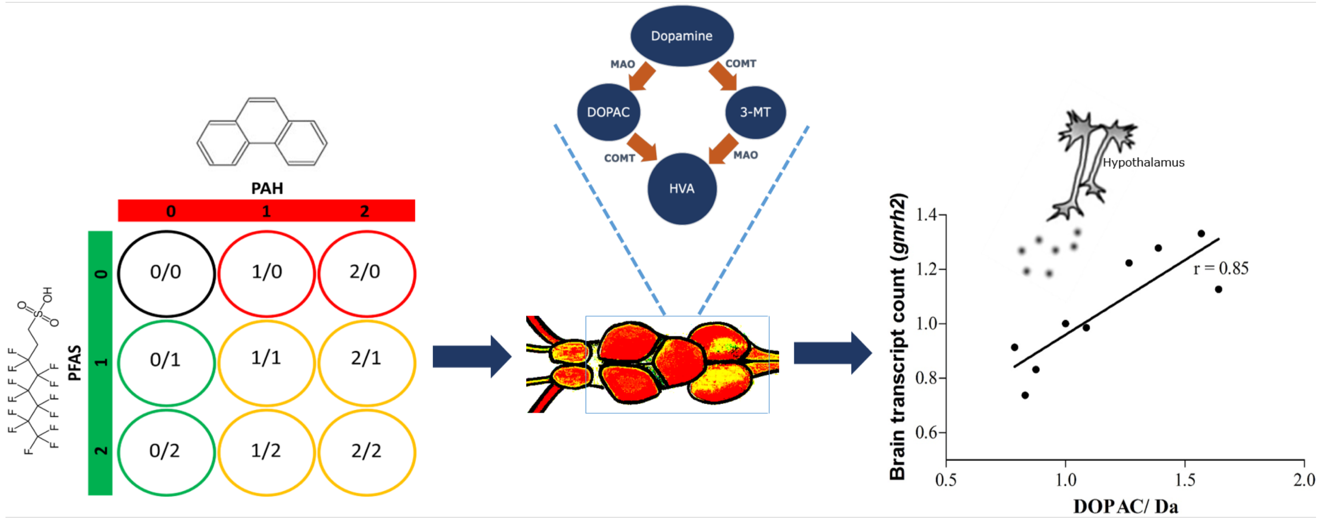

PAH
PFAS
0/0
1/0
2/0
0/1
1/1
2/1
0/2
1/2
2/2
Dopamine
MAO
COMT
DOPAC
3-MT
HVA
Hypothalamus
Brain transcript count (gnrh2)
DOPAC/ Da
r = 0.85

Modulation of neuro-dopamine homeostasis in juvenile female Atlantic cod (*Gadus morhua*) exposed to polycyclic aromatic hydrocarbons and perfluoroalkyl substances

Essa A. Khan[1], Luisa B. Bertotto[2], Karina Dale[3], Roger Lille-Langøy[3], Fekadu Yadetie[3], Odd André Karlsen[3], Anders Goksøyr[3], Daniel Schlenk[2], Augustine Arukwe[1*]

[1]Department of Biology, Norwegian University of Science and Technology (NTNU), Høgskoleringen 5, N-7491 Trondheim, Norway

[2] Department of Environmental Sciences, University of California-Riverside, California, USA

[3] Department of Biological Sciences, University of Bergen, N-5020 Bergen, Norway

***Corresponding author:**

E-mail: augustine.arukwe@ntnu.no

27 **Abstract**

28 The dopaminergic effect of PAH and PFAS mixtures, prepared based on environmental levels has
29 been studied in juvenile female Atlantic cod (*Gadus morhua*). Benzo[a]pyrene, dibenzothiophene,
30 fluorene, naphthalene, phenanthrene and pyrene were used to prepare a PAH mixture, while PFNA,
31 PFOA, PFOS and PFTrA were used to prepare PFAS mixture. Cod were injected intraperitoneally
32 twice, with either a low (1x) or high dose (20x) of each compound mixture or various combinations.
33 After two week of exposure, levels of plasma 17β-estradiol (E2) were significantly high in high
34 PAH/high PFAS treated groups. Dopamine: metabolite ratios (DOPAC/dopamine and
35 HVA+DOPAC/dopamine) in brain homogenate changes with the levels of E2 in plasma except for
36 high PAH/low PFAS and low PAH/high PFAS treated groups. In general, *th* mRNA levels inversely
37 correlated with dopamine: metabolite ratios and *gnrh2* mRNA levels. Respective decreases and
38 increases of *dr1* and *dr2a* after exposure to the high PAH dose were observed. Whereas, high PFAS
39 exposure decreased both *drs*, leading to high plasma E2 concentrations. Other investigated endpoints
40 suggest that these compounds at different doses and combinations have different toxicity threshold
41 and mode of actions. These effects indicate potential alternations in feedback signalling processes
42 within dopaminergic pathway by these contaminants.

43

44 **Keywords:** PAH; PFAS; Dopaminergic pathway; Estrogenic pathway; Feedback control; Atlantic
45 cod.

46

47

48

49

50

51

52

53

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) and perfluoroalkyl substances (PFASs) are among the most common xenobiotics found in the environment.[1,2] PAHs are generally categorized into petrogenic and pyrogenic hydrocarbon groups, depending on whether they are produced by the incomplete combustion of petroleum or by irreversible temperature-mediated change of chemical composition.[3] PFASs have been used in a wide variety of products, including fire-fighting foams, ink, paper coating and textile, and as water repellents.[4,5] Both PAHs and PFASs are found in the aquatic environment and may threaten marine organisms.[6,7] Concentrations of PAHs as high as 10 µg/g dry weight of sediment have been previously reported in the Seine estuary, Normandy, France.[8] PFAS concentrations have been observed at ng/L and ng/g levels in surface waters and sediments respectively.[9-14] Co-occurrence of both PAHs and PFASs at toxic levels has also been reported in tidal flats and costal ecosystems of the Ariake Sea, Japan.[7,15]

The concentrations of these compounds in the aquatic environment might not significantly affect survival, but may severely alter reproductive capacity and endocrinology in fish. PAHs have been shown to disrupt the endocrine system through binding and activating the aryl hydrocarbon receptor (AhR) and produce anti-estrogenic responses.[16-18] PAHs may also weakly bind to the estrogen receptor (ER) and have estrogenic properties.[17] PFASs have also been reported to possess estrogenic properties. For example, *in vivo* and *in vitro* exposure to PFOS produced an up-regulation of vitellogenin (*vtg*) mRNA expression in fish liver.[19,20] In contrast, other studies did not find significant changes in Vtg levels[21] or reported downregulation of *vtg* mRNA expression.[22] Further investigation is required to better understand the mechanism of action of PAHs, PFASs, and the combination of both compound classes on the reproductive system of teleosts.

Biosynthesis of estrogen is regulated through the hypothalamus-pituitary-gonadal (HPG) axis.[23] The hypothalamus produces gonadotropin-releasing hormone (GnRH) that controls the release of gonadotropins (GtHs): follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the pituitary gland. Gonadotropins induce oocyte development, maturation, and production of 17β-estradiol (E2) in gonads. Among different feedback mechanisms, the dopaminergic system plays an

81 essential role in controlling GnRH and GtH releases. High levels of E2 activate dopaminergic neurons
82 generally to reduce the production of these hormones.[23] However, increases in the production of GtHs
83 may also occur.[24,25] Dopamine can also regulate brain aromatase (Cyp19b), an enzyme that catalyzes
84 the production of estrogen in the brain. Unlike GnRH, brain aromatase responds differently to
85 dopaminergic agonists.[26] Fontaine et al.[27] showed that co-exposure of female fish to the dopamine
86 receptor (Dr2a) antagonist domperidone and a GnRH agonist, resulted in an increased expression of
87 GtH mRNA, suggesting that removal of dopamine inhibitory effects allow the hypothalamus to
88 produce GnRH and consequently modulate plasma E2 levels.

89 The production of dopamine is initiated by tyrosine hydroxylase (TH), the rate-limiting
90 enzyme that converts tyrosine to 3,4-dihydroxy-L-phenylalanine (L-DOPA) which is subsequently
91 metabolized by DOPA decarboxylase (DDC) to dopamine.[28] Once released in the synaptic cleft,
92 dopamine binds to two dopamine receptor (DR) families DR1 and DR2, which activate specific G
93 proteins. For example, DR1 is coupled to $G\alpha_s$ and activates adenylyl cyclase (AC) which increases
94 the concentrations of cyclic adenosine monophosphate (cAMP) and calcium ions. Conversely, DR2
95 is coupled to $G\alpha_{i/o}$ and inhibits AC. In excess, dopamine is reabsorbed through the dopamine active
96 transporter (Dat) in presynaptic neurons and further catabolized into 3,4-dihydroxyphenyl acetic acid
97 (DOPAC) and homovanillic acid (HVA) by monoamine oxidase (MAO) and catechol-O-
98 methyltransferase, respectively.[28,29] Exposure of mice to endocrine disruptive chemicals (EDCs),
99 such as bisphenol A (BPA) and 2,4-dichlorophenoxyacetic acid, altered the expression of DRs and
100 DAT, modulating dopamine synthesis, release and turnover in mice and rats.[30,31] Similar results were
101 observed in zebrafish and rainbow trout where bifenthrin altered E2 concentrations and dopaminergic
102 systems.[32]

103 Despite reports showing estrogenic responses of PAHs and PFASs, either individually or in
104 combination, there is limited data concerning the effects of these compounds on dopaminergic
105 signaling pathways. Therefore, the aim of this study was to investigate changes in dopaminergic
106 signaling and endocrine function after *in vivo* exposure of Atlantic cod (*Gadus morhua*) to a low dose
107 chosen based on environmental levels (low) and a higher (20 x low) dose (high) of PAHs and PFASs

108 alone or in combination. In the North Atlantic, Atlantic cod is major fisheries species, and is important
109 in costal as well as oceanic ecosystems. It has been used as an indicator as well as model organism in
110 environmental monitoring and toxicological studies respectively.[33-35] Therefore, cod is a valuable tool
111 for ecotoxicological studies and risk assessment.
112
113 **2. Materials and methods**
114 2.1. Chemicals and reagents
115 Direct-zol™ RNA isolation and MiniPrep kit from Zymo Research Corporation (Irvine, CA, USA),
116 iTaq SYBR Green Supermix with ROX and iScript cDNA synthesis Kit from Bio-Rad Laboratories
117 (Hercules, CA, USA). 17β-estradiol (E2) enzyme immunoassay (EIA) kits (Cat. No. 582251 and
118 582701) purchased from Cayman chemical company (Ann Arbor, MI, USA).
119
120 2.2. Animals
121 Juvenile Atlantic cod (*G. morhua*), approximately 5 months old were obtained from
122 Havbruksstasjonen in Tromsø AS (Tromsø, Norway) and reared at Industrilaboratoriet in Bergen
123 (ILAB, Bergen, Norway) in 500 L tanks supplied with seawater at 8 to 10 °C, 34 ppt salinity. The
124 cod were held at a 12:12 h light/dark cycle and fed with a commercial marine diet (Amber Neptune,
125 Skretting, Stavanger, Norway). At the start of the exposure the cod were approximately 18 months
126 with average bodyweight of 172 ± 34 g. The experimental setup was approved by the Norwegian
127 Food Safety Authorities (FOTS # 11730/17/18948) and performed accordingly.
128
129 2.3. Exposure and sampling
130 Cod were exposed for two weeks and were injected intraperitoneally once per week (day 0 and day
131 7) with two different doses: low (1x) and high (20x) dose of PAH and PFAS (Table 1), individually
132 and in various combinations consisting of the following groups; vehicle control, low PAH, low PFAS,
133 high PAH, high PFAS, low PAH/low PFAS, high PAH/low PFAS, low PAH/high PFAS and high
134 PAH/high PFAS (Fig. 1). The stock solutions were prepared in a 1:1 (v/v) mixture of rapeseed oil

135 (Eldorado rapsolje) and PBS and injected at 1 mL/100 g fish. Control cod were injected with solvent
136 vehicle (1:1 of oil and PBS). The PAH concentrations (1x) were chosen based on PAH levels detected
137 in Atlantic cod from Tampen and Egersund in the monitoring report of the Institute of Marine
138 Research (IMR) from 2012.[36] The PFAS concentrations (1x) were chosen based on reported values
139 in cod samples from the Nordic environment and northern Norwegian mainland.[37, 38] Following two
140 weeks of exposure, the cod were euthanized and tissue samples were collected and frozen in liquid
141 nitrogen before being transferred to -80 °C for downstream analyses.
142
143 2.4. Quantitative (real-time) PCR
144 Total RNA was extracted from brain tissues using the Direct-zol™ RNA kit, following the
145 manufacturer's protocol. Quality of RNA was confirmed by formaldehyde agarose gel electrophoresis
146 and spectrophotometric analysis. cDNA was generated by following the instruction of the iScript
147 cDNA synthesis kit (Bio-Rad) and transcripts were amplified using Mx3000P real-time PCR machine
148 (Stratagene, La Jolla, CA), details are presented in section 1.1 of the supplementary information (SI).
149
150 2.5. Steroid hormone analysis
151 Enzyme immunoassay (EIA) was used to measure the concentration of 17β-estradiol (E2) in plasma
152 using EIA kit (Cayman Chemical Company, Ann Arbor, MI, USA). Detailed description of hormone
153 extraction and quantification are presented in section 1.2 of SI.
154
155 2.6. Ultra-Performance Liquid chromatography-mass spectrometry (UPLC-MS/MS)
156 To measure dopamine and its metabolites, samples were prepared and ran, following the protocol of
157 Bertotto et al.[39] Detailed procedure is presented in section 1.3 of SI.
158
159 2.7. Statistics
160 Statistical analysis was performed on RStudio (version 1.1.456), the statistical difference between
161 control and exposure groups were determined through one-way ANOVA (and a post-hoc Dunnett's

162 test). To investigate interaction of compounds (and corresponding to mixture exposure), a two-way
163 analysis of variance (ANOVA), followed by Dunnett's post-hoc test was performed on a linear model
164 with the significance level set at $p \leq 0.05$. Details of the performed calculations and statistical tests
165 can be found in section 5 of SI. Relationship between biological parameters and their response to
166 chemical exposures were visualized in a principle component analysis (PCA). The first two principle
167 components and their factor scores were summarized in a biplot using XLSTAT. Data Analysis and
168 Statistical Solution for Microsoft Excel. Addinsoft, Paris, France (2017).
169

170 **3. Results**

171 3.1. Effects of PAH and PFAS on plasma E2
172 The plasma concentrations of E2 showed apparent dose-specific effects for both PAH and PFAS
173 exposure groups (Fig. 2). The low PAH dose, increased E2 levels, while no change was observed in
174 high dose exposure group. For PFAS, a non-significant increase in E2 levels was observed in the high
175 dose exposure group, but, a decrease was noted in the low dose treatment (Fig. 2). Combined exposure
176 to low PAH/low PFAS, low PAH/high PFAS and high PAH/high PFAS showed an increasing trend,
177 significantly so at the later value (Fig. 2).
178

179 3.2. Effects of PAH and PFAS on brain dopamine pathways
180 The concentrations of brain dopamine showed a decreasing trend except to high PAH/high PFAS
181 exposure. Cod exposed to low PAH, high PFAS and low PAH/low PFAS showed a significant
182 decrease in dopamine levels (Table 2). One of the major dopamine metabolite, DOPAC was
183 significantly decreased in cod exposed to high PAH dose. A decreasing trend (non-significant) of
184 DOPAC was observed in other exposure groups, except for cod exposed to low PAH/low PFAS, high
185 PAH/low PFAS and high PAH/high PFAS, only the latter showed a significant increase (Table 2).
186 The second most abundant dopamine metabolite (HVA) did not show any significant change after
187 exposure to PAH and PFAS, singly, at different doses and their various combinations (Table 2). In

188 addition, a significant increase in DOPAC-dopamine and DOPAC+HVA-dopamine ratios were
189 observed in cod exposed to low PAH/low PFAS and high PAH/low PFAS (Table 2).

