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Developmental exposure to environmentally relevant PFOS and PFBS disrupts adult behaviour, reproductive fitness, and lipid metabolism in Zebrafish

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Abstract

Background Poly- and perfluoroalkyl substances (PFAS) are persistent pollutants affecting wildlife and biodiversity. Perfluorooctane sulfonic acid (PFOS) and one of its short-chain substitutes, perfluorobutane sulfonic acid (PFBS), are widely found in environmental components, especially in water. PFOS has been highlighted as causing deleterious effects on various organisms while PFBS adversity is suspected but requires further investigation. In this study, zebrafish embryos were exposed from 2 h post-fertilization to 28 days post-fertilization to two different concentrations (0.2 µg/L and 2 µg/L) of PFOS or PFBS. We then investigated the impacts of these early exposures later in life on adult fish fitness, growth, morphology, behaviour, and liver lipidomic profiles.

Results PFOS exposure significantly reduced egg production, and both PFOS and PFBS altered growth patterns, organ development, and anxiety-like behaviour. Lipidomic analyses revealed persistent shifts in liver lipid composition that correspond to these phenotypic changes.

Conclusions Taken together, our findings indicate that early-life exposure to low levels of PFOS and PFBS leads to long-term, sex-specific impairments in zebrafish physiology and behaviour, with disruptions in lipid metabolism emerging as a potential underlying mechanism.

Keywords PFAS, Pollution, Zebrafish, Behaviour, Reproduction, Adverse effects, Lipidomic

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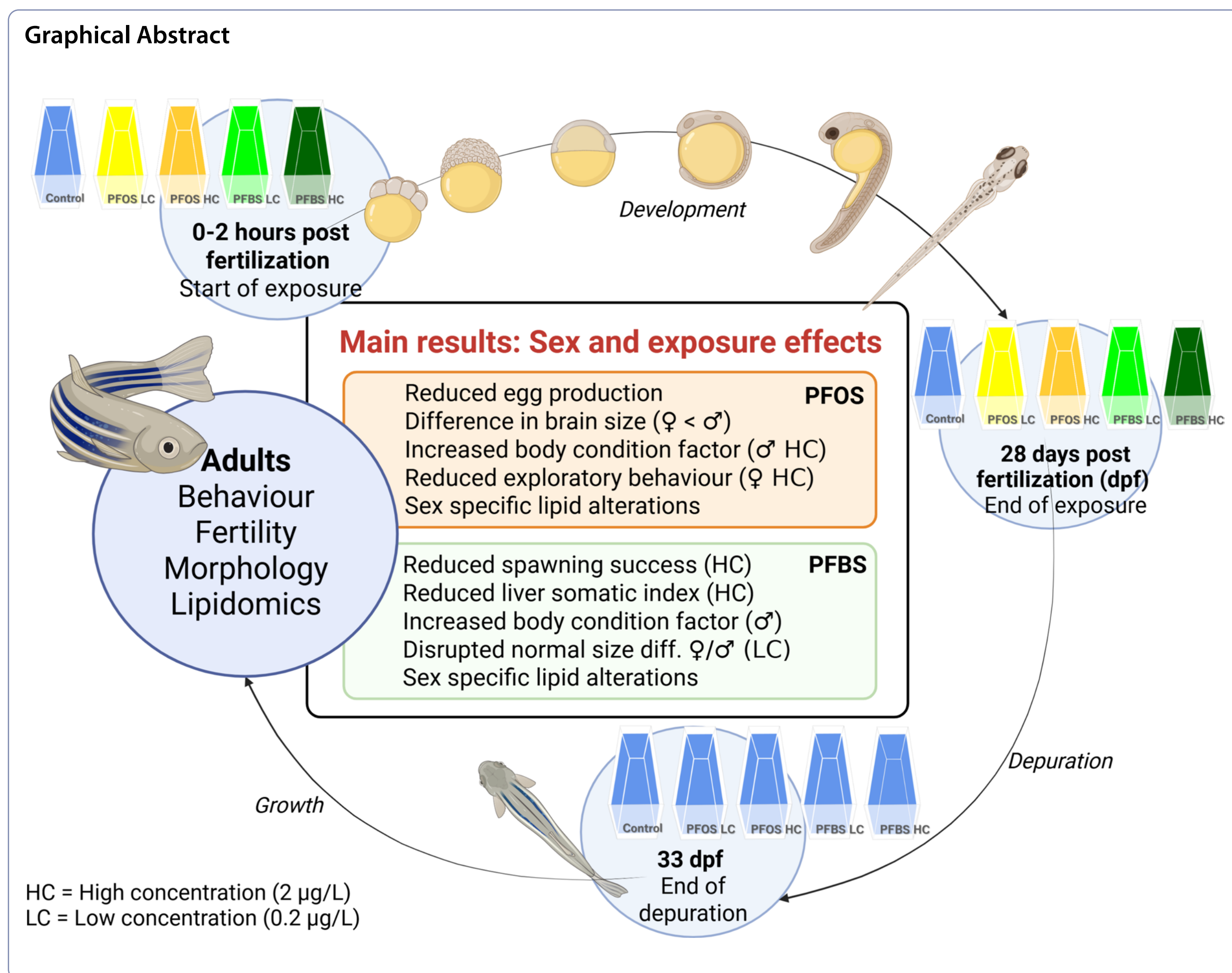

Background

Poly- and perfluoroalkyl substances (PFAS) have received worldwide attention due to their persistence, toxicity, and bioaccumulative potential [1, 2]. Among PFAS, perfluorooctane sulfonic acid (PFOS) has been extensively studied as it represents a major portion of PFAS contamination [1, 3]. PFOS is a precursor of various other PFAS and emerges through metabolomic or environmental degradation [4]. Its extreme persistence, toxicity, bio-accumulation, and transport across all environmental media has led to strict regulation under Annex B of the Stockholm Convention [1, 5]. However, long-chain PFAS, including PFOS, are still emitted through precursors degradation, historical products, and remobilization from soils and sediments [1, 6]. PFOS concentrations in aquatic systems range from 10 to 730 ng/L, reaching up to 8000 ng/L in Swedish water supplies [3].

PFOS has been shown to biomagnify, exhibiting concentrations in benthic invertebrates up to 1000-fold higher than in water [7], and causing developmental and neuro-toxicity in different vertebrates [4, 8–10]. In zebrafish embryos and larvae, PFOS exposure results in various deformities, decreased heart rate, and neurotoxicity including impaired neuronal growth, brain necrosis and behavioural alterations [11–13]. PFOS also affects liver lipid metabolism [14, 15], induces endocrine disruption [10, 16], and causes immunotoxicity [17, 18]. In humans, PFOS has been detected in blood, breast milk, and in various tissues including placenta and foetal organs [2, 19], which has been linked to reduced birth weight, impaired immune response, and metabolic changes [20, 21].

Perfluorobutane sulfonic acid (PFBS), a short-chain PFAS, has been introduced as a PFOS alternative due to its lower toxicity and shorter half-life in humans (ca. 1 month vs. 5.4 years) [22–25]. PFBS is widely used in industrial and consumer products. Its environmental persistence and mobility contribute to frequent detection in groundwater and surface waters [26], as well as in biota [27–30]. Moreover, studies have reported PFBS

concentrations ranging from 0.01 to 4520 ng/L in aquatic systems [31]; altogether resulting in a classification of PFBS and its salts as "Substances of very high concern" by the European Chemicals Agency.

Though PFBS is considered less toxic than PFOS, studies show that PFBS can induce developmental and reproductive toxicity, as well as disrupt endocrine function in zebrafish and other model organisms [22, 32–34]. Notably, PFBS elicits the most substantial behavioural alterations in zebrafish among various PFAS [35]. Despite these findings, the long-term effects following early-life exposure to low environmental concentrations of PFBS are poorly understood.

Previous studies have demonstrated the potential of PFOS and PFBS to alter lipid metabolism in fish [36, 37]; however, the adverse outcomes of such lipidomic changes directly remain largely unknown. Additionally, prior studies often used high concentrations and short-term exposure, which fail to reflect real-life exposure scenarios. To date, only one study has investigated low PFOS concentrations in fish but used only a short-term exposure over 5 days [38]. To our knowledge, no comparable studies exist for PFBS, nor have any examined effects of longer-term developmental exposures to either compound.

To address these knowledge gaps, this study investigates the long-term effects of subchronic (28 days) early-life exposure to environmentally relevant concentrations of PFOS and PFBS in zebrafish. We assessed adult behaviour, reproduction, morphology, and liver lipid profiles to identify persistent and sex- and exposure-specific adverse toxicological outcomes. Our findings demonstrate that even low-concentration developmental exposure can lead to persistent physiological and behavioural consequences later in life, with lipid metabolism emerging as a potential mechanistic link.

Methods

Pre-testing

PFOS (potassium salt, CAS 2795-39-3) and PFBS (tetrabutylammonium salt, CAS 108427-52-7), both with purity > 98%, were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Stock solutions (1050 µM for PFOS; 825 µM for PFBS) were prepared in aquarium water and stored at room temperature. Concentrations of working solutions were verified using liquid chromatography-mass spectrometry (LC-MS), yielding 2.41 µM PFOS and 2.64 µM PFBS.

Exposure concentrations were selected to avoid lethality during chronic exposure of 28 days. No-effect-concentrations (NOECs) were determined using a simplified Fish Embryo Toxicity (FET) test [39]. PFOS concentrations were ranging from 0.75 nM to 6.00 nM. Lethal endpoints including coagulation, lack of somite formation, non-detachment of the tail, and lack of heartbeat were monitored daily from 24 to 120 hpf. As a result, two concentrations were selected for subsequent chronic exposures: 3.716 nM (2 µg/L) and 0.37 nM (0.2 µg/L) designated as the High Concentration (HC) and Low Concentration (LC), respectively. Similar concentrations were used for PFBS: 6.6 nM (2 µg/L; HC) and 0.67 nM (0.2 µg/L; LC).

Fish husbandry

Wild-type zebrafish AB (*Danio rerio*; ZFIN ID: ZDB-GENO-960809-7) were housed at 26 ± 1 °C, with a 14:10-h light:dark cycle in a recirculating aquarium system at Örebro University. Water parameters were regulated by a Profilux 3.1 controller (GHL, Advanced Technology, Germany). Fish were fed twice daily (flakes in the morning and Artemia in the afternoon) [40].

After exposure, fish were maintained in a flow-through system under the same conditions.

