

Is minimally-invasive sampling the future of persistent organic pollutant (POP) research in birds? A meta-analysis on tissue comparisons

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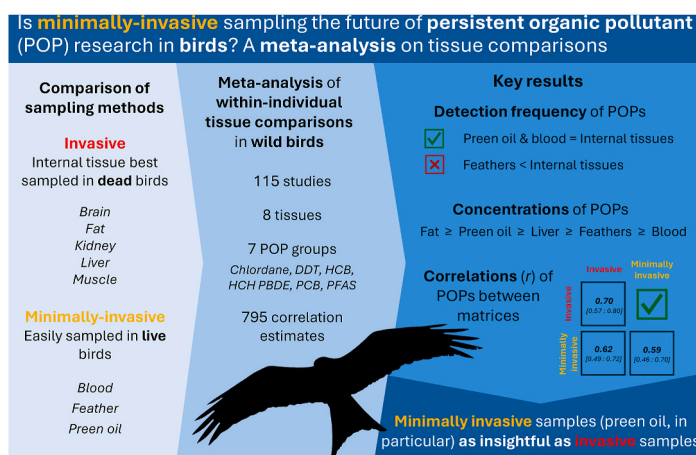
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HIGHLIGHTS

- Compare blood, feather and preen oil against invasive sampling matrices.
- No differences between invasive and minimally-invasive sampling correlations.
- Low concentrations in blood but detection frequency similar to liver & kidney.
- Feathers demonstrated lower detection frequency than all other matrices.
- Preen oil concentration lower than fat but detection frequency is similar.

GRAPHICAL ABSTRACT



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ABSTRACT

Persistent organic pollutants (POPs) bioaccumulate in the food chain and can cause ecotoxicity. In wild bird populations, various tissues are used to determine POP levels, including invasive (e.g., brain, fat, kidney, liver, muscle) and minimally-invasive tissues (e.g., blood, feather, preen oil). Minimally-invasive sampling, which does not require the death of the animal, opens new prospects for sampling birds as sentinels of environmental pollution and its consequences on fitness. However, POP variability between tissues is understudied, which is an essential prerequisite for making a reasoned choice about which tissues to sample. Here, we performed a meta-analysis of eight tissues across 115 studies comparing tissues across POP groups. We demonstrate increased use of minimally-invasive measures between 1974 and 2020. When grouping tissue correlations into three groups, “invasive:invasive”, “invasive:minimally-invasive” and “minimally-invasive:minimally-invasive”, we found that all three groups produced moderate to strong positive correlations with no difference seen between comparison groups. We demonstrate (1) lower POP concentrations in preen oil than fat, but no difference in detection frequencies, supporting preen oil use; (2) blood showed high concentration variability dependent on POP group but detection frequencies were comparable to liver and kidney; and (3) feathers demonstrated a significantly lower

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detection frequency than other matrices measured. By further researching minimally-invasive tissues, we increase our understanding of whether minimally-invasive tissues are ecologically representative of body-level toxicity. Our study supports blood and preen oil as substitutes for invasive measures when sampling living bird populations as they represent internal POP concentrations and provide significant benefits both practically and ethically.

1. Introduction

Persistent organic pollutants (POPs) are man-made chemicals that are persistent and resistant to degradation (Cousins et al., 2019; Rüdel et al., 2020). Although the international community has called for actions to reduce and eliminate the production, use and release of these chemicals, with many of those chemicals already banned from the 1970s, these contaminants are still globally distributed and can be toxic to humans (Alderete et al., 2019; Lin et al., 2019) as well as terrestrial (e.g., birds (McCarty and Secord, 1999) and marine wildlife (Madgett et al., 2022)). Indeed, above a certain concentration threshold in an organism, POPs have numerous adverse effects on the health of organisms (e.g. immunotoxicity (Desforges et al., 2016; Weisglas-Kuperus et al., 2000), endocrine disruption (Han et al., 2019; Monclús et al., 2018) and deregulation of the oxidative balance (Kumar et al., 2014; Lee et al., 2021), leading to changes in reproductive success and survival. Due to their lipophilic properties, POPs are stored in fat and thus bioaccumulate up the food chain during the lifetime of an individual. Bird species are useful in analysing POPs in the wild as they (1) are found worldwide; (2) have a high prevalence in most habitats; (3) are sensitive to environmental change; (4) allow for both within and between population comparison (e.g., high and low POP exposure, migratory/non-migratory or breeding/non-breeding); and (6) we can measure reproductive success as nests are typically easily accessible in many bird species. Thus, bird species can serve as a “sentinel” to reflect the ecosystem’s health and serve as risk indices for POP pollution (Carere et al., 2010; Champoux et al., 2002; Pakzad Toocheaei et al., 2019).

Research into wild bird populations has changed over the years with an increase in animal welfare interests (Marchant-Forde, 2015). In POP research, samples of the bird can either require invasive sampling (such as fat, muscle, and organs), which are usually taken from animals found dead or euthanised for sampling purposes. In contrast, minimally-invasive sampling (such as blood, feathers and preen oil) can be taken in the wild with little detriment to the animal’s welfare. In this paper, we termed this “invasive” and “minimally-invasive” respectively. Instead of harvesting wild animals for invasive POP measures, researchers may be moving towards the use of minimally-invasive measures, not only to improve animal welfare but to allow the sampling of long-term study populations and endangered species (Dauwe et al., 2005; González-Gómez et al., 2020). However, the impact of tissue choice on our ability to measure and assess POPs in wild bird populations is currently unclear. We, therefore, must first understand how each tissue differs in both the exposure and retention of POPs residing within these tissues.

Comparing avian tissues for their usefulness in the environmental monitoring of POPs is complex due to the interaction between differences in POP characteristics and tissue physiology (Wan et al., 2006; Zheng et al., 2018). Differences in POP characteristics can be seen by comparing concentrations across different POPs within the same tissue. Within a tissue, the sorption of newly ingested POPs and the retention of both new and previously retained POPs, is largely dictated by the kinetics and equilibrium of the sorption/desorption of these compounds (Kubota et al., 2004). The concentration of different POPs within the same tissue is often summarised by using both the octanol-water partition coefficient (i.e., K_{ow}) and the lipid content of that tissue. Combining both gives us an understanding of the bioaccumulation ability of that specific tissue for that particular POP. Alternatively, if we compare the difference between tissues from the same POP, both tissue

physiology and individual state (e.g. starving, migrating, reproducing) of the sampled birds must be considered. For example, blood is a common choice of tissue as a representation of the most recent POP exposure, but the state of the individual can confound this through their fat metabolism (Bustnes et al., 2012). As POPs are fat soluble, they are stored within the animal’s fat reserves. When fat is metabolised, for example during periods of low food availability, these POPs are then remobilised in the blood, introducing a temporal confound if a blood sample is taken. The movement of POPs between fat and blood also varies depending on the POP in question (Polischuk et al., 2002; Bigsby et al., 1997). In addition, a target tissue should be ecologically relevant for assessing POPs and the POPs concentration should be above the limit of detection (LOD). As such, a target tissue should be assessed for suitability by comparing its detection frequency (DF) against those of other tissue choices. To evaluate systemic toxicity caused by POPs, we need a target tissue that is representative of the true pollution burden and not a tissue that retains very low levels of POPs (Kocagöz et al., 2014). Therefore, not considering the individual state of each bird and the tissue physiology of the sample can result in inconsistencies when correlating tissue concentrations together and can largely impact our ability to look at the impacts of POPs on population-level health.