190 In this study, an interactive effect of PAH and PFAS was investigated on the level of dopamine
191 and its metabolites as being represented in Fig. S5 and Table S3. PAH and PFAS at low dose produced
192 a strong interactive effect on the levels of DOPAC. In single exposure, there is a mild decrease in
193 levels of DOPAC, however in the mixture, the level was similar to the control group (Fig. S5D and
194 Table S3). High PAH with low and high dose of PFAS in a mixture also produced an interactive
195 effect on DOPAC levels (Fig. S5E and F and Table S3). For HVA, significant interaction was
196 observed between high PAH and high PFAS in a mixture (Fig. S5C and Table S3). Exposure in the
197 mixture of low PAH/high PFAS and high PAH/high PFAS showed strong interactive effects on the
198 levels of dopamine. High PFAS and Low PAH single exposure significantly decreased dopamine
199 level. However in the mixture, the level was similar to the control group (Fig. S5A and B and Table
200 S3).

201

202 3.3. Effects on brain dopaminergic and estrogenic signaling

203 Significant changes were observed in the expression of dopaminergic (*dat*, *dr1,* and *dr2a*) and
204 estrogenic (*esr1*, *esrrb* and *cyp19a1b*) signaling genes in cod brain following exposure to PAH and
205 PFAS, given alone or in combination (Fig. S3 and S4). The high PAH dose decreased *dr1* mRNA
206 and increased of *dr2a* transcripts. On the other hand, high PFAS decreased both *drs*. Whereas, the
207 combined exposure scenarios did not change the expression of *dr2a* mRNA. (Fig. S3C and D).
208 Exposures in the mixture of low PAH/high PFAS and high PAH/high PFAS produced interactive
209 effects on the expression of *dr1*. In an individual exposure, high PFAS significantly decreased the
210 expression of *dr1* than both doses of PAH. However, when they occurred in a mixture, the expression
211 was similar to the control group (Fig. S6G and H and Table S4). In contrast, expression of *dat* was
212 significantly increased after exposure to high PAH/high PFAS (Fig. 3B and D).

213 The expression of *esr1* was significantly decreased after exposure to high PAH and high PFAS
214 doses, while combined exposures, including high PAH/low PFAS and low PAH/high PFAS,

215 significantly reduced *esr1* expression (Fig. S4A). Both exposures, in the mixtures of low PAH/high
216 PFAS and high PAH/high PFAS produced interactive effects, but the former showed a significant
217 decrease compared to control (Fig. S6A and B and Table S4). Expression of *esrrb* mRNA was
218 significantly decreased after low PAH, high PFAS and high PAH/low PFAS treatments (Fig. S4B).
219 However, other combined exposures including low PAH/low PFAS, low PAH/high PFAS and high
220 PAH/high PFAS showed significant interactive effect on *esrrb* expression (Fig. S6C, D and E and
221 Table S4). Brain aromatase (*cyp19a1b*) gene expression was also significantly decreased in fish
222 exposed to both low and high PAH dose and combined exposure of low PAH/high PFAS (Fig. S4C).
223
224 3.4. Principle component analysis (PCA)
225 The relationship between levels of all analysed observation and different exposure groups showed
226 that the first two factor score (F1 and F2) could accounted 69.12 % of the total variance in the dataset
227 (Fig. 3). Respectively, F1 and F2 covered 50.5 % and 18.59 % of the total variability, showing that
228 low PAH, high PFAS and combination exposure including, low PAH/low PFAS, high PAH/low
229 PFAS and high PAH/high PFAS has a strong positive relationship with dopamine: metabolite ratios
230 (Fig. 3 and Table S2). Despite of their strong positive association with dopamine: metabolite ratio,
231 only high PAH/high PFAS exposure showed significant increase in E2 level. The expression levels
232 of *th*, *dr1*, *esr1*, *esrrb* and *cyp19a1b* mRNA showing a negative relationship with all exposure groups
233 and strong particularly to those that has highest dopamine: metabolite ratios. F1 and F2, showed
234 strong negative relationship between *th* (-1.886, 0.022), *dr1* (-1.703, 0.911), *esrrb* (-2.306, 0.139)
235 mRNA expression and low PAH (0.955, 0.061), high PFAS (0.886, -0.408), low PAH/low PFAS
236 (0.879, -0.342) and high PAH/low PFAS (0.788, -0.282) exposure groups. In general, *th* showed a
237 decreasing trend and was negatively correlated to the expression of brain *gnrh2* with F1 value of -
238 1.886 and 0.142 respectively (Pearson's correlation coefficient, r = -0.54) (Fig. 3, Table S2 and S2.2).
239
240
241

242 **4. Discussion**

243 Several *in vivo* and *in vitro* studies have shown that PFASs and PAHs are respective agonist and
244 antagonists of ER and subsequently affect ER-mediated responses in vertebrate species.[40,41] To better
245 understand the underlying processes, alternative pathways by which PAH and PFAS may affect
246 cellular E2 levels and E2-mediated downstream responses should be investigated. For example, PFOS
247 and PFOA, despite their weak affinities to ER, have been shown to increase E2 production through
248 modification of steroidogenic pathways.[42,43] In addition, Cyp pathways contribute to estrogenic
249 properties displayed by B[a]P, where hydroxylated metabolites such as 9-OH-B[a]P displayed
250 estrogenic properties or may undergo subsequent metabolic steps to form more estrogenic species.[44]
251 Nevertheless, there are few studies examining the effects of PAH and PFAS on E2 clearance,
252 biosynthesis or physiological control mechanisms, including feedback processes. Consequently,
253 additional biochemical processes that regulate neuronal and gonadal E2 homeostasis may be involved
254 in previously reported estrogenic and anti-estrogenic responses of PAH and/or PFAS.

255 Herein, the low PAH dose and high PFAS dose produced non-significant increases of plasma
256 E2 levels. Previous studies have demonstrated an association between liver ER expression and plasma
257 E2 concentrations, in contrast to brain ER expression patterns after exposure to a xenoestrogen
258 (nonylphenol, NP), showing that *er* isotype expression in various tissues paralleled other
259 xenoestrogen biomarkers (such as Vtg) in liver or plasma samples of Atlantic salmon (*Salmo salar)*.[45]
260 In addition, the authors reported differential expression pattern of *er* isotypes in liver and brain of
261 NP-exposed fish.[45] Regression analysis of brain and liver *er-α* and *er-β* transcripts and *vtg* expression
262 levels showed a linear relationship between liver *er-α* and *vtg* mRNA, whereas brain *er-β* had limited
263 linearity with liver *vtg*. Unlike liver *er-α*, both brain and liver *er-β* showed a non-linear relationship
264 with *cyp19* isotypes in the brain.[45] Despite the high plasma E2 levels measured at low PAH dose,
265 there was a decrease in the levels of both brain *esr1* and *esrrb* mRNA. In addition, we observed that
266 the high PFAS dose produced elevated plasma E2 concentrations, while the brain showed decreased
267 levels of both *esr1* and *esrrb* transcripts. The fact that brain *ers* have limited association with
268 dopaminergic and estrogenic signaling, mixture exposure scenarios may help in understanding

269 interactions among chemicals. For example, single exposures of high PAH and PFAS significantly
270 decreased the expression of *esr1*, compared to their mixture, suggesting antagonistic interactions
271 among these chemicals at the tested doses. On the other hand, exposure to low PAH/high PFAS
272 significantly reduced *esr1* mRNA. Similar antagonistic and chemical masking effects were observed
273 with *esrrb* by low PAH/low PFAS, low PAH/high PFAS and high PAH/high PFAS mixtures,
274 suggesting a complex interaction mode by these chemicals.

275 Inconsistency in relationship between brain ERs expression and plasma E2 levels may suggest
276 that other cellular pathways play important roles in regulating GnRH2 release in the brain or that
277 neuronal ER expression does not parallel cellular E2 function and regulation. This speculation is
278 supported by our observation that changes in *gnrh2* expression paralleled changes in plasma E2
279 levels, with Pearson's correlation coefficient between dopamine metabolite ratio and gnrh2
280 expression showing an $r = 0.86$ and 0.81 (Section 2.2 SI). Modulation of dopamine and its metabolites
281 after exposure to PAH and PFAS, singly or in combination, suggests a disruption in dopaminergic
282 function, which may subsequently disrupt E2 synthesis and regulation through feedback
283 mechanisms.[23] The high PAH treatment decreased the expression of *dr1* with a concomitant increase
284 in *dr2a*. A decrease in *gnrh2* mRNA was observed in the brain. In rainbow trout (*O. mykiss*), binding
285 of dopamine to Dr2a inhibited the production of GnRH-stimulated gonadotropin release from the
286 pituitary.[46-50] Elsewhere in goldfish (*Carassius auratus*), the release of GnRH-activated LH was
287 blocked through Dr2a activation.[51] In contrast, dopaminergic signaling via Dr2a was reported in
288 *Tilapia zillii*, where an increase of *dr2a* mRNA paralleled increase in plasma E2 levels[47,48,52],
289 suggesting the presence of ER responsive elements (ERE) in the promoter of *dr2a*.[53] In the present
290 study, we observed a decrease in *dr2a* mRNA with a corresponding increase in plasma E2 for high
291 PFAS and low PAH/ low PFAS treatment. Other combined exposures (high PAH/low PFAS, low
292 PAH/high PFAS, and high PAH/high PFAS) did not produce any change in the expression of brain
293 *dr2a* transcripts.

294 For dopaminergic signaling, we observed a reciprocal association between expression of *dr1*
295 and *dr2a* in the high PAH exposure group. Combined exposure, especially high PAH/low PFAS also

296 decreased the expression of *dr1*. In previous studies using rat prefrontal cortex, reduced expression
297 of *Dr1* was observed which paralleled an increase in DR1 protein after exposure to PFOS.[54] In
298 contrast, Pereiro et al.[55] reported a respective decrease and increase of *Dr1* mRNA and protein
299 expressions in rat hippocampus after exposure to PFOS. Potential inconsistencies in the expression
300 of transcript and protein might be due to an inhibitory effect of microRNA (miR-142-3p) that post-
301 transcriptionally regulates *Dr1*.[56] This assumption is supported by recent studies showing that other
302 regulatory micro RNAs, such as miR-326 and miR-9, which also control *DR* expression, were
303 inhibited after exposure to PFAS.[57] A significant interaction among chemicals also affected the
304 expression of *dr1*. High PFAS significantly decreased *dr1* expression. However, combined with
305 PAHs in the mixtures of low PAH/high PFAS and high PAH/high PFAS, PAHs masked the inhibitory
306 effect of high PFAS, enhancing *dr1* expression which was similar to control.

307 Modulation of *dr* transcription may ultimately affect dopamine and its metabolites (HVA and
308 DOPAC) in the brain. In humans, alteration of dopaminergic-signaling was assessed by measuring
309 dopamine metabolites in plasma and urine.[58] Thus, the ratio of DOPAC:dopamine, as well as
310 DOPAC+HVA:dopamine was used in the present study, to estimate the release and turnover of
311 dopamine. The low PAH/low PFAS exposure significantly increased these ratios and paralleled an
312 increase in plasma E2. Except for high PAH/low PFAS and low PAH/high PFAS, all exposure groups
313 produced similar, but non-significant patterns between E2 and dopamine metabolite ratios. Contrary
314 to individual chemicals, mixture exposures might have interactive effects at specific concentrations
315 that regulate dopamine metabolism differently, thus violating their association with dopamine
316 turnover. Previously, Bertotto et al.[39,59] used these ratios to determine dopamine turnover in zebrafish
317 embryos and juveniles, showing that a low ratio of dopamine and its metabolites demonstrated a
318 relationship with dopamine turnover.

