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Variation in concentrations of organochlorines and brominated flame retardants among eggs in abandoned clutches of a terrestrial raptor

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HIGHLIGHTS

- Tawny owl clutches abandoned by the parents were collected.
- Chlorinated and brominated compounds were analyzed in all eggs.
- Concentrations were similar among eggs within clutches.
- Abandoned eggs seem to provide a relatively reliable measurement of lipophilic pollutants.

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ABSTRACT

Bird eggs are often used to measure various pollutants in monitoring programs. In protected species, however, it might only be possible to collect eggs abandoned by the parent birds. In order to assess the appropriateness of abandoned eggs for contaminant monitoring we examined intra-clutch variation in concentrations of 24 halogenated organic pollutants (16 organochlorines [OCs] and 8 brominated flame retardants) in six abandoned tawny owl (*Strix aluco*) clutches from central Norway. The variation among eggs within a clutch was low for nearly all compounds (intra-class correlation >0.9 both for lipid- and wet weight). This study suggests that abandoned eggs provide a relatively reliable measurement of lipophilic pollutants in clutches of terrestrial raptors such as the tawny owl.

1. Introduction

Bird eggs, especially from raptors and aquatic birds have been used to monitor persistent organic pollutants (POPs) for several decades (Hebert et al., 1999; Helander et al., 2002; Klein et al., 2012; Bustnes et al., 2007). However, in protected species it is often only possible to collect eggs abandoned by the parents (e.g. Helander et al., 2002; Vorkamp et al., 2005; Bustnes et al., 2007). Such eggs may be unfertilized, the embryo may have started to develop or they may be addled. In addition, embryo development potentially cause alterations in the concentration and composition of POPs in the egg (Klein et al., 2012). If abandoned eggs should be appropriate for monitoring persistent POPs, they must reflect the concentrations in the clutch as a whole. We are, however, aware of no studies that have investigated the intra-clutch variation of POPs in abandoned clutches of terrestrial raptors such as owls. In this study we analyzed intra-clutch variation in organochlorines (OCs) and polybrominated diphenyl ethers (PBDEs) in tawny owl (*Strix aluco*) from Central Norway, in order to document intra-clutch variability. Six whole clutches (4–5 eggs in each; 26 eggs in total) abandoned by the parent birds were collected and analyzed for 24 different organic compounds (Table 1).

2. Materials and methods

2.1. Study area and sample collection

The study was carried out in the area surrounding the city of Trondheim (63.42°N, 10.23°E) in Sør-Trøndelag County, Central Norway. More than 100 tawny owl nest boxes have been deployed. The boxes were visited in mid-May when the young had hatched,

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and all non-hatching eggs were collected. In some cases, nests were abandoned and then whole clutches were collected. Eggs were kept at the ambient temperature until frozen, which usually took place within 10 h of collection. In this study eggs from six whole clutches were analyzed (2 with 5 eggs and 4 with 4 eggs). The clutches were collected in 1993 (1), 1994 (1), 1995 (1), 1999 (1) and 2001 (2). Stage of embryonic development was classified for each egg as: no embryo development, low development, medium development and fully developed chick.

2.2. Chemical analyses

The chemical analyses were carried out at the Laboratory of Environmental Toxicology at the Norwegian School of Veterinary Science by gas chromatography (GC)/mass spectrometry (MS) and or GC/electron capture detector. All details about the analyses have been described elsewhere (Bustnes et al., 2007; Yoccoz et al., 2009). The laboratory is accredited for analyzing the components reported here according to the requirements of NS-EN ISO/IEC 17025:2000. The analytical quality of the laboratory has been demonstrated in several intercalibration tests. Standard procedure recoveries of spiked samples, blanks and reference samples were analyzed in each series and acceptable results were achieved.

The following polychlorinated biphenyl (PCB) congeners were analyzed; −101, −99, −118, −153, −138, −187, −180, −170, −194 (−28 and −52 were not detected). Other organochlorines analyzed were hexachlorobenzene (HCB), *trans*-nonachlor, oxychlordane, 2,2-bis(4-chlorophenyl)-1,1-dichloroethene(*p,p*′-DDE), and α-, β- and γ-hexachlorocyclohexane (HCH). Polybrominated diphenyl ethers (PBDEs) congeners analyzed included: −28, −100, −47, −99, −153, −154, and −209. In addition we also analyzed hexabromocyclododecane (HBCD). Lipid content was analyzed gravimetrically. Since lipid corrected concentrations are frequently used in monitoring of POPs, we focused primarily on lipid weight concentrations. However, we also included results for wet weight concentrations for comparison.