Here, we performed a meta-analysis of previously published studies that measured POPs in two or more tissues of wild bird species. We collected data from publications with POP tissue comparisons at both a population-level (e.g., means and SD) and an individual-level (e.g. correlations) from individually and summed POP concentrations; 1) we explored the gathered dataset by looking at the change in publication year against tissue type to see if this dataset mirrors the increase in minimally-invasive measures over time; 2) we looked at the percentage DFs, where available, to research the probabilities of discovering POPs within each tissue; 3) we collected population-level POP comparisons to research how concentration information differ across tissues and POP groups and; 4) we collected individual-level tissue comparisons and investigated how POP concentrations correlate across tissues of the same individual. We expected to see the following: 1) a gradual shift over the years towards greater use of minimally invasive tissues in publication; 2) DFs to be higher for tissues of higher lipid content, i.e. fat and preen oil to yield higher DFs than the other included tissues; 3) lowest level DFs to be expected in feathers due to their limited time to build POP stores whilst connected to the bloodstream during growth; 4) and that individual-level measures should broadly correlate between different tissues.

2. Methods

2.1. Literature search and data compilation

Peer-reviewed publications were systematically searched in July 2020 using the online database Scopus (<http://www.scopus.com>). Study title, abstract and keywords were searched (Scopus field ‘TITLE-ABS-KEY’) using terms for POPs, birds, and wild populations (Figure A.1). We also conducted a forward search within Scopus, which yielded a large number of studies (~15,000 papers). To help increase our efficiency, we reduced this using the calculated list of most common keywords produced by Scopus: ‘aves’, ‘bird’, ‘birds’, ‘seabird’, ‘and ‘raptor’.

The title and abstract of identified publications were screened using seven criteria: (1) measure a POP accepted within the Stockholm Convention (<http://www.pops.int/>); (2) measure a wild bird

population, all bird species were accepted; (3) be an observational study, studies with experimental manipulation were excluded; (4) sample more than one tissue; (5) give two or more within-individual tissue measures, measures from tissues taken from different sampling groups were not considered; (6) useable statistics or the data available for further analysis; and (7) the full article was available in English. Our initial search yielded ~2900 papers, and, coupled with our subsequent forward search and search criteria, we retained 183 studies (Figure A.2).

2.2. Tissue and POP refinement

We refined our tissue comparison and POP selection from the collection of studies chosen dependent on data availability. In total, 27 tissues and 23 POP groups were identified. Of these, we retained eight tissues and seven POP groups which contained enough studies reporting comparisons between invasive and minimally-invasive matrices to allow for subsequent comparisons (Figure A.4). The eight selected matrices included five invasive (brain, fat, kidney, liver, and muscle) and three minimally-invasive matrices (blood, feather and preen oil; Figures A.3–A.4). The seven POP groups consisted of chlordane, dichlorodiphenyltrichloroethane (DDT), hexachlorobenzene (HCB), hexachlorocyclohexane (HCH), polybrominated diphenyl ether (PBDE), polychlorinated biphenyls (PCB) and per- and polyfluoroalkyl substances (PFAS) (Table A.1; Figure A.4). After tissue and POP refinement, we had a pool of 115 studies for our analyses (Appendix B).

2.3. Data extraction and treatment

We extracted the following data from our pool of 115 studies when available: detection frequency (DF); population-level measures (e.g. mean and standard deviation/standard error (SD/SE)), and individual-level measures (e.g. correlation). We used raw data to calculate estimates when required/available. Web Plot Digitiser was used to extract data from figures (Rohatgi, 2019). Effect sizes (ES) were extracted at the individual POP level for all measures (e.g. PCB-62, pp-DDE). Summed POP data was excluded for detection frequency and population-level measures as these measures are skewed depending on the number of individual POPs included in the group ($n = 6$ and 19 studies excluded respectively). When studies reported effect sizes for different groups (including age group, location, sex, species, and year), the effect sizes were not combined as POP exposure cannot be assumed to be consistent between groups. Age was grouped into nestlings (pre-fledging), juveniles (post-fledging but not of reproducing age), and adults (assumed to be fully mature) when necessary.

Population-level measures were converted into nanogram per gram on a wet weight basis (ng/g ww) except for POP concentrations in blood (ng/ml). When only median and/or range were given for a population level measure, mean and standard deviations (SD) were estimated using methods provided by Wan et al. (2014). Conversions from lipid or dry weight to wet weight basis require tissue lipid or water tissue percentages, respectively (U.S. EPA, 2011). Tissue lipid percentages were extracted from studies when available, and an average was taken for each tissue (Table A.2). Water percentages of the tissues were provided in too few studies to be sufficient for the calculation. Thus, additional studies were located to calculate an average water percentage per tissue (Table A.2).

2.4. Statistical analysis

All analyses were conducted in R version 4.0.4. (R Core Team, 2019). Data format, model, study number and outcome number included in each analysis can be found in Table A.3. Further information regarding study and outcome numbers can be found in Table A.4 – A.6.

2.4.1. Detection frequency (DF)

DFs for summed POPs were excluded from the analysis as DF is

biased towards POP groups dependent on the number of individual POPs ($n = 6$ studies excluded). Binomial logistic regression by Generalised Linear Mixed Model (lme4 package; Bates et al., 2015) was used to investigate how tissue POP DF varies. Tissue and POP group were included as fixed effects and POP as a random effect. False discovery rate (FDR) post-hoc comparisons were carried out to extract tissue estimations (predicted mean and 95% confidence intervals (95%CI)).

2.4.2. Network meta-analyses

We performed all network meta-analyses with multi-level random effects (MLRE) models using restricted maximum likelihood estimation in the function *rma.mv* (metafor package; Viechtbauer, 2010) unless otherwise stated.

2.4.2.1. POP concentrations. Log transformed means of POP concentrations were calculated using the *escalc* function (metafor package; Viechtbauer, 2010). An interaction between tissue and POP group was included as a moderator in the MLRE model to account for differences in POP characteristics between POP groups. Study was included as a random term. Predicted values and standard error (SE) were calculated for each tissue and POP group using the *predict.rma* function (metafor package; Viechtbauer, 2010).

2.4.2.2. Tissue correlations. Fisher's Z correlation coefficients were calculated using the *escalc* function (metafor package; Viechtbauer, 2010). A tissue matrix was included as moderators in the model. The POP group was nested inside the study and used as a random effect. For each study and POP group, individual POPs were prioritised, with summed POP measures only kept if individual POP measures were not available. We ran the individual-level network meta-analysis with and without summed POP correlations. The model included summarised POP correlations as they did not change the conclusions drawn.

We ran two additional MLRE models using Fisher's Z correlation coefficients to compare between; (1) invasive and minimally-invasive tissue choice, and (2) life stages. First, we compared invasive (brain, fat, kidney, liver and muscle) and minimally-invasive measures (blood, feather and preen oil) by categorising tissue correlations into invasive versus invasive (e.g., liver vs muscle), invasive versus minimally-invasive (e.g., fat vs preen oil) and minimally-invasive versus minimally-invasive comparisons (e.g., blood vs feathers) and including this as a moderator in the model. The POP group was nested inside the study and used as a random effect. Second, we compared grouped life stages: nestling, juvenile and adult. In this, we included life stage as a moderator and tissue comparison and study as random effects.

2.4.2.3. Individual POP estimations. We calculated an estimated concentration for each POP for each tissue. We ran a model of single means for each tissue-POP group combination using the *metamean* function (meta package; Balduzzi and Schwarzer, 2019). The study was included as a random effect and individual POP was included as a subgroup. Only identified POP concentrations were included in the model (i.e. zeros were excluded).

2.4.3. Publication bias

We assessed publication bias using Egger's regression test. We modified the models to include the sampling variance or the inverse sample size as a moderator when analysing log transformed means or Fisher's Z correlation coefficients respectively. Sampling variance was not used in the Egger's regression test when using correlations due to the inherent correlation between correlations and their sampling variance.