Water chemical analysis

Triplicate water samples were collected on days 1, 7, 14, 21, and 28, and stored at − 20 °C. PFOS and PFBS concentrations were quantified using ultra-high-performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS) coupled with a XEVO-TQS (Waters Corp., Milford, MA) [41]. Analytical standards, including potassium salts of PFOS linear and branched PFOS and PFBS, mass-labelled standards of PFOS and PFBS (purity > 98%), were obtained from Wellington Laboratories (Guelph, ON, Canada). The purities of analytical standards were over 98%. Ammonium acetate (> 99%) and LC-grade methanol (99.9%) were purchased from Fluka (Steinheim, Germany) and Fisher Scientific (Leicestershire, UK), respectively.

The concentrations of PFOS and PFBS in the spiked water were measured using ultra-high-performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS) after adding mass-labeled standards, with a 50:50 volume-to-volume ratio of sample water to methanol containing the mass-labeled standard. The UPLC-MS/MS was a Waters Acquity UPLC coupled to a triple quadrupole mass spectrometer XEVO-TQS (UPLC-MS/MS, Waters Corp., Milford, MA); the column was a C18 BEH column (100 mm × 2.1 mm, 1.7 µm). Details of the chromatographic separation conditions are available in Uwayezu et al. [41]. The L-PFOS was detected as an individual isomer whereas the sum of Br-PFOS isomers of 3/4/5-PFOS and 6/2-PFOS were reported.

Limits of quantification (LOQs) were determined as three times the signal in the negative control; and in the absence of the analyte in the blank, the lowest point in

the calibration curve: 0.080 ng/mL − 1 for 3-/4-/5-PFOS, 0.098 ng/mL for 6-/2-PFOS, and 0.01 ng/mL for L-PFOS, 0.01 ng/mL for PFBS.

To ensure stable sensitivity over the entire instrumental analysis, a quality assurance (QA) sample made of PFAS standards was injected between every eight samples; a small deviation (< 10%) of QA signal was observed throughout all batch experiments. If the deviation was found to be greater than 10%, the batch was re-analyzed after maintenance of the LC–MS/MS.

Fish exposure and sampling

Six breeding groups (3 males and 2 females) spawned eggs that were transferred at 1 hpf into 2 L tanks with exposure solutions. Embryos were exposed under semi-static conditions at 26 ± 1 °C, on a 14:10 h light: dark cycle and aeration. Larvae from 5 days post-fertilization (dpf) were fed thrice daily with appropriate diets. From 28 to 35 dpf, exposure solutions were progressively replaced with aquarium water, after which juveniles were transferred in 4 L tanks in the flow-through system and maintained as described previously. Adult fish (> 3 months) were sampled for gonad, liver, and brain tissues for further analysis and stored at − 80 °C.

Phenotypical and functional analyses

Teratogenic and developmental effects

Developmental effects were assessed using a simplified FET test (OECD 236). For each treatment (control, PFOS LC, PFOS HC, PFBS LC, and PFBS HC), three technical replicates of 4 × 96-well plates were used with 16 embryos per condition. Developmental endpoints (e.g., coagulation, somite formation, and absence of heartbeat and blood circulation) were monitored from 24 to 120 hpf. Survival analyses were computed using the Kaplan–Meier method and health probability between conditions were analysed using a Cox proportional hazards regression model utilizing the R package "survival" (v4.3.1, R Core Team, 2013) [42].

Reproductive success

Reproductive success was investigated in six mating pairs per condition. After initial pair bonding and starting from the third successful breeding event, egg production was recorded over multiple breeding events (N = 30 per condition). Moreover, egg quality (healthy, coagulated, unfertilized) was documented.

Data were analysed using least-squares linear model (lm) with Condition as a fixed factor. Residuals were checked for normality (Shapiro–Wilk), equal variance (Breusch–Pagan; Levene), and influence (Cook's D > 4/n; hat values). When satisfied, we estimated treatment effects via Dunnett-adjusted contrasts (emmeans) from the lm; when violated, we used a robust Huber M-estimator (MASS::rlm) and applied the same contrasts.

Growth and morphology analysis

Adult fish and organs (brain, liver, and gonads) were weighed. Respective data were analysed following the same procedure as described in the previous paragraph. For the evaluation of morphological changes, Somatic Indices (SI) were investigated.

$$\text{Body Condition Factor (BCF)}: \text{BCF} = \text{BW}/\text{L}^3 (\text{BW} = \text{body weight, L} = \text{body length})$$

$$\text{Gonadosomatic Index (GSI)}: \text{GSI} = \text{GW}/\text{BW} \left(\text{GW} = \text{gonads weight}\right)$$

$$\text{Brainsomatic Index (BSI)}: \text{BSI} = \text{BrW}/\text{BW} \left(\text{BrW} = \text{brain weight}\right)$$

$$\text{Hepatosomatic Index (HSI)}: \text{HSI} = \text{LW}/\text{BW} \left(\text{LW} = \text{liver weight}\right).$$

For each index, nested models were compared: (i) index ~ Condition, (ii) index ~ Condition + Sex, and (iii) index ~ Condition * Sex, using ANOVA to identify the best fit. Then, the index was log-transformed to improve distribution. After transformation, residuals were assessed using Shapiro–Wilk for normality, Breusch–Pagan for homoscedasticity, and Cook's distance for influential points. If assumptions were met or not, ordinary least-squares fit (lm) or refit with robust regression (rlm) to down-weight outliers were applied, respectively. Finally, pairwise contrasts versus control were computed with Dunnett adjustment.

Behavioural experiments

Anxiety-like behaviour was assessed using the novel tank diving test (NTT) [43, 44]. Thirty fish per treatment (15/sex) were investigated. Fish were acclimated overnight in 3 L isolation tanks and tested in a 1.5 L novel trapezoid tank (Aquatic Habitats, USA). Vertical activity was recorded for 4 min, and the behaviour was analysed using EthoVision® XT. The novel tank was divided into bottom and top zones for the evaluation of the activity. For each endpoint, we compared each exposure to Control using two-sided Wilcoxon rank-sum tests with Benjamini–Hochberg FDR adjustment (R v4.3.1).

Lipidomic analysis

Lipidomic extraction and liquid chromatography

Livers from six females and six males for each condition, except PFOS HC (six/five), PFBS LC (five/five), and PFBS HC (6/4) were prepared for lipidomic analysis. Livers were weighed and lysed in 350 µL of RP1 buffer (Triprep extraction kit, Macherey–Nagel, France). Total lipid extraction was performed as described in Blanc

et al. 2021 [45]. Analysis was done by using an ultra-high-performance liquid chromatography quadrupole time-of-flight mass spectrometry (UHPLC-Q-TOF/MS) previously described [46]. Additional information can be found in Supplemental Material.

Data analysis

Data analysis was performed using MetaboAnalyst 6.0 [47] and R (version 4.3.1). The dataset was pre-processed by applying $\log_{10}$ transformation and autoscaling (i.e., scaling to zero mean and unit variance). First, we fitted a limma-voom model (sex ~ condition) on the full dataset, then performed fold-change and *t*-tests ($p < 0.05$) for each control-vs-exposed, stratified by sex. Significant m/z features ($p < 0.05$) were used for functional analysis, employing both the Mummichog and GSEA algorithms (KEGG + MTF libraries, *Danio rerio*) considering the following adducts: M + H[1 +], M + Na[1 +], M + K[1 +], M + H2O + H[1 +], M-H2O + H[1 +], M-NH3 + H[1 +], M + HCOONa[1 +], and M-HCOONa + H[1 +]. Bubble plots were generated in R to depict significant pathway alterations (Mummichog and GSEA cumulated *p*-value < 0.05) between each condition and the control for each sex.

In parallel, to aid interpretation, lipids were grouped into biological classes, including ceramides (Cer), phosphatidylcholines (PC), cholesteryl esters (CE), phospholipids (PL), phosphatidylethanolamines (PE), sphingomyelins (SM), lysophosphatidylcholines (LPC), retinoids (RE), triglycerides (TG), amino acid derivatives (24-diaminobutyric acid and 4-amino-1-piperidinecarboxylic acid, AA), and unidentified metabolites (unknown). Using these class-level abundances, we then performed PLS-DA separately for each sex to compare conditions and identify the lipid classes that most strongly discriminate between them.

Correlation analyses

Spearman correlations were conducted between lipidome changes and phenotypic endpoints (morphology, reproduction, and behaviour). Morphology and lipids were measured in the same individuals; reproduction and behaviour came from separate cohorts. To integrate datasets, we averaged each lipid within sex x exposure-condition groups and assigned those group means to reproduction and behaviour records with the matching sex and condition. Therefore, four correlation matrices were designed: lipids versus morphology, egg output, spawning success, and behavioural endpoints. For each, partial Spearman correlations were conducted for all exposure conditions and both sexes to identify sex-specific relationships.

Heatmaps and bubble plots visualized class-level trends and intersected lipids linked to both PFAS exposure and phenotypic effects. Significantly intersected lipids ($p < 0.05$ and FDR-adjusted $q < 0.05$) were taken forward for pathway enrichment using both Mummichog and GSEA (cumulated $p < 0.05$).

Results

To investigate long-term effects of PFAS, zebrafish embryos were exposed for 28 days to control, PFOS LC, PFOS HC, PFBS LC, and PFBS HC. Exposures were verified through assessments of embryonic health and chemical analysis (Sect. "Verification of exposure conditions"). Adult zebrafish (> 3 months) were evaluated for alterations in behaviour, hepatic lipid composition, reproductive capacity, and morphological alterations. Raw data for the various analysis can be found in the supplemental materials.

Verification of exposure conditions

No significant difference in embryo health (24-120hpf) was observed between the control and the different exposure conditions, confirming the concentrations suitability (Sup. Fig. S1).

Mean concentrations of both chemicals in the control was < 10 ng/L. Measured PFOS and PFBS concentrations in the tanks matched nominal values with minor variability (Sup. Tab. S1).

Sex differences for both phenotypical and lipidomic endpoints

As expected, significant differences were found between males and females (Sup. Tab. S2) for the condition factor GSI, and BSI (*t*-test, all p.adj < 0.05) but not for the LSI (*t*-test, *p.adj* = 2.06e-01). Liver lipidomic analysis revealed 317 differentially expressed lipids between sexes (*p.adj* < 0.05; Sup. Fig. S2 and Sup. Tab. S3). For the behavioural analyses, no sex effect was observed overall.

PFOS exposures alter adult phenotypes in a sex-dependent manner

Reproduction, morphological indices, and behaviour

PFOS LC and PFOS HC exposure significantly reduced egg production (p.adj = 2.533e-04, 8.35e-03; Fig. 1a). Adult fish (> 5 months) were investigated for length, brain, gonads, liver, and total weight. Results showed non-significant increase of the BCF in PFOS HC (Sup. Fig. S3a). However, sex-specific analysis showed a significant increase in BCF for males exposed to PFOS HC (*p.adj* = 2.06e-02) (Fig. 1b).

For the GSI, no significant difference was observed in both sexes (Sup. Fig. S3b).

