight carbon atoms, are considered to be the representative legacy PFAS and have been investigated widely.

Longer-term use of AFFF at airports, military sites, firefighting training sites, and industrial facilities has resulted in widespread contamination of soils and ground waters by PFAS. This is particularly true at current and former airports in the Canadian Arctic. In addition to the more well-studied PFOS and PFOA, hundreds of other PFAS have been used to formulate complex mixtures present in some AFFF formulations (Barzen-Hanson et al., 2017; Liu et al., 2019). Exposure to these mixtures is inevitable as evidenced by biomonitoring studies suggesting exposure of humans and wildlife to AFFF (Oakes et al., 2010; Dobraca et al., 2015; Nair et al., 2021). While studies have been conducted to assess ecotoxicities of legacy PFAS including PFOS and PFOA and a few others, limited information is available on potential environmental effects of the broader set of perflourinated substances, including those being used as replacements for PFOS and PFOA (Jantzen et al., 2016; Chen et al., 2018; Khan et al., 2019). Studies by research groups, including ours, have revealed that some PFAS, such as fluorotelomer carboxylic acids and sulfonamides, can have greater toxic potencies than those exhibited by PFOS or PFOA (Teuschler, 2007; Phillips et al., 2010; Dasgupta et al., 2020; Goodrum et al., 2021; Han et al., 2021).

Various studies involving monitoring of the environment and studies of toxic potencies have led to the phase-out of production and use of PFOS and PFOA. The concern surrounding exposure of and potential effects on humans and wildlife has led to some manufacturers voluntarily phasing out production of legacy PFAS. In 2009, PFOS and related compounds were listed as persistent organic pollutants under the Stockholm Convention, and PFOA and related compounds were also added in 2019 (UNEP, 2009, 2019). Since then, most research on effects in the environment has focused on two chemical classes of PFAS: perfluoroalkyl sulfonic acids (PFSAs) and perfluoroalkyl carboxylic acids (PFCAs), as well as their precursors (Buck et al., 2011; USEPA, 2017; Lee et al., 2020). However, thousands of PFAS compounds are still available on the open market, and compounds with known modes of toxic action are still being manufactured around the globe (Organization for Economic Co-operation and Development, 2020). While blanket bans on PFAS substances could be employed in the future, PFAS will still continue to be produced for use in industries that require their unique characteristics (Cousins et al., 2020; Glüge et al., 2020).

Due to the unique chemical properties of PFAS chemicals, and recent limitations on uses of typical, medium-length chain PFAS, shorter-chain PFAS and other novel PFAS substitutes are increasingly being developed and used (Ankley et al., 2021). The Organization for Economic Co-operation and Development, defines short-chain PFAS as PFCAs containing fewer than 8 carbon atoms and PFSAs containing fewer than 6 carbon atoms (Organization for Economic Co-operation and Development, 2011). Examples of short-chain PFAS are perfluorobutanesulfonic acid (PFBS), perfluorobutanoic acid (PFBA). In addition to industrial production, degradation of longer-chain homologues also contributes to the prevalence of the short-chain PFAS (Vaalgamaa et al., 2011; Wang et al., 2011; Codling et al., 2018a). Other novel PFAS substitutes include perfluoroalkyl and polyfluoroalkyl ether carboxylic acids, perfluoroalkyl phosphinic acids (PFPiAs), perfluoroalkyl ether sulphonic acids (PFESAs) and so on, like 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy) propanoic acid (GenX), 6:2 chlorinated polyfluorinated ether sulfonate (F53B), salts of 6:2 fluorotelomer sulfonic acid (Buck et al., 2011; Sheng et al., 2018).

These short-chain and other novel PFAS have been reported to demonstrate resistance to degradation and biotransformation that is similar to or greater than those of legacy PFAS. Compared to the longer-chain legacy PFAS, short-chain PFAS are more water soluble and less likely to adsorb to particulates, which can result in greater mobility in the environment (Wang et al., 2015; Li et al., 2020). These short-chain and other novel PFAS have been detected in several environmental media globally (Codling et al., 2018b). Due to their greater solubilities in water, short-chain PFAS are assumed to be less bioaccumulative than longer-chain PFAS (Brendel et al., 2018; Li et al., 2020). Although most perfluoroalkyl acids (PFAAs) have lesser vapor pressures, their gaseous precursors can migrate relatively long distances, to be subsequently degraded by oxidation in the atmosphere to their respective terminal products and deposited in remote areas, such as Northern Canada (Ellis et al., 2004; Butt et al., 2010). Together with oceanic currents containing PFAS emitted elsewhere, these transportation phenomena can introduce PFAS to the Arctic (Stock et al., 2007; Wania, 2007). PFAS can remain in large water bodies for extended periods, during which they might present risks to aquatic organisms. PFAS have been found in benthic and pelagic invertebrates, muscle of juvenile and adult Arctic char *(Salvelinus alpinus)* from six lakes of the Canadian Arctic (Lescord et al., 2015). Total concentrations of a sum of 19 individual PFAS were greater in muscle of adult char from Resolute (122 ± 65 ng/g) and Meretta lakes (27 ± 6.8 ng/g) than those from other lakes (Lescord et al., 2015). During the period 1984–2006, PFOS, PFOA, perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnA), perfluorododecanoic acid (PFDoA) and perfluorotridecanoic acid (PFTrA) found in the livers of East Greenland polar bears significantly increased, with the total amount of 3359 ng/g wet weight (the sum of medians) in year 2006 (Dietz et al., 2008). Aquatic invertebrates and fish are exposed to PFAS, mainly through the skin or gills, and the reproductive development of offspring may also be affected through transgenerational exposure (Abercrombie et al., 2019; Chen et al., 2019). Fishes in early life stages are more sensitive to the toxic effects of PFAS exposure (Embry et al., 2010). However, the lack of monitoring data in northern environments as well as the lack of toxicological data on these novel PFAS in northern organisms makes it difficult to estimate the ecological risks.

Based on results of previous studies (Raine et al., 2021), exposure to chemicals before fertilization and subsequent water-hardening can deliver predictable amounts of PFAS to the yolk of salmonid eggs. In the current study, oocytes of Arctic char (*Salvelinus alpinus*) and Rainbow trout *(Oncorhynchus mykiss*) were exposed to PFAS before fertilization, using Cortland's solution as the exposure medium. The Arctic char is a culturally and economically significant species in Northern Canada while the rainbow trout is commonly used model salmonid species, which has been studied in other toxicological experiments and can act as a bridge to compare the results from this and other studies. Before fertilization, oocytes of the two salmonid species were exposed to three short-chain or other novel PFAS, PFBA, PFBS and GenX, and two historically used, perfluorinated compounds, PFOS and PFOA as positive, reference materials. After fertilization, eggs were incubated until swim-up. Concentrations of PFAS in egg yolks, whole eggs and fry were measured at several stages of development.

