Ecotoxicology and Environmental Safety 222 (2021) 112495

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

# Thyroid function and immune status in perch (*Perca fluviatilis*) from lakes contaminated with PFASs or PCBs

Lina Birgersson [a], Justin Jouve [a], Elisabeth Jönsson [a], Noomi Asker [a], Fredrik Andreasson [b], Oksana Golovko [c], Lutz Ahrens [c], Joachim Sturve [a,*]

[a] Department of Biological and Environmental Sciences, University of Gothenburg, Box 463, SE-405 30 Göteborg, Sweden
[b] Department for Nature and Climate, County Administrative Board of Blekinge, SE-371 86 Karlskrona, Sweden
[c] Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences (SLU), SE-75 007 Uppsala, Sweden

ARTICLE INFO

Edited by: Dr. Caterina Faggio

*Keywords:*
European perch
Thyroid disruption
Immunotoxicity
PFASs
PCBs

ABSTRACT

The environment contains a multitude of man-made chemicals, some of which can act as endocrine disruptors (EDCs), while others can be immunotoxic. We evaluated thyroid disruption and immunotoxic effects in wild female perch (*Perca fluviatilis*) collected from two contaminated areas in Sweden; one site contaminated with per- and polyfluoroalkyl substances (PFASs) and two sites contaminated with polychlorinated biphenyls (PCBs), with one reference site included for each area. The hepatic mRNA expression of thyroid receptors α and β, and the thyroid hormone metabolising iodothyronine deiodinases (*dio1, dio2* and *dio3*) were measured using real-time PCR, while the levels of thyroid hormone T3 in plasma was analysed using a radioimmunoassay. In addition, lymphocytes, granulocytes, and thrombocytes were counted microscopically. Our results showed lower levels of T3 as well as lower amounts of lymphocytes and granulocytes in perch collected from the PFAS-contaminated site compared to reference sites. In addition, expressions of mRNA coding for thyroid hormone metabolising enzymes (*dio2* and *dio3*) and thyroid receptor α (*thra*) were significantly different in these fish compared to their reference site. For perch collected at the two PCB-contaminated sites, there were no significant differences in T3 levels or in expression levels of the thyroid-related genes, compared to the reference fish. Fish from one of the PCB-contaminated sites had higher levels of thrombocytes compared with both the second PCB lake and their reference lake; hence PCBs are unlikely to be the cause of this effect. The current study suggests that lifelong exposure to PFASs could affect both the thyroid hormone status and immune defence of perch in the wild.

## 1. Introduction

Numerous studies have shown widespread contamination by anthropogenic chemicals in the environment. Many of these chemicals can affect the endocrine status of humans and wildlife (Diamanti-Kandarakis et al., 2009) and are regarded as endocrine disrupting chemicals (EDCs), while others may affect the immune system. Both the endocrine and immune systems are especially sensitive to disruption by exogenous chemicals during early development and some EDCs can have immunotoxic effects (DeWitt and Patisaul, 2018). Aquatic environments often act as sinks for pollutants and can contain mixtures of chemicals originating from agricultural runoff, industrial effluents, and household waste, which can bioaccumulate in biota and sediments (van der Oost et al., 2003). While EDCs may affect many different endocrine systems, chemicals acting as thyroid disruptors are of special concern because thyroid hormones (THs) are essential for normal vertebrate growth and development (Mullur et al., 2014; Williams, 2008). Even small changes in thyroid hormone homeostasis during early developmental stages can have detrimental effects on several life stages of an organism (Korevaar et al., 2016). In fish, THs play a critical role in processes such as fish larval metamorphosis (Taillebois et al., 2011), smoltification (Björnsson et al., 2011), osmoregulation (Peter, 2011) and behaviour (e.g., Imbert et al., 2008) as well as growth, metabolism, and neurodevelopment (Blanton and Specker, 2007; Power et al., 2001;

*Abbreviations:* AFFFs, aqueous film forming foams; CF, condition factor; EDCs, endocrine disrupting chemicals; LSI, liver somatic index; PCBs, polychlorinated biphenyls; PFASs, per- and polyfluoroalkyl substances; PFHxS, perfluorohexane sulfonate; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate; T3, triiodothyronine; T4, thyroxine; TH, thyroid hormone; RIA, radioimmunoassay.

* Corresponding author.
*E-mail address:* joachim.sturve@bioenv.gu.se (J. Sturve).

https://doi.org/10.1016/j.ecoenv.2021.112495
Received 6 February 2021; Received in revised form 2 July 2021; Accepted 4 July 2021
Available online 12 July 2021

Walpita et al., 2009).

EDCs can disrupt the thyroid system at many different levels of the hypothalamic–pituitary–thyroid (HPT) axis, which regulates the synthesis and activity of the thyroid hormones thyroxine (T4) and triiodothyronine (T3). This includes disruption of the thyroid gland, thyroid receptors (Thra and Thrb), transport proteins, and regulatory enzymes, such as the deiodinases, which are involved in regulation of the availability and disposal of THs, including conversion of T4 to T3 (Brown et al., 2004; Zoeller, 2007). A range of compounds prevalent in the environment have documented effects on thyroid homeostasis, including emerging contaminants that have not yet or only recently been regulated, such as per- and polyfluoroalkyl substances (PFASs) as well as legacy contaminants like the many banned persistent organic chemicals such as dioxins and polychlorinated biphenyls (PCBs) (Boas et al., 2009; Mughal et al., 2018).

PFASs are highly persistent, surface-active anthropogenic compounds, which are able to bioaccumulate through food webs and can have severe effects on the health of aquatic organisms (Houde et al., 2011). Due to their stability and surface-tension-lowering properties, PFASs have several industrial and commercial applications, for instance as surfactants in paper and textile products as well as in aqueous film-forming foams (AFFFs) for fire suppression (Kissa, 2001). PFASs are widely distributed throughout the environment and have been detected in both animals and humans (Lau et al., 2007). PFASs can disrupt TH-related mRNA expression and serum levels of THs and can have effects on behaviour and immunotoxicity in fish (Han et al., 2012; Shi et al., 2009). Elevated PFAS contamination of biota and water can be detected in areas close to airfields, where large quantities of PFAS-containing AFFFs have been used for firefighting training (Ahrens et al., 2015; Gewurtz et al., 2014). This is the case in Kallinge, a town located in south-eastern Sweden, where a fire training facility at an airfield was identified as a point source for PFASs in nearby groundwater, streams, and lakes that caused elevated serum levels of PFASs in the local human population (Andersson et al., 2019; Li et al., 2018, 2020).

PCBs are lipophilic, highly stable anthropogenic chemicals, which are still pervasive in the environment due to their resistance to degradation. PCBs were used in industrial products including hydraulic fluids, heat transfer fluids, and lubricants, and due to their high levels of persistence they are still widespread in the environment and in living organisms decades after being banned (Henry, 2015; Maisano et al., 2016). PCBs have well-documented effects on TH levels and animal studies suggest a link between PCB exposure and reduced TH levels in the serum of birds and mammals (Ahmed, 2013; Katarzyńska et al., 2015; Smits et al., 2002). As PCBs are structurally similar to T4, they may act as TH analogues, disrupting the TH system by acting on or binding to the thyroid receptor (Boas et al., 2012; Zoeller, 2007). In addition, exposure to PCBs is known to have adverse effects on the immune system in mammals (Lyche et al., 2004), including marine mammals (Desforges et al., 2016; Sørmo et al., 2009). In fish, most studies of PCB exposure and its negative effects on the immune system have been performed in laboratory settings (reviewed by Henry, 2015). In 2013, a regional survey revealed high levels of PCBs in perch from the lake Oxundasjön, north of the town of Upplands Väsby, Sweden (Karlsson and Viktor, 2014). Later, large amounts of PCBs were found in the sediment of the lake, estimated to be as much as five tonnes and therefore unique of its kind in Sweden (Hållén et al., 2017). Further studies also detected high levels of PCBs in the downstream Lake Mälaren at the bay of Rosersbergsviken (Karlsson, 2014; Karlsson et al., 2014). The source of the contamination has been localised to the town of Upplands Väsby, where industries in the area have most likely used and released PCBs into the environment for a long period of time, predominantly during the 1960–80s based on chemical analysis of sediment cores in the lake Oxundasjön (Hållén et al., 2017).