319 In the HPG axis, feedback mechanisms regulate the endocrine physiology of vertebrates,
320 including teleosts. Exposure to low PAH and high PFAS and combined low PAH/low PFAS triggered
321 a mild increase in plasma E2 levels and subsequently decreased brain dopamine concentrations. On
322 the other hand, high PAH and low PFAS exposures did not produce significant changes that

323 corresponded with plasma E2 levels. It should be noted that tyrosine hydroxylase (Th) plays an
324 important role as the rate-limiting enzyme in the production of dopamine.[28] We observed a reciprocal
325 association between dopamine metabolite ratios and *th* expression in fish exposed, either to a low and
326 high PAH and PFAS singly or in their various combinations (Pearson's correlation coefficient, r = -
327 0.652 and -0.701, section 2.2 SI). For example, combined high PAH/low PFAS produced a decrease
328 of *th* mRNA with a concomitant increase of both DOPAC:dopamine and DOPAC+HVA:dopamine
329 levels. In contrast, there is a direct relationship between the expression of *th* and dopamine
330 concentration in the brain. Elsewhere, Kumer and Vrana[60] reported that the expression of *th* is
331 regulated through a negative feedback loop in dopaminergic signaling, where elevated concentrations
332 of catecholamine down-regulated *th* mRNA expression, leading to a decrease in dopamine levels.

333 Locally produced brain E2 through aromatase (Cyp19b) activity plays an important role in
334 reproductive- and neuroendocrine functions, and socio-sexual behavior in fish.[61, 62] The entire process
335 involves multiple factors, including E2, dopamine and their receptors which regulate the expression
336 of *cyp19a1b*.[26] Exposure of radial glial cells (RGCs) to a Dr1 agonist upregulated *cyp19a1b*
337 expression through the phosphorylation of cyclic AMP response element binding protein (Creb).[63]
338 This effect was shown to be enhanced using low E2 (100 nM) concentrations.[63] However, exposure
339 to high E2 concentrations decreased *cyp19a1b* expression through a classical negative feedback
340 mechanism.[26] As discussed above, the observed differences between dopamine receptor transcript
341 and protein expression data and patterns of Cyp19b regulation reported by Xing et al.[63], did not
342 parallel our findings. These differences might be attributed to the direct exposure to E2 that may have
343 suppressed the physiological feedback loop and should be investigated in more detail.

344 In conclusion, we have demonstrated that exposure to these compounds altered dopaminergic
345 signaling, including the modulation of dopamine biosynthesis, catabolism and its receptor expression
346 in the brain of juvenile female Atlantic cod. These changes may affect the HPG axis and apical
347 endpoints such as reproduction or behavior. Overall, our findings contribute to the understanding of
348 novel cellular pathways that control steroidogenesis after exposure to PAHs and PFASs, and in
349 complex contaminant mixture scenarios.

350

351 **Conflict of interest**

352 The authors have no competing interests.

353

354 **Acknowledgments**

355 We thank the Department of Biology, NTNU for strategic IBI PhD research travel grant and 356 Department of Environmental Sciences, University of California-Riverside, USA for hosting this trip 357 and provide research facilities to conduct UPLC-MS/MS experiment. We would like to acknowledge 358 Nathalie Briels for helping in statistical analysis. The results of this contribution were presented at 359 2nd annual conference of Digital Life Norway Research School in Stiklestad, Norway. Support was 360 also provided through the UCR/Agricultural Experiment Station Resource Allocation Program.

361

362 **Funding**

363 This work was supported by the Research Council of Norway to the Centre for Digital Life Norway 364 (DLN) project dCod 1.0 [grant number 248840], the CAPES Foundation, Ministry of Education of 365 Brazil, through the program Science without Borders (grant n. BEX 99999.013554/2013-01) to LBB.

366

367 **References**

368 1. Varanasi, U.; Stein, J. E., Disposition of xenobiotic chemicals and metabolites in marine 369 organisms. *Environ. Health Perspect.* **1991,** *90*, 93-100.
370 2. Lau, C.; Anitole, K.; Hodes, C.; Lai, D.; Pfahles-Hutchens, A.; Seed, J., Perfluoroalkyl Acids: A 371 Review of Monitoring and Toxicological Findings. *Toxicol. Sci.* **2007,** *99*, (2), 366-394.
372 3. Latimer, J.; Zheng, J., The Sources, Transport And Fate Of Pahs In The Marine 263 Environment. 373 *PAHs: An Ecotoxicol.Perspect. 264*, 10-22.
374 4. Giesy, J. P.; Kannan, K., Global distribution of perfluorooctane sulfonate in wildlife. *Environ.* 375 *Sci. Technol.* **2001,** *35*, (7), 1339-1342.
376 5. Giesy, J. P.; Kannan, K., Peer reviewed: perfluorochemical surfactants in the environment. In 377 ACS Publications: 2002.
378 6. Hussain, K.; Hoque, R. R.; Balachandran, S.; Medhi, S.; Idris, M. G.; Rahman, M.; Hussain, F. 379 L., Monitoring and Risk Analysis of PAHs in the Environment. In *Handbook of Environmental* 380 *Materials Management*, Hussain, C. M., Ed. Springer International Publishing: Cham, 2018; pp 381 1-35.
382 7. Nakata, H.; Kannan, K.; Nasu, T.; Cho, H.-S.; Sinclair, E.; Takemura, A., Perfluorinated 383 Contaminants in Sediments and Aquatic Organisms Collected from Shallow Water and Tidal Flat 384 Areas of the Ariake Sea, Japan: Environmental Fate of Perfluorooctane Sulfonate in Aquatic 385 Ecosystems. *Environ. Sci. Technol.* **2006,** *40*, (16), 4916-4921.

386 8. Cachot, J.; Geffard, O.; Augagneur, S.; Lacroix, S.; Le Menach, K.; Peluhet, L.; Couteau, J.;
387 Denier, X.; Devier, M. H.; Pottier, D.; Budzinski, H., Evidence of genotoxicity related to high
388 PAH content of sediments in the upper part of the Seine estuary (Normandy, France).
389 *Aquat.Toxicol.* **2006,** *79*, (3), 257-67.
390 9. Saito, N.; Harada, K.; Inoue, K.; Sasaki, K.; Yoshinaga, T.; Koizumi, A., Perfluorooctanoate and
391 perfluorooctane sulfonate concentrations in surface water in Japan. *J. Occupat. health* **2004,** *46*,
392 (1), 49-59.
393 10. So, M.; Taniyasu, S.; Yamashita, N.; Giesy, J.; Zheng, J.; Fang, Z.; Im, S.; Lam, P. K.,
394 Perfluorinated compounds in coastal waters of Hong Kong, South China, and Korea. *Environ.*
395 *Sci. Technol.* **2004,** *38*, (15), 4056-4063.
396 11. Lu, G.-H.; Yang, Y.-L.; Taniyasu, S.; Yeung, L. W.; Pan, J.; Zhou, B.; Lam, P. K.; Yamashita,
397 N., Potential exposure of perfluorinated compounds to Chinese in Shenyang and Yangtze River
398 Delta areas. *Environ. Chem.* **2011,** *8*, (4), 407-418.
399 12. Yang, L.; Zhu, L.; Liu, Z., Occurrence and partition of perfluorinated compounds in water and
400 sediment from Liao River and Taihu Lake, China. *Chemosphere* **2011,** *83*, (6), 806-814.
401 13. Sepulvado, J. G.; Blaine, A. C.; Hundal, L. S.; Higgins, C. P., Occurrence and fate of
402 perfluorochemicals in soil following the land application of municipal biosolids. *Environ. Sci.*
403 *Technol.* **2011,** *45*, (19), 8106-8112.
404 14. Wang, T.; Chen, C.; Naile, J. E.; Khim, J. S.; Giesy, J. P.; Lu, Y., Perfluorinated compounds in
405 water, sediment and soil from Guanting Reservoir, China. *Bull. Environ. Contam. Toxicol.* **2011,**
406 *87*, (1), 74.
407 15. Nakata, H.; Sakai, Y.; Miyawaki, T.; Takemura, A., Bioaccumulation and Toxic Potencies of
408 Polychlorinated Biphenyls and Polycyclic Aromatic Hydrocarbons in Tidal Flat and Coastal
409 Ecosystems of the Ariake Sea, Japan. *Environ. Sci. Technol.* **2003,** *37*, (16), 3513-3521.
410 16. Santodonato, J., Review of the estrogenic and antiestrogenic activity of polycyclic aromatic
411 hydrocarbons: Relationship to carcinogenicity. *Chemosphere* **1997,** *34*, (4), 835-848.
412 17. Clemons, J. H.; Allan, L. M.; Marvin, C. H.; Wu, Z.; McCarry, B. E.; Bryant, D. W.;
413 Zacharewski, T. R., Evidence of Estrogen- and TCDD-Like Activities in Crude and Fractionated
414 Extracts of PM10 Air Particulate Material Using in Vitro Gene Expression Assays. *Environ. Sci.*
415 *Technol.* **1998,** *32*, (12), 1853-1860.
416 18. Tran, D. Q.; Ide, C. F.; McLachlan, J. A.; Arnold, S. F., The anti-estrogenic activity of selected
417 polynuclear aromatic hydrocarbons in yeast expressing human estrogen receptor. *Biochem.*
418 *Biophy. Res. Comm.* **1996,** *229*, (1), 101-8.
419 19. Du, Y.; Shi, X.; Liu, C.; Yu, K.; Zhou, B., Chronic effects of water-borne PFOS exposure on
420 growth, survival and hepatotoxicity in zebrafish: A partial life-cycle test. *Chemosphere* **2009,** *74*,
421 (5), 723-729.
422 20. Liu, C.; Du, Y.; Zhou, B., Evaluation of estrogenic activities and mechanism of action of
423 perfluorinated chemicals determined by vitellogenin induction in primary cultured tilapia
424 hepatocytes. *Aquat. Toxicol.* **2007,** *85*, (4), 267-277.
425 21. Ankley, G. T.; Jensen, K. M.; Durhan, E. J.; Makynen, E. A.; Butterworth, B. C.; Kahl, M. D.;
426 Villeneuve, D. L.; Linnum, A.; Gray, L. E.; Cardon, M.; Wilson, V. S., Effects of two fungicides
427 with multiple modes of action on reproductive endocrine function in the fathead minnow
428 (Pimephales promelas). *Toxicol. Sci.* **2005,** *86*, (2), 300-8.
429 22. Hagenaars, A.; Knapen, D.; Meyer, I. J.; van der Ven, K.; Hoff, P.; De Coen, W., Toxicity
430 evaluation of perfluorooctane sulfonate (PFOS) in the liver of common carp (Cyprinus carpio).
431 *Aquat.Toxicol.* **2008,** *88*, (3), 155-63.
432 23. Zohar, Y.; Muñoz-Cueto, J. A.; Elizur, A.; Kah, O., Neuroendocrinology of reproduction in
433 teleost fish. *Ge. Comp. Endocrinol.* **2010,** *165*, (3), 438-455.
434 24. Yu, K. L.; Rosenblum, P. M.; Peter, R. E., In vitro release of gonadotropin-releasing hormone
435 from the brain preoptic-anterior hypothalamic region and pituitary of female goldfish. *Gen.*
436 *Comp. Endocrinol.* **1991,** *81*, (2), 256-267.
437 25. Chang, J. P.; Yu, K. L.; Wong, A. O.; Peter, R. E., Differential actions of dopamine receptor
438 subtypes on gonadotropin and growth hormone release in vitro in goldfish. *Neuroendocrinol.*
439 **1990,** *51*, (6), 664-74.