2. Methods

2.1. Exposure studies

To approximate the normally longer-term, low-level of maternal transfer of PFAS to oocytes based on our previous results (Raine et al., 2021), media containing 1, 10, 100 or 1000 μg/mL of either PFBA, PFBS, GenX or PFOS or 1, 10 or 100 μg/mL PFOA (SynQuest Laboratories, FL, USA) were prepared in Cortland's Solution (124.1 mM NaCl, 5.1 mM KCl, 1.0 mM $MgSO_4 \cdot 7H_2O$, 1.6 mM $CaCl_2 \cdot 2H_2O$, 5.6 mM glucose, 20 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethane-sulfonic acid). The pHs of exposure and control solutions were adjusted to 8.5, the natural pH of salmonid coelomic fluid. Aliquots of exposure solutions and controls were stored at −20 °C for subsequent quantification of PFAS.

About 300 unfertilized oocytes collected from 10 female Arctic Char (Miracle Springs Inc., BC, CA) or 9 female Rainbow Trout (Troutlodge, WA, USA) were distributed into polypropylene jars and were then covered by the exposure solutions for 3 h at 6 °C, with gentle shaking every 20 min. Based on the number of oocytes available, char eggs were exposed in triplicate batches to one of five chemicals: PFBA, PFBS, GenX, PFOA or PFOS, while the trout eggs were exposed in triplicate batches to PFBA, PFBS, GenX or PFOS. Nine exposure replicates of unexposed controls were made for char, with twelve control replicates for trout. After exposure, oocytes were fertilized with combined milt from 6 male char or 11 male trout, and were then washed with cold water (6 °C) three times to remove milt and exposure chemicals. After fertilization and water hardening, about 40 embryos were selected from each batch and the yolk was aspirated from half of these 40 embryos. Samples of aspirated yolk and the remaining 20 fertilized eggs were stored at −20 °C for chemical analysis. For Arctic char the remaining embryos were randomly allocated to Heath Trays for further incubation until the post-hatch sac-fry stage. Sac-fry were then transferred to a zebrafish rack system and grown to swim-up. Rainbow Trout embryos were incubated in Heath Trays until swim-up stage. The water temperature was maintained at 6 °C for the duration of the development process, and the other water quality parameters were monitored daily. The char started to hatch 76 days post fertilization (DPF), and reached 50% hatching at 85 DPF. The trout started to hatch around 56 DPF, and reached 50% hatching at 58 DPF. During incubation, 10–12 eggs of different stages (char: 30 DPF; trout: 3, 7, 14, 28 DPF) were sampled. Half of the sampled char egg samples (30 DPF) was aspirated for yolk immediately. The yolk samples, egg samples, together with the fry samples were kept at −20 °C until further dentification and quantification of PFAS.

2.2. Chemical analysis

Fifty mg of freeze-dried egg samples or 150 μL of freeze-dried yolk samples or 5 freeze-dried fry, fortified with internal standards (Wellington Laboratories, Ontario, CA) were extracted and PFAS concentrations were analyzed, using previously described methods (Raine et al., 2021). In brief, extraction was carried out with 2 mL 0.01 N KOH in methanol (MeOH) for 16 h at room temperature. One mL of the supernatant was then diluted with 100 mL LC/MS grade H_2O, followed by solid phase extraction (SPE) to purify extracts. Target PFAS were eluted with 4 mL 0.1% NH_4OH in MeOH. The eluates were concentrated under high purity nitrogen and filtered through 0.2 μm polypropylene filters for subsequent analysis. Exposure solutions were analyzed by adding 40–100 μL of the solution directly to the 100 mL H_2O and the subsequent steps were the same as those used for samples.

Quantifications of PFAS were conducted using high performance liquid chromatography (Vanquish UHPLC, Thermo Scientific, Mississauga, ON) coupled to ultra-high resolution mass spectrometry (Q Exactive HF, Thermo Scientific, Mississauga, ON) as described previously (Raine et al., 2021). The analytical column was a Betasil C18, 2.1 × 100 mm, 5 μm and the solvent trapping column was a Betasil C18, 2.1 × 10 mm, 3 μm. The flowrate was 0.3 mL/min with the temperature of 40 °C. All target PFAS were scanned using negative ion electrospray in full scan mode for quantification with simultaneously acquired parallel reaction monitoring MS/MS for compound confirmation.

2.3. Quality control

Authentic standards were added to some samples (whole egg, yolk and fry) and some procedural blanks before extraction. Matrix spike recoveries of PFBA, PFBS, GenX, PFOA and PFOS for whole egg, yolk and fry were all between 80%–120%. The method detection limits (MDL) for the five PFAS are provided in the supporting information (Table S1). Procedural blanks were repeated every 10 samples and the blank value was subtracted from the sample results. Recoveries of mass-labeled surrogates added before extraction were all between 70% and 120%.

2.4. Data analysis

IBM SPSS Statistics (IBM, USA) was used to run the statistical analyses. The Shapiro-Wilks test was used to test for normality and Levene's test was used to test for homogeneity of variance. The paired *t*-test was used for comparison between two related groups, with the randomized blocks analysis of variance for more than two related groups, and the independent t-test was run for two unrelated groups (comparison of ratios of PFAS concentrations in egg yolks to the actual PFAS concentrations in exposure solutions between two species). The significance level 0.05 was selected for all the statistical tests. In some cases, the above parametric tests were applied even when the normality assumption was not met, considering the small sample size. Linear regressions were based on log-transformed PFAS concentrations in egg yolks and exposure solutions.