While it is known that PFASs and PCBs have adverse effects on living organisms, as shown in laboratory studies, there are still knowledge gaps regarding their potential effects on wild fish that have been chronically exposed to pollutants in their natural environment. The aim of our study was to examine the effects of PFASs and PCBs on thyroid- and immune-related parameters in perch that have been living in contaminated environments for their entire lives. Female European perch (*Perca fluviatilis*) were caught in two contaminated areas in Sweden that have been polluted for decades: one PFAS-polluted site close to Kallinge, and two sites polluted with PCBs. While there is existing data on PCB levels in perch muscle from monitoring surveys for the PCB area, no previous studies have been conducted on fish from the PFAS-contaminated site. Effects on the thyroid system were assessed by measuring thyroid hormone levels in plasma as well as levels of mRNA coding for thyroid-related genes. In addition, the levels of immune-relevant blood cells in the fish were analysed to determine whether the immune system had been affected by long-term exposure to the contaminants. In addition, we evaluated deiodinase expression as markers for exposure to thyroid disrupting chemicals in fish.

## 2. Material and methods

### 2.1. Sampling sites and chemical data

Female perch were sampled at five sites from two main areas in Sweden during the autumn of 2016: one area including a PFAS-contaminated lake and a reference lake, and one area including two PCB-contaminated sites and a reference lake (Fig. 1). The studied areas were selected based on existing chemical data. The lake Sänksjön near Kallinge has been contaminated for decades by PFASs (i.e.,

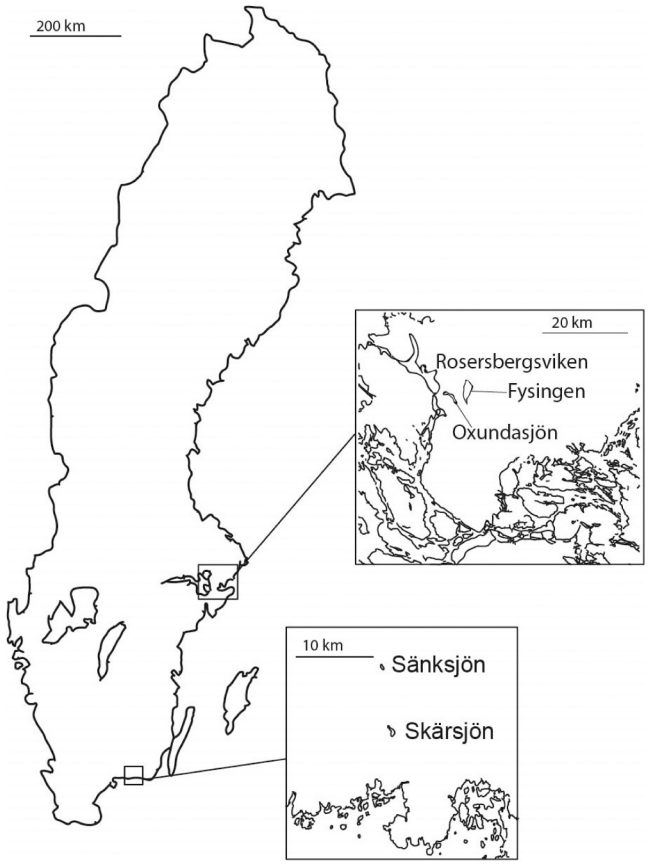


**Fig. 1.** Map showing the five sampling sites in Sweden where perch were caught during autumn 2016. Sänksjön has been contaminated with PFASs from Ronneby airport, whereas Oxundasjön and Rosersbergsviken have been contaminated with PCBs. Skärsjön was used as a reference site for Sänksjön and Fysingen was used as a reference site for Oxundasjön and Rosersbergsviken.

perfluorooctane sulfonate (PFOS), perfluorohexane sulfonate (PFHxS), and to a lesser extent perfluorooctanoate (PFOA)) (Andersson et al., 2019; Li et al., 2018) and was selected based on PFAS content in the water (Niras Sweden, 2016). The PFASs originated from a fire training facility at the nearby airfield. The lake Oxundasjön and the bay Rosersbergsviken were selected based on the elevated levels of PCBs in the water and biota, including perch, from these sites (Karlsson, 2014; Karlsson et al., 2014; Karlsson and Viktor, 2014), originating from several surrounding industries that have been active for the last century. Since PCB content in perch from Oxundasjön and Rosersbergsviken had already been determined, we did not measure this in the current study. In contrast, chemical analysis for the levels of PFASs in perch from the lakes Sänksjön and Skärsjön was not previously available, therefore PFAS levels in muscle samples were measured for these perch samples (see Section 2.7. Chemical analysis). Lake Skärsjön was included as a reference site for the PFAS area as this lake is part of a separate catchment area, which is not connected to the PFAS-contaminated lake. Lake Fysingen was included as a reference site for the PCB area since this site is known to be pristine, and previous measurements have shown low PCB levels in perch muscle from this site (Hållén, 2016).

### 2.2. Fish sampling

Sexually mature female perch were sampled from the five sites in October 2016 (n = 11–22 per lake). Reproductive status was based on gonadal size. In Sänksjön and its reference lake Skärsjön, fish were caught by fishing rod and then kept in corves at the sampling location for one day prior to sampling. Temperatures in Sänksjön and Skärsjön were 10.8 and 10.5 °C, respectively. In Oxundasjön, Rosersbergsviken and the reference lake Fysingen, fish were caught with gill nets and then kept in corves at the sampling location for three days prior to sampling. Temperatures in Oxundasjön, Rosersbergsviken and Fysingen were 9.8, 9.6 and 10.2 °C, respectively. Before sampling, the fish were killed by a sharp blow to the head. All fish were then weighed (g) and measured (mm). Blood was drawn from the caudal vein with a heparinised syringe (Bojarski et al., 2018). Fresh blood was immediately used to produce smears for blood cell counts, and to measure the erythrocyte volume fraction (haematocrit), glucose concentration, and haemoglobin concentration. The rest of the blood was centrifuged at 6000 rpm for two min, the plasma was then collected, aliquoted and stored at − 80 °C until analysis. The liver was rapidly dissected, and the bile was collected with a syringe. The liver was weighed and cut into smaller pieces that were flash frozen and stored in liquid nitrogen until sample preparation. Eight to nine perch samples were analysed from each site for thyroid-related hepatic mRNA expression and thyroid hormone measurement in plasma. Four individuals from Skärsjön and five from Sänksjön were randomly selected for PFASs analysis in fish muscle tissue. Fish were treated and sampled according to animal ethics regulation in Sweden and as outlined in the ethical permit (274–2011).

### 2.3. Morphometric indices and age

Condition factor (CF), and liver somatic index (LSI) were calculated as follows: CF = total weight (g) × 100/length$^3$ (cm) and LSI = total liver weight (g) x 100/total weight (g). The age of the fish was determined by the otolith structures, as previously described (Svedäng et al., 1998).

### 2.4. Blood parameters

Haematocrit was measured using haematocrit capillary tubes that were centrifuged in a haematocrit capillary centrifuge for 2 min and then read with a microhematocrit reader (n = 10–20). The haemoglobin and glucose concentrations in blood were measured using a cuvette system from Hemocue, with assayed haemoglobin (HemoTrol; Eurotrol) and glucose (GlucoTrol-AQ; EuroTrol) as quality controls. To determine the amounts of blood cells, blood smears were prepared on glass slides and stained as previously described (Asker et al., 2015). The number of lymphocytes, granulocytes, and thrombocytes were counted microscopically and are presented as a percentage of the total number of counted blood cells. The total percentage of white blood cells (WBCs) was calculated as the sum of the lymphocytes, granulocytes, and thrombocytes (Asker et al., 2015).

### 2.5. RNA isolation and real time RT-PCR

For the mRNA expression analysis of selected thyroid-related mRNA transcript levels, RNA was isolated from the liver of eight or nine individual perch females from each site. Samples were homogenised using a TissueLyser (Qiagen) and total RNA was isolated from homogenised liver samples using the RNeasy® Plus Mini Kit (Qiagen) according to the manufacturer's instructions. The RNA concentration of each sample was assessed using spectrophotometry (NanoDrop, Thermo Fisher Scientific). Complementary DNA was synthesised from 1 μg total RNA using the iScript cDNA synthesis kit (BioRad). RT-qPCR was carried out in a total volume of 10 μL per reaction (4 μL cDNA diluted 1/20, and 5 μL SsoAdvanced Universal SYBR-Green Supermix (BioRad)). Target gene transcripts were normalised to the expression of the geometric mean of two housekeeping genes (ubiquitin and elongation factor 1α) to obtain delta Ct values (dCt). qPCR data is presented as (2^-dCt) x 1000 and statistical analysis was performed on delta Ct values. Primers were designed with NCBI primer-BLAST using sequenced perch transcriptome data (Förlin et al., 2019). Information regarding primer sequences and annealing temperatures is provided in Table S1.

