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# Exploring avian exposure to parent polycyclic aromatic hydrocarbons (PAHs): Using the common eider *Somateria mollissima* in a global context

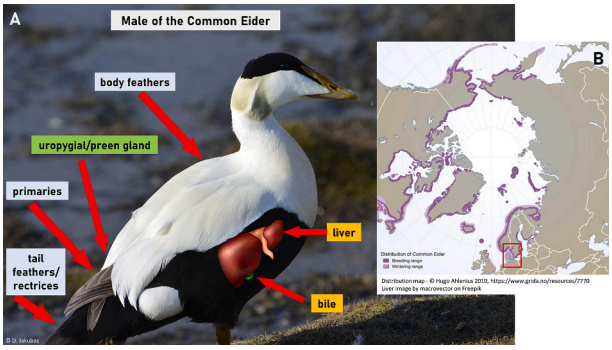
Aneta Dorota Pacyna-Kuchta [a,*], Juliana Souza-Kasprzyk [b], Svend Erik Garbus [c], Igor Eulaers [d], Christian Sonne [e], Dariusz Jakubas [f]

[a] Gdańsk University of Technology, Faculty of Chemistry, Department of Biotechnology and Microbiology, 11/12 Narutowicza Street, 80-233 Gdańsk, Poland
[b] Adam Mickiewicz University, Faculty of Chemistry, Department of Analytical Chemistry, 8 Uniwersytetu Poznańskiego Street, 61-614 Poznań, Poland
[c] Dyrlægehuset Randers (The Veterinary House Randers), Sallingvej 5, 8940 Randers, SV, Denmark
[d] FRAM Centre, Norwegian Polar Institute, 9007 Tromsø, Norway
[e] Department of Ecoscience, Aarhus University, Arctic Research Centre; Frederiksborgvej 399, DK-4000 Roskilde, Denmark
[f] University of Gdańsk, Faculty of Biology, Department of Vertebrate Ecology and Zoology, Wita Stwosza 59, 80-308 Gdańsk, Poland

## HIGHLIGHTS

- Phenanthrene was the main compound found, and it was detected in all sample types.
- Differences in PAH concentrations were observed between sample types.
- Highest concentrations were found in preen oil and liver.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Compared to other organic contaminants, birds are rarely studied for their exposure to polycyclic aromatic hydrocarbons (PAHs), mainly due to their effective metabolization of parent PAHs. However, as some studies suggest, exposure to PAHs may result in adverse health effects including decreased survival, especially following oil spills. In the present study, we analyzed samples from a sea duck, the common eider *Somateria mollissima* including feathers, preen oil, blood, liver and bile, to evaluate whether non- lethally collected samples could be reliably used for avian biomonitoring strategies. Phenanthrene was the only individual PAH detected across sample types, with the highest concentration found in preen gland and the lowest in blood. Significant differences in concentrations were observed between bile vs preen gland and liver vs preen gland, while for most compounds neither blood nor feathers showed detectable levels of parent PAHs. Therefore, the utility of those sample types for PAH exposure assessment may be limited and should be interpreted with caution, moreover as several physiological factors may affect them. Additionally, we also provide a comparison with the available literature to review current avian PAH exposure assessment and outline future research focused needs.

* Corresponding author.
*E-mail address:* aneta.kuchta@pg.edu.pl (A.D. Pacyna-Kuchta).

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## 1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of ubiquitous compounds in the environment, originating from both natural and anthropogenic emissions. The primary sources of PAHs in the marine environment include inputs from natural seafloor oil seeps, anthropogenic runoff from shipping, emissions from oil industry, production water discharges from offshore oil and gas platforms, as well as rivers and urban runoff (Troisi et al., 2006; McConnell et al., 2015; Hodson et al., 2020). Other sources include forest fires, volcanic eruptions, waste incineration and asphalt production (Pereira et al., 2009). Accidents, such as the Prestige oil spill in November 2002 in Europe, the Deepwater Horizon oil spill in 2010 in the Gulf of Mexico and other incidental releases of oil and diesel fuel increase availability of petrogenic PAHs to the aquatic ecosystem (Troisi et al., 2006; Alonso-Alvarez et al., 2007; Paruk et al., 2014).

PAHs can be divided into groups based on their sources. Biogenic PAHs are generated by biological processes or during diagenesis in marine sediments, while pyrogenic PAHs are formed through incomplete combustion of organic matter such as coal and petrogenic PAHs are originating from fossil fuels including crude oil and its derivatives (e.g. gasoline and diesel fuel) (AMAP Assessment, 2007; Antunes et al., 2013; McConnell et al., 2015). PAHs can also be categorized based on their chemical structure. High molecular weight (HMW) PAHs, which contain four or more aromatic rings, are usually more associated with pyrogenic sources. Low molecular weight (LMW) PAHs, which have less than four aromatic rings that are more associated with petrogenic processes (Pereira et al., 2009). They also tend to be more volatile and easily re-released into air or water from soil or sediment (Wallace et al., 2020).

In vertebrates, PAHs are quickly metabolized by mixed-function oxidases and transformed into more water-soluble metabolites, which eliminate more easily from the body (Pereira et al., 2009; Barreto et al., 2020). They may also undergo trophic dilution in the marine food web (Broman et al., 1990; Wan et al., 2007). Nevertheless, several reports have shown detectable amount and sometimes high levels of parent (i.e., non-alkylated) PAHs and/or their metabolites in multiple tissues collected from vertebrates, such as avian liver and muscle (Troisi et al., 2006; Fernie et al., 2018), fish bile (Kammann et al., 2017) or subcutaneous blubber of aquatic mammals (Marsili et al., 2001).

When PAHs enter water bodies, they can become incorporated into sediments with a long half-life, affecting bottom-dwelling species (Roscales et al., 2011). It is known that shellfish lack the ability to metabolize and excrete PAHs, potentially leading to the bioaccumulation of PAHs over a longer period (Eisler, 1987). Birds that rely on shellfish as a primary food source are therefore susceptible to possible PAH contamination (Bustnes, 2013; McConnell et al., 2015). In addition to diet, birds may be exposed to PAHs through water or via respiratory uptake of atmospheric PAHs (Fernie et al., 2018). Seabirds may face additional exposure risks because of marine oil spills, which increase the availability of petrogenic PAHs (Paruk et al., 2014; Power et al., 2021). Furthermore, active mining-related activities and refinery sites are sources of PAHs, and birds nesting in proximity of such sites have shown higher PAH concentrations compared to reference sites (Custer et al., 2001; Fernie et al., 2018).

PAHs are lipophilic, which means they tend to accumulate over the short-term in lipid-rich organs such as the liver (McConnell et al., 2015). However, the distribution of PAHs in tissues appears to be compound-specific, rather than solely dependent on passive partitioning according to their lipophilicity (Wallace et al., 2020). It is also affected by the biotransformation potential of the compounds (Pei et al., 2017). The effect of PAH contamination on birds may be acute, particularly in exposure to low molecular weight PAHs, for example hemolytic anemia; or chronic, associated with high molecular weight PAHs, causing long-lasting health problems with carcinogenic, mutagenic, or teratogenic properties (Eisler, 1987; Troisi et al., 2007; Bustnes, 2013). While acute effects may increase mortality of oiled seabirds, long-term sub-lethal exposures may affect avian health and their over-winter survival chances (Alonso-Alvarez et al., 2007). For instance, PAH ingestion may interfere with lipid absorption and impair pre-migratory fueling, thereby reducing their chances of survival during migration (Bianchini and Morrissey, 2018).

The common eider (*Somateria mollissima*) is the largest sea duck in the Northern Hemisphere that nests mainly in polar or subpolar coastal areas, but also in the Baltic and Wadden Seas (Matson et al., 2004; Fenstad et al., 2017; Ma et al., 2020). It has been recognized as an important bioindicator of inshore marine pollution (Meattey et al., 2014). The Baltic Sea is a semi-enclosed sea, with limited water exchange and many sources of contaminants, including high ship traffic and oil pollution, river runoff, and contamination from industries in the well-developed countries surrounding the Baltic Sea (Ruszyńska et al. 2011). Previous research has reported genotoxic effects including elevated chromosomal damage in the Baltic population of the common eider, likely caused by environmental contamination (Matson et al., 2004). Estimates indicate a decline of over 30 % in the Baltic common eider population since the 1990s (Fenstad et al., 2017). Several factors contribute to this decline, with changes in food web dynamics and exposure to pollution being particularly significant (Sonne et al., 2020; Ma et al., 2020).

The diet of common eiders consists mainly of molluscs, crustaceans, and echinoderms collected from the benthic zone at depths of 10 to 20 m. In the Baltic Sea they feed primary on the blue mussel (*Mytilus edulis*), which may bioaccumulate hydrophobic contaminants such as PAHs, polychlorinated biphenyls (PCBs), and polychlorinated dibenzo-p-dioxin and dibenzofurans (PCDD/Fs) (Matson et al., 2004). The common eider can consume up to 2–3 kg of mussels and other invertebrates daily (Bustnes, 2013). A previous study shows that PAH concentrations in blue mussels in the Baltic area are affected by different sets of environmental variables including bioturbation and salinity (Ek et al., 2021).

PAHs are listed as priority pollutants by many authorities, including the World Health Organization (World Health Organization, 2021). In 1976, the U.S. Environmental Protection Agency published a list of 16 priority PAH pollutants as representatives of regulated PAHs. This list is often further used by researchers for comparative purposes (Keith, 2014). Due to their efficient metabolization in the body by cytochrome P450 enzymes, PAHs compounds are not classified as classical persistent organic pollutants (POPs) (Hodson et al., 2020). However, they share several characteristics with POPs, such as lipophilicity, high prevalence in the environment, toxicity and bioaccumulation capacity (Luzardo et al., 2014; Hodson et al., 2020). Some of the PAHs, e.g., anthracene, are classified by the European Chemicals Legislation (REACH) as PBT (persistent, bioaccumulative, toxic) or CMR (carcinogenic, mutagenic, toxic for reproduction) compounds (Kleinteich et al., 2018).