The sensitivity of the meta-analytic models was assessed by running MLRE models with and without values of high influence. This allows us to assess whether the conclusions drawn are dependent on values of high influence. Highly influential values were identified as effect sizes with hat values two times greater than the average hat value and/or

studentised residuals that exceed the 95% confidence interval.

3. Results

3.1. Datasets and diagnostics

There were 115 peer-reviewed studies included in our dataset, published between 1974 and 2020. More studies reported population-level (72%, $n = 83$ studies) than individual-level measures (37%, $n = 43$ studies). We collated a total of 5437 effect sizes across five datasets (Table A.3), including individual POP estimations (Tables A.4–A.5).

We identified publication bias in all meta-analytic models using correlations, demonstrated using the Egger's regression test (Tissue correlations: $P < 0.001$, Invasive versus minimally-invasive: $P = 0.002$, Age group: $P = 0.017$). The sensitivity of meta-analytic studies identified high influence values in all MLRE models (Invasive versus minimally-invasive: $n = 98$, Age group: $n = 97$, Tissue correlations: $n = 97$, POP concentration: $n = 696$). All MLRE models were re-analysed without the high influence values to demonstrate the reliability of the conclusions drawn (Fig. 4b, 5b, 6b, A.5). Throughout the results, any variability in conclusions drawn, outlined by the model sensitivity analysis, was identified.

3.2. Change in tissue comparisons in birds over time

Minimally-invasive tissues had a later publication date than invasive measures (Wilcoxon test; $W = 6346$, $P < 0.001$, $n = 115$ studies). All three minimally-invasive tissues had later average publication years than our invasive measures (minimally-invasive tissues: blood 2009, feather 2009, preen oil 2011; invasive tissues: brain 1999, fat 2001, kidney 2002, liver 2004 and muscle 2003; Fig. 1).

3.3. Tissue comparisons

3.3.1. Detection frequency

Detection frequency (DF) was reported in 50% of studies ($n = 57$ of 115 studies). In total, 51 studies reported DFs for individual POPs, with DF sample sizes ranging from 4 to 38 studies (Fig. 2). The DF of fat and preen oil were the highest out of all tissues (DF = 0.95 and 0.93, respectively). Although fat and preen oil had a noticeable difference in lipid content (fat ~70% and preen oil ~55%; Fig. 2), the DF did not significantly differ between these tissues (Fig. 2). Blood, kidney, and liver DF did not differ significantly (DF = 0.89, 0.88 and 0.87, respectively). Feathers had a significantly lower DF than all other tissues (DF = 0.66). However, feather lipid content was not noticeably different from other tissues (Figure A.6). Interestingly, feathers also had the largest variability of all tissues (SE: feather 0.109, muscle 0.076, brain 0.072; Fig. 2).

3.3.2. POP concentration

POP concentrations of individual POPs were reported in 72% of studies ($n = 83$ of 115 studies). POP concentrations included 98 bird species across 16 orders. Predicted POP concentrations were highest in fat and second-highest in preen oil for all POP groups except PFAS (Fig. 3). PFAS showed little difference between tissues, which could be explained by lack of power as PFAS had the fewest studies reporting population-level measures (Table A.6). Alternatively, PFAS congeners have been demonstrated to bind to blood proteins, unlike other POPs in this study which bind to lipid-rich tissues, which may explain the overall lower concentrations in fat and preen oil (Bossi et al., 2005; Jones et al., 2003). Blood and feather were highly varied across POP groups. Individual POP concentrations by tissue are provided in Appendix A (Figure A.7 – A.13).

3.3.3. Tissue correlations

Individual-level measures were reported in 37% of studies ($n = 43$ of

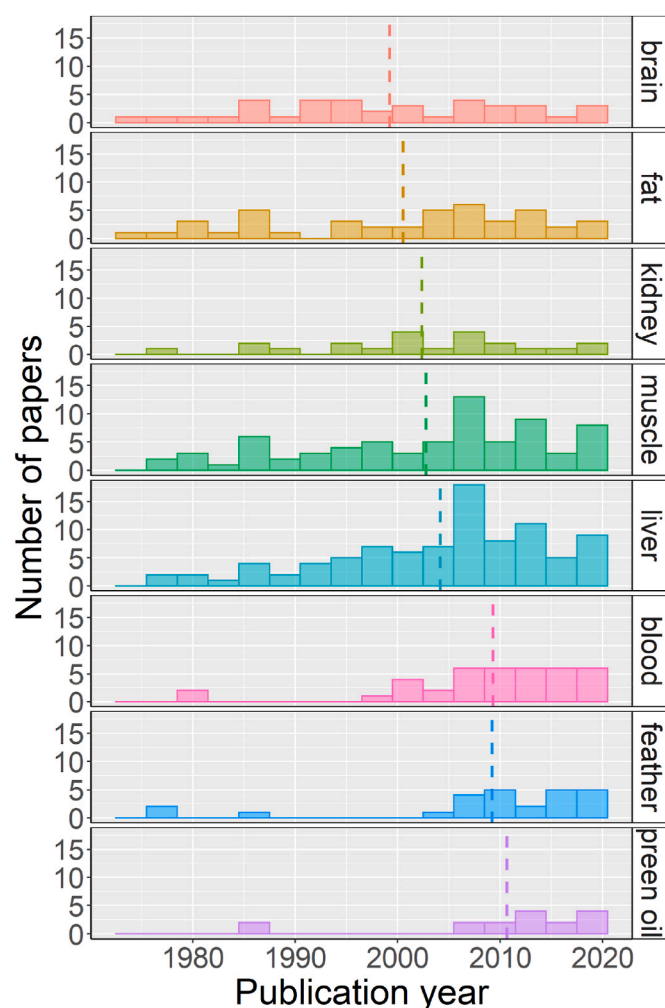


Fig. 1. Changes in tissue choice by publication year for POP analysis in wild bird populations. Histogram of publication year for our meta-analytic study pool. Vertical dashed lines denote average publication year for each tissue. Y-axis shows the number of papers published.

115 studies). Individual-level measures included 39 bird species across 11 orders. The number of estimates for a given pairwise tissue correlation was low with an average of 28 estimates and three studies per comparison (Fig. 4a). There was a maximum of 40 estimates and 11 studies found in the liver-muscle comparison. Five tissue comparisons are missing from our dataset (brain-feather, brain-preen oil, fat-preen oil, feather-kidney, kidney-preen oil). Thus, the predicted values for these comparisons were removed.

All the predicted correlations for tissue comparisons that are significant (i.e. do not cross zero) are positively correlated (Fig. 4a). The strongest significant correlation is fat and muscle ($r = 0.329$). All significant correlations were weakly positively correlated, with 65% of studies reporting correlations in adults ($n = 28$ studies; nestling: 23%, $n = 10$ studies; juvenile: 23%, $n = 10$ studies).