### 2.6. Thyroid hormone measurement

The levels of total thyroid hormone triiodothyronine (T3) in the blood plasma of female perch (n = 8–9) were quantified using a radioimmunoassay (RIA) protocol as described previously (Einarsdóttir et al., 2006; Rotllant et al., 2003), but with 0.25% bovine serum albumin (BSA) in the RIA buffer. The T3 label was purchased from Perkin Elmer (Waltham, USA). A test of parallelism was carried out to validate the RIA method for perch prior to measurements of samples and showed that the standard and plasma slopes were parallel (Fig. S1). Each plasma sample was diluted 1:10 in the RIA buffer and kept in the refrigerator until the start of the assay. Levels of T4 in perch plasma were not measurable (signal not detected) with two applied methods, a T4-specific RIA developed in-house and a T4-specific ELISA method (Perkin Elmer). We were also unable to measure TSH in plasma.

### 2.7. Chemical analysis

#### 2.7.1. Chemicals and standards

All the analytical standards used for quantification were of high purity grade (>95%) and all were purchased from Sigma-Aldrich (Sweden). The mass-labelled internal standards for PFASs were purchased from Wellington Laboratories (Canada). Working mixtures of native compounds (1 μg/mL) and internal standards (0.5 μg/mL) were prepared in methanol and stored at − 20 °C. C6 and C8 perfluoroalkyl sulfonates (PFSAs) (PFHxS, PFOS) and C4-C11 and C13 perfluorocarboxylates (PFCAs) (PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTeDA), and perfluorooctane sulfonamide FOSA, were analysed in perch muscle tissue extracts. Detailed information about internal and native standards can be found elsewhere (Rostvall et al., 2018). Ultrapure water was generated by a Milli-Q (MQ) Advantage Ultrapure Water purification system and filtered through a 0.22 μm Millipak Express membrane and an LC-Pak polishing unit (Merck Millipore, Billercia, MA). Methanol, acetonitrile, ammonium acetate, and formic acid of high analytical grade were acquired from Sigma-Aldrich (Sweden).

#### 2.7.2. Fish sample pre-treatment and extraction

PFASs were analysed in perch muscle tissue from Sänksjön (n = 5) and Skärsjön (n = 4) as described in Grabicova et al. (2018). Briefly, 0.5 g of fish muscle tissue spiked with internal standard mixture (5 ng per sample) was extracted and homogenised with 0.5 mL of acetonitrile acidified with 1% of formic acid at 5000 rpm 2 times for 40 s each, with a 20 s break (Precellys Evolution Homogenizer, Bertin Technologies, Paris, France). The extract was then centrifuged at 3900 rpm for 15 min in an Eppendorf Centrifuge 5810 R (Eppendorf AG, Hamburg, Germany). The supernatant was filtered through a syringe filter (0.45 μm pore size, regenerated cellulose) and frozen for 24 h to ensure protein precipitation. The method for PFAS analysis in biota was validated for linearity, repeatability, quantification limit (LOQ), and recovery (Cerveny et al., 2016). The final extract was then centrifuged at 10,000 rpm for 3 min and an aliquot was taken and analysed using an ultra-high-pressure liquid chromatography (UPLC) system coupled to a triple quadrupole mass spectrometer (MS/MS) (UPLC-MS/MS).

#### 2.7.3. UPLC-MS/MS analysis

The perch muscle tissue extracts were analysed using a DIONEX UltiMate 3000 UPLC system (Thermo Scientific, Waltham, MA, USA) coupled to an MS/MS (TSQ QUANTIVA, Thermo SCIENTIFIC, Waltham, MA, USA). An Acquity UPLC BEH-C18 column (Waters, 100 mm × 2.1 i.d., 1.7 μm particle size from Waters Corporation, Manchester, UK) was used as an analytical column. Injection volume was 10 μL for all samples. A heated electrospray ionisation (H-ESI) was used to ionise the target compounds. The spray voltage was set to positive ion with 3500 V. Nitrogen (purity >99.999%) was used as a sheath gas (50 arbitrary units), auxiliary gas (15 arbitrary units), and sweep gas (2 arbitrary units). The vaporiser was heated to 400 °C and the capillary to 325 °C. Two selected reaction monitoring (SRM) transitions were monitored for all analytes. The mobile phase consisted of ultrapure water with 5 mM ammonium acetate and acetonitrile. The flow rate was 0.5 mL/min and run time was 15 min (negative electrospray ionisation mode). The obtained data were evaluated using TraceFinderTM 3.3. software (Thermo Fisher).

### 2.8. Statistical analysis

Statistical analyses were performed using GraphPad Prism 8 (GraphPad Software, La Jolla, CA, USA). Results are presented as mean ± SEM per group. The normality of the data was tested with the Shapiro-Wilk test. Due to the spatial distance between the two studied areas, the results from the two areas were statistically analysed separately. For the PFASs area, *t*-tests were used to determine significant differences between the two sites. For the PCB area, a one-way ANOVA was used to compare the means between the three sites, followed by Tukey's post hoc test or Kruskal Wallis' test. Differences were considered significant at $p < 0.05$. Where applicable, outliers were identified and removed from the data following a ROUT analysis (Q = 0.05).

## 3. Results and discussion

In this study, we used perch from two areas in Sweden known to be contaminated with specific groups of chemicals in order to see if the effects of the contaminants observed in laboratory studies could also be detected in wild fish from real environmental conditions. Laboratory studies have indicated that PFASs and PCBs have effects on both the thyroid and immune status of fish. However, very few studies have demonstrated this in naturally occurring fish populations in the field. In this study, we used long-term polluted lakes as experimental lakes in order to study the effects of PFASs and PCBs on natural fish populations. To our knowledge, this is one of the first studies to have examined these parameters in wild perch from sites contaminated with PFASs or PCBs.

### 3.1. Effects of PFASs on the thyroid system

The hepatic mRNA expression levels of five selected, thyroid-related genes: *thra, thrb, dio1, dio2*, and *dio3*, in nine individual females per site were measured to investigate effects on the thyroid system at the contaminated sites (Fig. 2). These genes were selected because they code for proteins that regulate the bioavailability and tissue responsiveness of the active form of thyroid hormone (T3). The expression of *thra* and *dio2* were significantly downregulated, while the expression of *dio3* was upregulated in fish from the PFAS-contaminated lake Sänksjön compared with the reference lake Skärsjön. This suggests that chronic exposure to PFASs in perch may affect deiodinase mRNA expression, and possibly also be translated into altered deiodinase activity. The concentration of thyroid hormones is in part regulated through deiodinases, which adjust the bioavailability of the active form of thyroid hormone (T3). Activation and synthesis of T3 is stimulated by type 2 deiodinase (*dio2*) through increased biosynthesis of the prohormone T4. Inactivation of THs is catalysed by type 3 deiodinase (*dio3*) through deiodination, transforming T4 and T3 into inactive metabolites (Bianco and Kim, 2006; Deal and Volkoff, 2020).

Alterations in deiodinase activity might be one explanation for the observed effects on the plasma levels of T3 (Fig. 3), which were significantly lower in perch caught at the PFAS-contaminated lake Sänksjön compared to its reference site Skärsjön. The significantly lower levels of thyroid hormone (T3) in perch from Sänksjön (Fig. 3) are consistent with the decreased *dio2* expression levels and increased *dio3* expression levels (Fig. 2). Taken together, the low levels of T3, low expression of *dio2* and high expression of *dio3* in perch from the PFAS-contaminated site indicate that chronic PFASs exposure may disrupt thyroid hormone homeostasis through several mechanisms of action in wild perch. The findings for Sänksjön are consistent with a number of studies showing effects of PFASs on thyroid hormone levels in marine mammals (e.g., Desforges et al., 2016; Fair et al., 2013) and in fish (Shi et al., 2009), further supporting the hypothesis that PFASs can act as TH-disruptors. In addition, acute PFOS exposure changed mRNA expression in the HPT axis of zebrafish and altered whole-body levels of T3 (Shi et al., 2009). PFOA exposure (250 and 500 mg/L) in zebrafish has been shown to reduce the whole-body levels of both T3 and T4 after five days (Wang et al., 2020). Chronic PFOS exposure in zebrafish of 250 μg/L (Chen et al., 2018) led to altered whole-body content of T4, as well as an altered *dio1* expression, but no changes in the levels of T3 (Shi et al., 2009). PFAS exposure may also affect thyroid hormone levels in humans. For example, decreased levels of T4 (but not T3) have been shown after PFAS exposure in humans (Melzer et al., 2010; Olsen et al., 2009).