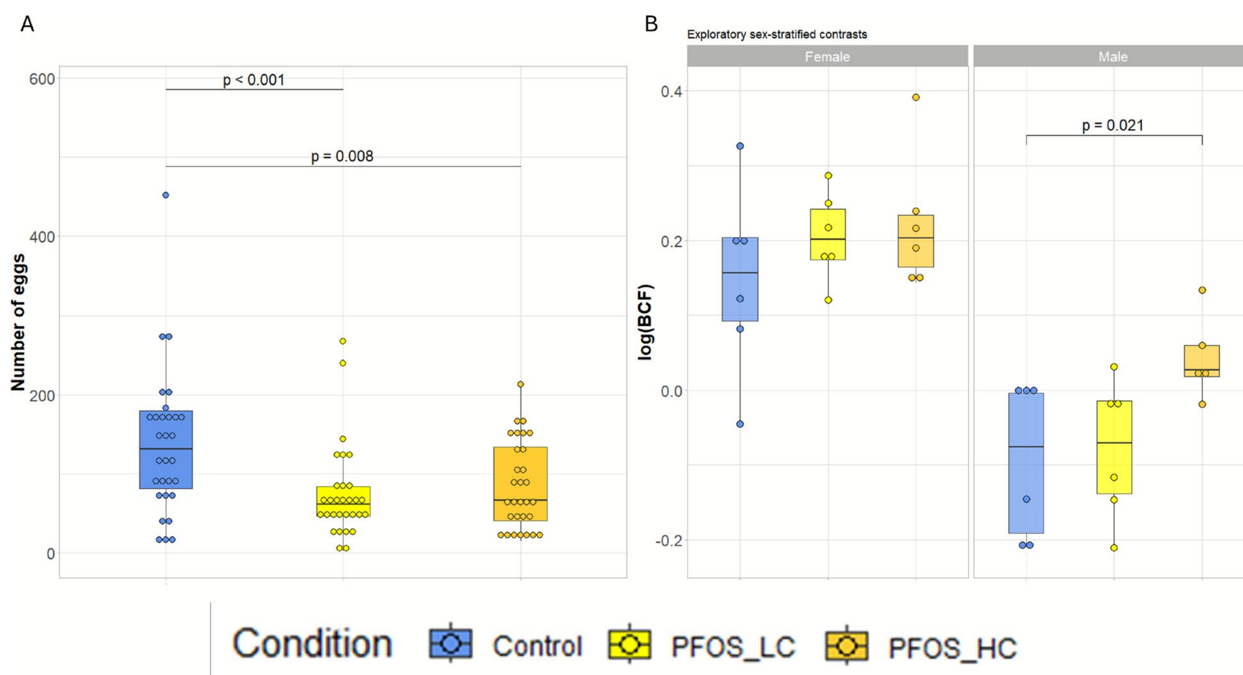

Fig. 1 **A**. Total number of eggs produced (y-axis) by each fish pair (N = 30). A significant difference was observed between control and PFOS LC (Dunnett *p.adj* = 2.53e-04) and control and PFOS HC (Dunnett *p.adj* = 8.35e-03). **B**. Log of fish body condition factor (y-axis) for females (left panel) and males (right panel) (N = 6 per condition) in the different exposure conditions. A significant increase was observed for PFOS HC males (Dunnett *p.adj* = 2.06e-02) compared to control males. For the whole figure, the colour code represents the different exposure conditions with control (blue), PFOS LC exposure (yellow), and PFOS HC exposure (orange)

BSI were significantly different between sexes under PFOS LC and HC exposures (*p.adj* = 1.78e-03, 1.53e-04), though the control group showed no difference (*p. adj* = 8.04e-02) (Fig. 2a).

PFOS HC exposure significantly increased latency to enter the top zone in the NTT (*p.adj* = 2.30e-02) (Fig. 2b). PFOS HC-exposed females exhibited a trend toward longer latency (*p.adj* = 6.20e-02) (Sup. Fig. S4a). Moreover, these females made significantly fewer entries into the top zone (*p.adj* = 1.70e-02), indicating increased anxiety-like behaviour. Males showed no change, and no significant effect was seen in the combined group for the number of entries to top zone (Sup. Fig. S4b).

Hepatic lipidomics

Untargeted UHPLC-QTOF analysis identified 595 distinct lipid species across all treatment groups for subsequent statistics and pathway analysis (Sup. Table S4).

Although no lipid passed the FDR correction, we observed robust nominal trends ($p < 0.05$) resulting in persistent hepatic lipidome changes after embryonic PFOS treatment (Sup. Tab. S5). PFOS LC elicited 31 altered lipids in males (13 up- and 18 down-regulated), and 20 in females (9 up- and 11 down-regulated). PFOS HC treatment caused 12 lipid changes in males (8 up- and 4 down-regulated), and 28 in females (9 up- and 19 down-regulated). In the female PFOS LC group, enrichment analysis highlighted fatty acid metabolism and C21 steroid biosynthesis (cumulated p = 3.30e-02), each driven by significantly altered cholesterol. PFOS HC females exhibited a strong perturbation of fatty acid oxidation (cumulated p = 5.31e-03), supported by 2,6-Dimethylheptanoyl-CoA (Fig. 3). PFOS exposure caused subthreshold shifts in bile acid synthesis (LC) and carnitine shuttle (HC) in adult females, suggesting an impairment of lipid homeostasis and mitochondrial oxidation.

PLS-DA (Partial least squares-discriminant analysis) showed separation between the control and PFOS LC female groups while PFOS HC and the control formed an overlapping cloud (Fig. 4a). PFOS LC caused a pronounced increase in retinoids, phosphatidylethanolamines, and sphingomyelins, whereas phosphatidylcholines (PC) and broader phospholipids (PL) dominate the PFOS HC profile (Fig. 4b). For males, PFOS LC samples deviated strongly from controls, while PFOS HC occupies an intermediate position (Fig. 4c). Group Variable Importance in Projection (VIP) scores revealed that primary discriminators for PFOS LC are

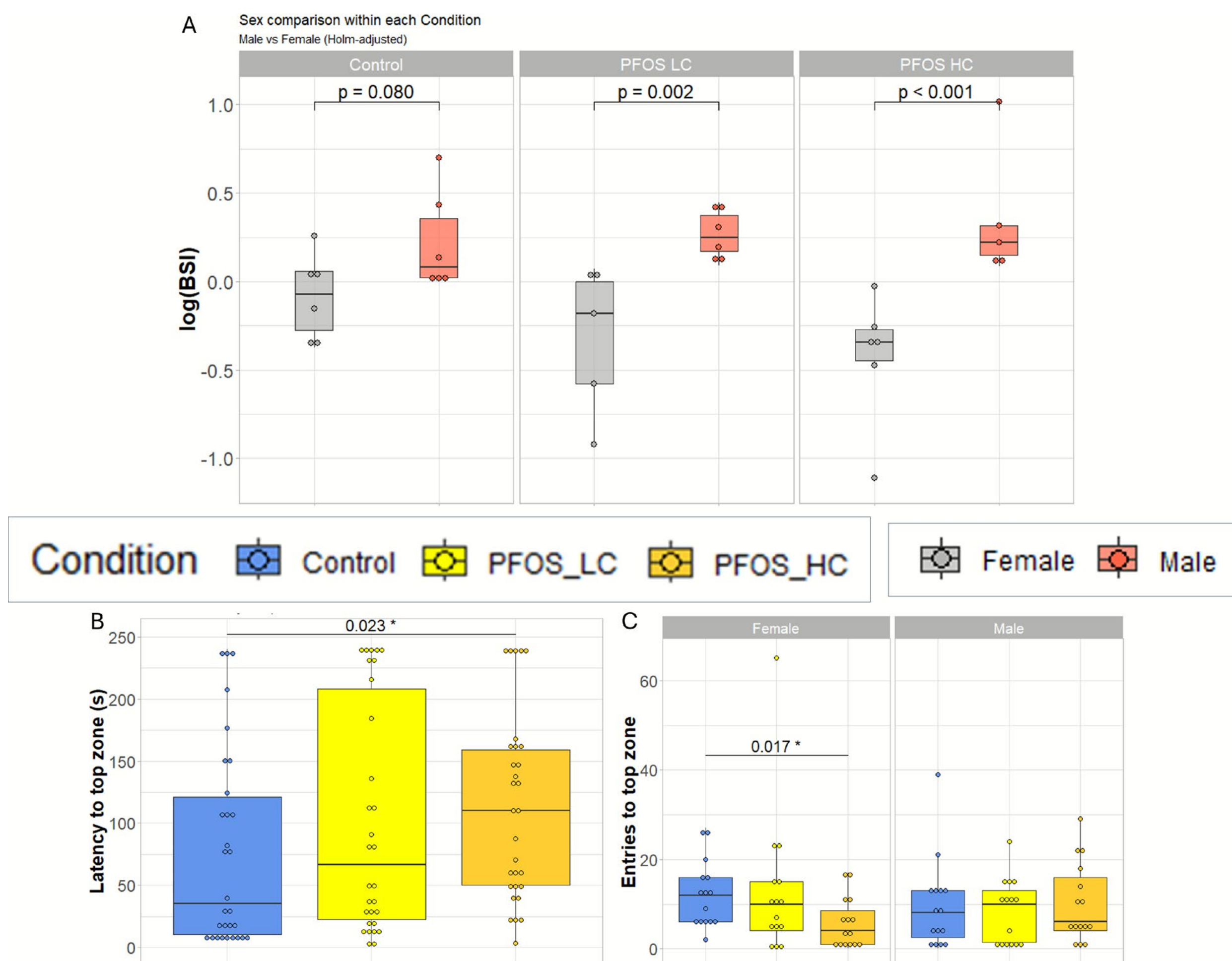

Fig. 2 Effects of developmental PFOS exposures on adult brain and behaviour. **A**. Brain somatic index comparison between sex (N = 6 per condition) in the different conditions. A significant reduction was observed in females compared to males for PFOS LC (*p*.adj = 1.78e-03) and for PFOS HC (*p*.adj = 1.53e-04). **B**. Latency for adult fish to reach the top zone (y-axis) during the NTT behavioural test in each condition. Exposure to PFOS HC led to an increase in the latency for fish to enter the top zone (Wilcox test, Control vs. PFOS HC, *p.val* = 2.30e-02) group. N = 30 fish per exposure condition. **C**. Number of entries into the top zone (y-axis) during the NTT behavioural test on adults for each condition. A significant reduction was observed in females exposed to PFOS HC compared to females in the control condition (Wilcox-test, *p.val* = 1.70e-02). N = 30 fish per exposure condition (15 males and 15 females)

amino-acid derivatives (AA), CE and PC (Fig. 4d), and for PFOS HC triglycerides (TG).