Environmental exposure assessment of those compounds is often evaluated using internal tissues. In vertebrates, due to minor concentrations of parent PAHs detected in tissues, authors often chose alternative techniques to assess exposure, such as PAH metabolite bile burden, the induction of cytochrome P450, or ethoxyresorufin-O-deethylase (EROD) activity in liver. However, these methods require sample collection from freshly killed animals or specialized team and equipment to perform a liver biopsy (Pérez et al., 2008; Willie et al., 2017; Perez-Umphrey et al., 2018).

To the present day, our understanding of the feasibility and use of non-lethal strategies for PAH exposure assessment remains limited. To fill this gap, in the present study, various tissues and organs of adult male common eiders were analyzed. Specifically, the PAH profile and detectability were compared between samples that can be collected non-lethally, such as blood, feathers, and preen oil, and postmortem collected samples, such as liver and bile. In addition, blood clinical-chemical parameters (BCCPs) were evaluated to assess the overall health of the birds, adding to dietary assessment based on stomach contents. Additionally, a comprehensive review of the birds PAHs exposure based on different sample types, including this case study on

the selected species, was conducted.

## 2. Methods

### 2.1. Study site, and sampling

The common eider population breeding in the Baltic Sea basin is estimated to be around 900,000 birds (Ma et al., 2020), migrating to Denmark, Germany and the Netherlands for the winter (Matson et al., 2004; Fenstad et al., 2017).

The analysis included 15 adult males shot in the Danish Straits and obtained from a licensed hunter during the hunting season in December 2015. The eiders' body mass was measured using a Pesola Spring balance, accurate to 10 g. Within minutes, 8–12 mL of intracardiac blood was drawn. This sample was then distributed between a 4 mL BD Vacutainer® tube containing Lithium Heparin and another with ethylenediaminetetraacetic acid (EDTA). Within the next 8 h, these tubes were centrifuged at 2500 rpm for a duration of 10 min (equivalent to ~839 G). Subsequently, the plasma from the top layer was transferred to a sterile Eppendorf® tube and stored at −20 °C, awaiting biochemical assessment. Another blood vial for PAHs and bile were collected immediately after the bird was shot and frozen at −20 °C until chemical analysis.

All birds were transported to the laboratory and dissected the same day. Analyses of different sample types, including blood, three types of feather and preen oil, were chosen as all of these can be collected non-lethally. Liver and bile samples were collected to represent postmortem samples, as they were expected to have the highest PAH concentrations due to their important role in PAH biotransformation. Liver samples were stored in aluminum foil. Entire preen gland was cut out from the body. Stomach content was placed in a separate plastic bag. Underlying body feathers were sampled from the back, outer primaries were taken from both wings and a couple of tail feathers were collected, all of them were packed separately in enclosed transparent plastic bags. All samples were stored in separate zip bags and immediately frozen at −20 °C.

### 2.2. Chemicals used

Reagents used for the PAHs analysis include acetone (purity ≥99.8 %, Sigma – Aldrich), dichloromethane (purity 99.9 %, Merck), *n*-hexane (purity ≥98.8 %, Merck), 30 % hydrochloric acid (Merck, Suprapure), isooctane (purity ≥99.8 %, Sigma – Aldrich), anhydrous sodium sulfate (≥99.0 %, Sigma-Aldrich) and silica gel (high purity grade, 60 Å, Sigma–Aldrich). Ultrapure water was produced from a Milli-Q Gradient A10 (Milipore, France). Analysis was performed for 15 parent PAHs referenced by the US-EPA Clear Water Act as priority ones: naphthalene (NaP), acenaphthylene (Ace), acenaphthene (A), fluorene (F), phenanthrene (Phe), anthracene (An), fluoranthene (Flu), pyrene (Py), benz[*a*]anthracene (BaA), chrysene (Chry), benzo[*b*]fluoranthene (BbF), benzo[*a*]pyrene (BaP), indeno-[1,2,3-cd]pyrene (IP), dibenz[*a*,*h*]anthracene (DBA), benzo[*ghi*]perylene (BghiP). A commercial PAH mixture and mass labelled internal standard (deuterated benzo(*a*)anthracene-d12 in dichloromethane) was provided by Restek Corporation (Bellefonte, PA, USA) and Supelco (Bellefonte, PA, USA) respectively.

### 2.3. Chemical analysis

#### 2.3.1. Blood plasma clinical–chemical parameters (BCCP)

The analysis for blood plasma clinical–chemical parameters (BCCPs) was conducted at the Veterinary Diagnostic Laboratory, Department of Veterinary Clinical Sciences at the University of Copenhagen (Table 1). The BCCPs composed three liver enzymes, which were alkaline phosphatase (ALP; U $L^{-1}$), alanine aminotransferase (ALAT; U $L^{-1}$) and gamma-glutamyltransferase (GGT; U $L^{-1}$). The digestive enzyme amylase (Amy; U $L^{-1}$) was targeted as well. Three groups of proteins were investigated, including total protein (TP; g $L^{-1}$), globulin (Glo; g $L^{-1}$) and albumin (Alb; g $L^{-1}$), one hepatic/erythrocyte metabolic product, total bilirubin (TB; μmol $L^{-1}$), and one carbohydrate, glucose (Glu; mmol $L^{-1}$). Moreover, two muscle and protein metabolic products, creatinine (Cre; μmol $L^{-1}$) and urea (Urea; mmol $L^{-1}$), cholesterol (Cho; mmol $L^{-1}$), and seven electrolytes, being inorganic phosphate (IP; mmol $L^{-1}$), calcium (Ca; mmol $L^{-1}$), magnesium (Mg; mmol $L^{-1}$), sodium (Na; mmol $L^{-1}$), potassium (K; mmol $L^{-1}$) and chloride (Cl; mmol $L^{-1}$) were measured as well. Analyses were routinely conducted at the laboratory using an automated spectrophotometric analyzer containing ion-selective electrodes (ADVIA 1800, Siemens). All assays were subjected to daily internal and quarterly external quality control. Information on the methods and their interpretations in clinical studies has been previously discussed (Table 1, Sonne, 2010; Sonne et al., 2012; Sonne et al., 2013).

#### 2.3.2. Whole blood

The method for blood analysis was based on Pleil et al. (2010) with minor modifications. Samples were left to thaw, and 10 mL of hexane and 10 μL of 0.1 μg $mL^{-1}$ internal standard (benzo(*a*)anthracene-d12) were added to 1 mL of blood. Samples were left to equilibrate for 5 min. Then, they were vortexed for 30 s, placed for 5 min in an ultrasound bath, then agitated at 400 rpm for 25 min on an orbital shaker and vortexed again for 30 s. Next, samples were centrifuged at 4000 rpm for 5 min, and frozen overnight for approximately 12 h. The following day, an organic layer was collected and reduced under high purity nitrogen

**Table 1**
Summary statistics (median ± *SD* and range) of body mass and blood plasma clinical–chemical parameters (BCCPs) in 14 adult male common eiders collected in the Western Baltic December 2015. For comparison, reference values from captive common eiders are included. Elevated values are bolded.

| Variable | Current study (n = 14) | Reference values[ISIS, 2015;Camphuysen et al., 2002; Laursen and Frikke, 2008] (*n* = 79) |
|---|---|---|
| ALAT, ukat/L (U $L^{-1}$) | **160.5** ± 123.7 (16–433) | 12 ± 9 (4–29) |
| Albumin, g $L^{-1}$ | 19.1 ± 3.6 (13.8–28.1) | 24 ± 9 (14–42) |
| ALP, ukat/L (U $L^{-1}$) | **1063** ± 1275 (320–5375) | 84 ± 59 (32–265) |
| Amylase, ukat/L (U $L^{-1}$) | **830** ± 376 (263–1630) | 553 ± 101 (436–692) |
| Body mass, g | 2400 ± 175 (2108–2708) | 2494 (A), 2166 (S) |
| Calcium, mmol $L^{-1}$ | 2.6 ± 0.4 (1.92–3.26) | 2.7 ± 0.2 (2.3–3.1) |
| Cholesterol, mmol $L^{-1}$ | 4.8 ± 0.8 (3.8–6.6) | 8.2 ± 2.4 (4.6–13.8) |
| Chloride mmol $L^{-1}$ | 119 ± 7 (109–132) | 118 ± 5 (108–128) |
| Creatinine, μmol $L^{-1}$ | 9.1 ± 2.5 (5–15) | 53 ± 18 (0–97) |
| GGT, ukat/L (U $L^{-1}$) | **5.1** ± 9.6 (0–38) | 3.0 ± 2 (1–6) |
| Globulin, nmol $L^{-1}$ (g $L^{-1}$) | 27.1 ± 3.8 (19.4–31.9) | 29 ± 7 (20–40) |
| Glucose, mmol $L^{-1}$ | 9.2 ± 1.8 (6.9–12.6) | 13.2 ± 2.4 (7.8–21.2) |
| Inorganic phosphate, mmol $L^{-1}$ | 2.1 ± 1.1 (0.8–4.6) | 1.2 ± 0.6 (0.5–2.2) |
| Potassium, mmol $L^{-1}$ | **6.8** ± 3.5 (3.3–16.4) | 2.1 ± 0.4 (1.6–3.2) |
| Sodium, mmol $L^{-1}$ | 156.8 ± 6.6 (147–172) | 163 ± 6 (148–178) |
| Total bilirubin, μmol $L^{-1}$ | 5.5 ± 1.8 (3–9.4) | 3.0 ± 3 (0–9) |
| Total protein, g $L^{-1}$ | 46.3 ± 6.7 (33.5–59.9) | 54 ± 12 (27–81) |
| Urea, mmol $L^{-1}$ | 1.8 ± 0.5 (1.3–3.3) | 1.1 ± 0.7 (0.36–2.86) |

ALAT: alanine aminotransferase. ALP: alkaline phosphatase. GGT: gamma glutamyltransferase.

gas to a final volume of 450 μL. The extract was transferred into an amber glass vial and kept under cold conditions until further analysis.

