The importance of age when studying persistent organic pollutants in white-tailed eagle nestlings

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Introduction

By occupying higher trophic levels, the white-tailed eagle (WTE; *Haliaeetus albicilla*) can accumulate a wide range of environmental contaminants, even at an early age [1–4]. The nestlings can be exposed to high levels of certain persistent organic pollutants (POPs) through maternal transfer to the eggs, and later on through diet. As the nestlings develop and grow, the concentrations of maternally-derived compounds will be diluted [1]. However, few studies are accounting for the biological factors of age and increase in body mass when monitoring POPs in nestlings.

Top predators are superb sentinel species for monitoring the presence of POPs in the environment and non-destructive sampling methods are most commonly applied due to ethical and species conservation aspects [5]. Collecting samples from adult avian top predators is impossible without physically trapping or catching the bird, with a high risk of inflicting stress or even serious injuries to both the collector and the bird. Thus, sampling of nestlings while in the nests without inflicting severe stress is a preferred method to reduce the disturbance of raptor species. Furthermore, sampling of feathers has been proposed as a non-destructive and non-invasive method for monitoring POPs nestlings [2-5]. It has been advised to use recently grown feathers which are still connected to the blood stream and to a lesser extent prone to external contamination [5]. Nevertheless, because the pollutant levels may decrease as a function of age and body mass due to growth dilution these factors may confound the interpretation of the results.

The aim of the present study was to investigate (1) effects of age and body mass, as well as sampling year on POPs accumulation in plasma and feathers, and to explore (2) correlations between feather and plasma concentrations of POPs in nestling WTEs.

Materials and methods

Feather and blood samples were obtained from nestling WTEs from Steigen (Norway) in 2015 and 2016. Age was estimated from tail feather length (mm); the tail feathers emerge at day 30 and grow at a rate of 4.95 mm per day [T. Nygård 2015, pers. comm.]. All chicks were sampled in the nest. POPs were analysed in plasma and feathers according to Eulaers *et al*. [4].

In total, 12 polychlorinated biphenyls (PCBs), 7 organochlorinated pesticides (OCPs) and 5 polybrominated diphenylethers (PBDEs) were quantified in plasma and 9 PCBs, 4 OCPs and 1 PBDE were detected in feathers. For statistical modelling we chose one representative compound which contributed the most within each

compound group; CB 153 (37 % in both feathers and plasma), *p,p'*-DDE (88 % in feathers, 76 % in plasma) and BDE 47 (100 % in both feather and plasma). To meet the requirement of normality, all POPs variables were $\log_e$ transformed. Correlations between explanatory variables, feather and plasma concentrations of the POPs variables were investigated using Pearson correlation tests with the level of significance at $\alpha = 0.05$. The mean age (± SE) at sampling in 2015 was 63.4 ± 3.4 days and 66.0 ± 2.5 days in 2016. A Welsh Two sample t-test showed no significant difference in age at sampling between 2015 and 2016 ($t_{df\ ,25.6} = 0.63$, $p = 0.53$).

Due to the structure of the WTE data, with two or three chicks in some nests, statistical tests from the *lme* package (Rstudio) were applied to control for the possible variation between the nests (nest factor). The correlation between body mass and age was low, but significant ($r_p = 0.34$, $p < 0.01$) and was taken into account by including their interaction. Linear mixed effect models were used to investigate which of the explanatory variables could explain most of the variation in POPs and nest was included as a random factor. The nest factor was included in all models as it explained 55-83 % and 14-25 % of the total variation of the POPs variables, in plasma and feathers, respectively. The most parsimonious models were selected based upon Akaike Information Criterion with a correction for finite sample sizes (AICc) and models with ΔAICc > 2 are discussed.

Results and discussion

The concentrations of CB 153, *p,p´*-DDE and BDE 47 are listed in Table 1. For all compounds, higher concentrations were detected in feathers compared to plasma. This was confirmed by the most parsimonious models for both feather and plasma for each compound.

Table 1: Plasma and feather concentrations in median and range of CB 153, *p,p'*-DDE and BDE 47 from white-tailed eagle nestlings from 2015 and 2016.