When controlling for model sensitivity, the majority of tissue correlations are strengthened, with the highest significant correlation found between blood and preen oil ($r = 0.802$; Fig. 4b). Six non-significant tissue correlations become a significant positive correlation when controlling for model sensitivity: (1) blood and fat ($r = 0.467$); (2) blood and liver ($r = 0.661$), (3) brain and liver ($r = 0.593$); (4) brain and muscle ($r = 0.758$); (5) fat and liver ($r = 0.281$) and; (6) feather and liver ($r = 0.267$). The correlation between fat and feather becomes non-significant when controlling for model sensitivity ($r = 0.015$). The correlation between blood and brain, and all tissue correlations involving kidney were

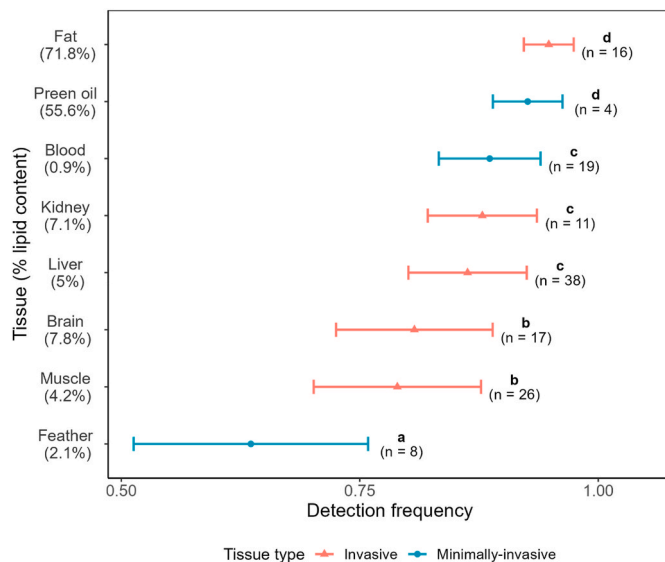


Fig. 2. Effect of tissue choice on detection frequency in wild bird populations. Detection frequency ($\pm$ SE) expressed as probability of detection for each tissue. Letters denote significance based on post-hoc comparisons. 'n' provides the number of studies with available DF. Average lipid content for each tissue is denoted as a percentage on the y axis (See Table A.2 for papers included in average lipid calculation).

removed when controlling for model sensitivity (Fig. 4b).

Tissue correlations compared between age groups (adult, juvenile and nestling) found that tissue correlations in juveniles are significantly higher than in adults (Fig. 5a). However, this result might be sensitive to publication bias as, although the trend remains when controlling for model sensitivity, the difference between groups becomes non-significant (Fig. 5b).

Correlation coefficients were compared between three groups: invasive versus invasive, invasive versus minimally-invasive, and minimally-invasive versus minimally-invasive. Overall correlation coefficients demonstrated moderate to strong positive correlations in all three groups and no significant differences were found between groups in pairwise comparisons (Fig. 6a). Although not significant, the results may suggest a stronger positive correlation between invasive tissues compared to other groups. However, when controlling for model sensitivity, this non-significant trend was removed (Fig. 6b).

4. Discussion

The current study provides a quantitative overview of POP distributions in both invasive and minimally-invasive tissues in wild bird populations based on the analysis of 115 studies in which more than one tissue was measured. The objective of this study was to better understand the extent to which POP measurements are comparable between different tissues and to examine the usefulness of minimally-invasive matrices in monitoring wild bird populations. In this meta-analysis, we focused on blood, feathers and preen oil as they were the most frequently reported minimally-invasive measures that allow for within-individual tissue comparisons. Other minimally-invasive measures include matrices that are not direct measures of internal POP concentrations (i.e. crop contents, regurgitated pellets and excrement), do not allow for within-individual tissue comparisons (i.e. added or unhatched eggs) or have little supporting literature (i.e. claw trimmings) (Espín et al., 2016; Pacyna-Kuchta, 2023). Consistent with the current push to minimise the impact of scientific procedures on animal welfare, the analysis of publication year shows a change in the choice of matrices when studying POPs, with more frequent use of minimally-invasive measures nowadays compared to the 80s and 90s. The analysis of DF

levels and POP concentrations in different tissues showed that; (1) fat and preen oil had the highest overall DF level even though concentrations (wet weight) of preen oil were consistently lower than fat in all POP groups; (2) blood, liver and kidney had equivalent DF levels, but when blood and liver concentrations are visually different for a POP group, blood concentrations are always lower than liver; and (3) feather had the lowest DF levels of all matrices included in this meta-analysis. Although few studies report correlations between tissues (37%; $n = 115$ studies), our meta-analysis showed positive correlations between different tissues. When grouping tissue correlations into three groups, "invasive and invasive", "invasive and minimally-invasive" and "minimally-invasive and minimally-invasive", all three groups produced moderate to strong positive correlations with no differences seen between comparison groups. Our study supports the use of blood and preen oil as substitutes for invasive measures when sampling living bird populations as they represent internal POP concentrations and provide significant benefits both practically and ethically.

4.1. Detection frequency (DF) and the water/fat content of tissues in POP studies

One of this study's aims was to identify gaps in data collection and reporting. The studies we collected on tissue comparisons for POP research in wild bird populations were extensive but likely incomplete. However, clear trends across our study pool allowed us to reflect and make recommendations for future research in the field. First, DF is invaluable when comparing tissues at a within-individual level. Within-individual tissue comparisons control for the confounding effect of varying POP exposure. Therefore, the strength of each tissue as a measure of contaminant level can be assessed by using DFs, as differences in DF between tissues can only be caused by internal differences in tissue physiology when sampled at a within-individual level. A tissue with a higher DF than other tissues encapsulates individuals with lower levels of POP exposure. Thus, choosing a tissue with a higher DF level, such as fat, preen oil, or blood (versus feathers with low DF) increases the ecological relevance of measured POP concentrations and also increases the power of any subsequent analysis. DF was reported in only half the studies in our study pool (50%; 57 studies), but we recommend that DF is reported in all studies moving forward. Second, to compare studies effectively, we need to be able to convert population-level measures (i.e. mean and SE) between dry, lipid and wet weight. Lipid and water content of tissues were underreported in our study pool (lipid: 27%; 31 studies; water: 3%; 3 studies), but both measures are essential for these conversions. We are aware that low sample quantity can be a limitation when working with minimally-invasive matrices, however we recommend that lipid and water content be reported in all future POP studies when possible. Increased reporting of tissue lipid and water content would allow future meta-analytic studies to convert POP concentrations using lipid and water content measures taken directly from the study in question when necessary.

4.2. Internal POP concentrations using minimally-invasive methods

Minimally-invasive measures have significant benefits when sampling living birds including (1) the opportunity to increase statistical power by sampling a larger number of individuals compared to invasive sampling, which requires culling or surgery; (2) repeated sampling of the same individuals to gain new insights on dynamics and bio-accumulation of POPs in the body; (3) easier investigation on the links between exposure to contaminants and fitness (reproduction and survival) since invasive approaches are likely to have consequences on fitness and thus introduce biases; (4) the opportunity for a more exhaustive sampling design compared to the sampling biases introduced when sampling already dead individuals; (5) sampling of endangered species and long-term study populations; and more generally (6) improving animal welfare and ethical practices (Cobb et al., 2003;