3. Results

3.1. Absorption of PFAS

3.1.1. PFAS concentrations in exposure solutions

Concentrations of PFOA, PFBA, PFBS and GenX in Cortland's solutions to which Arctic Char were exposed, were near nominal concentrations, while actual concentrations of PFOS were somewhat less than nominal concentrations (Table 1). This was the same for exposure solutions used for the Rainbow Trout. Exposure solutions of PFOS greater than 10 μg/mL were adsorbed by the polypropylene container. MeOH was used to extract the PFOS adsorbed by the polypropylene container and the total PFOS concentrations were reported here as the actual exposure solution. However, since the exposure solutions were prepared 12 h before the exposure started, some portion of the PFOS had probably already been adsorbed during this time. In addition, 1000 μg/mL PFOS could not be completely dissolved in Cortland's solution. Arctic Char and Rainbow Trout oocytes were directly exposed to 1000 μg PFOS/mL containing both dissolved and undissolved PFOS.

Table 1
Measured PFAS concentrations in exposure solutions for char and trout.

	Nominal concentration, μg/mL	Actual concentration, μg/mL				
		PFOA	PFOS	PFBA	PFBS	GenX
Char	Control	ND	ND	ND	ND	ND
	1	0.97	0.66	1.02	1.24	1.19
	10	9.62	7.85	10.1	11.9	10.6
	100	97.6	64.3	98.4	119	104
	1000		800	1047	1229	761
Trout	Control		ND	ND	ND	ND
	1		0.80	0.98	1.22	1.08
	10		9.86	10.0	12.7	10.7
	100		71.2	97.8	120	100
	1000		916	832	1274	745

ND: less than MDL.

Table 2
Ratios of PFAS yolk concentrations to actual exposure concentrations.

	Nominal concentration, μg/mL	Yolk concentration/actual exposure concentration				
		PFOA	PFOS	PFBA	PFBS	GenX
Char	1	0.42% ± 0.03%	0.53% ± 0.37%	0.18% ± 0.07%	0.25% ± 0.10%	ND
	10	0.43% ± 0.05%	1.13% ± 0.79%	0.11% ± 0.05%	0.24% ± 0.02%	0.08% ± 0.04%
	100	0.38% ± 0.03%	0.51% ± 0.15%	0.24% ± 0.18%	0.25% ± 0.02%	0.30% ± 0.21%
	1000		0.52% ± 0.09%	0.11% ± 0.04%	0.21% ± 0.09%	0.26% ± 0.11%
Trout	1		0.60% ± 0.14%	0.32% ± 0.26%	0.27% ± 0.01%	0.09% ± 0.06%
	10		1.24% ± 0.79%	0.18% ± 0.02%	0.27% ± 0.08%	0.46% ± 0.38%
	100		0.63% ± 0.30%	0.17% ± 0.05%	0.45% ± 0.26%	0.26% ± 0.02%
	1000		0.54% ± 0.12%	0.26% ± 0.02%	0.28% ± 0.05%	0.31% ± 0.02%

Expressed as mean ± standard deviation.
ND: less than MDL.

3.1.2. PFAS concentrations in egg yolk

Ratios of concentrations of PFAS in egg yolks (ng/mL) to the actual PFAS concentrations in exposure solutions (ng/mL) were determined (Table 2). When exposed from 1.0 to 1000 μg/mL, the ratios of PFBA, PFBS, GenX and PFOS, respectively, were in the range of 0.11–0.24%, 0.21–0.25%, 0.08–0.30% and 0.51–1.13% for Arctic Char, while they were in the range of 0.17–0.32%, 0.27–0.45%, 0.09–0.46% and 0.54–1.24% for Rainbow Trout. When char eggs were exposed from 1.0 to 100 μg/mL PFOA, the ratio was from 0.38% to 0.43%. Concentrations of PFAS in exposure solutions were relatively great, however, only a small portion of the dissolved PFAS masses were absorbed into the salmonid oocytes after the 3 h pre-fertilization exposure. No significant differences ($p > 0.05$) in ratios were observed between the two salmonid species, except the ratio when the exposure concentration was 1000 μg/mL PFBA (0.11% for char and 0.26% for trout, $p = 0.005$).

Slopes of linear regressions of log transformed concentrations of PFAS in yolks (ng/mL) to the log transformed actual PFAS amounts in exposure solutions (ng/mL), were generally near 1 for PFBA, PFBS, PFOS and PFOA in both species (Figs. 1, 2). This result indicated a linear relationship between external exposure concentrations and amounts absorbed by oocytes. For the Arctic Char, slopes of PFBA, PFBS, PFOS and PFOA were 0.968, 0.977, 0.969 and 0.977, respectively, while slopes for Rainbow Trout exposed to PFBA, PFBS or PFOS were 0.967, 1.027, and 0.962, respectively. No significant differences ($p > 0.05$) in slopes were observed among the studied PFAS, nor between the two salmonid species. When exposed to GenX, slopes (not significant) were calculated based on the actual concentrations in the 10, 100 and 1000 μg/mL exposure solutions and the corresponding yolk concentrations, and were 1.293 for char and 0.904 for trout.

3.1.3. Differences of PFAS content in egg and yolk

Besides the concentrations of PFAS in egg yolks, their concentrations in whole eggs were also determined (Table 3). In both char and trout eggs for all five PFAS, mean concentrations ($C_{0d\text{-}egg}$, ng/g) in whole eggs were