While there is a lack of studies on the effects of lifelong exposure to PFASs on TH levels or thyroid-hormone-related mRNA expression in fish, some previous short-term laboratory experiments also suggest the thyroid-disruptive effects of PFASs. Acute exposure to PFASs affected the expression of the thyroid-related genes thyroglobulin and thyroid-stimulating hormone, as well as corticotropin-releasing factor in zebrafish after 15 days (Shi et al., 2009) and genes related to early thyroid development (*hhex* and *pax8*) in zebrafish embryos (Shi et al., 2008). A study using mixtures of EDCs containing PFASs, phthalates, and BPA or triclosan was shown to have thyroid-disruptive effects on TH-related mRNA expression (*thra, thrb, dio1,* and *dio2*) in zebrafish larvae after 48 h acute exposure to relatively low concentrations of a mixture of EDCs relevant to the environment. Similarly to the results in the present study, the expression of *thra* and *dio2* was significantly altered by EDC exposure. These mixtures contained several PFASs and phthalates, both of which are compounds with suspected thyroid disrupting properties (Birgersson et al., 2017).

It remains to be elucidated whether the low T3 levels demonstrated here are caused by direct effects of PFASs on the transcription of *dio2* and *dio3* and/or on other parts of the HPT axis. PFOS is also known to have high affinity for the TH transport protein Transthyretin (TTR)

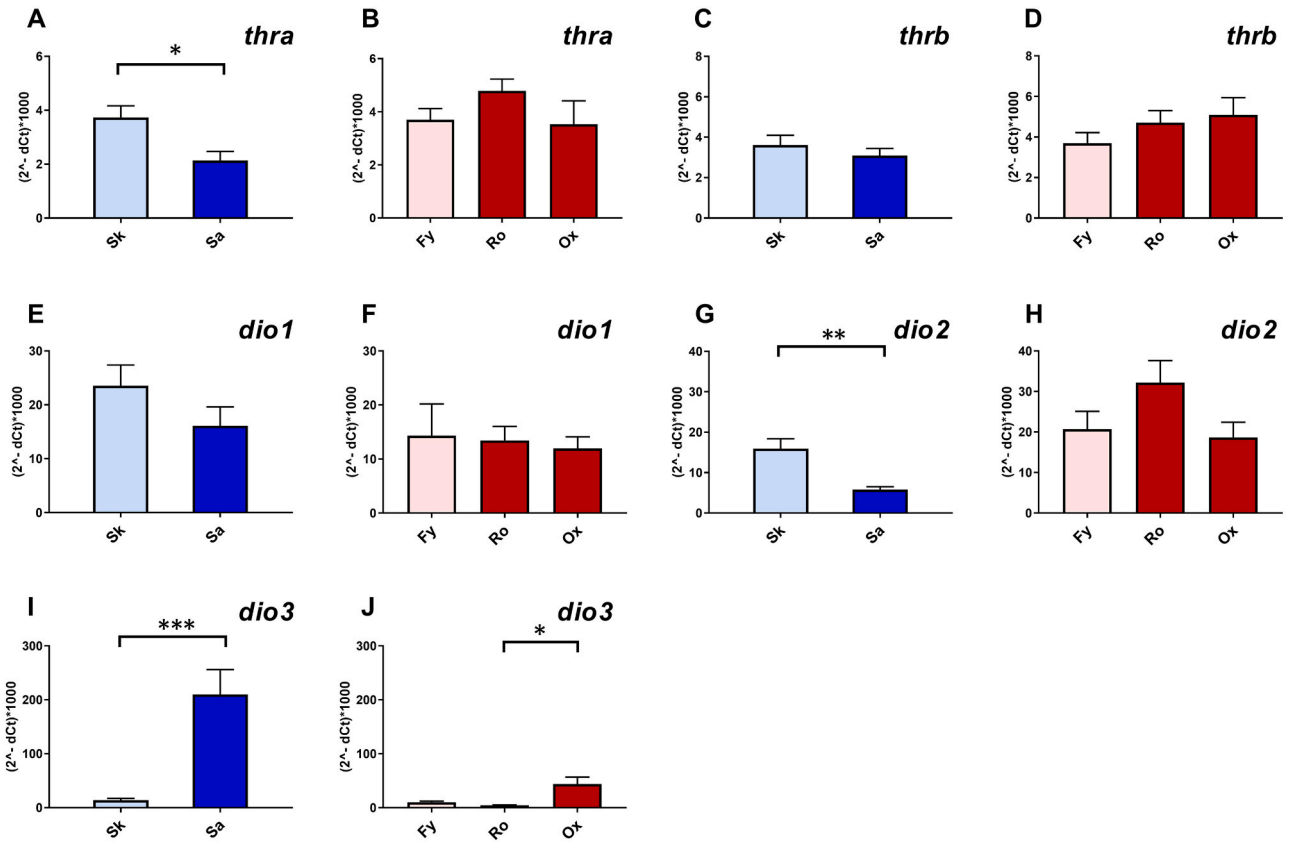


**Fig. 2.** RT-qPCR results for relative mRNA expression levels of (A–D) thyroid receptors (*thra* and *thrb*), and (E–J) deiodinases (*dio1, dio2* and *dio3*) in liver samples from female perch from the PFAS-contaminated lake Sänksjön (Sa) and the reference lake Skärsjön (Sk), and the PCB.contaminated lakes Oxundasjön (Ox) and Rosersbergsviken (Ro) and the reference lake Fysingen (Fy) (n = 8–9 per site) presented as (2ˆ-dCt) x 1000. Results are presented as mean ± standard error and the statistical analysis was performed separately for the two different areas (PFASs vs PCBs). Asterisks (*) indicate statistical significance between the groups ($p < 0.05$).

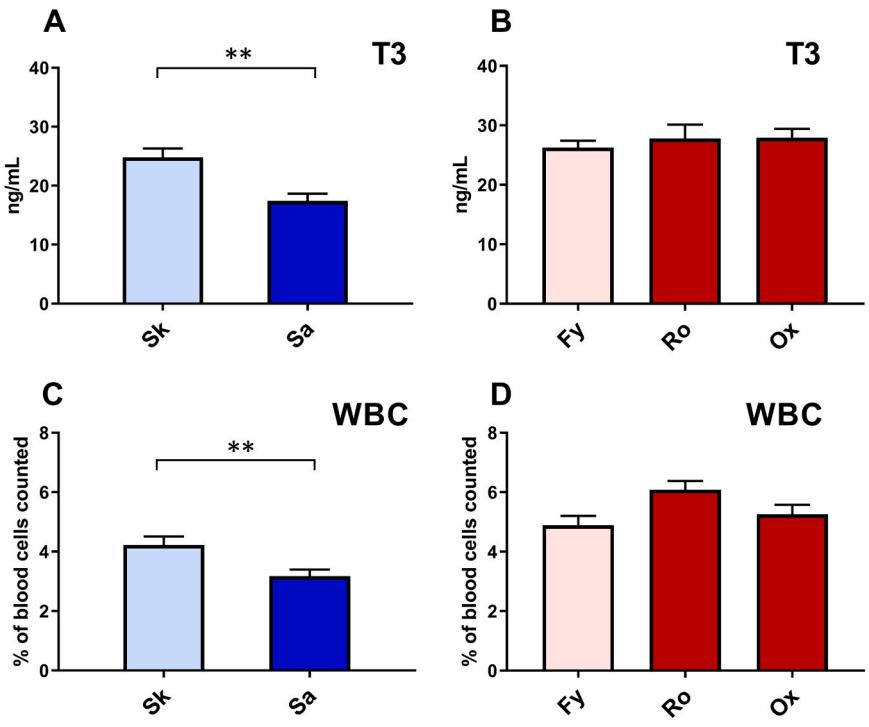


**Fig. 3.** Differences in (A and B) total triiodothyronine (T3) levels in plasma and (C and D) white blood cell (WBC) counts from the blood of perch (lymphocytes, granulocytes, and thrombocytes) from the PFAS-contaminated lake Sänksjön (Sa) and the reference lake Skärsjön (Sk), and the PCB-contaminated sites Oxundasjön (Ox) and Rosersbergsviken (Ro) and the reference lake Fysingen (Fy). T3 levels were measured using RIA in plasma samples (n = 8–9 per site) and white blood cell counts were performed on blood smears from 6 to 20 individuals per site. Data are presented as mean ± S.E.M. and the statistical analysis was performed separately for the two different areas (PFASs vs PCBs). Asterisks (*) indicate statistical significance between the groups ($p < 0.05$).