PFBS exposures induces sex-specific alterations in adult fish

Reproduction, body indices, and behaviour

Significantly fewer successful reproductive events were observed in PFBS HC (*p.adj* = 2.68e-02) (Fig. 5a).

PFBS HC exposed fish showed a significant reduction in LSI (*p.adj* = 1.74e-02) (Fig. 5b) while PFBS LC-exposed males exhibited a significant increase in BCF (*p.adj* = 2.53e-02) and PFBS-HC-exposed males followed a similar trend (*p.adj* = 7.58e-02) (Fig. 5c). Natural sex-based difference in BCF, disappeared under PFBS-LC exposure (*p.adj* = 0.80) (Fig. 5d).

For the BSI, PFBS HC caused a significant difference between males and females (p.*adj* = 2.41e-03); however, a sex-difference in control fish or fish exposed to PFBS LC was not observed (Fig. 6a). The behavioural analysis revealed that PFBS LC-exposed females spend significantly more time at the tank bottom than their male counterparts (p.*adj* = 2.30e-03, Fig. 6b) and trended toward fewer top zone entries compared to control females (*p.adj* = 9.60e-02) (Sup. Fig. S5). PFBS-exposed males showed a non-significant increase in entries into the top zone, under both PFBS LC and PFBS

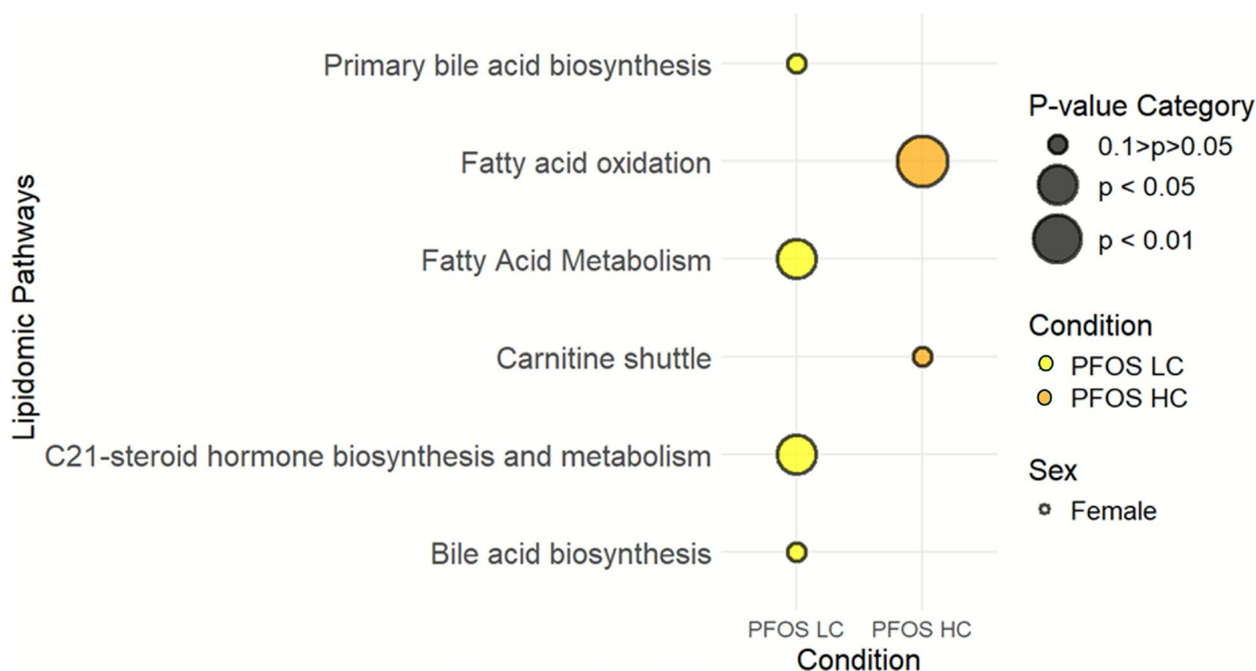

Fig. 3 Lipidomic pathway alterations in adult liver following developmental PFOS exposures. Lipidomic pathways altered in PFOS LC (yellow) or PFOS HC exposure (orange) compared to control. Alterations were observed only in females (circles). The size of the shapes corresponds to the significance of the p-value. Significant alterations in fatty acid metabolism (*pval* = 3.30e-02) and C21-steroid hormone biosynthesis and metabolism (*pval* = 3.30e-02) were noted in response to PFOS LC exposure, while changes in fatty acid oxidation were observed in PFOS HC-exposed females (*pval* = 5.31e-03). Finally, several pathways showed trends toward alterations, including bile acid biosynthesis and primary bile acid biosynthesis in LC and carnitine shuttle in HC. For the whole figure, $N_{control}$ = 12 (6 females and 6 males), $N_{PFOS\ LC}$ = 12 (6 females and 6 males) and $N_{PFOS\ HC}$ = 11 (6 females and 5 males)

HC exposure (PFBS LC: *p.adj* = 8.10e-02; PFBS HC: *p.adj* = 6.20e-02, Sup. Fig. S5).

Hepatic lipidomics

Like PFOS, PFBS exposure altered liver lipid profiles (Sup. Tab. S5). PFBS LC caused 22 altered lipids in males (9 up- and 13 down-regulated), and 13 in females (11 up- and 2 down-regulated). PFBS HC produced 20 altered lipids in males (12 up- and 8 down-regulated species) and 24 in females (16 up- and 8 down-regulated).

Enriched pathways revealed concentration- and sex-specific patterns (Sup. Tab. S6). In PFBS HC males, phosphatidylinositol phosphate metabolism was significantly altered (cumulated *p.val* = 2.34e-02), a signal driven by an Acyl-CoA species. PFBS HC females showed significant changes in glycosphingolipid metabolism (cumulated *p.val* = 2.74e-02) supported by N-Acetylneuraminyl-galactosylceramide, and squalene/cholesterol biosynthesis (cumulated *p.val* = 4.99e-02) driven by cholesterol. Several non-significant trends indicated potential dysregulation of steroid biosynthesis in females (cumulated *p.val* = 5.12e-02), glycosylphosphatidylinositol (GPI)-anchor biosynthesis (cumulated *p.val* = 8.41e-02) and bile acid biosynthesis (cumulated *p.val* = 9.20e-02) in males (Fig. 7).

PLS-DA analysis revealed that PFBS LC showed the strongest deviation from control while PFBS HC occupied an intermediate position in both sexes (Fig. 8a, c). In females, the VIP scores (Fig. 8b) identified LPC as the main discriminator in PFBS HC (VIP score > 2.5). Phospholipids were the major differentiating lipids between control and PFBS LC (VIP score > 1). TG and SM also contributed to a lesser extent.

In males, reduced amino acids-derived lipid levels led separation in the PFBS LC group (VIP score > 1.6) followed by reduction in LPC and retinoids in PFBS HC. PC increased moderately in PFBS LC (VIP ≈ 1.2). Together, these findings show that early-life PFBS exposure results in lasting sex-specific alterations in hepatic lipid composition, particularly affecting membrane integrity and energy–storage species.

Phenotypic endpoints correlate with the hepatic lipidome

Spearman correlations were conducted between liver lipid abundances per class (Sup. Tab. S7) and each phenotypic endpoint, first across the entire cohort (with sex

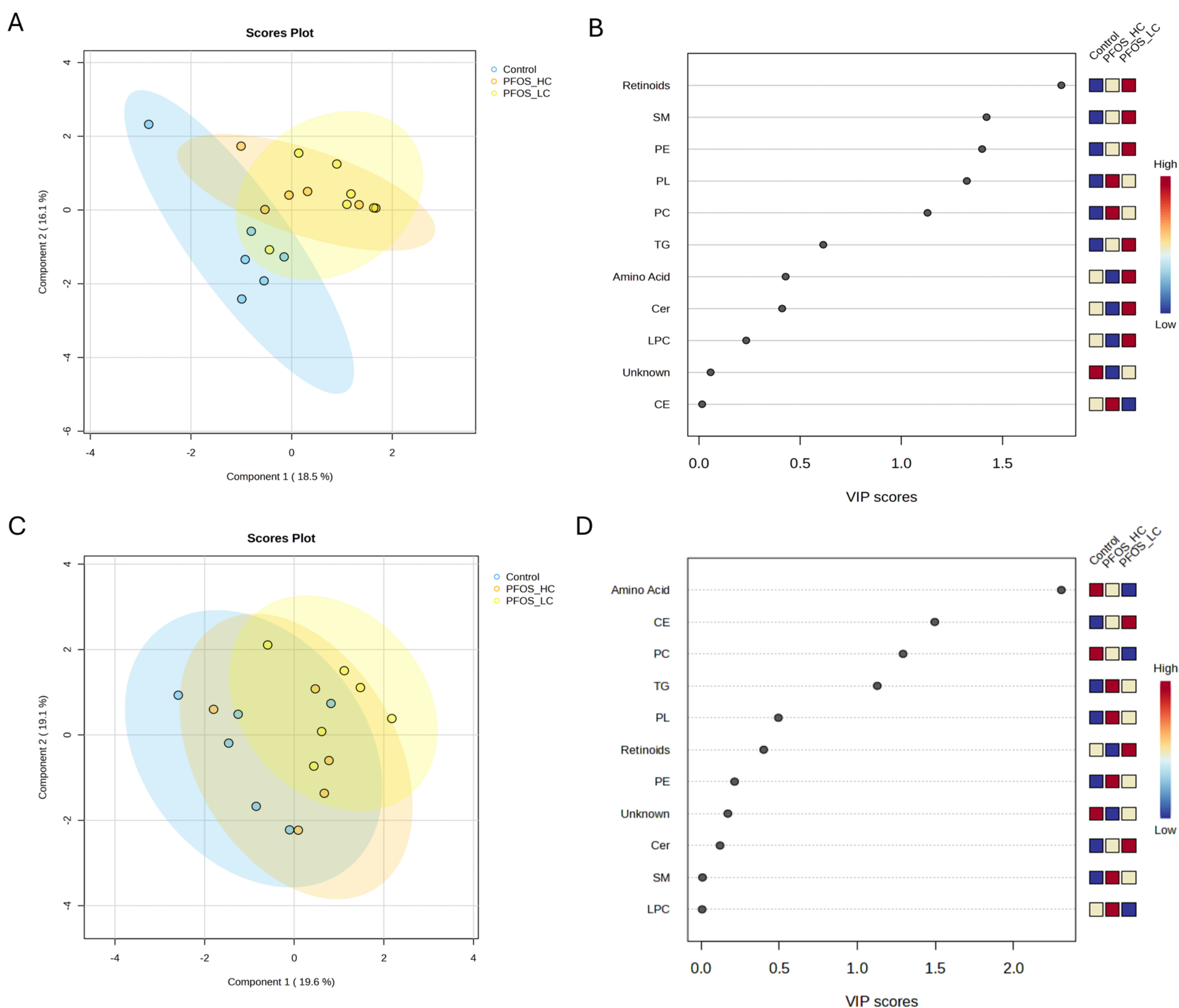

Fig. 4 Lipidomic alterations in adult liver following developmental PFOS exposures. **A**. 2D score plot from PLSDA analysis for females, highlighting the segregation of samples between the different exposure conditions. **B**. Variable Importance in Projection (VIP) plot for females displaying the top 15 lipid classes that contribute most to the observed differences between conditions. The x-axis shows the VIP scores, which are based on the weighted sum of the absolute regression coefficients. Lipid classes with high VIP scores play a significant role in distinguishing the conditions. The color-coded boxes to the right indicate the relative concentrations of these lipid classes across the different groups, providing further insight into which metabolites are most abundant and may contribute to the observed segregation in the score plot. **C**. 2D score plot from PLSDA analysis for males. D. Variable Importance in Projection (VIP) plot for males. For the whole figure, $N_{control} = 12$ (6 females and 6 males), $N_{PFOS\ LC} = 12$ (6 females and 6 males) and $N_{PFOS\ HC} = 11$ (6 females and 5 males)

and exposure as covariates) and then within each sex (Sup. Tab. S8).