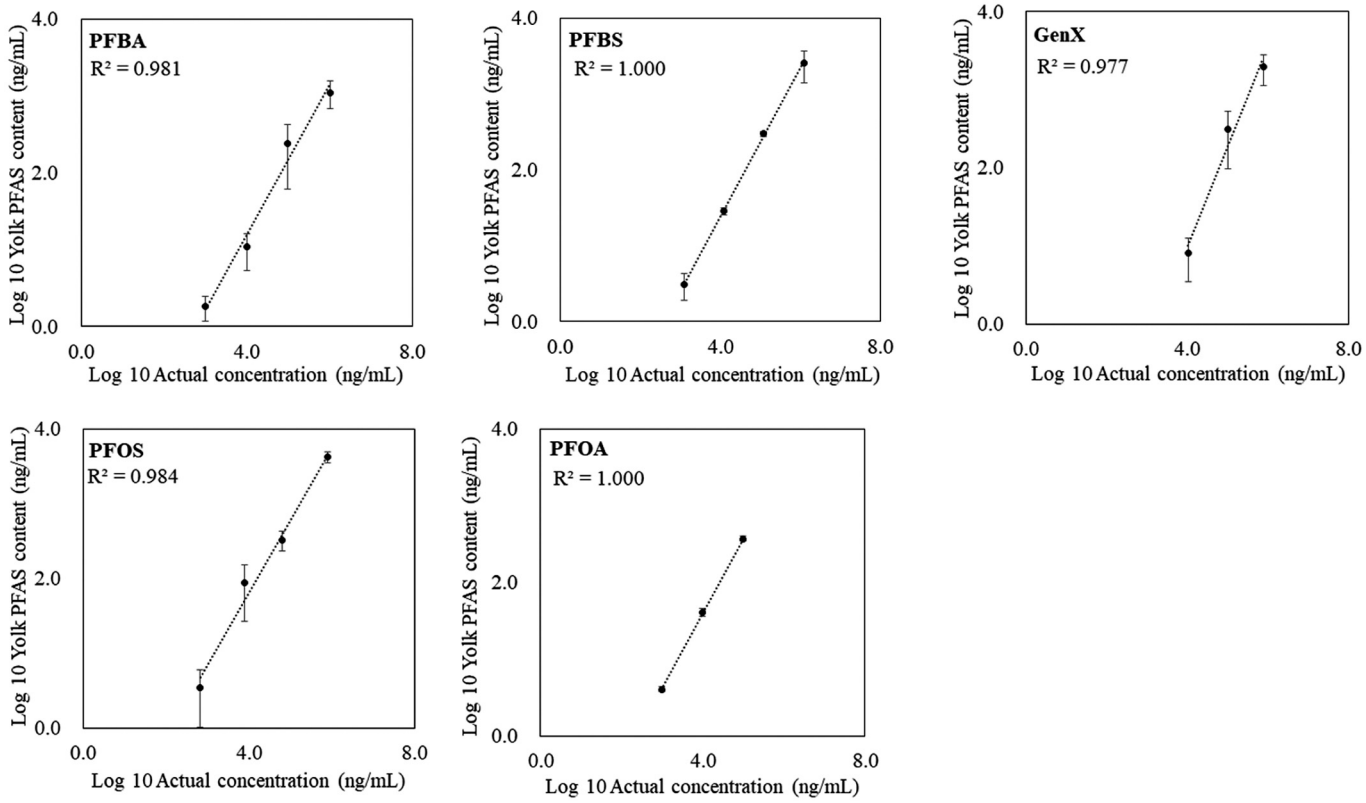

Fig. 1. Linear relationship between concentrations of PFBA, PFBS, GenX, PFOS and PFOA in exposure solutions and in the char egg yolk. Equations for PFBA, PFBS, GenX, PFOS and PFOA were $y = 0.968 \times -2.682$ ($R^2 = 0.981$, $p = 0.009$), $y = 0.977 \times -2.519$ ($R^2 = 1.000$, $p < 0.001$), $y = 1.293 \times -4.201$ ($R^2 = 0.977$, $p = 0.097$), $y = 0.969 \times -2.064$ ($R^2 = 0.984$, $p = 0.008$) and $y = 0.977 \times -2.298$ ($R^2 = 1.000$, $p = 0.011$), respectively. The error bar represents standard deviation.

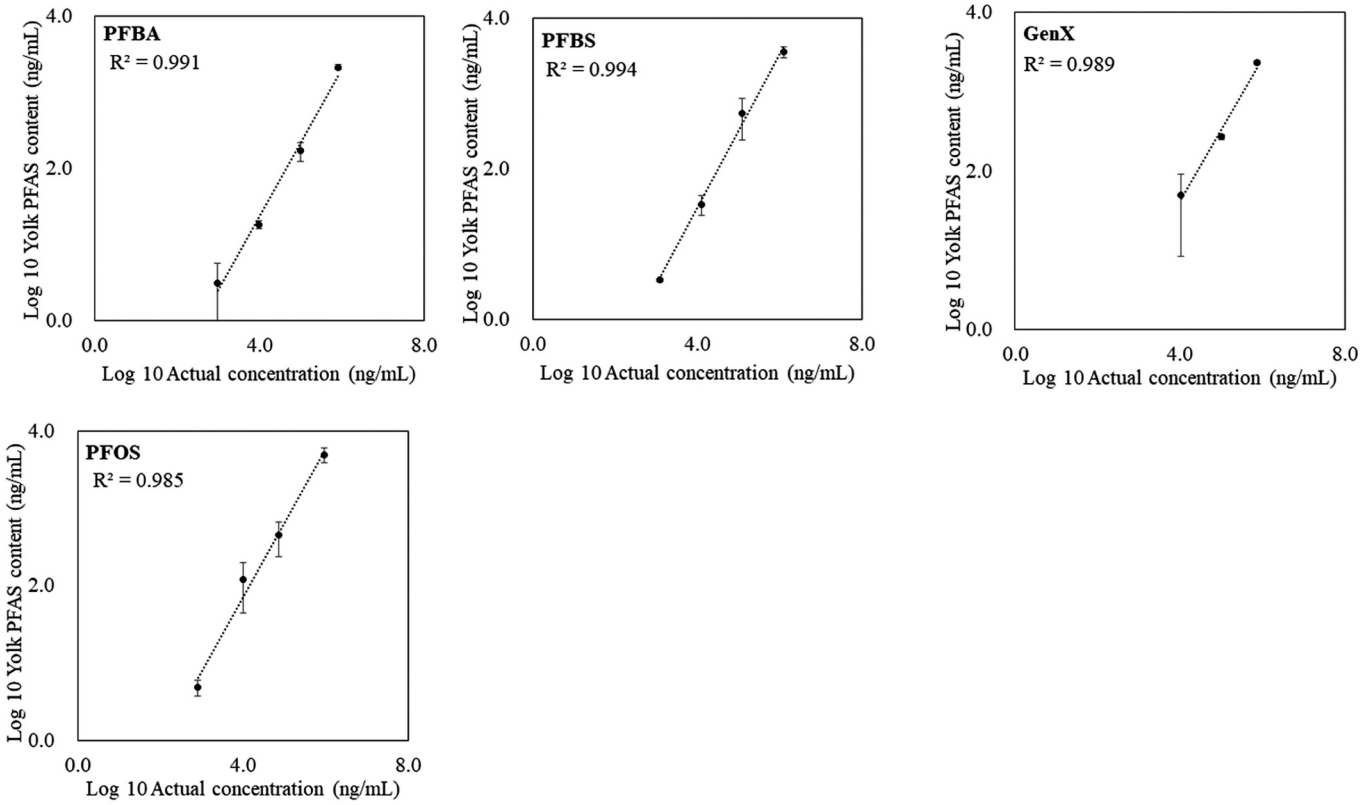

Fig. 2. Linear relationship between concentrations of PFBA, PFBS, GenX and PFOS in exposure solutions and in the trout egg yolk. Equations for PFBA, PFBS, GenX and PFOS were $y = 0.967 \times -2.499$ ($R^2 = 0.991$, $p = 0.005$), $y = 1.027 \times -2.633$ ($R^2 = 0.994$, $p = 0.003$), $y = 0.904 \times -1.999$ ($R^2 = 0.989$, $p = 0.066$) and $y = 0.962 \times -1.982$ ($R^2 = 0.985$, $p = 0.008$), respectively. The error bar represents standard deviation.