(Weiss et al., 2009), which may affect TH-binding to this transporter, leading to altered TH levels. The issue of compensation is important to consider for thyroid disruption (Zoeller, 2010), because when levels of thyroid hormones decline, systematic responses are activated as a response to decreasing TH levels in order to restore TH homeostasis or ameliorate the effects caused by the decreased TH levels (Zoeller, 2010). However, there is still a risk of adverse effects if the exposure duration is long or if it occurs during sensitive windows of development, such as organogenesis. The perch in this study have been exposed for several generations to relatively high levels of PFASs (contamination started in the mid-1980 s) and do not seem to have adapted to these conditions in terms of thyroid endocrinology. In order to maintain TH homeostasis and compensate for decreased T3 levels, it might be expected that *dio2* would be upregulated and *dio3* down-regulated, resulting in an increase in T3 levels, which is not the case here. Even though the results showed that the genes involved in thyroid hormone metabolism were significantly different in the PFAS-contaminated lake, and that T3 levels were lower, no differences in size or morphology were observed in the studied fish. Fish caught at the PFAS-contaminated lake Sänksjön were smaller compared to the other sites due to the fact that they were younger. However, all the perch included in the study were adults and sexually mature, and the condition factor (CF) of the fish did not differ between any of the studied lakes (Table S2), indicating that the growth pattern and/or fat deposition was not affected by the PFAS or PCB exposure.

### 3.2. Effects of PCBs on the thyroid system

Little is known about baseline data for thyroid related parameters in perch, so the PCB-area provided an opportunity to gain another reference site to compare to the PFAS-area in the current study. The hepatic mRNA expression levels of thyroid related genes were also measured for perch from the PCB-area. Fish from the most heavily PCB-contaminated lake, Oxundasjön, showed a clear upregulation in hepatic *dio3* expression compared with the less contaminated site at Rosersbergsviken, and to some extent also compared to the reference lake Fysingen (Fig. 2). There were no significant differences in levels of *dio1, dio2, thra* or *thrb* in fish from either of the PCB-contaminated sites compared to their reference lake.

Furthermore, the total T3 levels did not differ between fish from the PCB-contaminated lakes compared with those from the reference site (Fig. 3). This is in contrast to the altered levels of both T3 and T4 that have been reported in previous studies of PCB-exposed humans (Darnerud et al., 2010) and animals (Ahmed, 2013; Brar et al., 2010; Katarzyńska et al., 2015; Smits et al., 2002), including fish (Brown et al., 2004). Effects of PCBs on the HPT axis have been shown in some studies of wild fish (e.g., Brar et al., 2010), while others have found that effects are within the compensatory scope of the thyroid system and may not be substantial enough to lead to negative effects on the physiology of the fish (Buckman et al., 2007; Henry, 2015).

Unfortunately, levels of thyroxine (T4) or thyroid stimulating hormone (TSH) could not be determined in perch plasma samples by RIA or ELISA, despite the fact that these methods have been successful for other species (such as eelpout (unpublished data) and Atlantic Halibut (Einarsdóttir et al., 2006)). The reason for this is not known and should be further investigated, since one of the most commonly reported adverse outcomes in humans exposed to PCBs is serum hypothyroxinemia, i.e. reduced T4 levels (Mughal et al., 2018).

### 3.3. Chemical analysis

PFOS was found to be the most prevalent PFAS in Sänksjön, with 72 ng/g ww in perch muscle (Table S3). The ∑PFAS concentration in surface water from Sänksjön has previously been determined to be 155 ng/L (Niras Sweden, 2016). PFOS was the most prevalent PFAS in both Skärsjön and Sänksjön with a contribution of 90% and 97% of the ΣPFASs. Some minor contributions of PFDA (3.2% of ΣPFASs) and PFUnDA (3.8%) were also found. Thus, the discussion relating to the PFAS-contaminated area is mainly focused on PFOS. For Oxundasjön and Rosersbergsviken, the PCB content in perch had already been determined and was not measured in the current study (see Section 2.1.). The PCB levels in perch muscle from Oxundasjön and Rosersbergsviken were determined to be 490 ng $\sum PCB_7$/g ww and 150 ng $\sum PCB_7$/g ww, respectively, which is well above the threshold of 125 ng $\sum PCB_6$/g ww in muscle set by the EU (Hållén, 2016; Karlsson, 2014; Karlsson et al., 2014; Karlsson and Viktor, 2014). PCB levels in the reference lake are considerably lower, for example $\sum PCB_7$ levels have previously been determined as 3.1 ng/g ww in Lake Fysingen, Sweden (Hållén, 2016).

### 3.4. Effects of PFASs and PCBs on blood cell counts

Haematological parameters such as blood cell count are widely used to assess the effects of exposure to environmental stressors such as pollutants (Burgos-Aceves et al., 2019; Bojarski and Witeska, 2020). In the present study, the relative percentages of lymphocytes, granulocytes, and thrombocytes were determined in female perch from the PCB- and PFAS-contaminated areas (Table 1). The sum of WBCs (lymphocytes, granulocytes, and thrombocytes) (Fig. 3) was significantly lower in perch from the PFAS-contaminated lake Sänksjön than in perch collected from its reference lake Skärsjön, predominantly due to a significantly lower number of lymphocytes and granulocytes (Table 1). As granulocytes and lymphocytes are involved in the recognition and elimination of pathogens, the lower levels of these immune-related cells in perch found in the PFAS-contaminated areas may lead to a higher susceptibility to infection in these fish (Rieger and Barreda, 2011). Immunotoxic effects of PFAS exposure, such as changes in the number of immune cells and immune-related mRNA expression, have previously been demonstrated in fish in laboratory studies, particularly for short exposures (days up to weeks) at concentrations in the mg/L range (Fang et al., 2013; Han et al., 2012; Huang et al., 2015; Yang, 2010).

In contrast to the PFAS-contaminated site, the total number of white blood cells did not differ significantly between fish collected in the PCB-contaminated sites and the reference lake (Fig. 3). However, the relative percentage of thrombocytes in perch from Rosersbergsviken was significantly higher in comparison to Oxundasjön as well as the reference site Fysingen (Table 1), suggesting a possible effect on the blood-clotting process in the examined fish (Tavares-Dias and Oliveira, 2009). The phagocytic properties of thrombocytes in non-mammalian vertebrates such as fish (Nagasawa et al., 2014) also indicated a potential effect on the innate immune system of fish collected in the PCB-contaminated Rosersbergsviken. PCB exposure in laboratory settings has previously been shown to affect the immunocyte function, immune response, or disease resistance of mammals, birds, and fish (Arkoosh et al., 1994; Iwanowicz et al., 2009; Smits et al., 2002). In some cases, effects have also been seen on wild fish from PCB-contaminated ecosystems, while others did not observe effects on immune parameters (Barron et al., 2000). More evidence is needed in order to determine whether PCB contamination adversely affects the health of wild fish (Henry, 2015).

## 4. Conclusions

This study looked at the effects on thyroid- and immune-related parameters in wild perch that have been living in lakes contaminated with PFASs or PCBs for their entire lives. Although these chemicals have been shown to have TH-disruptive effects in laboratory studies, there is a lack of knowledge about the effects on thyroid and immune status in wild fish in their natural habitat. In summary, we observed that lifelong exposure to PFASs had effects on both the levels of T3 and the expression of TH-related genes (*dio2, dio3,* and *thra*). The numbers of immune-relevant blood cells were also significantly different from reference fish, and further studies are warranted to explore the potential effects on

**Table 1**
Frequency of blood cells of total number of blood cells counted[a].

| | PFASs | | PCBs | | |
|---|---|---|---|---|---|
| | Skärsjön | Sänksjön | Fysingen | Rosersbergsviken | Oxundasjön |
| Lymphocytes (%) | 1.9 ± 0.26 A | 1.2 ± 0.08 B | 2.1 ± 0.16 A | 2.5 ± 0.19 A | 2.2 ± 0.16 A |
| Granulocytes (%) | 1.1 ± 0.06 A | 0.7 ± 0.08 B | 1.1 ± 0.12 A | 1.2 ± 0.08 A | 1.4 ± 0.13 A |
| Thrombocytes (%) | 1.2 ± 0.11 A | 1.1 ± 0.13 A | 1.6 ± 0.12 A | 2.3 ± 0.13 B | 1.7 ± 0.13 A |

[a] Cell counts were performed on blood smears from 6 to 20 individuals per site. Results are presented as mean ± standard error and the statistical analysis was performed separately for the two different areas (PFASs vs PCBs). Uppercase letters indicate significant differences between the sites from each area ($p < 0.05$).

immune function. To our knowledge, our study is the first to suggest that lifelong exposure to PFASs in the ng/L range may have adverse effects on thyroid status and the cellular immune defences of wild fish. We also found that lifelong exposure to PCBs might have a slight effect on *dio3* expression in wild perch. In addition, our results indicate that differences in the expression of deiodinase genes, especially *dio2* and *dio3*, are biological effects that can potentially be used as markers for thyroid disrupting chemicals, such as PFASs, in environmental monitoring. Although the possibility of other contributing contaminants needs to be taken into consideration, we believe that it is still valuable to study effects not just in laboratory setups, but also under real environmental conditions, which can provide insight into the effects of these chemicals on wild fish. However, further studies of both PFASs and PCBs in both wild fish populations and in laboratory settings are needed to confirm the present findings.