#### 2.3.3. Bile

Similar to blood, samples were thawed, and 10 mL of hexane and internal standard were added to 0.2–1 mL of bile. Samples were left to equilibrate for 5 min. Then, samples were vortexed for 30 s, agitated at 400 rpm for 25 min on an orbital shaker, and vortexed again. Next, samples were centrifuged at 4000 rpm for 5 min, and frozen overnight. The next day, an organic layer was collected and reduced under high purity nitrogen gas to approx. 2 mL. Clean-up was performed using silica solid-phase extraction (JBaker) with 3 mL of hexane used for conditioning, and elution performed with 5 mL of hexane. The eluted samples were collected and reduced to a final volume of 400 μL.

#### 2.3.4. Body, primary, and tail feathers

All feather samples were firstly washed 3 times with deionized water, dried overnight and cut with clean, acetone-rinsed stainless scissors. For tail and primary feathers the calamus was removed. Samples (mean 237.0 ± 47.9 mg of tail feathers; 222.0 ± 37.5 mg of body feathers; 335.0 ± 55.8 mg of primary feathers) were incubated overnight in 40 °C with 10 mL hexane:acetone (2:1) (18 mL for primary feathers), 7 mL 15 % HCl (10 mL in primary feathers) and 10 μL (0.1 μg $mL^{-1}$) of internal standard. Next day, samples were put in an ultrasound bath for 10 min, vortexed and an organic layer was collected. Fresh solvent (hexane: acetone 2:1; *v:v*) was added, samples were vortexed again, and the organic layer was collected and combined with the previous one. Volume was reduced until approx. 2 mL. Clean-up was performed on solid-phase extraction (SPE) columns filled from the bottom with activated silica gel and anhydrous $Na_2SO_4$. Columns were conditioned with hexane, and then sample extract was added and eluted using a mixture of DCM: hexane (3:7; *v:v*). The volume was reduced to 300 μL under a nitrogen stream.

#### 2.3.5. Liver and preen gland

Liver and preen gland were left to thaw, weighted (mean 4770 ± 1160 mg wet weight of liver; 589 ± 104 mg wet weight of preen gland) and extracted using pressurized liquid extraction (PLE, FMS model). Samples were firstly homogenized with anhydrous magnesium sulphate and 50 μL of 10 μg $mL^{-1}$ of internal standard. Then, diatomaceous earth was added to the sample, all were mixed and transferred to the extraction cell. Two extraction cycles were used, performed in 120 °C and 1500 psi. Samples were extracted with hexane:DCM (50:50; *v:v*). Obtained extracts were collected and the volume was subsequently reduced to approx. 2 mL. Clean-up was performed on SPE columns, filled from the bottom with activated silica gel, activated $Al_2O_3$ and anhydrous $Na_2SO_4$. The columns were firstly conditioned with hexane, then, the sample extract was added, and eluted using a mixture of DCM:hexane (1:1; *v:v*). Next, the sample was evaporated under a nitrogen stream until almost dry and reconstituted using 300 μL of hexane.

### 2.4. Quality control and quantification

Laboratory analyses were performed in 2016/2017. All equipment used was firstly washed with hexane. Standard Reference Material 2977 Mussel Tissue (NIST; National Institute of Standards and Technology, USA) was used to check method accuracy. A procedural blank was included in each analytical batch to determine laboratory background levels. All compounds were blank corrected by a mean procedural blank value. Limits of detection (LOD) and quantification (LOQ) were calculated based on the standard deviation of the response (s) and the slope of the calibration curve (b). This was according to the formula: LOD =3.3 (s/b), LOQ =10(s/b). The results were corrected for sample weights and reported as nanograms per ml (ng $mL^{-1}$) for blood and bile; ng $g^{-1}$ dry weight (dw) for all feather types; and ng $g^{-1}$ wet weight (ww) for liver and preen gland. LOD values vary between 0.01 and 0.10 ng $mL^{-1}$ for blood and bile; 0.01–0.39 ng $g^{-1}$ for feathers; 0.01–0.17 ng $g^{-1}$ ww for preen gland and liver. Values below LOD were replaced with half the LOD value. For statistical analysis, the arithmetic mean was calculated if at least 65 % of the samples had concentrations of the compound >LOD. Samples were collected from 15 individuals, however, in certain instances, there was either insufficient material available for analysis or the samples were too contaminated to be used (some samples got post-contaminated with blood after collection; for the number of individuals for each sample type see Supplementary materials, Table 1). Recovery was above 65 %.

PAHs were determined using a Shimadzu GC–MS-TQ8050 equipped with autosampler AOC-20i in MRM mode. The column used for separation was Zebron ZB-Semivolatiles 20 m L x 0.18 mm I.D. x 0.18 μm df, coated with 5 % polysilarylene – 95 % Polydimethylsiloxane provided by Phenomenex. 2 μL of the extract was injected at a temperature of 280 °C in a splitless mode under a constant He flow rate of 1 mL $min^{-1}$ (purity of carrier gas 99.999 %). The interface and ion source temperatures were 320 °C and 230 °C, respectively (ion source EI). The column temperature was initially held at 50 °C for 1 min, raised to 260 °C at the rate of 20 °C $min^{-1}$, then to 300 °C at the rate of 5 °C $min^{-1}$, finally to 320 °C at the rate of 20 °C $min^{-1}$, held at final temperature for 15 min. Equilibration time between analysis was set for 3 min. Argon was used as a collision gas. A solvent delay of 4 min was used to prevent filament damage. Finally, the detector voltage was 1.5 kV. MRM transition for each compound can be found in Supplementary materials, Table. 2.

### 2.5. Statistical analysis

Due to relatively high number of samples with PAHs below detection level, concentrations of only 8 PAHs (NaP, Ace, A, F, Phe, An, Flu, Py) in three types of samples (liver, bile, preen gland) were statistically analyzed using multivariate analysis.

To investigate variation in the qualitative and quantitative composition of PAHs in three types of samples, a principal component analysis (PCA) was used. This technique identifies hypothetical variables (components) accounting for as much as possible of the variance in the multivariate data. PCA was applied to reduce the number of variables to a few new factors representing groups of PAHs with significantly intercorrelated concentrations. As all variables (PAHs) were measured in the same or similarly ranged units, the variance-covariance matrix was used (Legendre and Legendre, 1998).

Discriminant Analysis of Principal Components (DAPC) was also used to investigate differences between three types of samples (liver, bile and preen gland). This procedure firstly transforms the data using PCA, and then performs a Discriminant Analysis on the retained principal components (Jombart et al., 2010).

To compare the qualitative and quantitative compositions of all PAHs between liver, bile and preen gland the following multivariate (including all PAHs) methods were applied:

1) PERMANOVA, i.e. permutational MANOVA is a non-parametric test of significant difference between two or more groups [here sample type (liver, bile, preen gland)], based on the Euclidean distance measure (Anderson, 2001). The significance is computed by permutation of group membership, with 9999 replicates. Pairwise PERMANOVAs between all pairs of groups was used as a *post-hoc* test with Bonferroni corrected *p* values.

2) test for multivariate dispersion (PERMDISP) of multivariate data; it starts from a standard Principal Coordinates Analysis (PCoA) of the complete data set using the selected distance measure. In the PCoA space, calculate the Euclidean distance from each point *to* its group centroid; these distances are subjected to a standard one-way, univariate ANOVA; the significance (*p* value) is estimated by a permutation test (Anderson, 2005). *Post-hoc* pairwise tests across groups are based on the original PCoA of the complete data set.

3) the similarity percentage breakdown (SIMPER) procedure to assess the average percentage contribution of individual factors to the

dissimilarity between objects in a Euclidean dissimilarity matrix (Clarke, 1993).

All multivariate analyses were performed on log(x + 1) transformed data. After multivariate analyses, unimodal tests, i.e., Kruskal–Wallis and then pairwise Wilcoxon (Mann–Whitney U) tests were performed to compare concentrations of particular PAHs among liver, bile and preen gland.

PERMANOVA, PERMDISP, SIMPER, and PCA analyses were performed using PAST 4.11 software (Hammer et al., 2001). Unimodal tests and Spearman correlations were performed in packages *ggpubr* (Kassambara, 2019), *corrplot* (Wei and Simko, 2021) in R (R Core Team, 2022). DAPC was performed in the *adegent* package (Jombart, 2008) in R. Microsoft Excel Office 365 (Microsoft Corporation, WA, USA) was used for data organization and GraphPad Prism 10 (GraphPad Software, San Diego, CA, USA) was utilized to generate Fig. 5.