greater than were mean concentrations in yolks ($C_{0d\text{-}yolk}$, ng/g), though not all pairs were significant due to the variation among three replicates. Ratios of $C_{0d\text{-}egg}$/ $C_{0d\text{-}yolk}$ were calculated. These ratios were all greater than 1.0, which indicated that concentrations of PFAS in/on egg chorions (ng/g) were greater than those in egg yolks (ng/g). After the 3 h exposure, eggs were rinsed three times with clean water to remove PFAS on the surface of the fertilized eggs. Results suggest that a portion of PFAS associated with the egg chorion cannot be easily rinsed off and was tightly bound to the chorion. This phenomenon was observed for both char and trout eggs, and for all 5 PFAS at various exposure concentrations.

Yolks ($C_{30d\text{-}yolk}$, ng/g) and whole eggs ($C_{30d\text{-}egg}$, ng/g) of char, collected 30 days after the exposure were also analyzed for PFAS (Table 3). The yolk and whole egg concentrations were nearly the same at 30 DPF in char (not significantly different, $p > 0.05$, except eggs exposed to 100 μg/mL PFBA). The values of $C_{30d\text{-}egg}/C_{0d\text{-}egg}$ were all less than values of $C_{30d\text{-}yolk}/C_{0d\text{-}yolk}$ for all the 5 PFAS at different exposure concentrations. This indicates that portions of the PFAS were eliminated from both the chorions and yolks with different elimination rates, causing the convergence of egg and yolk PFAS concentrations. During the 30 days of incubation, all the char eggs were incubated in trays with flowing water (internal recirculation) with 2/3 of the water changed each day.

3.2. Elimination of PFAS

At 3, 7, 14 and 28 DPF, trout eggs were sampled and concentrations of PFAS in whole eggs were analyzed. Considering possible differences in masses of trout eggs at various developmental stages, the results were expressed as amounts of PFAS per egg (ng/egg) (Fig. 3). During the period of 3–28 DPF, PFBA (100 μg/mL), PFBS (10, 100, 1000 μg/mL), GenX (100, 1000 μg/mL) and PFOS (1, 10, 100, 1000 μg/mL) amounts in trout eggs were stable, showing no significant differences at various times ($p > 0.05$). When exposed to the greatest concentration, PFBA, PFBS, GenX ($p = 0.005$, $p = 0.008$, $p = 0.001$) and PFOS ($p > 0.05$, not significant) amounts in trout eggs decreased after 3 or more days of incubation compared with PFAS amounts in trout eggs just after fertilization. This result demonstrates that the major portion of elimination of PFBA, PFBS, GenX and PFOS took place in the first three days post-fertilization. During the period from day 3 through 28, mean amounts of PFBA represented 1.4% and 1.0% of initial amounts in fertilized trout eggs, when exposed at concentrations of 100 and 1000 μg/mL, while ratios for GenX were 2.9% and 3.8%. Mean ratios when exposed to 10, 100 or 1000 μg/mL were 8.3%, 6.4%, 10% for PFBS, and 68%, 69%, 27% for PFOS. Based on the above results, rates of elimination of PFBA and GenX were greater than for PFBS and PFOS. PFOS exhibited the slowest rate of elimination.

To evaluate elimination of PFAS in salmonid fishes during development from embryo to the swim-up, char eggs were collected 30 DPF, while trout eggs were sampled 28 DPF and their respective fry were sampled at swim-up (Table 4). No significant differences ($p > 0.05$) were observed between PFOS/PFOA amounts in salmonid eggs sampled a month after exposure and PFOS/PFOA amounts in fry at the swim-up stage for any of the exposure concentrations. This indicates that no additional PFOS or PFOA was excreted during the period of development from the embryo (1 month after fertilization), through the sac-fry stage, and up to the swim-up stage at which time the yolk sac was totally absorbed. Significantly ($p < 0.05$) lesser amounts of PFBA, PFBS and GenX were present in swim-up stage fry compared to amounts in respective eggs sampled a month post-fertilization. This pattern of elimination of PFBA, PFBS, GenX and PFOS during development was observed in both species and was also observed for the elimination of PFOA during development in char.

Amounts of PFAS in fry at the swim-up stage ($C_{swim\text{-}up}$, ng/fry) were compared with amounts of PFAS in eggs collected just after fertilization ($C_{0d\text{-}egg}$, ng/egg) and the $C_{swim\text{-}up}/C_{0d\text{-}egg}$ ratio was calculated (Table 4). The $C_{swim\text{-}up}/C_{0d\text{-}egg}$ values of PFBA, GenX, PFBS, PFOS and PFOA in char (trout) ranged from 0.135%–0.145% (0.42%–0.72%, 100–1000 μg/mL),

Table 3
Concentrations of PFAS in whole eggs and egg yolks.