## Supplementary material

Figure S1 Binding inhibition curves for radioimmunoassay. Table S1 Primer sequences used for RT-qPCR. Table S2 Biometric data and blood parameters. Table S3 Average PFASs concentration in muscle.

## Funding Sources

This work received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 634880, EDC-MixRisk. The funding source had no involvement in the study design, data collection, data analysis and interpretation, or in the writing and submission.

## CRediT authorship contribution statement

**Lina Birgersson:** Investigation, Data analysis, Writing – original draft, Writing – review & editing. **Justin Jouve:** Investigation. **Elisabeth Jönsson:** Supervision, Validation, Writing – original draft, Writing – review & editing. **Noomi Asker:** Supervision, Validation, Writing – original draft, Writing – review & editing. **Fredrik Andreasson:** Resources, Writing – review & editing. **Oksana Golovko:** Investigation, Data analysis, Writing – review & editing. **Lutz Ahrens:** Investigation, Data analysis, Validation, Writing – review & editing. **Joachim Sturve:** Conceptualisation, Supervision, Data analysis, Validation, Writing – original draft, Writing – review & editing. Funding acquisition.

## Author Contributions

J.S sampled the fish. J.J and L.B carried out the experiments, including RT-qPCR and radioimmunoassay. L.B and J.S analysed this data. L.A and O.G performed the chemical analysis and analysed this data. F.A was a local advisor in the Kallinge area. L.B, N.A E.J and J.S wrote the paper. N.A, E.J, F.A, L.A and O.G contributed to discussions and critical reading of the manuscript. J.S designed and supervised the study, N.A and E.J contributed to the study design.

## Declaration of Competing Interest

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

## Acknowledgement

This work received funding from the European Union's Horizon 2020 Research and Innovation Programme under grant agreement No. 634880, EDC-MixRisk. We thank the Department for Nature and Climate, County Administrative Board of Blekinge for their support during sampling.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2021.112495.

## References

Ahmed, R.G., 2013. Early weaning PCB 95 exposure alters the neonatal endocrine system: thyroid adipokine dysfunction. J. Endocrinol. 219, 205–215. https://doi.org/10.1530/JOE-13-0302.

Ahrens, L., Norström, K., Viktor, T., Cousins, A.P., Josefsson, S., 2015. Stockholm Arlanda Airport as a source of per- and polyfluoroalkyl substances to water, sediment and fish. Chemosphere 129, 33–38. https://doi.org/10.1016/j.chemosphere.2014.03.136.

Andersson, E.M., Scott, K., Xu, Y., Li, Y., Olsson, D.S., Fletcher, T., Jakobsson, K., 2019. High exposure to perfluorinated compounds in drinking water and thyroid disease. A cohort study from Ronneby, Sweden. Environ. Res. 176, 108540 https://doi.org/10.1016/j.envres.2019.108540.

Arkoosh, M.R., Clemons, E., Myers, M., Casillas, E., 1994. Suppression of B-cell mediated immunity in juvenile chinook salmon (*Oncorhynchus tshawytscha*) after exposure to either a polycyclic aromatic hydrocan or to polychlorinated biphenyls. Immunopharmacol. Immunotoxicol. 16, 293–314.

Asker, N., Carney Almroth, B., Albertsson, E., Coltellaro, M., Bignell, J.P., Hanson, N., Scarcelli, V., Fagerholm, B., Parkkonen, J., Wijkmark, E., Frenzilli, G., Förlin, L., Sturve, J., 2015. A gene to organism approach—assessing the impact of environmental pollution in eelpout (*Zoarces viviparus*) females and larvae. Environ. Toxicol. Chem. 34, 1511–1523. https://doi.org/10.1002/etc.2921.

Barron, M.G., Anderson, M.J., Cacela, D., Lipton, J., Teh, S.J., Hinton, D.E., Zelikoff, J.T., Dikkeboom, A.L., Tillitt, D.E., Holey, M., Denslow, N., 2000. PCBs, Liver Lesions, and Biomarker Responses in Adult Walleye (*Stizostedium vitreum vitreum*) Collected from Green Bay, Wisconsin. J. Gt. Lakes Res 26, 250–271. https://doi.org/10.1016/S0380-1330(00)70691-5.

Bianco, A.C., Kim, B.W., 2006. Deiodinases: implications of the local control of thyroid hormone action. J. Clin. Invest. 116, 2571–2579. https://doi.org/10.1172/JCI29812.

Birgersson, L., Borbely, G., Caporale, N., Germain, P.-L., Leemans, M., Rendel, F., D'Agostino, G., Bressan, R.B., Cavallo, F., Chorev, N.E., Kos, V.M., Lazzarin, M., Pollard, S., Sundstrom, B., Tobon, A.L., Trattaro, S., Zanella, M., Bergman, A., Damdimopoulou, P., Jonsson, M., Kiess, W., Kitraki, E., Kiviranta, H., Oberg, M., Rantakkoko, P., Ruden, C., Soder, O., Bornehag, C.-G., Demeneix, B., Fini, J.-B., Gennings, C., Nanberg, E., Ruegg, J., Sturve, J., Testa, G., 2017. From cohorts to molecules: adverse impacts of endocrine disrupting mixtures. bioRxiv. https://doi.org/10.1101/206664.

Björnsson, B.T., Stefansson, S.O., McCormick, S.D., 2011. Environmental endocrinology of salmon smoltification. Gen. Comp. Endocrinol. 170, 290–298. https://doi.org/10.1016/j.ygcen.2010.07.003.

Blanton, M.L., Specker, J.L., 2007. The hypothalamic-pituitary-thyroid (HPT) axis in fish and its role in fish development and reproduction. Crit. Rev. Toxicol. 37, 97–115. https://doi.org/10.1080/10408440601123529.

Boas, M., Feldt-Rasmussen, U., Main, K.M., 2012. Thyroid effects of endocrine disrupting chemicals. Mol. Cell. Endocrinol. 355, 240–248. https://doi.org/10.1016/j.mce.2011.09.005.

Boas, M., Main, K.M., Feldt-Rasmussen, U., 2009. Environmental chemicals and thyroid function: an update. Curr. Opin. Endocrinol. Diabetes Obes. 16, 385–391. https://doi.org/10.1097/MED.0b013e3283305af7.

Bojarski, B., Kondera, E., Witeska, M., Ługowska, K., 2018. Differences in hematological values of common carp between cardiac and venous blood. Bull. Eur. Assoc. Fish. Pathol. 38 (6), 234–239.

Bojarski, B., Witeska, M., 2020. Blood biomarkers of herbicide, insecticide, and fungicide toxicity to fish—a review. Environ. Sci. Pollut. Res. 27 (16), 19236–19250. https://doi.org/10.1007/s11356-020-08248-8.

Brar, N.K., Waggoner, C., Reyes, J.A., Fairey, R., Kelley, K.M., 2010. Evidence for thyroid endocrine disruption in wild fish in San Francisco Bay, California, USA. Relationships to contaminant exposures. Aquat. Toxicol. 96, 203–215. https://doi.org/10.1016/j.aquatox.2009.10.023.

Brown, S.B., Evans, R.E., Vandenbyllardt, L., Finnson, K.W., Palace, V.P., Kane, A.S., Yarechewski, A.Y., Muir, D.C.G., 2004. Altered thyroid status in lake trout (*Salvelinus namaycush*) exposed to co-planar 3,3′,4,4′,5-pentachlorobiphenyl. Aquat. Toxicol. 67, 75–85. https://doi.org/10.1016/j.aquatox.2003.12.002.

Burgos-Aceves, M.A., Lionetti, L., Faggio, C., 2019. Multidisciplinary haematology as prognostic device in environmental and xenobiotic stress-induced response in fish. Sci. Total Environ. 670, 1170–1183. https://doi.org/10.1016/j.scitotenv.2019.03.275.

Buckman, A.H., Fisk, A.T., Parrott, J.L., Solomon, K.R., Brown, S.B., 2007. PCBs can diminish the influence of temperature on thyroid indices in rainbow trout (*Oncorhynchus mykiss*). Aquat. Toxicol. 84, 366–378. https://doi.org/10.1016/j.aquatox.2007.06.016.