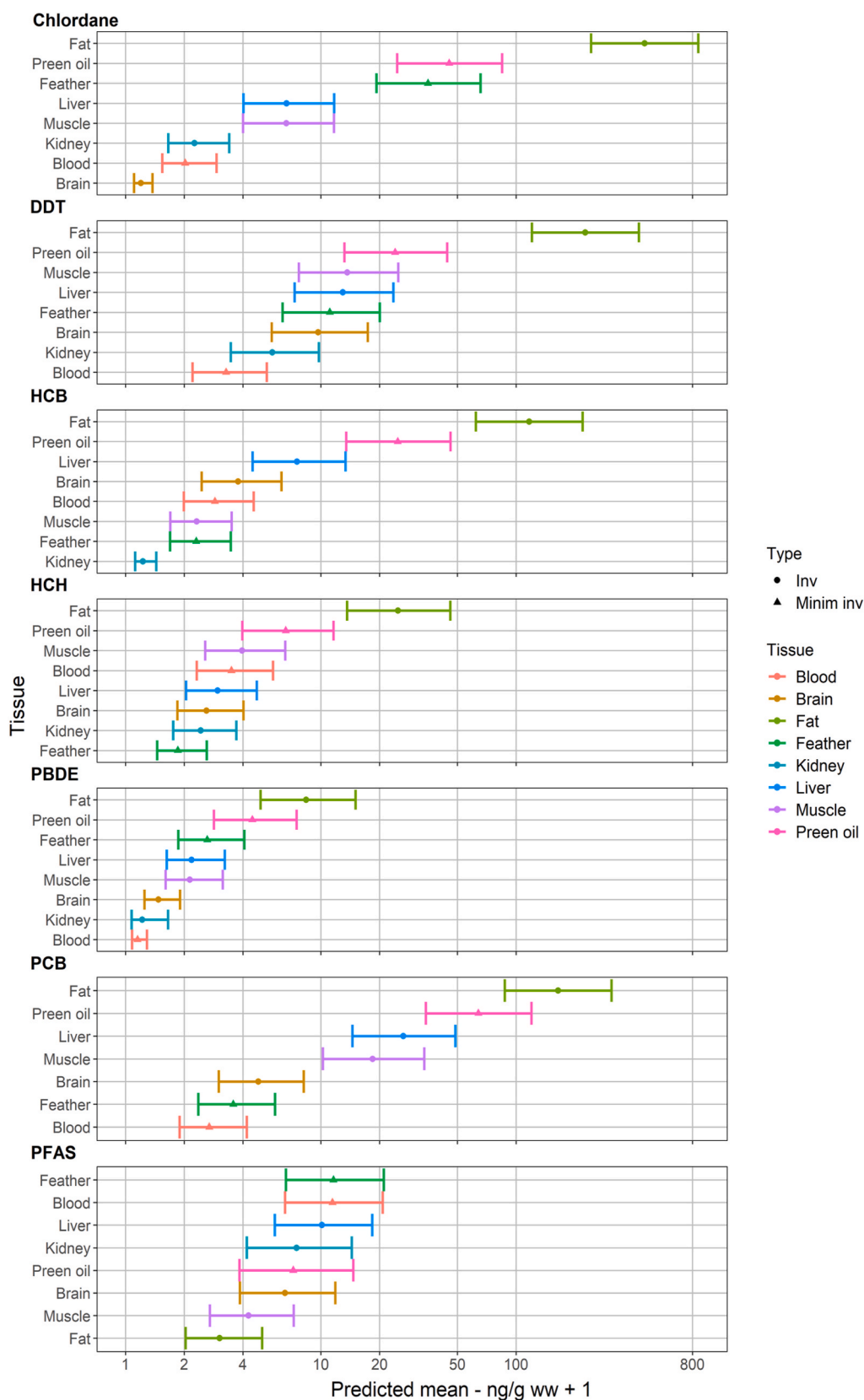


Fig. 3. Effect of tissue choice on POP concentrations in wild bird populations. POP concentrations ($\pm 95\%$ CI, ng/g ww + 1) predicted by the population-level network meta-analysis for each tissue, broken down by POP group; (a) Chlordane, (b) DDT, (c) HCB, (d) HCH, (e) PBDE, (f) PCB, and (g) PFAS. Invasive and minimally-invasive tissues are denoted with filled circles and triangles, respectively. + 1 was added to predicted measures for a log transformation. Alternative graph with results grouped by tissue is available (Figure A.14).

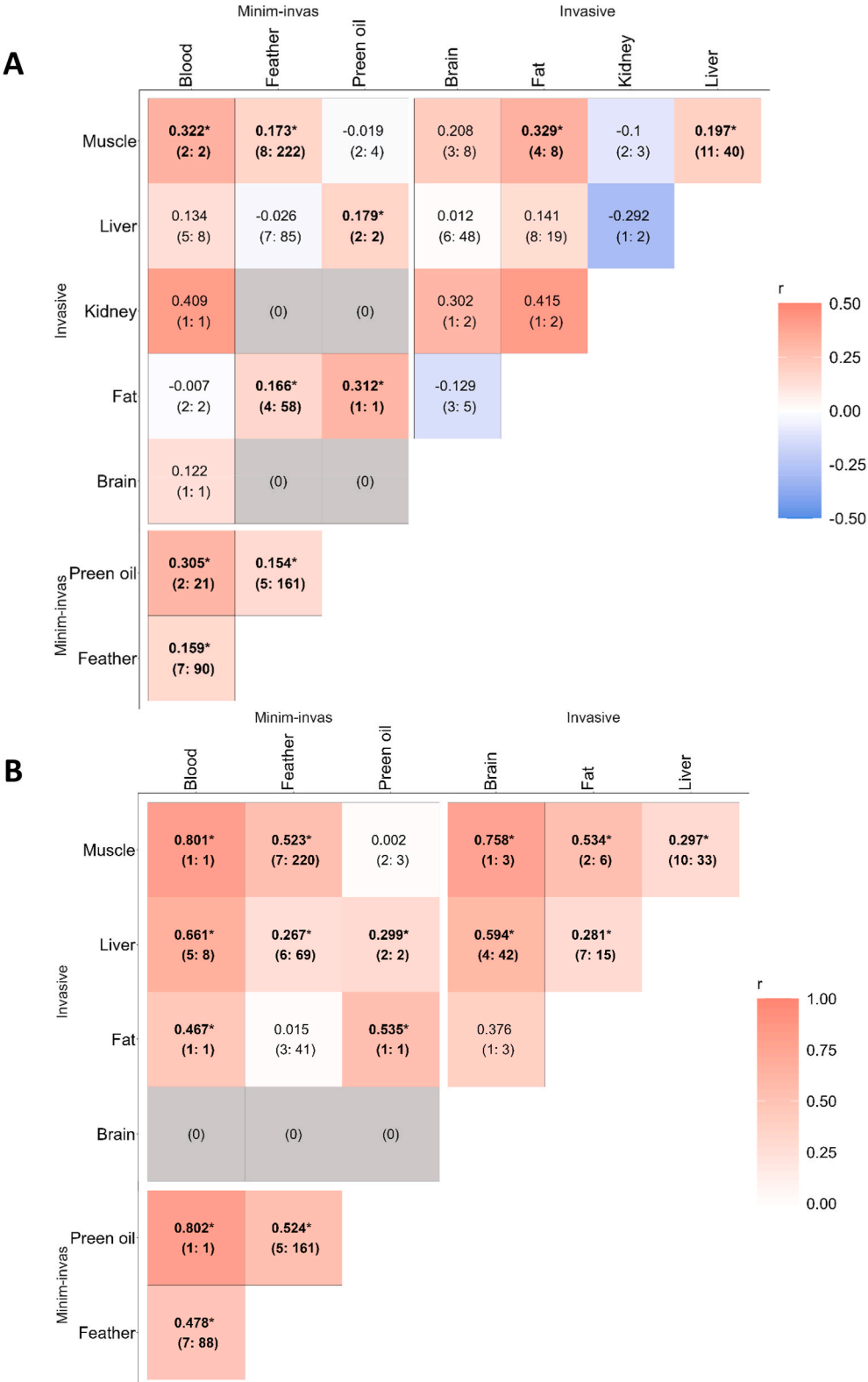


Fig. 4. Tissue comparison correlations for POP concentrations in wild bird populations, (A) before and (B) after controlling for model sensitivity. Heatmap of tissue correlation predictions (r) from the individual-level network meta-analysis, clustered by invasive and minimally-invasive measures. Predicted correlations are given and significance (i.e. if the predicted value did not cross 0) is denoted with an asterisk (*). Number of studies: number of effect sizes included in the model is given in parenthesis.