	CB 153		*p,p'*-DDE		BDE 47	
Years	plasma (ng/ml)	feather (ng/g)	plasma (ng/ml)	feather (ng/g)	plasma (ng/ml)	feather (ng/g)
2015 n = 14	2.05 (1.12-26.27)	12.59 (5.48-33.16)	3.95 (2.18-47.61)	26.59 (12.38-94.38)	0.19 (0.06-1.82)	1.73 (0.63-2.96)
2016 n = 21	1.75 (0.43-10.16)	4.15 (0.92-9.73)	1.45 (0.48-8.64)	3.74 (1.14-8.81)	0.09 (0.01-0.36)	0.45 (0.14-1.06)

CB 153

The best model (ΔAICc = 0) in explaining the variation in plasma of CB 153 included age, while the second best (ΔAICc = 0.68) included age and year. Both models estimate a significant negative effect of age on the CB 153 concentration, with a 1 % decrease in concentration per day ($p = 0.05$). The second model also showed that the concentrations of CB 153 were 15 % lower in 2016, compared to 2015, however this difference was not statistically significant ($p = 0.25$). The best model (ΔAICc = 0) in explaining the variation in feather of CB 153 included age and year, and showed a significant -24 % difference in CB 153 in the feathers from 2016, when compared to 2015 ($p < 0.01$), as well as an estimated decrease of 0.7 % per day ($p < 0.01$).

p,p'-DDE

The best model (ΔAICc = 0) in explaining the variation in plasma and feather of *p,p'*-DDE included age and

year. For plasma it showed a significant -35 % difference in *p,p*-DDE in the samples from 2016, when compared to 2015 ($p < 0.01$), while in feathers the difference was -39 %. It also estimates that the concentration of *p,p'*-DDE decreases by 0.8 % per day ($p = 0.03$) in plasma and 0.5 % per day ($p < 0.01$) in feathers.

BDE 47

The best model (ΔAICc = 0) in explaining the variation in plasma BDE 47 included year, showing a 69 % lower concentration detected in 2016, compared to 2015 ($p = 0.03$). However, the best model in explaining the variation in feathers included both year and age. Showing on average a 79 % lower concentration of BDE 47 detected in 2016, compared to 2015 ($p < 0.01$), as well as a 1 % decrease in BDE 47 per day ($p < 0.01$).

It is important to emphasize that these estimates are from models, and that only one sample is available from each individual. However, the calculated age of the nestlings is important in understanding the variation in the concentrations of the compounds. In both years, there are nestlings sampled at varying ages, with on average 3 days younger individuals in 2015. Our data clearly indicate an effect of age on the concentrations of these compounds, with lower concentrations with increasing age at sampling. This is consistent with a study from Bustnes et al. [1] who investigated to which extent plasma POP concentrations from WTE nestlings late in the nestling period originated from maternal or dietary input. They sampled twice from the same individuals with only a few weeks difference and found that POPs concentrations were declining with age. However, when controlling for growth rate and dilution the nestlings were still accumulating more than expected [1].

Correlations between plasma and feather concentrations

In order to validate nestling feathers for biomonitoring of POP concentrations, it is important that feather concentrations correlate well with internal body concentrations, e.g. in plasma. For all selected POPs, feather and plasma concentrations correlated significantly; CB 153 ($r_P = 0.74$, $p < 0.001$), *p,p'*-DDE ($r_P = 0.83$, $p < 0.001$) and BDE 47 ($r_P = 0.81$, $p < 0.001$). The correlations between feather and plasma concentrations are shown in Figure 1.