Cerveny, D., Grabic, R., Fedorova, G., Grabicova, K., Turek, J., Kodes, V., Golovko, O., Zlabek, V., Randak, T., 2016. Perfluoroalkyl substances in aquatic environment-comparison of fish and passive sampling approaches. Environ. Res. 144, 92–98. https://doi.org/10.1016/j.envres.2015.11.010.

Chen, J., Zheng, L., Tian, L., Wang, N., Lei, L., Wang, Y., Dong, Q., Huang, C., Yang, D., 2018. Chronic PFOS exposure disrupts thyroid structure and function in zebrafish. Bull. Environ. Contam. Toxicol. 101, 75–79. https://doi.org/10.1007/s00128-018-2359-8.

Darnerud, P.O., Lignell, S., Glynn, A., Aune, M., Törnkvist, A., Stridsberg, M., 2010. POP levels in breast milk and maternal serum and thyroid hormone levels in mother–child pairs from Uppsala, Sweden. Environ. Int. 36, 180–187. https://doi.org/10.1016/j.envint.2009.11.001.

Deal, C.K., Volkoff, H., 2020. The role of the thyroid axis in fish. Front Endocrinol. (Lausanne) 11, 596585. https://doi.org/10.3389/fendo.2020.596585.

Desforges, J.-P.W., Sonne, C., Levin, M., Siebert, U., De Guise, S., Dietz, R., 2016. Immunotoxic effects of environmental pollutants in marine mammals. Environ. Int. 86, 126–139. https://doi.org/10.1016/j.envint.2015.10.007.

DeWitt, J.C., Patisaul, H.B., 2018. Endocrine disruptors and the developing immune system. Curr. Opin. Toxicol. 10, 31–36. https://doi.org/10.1016/j.cotox.2017.12.005.

Diamanti-Kandarakis, E., Bourguignon, J.-P., Giudice, L.C., Hauser, R., Prins, G.S., Soto, A.M., Zoeller, R.T., Gore, A.C., 2009. Endocrine-disrupting chemicals: an endocrine society scientific statement. Endocr. Rev. 30, 293–342. https://doi.org/10.1210/er.2009-0002.

Einarsdóttir, I.E., Silva, N., Power, D.M., Sámaradóttir, H., Björnsson, B.T., 2006. Thyroid and pituitary gland development from hatching through metamorphosis of a teleost flatfish, the Atlantic halibut. Anat. Embryol. 211, 47–60. https://doi.org/10.1007/s00429-005-0055-z.

Fair, P.A., Romano, T., Schaefer, A.M., Reif, J.S., Bossart, G.D., Houde, M., Muir, D., Adams, J., Rice, C., Hulsey, T.C., Peden-Adams, M., 2013. Associations between perfluoroalkyl compounds and immune and clinical chemistry parameters in highly exposed bottlenose dolphins (*Tursiops truncatus*). Environ. Toxicol. Chem. 32, 736–746. https://doi.org/10.1002/etc.2122.

Fang, C., Huang, Q., Ye, T., Chen, Y., Liu, L., Kang, M., Lin, Y., Shen, H., Dong, S., 2013. Embryonic exposure to PFOS induces immunosuppression in the fish larvae of marine medaka. Ecotoxicol. Environ. Saf. 92, 104–111. https://doi.org/10.1016/j.ecoenv.2013.03.005.

Förlin, L., Asker, N., Töpel, M., Österlund, T., Kristiansson, E., Parkkonen, J., Haglund, P., Faxneld, S., Sturve, J., 2019. mRNA expression and biomarker responses in perch at a biomonitoring site in the Baltic Sea–possible influence of natural brominated chemicals. Front. Mar. Sci. 6, 316.

Gewurtz, S.B., Bhavsar, S.P., Petro, S., Mahon, C.G., Zhao, X., Morse, D., Reiner, E.J., Tittlemier, S.A., Braekevelt, E., Drouillard, K., 2014. High levels of perfluoroalkyl acids in sport fish species downstream of a firefighting training facility at Hamilton International Airport, Ontario, Canada. Environ. Int. 67, 1–11. https://doi.org/10.1016/j.envint.2014.02.005.

Grabicova, K., Vojs Staňová, A., Koba Ucun, O., Borik, A., Randak, T., Grabic, R., 2018. Development of a robust extraction procedure for the HPLC-ESI-HRPS determination of multi-residual pharmaceuticals in biota samples. Anal. Chim. Acta 1022, 53–60. https://doi.org/10.1016/j.aca.2018.04.011.

Hållén, J., 2016. PCB i Oxundasjön och Rosersbergsviken: Prediktiv modellering av återhämtningsscenarier. diva-portal.org.

Hållén, J., Karlsson, M., Hansson, K., 2017. PCB-undersökningar i Oxundasjön 2016. IVL Rapport U5846.

Han, Z., Liu, Y., Wu, D., Zhu, Z., Lü, C., 2012. Immunotoxicity and hepatotoxicity of PFOS and PFOA in tilapia (*Oreochromis niloticus*). Chin. J. Geochem. 31, 424–430. https://doi.org/10.1007/s11631-012-0593-z.

Henry, T.B., 2015. Ecotoxicology of polychlorinated biphenyls in fish—a critical review. Null 45, 643–661. https://doi.org/10.3109/10408444.2015.1038498.

Houde, M., De Silva, A.O., Muir, D.C.G., Letcher, R.J., 2011. Monitoring of perfluorinated compounds in aquatic biota: an updated review: PFCs in aquatic biota. Environ. Sci. Technol. 45, 7962–7973.

Huang, Q., Chen, Y., Chi, Y., Lin, Y., Zhang, H., Fang, C., Dong, S., 2015. Immunotoxic effects of perfluorooctane sulfonate and di(2-ethylhexyl) phthalate on the marine fish *Oryzias melastigma*. Fish. Shellfish Immunol. 44, 302–306. https://doi.org/10.1016/j.fsi.2015.02.005.

Imbert, H., Arrowsmith, R., Dufour, S., Elie, P., 2008. Relationships between locomotor behavior, morphometric characters and thyroid hormone levels give evidence of stage-dependent mechanisms in European eel upstream migration. Horm. Behav. 53, 69–81. https://doi.org/10.1016/j.yhbeh.2007.06.011.

Iwanowicz, L.R., Blazer, V.S., McCormick, S.D., Vanveld, P.A., Ottinger, C.A., 2009. Aroclor 1248 exposure leads to immunomodulation, decreased disease resistance and endocrine disruption in the brown bullhead, *Ameiurus nebulosus*. Aquat. Toxicol. 93, 70–82. https://doi.org/10.1016/j.aquatox.2009.03.008.

Karlsson, M., 2014. PCB i nedre Oxundaåsystemet. IVL-Rapp. U 4925.

Karlsson, M., Sjöholm, L., Viktor, T., 2014. Metaller och stabila organiska ämnen i Oxundaåsystemet. Rapport U4769 from the Swedish Environment Research Institute IVL.

Karlsson, M., Viktor, T., 2014. Miljöstörande ämnen i fisk från Stockholmsregionen 2013. Rapport B2214 from the Swedish Environment Research Institute IVL.

Katarzyńska, D., Hrabia, A., Kowalik, K., Sechman, A., 2015. Comparison of the in vitro effects of TCDD, PCB 126 and PCB 153 on thyroid-restricted gene expression and thyroid hormone secretion by the chicken thyroid gland. Environ. Toxicol. Pharmacol. 39, 496–503. https://doi.org/10.1016/j.etap.2015.01.016.

Kissa, E., 2001. Fluorinated Surfactants and Repellents. CRC Press.

Korevaar, T.I.M., Muetzel, R., Medici, M., Chaker, L., Jaddoe, V.W.V., de Rijke, Y.B., Steegers, E.A.P., Visser, T.J., White, T., Tiemeier, H., Peeters, R.P., 2016. Association of maternal thyroid function during early pregnancy with offspring IQ and brain morphology in childhood: a population-based prospective cohort study. Lancet Diabetes Endocrinol. 4, 35–43. https://doi.org/10.1016/S2213-8587(15)00327-7.

Lau, C., Anitole, K., Hodes, C., Lai, D., Pfahles-Hutchens, A., Seed, J., 2007. Perfluoroalkyl acids: a review of monitoring and toxicological findings. Toxicol. Sci. 99, 366–394. https://doi.org/10.1093/toxsci/kfm128.