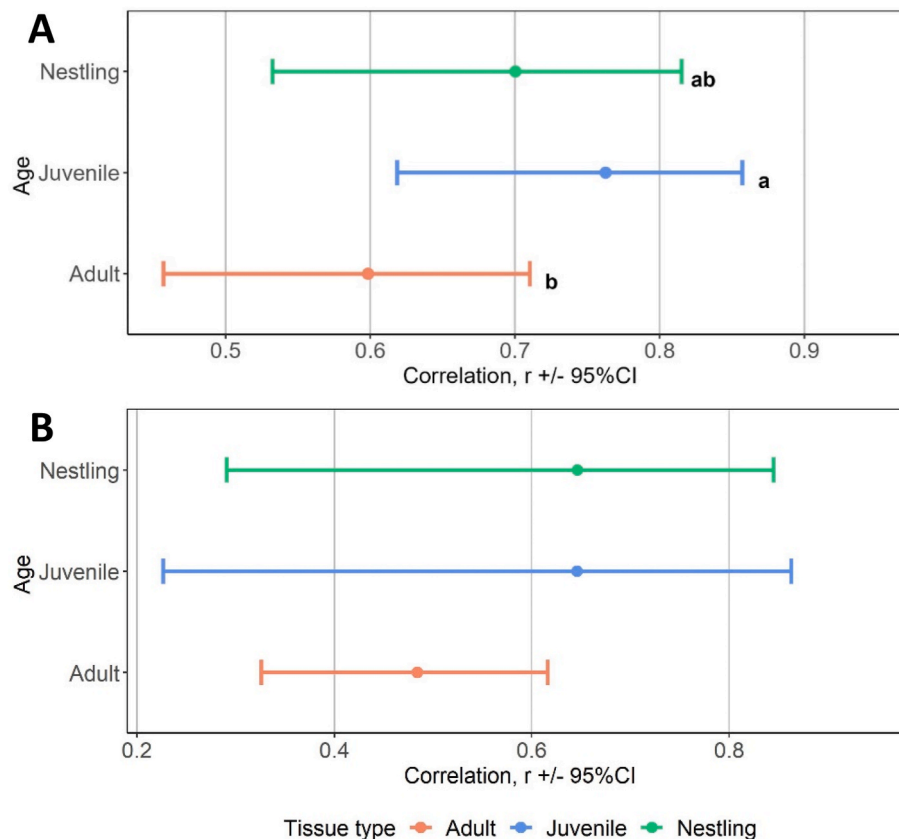


Fig. 5. Effect of age group on tissue correlations for POP concentrations in wild bird populations, (A) before and (B) after controlling for model sensitivity. Age group tissue correlations (r) predicted with correlations between tissues grouped by adult ($n = 28$ studies), juvenile ($n = 9$ studies) and nestling ($n = 10$ studies). Significant differences between age groups denoted by asterisks.

Pacyna-Kuchta, 2023). To benefit from minimally-invasive measures, however, we need to understand how minimally-invasive measures relate to internal POP concentrations. To achieve this, we can compare minimally-invasive measures against measures from more well-studied invasive tissues.

When comparing tissues at within-individual level in a wild population, the most informative comparison is correlation data, which provides information on how tissues relate to each other across sampled individuals. Overall, POP measurements were positively correlated between tissues, but the strength of these pairwise correlations between tissues remained weak to moderate. However, sensitivity analyses showed that these results were robust, and therefore that sampling one tissue allows extrapolating to POP levels in other tissues for the same individual. In addition, it is interesting to note that we found no overall significant difference between invasive and minimally-invasive matrices in pairwise comparisons. This highlights that minimally-invasive matrices can be used adequately to represent internal POP concentrations. However, the number of papers reporting individual-level tissue comparisons remain low ($n = 43$ of 115 studies), coupled with high levels of variation both within and between bird populations. Therefore, further efforts to investigate the correlation of POPs between invasive and minimally-invasive matrices are still needed to confidently conclude that minimally invasive matrices can be used as substitutes for invasive matrices. Given that, until now, toxicity thresholds have been primarily based on measurements in invasive matrices, information on the relationships between matrices is also essential for extrapolating toxicity thresholds from invasive matrices to minimally-invasive matrices.

Variation within and between bird populations is high as POP concentrations have been shown to differ due to species, season and geographical location (Eulaers et al., 2013). Furthermore, challenges in assessing minimally-invasive matrices as measures of internal POP

concentrations could be dampened by differences in results dependent on the age group of individuals sampled. POP exposure is likely more constant in nestlings than adults as POP exposure in nestlings is less confounded by factors such as movement, age, bioaccumulation, migratory activity and reproductive output (Eulaers et al., 2011a, 2011b). Consequently, we expected tissue correlations to be higher in nestlings than in adults, but we found little support for this prediction. Juveniles had higher tissue correlations than adults and nestlings, although this result did not remain significant when controlling for model sensitivity. Lower tissue correlations in nestlings and adults could be due to differences in pre- and post-hatching exposure and differing temporal exposure in tissues, respectively (Martinez-Lopez et al., 2007; Pacyna-Kuchta, 2023; Zhao et al., 2019). However, to understand how age group influences tissue correlations in this study, we need to consider each matrix individually rather than grouping them into invasive and minimally-invasive measures. Unfortunately, we did not have enough data to do this. To understand whether minimally-invasive matrices represent internal POP concentrations, the following sections discuss each minimally-invasive matrices included in this study separately (blood, feather and preen oil) and its potential as a measure of internal POP concentrations.

Preen oil

Fat and preen oil act as a POP sink due to their high lipid content (Eulaers et al., 2011b). POPs have lipophilic properties and accumulate in lipid-rich tissues, which makes fat and preen oil suitable matrices for measures of POP concentrations (Hela et al., 2006). Accordingly, fat and preen oil had the highest DF levels compared to the other matrices retained in our analyses. However, preen oil's lipid percentage was lower than fat, which was reflected by a lower POP concentration in preen oil than fat found in this study. Yamashita et al. (2007) and

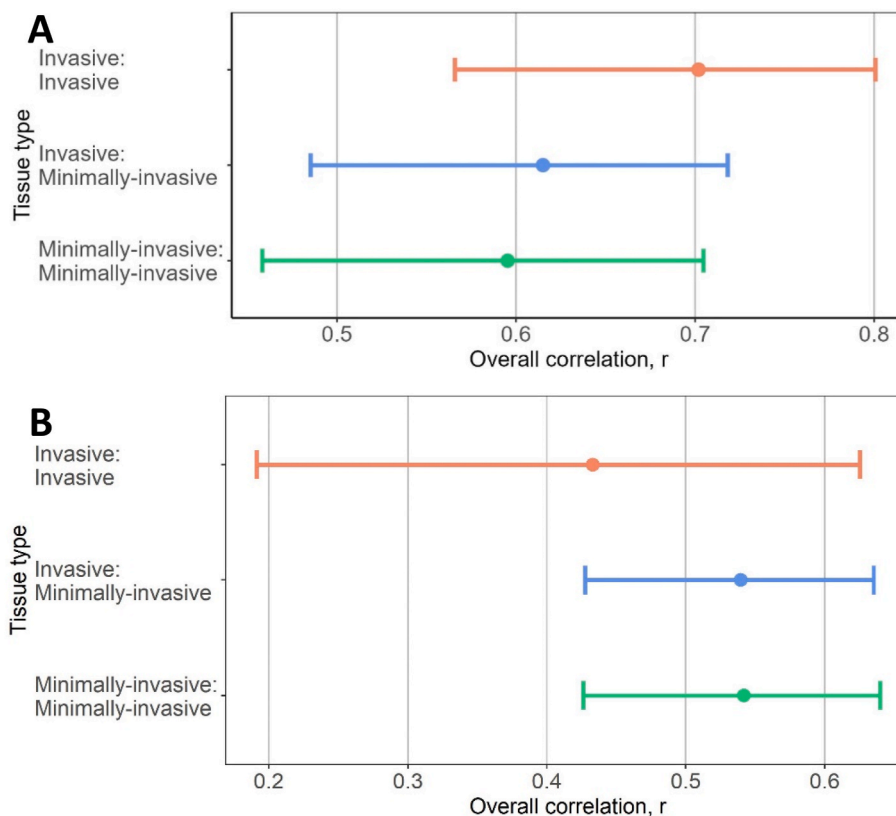


Fig. 6. Tissue type correlations for POP concentrations in wild bird populations, (A) before and (B) after controlling for model sensitivity. Tissue type correlations (r) predicted with correlations between tissues grouped into “invasive and invasive” ($n = 19$ studies), “invasive and minimally-invasive” ($n = 19$ studies) and “minimally-invasive and minimally-invasive” ($n = 12$ studies).