	Nominal Concentration, μg/mL	PFOA, ng/g		PFOS, ng/g		PFBA, ng/g		PFBS, ng/g		GenX, ng/g	
		Egg	Yolk	Egg	Yolk	Egg	Yolk	Egg	Yolk	Egg	Yolk
Char-0 day	Control	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	1	10.9* ± 2.1	4.06 ± 0.31	9.22* ± 3.11	3.66 ± 2.57	2.54* ± 0.69	1.95 ± 0.73	5.75 ± 1.31	3.28 ± 1.25	0.93 ± 0.14	ND
	10	78.6 ± 9.7	42.2 ± 3.2	141 ± 93	92.8 ± 64.3	16.6* ± 7.3	11.4 ± 5.6	51.4 ± 7.4	31.2 ± 3.3	21.7* ± 9.4	8.46 ± 4.83
	100	714 ± 99	363 ± 29	624 ± 218	342 ± 97	313 ± 157	257 ± 186	538* ± 74	327 ± 25	673 ± 473	335 ± 229
	1000			11250* ± 3170	4517 ± 821	1524 ± 356	1222 ± 486	5642* ± 846	2730 ± 1150	3575 ± 2344	2132 ± 881
Trout-0 day	Control			0.51 ± 0.23	0.59 ± 0.28	ND	ND	ND	ND	ND	ND
	1			6.29* ± 1.07	4.75 ± 1.09	4.20* ± 2.39	3.15 ± 2.51	5.35 ± 2.31	3.41 ± 0.17	3.95 ± 2.53	1.02 ± 0.74
	10			126 ± 57	121 ± 78	26.9* ± 2.8	19.1 ± 2.5	63.3 ± 8.8	35.9 ± 10.3	51.9 ± 40.3	51.9 ± 42.6
	100			505 ± 263	453 ± 218	251* ± 59	177 ± 48	864* ± 269	566 ± 327	504 ± 183	278 ± 24
	1000			10,697 ± 5969	5186 ± 1379	3102* ± 520	2229 ± 212	4886* ± 700	3636 ± 554	3736* ± 184	2400 ± 211
Char-30 day	Control	ND	ND	ND	4.19 ± 2.71	–	–	ND	ND	ND	ND
	1	2.66 ± 0.28	2.62 ± 0.14	4.30 ± 1.77	–	–	–	–	–	–	–
	10	34.5 ± 6.5	27.9 ± 1.8	–	–	ND	ND	3.22 ± 0.30	3.35 ± 0.42	ND	ND
	100	153 ± 34	135 ± 10	203 ± 80	219 ± 85	1.19* ± 0.07	1.73 ± 0.06	28.2 ± 10.0	28.2 ± 3.1	6.71 ± 2.77	6.10 ± 2.77
	1000			988 ± 147	1029 ± 81	13.4 ± 3.2	14.9 ± 5.8	206 ± 48	218 ± 35	68.8 ± 22.3	70.2 ± 3.6
Char-30 day/ Char-0 day	1	25%	64%	47%	114%	–	–	–	–	–	–
	10	44%	66%	–	–	–	–	6.3%	11%	–	–
	100	21%	37%	33%	64%	0.4%	0.7%	5.2%	8.6%	1.0%	1.8%
	1000			8.8%	23%	0.9%	1.2%	3.7%	8.0%	1.9%	3.3%

Expressed as PFAS wet mass (ng/g), mean ± standard deviation.
ND: less than MDL.
* Concentrations of PFAS in whole eggs significantly greater than concentrations of PFAS in egg yolks, $p < 0.05$.

0.19%–0.47% (1.1%–1.7%, 100–1000 μg/mL), 0.27%–0.51% (1.4%–2.2%, 10–1000 μg/mL), 29%–53% (67%–73%, 1–100 μg/mL) and 15%–26% (1–100 μg/mL) at various exposure concentrations. When exposed to 1000 μg/mL PFOS, the $C_{swim\text{-}up}/C_{0d\text{-}egg}$ value was 8.0% for char and 18% for trout. The lesser $C_{swim\text{-}up}/C_{0d\text{-}egg}$ of PFBA, compared with PFBS, and PFOA compared with PFOS, indicated a greater rate of elimination of carboxylic acids compared to sulfonic acids in the two species studied. In addition, lesser values of $C_{swim\text{-}up}/C_{0d\text{-}egg}$ were observed for PFBA and PFBS, compared to PFOA and PFOS, respectively, indicating greater rates of elimination for these short-chain PFAS. The rate of elimination of GenX was greater than that of PFOA, but somewhat less than that of PFBA. For the two salmonid species, the percentages of residual PFAS in char fry at swim-up were generally less than trout.

In general, amounts of PFOS in each egg/fry were similar in eggs at 3, 7, 14, and 28 DPF as well as in fry at the swim-up stage, but were less than amounts of PFOS in eggs immediately post-fertilization. Therefore, PFOS in exposed salmonid eggs was mainly eliminated over the first 3 days post-fertilization, but was fairly stable during subsequent development through to the swim-up stage. Even though amounts of PFOA in eggs of 3, 7 and 14 days post-fertilization were not known, its elimination pattern was predicted to be similar with PFOS. For PFBA, PFBS and GenX, similar amounts were also found in eggs from 3 to 28 days post-fertilization. While these amounts were less than the respective amounts in fertilized eggs, they were greater than amounts in fry at swim-up. Thus, PFBA, PFBS and GenX were eliminated over two time periods; one during the first 3 days post-fertilization and the other from one month post-fertilization to the swim-up stage. Greater elimination rates of PFBA, PFBS, GenX and PFOS were found in the Arctic Char, compared with Rainbow Trout.

4. Discussion

All five PFAS at various exposure concentrations were absorbed by char and trout oocytes during a three hour pre-fertilization exposure. Concentrations of PFAS on chorions of eggs, which was estimated by measuring the total egg concentration, were observed to be greater than concentrations in egg yolks, for char and trout. This pre-fertilization exposure method has been previously demonstrated to efficiently introduce legacy PFAS, including PFOS, PFHxA and PFOA, into oocytes before water hardening and formation of the chorion (Raine et al., 2021), and was also effective for uptake of the legacy PFAS substitutes, PFBA, PFBS and GenX.

Unlike other persistent, organic pollutants, PFAS have greater affinity for proteins rather than for lipids (Jones et al., 2003; Bossi et al., 2015). PFAS are known to bind with serum albumin, fatty acid binding proteins and organic anion transporter proteins, which results in accumulation in blood, liver and kidney (Ng and Hungerbühler, 2013). Interactions between the PFAS studied here and the proteins of the egg chorion/yolk probably caused accumulation of PFAS both in/on the chorion and in the yolk. It seems that these interactions are not specific to different PFAS or fish species.

Greater concentrations of PFAS in whole eggs immediately post-fertilization were found to finally approach the yolk PFAS concentrations after 30 days of incubation. This is presumed to be caused by different rates of elimination of PFAS from the chorion and from the yolk. PFAS on outer surfaces of chorions were probably more easily influenced by the flowing water system. The greater rate of elimination from the chorion might also represent some redistribution of PFAS to the yolk as incubation proceeds. Finally, the relative amounts of the most retained PFAS (PFOS), which were eliminated were greater at the greatest exposure concentrations. This suggests potential for some lesser affinity binding processes to occur at the greatest exposure concentrations.