440 26. Xing, L.; Esau, C.; Trudeau, V. L., Direct Regulation of Aromatase B Expression by 17beta-
441 Estradiol and Dopamine D1 Receptor Agonist in Adult Radial Glial Cells. *Frontiers Neurosci.*
442 **2015,** *9*, 504.
443 27. Fontaine, R.; Affaticati, P.; Yamamoto, K.; Jolly, C.; Bureau, C.; Baloche, S.; Gonnet, F.;
444 Vernier, P.; Dufour, S.; Pasqualini, C., Dopamine inhibits reproduction in female zebrafish
445 (Danio rerio) via three pituitary D2 receptor subtypes. *Endocrinol.* **2013,** *154*, (2), 807-18.
446 28. Ashcroft, G. W., Amine Metabolism in Brain. *Proceed. Royal Soc. Med.* **1969,** *62*, (11 Pt 1),
447 1099-1101.
448 29. Beaulieu, J. M.; Gainetdinov, R. R., The physiology, signaling, and pharmacology of dopamine
449 receptors. *Pharmacol. Rev.* **2011,** *63*, (1), 182-217.
450 30. Tanida, T.; Warita, K.; Ishihara, K.; Fukui, S.; Mitsuhashi, T.; Sugawara, T.; Tabuchi, Y.;
451 Nanmori, T.; Qi, W.; Inamoto, T.; Yokoyama, T.; Kitagawa, H.; Hoshi, N., Fetal and neonatal
452 exposure to three typical environmental chemicals with different mechanisms of action: Mixed
453 exposure to phenol, phthalate, and dioxin cancels the effects of sole exposure on mouse midbrain
454 dopaminergic nuclei. *Toxicol. Lett.* **2009,** *189*, (1), 40-47.
455 31. Suzuki, T.; Mizuo, K.; Nakazawa, H.; Funae, Y.; Fushiki, S.; Fukushima, S.; Shirai, T.; Narita,
456 M., Prenatal and neonatal exposure to bisphenol-A enhances the central dopamine D1 receptor-
457 mediated action in mice: enhancement of the methamphetamine-induced abuse state. *Neurosci.*
458 **2003,** *117*, (3), 639-44.
459 32. Crago, J.; Schlenk, D., The effect of bifenthrin on the dopaminergic pathway in juvenile rainbow
460 trout (Oncorhynchus mykiss). *Aquat. Toxicol.* **2015,** *162*, 66-72.
461 33. Sundt, R. C.; Ruus, A.; Jonsson, H.; Skarphéðinsdóttir, H.; Meier, S.; Grung, M.; Beyer, J.;
462 Pampanin, D. M., Biomarker responses in Atlantic cod (Gadus morhua) exposed to produced
463 water from a North Sea oil field: Laboratory and field assessments. *Mar. Pollut. Bull.* **2012,** *64*,
464 (1), 144-152.
465 34. Vethaak, A. D.; Davies, I. M.; Thain, J. E.; Gubbins, M. J.; Martínez-Gómez, C.; Robinson, C.
466 D.; Moffat, C. F.; Burgeot, T.; Maes, T.; Wosniok, W.; Giltrap, M.; Lang, T.; Hylland, K.,
467 Integrated indicator framework and methodology for monitoring and assessment of hazardous
468 substances and their effects in the marine environment. *Mar. Environ. Res.* **2017,** *124*, 11-20.
469 35. Star, B.; Nederbragt, A. J.; Jentoft, S.; Grimholt, U.; Malmstrøm, M.; Gregers, T. F.; Rounge, T.
470 B.; Paulsen, J.; Solbakken, M. H.; Sharma, A.; Wetten, O. F.; Lanzén, A.; Winer, R.; Knight, J.;
471 Vogel, J.-H.; Aken, B.; Andersen, Ø.; Lagesen, K.; Tooming-Klunderud, A.; Edvardsen, R. B.;
472 Tina, K. G.; Espelund, M.; Nepal, C.; Previti, C.; Karlsen, B. O.; Moum, T.; Skage, M.; Berg, P.
473 R.; Gjøen, T.; Kuhl, H.; Thorsen, J.; Malde, K.; Reinhardt, R.; Du, L.; Johansen, S. D.; Searle,
474 S.; Lien, S.; Nilsen, F.; Jonassen, I.; Omholt, S. W.; Stenseth, N. C.; Jakobsen, K. S., The genome
475 sequence of Atlantic cod reveals a unique immune system. *Nature* **2011,** *477*, 207.
476 36. Bjørn Einar Grøsvik, E. K., Li Liu, Guri Nesje, Kjell Westrheim, Marc H.G. Berntssen, Jérémie
477 LeGoff, Sonnich Meier *Condition monitoring in the water column 2011: Oil hydrocarbons in*
478 *fish from Norwegian waters*; **2012**.
479 37. Roland Kallenborn, U. B. a. U. J. *Perfluorinated Alkylated Substances (PFAS) in the Nordic*
480 *environment*; **2004**.
481 38. Dorte Herzke, M. H., Athanasios Katsoyiannis, Anders R.; Borgen, E. K. E., Nick Warner,
482 Eldbjørg S.; Heimstad, F. G., Håkon Gregersen, Jannike Gry Bettum; Jensen, Ø. P. H., Janne
483 Erath Horn, Kjetil Sagerup, Guttorm N. Christensen, Geir Wing Gabrielsen, Sveinn Are Hansen,
484 Kjetil Sagerup *Perfluorinated alkylated substances, brominated flame retardants and chlorinated*
485 *paraffins in the Norwegian Environment - Screening 2013*; **2013**.
486 39. Bertotto, L. B.; Dasgupta, S.; Vliet, S.; Dudley, S.; Gan, J.; Volz, D. C.; Schlenk, D., Evaluation
487 of the estrogen receptor alpha as a possible target of bifenthrin effects in the estrogenic and
488 dopaminergic signaling pathways in zebrafish embryos. *Sci. Total Environ.* **2019,** *651*, 2424-
489 2431.
490 40. Kjeldsen, L. S.; Bonefeld-Jorgensen, E. C., Perfluorinated compounds affect the function of sex
491 hormone receptors. *Enviro. Sci. Pollut. Res. Internat.* **2013,** *20*, (11), 8031-44.
492 41. Sartor, M. A.; Schnekenburger, M.; Marlowe, J. L.; Reichard, J. F.; Wang, Y.; Fan, Y.; Ma, C.;
493 Karyala, S.; Halbleib, D.; Liu, X.; Medvedovic, M.; Puga, A., Genomewide analysis of aryl

494 hydrocarbon receptor binding targets reveals an extensive array of gene clusters that control
495 morphogenetic and developmental programs. *Environ. Health Perspect.* **2009,** *117*, (7), 1139-
496 1146.
497 42. Du, G.; Huang, H.; Hu, J.; Qin, Y.; Wu, D.; Song, L.; Xia, Y.; Wang, X., Endocrine-related
498 effects of perfluorooctanoic acid (PFOA) in zebrafish, H295R steroidogenesis and receptor
499 reporter gene assays. *Chemosphere* **2013,** *91*, (8), 1099-106.
500 43. Du, G.; Hu, J.; Huang, H.; Qin, Y.; Han, X.; Wu, D.; Song, L.; Xia, Y.; Wang, X., Perfluorooctane
501 sulfonate (PFOS) affects hormone receptor activity, steroidogenesis, and expression of
502 endocrine-related genes in vitro and in vivo. *Environ. Toxicol.Chem.* **2013,** *32*, (2), 353-60.
503 44. Fertuck, K. C.; Matthews, J. B.; Zacharewski, T. R., Hydroxylated Benzo[a]pyrene Metabolites
504 Are Responsible for in Vitro Estrogen Receptor-Mediated Gene Expression Induced by
505 Benzo[a]pyrene, but Do Not Elicit Uterotrophic Effects in Vivo. *Toxicol. Sci.* **2001,** *59*, (2), 231-
506 240.
507 45. Meucci, V.; Arukwe, A., Transcriptional modulation of brain and hepatic estrogen receptor and
508 P450arom isotypes in juvenile Atlantic salmon (Salmo salar) after waterborne exposure to the
509 xenoestrogen, 4-nonylphenol. *Aquat. Toxicol.* **2006,** *77*, (2), 167-77.
510 46. Dufour, S.; Weltzien, F. A.; Sebert, M. E.; Le Belle, N.; Vidal, B.; Vernier, P.; Pasqualini, C.,
511 Dopaminergic inhibition of reproduction in teleost fishes: ecophysiological and evolutionary
512 implications. *Ann. New York Acad. Sci.* **2005,** *1040*, 9-21.
513 47. Peter, R. E.; Chang, J. P.; Nahorniak, C. S.; Omeljaniuk, R. J.; Sokolowska, M.; Shih, S. H.;
514 Billard, R., Interactions of catecholamines and GnRH in regulation of gonadotropin secretion in
515 teleost fish. *Rec. Prog. Hormone Res.* **1986,** *42*, 513-48.
516 48. Levavi-Sivan, B.; Safarian, H.; Rosenfeld, H.; Elizur, A.; Avitan, A., Regulation of gonadotropin-
517 releasing hormone (GnRH)-receptor gene expression in tilapia: effect of GnRH and dopamine.
518 *Biol. Reprod.* **2004,** *70*, (6), 1545-51.
519 49. Kumakura, N.; Okuzawa, K.; Gen, K.; Kagawa, H., Effects of gonadotropin-releasing hormone
520 agonist and dopamine antagonist on hypothalamus-pituitary-gonadal axis of pre-pubertal female
521 red seabream (Pagrus major). *Gen. Comp. Endocrinol.* **2003,** *131*, (3), 264-73.
522 50. Vacher, C.; Ferriere, F.; Marmignon, M. H.; Pellegrini, E.; Saligaut, C., Dopamine D2 receptors
523 and secretion of FSH and LH: role of sexual steroids on the pituitary of the female rainbow trout.
524 *Gen. Comp. Endocrinol.* **2002,** *127*, (2), 198-206.
525 51. Chang, J. P.; Johnson, J. D.; Van Goor, F.; Wong, C. J.; Yunker, W. K.; Uretsky, A. D.; Taylor,
526 D.; Jobin, R. M.; Wong, A. O.; Goldberg, J. I., Signal transduction mechanisms mediating
527 secretion in goldfish gonadotropes and somatotropes. *Biochem. Cell Biol.* **2000,** *78*, (3), 139-53.
528 52. Levavi-Sivan, B.; Biran, J.; Fireman, E., Sex Steroids Are Involved in the Regulation of
529 Gonadotropin-Releasing Hormone and Dopamine D2 Receptors in Female Tilapia Pituitary1.
530 *Biol. Reprod.* **2006,** *75*, (4), 642-650.
531 53. Nocillado, J. N.; Levavi-Sivan, B.; Avitan, A.; Carrick, F.; Elizur, A., Isolation of dopamine D(2)
532 receptor (D (2)R) promoters in Mugil cephalus. *Fish Physiol. Biochem.* **2005,** *31*, (2-3), 149-52.
533 54. Salgado, R.; Lopez-Doval, S.; Pereiro, N.; Lafuente, A., Perfluorooctane sulfonate (PFOS)
534 exposure could modify the dopaminergic system in several limbic brain regions. *Toxicol. Lett.*
535 **2016,** *240*, (1), 226-35.
536 55. Pereiro, N.; Moyano, R.; Blanco, A.; Lafuente, A., Regulation of corticosterone secretion is
537 modified by PFOS exposure at different levels of the hypothalamic-pituitary-adrenal axis in adult
538 male rats. *Toxicol.Lett* **2014,** *230*, (2), 252-62.
539 56. Tobon, K. E.; Chang, D.; Kuzhikandathil, E. V., MicroRNA 142-3p mediates post-transcriptional
540 regulation of D1 dopamine receptor expression. *PloS One* **2012,** *7*, (11), e49288.
541 57. Shi, S.; Leites, C.; He, D.; Schwartz, D.; Moy, W.; Shi, J.; Duan, J., MicroRNA-9 and microRNA-
542 326 regulate human dopamine D2 receptor expression, and the microRNA-mediated expression
543 regulation is altered by a genetic variant. *J. Biol. Chem.* **2014,** *289*, (19), 13434-44.
544 58. Kung, T. S.; Richardson, J. R.; Cooper, K. R.; White, L. A., Developmental Deltamethrin
545 Exposure Causes Persistent Changes in Dopaminergic Gene Expression, Neurochemistry, and
546 Locomotor Activity in Zebrafish. *Toxicol. Sci.* **2015,** *146*, (2), 235-243.

547 59. Bertotto, L. B.; Richards, J.; Gan, J.; Volz, D. C.; Schlenk, D., Effects of bifenthrin exposure on
548 the estrogenic and dopaminergic pathways in zebrafish embryos and juveniles. *Environ. Toxicol.*
549 *Chem.* **2018,** *37*, (1), 236-246.
550 60. Kumer, S. C.; Vrana, K. E., Intricate regulation of tyrosine hydroxylase activity and gene
551 expression. *J. Neurochem.* **1996,** *67*, (2), 443-62.
552 61. Azcoitia, I.; Sierra, A.; Veiga, S.; Garcia-Segura, L. M., Aromatase expression by reactive
553 astroglia is neuroprotective. *Ann. New York Acad. Sci.* **2003,** *1007*, 298-305.
554 62. Ubuka, T.; Haraguchi, S.; Tobari, Y.; Narihiro, M.; Ishikawa, K.; Hayashi, T.; Harada, N.;
555 Tsutsui, K., Hypothalamic inhibition of socio-sexual behaviour by increasing neuroestrogen
556 synthesis. *Nature Comm.* **2014,** *5*, 3061.
557 63. Xing, L.; McDonald, H.; Da Fonte, D. F.; Gutierrez-Villagomez, J. M.; Trudeau, V. L., Dopamine
558 D1 receptor activation regulates the expression of the estrogen synthesis gene aromatase B in
559 radial glial cells. *Front. Neurosci.* **2015,** *9*, 310.
560
561
562
563

564 **Figure 1.** Three-level design of the study with two factors, PAH and PFAS. Each digit represents the
565 dose (0=absent, 1=low, and 2=high dose) and values inside circle indicate the contribution of both
566 factors in one exposure group.

567

568 **Figure 2.** Plasma concentration of 17β-estradiol (E2) in juvenile female Atlantic cod exposed to two
569 different doses (low and high) of PAH and PFAS, given singly or in combinations (low PAH/low
570 PFAS, high PAH/low PFAS, low PAH/high PFAS and high PAH/high PFAS). Data are presented as
571 ng/mL of n=3-8 ± standard error of the mean (SEM). Groups marked with asterisks (*) are
572 significantly different compared with control ($p<0.05$).

573

574 **Figure 3.** Principle Component Analysis (PCA) of measured biological parameter, two-
575 dimensionally visualized on an x-y scatter plot, with a combined factorial score of 69.12%. The data
576 included is plasma 17β-estradiol levels, brain dopamine metabolite ratios and expression of
577 dopaminergic and estrogenic signalling gene in the brain of juvenile Atlantic cod following exposure
578 to PAHs and PFASs at different doses and their various combinations.

579

580

581

582

583

584

585

586

587

588

589

590 **Table 1:** Overview of exposure chemicals* and their concentrations for *in vivo* exposure.

	Compound	CAS No.	µg/kg		% of total
			Low Dose (1x)	**High Dose (20x)**	
PAH	Naphthalene	50-32-8	12.64	252.8	31.6
	Phenanthrene	132-65-0	8.38	167.6	21.0
	Dibenzothiophene	86-73-7	0.58	11.6	1.4
	Pyrene	91-20-3	1.45	29.0	3.6
	BaP	85-01-8	1.93	38.5	4.8
	Fluorene	129-00-0	15.03	300.5	37.6
	Total dose		**40**	**800**	**100**
PFAS	PFOS	2795-39-3	25	500	48.3
	PFTrA	375-95-1	16.95	339	32.8
	PFNA	335-67-1	5.925	118.5	11.5
	PFOA	72629-94-8	3.825	76.5	7.4
	Total dose		**51.7**	**1034**	**100**

591 * All chemicals were purchased from Sigma Aldrich (St. Louis, MO, USA)

592
593
594
595
596
597
598
599
600
601
602
603
604
605
606

607 **Table 2:** Concentration of dopamine, homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid
608 (DOPAC) as well as dopamine metabolite ratio (DOPAC/Da and DOPAC+HVA/Da) in the brain of
609 female juvenile Atlantic cod exposed to different doses of PAH and PFAS, singly or in combination,
610 analysed by UPLC-MS/MS.