## 3. Results

### 3.1. Levels and profiles of PAHs

Concentrations of PAHs found in common eiders were generally low. Levels in blood and feathers were mostly below detection limits, except for tail feathers. Bile and liver were expected to have the highest concentrations of parent compounds, and indeed both detectability and concentration were significantly higher in them compared to blood and feathers. Eight compounds were detected in bile, with phenanthrene and pyrene found at the highest concentrations (mean 1.19 ± 1.31 and 2.25 ± 2.13 ng $mL^{-1}$, respectively). In the liver, mean pyrene and benzo[*ghi*] perylene were present at the highest concentration (1.06 ± 0.80 and 1.32 ± 1.27 ng $g^{-1}$ ww, respectively). The liver was the only tissue where most compounds, beside benz[*a*]anthracene and chrysene, were detected. Preen gland was characterized by the highest concentration of compounds, with 10 out of 15 analyzed compounds present at detectable concentration and profile dominated by naphthalene (13.6 ± 7.80 ng $g^{-1}$ ww; for individual compound concentrations see Supplementary materials, Table 1) (Fig. 1).

### 3.2. Differences in PAHs accumulation among bile, liver and preening gland

A principal component analysis (PCA) on log-transformed data revealed that 85.0 % of the total variance was explained by three axes. PC1 explained 49.1 % of the total variance and was moderately positively correlated with Phe ($r = 0.55$), and weakly with F ($r = 0.45$) and A ($r = 0.41$) (Supplementary material, Table 3). PC2 explained 25.6 % of the total variance and was moderately positively correlated with An ($r = 0.67$) and NaP ($r = 0.59$), and weakly negatively with PKE ($r = -0.42$). PC3 explained 10.2 % of the total variance and was moderately positively correlated with A ($r = 0.58$), moderately negatively with An ($r = -0.52$), and weakly negatively with PHE ($r = -0.47$). Liver and bile clustered in a similar position in relation to the y-axis (in range of negative PC2 values) when preen gland was in a separate position (in range of positive PC2 values) in the PCA plot with almost no overlap between liver-bile and preen gland groups (Fig. 2).

In a discriminant analysis of principal components (DAPC), three principal components and two discriminant functions were retained (Fig. 3). Bile and liver clustered closely together, with significant overlap when the majority of preen gland samples clustered in a different position (Fig. 3).

The concentrations of all combined studied PAHs were significantly affected by sample type (multivariate one-way PERMANOVA, similarity measure: Euclidean, $F = 5.90$; $p < 0.01$). Pairwise comparisons with Bonferroni-corrected values revealed significant differences in PAH concentrations between bile and preen gland ($p < 0.01$), and liver and preen gland ($p < 0.01$), while concentrations in bile and liver were similar ($p = 0.47$).

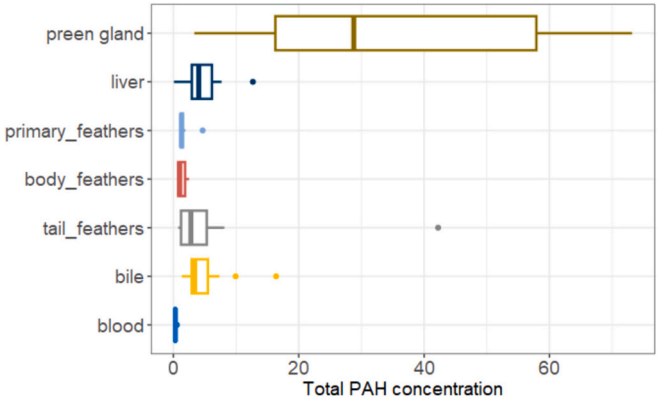


**Fig. 1.** Concentration of $\Sigma_{15}$PAHs in tissues of male common eiders wintering in Denmark. Concentration units are ng $g^{-1}$ ww for liver and preen gland; ng $g^{-1}$ for feathers and ng $mL^{-1}$ for bile and plasma, respectively. Box plots show the median (band inside the box), the first (25 %) and third (75 %) quartile (box), the lowest and the highest values within 1.5 interquartile range (whiskers), and outliers (dots).

The test for multivariate dispersion revealed significant differences among sample types (PERMDISP, similarity measure: Euclidean; $F = 20.31$; $p < 0.01$). Pairwise comparisons with Bonferroni-corrected values revealed significant differences in variation of PAHs concentrations between bile and preen gland ($p < 0.01$) and liver and preen gland ($p < 0.01$) with preen gland characterized by the highest distance to the centroid. Again, variation in concentrations in bile and liver were similar ($p = 0.37$).

SIMPER analysis showed that the following PAHs contributed the most (> 15 %) to the pattern of dissimilarity between the studied types of samples: An, Phe, NaP for overall dissimilarity (14.4 %), Phe, A and An to bile vs liver dissimilarity (6.1 %), An, Phe and NaP for bile vs preen gland dissimilarity (17.2 %), and An, NaP and Phe for liver vs. preen gland dissimilarity (18.8 %; Supplementary material, Table 4).

Univariate analyses revealed that levels of NaP and An differed significantly between the considered types of samples (Kruskal-Wallis test; $p < 0.01$ and $p < 0.01$, respectively) with preen gland having higher values compared to liver and bile (Wilcoxon test; $p < 0.01$; Fig. 4). Levels of other PAHs were similar among the studied tissues (Kruskal-Wallis test, Ace: $p = 0.70$; A: $p = 0.10$; F: $p = 0.86$; Phe: $p = 0.95$; Flu: $p = 0.81$; Py: $p = 0.24$).

### 3.3. Stomach content

The stomach contained clam shells (66.7 %), and the visible remains of crabs (20 %). Two stomachs (13.3 %) were empty or contained only small stones.

### 3.4. Blood plasma clinical–chemical parameters

One male common eider displaying a non-active focal granuloma had ALP levels increased by thrice and ALAT values seven times higher than the mean figures in the control group (Table 1). Such readings point towards liver abnormalities, although they were less pronounced than in another individual with an active granuloma and distended intestines. The eider presenting an active focal granuloma had ALP values rising ten times and ALAT increasing by 35 times, marking the most significant readings in this research, compared to the control group's mean. This suggests acute liver cell injury. Additionally, the individual showing distended intestines demonstrated amplified levels of ALP (63 times), ALAT (18 times), GGT (12 times), and Amylase (2.5 times) compared to the control group (Table 1).

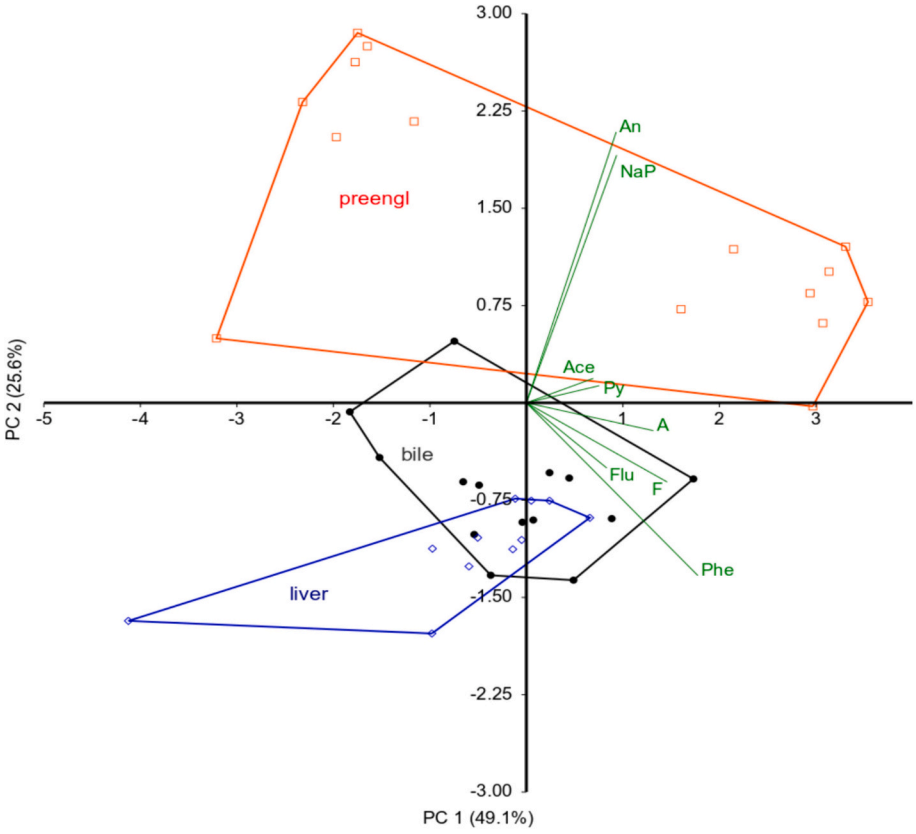


**Fig. 2.** Principal component analysis (PCA) biplot of PAH concentration in liver, bile and preen gland (preengl) of common eiders males wintering in Denmark based on log(x + 1)- transformed data. Convex hulls contain all samples of one type.

## 4. Discussion

### 4.1. Common eiders diet and health

Common eiders are marine ducks, and their PAH exposure is mostly through their food sources and water they inhabit. Shellfish lack the ability to metabolize and excrete PAHs, potentially leading to their bioaccumulation over a longer period (Eisler, 1987). Thus, the wild mussels (*Mytilus edulis*), an important diet component of common eiders, can accumulate relatively high concentrations of PAHs (Broman et al., 1990). For example, mussels sampled in the Belgian coast had a mean of 15 EPA-priority PAHs ranging from 64.1 to 306.8 ng $g^{-1}$ ww (De Witte et al., 2014), and those sampled in the Baltic Sea had $\Sigma_{15}$PAHs concentrations between 173 ng $g^{-1}$ dw and 238 ng $g^{-1}$ dw, with predominance of 4-ring PAHs (40–54 %) (Ruczyńska et al., 2011). However, PAHs are effectively transformed by vertebrates and may undergo trophic dilution. Common eiders and other birds have a high ability to biotransform PAHs, especially compared to mussel and seston (Broman et al., 1990; Wan et al., 2007). Thus, it is important to know which sample type may be the most appropriate to uncover avian exposure to PAHs. In addition to mussel shells, crab remains were found in stomach samples of dissected individuals. Crabs may bioaccumulate PAHs, including in exoskeleton, with tissue-specific bioaccumulation (Lee et al., 2023). However, data on crabs' exposure to PAHs in the Baltic area remain very scarce. When sampled from the same location in China, they had lower concentrations compared to clams and scallops (Wan et al., 2007).