In females, LSI correlated positively with a broad set of lipid classes: lysophosphatidylcholines, retinoids, amino-acid–conjugated lipids, sphingomyelins, phosphatidylcholines, phosphatidylethanolamines, and other phospholipids (Sup. Fig. S6a). In contrast, male LSI showed no such associations. Male GSI correlated exclusively with cholesteryl esters, while female GSI exhibited weaker association with retinoids and amino-acid lipids. Male BCF correlated with retinoid and amino-acid–conjugated lipid levels.

Egg production demonstrated a positive correlation with ceramide levels, whereas spawning success showed no lipid associations (Sup. Fig. S6b). In the NTT, males' exploratory behaviour (measured by entries into the top zone) correlated positively with phosphatidylcholines and phosphatidylethanolamines, while in females it was linked to amino-acid–conjugated lipids. Conversely, females' entries into the top-zone were negatively

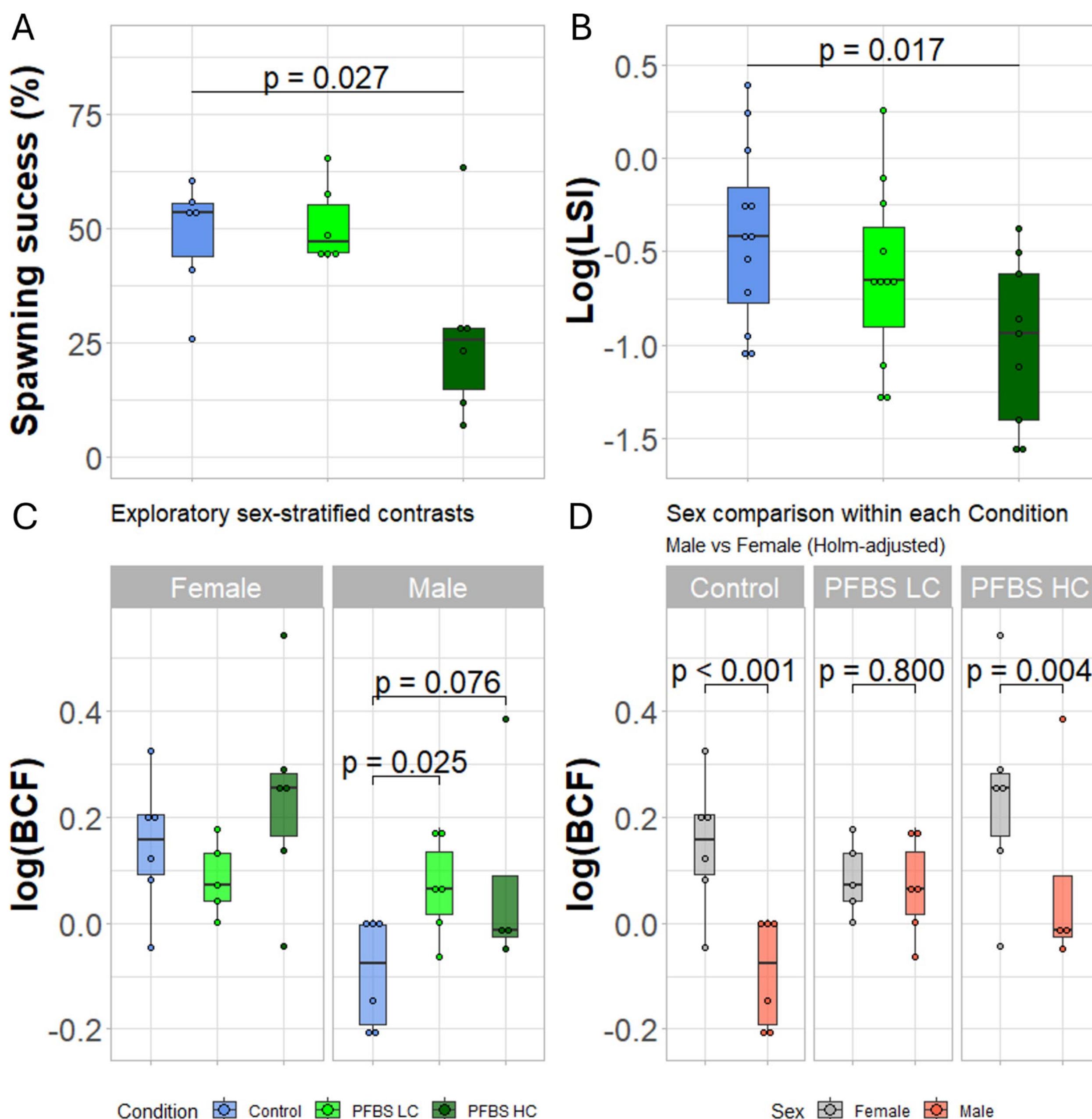

Fig. 5 Effects of PFBS developmental exposures on adult fertility and morphology. **A**. Percentage of spawning events leading to egg production (spawning success) (y-axis) by each fish pair (N = 6) in the control (blue), the PFBS LC exposure (green), and the PFBS HC exposure (dark green). A significant difference was identified with a reduction in spawning success in fish exposed to PFBS HC (*p.adj* = 2.68e-02). **B**. Liver somatic index comparison between conditions (N = 12 per condition) in the control (blue), the PFBS LC exposure (green) and the PFBS HC exposure (dark green). A significant reduction was observed in fish exposed to PFBS HC compared to control (*p.adj* = 1.74e-02). **C**. Fish body condition factor (y-axis) for females (left panel; N = 6 per condition) and males (right panel; N = 6 per condition) in the control (blue), the PFBS LC exposure (green), and the PFBS HC exposure (dark green). A significant increase was observed in males only, for PFBS LC (*p.adj* = 2.53e-02) and a trend toward increase was observed also in PFBS HC-exposed males (*p.adj* = 7.58e-02) compared to control. **D**. Body condition factor comparison between sex (N = 6 per condition) in the control (left panel), the PFBS LC exposure (middle panel) and the PFBS HC exposure (right panel) group. The significant difference between sex observed in control condition (*p.adj* = 6.66e-03) disappeared in fish exposed to PFBS LC condition (*p.adj* = 0.80)

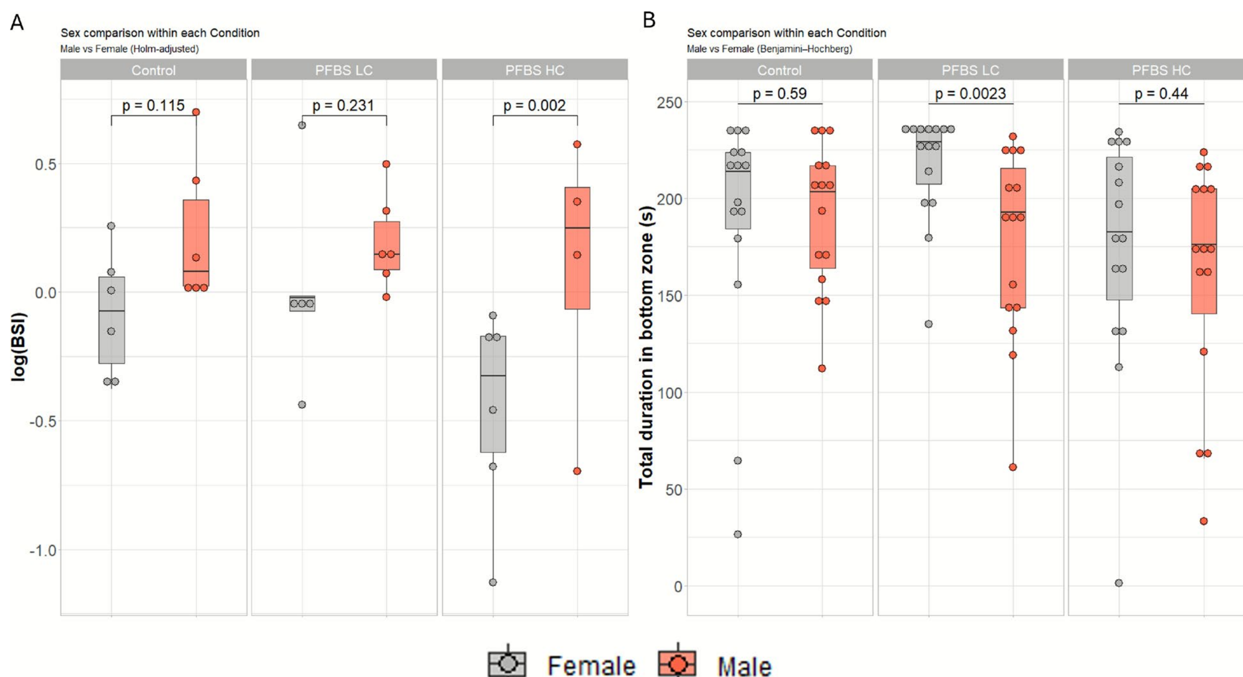

Fig. 6 Effects of PFBS developmental exposures on adult brain and behaviour. **A**. Brain somatic index comparison between sex (N = 6 per condition) in the control (left panel), the PFBS LC exposure (middle panel) and the PFBS HC exposure (right panel) group. A significant difference was observed between females and males exposed to PFBS HC (*p.adj* = 2.41e-03). **B**. Total duration spent in the tank bottom zone differentiated by sex in each condition. A significant difference in the time spent in the bottom zone was observed in PFBS LC between females and males. N = 30 fish per exposure condition (15 males and 15 females)

correlated with ceramides. Males' behaviour was negatively associated with LPC levels. Moreover, females' anxiety behaviour reflected in time spent at the tank bottom was positively related to ceramide concentrations and negatively to amino-acid lipids.