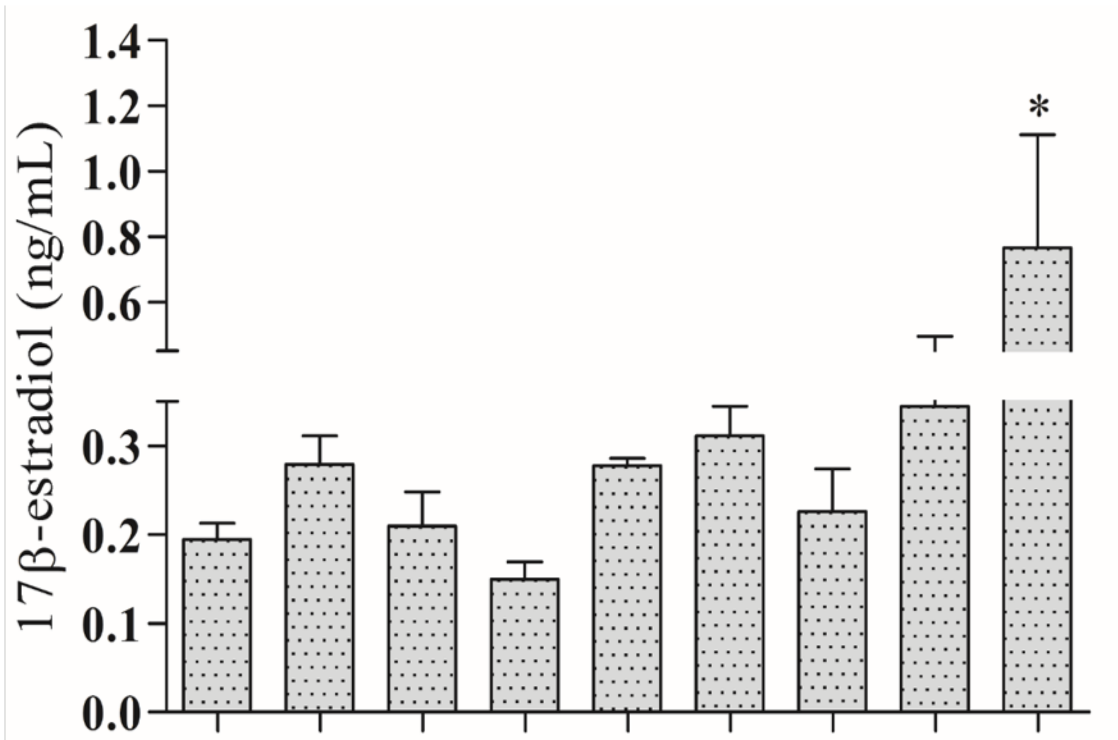
	pg/mg		ng/mg	Ratio	
Exposure group	**Dopamine**	**HVA**	**DOPAC**	**DOPAC/Da**	**DOPAC+HVA/Da**
Control	49.39 ± 4.39	182.53 ± 13.22	28.68 ± 1.56	0.615 ± 0.06	0.699 ± 0.08
Low PAH	**36.28 ± 1.46***	178.60 ± 11.90	23.55 ± 1.57	0.669 ± 0.126	0.809 ± 0.12
High PAH	38.79 ± 3.24	198.61 ± 16.65	**18.23 ± 1.34***	0.484 ± 0.06	0.638 ± 0.056
Low PFAS	46.56 ± 2.32	184.33 ± 35.59	24.33 ± 1.81	0.539 ± 0.041	0.629 ± 0.051
High PFAS	**33.93 ± 1.13***	143.84 ± 5.65	28.79 ± 1.66	0.855 ± 0.06	0.991 ± 0.058
Low PAH/Low PFAS	**32.51 ± 3.59***	133.59 ± 7.13	30.77 ± 1.09	**1.01 ± 0.106***	**1.174 ± 0.139***
High PAH/Low PFAS	38.80 ± 0.43	156.48 ± 13.29	36.80 ± 1.57	**0.965 ± 0.09***	**1.076 ± 0.10***
Low PAH/High PFAS	44.41 ± 3.27	199.74 ± 8.91	21.60 ± 1.58	0.512 ± 0.054	0.625 ± 0.063
High PAH/High PFAS	56.39 ± 3.43	258.15 ± 30.62	**43.52 ± 1.56***	0.779 ± 0.065	0.868 ± 0.056

611

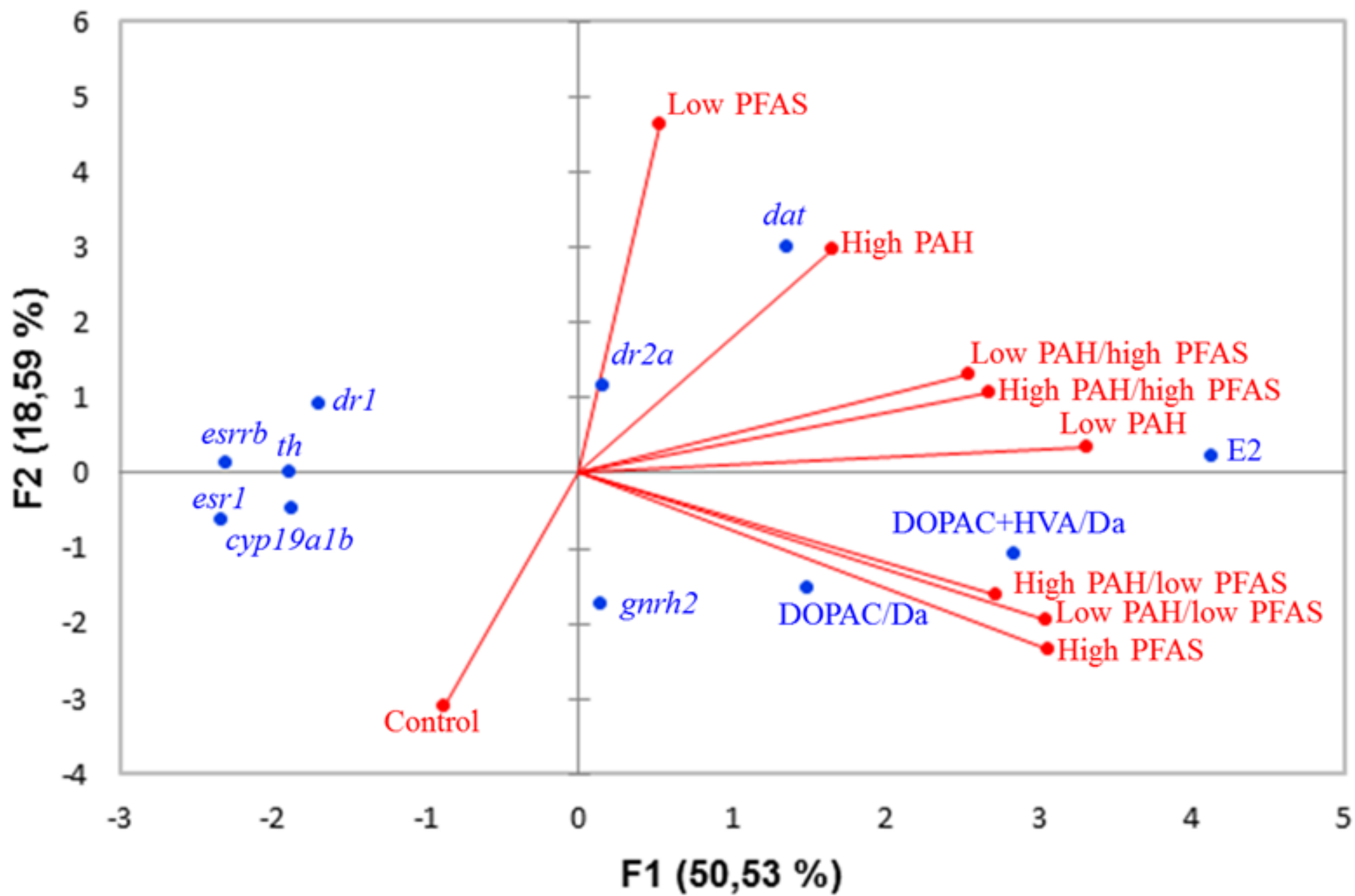
612 Concentration of both dopamine and HVA are given in pg/mg, while DOPAC is given in ng/mg. Each
613 value represents the mean (n = 6-9 ± standard error of the mean [SEM]). Ratio data are presented in
614 decimal of thousandth digit. Asterisk represent significant difference between control and exposure
615 groups at $p \leq 0.05$.

616
617
618
619
620
621
622
623
624

	PAH (Factor A)		
PFAS (Factor B)	0	1	2
0	0/0	1/0	2/0
1	0/1	1/1	2/1
2	0/2	1/2	2/2



17β-estradiol (ng/mL)
1.4
1.2
1.0
0.8
0.6
0.3
0.2
0.1
0.0
*
PAH Low - + - - - + - + -
High - - + - - - + - +
PFAS Low - - - + - + + - -
High - - - - + - - + +



Biplot (axes F1 and F2: 69,12 %)
F2 (18,59 %)
F1 (50,53 %)
6
5
4
3
2
1
0
-1
-2
-3
-4
-3
-2
-1
0
1
2
3
4
5
Low PFAS
dat
High PAH
dr2a
Low PAH/high PFAS
High PAH/high PFAS
Low PAH
E2
dr1
esrrb
th
esr1
cyp19a1b
DOPAC+HVA/Da
High PAH/low PFAS
Low PAH/low PFAS
High PFAS
gnrh2
DOPAC/Da
Control
Active variables
Active observations

1 **Supporting information (SI)**

2 **Neuro-dopamine homeostasis of juvenile female Atlantic cod (*Gadus morhua*) exposed**
3 **to polycyclic aromatic hydrocarbons and perfluoroalkyl substances**

4

5 Essa A. Khan[1], Luisa B. Bertotto[2], Karina Dale[3], Roger Lille-Langøy[3], Fekadu Yadetie[3], Odd
6 André Karlsen[3], Anders Goksøyr[3], Daniel Schlenk[2], Augustine Arukwe[1*]

7

8 [1]Department of Biology, Norwegian University of Science and Technology (NTNU),
9 Høgskoleringen 5, N-7491 Trondheim, Norway
10 [2] Department of Environmental Sciences, University of California-Riverside, Riverside,
11 California, USA
12 [3] Department of Biological Sciences, University of Bergen, N-5020 Bergen, Norway

13

14 ***Corresponding author:**
15 Augustine Arukwe
16 Department of Biology
17 Norwegian University of Science and Technology (NTNU)
18 Høgskoleringen 5, N-7491 Trondheim, Norway
19 Telephone: +47 99552728
20 E-mail: augustine.arukwe@ntnu.no

21
22
23
24
25
26
27
28

29 **1. Methodology**

30 **1.1. Biometric data**

31

32 **Table S1.** Biometric data of experimental fish used in the present study. Data are presented as
33 mean (n = 6-9) ± standard error of the mean (SEM).

	Biometric data		
Exposure group	Fish weight (g)	Fish length (cm)	k-factor
Control	177.54 ± 5.56	26.84 ± 0.26	0.91 ± 0.02
Low PAH	167.27 ± 4.94	26.87 ± 0.21	0.85 ± 0.01
High PAH	174.18 ± 7.79	26.72 ± 0.36	0.90 ± 0.01
Low PFAS	180.81 ± 8.72	26.81 ± 0.32	0.92 ± 0.02
High PFAS	154.68 ± 6.27	25.93 ± 0.32	0.88 ± 0.03
Low PAH/Low PFAS	175.14 ± 7.07	26.81 ± 0.35	0.90 ± 0.02
High PAH/Low PFAS	155.90 ± 7.41	25.89 ± 0.35	0.88 ± 0.01
Low PAH/High PFAS	172.33 ± 7.53	26.59 ± 0.32	0.90 ± 0.01
High PAH/High PFAS	182.75 ± 7.43	27.13 ± 0.32	0.90 ± 0.01

34

35

36 **1.2. Quantitative (real-time) PCR**

37 Specific primer pair sequences (Table S2) for th, *dat*, *drd1*, *drd2a*, *er-α*, *esrrb*, *cyp19a1b* and
38 *gnrh2* genes were amplified using the Mx3000P real-time PCR machine (Stratagene, La Jolla,
39 CA). The primer pairs were tested by analyzing single amplified product of expected size for
40 individual genes. A parallel control, lacking cDNA template was used to validate the specificity
41 and target sequence amplification. PCR program includes an enzyme activation step at 95 °C
42 (4 min) followed by 40 cycles of 95 °C (15 s), 60 °C (30 s) and 72 °C (15 s) and last step
43 temperature profile include 95 °C (60 s), 65 °C (30 s) and 95 °C (30 s). Expression of each
44 gene was determined by following the well-validated procedure of absolute quantification in
45 our laboratory.[1] A known amount of plasmid cloned with an amplicon of interest used to

generate a standard curve. The pre-made standard plot of cycle threshold (Ct) versus log copy number were used to quantify the expression of the target gene in unknown samples.

Table S2: Primer pair sequences with amplicon size and annealing temperature conditions for genes quantified with real-time PCR.

Target gene	Accession no.	Primer sequence*		Amplicon size (bp)	Annealing temp. (°C)
		Forward	Reverse		
th	ENSGMOG00000017881	ACCAGTGGCTGGTTTGTT	GTGACCCAGAAGCTCATGTAT	142	62
dat	ENSGMOG00000006703	CTCCAAGCTATGGTTCGTACAC	GCTATTCTATCGCAGAACTTCCC	142	62
drd1	ENSGMOG00000007704	CTTCATCCTCAACTGCATGGT	GCGTTGAAGGCGTAGATGAT	147	62
drd2a	ENSGMOG00000006531	CCACCTCGCTGAAGGATAAG	CTCATCCAGTTCCAGGTCTTC	152	62
er-α	ENSGMOG00000014898	ATCTTCGCACAAGACCTCATC	CCTTGAGACAGACAAACTCCTC	142	62
esrrb	ENSGMOG00000015180	AAGCGGCAGGAGGAGAG	GGATGCTCCGCTTGAAGAA	146	62
cyp19a1b	ENSGMOG00000010165	CTGGAAGAAAGTGAGGGCATATTT	CACAGATCCCCACGGTTCTC	145	51
gnrh2	ENSGMOG00000009002	TACCCTGGAGGAAAGAGAGAG	TGGCCAGGACATCCATAAAG	145	62

* Primer sequences in 5´to 3´ direction.