Comparison of BCCPs for 14 studied adult common eiders with the control group from other studies (ISIS, 2015; Camphuysen et al., 2002; Laursen and Frikke, 2008) revealed a consistent increase is observed across all three liver-related enzymes, even in eiders without discernible liver abnormalities. Specifically, elevated concentrations of ALP (92 %), ALAT (93 %), and GGT (41 %) suggest abnormalities in liver and biliary epithelial cells in these wild common eiders (Lumeij, 2008). Additionally, a rise in TB concentrations (45 %) indicates potential cholestasis, augmented liver metabolites, and enteritis. Possible conditions that might lead to the elevation of these enzymes in birds include cholangiohepatitis (inflammation of the liver and bile ducts), liver damage due to ingestion of toxic substances, viral and bacterial infections, physical injuries to liver or tumors (Harr, 2006; Fudge, 2000; Williams et al., 2012).

In avian medicine, the interpretation of blood tests, particularly in the presence of potential hemolysis, demands meticulous scrutiny. Hemolysis in the blood samples collected from gunshot eiders may influence the BCCPs. This phenomenon can lead to an elevation in plasma potassium levels, alanine aminotransferase (ALAT, although less significant in avian species) phosphorus, and total bilirubin, along with alkaline phosphatase (ALP). Conversely, glucose levels may exhibit a decline (Lumeij, 2008). Hemolysis can significantly confound the accurate measurement of various blood parameters. Therefore, for a comprehensive and precise evaluation, veterinarians typically rely on an integrative approach that encompasses laboratory results, clinical manifestations, and if possible the birds' clinical history, which is not possible in the present study. Therefore these results should be interpreted carefully.

### 4.2. Profile differences between sample types for PAHs

In the present study, significant differences in detectability between sample types were observed. For most compounds neither blood nor feathers showed detectable levels of parent PAHs. Phenanthrene was the only compound detected in all sample types, with the highest concentration found in preen gland (mean 3.07 ± 4.10 ng $g^{-1}$ ww) and the lowest concentration in blood (mean 0.06 ± 0.05 ng $mL^{-1}$). Congeners

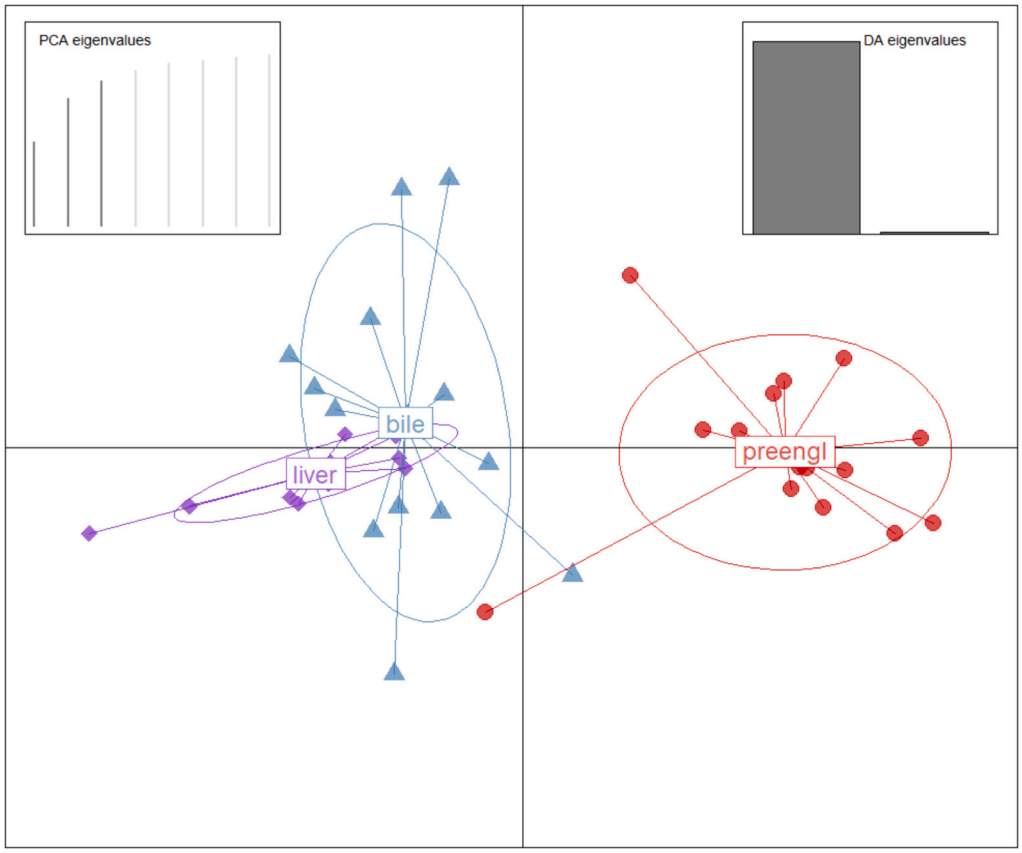


**Fig. 3.** Results of Discriminant Analysis of Principal Components (DAPC) analysis based on *log*(x + 1)- transformed data showing individuals as dots and the groups as inertia ellipses (liver, bile and preen gland). Eigen values of the analysis are displayed in inset (for PCA and DA in left and right, respectively).

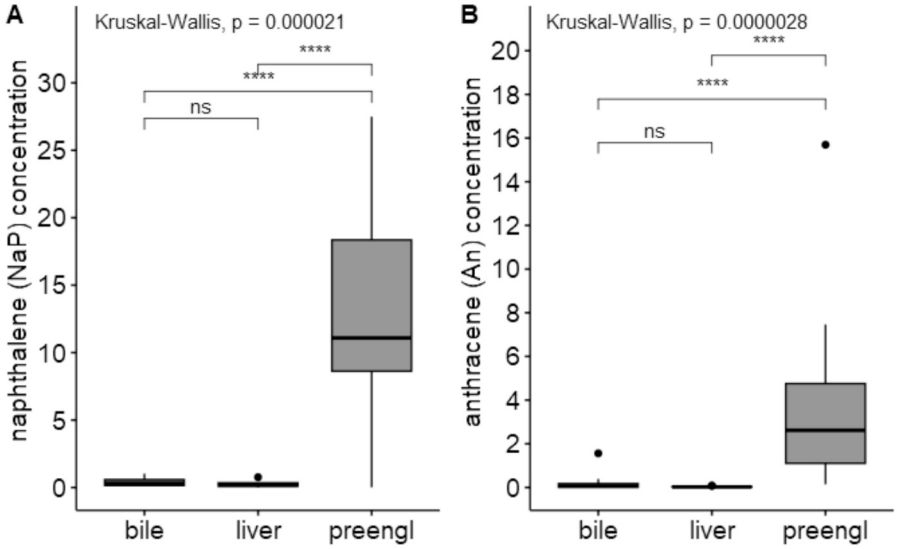


**Fig. 4.** Concentrations of naphthalene (A) and anthracene (B) in bile [ng $mL^{-1}$], liver and preen gland (preengl) [ng $g^{-1}$ww] in common eiders wintering in Denmark. Boxplots show the median (band inside the box), the first (25 %) and third (75 %) quartile (box), the lowest and the highest values within 1.5 interquartile range (whiskers), and outliers (dots). Lines show significant inter-year differences (*** - $p < 0.001$, Wilcoxon pairwise tests with Benjamini & Hochberg correction).

with 5- and 6 rings were mainly detected in the liver.

In the present study, we collected two types of samples – the ones that can be collected non-lethally (i.e., blood, feathers and preen oil) and postmortem (i.e., liver and bile) which are expected to have highest PAH concentration. The results suggest that when analyzing blood or feathers alone, recent PAH exposure was low or compounds were already transformed. Therefore, the utility of those sample types for PAHs exposure assessment may be limited and should be cautiously interpreted, as several factors may have an impact on them. Preen oil offers an interesting alternative for those aiming to avoid bird sacrifice. Previous studies have shown that the concentration of organic compounds, such as organochlorine pesticides or PCBs, may be higher in preen oil

than in liver, feathers or blood (e.g. Solheim et al., 2016; Briels et al., 2019). However, while preen oil was previously used for organic contaminants (see review Pacyna-Kuchta, 2023), its suitability for PAHs exposure assessment remains understudied. We observed several differences between PAHs in preen oil and liver or bile, especially for naphthalene and anthracene.

### 4.3. Comparison to other studies and species

#### 4.3.1. Blood

Recent exposure of living individuals to PAHs can be assessed through blood analysis, using whole blood, plasma or red blood cells alone. Circulating blood and internal tissues can quickly attain equilibrium with each other for many lipophilic POPs, however, this principle may not apply to compounds easily metabolized or eliminated (Bustnes et al., 2001). The concentration of these compounds may be additionally affected by many factors including recent diet, bird condition and remobilization of lipid stores (Bustnes et al., 2017). Furthermore, some compounds are also more sensitive to long-term storage and even storing them in the refrigerator may affect their stability. A detailed examination of the advantages and drawbacks of using blood for organic compounds analysis has been provided in a previous paper (Pacyna-Kuchta, 2023).