Renzoni et al. (1986) found a lower concentration of PCBs in preen oil/urophygial gland than fat, which supports our result. Preen oil had higher concentrations than fat in Eulaers et al. (2014a) and Jaspers et al. (2013a), reporting PBDEs and PCBs, respectively, likely due to the concentrations reported in lipid weight. Although preen oil had a lower concentration than fat in all POP groups in the current study, the detection frequencies of fat and preen oil were equivalent. Similar DFs between fat and preen oil is encouraging as it would suggest that fat and preen oil have similar benefits regarding maximising data quantity. The similarities between fat and preen oil are further supported by Yamashita et al. (2007), who found a weak but significant correlation between fat and preen oil (Sum PCBs: $r = 0.19$, $n = 30$). The correlation was calculated across 13 bird species, which may have weakened the correlation as POP exposure is likely to vary between species (Yamashita et al., 2007). Jaspers et al. (2013b) found a much stronger correlation between fat and preen oil in the barn owl (*Tyto alba*), but this was not included in our analyses due to the low sample size (PFOS: $r = 0.99$, $n = 3$). Differences in tissue physiology between fat and the uropygial gland (i.e., the gland which produces and stores preen oil) will always affect correlations in POP concentrations between the two tissues. The main physiological difference between fat and preen oil is that fat is stored and preen oil is continuously replenished (Acampora et al., 2017). Lower-chlorinated PCB congeners are found in higher concentrations in preen oil than fat, as POPs in preen oil spend less time in the body and thus have less metabolic transformation (Jaspers et al., 2013a; Larsson and Lindegren, 1987; Yamashita et al., 2007). Furthermore, as preen oil is excreted and in contact with the external environment during sampling, it is also subject to greater risk of contamination by the external environment than fat. However, the importance of sampling procedures in explaining differences in POP concentrations between tissues remains overlooked and should be given greater attention in order to establish

clear guidelines and standardised practices, particularly when collecting preen oil.

The POP profiles are similar between blood and preen oil (Briels et al., 2019; Yamashita et al., 2007), as blood and preen oil are continuously replenished and the uropygial gland is well-vascularised (Aslan et al., 2000). The similar characteristics between blood and preen oil are demonstrated by a multitude of studies which find a correlation between blood and preen oil (Briels et al., 2019; Eulaers et al., 2011b; Kocagöz et al., 2014). Overall, our analysis of the correlations between blood and preen oil showed a significant meta-correlation of 0.305, which increased to 0.802 after controlling for model sensitivity. However, a slight inverse correlation between PCB concentrations in blood and preen oil were seen in Wang et al. (2015), with more highly chlorinated congeners seen in preen oil compared to blood.

Blood

Blood is well-regarded as a measure of internal POP concentrations (Henriksen et al., 1998; Van den Steen et al., 2007). Internal tissues with high blood flow, such as liver and kidney, have shown correlations with blood in both laboratory studies (Henriksen et al., 1998; Van den Steen et al., 2007) and wild bird populations (Voorspoels et al., 2006). In our study, we did not find any significant correlations between blood, liver, and kidney. Although, a significant positive correlation between blood and liver arose after controlling for model sensitivity ($r = 0.661$). Furthermore, kidney correlations were removed during model sensitivity analysis and thus may be sensitive to publication bias. Although we would expect positive correlations between blood, liver, and kidney due to similar tissue physiology, this is not always the case. For example, a negative correlation between blood and liver is seen for PBDEs in yellow-legged gulls and the Eurasian coot (Kocagöz et al., 2014). The negative correlation between blood and liver found in Kocagöz et al.

(2014) is likely due to high within and between-species differences in POP metabolism and tissue physiology (Hela et al., 2006; Yamashita et al., 2007, 2018). As fat metabolism results in the remobilisation of POPs back into the blood, there have also been studies to show correlations between blood and fat (Capent and Leiker, 1979; Hela et al., 2006; Henriksen et al., 1998; Van den Steen et al., 2007). We did not find a positive meta-correlation between blood and fat in the original analysis. Although, the positive correlation became significant after controlling for model sensitivity ($r = 0.467$). However, we did find a positive meta-correlation between blood and preen oil ($r = 0.305$; 0.802 after controlling for model sensitivity), which can be explained by both blood and preen oil being continuously replenished, unlike fat, which can act as a POP sink, introducing differences in time of exposure between tissues (Acampora et al., 2017; Aslan et al., 2000).

When compared against other internal tissues, pollutant measures in blood have often been shown to be lower than internal tissue (Dauwe et al., 2007; Jaspers et al., 2013a; Martenson et al., 2016; Plourde et al., 2013; Voorspoels et al., 2006). The lower POP concentrations in blood could deter researchers from choosing blood as a POP matrix, with the review by Espín et al., 2016 concluding that higher volumes of blood could be needed to achieve an analytical detection level due to blood's lower lipid content. Although, we acknowledge that safe amounts of blood collection can impede the analysis of blood samples in smaller bird species (<1% of individual body mass). Despite the lower POP concentrations and lipid content, we demonstrated a higher overall DF level for blood than the internal organs measured (i.e. brain, kidney, liver, and muscle). The lower lipid content of blood may not influence blood POP concentrations as directly as other tissues because, once absorbed, POPs enter the body via the blood, circumventing the blood's need for a higher lipid content. However, blood has been suggested to be highly variable in POP measures as it is more dependent on current POP measures (Espín et al., 2016). Our study supports this finding as we found large differences in concentrations seen between POP groups with blood ranking even lower in POP concentration compared to other tissues after controlling for model sensitivity. As such, more research needs to be done to understand how DF levels differ between POP groups in comparison to internal DF measures in blood.

Feather

Although feathers have been used as a measure of POP concentration in wild birds since the 1970's (Kwon et al., 1976; Vannucchi et al., 1978), the use of feathers only came to the forefront of minimally-invasive research in recent years (Dauwe et al., 2005; Pacyna-Kuchta, 2023). Endogenous deposition of POPs in feathers only occurs during growth when feathers are connected to the bloodstream (Dauwe et al., 2005; Jaspers et al., 2004). Therefore, POP concentrations within feathers are a measure of internal POP concentrations dependent on feather moulting pattern (Dauwe et al., 2005; García-Fernández et al., 2013). Newly grown feather and blood pollutant measures increase proportionally (Van den Steen et al., 2007) and many studies have demonstrated correlations between feathers and internal tissues when accounting for the moulting pattern (Briels et al., 2019; Eulaers et al., 2011a; Gómez-Ramírez et al., 2017; Løseth et al., 2019). We found correlations between feathers and blood, fat, muscle, and preen oil ($r = 0.159$, 0.166 , 0.173 and 0.154 respectively), although the significant positive correlation between fat and feather was removed during model sensitivity analysis. We could not account for the moulting pattern in our analysis due to a lack of moulting reporting, which could inhibit our ability to detect correlations between feathers and other matrices. Without controlling for moulting pattern (i.e. POP concentrations could be temporally different from other matrices), positive correlations between feathers and other matrices could occur if POP exposure is constant over time, and/or external contamination of preen oil improves correlations (Jaspers et al., 2008). Furthermore, more research is needed to test these correlations dependent on species as interspecific variation in feather physiology and the moulting pattern is high (Bortolotti,

2010). The variability in DF of feathers was higher than other tissues in the current study, which may demonstrate a high variability between studies. Nevertheless, it is vital to have an in-depth understanding of a species' moulting pattern and migration patterns before using their feathers to measure POP concentrations (Jaspers et al., 2008).