Pathways enrichment analysis based on these correlations pointed to several functionally relevant metabolic routes (Sup. Fig. S6c). Vitamin A metabolism was correlated with GSI in both sexes and with LSI in females. BSI was associated with fatty acid activation and de novo fatty acid biosynthesis in males. Fatty acid metabolism and C21-steroid hormone biosynthesis were linked to GSI in males. Lipids involved in phosphatidylinositol phosphate metabolism were associated with the number of entries to the top zone in both sexes.

To identify lipids that may mediate the observed phenotypes (raw $p < 0.05$ vs. control), we intersected the list of PFAS-exposure affected lipids with those significantly correlated to each phenotypic endpoint.

In PFOS exposed females, fertility was inversely correlated with several under-expressed species, most notably PC (40:5), TG (53:3), and TG (18:0/18:1/20:4), alongside several unannotated features. Reduced exploratory behaviour in PFOS HC females correlated with the downregulation of various triglycerides and multiple unknown lipids. In contrast, no lipid features met both criteria for BCF in PFOS HC males and for BSI differences in females, despite the phenotypic alterations.

Applying this intersected lipid set to pathway enrichment recapitulated the key sterol and fatty acid routes implicated above, reinforcing their mechanistic relevance (Fig. 9).

In the PFBS exposed cohort, three uncharacterized lipids were linked to reduced spawning success in HC males. In females, seven lipid features, including TG (20:4/14:0/18:3) and TG (18:0/20:1/20:4) were associated with reproductive impairment. For PFBS LC males, two non-identified lipids (m/z 213.1628 and 392.2916) were associated with the BCF, while in females, exploratory behaviour correlated with GlcCer (d18:1/26:1) and several unknown features (m/z 575.5017, 899.7078, 946.8439, 971.7440). PFBS HC males also showed associations between behavioural endpoints and both PE (20:1/22:6) and several unknown compounds.

The pathway analysis of these intersected lipids confirmed that a core group of lipid pathways, such as those related to membrane structure, sterol metabolism and energy balance were consistently affected by PFAS

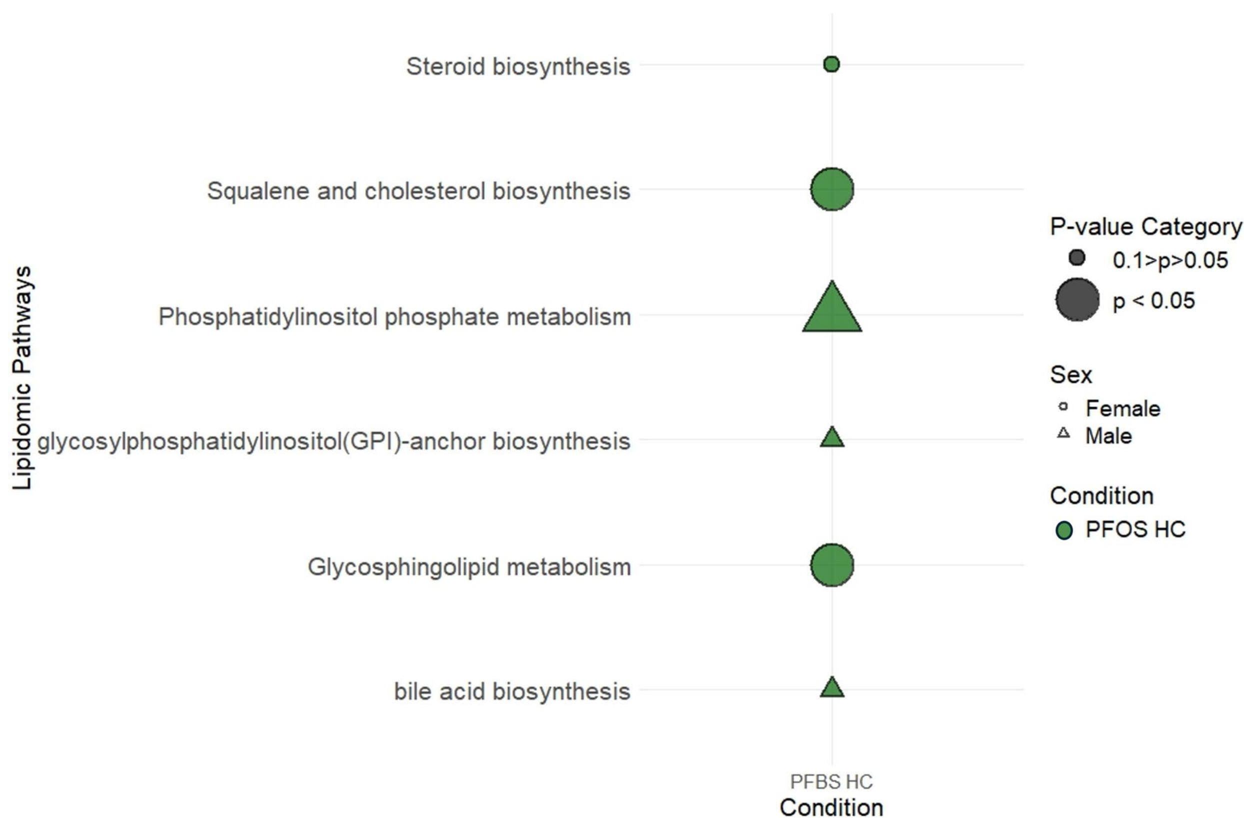

Fig. 7 Lipidomic alterations in adult liver following developmental PFBS exposures. Lipidomic pathways altered in PFBS exposure compared to control. No significant pathway was identified for PFBS LC exposure, therefore only PFBS HC exposure is represented (dark green). Males are represented by triangles and females by circles. The size of the shapes corresponds to the significance of the p-value. Males exposed to PFBS HC during development showed significant alteration in phosphatidylinositol phosphate metabolism (*cumulated pval* = 2.34e-02) as well as a trend toward alteration of glycosylphosphatidylinositol (GPI)-anchor biosynthesis *(cumulated p.val* = 8.41e-02) and bile acid biosynthesis (*cumulated p.val* = 9.20e-02). Females exposed to PFBS HC showed significant alterations in glycosphingolipid metabolism (*cumulated p.val* = 2.74e-02) and squalene and cholesterol biosynthesis (*cumulated p.val* = 4.99e-02) together with a trend toward changes in steroid biosynthesis (*cumulated p.val* = 5.12e-02). For the whole figure, $N_{control}$ = 12 (6 females and 6 males), $N_{PFBS\ LC}$ = 11 (5 females and 6 males) and $N_{PFBS\ HC}$ = 10 (6 females and 4 males)

exposure and linked to PFAS-driven phenotypic alterations (Fig. 9).

A summary of the results can be found in the Supplemental Material.

Discussion

PFOS-induced phenotypes

Previous research demonstrated that PFOS induces oxidative stress [48, 49], metabolic dysregulation [50] and is identified as a potential endocrine disruptor [51–53]. Divergent outcomes across PFOS studies, driven by differences in exposure duration, concentration, and whether effects are measured immediately versus after a recovery period [54, 55], suggest that many adverse responses only emerge or persist right after the initial insult. Short-term assays may miss delayed or lasting effects that develop in adulthood or differ by sex. Therefore, following organisms through to maturity after a defined developmental exposure is essential to capture the full spectrum of PFOS's long-term, sex-specific toxicities. In our study, PFOS exposure produced notable morphological differences with males exhibiting an increased body condition factor (BCF), while brain volume declined in females and rose slightly in males, creating a brain-size difference between the sexes that was absent in controls.

Reproductive fitness was clearly compromised as both PFOS LC and HC exposures led to significant reductions in egg output among females. This finding is consistent with PFOS bioaccumulation in gonadal tissue [56] and evidence that PFOS disrupts oocyte maturation by interfering with steroid hormones [52, 57]. However, we cannot dismiss a male contribution to this fertility loss as PFOS has been shown to impair sperm motility and dysregulate estrogenic signalling in males [58, 59], conditions that can weaken the hormonal and behavioural cues