In this research, we opted to use whole blood to avoid losing compounds attached to the cellular fraction. However, in most samples only phenanthrene was detected above the detection limit. This suggests that birds were either not recently exposed to PAHs or had already metabolized them.

In the literature, blood is often used for PAH analysis in oiled birds affected by oil spills and/or when recent high exposure to PAHs is expected. In an experimental approach involving seagulls fed with oil supplement, a 30 % increase in PAHs blood concentration has been observed compared to controls, supporting the claim that PAH levels in blood are, to some extent, directly linked to oil ingestion (Pérez et al., 2008). However, heavier PAHs had lower concentrations in blood, suggesting that the metabolism of the compounds is highly dependent on the number of rings or their molecular weight (Pérez et al., 2008). In field research, Paruk et al. (2014) analyzed plasma levels of several PAHs, both parent and alkylated, in common loons (*Gavia immer*) up to two years after the Deepwater Horizon spill. They found relatively low concentrations of 16 parent PAHs, with only anthracene and fluoranthene detected (mean PAHs 4.82 ± 0.36 ng $g^{-1}$ in 2012; 2.03 ± 0.17 ng $g^{-1}$ in 2011). Comparatively, alkylated PAHs were found at much higher concentration (26.9 ± 8.86 ng $g^{-1}$), suggesting that parent compounds could have already been metabolized. In a subsequent study by Paruk et al. (2016) assessing the long-term exposure of common loons to PAHs after the Deepwater Horizon spill, PAH concentrations over five years were analyzed. No consistent pattern in blood PAH concentrations from year to year has been observed. Surprisingly, higher PAH concentrations have been reported in the third year post-spill compared to the year following the spill. It has been attributed to an atypical event, such as Hurricane Isaac, which might have remobilized sediments previously trapped (Paruk et al., 2016). In another study conducted after the Deepwater Horizon oil spill, the authors have reported an increased incidence of PAH detection and higher PAH concentrations in the blood of tundra peregrine falcons (*Falco peregrinus tundrius*) (Seegar et al., 2015). The study on PAH concentrations in shallow sediment, water, fish and seabird samples from Cispata Bay in Colombia, an area susceptible to spills during oil transport at a nearby oil port, has revealed the highest average of 16 PAHs in seabirds blood (66 ng $g^{-1}$), with both low and high molecular weight PAH present in blood samples (Burgos-Núñez et al., 2017).

In studies on adult yellow-legged gulls (*Larus michahellis*) considerably high parent PAH concentrations have been found in the blood cells (Alonso-Alvarez et al., 2007; Pérez et al., 2008). Birds were captured from both unoiled and heavily oiled colonies, 17 months after the Prestige oil spill. Individuals sampled in oiled colonies had approximately two times higher total PAH concentrations compared to birds from unoiled colonies [ΣPAHs range in unoiled colonies: 72–101 ng $g^{-1}$; in oiled colonies: 132–228 ng $g^{-1}$ (Alonso-Alvarez et al., 2007; Pérez et al., 2008)]. Moreover, the colony most heavily affected by the oil spill had the highest ΣPAHs concentrations (Pérez et al., 2008). The PAHs were present in both adults (156.8 ± 27.3 ng $g^{-1}$) and in chicks (167.5 ± 21.9 ng $g^{-1}$), indicating that these pollutants were incorporated from their diet (Alonso-Alvarez et al., 2007).

Those results support the notion that blood can serve as a viable monitoring tool for assessing oil exposure in the cases where high PAH concentrations are expected, such as in areas previously affected by oil spill events. It is worth noting that avian red blood cells have a lifespan of around 30 days, meaning that any PAHs detected in them have been recently mobilized (Pérez et al., 2008). The process of incorporating ingested PAHs into blood cells is complex and may differ between compounds due to differences in metabolism, potentially resulting in an underestimation of exposure to heavier PAHs (Pérez et al., 2008).

Blood analysis may be challenging due to the relatively large volume of blood required to detect PAHs above LOD (Paruk et al., 2014). Typically, about 1 mL of sample is needed for PAHs analysis. However, in the case of lower-weight avian species, collecting this amount from live individuals might pose safety risks for them. Nonetheless, the established guidelines suggest that it is permissible to collect a blood volume equating to up to 1 % of avian body weight without causing harm (Lumeij, 1987). In the case of common eiders, which typically weigh between 2.5 and 3 kg, this translates to a permissible blood draw of 25–30 mL (Garbus et al., 2020). This quantity significantly exceeds the volume required for most diagnostic purposes.

New methods have recently been developed to quantify PAHs in smaller blood volumes. For instance, Morin-Crini et al. (2022) successfully analyzed PAHs in 300 μL of whole blood in the red kite (*Milvus milvus*). In another study, 50 mg of whole freeze-dried blood was used to demonstrate greater flamingo chicks (*Phoenicopterus roseus*) exposure to PAHs (ΣPAHs 31.0 ± 8.9 ng $mL^{-1}$; Dulsat-Masvidal et al., 2023). In the case of heavily oiled birds, even smaller amounts of blood samples have been used, for example, 50 μL in oiled common guillemots (*Uria aalge*) at the rehabilitation center, with a total PAHs concentration ranging from 10 to 184 ng $mL^{-1}$ (Troisi and Borjesson, 2005; Troisi et al., 2007).

#### 4.3.2. Bile

When PAH are transformed in the body, more water-soluble metabolites can accumulate in the bile and urine (Barreto et al., 2020). Although there is limited available data about birds PAH exposure assessment using bile as a biomarker of exposure, this type of sample was previously successfully used to determine PAH metabolites and assess recent PAH exposure in fish (e.g. Kammann et al., 2017; Albergaria-Barbosa et al., 2018). For birds though, collecting bile is more difficult than collecting blood, and requires freshly deceased individuals.

In the present study, 8 out of 15 tested parent PAHs (ΣPAHs 4.87 ng $mL^{-1}$) have been detected in bile. Heavier PAHs with more than four rings were mostly below LOD. Barreto et al. (2020) evaluating the bioavailability of naphthalene, phenanthrene and benzo[*a*]pyrene metabolites for the Magellanic penguin (*Spheniscus magellanicus*) found total concentration in the bile samples ranging from below method quantification limit to 270 μg $g^{-1}$ of bile. The authors detected naphthalene metabolites in 99 % of the samples, phenanthrene in 66 %, benzo[*a*]pyrene was mostly undetected (Barreto et al., 2020). The study on concentration of naphthalene and benzo[*a*]pyrene metabolites in bile of chicks of double crested cormorant (*Phalacrocorax auritus*) and herring gull (*Larus argentatus*) revealed consistently higher naphthalene metabolites concentrations across two study sites and years (Bishop et al., 2016). These studies collectively suggest that heavier PAHs tend to be less prevalent in bile samples, implying potential differences in their bioavailability or metabolism across these bird species. However, this

may not be the case for birds exposed to industrial pollution (King et al., 2014). As data are still very limited, any interpretations should be made with caution, especially in case of sites with more severe pollution sources.

#### 4.3.3. Feathers

Feathers, although not usually used for PAH determination, can serve as a valuable proxy for several POPs (Pacyna-Kuchta, 2023). They offer significant advantages, including their long-term storage potential, even at ambient temperature, as the compounds they stored are not prone to metabolization. Additionally, feathers are also easy to collect non-lethally and provide information about birds' exposure during the time of their formation. As a disadvantage, it is important to point out that feathers are highly susceptible to external contamination on their surface, which can be difficult to remove without affecting internal concentration of pollutants (Pacyna-Kuchta, 2023). Water can effectively remove exogenous contaminants such as dust and other adhering particles, but leaving a preen oil on the surface (Jaspers et al., 2008). The affinity of contaminants for keratin, the main component of feathers, varies among different contaminant groups and in the case of PAHs, this mechanism is not well documented and requires further research. Moreover, the sequestration time may vary, especially between feathers types, depending on the molting patterns of the species. For sea ducks feathers are in constant contact with water, which may also influence the presence of weakly bound compounds.

In this study, most compounds were not detected in primary and body feathers, except for naphthalene and phenanthrene. A higher number of individual compounds were found in tail feathers, with 3-ring compounds predominating the feather profile. While all feather types underwent the same cleaning method, which effectively removed most dust and external contamination, it cannot be excluded that some preen oil remained on the surface of the feathers. Due to the proximity to preen gland, tail feathers may have more preen oil deposited on the surface and thus show higher concentrations of PAHs. However, as the presence of PAHs in this oil indicates an internal source, its presence can be seen as an integrated contribution (Jaspers et al., 2008).

Few relevant studies have investigated PAHs in birds comprehensively. For example, a study examining feathers of wild red-billed choughs (*Pyrrhocorax pyrrhocorax*) from Spain, Canary Islands and Italy for levels of several organic contaminants, including 16 PAHs has found detectable PAH levels only in three subsamples from Italy (ΣPAHs between 39.3 and 54.6 ng $g^{-1}$ ww) (De Sanctis et al., 2013). Birds living in urban areas are often more exposed to PAHs, due to higher traffic-related air pollution (González-Gómez et al., 2020). Consequently, atmospheric exposure becomes an important source of PAHs for urban-dwelling birds (Pei et al., 2017). For example, analyses of several organic pollutants in the body feathers of feral pigeons (*Columba livia domestica*) living in urban areas, have revealed that PAHs were the most prominent group of contaminants, with mean values of 32 ± 15 ng $g^{-1}$; (González-Gómez et al., 2020). Among them pyrene (9.2 ± 4.8 ng $g^{-1}$), fluoranthene (7.1 ± 4.6 ng $g^{-1}$), chrysene (5.6 ± 5.2 ng $g^{-1}$) and benzo [*a*]anthracene were the most prevalent (5.5 ± 2.7 ng $g^{-1}$) (González-Gómez et al., 2020).