1.3. Steroid hormone analysis

Steroid hormones were extracted from plasma with organic solvent. Plasma sample (100 µL) was mixed thoroughly with diethyl ether (4:1 volume of plasma), and two phases were allowed to separate by vortexing. The organic phase containing steroid hormones was transferred into new glass tube whereas the frozen aqueous phase was extracted again. The combined extract was allowed to evaporate at 30 °C, and the dry extract was reconstituted in 100 µL EIA buffer by vortexing and stored at 80 °C until analysis. EIA for E2 was performed by following the manufacturer's guideline. The plate was read using a Bio-Tek Synergy HT microplate reader (Bio-Tek Instruments, Winooski, VT, USA) at 410 nm. A standard curve was made by a 4-parameter logistic fit between log concentration and logit transformation of B/Bo (Bound sample/maximum bound) and expressed as ng/mL.

1.4. Ultra-performance Liquid chromatography-mass spectrometry

The brain was homogenized using 0.1 % ice-cold formic acid (LC-MS grade, Sigma-Aldrich) with 1 ng DA-d4 and 2 ng of HVA-d5 per mg tissue as an internal standard. Following

68 centrifugation, the supernatant was subjected to solid-phase extraction with Starta X polymeric
69 reverse-phase cartridges (33 µm, 60 mg, 3 mL; Phenomenex) preconditioned with 0.1 % formic
70 acid in different solvents. The order in which cartridge was conditioned includes 0.1 % formic
71 acid in acetonitrile, followed by 0.1 % formic acid in methanol and 0.1 % formic acid in water.
72 The analyte was eluted with 0.1 % formic acid in acetonitrile/methanol (1:1, v/v), evaporate
73 the organic solvent with a stream of nitrogen gas and finally reconstituted in 0.1 % formic acid
74 in water. The extract was ready for analysis through Waters ACQUITY ultra-performance
75 liquid chromatography (UPLC) coupled with Micromass Triple Quadrupole mass spectrometer
76 (qQq) equipped with an electrospray ionization (ESI) interface (Waters, Milford, MA). An
77 injection volume of 5 µL was passed through ACQUITY UPLC HSS T3, 2.1 mm × 100 mm,
78 1.7 µm column at the flow rate of 0.3 mL min1 with the gradient of two solvents; solvent A
79 (0.1 % formic acid in DI water, 18Ω) and solvent B (0.1 % formic acid in an equal volume of
80 acetonitrile/methanol). The gradient program starts from 95 % A and 5 % B for 1 min and
81 ramped to 40 % A over the course of 1 min, it was further decreased to 10 % for 1.5 min then
82 linearly decreased to 0 % for 0.5 min and finally ramped back to 95 % A involving intermediate
83 step in which there is linearity increase to 10 % for 0.5 min and stayed at 95 % A, 0.5 min for
84 equilibration. The specific instrument setting was as follows: source temperature 150°C,
85 desolvation temperature 600 °C, capillary source voltage 3.00 kV, dwell time 0.028 s, cone gas
86 150 L h-1 and desolvation gas 1200 L h-1, the collision gas was 99.9% pure argon. Cone and
87 collision voltage of 50 V and 20 V respectively were generated using IntelliStart software
88 (Waters). Individual compound peaks were detected and integrated using TargetLynx XS
89 software.
90 A linear calibration curve in a range of 0.2 - 5 ppb for dopamine, from 5 – 200 ppb for
91 HVA and from 1 – 15 ppm for DOPAC (Sigma-Aldrich), purity >98 %) with r2 > 0.97 was
92 used to quantify levels of these metabolites in the brain (Figure S1A, B and C). A deuterated
93 derivative of dopamine-1,1,2,2-d4 (DA-d4) and 4-hydroxy-3methoxyphenyl-d3-acetic-d2 acid
94 (HVA-d5) were purchased from Sigma-Aldrich and used as an internal standard for dopamine
95 and HVA respectively (Figure S2A, B). The same HVA-d5 standard was used for DOPAC.
96 Recoveries were determined by spiking internal standard at a concentration of 4ppm of DA-d5
97 and 8 ppm of HVA-d4 per 100 mg of brain tissue.

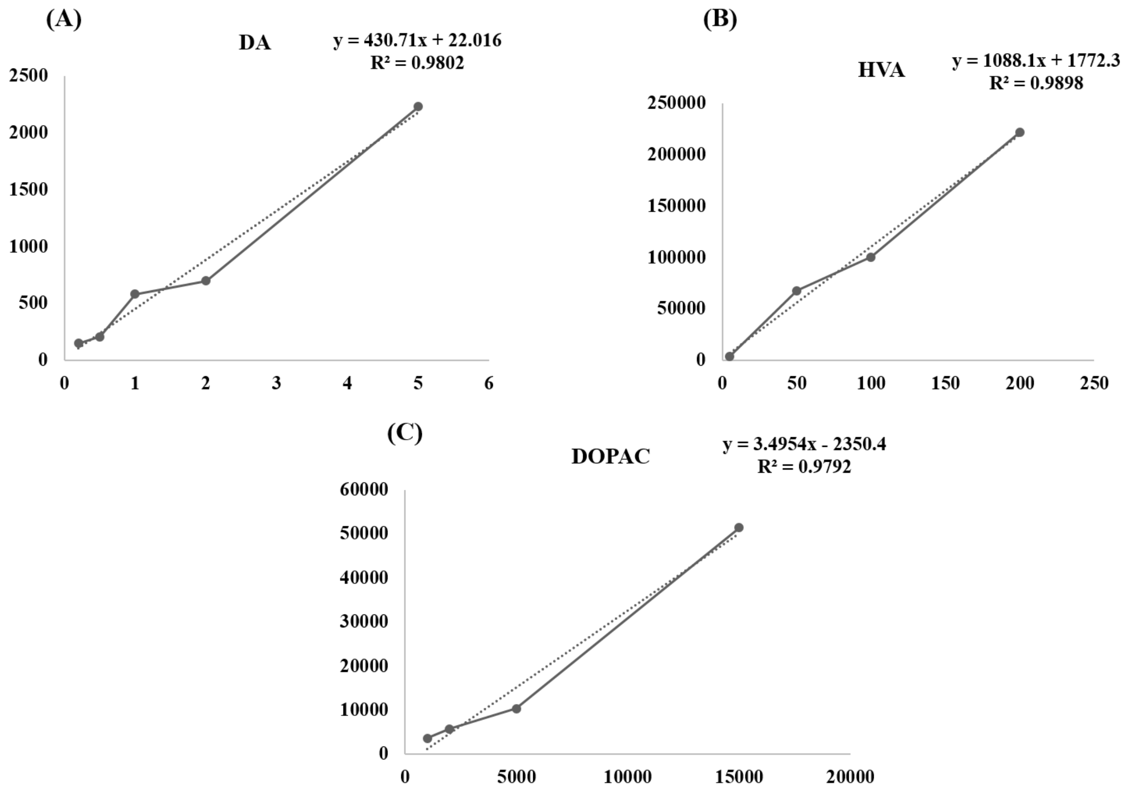


Figure S1: Calibration curve of DA, HVA and DOPAC spiked at specific concentration range through UPLC-MS/MS with r2 > 0.97.

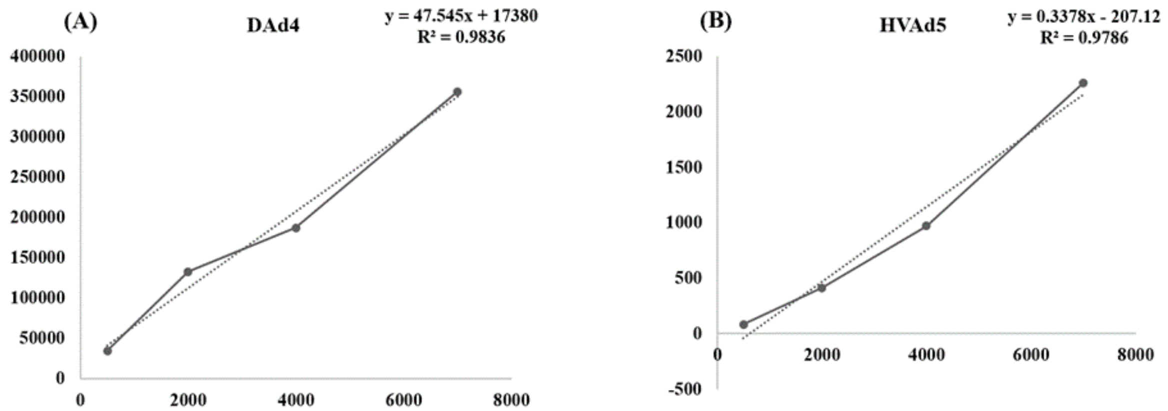
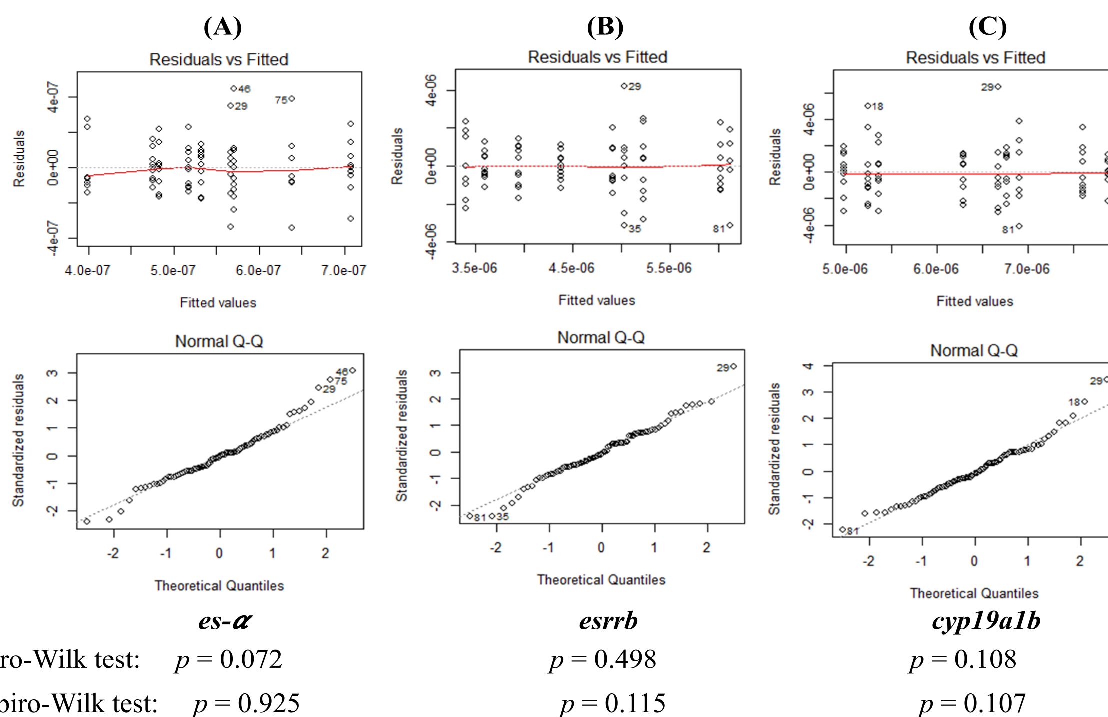


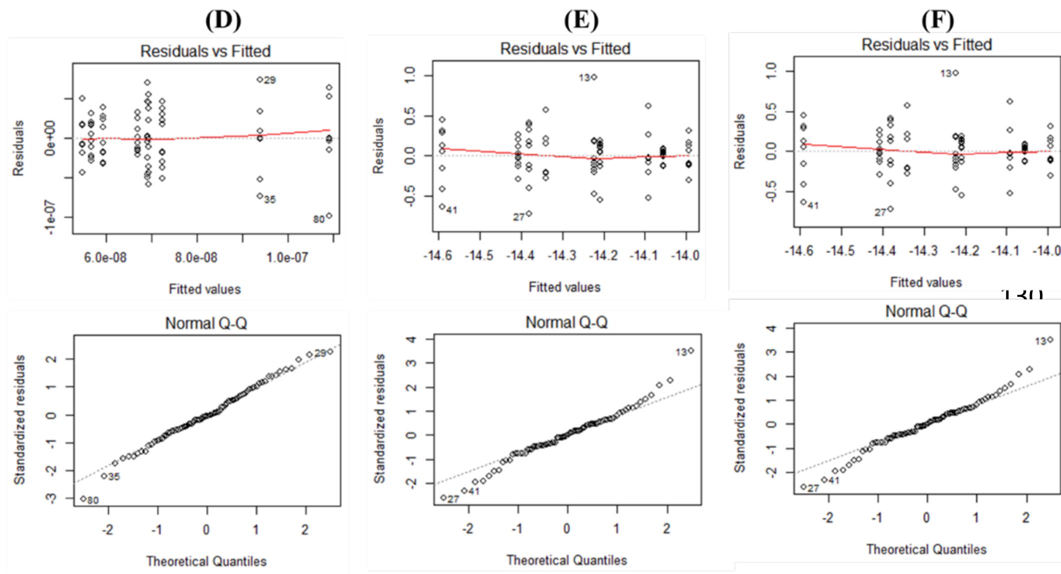
Figure S2: Calibration curve of deuterated derivative, DA-d5 and HVA-d4 spiked at specific concentration range through UPLC-MS/MS with r2 > 0.97.