PFBA, PFBS, GenX, PFOA and PFOS are generally not metabolized in organisms and are only eliminated through urine, feces, lactation, menstruation etc. (Pizzurro et al., 2019). Rates of elimination of PFAS were largely associated with properties of individual PFAS and species. Longer half-lives of PFOS and PFOA in humans than monkeys and rodents have been observed (Harada et al., 2007; Pizzurro et al., 2019). Generally, shorter-

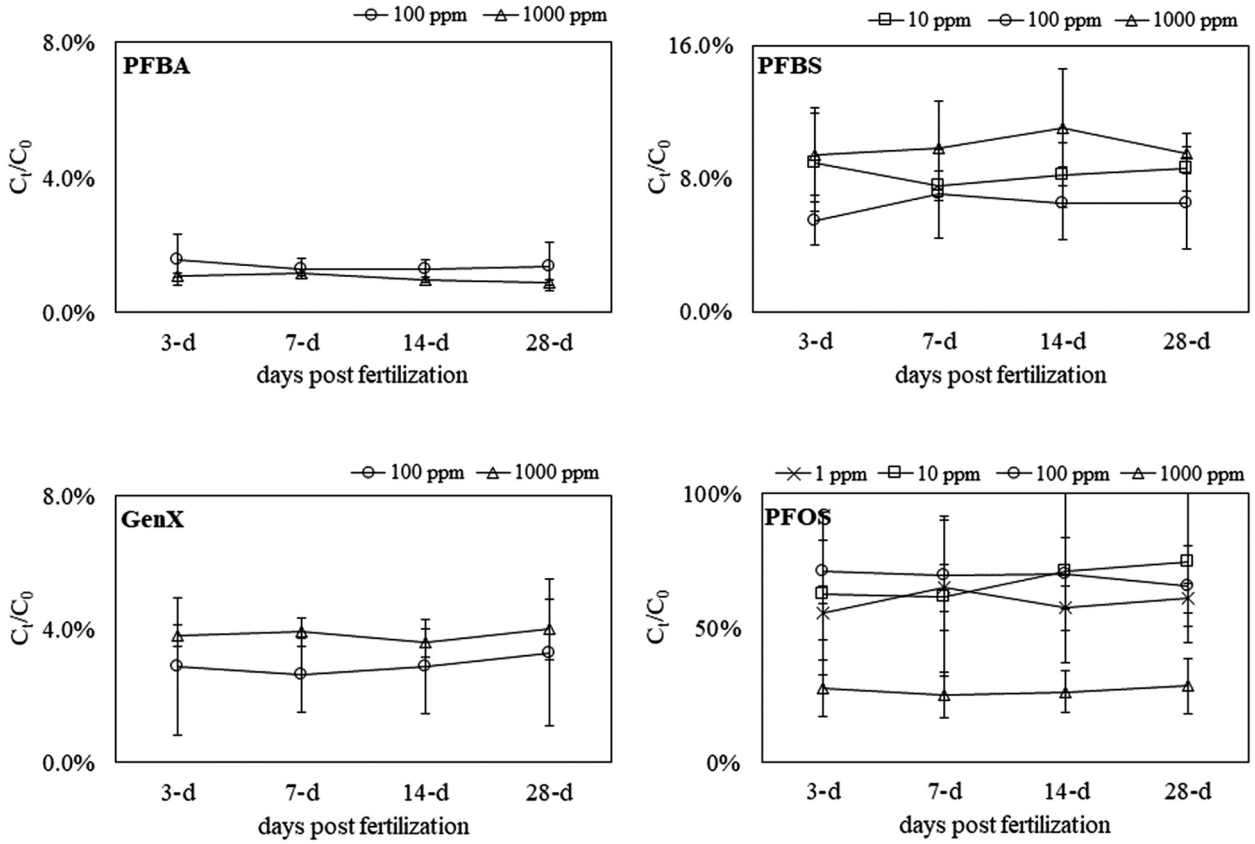

Fig. 3. Trends of PFAS amounts in trout eggs during 3–28 days post fertilization. C_t, concentrations of PFAS in whole eggs sampled at t days post fertilization, ng/egg; C_0, concentrations of PFAS in whole eggs sampled just after fertilization, ng/egg. The error bar represents standard deviation.

chain PFAS have been found to have shorter half-lives both in animals and humans (Wang et al., 2013). Consistent with these findings, results of the current study demonstrated that the rates of elimination of PFBA and PFBS were greater compared to PFOA and PFOS, respectively. Lesser bioaccumulation of shorter-chain PFAS can be related to their greater water solubility and weaker interaction with renal transport proteins resulting in increased elimination rates (Han et al., 2012). Similarly, studies of other novel PFAS substitutes like perfluoroether carboxylic acids (PFECAs)

Table 4
PFAS amounts in salmonid eggs or fishes sampled at different developmental stages.

	Nominal Concentration, μg/mL	Char, ng/egg or fry				Trout, ng/egg or fry	
		$C_{30\text{-d}}$	$C_{swim\text{-}up}$	$C_{swim\text{-}up}/C_{0d\text{-}egg}$	$C_{28\text{-d}}$	$C_{Swim\text{-}up}$	$C_{swim\text{-}up}/C_{0d\text{-}egg}$
PFOA	Control	ND	ND				
	1	0.25 ± 0.01	0.18 ± 0.04	17%			
	10	2.96 ± 0.61	1.72 ± 0.28	26%			
	100	13.9 ± 2.47	10.3 ± 1.97	15%			
PFOS	Control	ND	0.06 ± 0.02		0.14 ± 0.06	0.14 ± 0.06	
	1	0.40 ± 0.18	0.47 ± 0.23	53%	0.36 ± 0.05	0.40 ± 0.08	68%
	10	–	6.18 ± 4.29	45%	9.68 ± 6.63	8.53 ± 5.77	73%
	100	19.2 ± 8.20	18.1 ± 8.38	29%	31.2 ± 13.3	33.1 ± 13.2	67%
	1000	87.8 ± 13.8	81.5 ± 9.23	8.0%	229 ± 34.9	169 ± 17.0	18%
PFBA	Control	ND	ND		ND	ND	
	100	0.11 ± 0.01	0.04	0.14%	0.28 ± 0.08	0.16 ± 0.05	0.72%
	1000	1.30 ± 0.38	0.21 ± 0.07	0.14%	2.46 ± 0.32	1.16* ± 0.28	0.42%
PFBS	Control	ND	ND		ND	ND	
	10	0.29 ± 0.02	0.02* ± 0.01	0.44%	0.51 ± 0.03	0.14* ± 0.05	2.2%
	100	2.55 ± 0.97	0.25 ± 0.06	0.51%	4.76 ± 0.82	1.13* ± 0.27	1.4%
	1000	18.3 ± 5.05	1.52* ± 0.29	0.27%	41.8 ± 0.61	9.80* ± 1.80	2.2%
GenX	Control	ND	ND		ND	ND	
	100	0.61 ± 0.26	0.11 ± 0.02	0.19%	1.30 ± 0.59	0.53 ± 0.18	1.1%
	1000	6.26 ± 1.40	1.67* ± 0.03	0.47%	14.6 ± 3.02	6.26 ± 1.27	1.7%