Acampora et al. (2017) have reported ΣPAH concentrations in feathers of 30 ± 4.1 ng $g^{-1}$ for common terns (*Sterna hirundo*) breeding in Ireland. The authors have detected twelve PAH congeners, including 5 and 6-ring congeners (Acampora et al., 2017). A similar concentration has been found in the feathers of the European storm petrel (*Hydrobates pelagicus*), with an average PAH content of 38.9 ± 3.59 ng $g^{-1}$ ww, which was the highest among other organic pollutants analyzed. All 15 PAHs analyzed were found in feathers, with the highest mean concentration reported to phenanthrene and pyrene (12.6 ± 9.3 and 9.6 ± 4.9 ng $g^{-1}$, respectively) (Acampora et al., 2018). Zhao et al. (2019) have found several similarities in PAHs composition in body feathers of the little egret (*Egratta garzetta*) chicks, their food and in abiotic soil and water samples. The sum of PAHs in feathers ranged between 25.7 and 70.4 ng $g^{-1}$ dw.

#### 4.3.4. Preen oil

Preen gland oil, especially in seabirds, can be used as a nondestructive biomonitoring tool. It often contains high concentrations of many organic compounds, and has already been successfully used for exposure assessment of PCBs, and organochlorine pesticides (e.g. Wang et al., 2015; Yamashita et al., 2018). Additionally, it has a high lipid content, and may be collected relatively easily compared to other sample types. However, there is limited comparative data available for its use in PAHs analysis. Preen oil usually is not externally contaminated, but the possibility of compound metabolization cannot be excluded. In the present study, the profile was notably dominated by the lightest of analyzed PAHs, naphthalene, which may suggest a petrogenic source of pollutants (Pérez et al., 2008).

In a study by Acampora et al. (2018), no correlation or a weak correlation has been found between preen oil and feathers congener profiles. Interestingly, the total PAHs concentration in preen oil (mean 7.10 ± 1.06 ng $g^{-1}$ ww) has been much lower than in feathers (38.9 ± 3.59 ng $g^{-1}$ ww). Moreover, preen oil has had fewer detected individual congeners, with phenanthrene dominating the profile (mean 4.36 ± 1.47 ng $g^{-1}$ ww). ΣPAH concentration found in preen oil collected from alive common terns (*Sterna hirundo*) was 10.5 ± 0.94 ng $g^{-1}$ ww, with phenanthrene having the highest concentration (3.89 ± 2.45 ng $g^{-1}$ ww). For dead birds, the authors have performed analysis on whole preen gland, detecting a much higher mean ΣPAH concentration (46.7 ± 2.13 ng $g^{-1}$ ww), and phenanthrene concentration (7.59 ± 3.36 ng $g^{-1}$ ww) (Acampora et al., 2017). ΣPAH concentration found in preen gland was higher than the concentration found in liver (27.6 ± 1.92 ng $g^{-1}$ ww). Phenanthrene also had the highest concentration (7.03 ± 8.92 ng $g^{-1}$ ww), but in general, profile differences were observed (Acampora et al., 2017).

#### 4.3.5. Liver

Liver is the most frequently collected organ for parent PAH determination, due to its crucial role in PAH metabolization. In this study, the liver was the only tissue with detectable amounts of 5- and 6-ring PAHs. In general, the concentration of alkylated PAHs in the livers of birds is often higher than parent PAHs (Provencher et al., 2020). Some groups of birds are clearly more exposed to PAHs. Differences among various species from a single region seem to be caused by differences in foraging ecology, or potentially differences in the way of metabolizing these compounds (Provencher et al., 2020).

Troisi et al. (2006) have analyzed 10 parent and metabolite PAHs in the livers of adult common guillemots found stranded after an oil spill. They have found lower concentrations of parent (250 ± 90 ng $g^{-1}$ ww) compared to metabolite (520 ± 140 ng $g^{-1}$ ww) PAHs. PAH composition was characteristic for petrogenic sources, with naphthalene having the highest concentration, accounting for >35 % of the total PAHs concentration; in general dominance of dicyclic and tricyclic PAHs over tetra- and pentacyclic PAHs was observed. In a study on stranded Magellanic penguins' liver samples from Brazil, 5-ring PAHs have dominated the profile. However, it should be noted that many of the analyzed PAHs were detected at very low levels, below the LOQ (Quinete et al., 2020). Higher PAH concentrations have been found in the liver compared to the muscle, and the detectability was also higher in the liver (Quinete et al., 2020).

The hepatic concentration of Σ16PAH in four seabird species breeding in Northern Canada mean ranged between 0.17 and 3.29 ng $g^{-1}$ ww (Provencher et al., 2020). Total concentration of PAHs in livers of coastal and pelagic foragers and waders from New Zealand ranged from 0 to 29.5 ng $g^{-1}$ ww (mean 2.49 ± 3.89), indicating 98 % of collected birds were exposed to PAH (McConnell et al., 2015).

Roscales et al. (2011) have found PAHs in livers of 5 pelagic seabird species, with several differences between PAHs profiles among species. Mean concentrations vary from 1.60 ± 0.82 ng $g^{-1}$ ww in Cory's

shearwater (*Calonectris borealis*) fledglings to 32.5 ± 18.2 ng $g^{-1}$ ww for Bulwer's petrel (*Bulweria bulwerii*) adults.

Relatively high PAH liver concentration (ranging from 110 ± 32.6 to 382 ± 90.1 ng $g^{-1}$) has been reported for seven species of terrestrial birds from India representing various feeding guilds (omnivores, carnivores and frugivores). However, phenanthrene, anthracene, and benzo [*b*]fluoranthene have not been detected in any of the studied species (Dhananjayan and Muralidharan, 2013a).

In a study involving six species of predatory birds, phenanthrene, fluoranthene, and pyrene have been found in the livers of all analyzed individuals (Luzardo et al., 2014). The highest concentration has been found for naphthalene (median 1152 ng $g^{-1}$ ww; min-max 2.2–63,240.8 ng $g^{-1}$ ww), variation has been observed among birds with different feeding pattern, with ΣPAHs higher concentrations found in those that feed on small mammals and reptiles (Luzardo et al., 2014). In the red kite (*Milvus milvus*) the highest PAHs concentrations in the liver have been found for naphthalene and phenanthrene (mean concentration 43.4 and 31.3 ng $g^{-1}$, respectively) (Morin-Crini et al., 2022).

Mean liver PAH concentration (4.90 ± 0.44 ng $g^{-1}$) found in the studied common eiders aligns with findings from most of the studies conducted on various avian species (Fig. 5). PAH concentrations in birds ranged from 0.17 ± 0.55 [black-legged kittiwake *Rissa tridactyla*, $n = 19$ (Provencher et al., 2020)] to 812 ng $g^{-1}$ [white-rumped vulture *Gyps bengalensis*, $n = 15$ |(Dhananjayan and Muralidharan, 2013b)] with most species (21 out of 35) presenting concentrations lower than 50 ng $g^{-1}$. Luzardo et al. (2014) have reported notably elevated concentrations for three species of predatory birds, two owls, i.e., long-eared owl *Asio otus* (3967 ng $g^{-1}$ ww, $n = 14$) and barn owl *Tyto alba* (6481 ng $g^{-1}$ ww, $n = 20$), and common buzzard *Buteo buteo* (53,995 ng $g^{-1}$ ww, $n = 12$). Such a large difference was unexpected, but occurred in birds, which feed mainly on small mammals and reptiles, and the profile was dominated by low-molecular-weight PAHs, especially naphthalene. When LMWs were excluded from the analysis, the results showed smaller differences between species (range from 10.5 to 35.5 ng $g^{-1}$ wet weight (Luzardo et al., 2014).

#### 4.3.6. Eggs

Egg serves as a good matrix for several organic contaminant's detection (Pacyna-Kuchta, 2023). PAHs can be transferred to eggs either as a result of maternal exposure during egg formation or through external contamination after egg laying (Hodson et al., 2020). However, they also may undergo relatively fast biotransformation. An experimental study has shown that PAHs injected into the yolk can be quickly metabolized, with 94 % of them being metabolized within 2 weeks (Näf et al., 1992). After liver samples, eggs are often the main preferable matrix for PAHs analysis in birds worldwide, serving as an important non-lethal means to assess avian contamination (Hebert et al., 2011).

The PAHs concentration in eggs concerns developing embryos because individual PAHs can be highly embryotoxic (Pereira et al., 2009; Bustnes, 2013). It can vary significantly between species. For instance, Pereira et al. (2009) investigating PAHs in the eggs of three species of predatory birds in Britain have found the highest number of PAHs in northern gannets (*Morus bassanus*) eggs, with phenanthrene being the most abundant. Phenanthrene was also a dominant compound found in eggs of four different seabird species from Ireland (Power et al., 2021). The lowest concentration of Σ15PAH has been observed in the common tern (0.8 ng $g^{-1}$), and the highest in the common guillemot (2.08 ng $g^{-1}$) (Power et al., 2021). The study of PAH presence in the common eiders eggs has revealed low but detectable amounts of fluoranthene (0.34 ± 0.084 ng $g^{-1}$ ww), naphthalene (0.89 ± 0.036 ng $g^{-1}$ ww) and phenanthrene (0.49 ± 0.103 ng $g^{-1}$ ww) (Franson et al., 2004). In a multi-year study on the California gull (*Larus californicus*) eggs from Canada, only naphthalene has been detected in samples from 1977 and 2009 (Hebert et al., 2011).