2. Statistical analysis

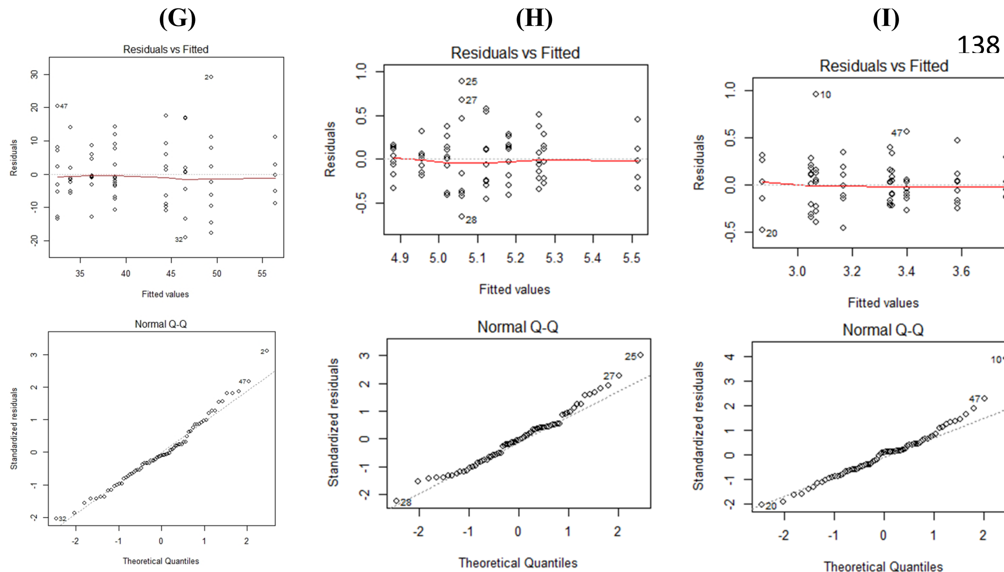
To determine effect of both compounds, separately and their interactions (corresponding to the mixtures), three-level factorial design, statistically analysed using R-Studio (version 1.1.456). A one-way analysis of variance (ANOVA) followed by a Dunnett's post-hoc test was performed to test effect of all treatment groups separately, comparison against a single control group (nine levels). A two-way analysis of variance (ANOVA) was performed on a linear model to test an interaction effect of compounds on parameter of interest. To normalize the residuals of model, data was transformed using natural logarithm and visually inspected using quantile-quantile and histogram plots, as well as Shapiro-Wilk test. Some of the groups belong to E2 and DOPAC data violated Shapiro-Wilk test, therefore applied Kolmogorov-Smirnov test was applied to check normality (Figure S3). Homogeneity of variance was determined by Levene's test. The data belongs to E2, HVA and *drd2a*, violated Levene's homogeneity test, therefore Brown-Forsythe and Welch's heteroscedastic F-test was applied. Gene expression data of *drd1*, an outlier belonging to the Low PAH/Low PFAS treatment group was removed from dataset before logarithm transformation. The dopamine catabolites, DOPAC, gene expression data of *drd1* and E2 data were logarithmically transformed before analysis.



	es-α	***esrrb***	***cyp19a1b***
Shapiro-Wilk test:	$p = 0.072$	$p = 0.498$	$p = 0.108$
Shapiro-Wilk test:	$p = 0.925$	$p = 0.115$	$p = 0.107$



	dat	*dr1*	*dr2a*
Shapiro-Wilk test:	$p = 0.925$	$p = 0.115$	$p = 0.107$

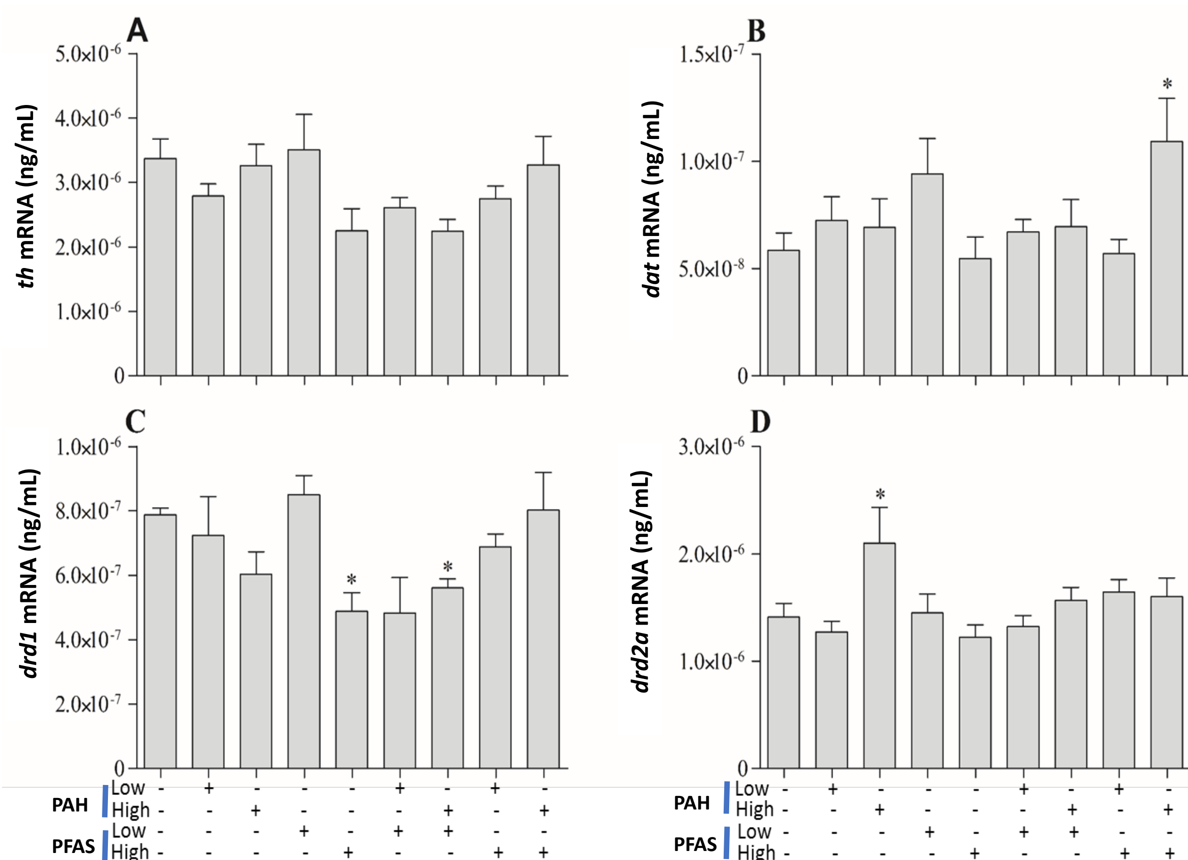


	Dopamine	**HVA**	**DOPAC**
Shapiro-Wilk test:	$p = 0.271$	$p = 0.338$	$p = 0.014$

Figure S3: Normal distribution of gene expression (*er-α*, *esrrb*, *cyp19a1b*, *dat*, *drd1*and *drd2a* in A, B, C, D, E and F panels respectively), dopamine and its metabolite data (dopamine, HVA and DOPAC in G, H and I panels respectively) visually inspected using quantile-quantile plot along with Shapiro-Wilk test, *p*-value.

154 **3. Results**

155 **3.1 Expression levels of genes measured in the present study.**

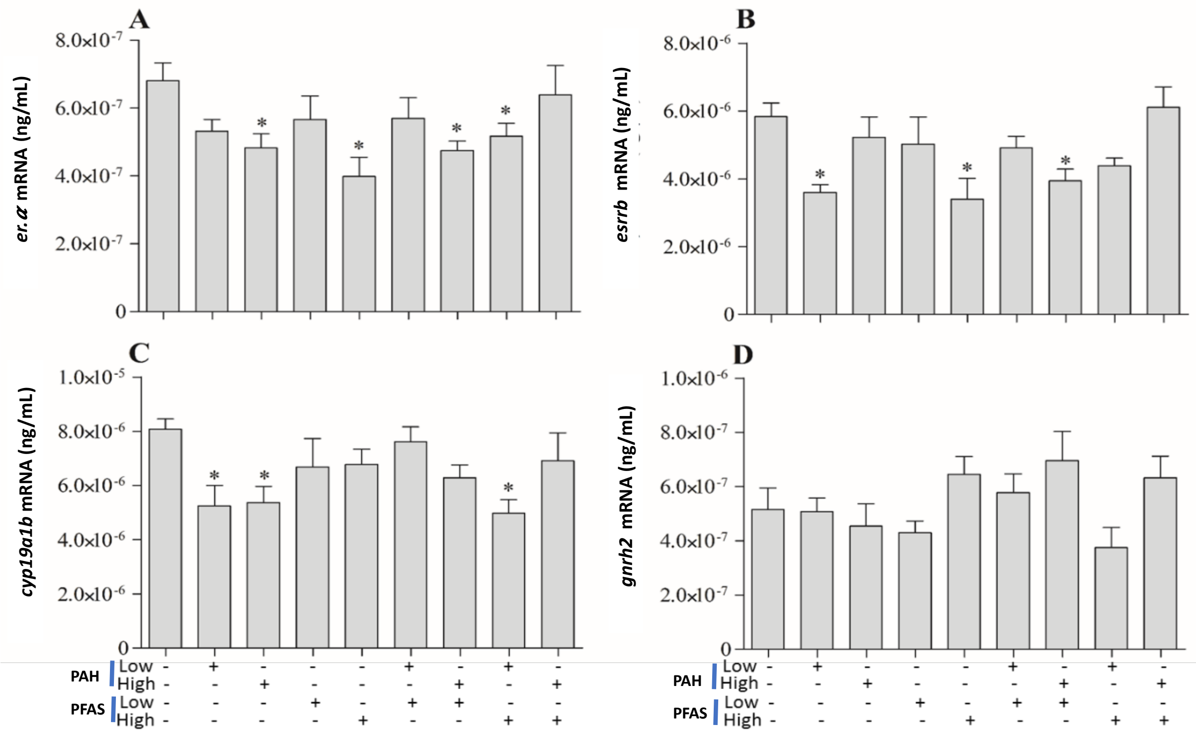


156

157 **Figure S4:** Transcriptional change of tyrosine hydroxylase 1 (*th*: **A**), dopamine active
158 transporter (*dat*: **B**), dopamine receptor (*drd1*: **C**) and (*drd2a*: **D**) in the brain of juvenile female
159 Atlantic cod exposed two different doses (low and high) of PAH and PFAS, given singly or in
160 various combinations (low PAH/low PFAS, high PAH/low PFAS, low PAH/high PFAS and
161 high PAH/high PFAS). Data are presented as mean (n = 6-9) ± standard error of the mean
162 (SEM). Groups marked with asterisks (*) are significantly different, compared with control
163 ($p<0.05$).

164

165



166 **Figure S5:** Transcriptional change of estrogen receptors (*er-a*: **A**, *esrrb*: **B**), *cyp19a1b* (**C**) and
167 *gnrh2* (**D**) in the brain of juvenile female Atlantic cod exposed two different doses (low and
168 high) of PAH and PFAS, given singly or in combinations (low PAH/low PFAS, high PAH/low
169 PFAS, low PAH/high PFAS and high PAH/high PFAS). Data are presented as mean (n = 6-9)
170 ± standard error of the mean (SEM). Groups marked with asterisks (*) are significantly
171 different, compared with control (p<0.05).

172

173

174

175

176

177

178

179

180

181

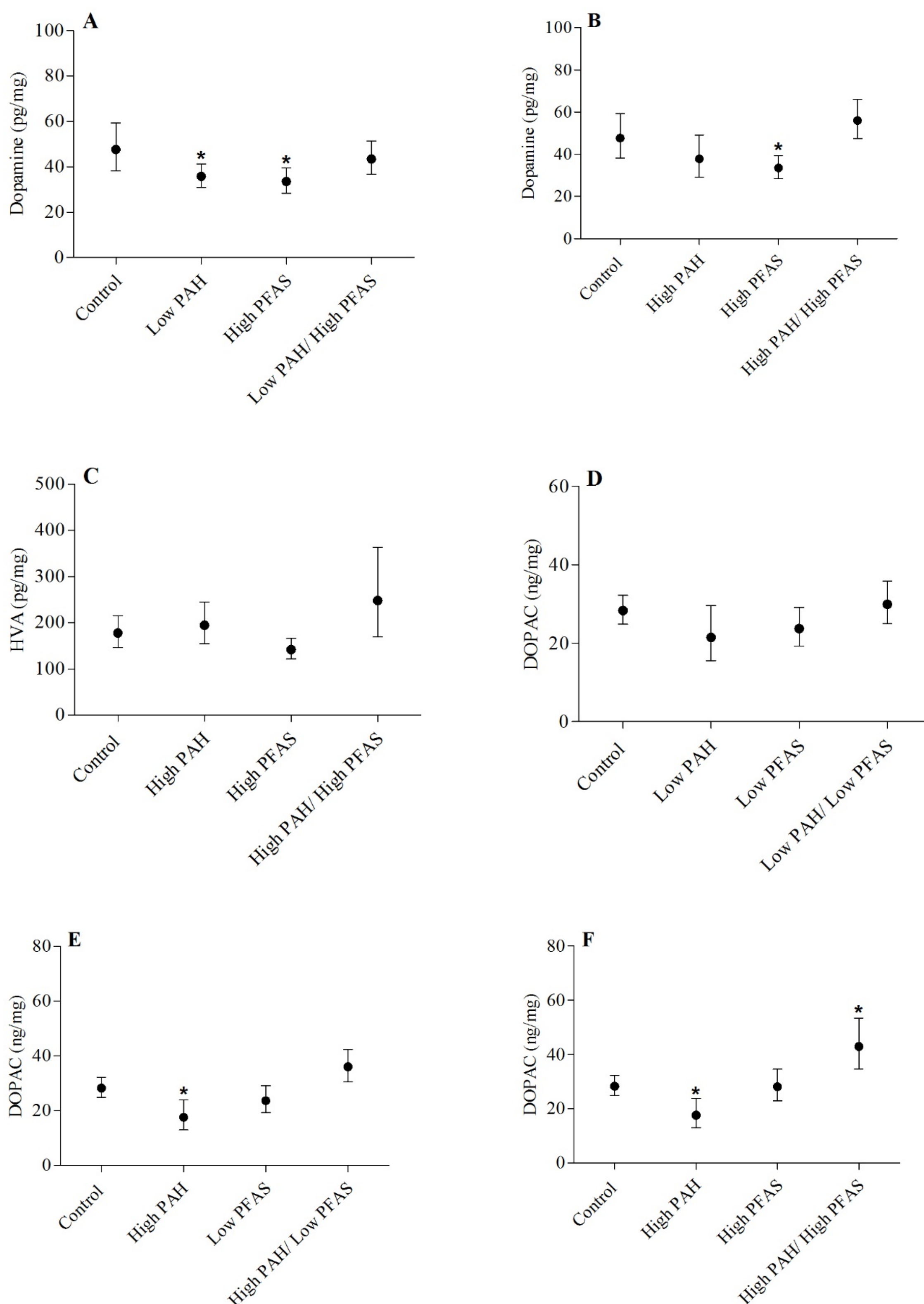
182

183

184

185

186 **3.2 Significant interaction effects between variables investigated in the present study**



187

188 **Figure S6:** PAH and PFAS interaction in mixture significantly affect levels of dopamine (p =
189 0.001 and 8.3e-05; **A** and **B**), HVA (p = 0.046; **C**) and DOPAC (p = 0.005, 9.7e-06 and 4.2e-
190 05; **D**, **E** and **F**). Data are presented as mean of concentration (n = 6-9) and 95 % confidence
191 interval. Group marked with asterisks (*) are significantly different, compared with control
192 (p≤0.05).