Li, Y., Fletcher, T., Mucs, D., Scott, K., Lindh, C.H., Tallving, P., Jakobsson, K., 2018. Half-lives of PFOS, PFHxS and PFOA after end of exposure to contaminated drinking water. Occup. Environ. Med. 75, 46–51. https://doi.org/10.1136/oemed-2017-104651.

Li, Y., Xu, Y., Fletcher, T., Scott, K., Nielsen, C., Pineda, D., Lindh, C.H., Olsson, D.S., Andersson, E.M., Jakobsson, K., 2021. Associations between perfluoroalkyl substances and thyroid hormones after high exposure through drinking water. Environ. Res. 194, 110647 https://doi.org/10.1016/j.envres.2020.110647.

Lyche, J., Larsen, H., Skaare, J.U., Tverdal, A., Dahl, E., Johansen, G., Ropstad, E., 2004. Effects of perinatal exposure to low doses of PCB 153 and PCB 126 on lymphocyte proliferation and hematology in goat kids. J. Toxicol. Environ. Health A 67, 889–904. https://doi.org/10.1080/15287390490443740.

Maisano, M., Cappello, T., Oliva, S., Natalotto, A., Giannetto, A., Parrino, V., Battaglia, P., Romeo, T., Salvo, A., Spano, N., Mauceri, A., 2016. PCB and OCP accumulation and evidence of hepatic alteration in the Atlantic bluefin tuna, *T. thynnus*, from the Mediterranean Sea. Mar. Environ. Res. 121, 40–48.

Melzer, D., Rice, N., Depledge, M.H., Henley, W.E., Galloway, T.S., 2010. Association between serum perfluorooctanoic acid (PFOA) and thyroid disease in the U.S. National Health and Nutrition Examination Survey. Environ. Health Perspect. 118, 686–692. https://doi.org/10.1289/ehp.0901584.

Mughal, B.B., Fini, J.-B., Demeneix, B.A., 2018. Thyroid-disrupting chemicals and brain development: an update. Endocr. Connect 7, R160–R186. https://doi.org/10.1530/EC-18-0029.

Mullur, R., Liu, Y.-Y., Brent, G.A., 2014. Thyroid hormone regulation of metabolism. Physiol. Rev. 94, 355–382. https://doi.org/10.1152/physrev.00030.2013.

Nagasawa, T., Nakayasu, C., Rieger, A.M., Barreda, D.R., Somamoto, T., Nakao, M., 2014. Phagocytosis by thrombocytes is a conserved innate immune mechanism in lower vertebrates. Front. Immunol. 5, 445. https://doi.org/10.3389/fimmu.2014.00445.

Niras Sweden, L., 2016. Bedömning av spridningsplym avseende PFAS Blekinge Flygflottilj, Delrapport 3 (No. FM2015–8456:12). Försvarsmakten/Miljöprovningsenheten.

Olsen, G.W., Butenhoff, J.L., Zobel, L.R., 2009. Perfluoroalkyl chemicals and human fetal development: an epidemiologic review with clinical and toxicological perspectives. Reprod. Toxicol. 27, 212–230. https://doi.org/10.1016/j.reprotox.2009.02.001.

Peter, M.C.S., 2011. The role of thyroid hormones in stress response of fish. Gen. Comp. Endocrinol. 172, 198–210. https://doi.org/10.1016/j.ygcen.2011.02.023.

Power, D.M., Llewellyn, L., Faustino, M., Nowell, M.A., Björnsson, B.T., Einarsdottir, I.E., Canario, A.V., Sweeney, G.E., 2001. Thyroid hormones in growth and development of fish. Comp. Biochem. Physiol. C Toxicol. Pharm. 130, 447–459.

Rieger, A.M., Barreda, D.R., 2011. Antimicrobial mechanisms of fish leukocytes. Dev. Comp. Immunol. 35 (12), 1238–1245.

Rostvall, A., Zhang, W., Dürig, W., Renman, G., Wiberg, K., Ahrens, L., Gago-Ferrero, P., 2018. Removal of pharmaceuticals, perfluoroalkyl substances and other micropollutants from wastewater using lignite, Xylit, sand, granular activated carbon (GAC) and GAC+ Polonite® in column tests–Role of physicochemical properties. Water Res. 137, 97–106.

Rotllant, J., Worthington, G.P., Fuentes, J., Guerreiro, P.M., Teitsma, C.A., Ingleton, P.M., Balment, R.J., Canario, A.V.M., Power, D.M., 2003. Determination of tissue and plasma concentrations of PTHrP in fish: development and validation of a radioimmunoassay using a teleost 1–34 N-terminal peptide. Gen. Compd. Endocrinol. 133, 146–153. https://doi.org/10.1016/S0016-6480(03)00166-7.

Shi, X., Du, Y., Lam, P.K.S., Wu, R.S.S., Zhou, B., 2008. Developmental toxicity and alteration of gene expression in zebrafish embryos exposed to PFOS. Toxicol. Appl. Pharmacol. 230, 23–32. https://doi.org/10.1016/j.taap.2008.01.043.

Shi, X., Liu, C., Wu, G., Zhou, B., 2009. Waterborne exposure to PFOS causes disruption of the hypothalamus–pituitary–thyroid axis in zebrafish larvae. Chemosphere 77, 1010–1018. https://doi.org/10.1016/j.chemosphere.2009.07.074.

Smits, J.E., Fernie, K.J., Bortolotti, G.R., Marchant, T.A., 2002. Thyroid hormone suppression and cell-mediated immunomodulation in American kestrels (*Falco sparverius*) exposed to PCBs. Arch. Environ. Contam. Toxicol. 43, 338–344. https://doi.org/10.1007/s00244-002-1200-9.

Sørmo, E.G., Larsen, H.J.R.S., Johansen, G.M., Skaare, J.U., Jenssen, B.M., 2009. Immunotoxicity of polychlorinated biphenyls (PCB) in free-ranging gray seal pups with special emphasis on dioxin-like congeners. J. Toxicol. Environ. Health A 72, 266–276.

Svedäng, H., Wickström, H., Reizenstein, M., 1998. Accuracy and precision in eel age estimation, using otoliths of known and unknown age. J. Fish. Biol. 53, 456–464.

Taillebois, L., Keith, P., Valade, P., Torres, P., Baloche, S., Dufour, S., Rousseau, K., 2011. Involvement of thyroid hormones in the control of larval metamorphosis in *Sicyopterus lagocephalus* (Teleostei: Gobioidei) at the time of river recruitment. Gen. Comp. Endocrinol. 173, 281–288. https://doi.org/10.1016/j.ygcen.2011.06.008.

Tavares-Dias, M., Oliveira, S.R., 2009. A review of the blood coagulation system of fish. Rev. Bras. De. Biociências 7, 205–224.

van der Oost, R., Beyer, J., Vermeulen, N.P.E., 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ. Toxicol. Pharmacol. 13, 57–149. https://doi.org/10.1016/S1382-6689(02)00126-6.

Walpita, C.N., Crawford, A.D., Janssens, E.D.R., Van der Geyten, S., Darras, V.M., 2009. Type 2 iodothyronine deiodinase is essential for thyroid hormone-dependent embryonic development and pigmentation in zebrafish. Endocrinology 150, 530–539. https://doi.org/10.1210/en.2008-0457.

Wang, J., Shi, G., Yao, J., Sheng, N., Cui, R., Su, Z., Guo, Y., Dai, J., 2020. Perfluoropolyether carboxylic acids (novel alternatives to PFOA) impair zebrafish posterior swim bladder development via thyroid hormone disruption. Environ. Int. 134, 105317 https://doi.org/10.1016/j.envint.2019.105317.

Weiss, J.M., Andersson, P.L., Lamoree, M.H., Leonards, P.E.G., van Leeuwen, S.P.J., Hamers, T., 2009. Competitive binding of poly- and perfluorinated compounds to the thyroid hormone transport protein transthyretin. Toxicol. Sci. 109, 206–216. https://doi.org/10.1093/toxsci/kfp055.

Williams, G.R., 2008. Neurodevelopmental and neurophysiological actions of thyroid hormone. J. Neuroendocrinol. 20, 784–794. https://doi.org/10.1111/j.1365-2826.2008.01733.x.

Yang, J.-H., 2010. Perfluorooctanoic acid induces peroxisomal fatty acid oxidation and cytokine expression in the liver of male Japanese medaka (*Oryzias latipes*). Chemosphere 81, 548–552. https://doi.org/10.1016/j.chemosphere.2010.06.028.

Zoeller, R.T., 2007. Environmental chemicals impacting the thyroid: targets and consequences. Thyroid 17, 811–817. https://doi.org/10.1089/thy.2007.0107.

Zoeller, T.R., 2010. Environmental chemicals targeting. Thyroid. Horm. 9, 28–40.