$C_{30\text{-d}}$, concentrations of PFAS in char eggs sampled 30 DPF, ng/egg, mean ± standard deviation.
$C_{28\text{-d}}$, concentrations of PFAS in trout eggs sampled 28 DPF, ng/egg, mean ± standard deviation.
$C_{swim\text{-}up}$, concentrations of PFAS in fry of the swim-up stage, ng/fry, mean ± standard deviation.
$C_{0d\text{-}egg}$, concentrations of PFAS in whole eggs sampled just after fertilization, ng/egg.
ND: less than MDL.
* $C_{swim\text{-}up}$ significantly less than $C_{30\text{-d}}$ or $C_{28\text{-d}}$, $p < 0.05$.

and PFESAs have been demonstrated to be eliminated more easily by biotic systems as a result of their greater hydrophilicity compared to the legacy PFAS (Wang et al., 2020). In the current study, GenX was also quickly eliminated from salmonid eggs with a rate similar to that of PFBA, and a considerably greater elimination rate compared to that of PFOA. When comparing PFAS containing the same number of carbon atoms, PFCAs were found to be more rapidly eliminated than PFSAs, indicated by the greater rates of elimination of PFBA and PFOA in comparison with PFBS and PFOS. This trend has also been observed in other studies, including humans, monkeys and rodents (Pizzurro et al., 2019; Xu et al., 2020).

For all PFAS studied, the apparent initial elimination happened during the first 3 days post-fertilization, while no obvious elimination occurred after that until a month post-fertilization. PFBA, PFBS and GenX were further eliminated during subsequent development until the swim-up stage, while amounts of PFOS and PFOA were relatively stable until swim-up. PFAS could be interacting with proteins both on the chorion and in the yolk, while there still could be free PFAS on the chorion and in the yolk, particularly at greater exposure concentrations, when the available protein binding sites could have reached saturation (NICNAS, 2005; Vogs et al., 2019). Rapid elimination just after fertilization probably represents the elimination of free PFAS and those bound to low affinity sites. PFAS bind with the proteins by electrostatic, ionic, and hydrogen bond interactions (De Silva et al., 2021). Loosely bound PFAS on the chorion and in the yolk might be eliminated during the first 3 days of incubation, while the more strongly bound PFAS take longer to be eliminated. The decreased elimination of PFAS between days 3 and 28 of incubation also demonstrates the decreased permeability of the chorion as it matures over the incubation period.

Fish were not fed during the incubation period and gained nutrients only from the sac yolk. At the swim-up stage, the yolk sac was totally absorbed and the fry would need to obtain external food. It is anticipated that going into the feeding phase elimination of PFAS in fishes would vary, and different patterns might be observed among various PFAS. Experiments including later development points, e.g. maturity and reproduction, are needed to investigate the longer term elimination characteristics of the strongly bound PFAS.

PFAAs are resistant to degradation including biodegradation, photolysis, hydrolysis, and are thus persistent in the environment (Brendel et al., 2018). Short-chain PFAS and some other novel substitutes have been demonstrated to be more resistant to degradation even by the artificial treatment processes (Li et al., 2020). The shorter half-lives in fishes and greater persistence in the environment of some short-chain PFAS cause rapid accumulation of these PFAS in the natural environment. In addition, their larger production nowadays further accelerates that accumulation. Other toxicological data will be needed to assess the ecological risks of long-term exposure to these low-dose short-chain PFAS.

In both char and trout eggs, concentrations of PFAS in whole eggs (ng/g) were greater than their concentrations (ng/g) in egg yolks for all the PFAS studied. PFAS in whole eggs were 1.2–2.7 fold greater for char eggs, while 1.0–2.1 fold greater for trout eggs. PFAS getting into the salmonid eggs were distributed both in egg chorions and yolks. However, their respective bioavailability is hard to know. Additional studies will be needed to determine whether PFAS amounts in the whole eggs or PFAS amounts in the egg yolks are a better indicator of internal bioavailable doses. Considering that PFAS amounts in egg yolks and whole eggs were similar a month after fertilization, and that PFAS amounts in salmonid eggs were relatively stable after 3 DPF, it can be supposed that PFAS amounts in whole eggs and egg yolks would be very similar in the salmonid eggs sampled 3 DPF. Since aspirating the yolk using needles is likely to introduce variability amounts of PFAS in whole eggs (after 3 days) provides a suitable metric of internal dose that can be related to toxic effects. Assessment of adverse effects on fry from the above experiments is currently underway.

5. Conclusion

All five PFAS, PFBA, PFBS, GenX, PFOA and PFOS, were accumulated into char and trout egg yolks at similar rates through 3 h pre-fertilization exposure. All five PFAS demonstrated relatively rapid elimination from eggs up to 3 DPF, thereafter elimination was minimal until 30 DPF. PFBA, PFBS and GenX were steadily eliminated during development from a month after fertilization to the swim-up stage, while PFOA and PFOS contents in salmonid fishes were still relatively constant from hatch until swim-up. In addition, the short-chain PFAS (PFBA and PFBS) and the PFECA (GenX) demonstrated greater rates of elimination in salmonid fishes studied here, compared with longer-chain, legacy PFAS (PFOS and PFOA).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.152547.

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Absorption and elimination of per and poly-fluoroalkyl substances substitutes in salmonid species after pre-fertilization exposure

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Table S1. Method detection limits of five PFAS in egg, yolk, fry samples and exposure solutions

	Egg, ng/g		Yolk, ng/mL	Fry, ng/fry	Exposure solution, ng/mL
	char	trout			
PFBA	0.948	0.975	0.887	0.027	0.665
PFBS	0.266	0.273	0.249	0.007	0.187
GenX	0.713	0.733	0.667	0.020	0.500
PFOA	0.168		0.157	0.005	0.118
PFOS	0.198	0.204	0.186	0.006	0.139