Eggs of three species: common eider, herring gull and European shag (*Phalacrocorax aristotelis)* from Norway were analyzed for a broad cocktail of contaminants, including PAHs (Huber et al., 2015). The analysis has shown the highest ΣPAHs concentration in herring gull eggs

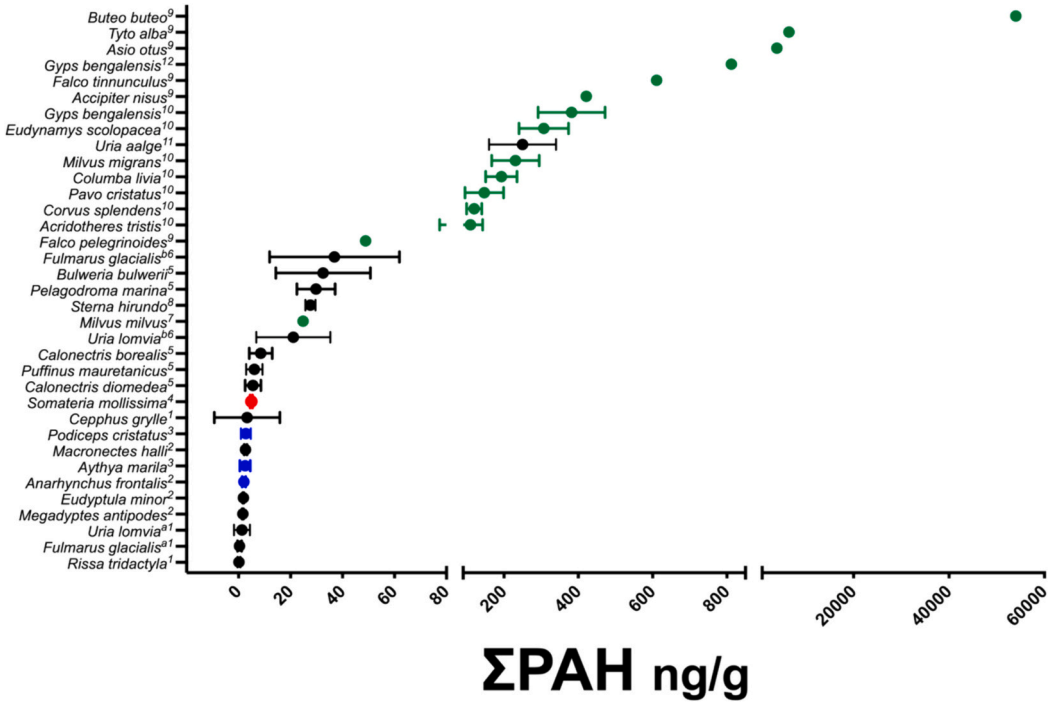


**Fig. 5.** Comparison of mean (bars indicate ± SD) or median ΣPAH concentrations (ng $g^{-1}$ ww) in the liver of selected avian species. [1] Provencher et al., 2020; [2] McConnell et al., 2015; [3] Waszak et al., 2021; Dhananjayan and Muralidharan, 2013a; [4] Present study; [5] Roscales et al., 2011; [6] Provencher et al., 2022; [7] Morin-Crini et al., 2022; [8] Acampora et al., 2017; [9] Luzardo et al., 2014; [10] Dhananjayan and Muralidharan, 2013a; [11] Troisi et al., 2006; [12] Dhananjayan and Muralidharan, 2013b. Black, blue and green circles represent marine, freshwater and terrestrial birds, respectively. The red circle highlights the results of the present study. Included only studies with a minimum sample size of 5 individuals.

sampled (7.63 ng $g^{-1}$ ww), while the concentrations found in eggs collected from common eider and European shag were much lower (ΣPAHs range 0.11–1.02 and < LOD-0.56 ng $g^{-1}$ ww, respectively) (Huber et al., 2015).

Eggs of the Kentish plover (*Charadrius alexandrinus*) breeding along the Iberian Atlantic coast were used to assess birds' exposure after the Prestige oil spill. PAH levels highly varied depending on the year of collection, firstly decreasing from 2004 to 2006 and then increasing in 2007, which coincides with severe forest fires in summer 2006 in the surrounding area. These findings indicate that birds may be affected by different sources of exposure, and eggs could show temporal contaminants variation (Vidal et al., 2011).

#### 4.3.7. Other sample types

A previous study on the Baltic common eiders examining the PAHs distribution in the gallbladder, liver, adipose tissue and eggs has revealed the highest concentration in gallbladder and the lowest in adipose tissue or liver (depending on whether lipid weight or ww was considered) (Broman et al., 1990).

Some studies have investigated the presence of parent PAHs in fat samples from Antarctic seabirds. For instance, Taniguchi et al. (2009) have reported very high concentration of parent PAHs, ranging from 1588 ± 654 ng $g^{-1}$ lipid weight in penguins (*Pygoscelis* spp.) to 5744 ± 2546 ng $g^{-1}$ lipid weight in Antarctic terns (*Sterna vittata*). Meanwhile, Montone et al. (2016) found PAH concentrations in the range of 60 to 239 ng $g^{-1}$ ww in samples from *Pygoscelis* spp. penguins. Both studies have identified the predominance of naphthalene and alkylnaphthalenes in these seabirds, suggesting that their origin is likely local contamination from petroleum derivative Diesel Fuel Arctic, which is used as fuel by research stations and tourism industry. The extent to which compounds reach adipose tissue is highly dependent on their rate of transformation and in the case of PAHs, this may result in smaller amounts ultimately accumulating in fat tissue (Pei et al., 2017).

Another sample type used for PAH determination is avian muscle. The total concentration of PAHs in the muscle tissues of Australian pelican (*Pelecnus conspicillatus*) and silver gull (*Larus novaehollandiae*) were 74.5 and 85 ng $g^{-1}$ ww, respectively. However, small sample size was used with only three samples being analyzed per species (Kayal and Connell, 1995). Nestling tree swallows (*Tachycineta bicolor*) from Canada showed much lower PAHs concentration in muscle (min-max 3.6–8.08 ng $g^{-1}$ ww; Fernie et al., 2018). Similarly low concentration of PAHs with predominance of 3-ring forms was found in greater scaup (*Aythya marila*) (1.5 ± 0.9 ng $g^{-1}$ ww) and great crested grebe (*Podiceps cristatus*) muscle (2.7 ± 1.9 ng $g^{-1}$ ww) sampled in the South coast of the Baltic Sea (Waszak et al., 2021). Muscle of the herring gull from Bohai Bay, North China contained 37.8 ± 12.5 ng $g^{-1}$ ww of PAHs (Wan et al., 2007). High parent PAHs concentration have been found in a muscle of a terrestrial species, the white-backed vulture (*Gyps bengalensis*) with min-max 57–565 ng $g^{-1}$ ww (Dhananjayan and Muralidharan, 2013b).

Respiratory uptake of PAHs can be assessed by lung analysis. For example, Falkowska et al. (2017) have conducted a comparison between alimentary and respiratory PAH exposure in herring gulls reporting similar ∑PAHs concentrations in lungs (26.4 ng $g^{-1}$ dw) and intestines (26.1 ng $g^{-1}$ dw). Interestingly, they have observed higher PAH concentrations in the lungs of adult birds compared to immatures and this appeared to be inversely related to the trophic level occupied by the birds (Falkowska et al., 2017). In the greater scaup the highest parent PAH concentrations has been found in the lungs (2-ring PAHs dominated), followed by the liver and kidneys (mean 6.4 ± 2.1 ng $g^{-1}$ ww; Waszak et al., 2021). In lungs of home pigeons, high concentrations of parent PAHs have been found (234–921 ng $g^{-1}$ lipid weight) (Pei et al., 2017).

Other sample types including feces (Fernie et al., 2018), kidney (Waszak et al., 2021), brain (Dhananjayan and Muralidharan, 2013b) or eggshells (Zhao et al., 2019) are used relatively rarely in PAH analysis. Despite this, high correlations between PAH concentrations in nestling tree swallows feces and muscle have been found, supporting the potential use of feces as a non-destructive tool for PAHs exposure (Fernie et al., 2018).

## 5. Conclusions

The present study contributes to a better understanding of using various avian samples in PAH studies by comparing PAH exposure across organs and tissues of common eiders, offering insights into their health status compared to other avian species worldwide. Due to the metabolizing capacity of the organisms for PAHs, estimating of PAH exposure on the basis of the presence of parent compounds in tissues/biomaterials is challenging. Most studies have focused on a single sample type which, while relevant, provides a limited overview of the PAH distribution. Literature data consistently shows the complexity of PAH exposure pathways and the importance of considering multiple factors in the assessment process. Our results contributes to understanding on the distribution patterns, aligning with existing literature to investigate the possibility to use non-lethally collected samples for PAH avian monitoring. Significant differences in concentrations were observed between bile, preen gland and liver, while neither blood nor feathers showed detectable levels of parent PAHs. This highlights that the use of various tissues needs to be carefully selected when using common eider for biomonitoring of the marine environment.

## CRediT authorship contribution statement

**Aneta Dorota Pacyna-Kuchta:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Juliana Souza-Kasprzyk:** Writing – review & editing, Writing – original draft, Visualization. **Svend Erik Garbus:** Writing – original draft, Formal analysis, Data curation. **Igor Eulaers:** Conceptualization, Resources, Project administration, Writing – review & editing. **Christian Sonne:** Resources, Project administration, Writing – review & editing. **Dariusz Jakubas:** Methodology, Formal analysis, Writing – original draft, Writing – review & editing.

## 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.

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.173801.

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