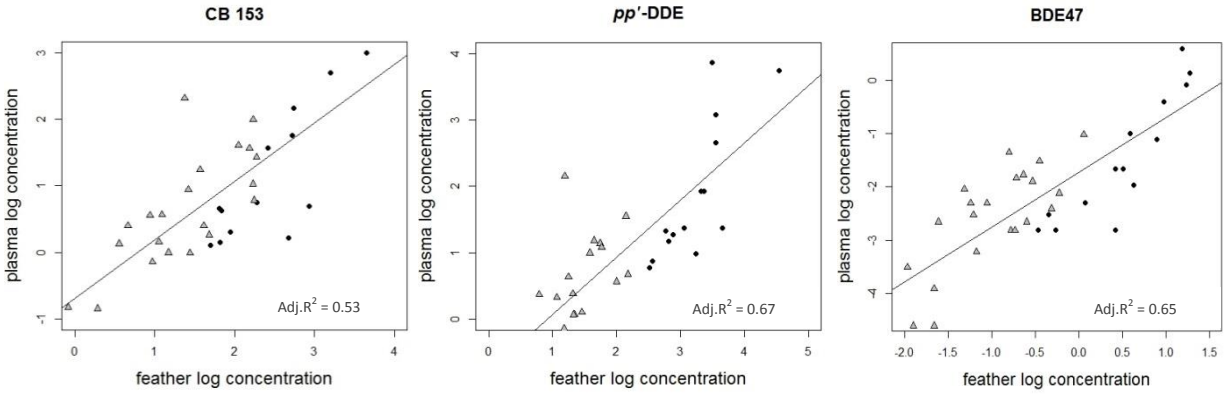

Figure 1: Correlations between $\log_e$ feather and $\log_e$ plasma concentration of CB 153, *p,p'*-DDE and BDE 47 in white-tailed eagle nestlings from Steigen (Norway). Samples from 2015 are black circles and samples from 2016 are grey triangles. The line in each plot shows the linear regression between feather and plasma $\log_e$ concentrations, and the adjusted R^2 is shown in each plot.

Our results are similar to previous studies, showing strong correlations between feather and plasma concentrations in WTE nestlings [3,4]. The correlation is strong and stable in the samples from both years, even from individuals sampled at varying ages.

Conclusions

Our results indicate that age of birds at sampling is important in understanding the variation in CB 153, *p,p'*-DDE and BDE 47 concentrations in WTE nestlings. Further studies should therefore record and take age into account when studying POPs in nestlings. In both feathers and plasma, the concentrations of the compounds are lower when nestlings are sampled at a later age. However, as there is no significant difference in age between the years, the difference in compound concentration between years may be better explained by an environmental decrease in the compounds or by varying ecological variables, such as diet. Further studies should therefore also include stable isotopes of $\delta^{13}C$ and $\delta^{15}N$ as proxies for diet and trophic level, respectively.

The strong correlations between feather and plasma of the selected compounds in this study are providing more evidence to the usefulness of feathers for environmental monitoring of POPs in WTE nestlings.

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References

1. Bustnes, J.O., Bårdsen, B.J., Herzke, D., Johnsen, T. V., Eulaers, I., Ballesteros, M., Hanssen, S.A., Covaci, A., Jaspers, V.L.B, Eens, M., Sonne, C., Halley, D.J., Moum, T., Nøst, T.H., Erikstad, K.E and Ims, R.A. (2013) *Environ Toxicol Chem*; 32(11): 2520–7.
2. Eulaers, I., Jaspers, V.L., Bustnes, J.O., Covaci, A., Johnsen, T.V., Halley, D.J., Moum, T., Ims, R.A., Hanssen, S.A., Erikstad, K.E., Herzke, D., Sonne, C., Ballesteros, M., Pinxten, R. and Eens M. (2013) *Environ Int* 57-58: 25-33.
3. Eulaers, I., Covaci, A., Hofman, J., Nygård, T., Halley, D.J., Pinxten, R., Eens, M. and Jaspers, V.L. (2011) *Sci Total Environ* 410-411: 258-65.
4. Eulaers, I., Covaci A., Herzke, D., Eens M., Sonne, C., Moum, T., Schung, L., Hanssen, S.A., Johnsen, T.V., Bustnes, J.O. and Jaspers, V.L.B. (2011) *Environ. Int.* 37(3): 622-630.
5. Espín, S., García-Fernández, A. J., Herzke, D., Shore, R. F., van Hattum, B., Martínez-López, E., Coeurdassier, M., Eulaers, I., Fritsch, C., Gómez-Ramírez, P., Jaspers, V. L. B., Krone, O., Duke, G., Helander, B., Mateo, R., Movalli, P., Sonne, C. and van den Brink, N. W.(2016) *Ecotoxicology*; 25(4): 777-801.