PFAAs levels, oxidative status and reproductive success in great tits (*Parus major*) inhabiting a contamination hot-spot.

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1. Introduction

Perfluoroalkyl acids (PFAAs) are substances which have been produced for more than five decades. Their unique properties of repelling both water and oil, make them suitable for many industrial and consumer applications such as water and dirt repellents for clothes and carpets, active components in firefighting foams or precursors in Teflon® production [1]. Its extensive use, together with their high persistence, has resulted in global contamination of the environment, wildlife and even humans [2,3]. This ubiquity contrasts sharply with the limited amount of available information about their effects on organisms.

2. Materials and methods

Nestboxes (n=115) were placed at five sampling sites, representing a distance gradient from a fluorochemical plant in Antwerp.

A first blood-sampling period was performed in the winter of 2015 (79 sampled adult birds). A second blood-sampling period of adult birds was performed in the rearing period, when chicks were 10 days old, parents were captured inside the nestbox (60 sampled birds). After that, when chicks were 14 days old, all the chicks in each nest were sampled (441 chicks from 79 nests). From these we selected 179 samples (two per nest, the lightest and the heaviest chicks) for PFAAs and Oxidative Stress (OS) parameters analysis.

During the breeding season, nestboxes were intensively monitored to assess key reproductive traits (first-egg laying date, egg characteristics, clutch size, hatching and fledgling success). From each nest, the third egg was collected (98 eggs) to analyse PFAAS content.

The determination of PFAAs in egg and plasma samples was performed by UPLC-TQD mass spectrometry. Eleven PFCAs (PFBA, PFPeA, PFHxA, PFHpA, PFNA, PFOA, PFDA, PFUdA, PFDoA, PFTrA and PFTeA) and four PFSAs (PFBS, PFHxS, PFOS and PFDS) were selected as target analytes. OS parameters (GHS, gssg, protein carbonyls, SOD, GPX, CAT and TAC) were studied in the red blood cells

To study the relationship between PFAA levels and the multiple biomarkers (reproductive and OS) we conducted Principal Component Analysis. Two Principal Components (PCs) were selected for adults and eggs and one for chicks.

3. Results and discussion

3.1. Blood and egg levels, gender-differences and maternal transfer.

Mean ∑PFAAs levels found at the plant are among the highest ever measured in wildlife in eggs (48,188 ng/g) and plasma (57,630 ng/ml). Although these levels decreased sharply at a short distance from the plant (1km away), they remained high at the furthest location (10 km away).

We found significantly higher PFOS and PFUdA plasma levels and prevalence in adult females than in males (all $p \leq 0.01$). These differences were consistent along the distance gradient and in both sampling seasons. In chicks, we found a trend of a higher prevalence of PFOS in females than in males ($p=0.058$). This result is the opposite of what has been found in birds so far and should be examined further.

Correlations were found between egg, mother and offspring levels for PFOS but not for other compounds. This means that maternal transfer is the main route of exposure for chicks to PFOS but not for other compounds. Moreover, higher levels of PFOA and PFBA were found in the offspring than in the mothers during the nestling period.

3.2. Correlation of PFAAs levels with reproductive success and OS parameters.

In eggs, high values of PC1 were associated with a reduced hatching success ($p<0.001$; Figure 1a) and thinner egg shells ($p<0.001$; Figure 1b), but also with an increased survival of hatched chicks ($p=0.027$). PC2 was negatively associated with laying date of the 1st egg ($p=0.005$). In addition, high PC2 values were significantly associated with the total failure of hatching ($p=0.025$) and thus reduced the total success of the breeding ($p=0.005$; Figure 1c).

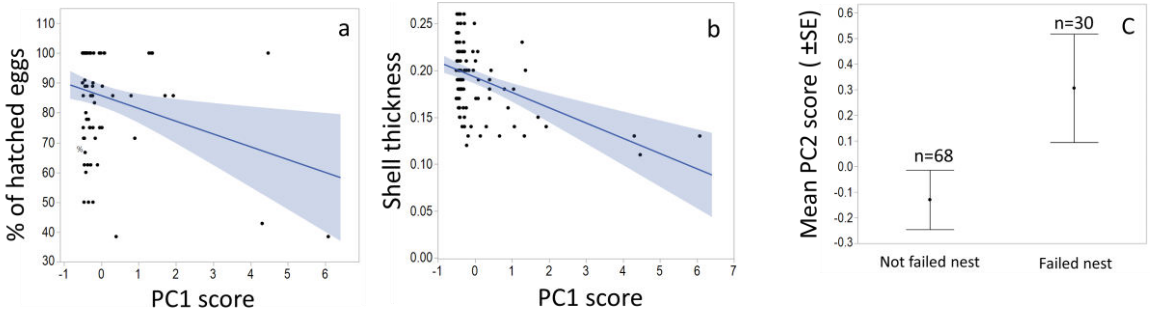

Figure 1: Correlations between the PFAAs levels in the eggs and reproductive parameters.

In plasma, the concentration of protein carbonyls (a measurement of protein damage) was positively correlated with Adults-PC1 ($p=0.08$; Figure 2a) and Adults-PC2 ($p=0.07$; Figure 2b) in a marginally significant way. Chicks' GPX and CAT activity were significantly correlated with Chicks-PC1 levels ($p=0.006$ and $p=0.05$ respectively).

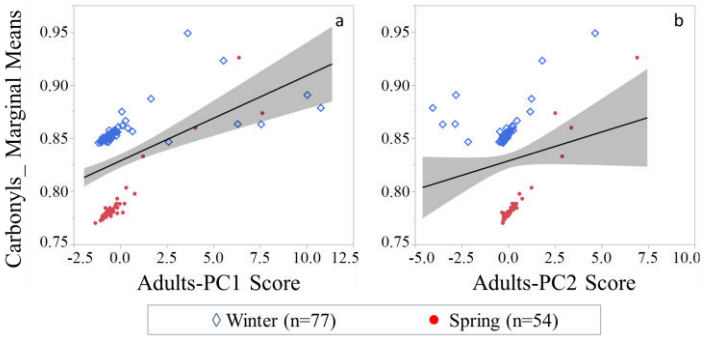

Figure 2. Relationship between Adults-PC1 and Adults-PC2 and protein carbonyl content in blood of adult birds sampled in winter and the nestling period (spring).

4. Conclusions

Our results provide evidences that Antwerp is one of the main hot-spots in the world for perfluorinated compounds pollution and highlight the need to i) keep monitoring long-chain PFAAs ii) identify and monitor currently used fluorinated alternatives and. iii) study the effects of these compounds in a holistic way; from molecular events to the final consequences for the individuals and populations. The obtained data represent an important step towards the understanding of the behaviour, effects and consequences of PFAAs in wild bird populations. A constant monitoring effort of exposure and effects in wild populations established in the area, together with controlled experimental exposure of captive birds, will be extremely useful for ecological and human risk assessment.

5. References

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