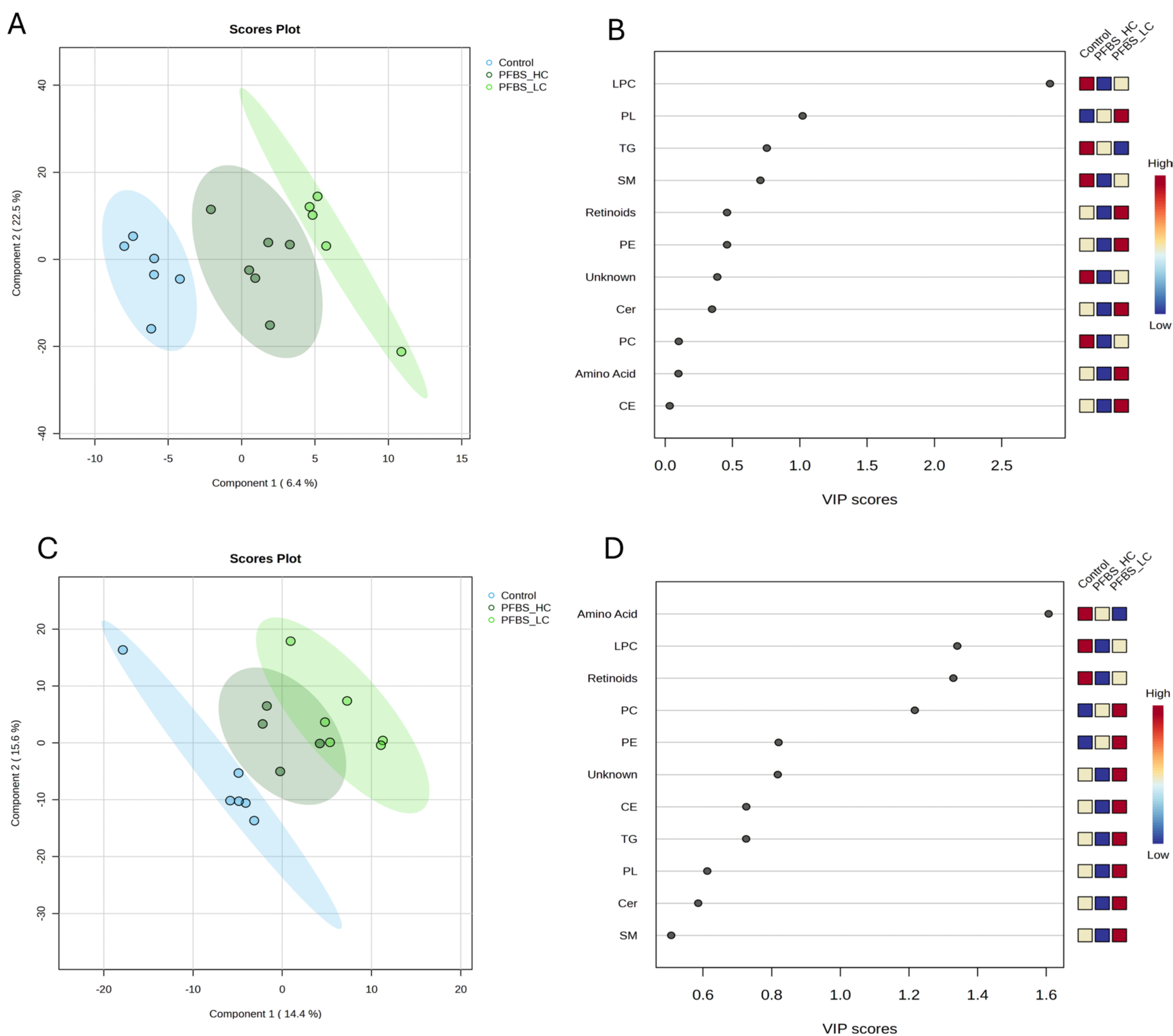

Fig. 8 Lipidomic alterations in adult liver following PFBS developmental exposures. **A**. 2D score plot from PLSDA analysis for females using the concentrations of 595 lipids aggregated in 11 classes, highlighting the segregation of samples between the different exposure conditions. **B**. Variable Importance in Projection (VIP) plot for females displaying the top 15 lipid classes that contribute most to the observed differences between conditions. The x-axis shows the VIP scores, which are based on the weighted sum of the absolute regression coefficients. Lipid classes with high VIP scores play a significant role in distinguishing the conditions. The color-coded boxes to the right indicate the relative concentrations of these lipid classes across the different groups, providing further insight into which metabolites are most abundant and may contribute to the observed segregation in the score plot. **C**. 2D score plot from PLSDA analysis for males. **D**. Variable Importance in Projection (VIP) plot for males

necessary to trigger female ovulation and spawning [60, 61].

In terms of behavioural impacts, PFOS-exposed females demonstrated reduced exploratory activity, aligning with prior studies that documented PFOS-induced neurological disruption [12]. In contrast, male behaviour appeared largely unaffected, underscoring sex-specific vulnerabilities in developmental neurotoxicity.

PFBS-driven effects

Although PFBS has been presented as a safer alternative to PFOS due to its shorter half-life and lower bioaccumulation potential, our results challenge this view. PFBS exposure led to subtle yet persistent sex-specific effects, including morphological changes, reduced reproductive success, and alterations in liver lipid metabolism. Males exposed to high concentrations of PFBS exhibited a

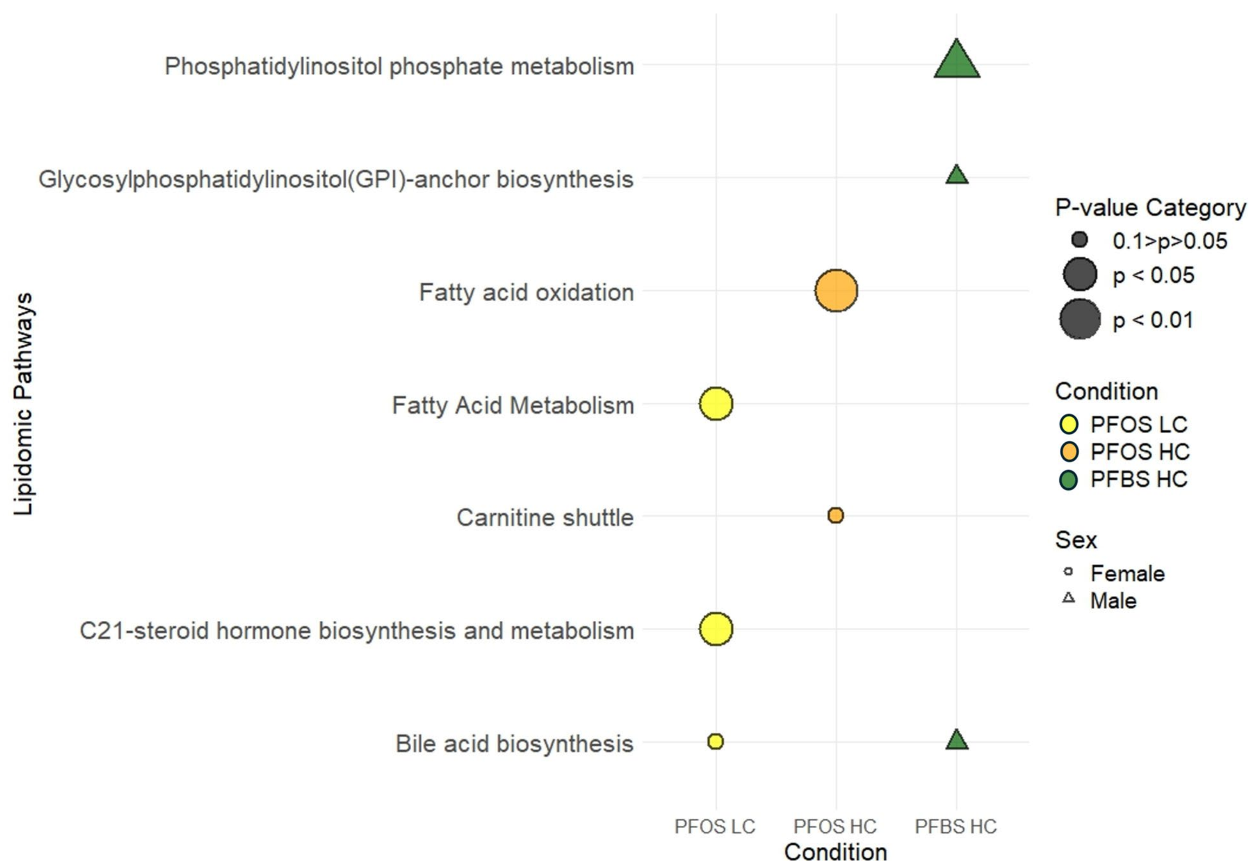

Fig. 9 Pathways analysis for lipids correlating with phenotypes and differentially expressed in response to the exposures. Three pathways were enriched for lipids associated with the studied phenotypes and being differentially expressed in PFOS LC in females only: Fatty acid metabolism (cumulated pval = 3.30e-02), C21-steroid hormone biosynthesis and metabolism (cumulated pval = 3.30e-02) and trend in bile acid biosynthesis (cumulated pval = 6.15e-02). In PFOS HC treated fish, only females showed enrichments in pathways linked to Fatty acid oxidation (cumulated pval = 5.31e-03) and trends in Carnitine shuttle (cumulated pval = 8.46e-02). Following PFBS HC exposure, this time, only males showed enriched pathways: Phosphatidylinositol phosphate metabolism (cumulated pval = 2.34e-02), and trends for Glycosylphosphatidylinositol (GPI)-anchor biosynthesis (cumulated pval = 8.41e-02) and Bile acid biosynthesis (cumulated pval = 9.20e-02)

significant reduction in liver somatic index (LSI), indicating hepatic stress.

We observed a significantly reduced spawning success in PFBS HC-exposed fish. The mechanistic basis of this impaired reproduction remains unresolved in zebrafish. However, work in other model systems, such as *C. elegans* [32], showed that PFBS can induce germ-cell apoptosis through oxidative pathways. While nematode findings cannot be extrapolated directly to fish, they point to oxidative stress and apoptosis as plausible routes worth testing in future zebrafish studies.

PFBS LC exposure abolished the normal sex dimorphism in BCF with females showing a slight, non-significant decline, whereas males displayed a significant increase, bringing both sexes to similar values. Such convergence is frequently observed in the context of endocrine disruption, most often reflecting male "feminization" via estrogenic or anti-androgenic activity. In teleosts, xenoestrogens like BPA can upregulate hepatic lipid synthesis in males, thus driving adiposity and secondary female-like traits [62, 63]. Our data indicate that PFBS may act in a similar way.

Previous work using high-concentration, multi-omics approaches has demonstrated that embryonic exposure to both PFBS and PFOS activates common neurotoxic pathways—namely oxidative stress, disruptions in lipid and energy metabolism, altered calcium signalling, and impaired bile acid synthesis [64]. Furthermore, acute PFBS treatment in zebrafish larvae was shown to elicit hyperactive swimming and perturb dopaminergic pathways [65], but these investigations have been limited to early-life effects and have not examined whether such changes endure into adulthood. In contrast, our results demonstrated that a sub-chronic, low-level embryonic PFBS exposure, at environmentally relevant concentrations, produces persistent, sex-specific changes in

adult zebrafish behaviour. PFBS exposed females exhibited increased bottom dwelling, a validated marker of heightened anxiety, whereas PFBS males displayed a complementary increase in top zone exploration. These divergent adult phenotypes suggest that PFBS can rewire neurodevelopment in a sex-dependent manner, long after the exposure has ceased, and extend the known PFBS toxicity profile from early-life molecular disruptions to enduring behavioural consequences.

Lipidomic disruptions and mechanistic links

The lipidomic analysis revealed sex-dependent alterations in hepatic lipid profiles following exposure to both PFOS and PFBS. Although FDR correction proved conservative for our sample sizes, nominal-trend analyses and PLS-DA uncovered coherent shifts. In PFOS-exposed females, disruptions in fatty acid metabolism, oxidation, and C21-steroid hormone biosynthesis were identified. These changes might interfere with energy production, hormonal regulation, and membrane stability, as reflected by trends in altered steroid hormones, cholesterol, squalene, bile acid biosynthesis, and the carnitine shuttle. Overexpression of retinoids, sphingomyelins (SM), phosphatidylethanolamines (PE), and phosphatidylcholines (PC) indicate a compensatory response in lipid metabolism, potentially aiming to adjust membrane composition and signalling [66–69]. This aligns with previous findings showing that PFOS alters membrane properties and may act as a chemosensitizer by inhibiting Abcb4 activity. Abcb4 is a membrane-associated protein that contributes to multi-drug or multi-xenobiotic resistance (MDR or MXR) by preventing a wide range of toxic compounds from remaining in the cell [5, 70, 71]. It operates optimally only within a narrow PC/SM and sterol milieu and perturbations of these lipids can slow its ATPase turnover and flipping activity [72–74]. The PC, SM and sterol shifts we observed therefore provide a biophysical context for the ABCB4 inhibition effect of PFOS reported previously [5]. Dedicated transporter assays will be needed to confirm this mechanism in our zebrafish model.