One of the key results in our study revealed that the overall DF of feathers was significantly lower than that of other matrices. When sampling the POP exposure of a wild bird population, using a matrix with a lower DF can reduce the ecological relevance of the study and the power of any subsequent analysis. Thus, we suggest that other minimally-invasive matrices may be more appropriate when measuring the current exposure level in lipophilic POPs. As PFAS compounds are proteinophilic rather than lipophilic, feathers may still remain a primary alternative to invasive measures (Jaspers et al., 2013b; Kannan et al., 2005). Although there is little difference in PFAS concentrations between tissues in our study, feathers had the highest PFAS concentrations compared to other tissues. Notwithstanding, feathers remain an excellent strategy for metal trace elements as these contaminants appear more easily incorporated into feathers than POPs (Abdullah et al., 2015; Rutkowska et al., 2018). Furthermore, as feathers only accumulate POPs during growth, studies can utilise the temporal disparity of old/fully-grown feathers to gain information on POP exposure from a different time point from the sampling time. The potential for temporal disparity between sampling and internal POP accumulation allows us to sample internal POP concentrations from different time points (Dauwe et al., 2005; Van den Steen et al., 2007) and geographical locations (Pacyna et al., 2019; Pacyna-Kuchta et al., 2020) that may have otherwise been unavailable to us. The potential temporal disparity between feather sampling and internal POP concentrations makes feathers irreplaceable in their use in POP research in wild bird populations (Bortolotti, 2010; García-Fernández et al., 2013; Meyer et al., 2009). Thus, it is vital to understand how feathers relate to internal POP concentrations and compare them to other tissues.

4.3. Additional considerations when choosing matrices for the monitoring of POP exposure in wild bird populations

In this discussion, we have primarily focused on how tissue physiology impacts matrix choice when measuring POP exposure in a wild bird population. Besides this, multiple additional factors can influence the validity of a chosen matrix of POP exposure. Firstly, internal POP concentrations are affected by current exposure but are also influenced by previous exposure (Kenntner et al., 2003; Subramanian et al., 1986). Fluctuations in POP exposure over time affect internal POP concentrations differently depending on the matrices chosen. Fat, for example, acts as a POP sink and results in a temporal difference of measured POP concentration dependent on when the sampled fat was stored. Secondly, the individual state, defined as "the condition of the individual at a given time", can affect the internal movement of POPs within the body, affecting the POP concentrations measured depending on the matrices used (Costantini et al., 2014). Individual state affects POP concentrations through fat metabolism, with the metabolism of fat resulting in the remobilisation of POPs stored in the fat. Thus, when fat is metabolised, POP concentrations in other matrices can increase, such as blood and liver (Bustnes et al., 2012; Thorstensen et al., 2021). Various factors that influence individual state have been suggested to affect POP concentrations measured, including metabolism, foraging behaviours and migratory habits (Borgå et al., 2005; Fisk et al., 2001; Hela et al., 2006; Henriksen et al., 1996, 1998; Monclús et al., 2018; Yamashita et al., 2007). Finally, practical considerations should be considered when choosing the most appropriate matrices for monitoring POPs in the chosen bird population. Table 1 reviews the pros and cons of the minimally-invasive measures considered in the current study.

Table 1

Pros and cons of using blood, feathers and preen oil for POP measures in wild bird populations. Adapted from Eulaers et al. (2011b) and Pacyna-Kuchta (2023).

Blood	Feathers	Preen oil
Pros		
- Minimally-invasive sampling - Year-round sample collection - Can be collected at all life stages - Well-studied matrix across POP groups - Ongoing sequestration – represents current POP burden - No external contamination	- Minimally-invasive sampling - Year-round sample collection - Can be collected at all life stages - Sequestration delay allows for “remote” sampling dependent on feather growth - Metabolically inactive after growth - No contact collection after moulting possible - Museum sampling possible - Transported and stored at room temperature	- Minimally-invasive sampling - Year-round sample collection - Can be collected at all life stages, as soon as preen oil is produced - High lipid content accumulates higher concentrations of (lipophilic) compounds - Sequestration time close to blood due to ongoing blood supply - No external contamination - Repeated sampling over short period of time
Cons		
- Training and practice required for sampling - Possible metabolism - Quantity required may not be suitable for smaller species and/or nestlings (<1% of individual body mass) - Cold conditions required for transport and storage	- Need detailed species’ moulting pattern and migration timings - Quantity required may not be suitable for smaller species and/or nestlings - External contamination of preen oil - Concentration variability across feather types	- Training and practice required for sampling - Possible metabolism - Cold conditions required for transport and storage

4.4. Strengths and limitations of our study

With all meta-analytic studies, there are recurring strengths and limitations (Borenstein et al., 2021; Cheung and Vijayakumar, 2016; Vetter et al., 2013). A key strength of our study is its purpose; to provide a global overview of the most commonly used matrices in the analysis of POP exposure in wild bird populations. A further strength of our study was an extensive search of the literature including over 3000 papers, although we acknowledge that our resulting study pool is likely not exhaustive. Another strength is how we handled publication bias which can impact the validity of conclusions drawn in a meta-analysis (Cassey et al., 2004). Although publication bias was present in our study pool, we have provided extensive sensitivity analyses to ensure that the conclusions drawn in this study were robust and independent of values of high influence.

Although we gained great insight into matrix uses and their representation for contaminant level, we need to place these results in the context of lack of data availability and methodological reporting in the published manuscripts used in this meta-analysis. To circumvent these limitations, we included POP group as a moderator in the analysis of POP concentrations, not individual POP congener. Sadly, due to a frequent lack of standard procedure descriptions, we did not include methodological considerations as moderators in our analysis. As such, we encourage future publications to include detailed methodology as methodological approaches can influence results. These can range from tissue sampling procedures that can lead to external contamination and thus increase or decrease correlations between tissues, to extraction protocols and analytical equipment that will affect the sensitivity of measures in different tissues.

4.5. Conclusion

Minimally-invasive measures have increased ethical and practical applications when sampling living birds (Abbasi et al., 2017; Eulaers et al., 2014b). Our meta-analysis provided an overview of minimally-invasive matrices comparisons for blood, feathers and preen oil and increased knowledge on how minimally-invasive measures reflect internal POP concentrations. Specifically, we highlight the following conclusions on minimally-invasive matrices: (1) DF between fat and preen oil were similar even though preen oil demonstrated overall lower POP concentrations than fat; (2) blood showed high variability in concentrations dependent on POP group but detection frequencies were comparable to liver and kidney; and (3) feathers demonstrated a significantly lower detection frequency than all other matrices measured in this study, suggesting that feathers may be better suited as a measure of POP exposure only when taking advantage of the temporal disparity feathers can provide. Furthermore, we suggest that all studies on POP concentrations in wild bird populations moving forward should report detection frequencies, lipid/water content of tissues and tissue correlations where possible. Our dataset demonstrates a shift towards more minimally-invasive measures over time. Whether this is due to minimally-invasive measures being more versatile or an increase in the awareness of ethical practices, we wholeheartedly support this shift.

CRediT authorship contribution statement

Francesca E. Gray: Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Davina Derous:** Writing – review & editing, Supervision. **Pierre Bize:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition.

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.

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.chemosphere.2024.142591>.

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