193

194 **Table S3:** Significant interaction effect of PAH and PFAS on dopamine and its metabolite
195 (DOPAC and HVA). All metabolic data are presented in concentrations, dopamine and HVA
196 (pg/mg) and DOPAC (ng/mg) with 95% confidence interval (n = 6-9).

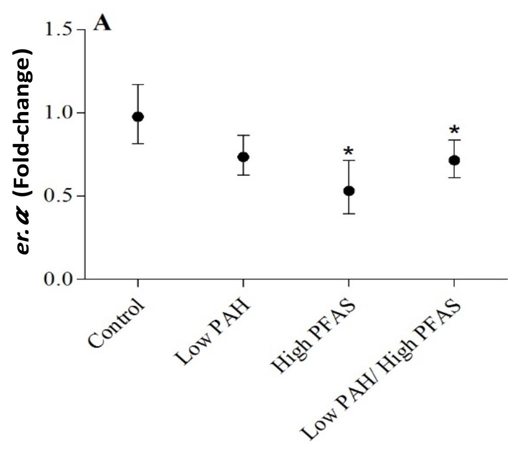
		Conc.	Low 95% CI	High 95% CI
Dopamine	Control	49.39	38.19	60.59
($p = 0.001$)	Low PAH	36.28	31.52	41.05
	High PFAS	33.93	27.7	40.16
	Low PAH/ High PFAS	44.41	36.87	51.95
($p = 8.3e-05$)	High PAH	38.79	28.46	49.12
	High PFAS	33.93	27.7	40.16
	High PAH/ High PFAS	56.39	46.84	65.94
HVA	Control	182.53	149.58	215.49
($p = 0.046$)	High PAH	198.61	153.75	243.48
	High PFAS	143.84	119.63	168.04
	High PAH/ High PFAS	258.15	154.02	362.28
DOPAC	Control	28.68	24.72	32.65
($p = 0.005$)	Low PAH	23.55	13.75	33.35
	Low PFAS	24.33	19.60	29.05
	Low PAH/ Low PFAS	30.77	24.20	37.34
($p = 9.7e-06$)	High PAH	18.23	13.11	23.35
	Low PFAS	24.33	19.60	29.05
	High PAH/ Low PFAS	36.80	30.10	43.39
($p = 4.2e-05$)	High PAH	18.23	13.11	23.35
	High PFAS	28.79	22.48	35.10
	High PAH/ High PFAS	43.52	33.43	53.61

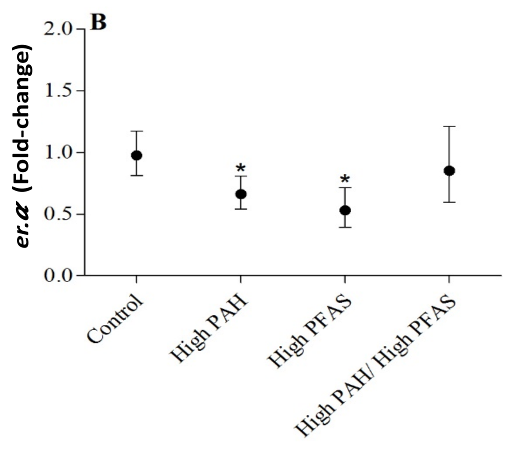
197

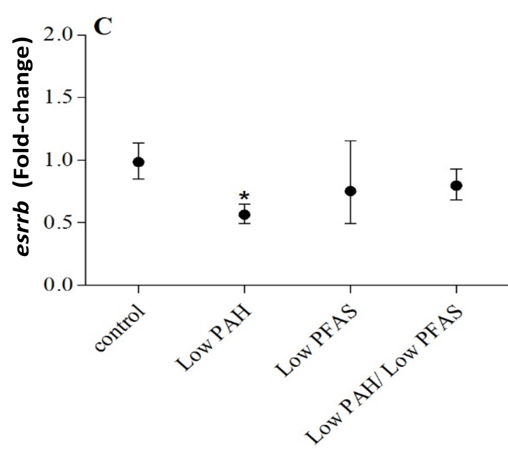
198

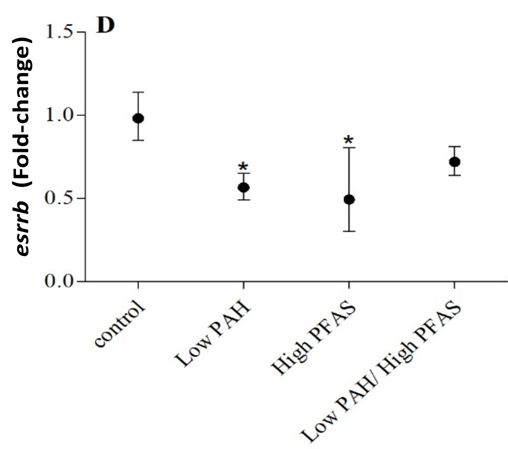
199

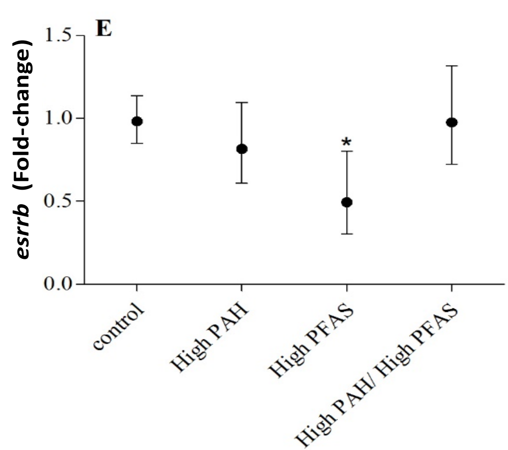
200

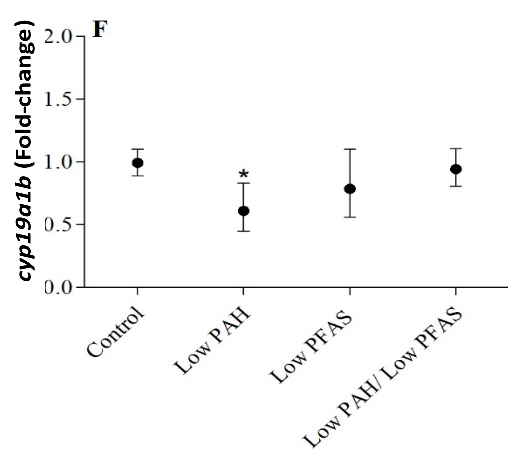




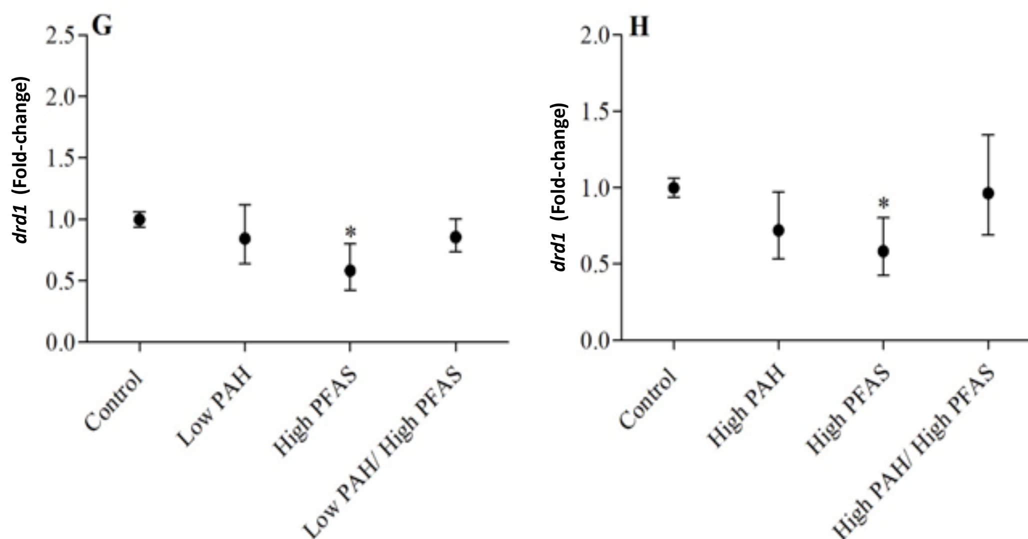








201



202

203 **Figure S7:** PAH and PFAS interaction in mixture significantly affect levels of *er-α* (*p* = 0.004
204 and 4.7e-05; **A** and **B**), *esrrb* (*p* = 0.015, 4.0e-04 and 6.0e-04; **C**, **D** and **E**), *cyp19a1b* (*p* =
205 0.008; **F**), *drd1* (*p* = 0.005 and 0.0001; **G** and **H**) and *gnrh2* at High PAH/Low PFAS (p =
206 0.031; not graphically presented). Data are presented as mean of fold change (n = 6-9) and 95%
207 confidence interval. Group marked with asterisks (*) are significantly different, compared with
208 control (p≤0.05).

209

210 **Table S4:** Significant interaction effect of PAH and PFAS on gene expression of *er-α*, *esrrb*,
211 *cyp19a1b* and *drd1*. All expressions are presented relative to the control (set at 1) with 95%
212 confidence interval (n = 6-9).

		Mean	**Low 95 % CI**	**High 95 % CI**
er-α	Control	1	0.83	1.16
(*p* = 0.0049)	Low PAH	0.73	0.64	0.86
	High PFAS	0.56	0.37	0.75
	Low PAH/ High PFAS	0.74	0.61	0.85
(*p* = 4.7e-05)	High PAH	0.68	0.54	0.81
	High PFAS	0.56	0.37	0.75
	High PAH/ High PFAS	0.90	0.61	1.19
esrrb	Control	1	0.84	1.15
(*p* = 0.015)	Low PAH	0.57	0.49	0.65
	Low PFAS	0.83	0.52	1.14

	Low PAH/ Low PFAS	0.84	0.68	0.94
(p = 4.0e-4)	Low PAH	0.57	0.49	0.65
	High PFAS	0.56	0.32	0.80
	Low PAH/ High PFAS	0.72	0.64	0.81
(p = 6.0e-4)	High PAH	0.86	0.63	1.09
	High PFAS	0.56	0.32	0.80
	High PAH/ High PFAS	1.01	0.77	1.25
cyp19a1b	Control	1	0.89	1.10
(p = 0.008)	Low PAH	0.61	0.44	0.88
	Low PFAS	0.84	0.53	1.16
	Low PAH/ Low PFAS	0.98	0.80	1.12
drd1	Control	1	0.94	1.06
(p = 0.005)	Low PAH	0.90	0.57	1.26
	High PFAS	0.61	0.44	0.79
	Low PAH/ High PFAS	0.87	0.75	0.98
(p = 0.0001)	High PAH	0.76	0.56	0.96
	High PFAS	0.61	0.44	0.79
	High PAH/ High PFAS	1.02	0.65	1.38

213

214

215 **4. Principal component analysis**

216 **4.1 Correlation matrix (Pearson)**

217 **Table S5:** Correlatonal matrix (Pearson) showing statistical interactions between studied
218 variable. Values in bold fonts are significantly different, compared with control at α≤0.05.

Observations	DOPAC/ Da	DOPAC+HVA/Da	*th*	*drd1*	*drd2a*	*er-α*	*esrrb*	*gnrh2*
DOPAC/ Da	**1**	**0.978**	**-0.652**	-0.620	-0.555	-0.150	-0.227	**0.856**
DOPAC+HVA/Da	**0.978**	**1**	**-0.701**	**-0.703**	-0.508	-0.277	-0.328	**0.811**
th	**-0.652**	**-0.701**	**1**	**0.798**	0.414	**0.706**	**0.802**	-0.543
drd1	-0.620	**-0.703**	**0.798**	**1**	0.127	**0.650**	0.509	-0.485
drd2a	-0.555	-0.508	0.414	0.127	**1**	-0.009	0.396	-0.367
er-α	-0.150	-0.277	**0.706**	**0.650**	-0.009	**1**	**0.827**	-0.205
esrrb	-0.227	-0.328	**0.802**	0.509	0.396	**0.827**	**1**	-0.159
gnrh2	**0.856**	**0.811**	-0.543	-0.485	-0.367	-0.205	-0.159	**1**

219

220

221 **Table S6:** Factor score of the variable and observation on the two factorial axes.

		Axes	
	Descriptors	**F1**	**F2**
Variable	Control	-0.256	-0.540
	Low PAH	0.955	0.061
	High PAH	0.479	0.521
	Low PFAS	0.153	0.809
	High PFAS	0.886	-0.408
	Low PAH/ Low PFAS	0.879	-0.342
	High PAH/ Low PFAS	0.788	-0.282
	Low PAH/ High PFAS	0.737	0.229
	High PAH/ High PFAS	0.771	0.189
Observation	DOPAC/Da	1.488	-1.541
	DOPAC + HVA/Da	2.835	-1.075
	17β-estradiol	4.136	0.231
	th	-1.886	0.022
	drd1	-1.703	0.911
	drd2a	0.162	1.159
	dat	1.352	2.990
	er-α	-2.341	-0.609
	esrrb	-2.306	0.139
	cyp19a1b	-1.879	-0.476
	gnrh2	0.142	-1.753

222

223

224

225

226

227

228

229

230 # References

231 1. Mortensen, A. S.; Arukwe, A., Effects of 17alpha-ethynylestradiol on hormonal responses and
232 xenobiotic biotransformation system of Atlantic salmon (*Salmo salar*). *Aquatic Toxicology* **2007**, 30;85,
233 (2), 113-23.