Although the levels of PC, Cer, and CE correlated with egg production across the entire dataset, PFOS-exposed females produced fewer eggs, likely due to disrupted steroid signalling, as steroid hormones are critical for oogenesis and ovulation [75]. PFOS exposure was also found to alter fatty acid metabolism, necessary for energy reserves in egg development [76], and neuroendocrine signalling that regulates reproductive hormones [77, 78]. The overexpression of membrane lipids suggests compromised cellular and oocyte function, which may hinder egg maturation and fertilization.

PFOS-induced liver dysfunction may have cascading effects on the brain. Altered cholesterol metabolism, for example, has been linked to synaptic changes [79], which could underlie the reduced brain size and anxiety-like behaviours observed in females. Indeed, PFOS-exposed females' dysregulation in brain lipids like PE, PC, and SM, can be linked to brain function as phosphatidylcholine content has been linked to cognitive function [80] and sphingomyelins are involved in neuronal functioning through the electrical insulation of axons and axon extension [81]. Additionally, disrupted retinoid signalling, essential for brain development and mature brain function [67], and impaired fatty acid metabolism may contribute to the observed brain size reduction. PFOS-induced changes in neuroendocrine pathways, particularly stress hormones like cortisol, could further impair brain development and behavioural responses. The altered lipid signalling and phosphatidylinositol phosphate metabolism may exacerbate these effects, impair synaptic function and contribute to the anxiety-like behaviours and reduced exploratory activity observed in PFOS-exposed females.

In males exposed to higher concentrations of PFOS during development, an increase in BCF was observed, as well as an overexpression of TG. This increase in TG suggests an adaptive response to excess lipid storage, likely due to disrupted lipid metabolism, where energy storage in the form of TG may compensate for impaired lipid homeostasis.

Interestingly, while most phenotypic effects were observed in fish exposed to higher concentrations of PFOS, lipid changes were more pronounced in those exposed to lower concentrations. This pattern mirrors classic non-monotonic concentration–responses documented for many endocrine-active chemicals [82]. For instance, PFOS can activate hepatic nuclear receptors such as peroxisome proliferator-activated receptor-α (PPARα) [83]. Low concentrations could subtly reprogram lipid signalling and cell function without triggering overt toxicity, whereas higher concentrations might saturate these pathways and instead provoke acute metabolic stress. Our female PFOS HC data showed perturbation of PC and fatty acid oxidation pathways whereas male PFOS LC signature showed widespread shifts across amino acid and PCs. Together, this body of work underscores that "concentration" and "exposure window" codetermine PFOS toxicity: low-level exposures may impact metabolism in ways that only manifest under challenge or with age, whereas high-level exposures produce more tractable, acute phenotypes.

PFBS exposure also induced significant lipidomic alterations. In females, dysregulation in squalene and cholesterol metabolism, glycosphingolipid metabolism,

and steroid biosynthesis pointed to compromised cellular homeostasis and energy regulation. These disruptions are particularly concerning because squalene and cholesterol are precursors for steroid hormones critical for reproductive health. Lowered expression of LPC, a lipid essential for membrane fluidity and neuronal differentiation [84], might contribute to the observed brain size reduction and altered behaviour. Conversely, lower concentrations of PFBS led to overexpression of PL, which may represent a compensatory response to membrane instability but could also impair synaptic plasticity.

In males, exposure to PFBS disrupted phosphatidylinositol phosphate (PIP) metabolism, a process that is also involved in cell signalling and membrane dynamics [85]. PIP metabolism was associated with exploratory behaviour across the entire dataset, and PFBS-exposed males displayed a tendency toward increased entries into the top zone that might be explained by PIP metabolism. PIPs are known to impact various aspects of neuronal function, including synaptic plasticity, neuroendocrine signalling, and motor control, all of which contribute to behaviour. Disturbances in the balance between different PIPs and PIP signalling are known to lead to neurodevelopmental and neurodegenerative disorders in mammals [86]. An increase in entries into the top zone could indicate an elevated response to disrupted signalling pathways involving PIPs.

Additional changes in glycosylphosphatidylinositol (GPI) anchor biosynthesis and bile acid production further highlight interferences with membrane composition [87], lipid metabolism, and excretion of toxic metabolites [88] which could adversely affect liver function and overall metabolic health, potentially contributing to the observed changes in liver function and lipid profiles in PFBS-exposed males. Lipid alterations, including changes in PC, LPC, retinoids, and acid amines, varied depending on the PFBS concentration.

At LC, males exhibited diminished expression of amino acids and LPC and overexpression of PC. These shifts might denote a fundamental re-patterning of membrane phospholipid composition in liver cells that extends to neuronal tissues, where similar lipid assemblies govern membrane fluidity, receptor conformation, and synaptic vesicle dynamics [89]. Particularly, LPC depletion may impair membrane curvature [90, 91] and neurotransmitter release [92] while reduced amino acids could disrupt neurotransmission [93]. These mechanisms are consistent with the enhanced exploratory behaviour we observed.

Elevated PC levels suggest dysregulated phosphatidylcholine turnover, potentially driven by PFBS interference with choline kinase mediated synthesis or phospholipase activity leading to impaired breakdown. A recent multi-omics study of zebrafish embryos exposed to PFBS reported neurochemical changes in the cholinergic system [64] supporting our interpretation that PFBS diverts choline into hepatic PC at the expense of neuronal transmitter pools.

Additionally, decreased expression of retinoids, which are important for neurodevelopment [94] and reproductive health [95, 96], could also impair neural function and reproductive success. Notably, BCF at this low concentration correlated negatively with hepatic retinoids and amino acid–conjugated lipids, indicating that depletion of these bioactive species coincides with increased somatic fat. The concomitant up-regulation of TG and redistribution of phospholipids might reflect a maladaptive shift toward energy storage triggered by PFBS-driven metabolic stress, as supported by the concurrent trend toward lower spawning success and broader metabolic disruption.

Although PFBS is considered to bioaccumulate far less than PFOS, our data show that even at low concentrations, developmental exposures are sufficient to disrupt hepatic lipid metabolism linked to phenotypic changes, including loss of normal BCF dimorphism, anxiety-like behaviour in females, and a reduction in spawning success.

Conclusion

Our findings demonstrate that developmental exposure to PFOS as well as to its alternative PFBS induces reproductive, morphological, and behavioural disturbances in adult zebrafish linked to disruptions in lipid metabolism. The endpoints affected by the chemicals are critical for fish survival, and may lead to substantial population declines, as observed for fathead minnow population exposed to an estrogenic compound [97], and in response to various compounds and chemical mixtures [98]. These findings can also suggest a trade-off between the metabolic cost of toxicant exposure and reproductive processes, a phenomenon previously noted in *Cyprinus carpio* following PFOS exposure [99]. Moreover, the observed changes were distinctly sex-specific, aligning with the recognized role of perfluoroalkylated substances as endocrine disruptors that interact with lipid metabolism in a sex-dependent manner [100, 101].

These outcomes emphasize the urgent need for regulatory frameworks to consider chronic exposure and nonlethal endpoints rather than relying solely on acute toxicity testing. In this study, we used environmentally relevant concentrations of PFOS and PFBS, reflecting moderately elevated levels found in impacted environments. However, as PFAS are known to exhibit non-monotonic dose–response relationships, it remains crucial to investigate

their long-term effects at even lower environmental concentrations.

Abbreviations

AA	Amino Acids derivatives
BCF	Body Condition Factor
BrW	Brain Weight
BW	Body Weight
CE	Cholesteryl Esters
Cer	Ceramides
Dpf	Days post fertilization
FET	Fish Embryo acute Toxicity
GPI	Glycosylphosphatidylinositol
GSI	Gonadosomatic Index
GW	Gonads Weight
HC	High Concentration
Hpf	Hours post fertilization
HSI	Hepatosomatic Index
L	Body length
LC	Low Concentration
LC-MS	Liquid Chromatography-Mass Spectrometry
Lm	Linear model
LOQs	Limits of Quantification
LPC	Lysophosphatidylcholines
MTM	Man-Technology-Environment Research Centre
NOEC	No-effect-concentration
NTT	Novel Tank diving Test
PC	Phosphatidylcholines
PE	Phosphatidylethanolamines
PFAS	Poly- and perfluoroalkyl substances
PFBS	Perfluorobutane sulfonic acid
PFOS	Perfluorooctane sulfonic acid
PIP	Phosphatidylinositol phosphate
PL	Phospholipids
PLS-DA	Partial least squares-discriminant analysis
QA	Quality Assurance
RE	Retinoids
Rlm	Robust linear model
SI	Somatic Indices
SM	Sphingomyelins
TG	Triglycerides
UHPLC-Q-TOF/MS	Ultra-high-performance liquid chromatography quadrupole time-of-flight mass spectrometry
UPLC-MS/MS	Ultra-high-performance liquid chromatography coupled to tandem mass spectrometry
VIP	Variable Importance in Projection
VS	Vibrational Stimulus

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12302-025-01281-9.

Additional file 1.
Additional file 2.
Additional file 3.
Additional file 4.
Additional file 5.
Additional file 6.
Additional file 7.
Additional file 8.
Additional file 9.
Additional file 10.
Additional file 11.
Additional file 12.
Additional file 13.
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Additional file 23.

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Author contributions

M.F., J.Z., C.Y., and M. D. C. performed the zebrafish experiments. M.F., R.A., and L.W.Y.Y. performed the water analysis. M.F. and C.Y. performed the FET analysis. M.F. and J.Z. performed the reproduction success analysis. M.F. performed the growth and morphological analysis. M.F., J.Z. and C.Y. performed behavioural analyses. M.F., D.B., and T.H. performed the lipidomic analyses. M.F., N.S., J.R., P.A. and S.H.K. designed experiments. M.F., J.Z., A.O., M.D.C., P.A., J.R. and S.H.K. interpreted the results. M.F., L.W.Y.Y., M.D.C., J.R., T.H. and S.H.K. wrote the manuscript. All authors read and approved the final manuscript.

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Data availability

All data generated or analysed during this study are included in this published article and its supplementary information files.

Declarations

Ethics approval and consent to participate

The study was conducted at the Man-Technology-Environment Research Centre (MTM) in compliance with national and international laws, regulations, and agreements. The aquarium facilities for husbandry and zebrafish breeding hold permissions from the Swedish Board of Agriculture, Jönköping, Sweden to use test animals (# 5.2.18-20142/2022) and for experimental animal breeding (# 5.2. 18-20144/2022). The research unit also holds ethical permission from Linköping's animal test ethics committee for animal testing (#13595-2023).

Consent for publication

Not applicable.

Use of generative AI and AI-assisted technologies in the writing process

During the preparation of this manuscript, the author(s) used ChatGPT (OpenAI) to enhance the readability of certain sections of the text. All content was subsequently reviewed and edited by the author(s), who take full responsibility for the final version of the article.

Competing interests

The authors declare no competing interests.

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