2.3. Statistical analysis

In order to assess the variability of POP concentrations in a clutch we compared the variation within-to the variation among clutches. To achieve this we used the intraclass correlation coefficient as a measure of the repeatability of measurements within clutches (Lessells and Boag 1987; Wolak et al., 2012). It is defined as the ratio of the between-clutch variance to the total variance (i.e., the sum of the within and between clutch variance). Intraclass correlation coefficients and their confidence intervals (CIs) were estimated using the R library ICC (Wolak et al., 2012), using the Searle (1971) method (Donner, 1986; Donner and Wells, 1986).

As estimates of variance components can be sensitive to the normality assumption, we investigated for each variable the optimality of the log transformation and the value of the constant added when some observations were equal to 0 using the Box and Cox (1964) transformation family (Venables and Ripley, 2002). For each variable, we assessed if the 95% CI of the λ parameter (as in $(y^{\lambda} - 1)/\lambda$ *if* $\lambda \neq 0$, $\log(y)$ if $\lambda = 0$) included 0, i.e., the log-transformation. When zeros were present, we tried first as a value for the constant the minimum non-zero value. In addition, residuals were systematically plotted and the constancy of the within-clutch variance was assessed. For two variables with very large concentrations for one clutch (PCBs), adding a relatively large constant resulted in approximately constant within clutch variance.

Too few clutches were available to make a formal analysis of the effect of embryo development. We calculated the coefficient of variation (SD/mean). Note that when variation is not too large, CV is equal to exp(SD_log) − 1, with SD_log being the standard deviation calculated on a logarithmic scale (e.g., Bland and Altman, 1996), so

Table 1

Mean concentrations (lipid-weight) and coefficient of variation (CV; %) for 24 organohalogenated compounds (organochlorines and brominated flame retardants) in 6 whole tawny owl clutches ($n = 26$ eggs) from central Norway. Embryo development in each egg was classified as no development (N), low development (L), medium development (M) and fully developed embryos (F).

Clutches (number and year of collection)												
	1 Embryo dev:Mean	1994 NNNNN CV	2 Embryo dev:Mean	1993 NNNN CV	3 Embryo dev:Mean	2001 NNNLL CV	4 Embryo dev:Mean	1995 LLMM CV	5 Embryo dev:Mean	1999 MMMM CV	6 Embryo dev:Mean	2001 FFFF CV
HCB	442.75	6.27	47.13	12.48	40.43	14.36	103.46	3.71	64.70	5.52	208.50	11.18
α-HCH	9.02	14.56	4.76	39.16	1.56	9.77	9.51	8.53	3.28	10.07	2.63	5.85
β-HCH	26.32	5.54	2.05	66.76	1.86	45.94	7.24	8.03	2.13	24.16	15.05	7.90
γ-HCH	2.70	18.90	1.56	44.83	0.76	5.30	1.63	30.25	2.47	17.68	1.08	8.22
Oxychlordane	413.64	7.09	10.33	25.14	20.22	23.55	46.72	1.46	47.46	13.11	52.47	8.43
trans-nonachlor	1906.03	8.98	101.70	23.84	14.45	10.52	220.01	13.25	198.55	28.97	29.27	16.46
p,p′-DDE	5903.91	5.72	298.72	18.62	1672.35	17.41	985.58	7.85	1437.72	13.61	2656.59	7.82
PCB101	30.77	12.74	4.23	70.63	2.91	5.30	11.41	10.55	5.30	29.76	19.12	13.08
PCB99	354.39	4.62	8.08	25.72	24.49	15.64	65.76	3.87	14.04	11.97	102.23	9.87
PCB118	142.37	6.26	6.82	58.16	35.17	10.15	62.02	3.83	23.64	12.49	100.37	10.43
PCB153	3608.07	6.85	104.61	17.55	524.93	10.96	504.28	4.07	189.88	6.02	806.22	9.33
PCB138	1373.04	5.09	43.05	23.74	201.02	12.51	258.58	3.75	72.37	5.57	552.13	8.27
PCB187	494.53	8.85	38.91	23.34	149.79	13.69	150.14	4.84	43.54	6.17	341.58	7.28
PCB180	2221.25	7.50	83.45	19.54	334.78	16.52	283.07	5.21	104.83	7.29	352.69	5.88
PCB170	687.54	7.95	19.78	22.17	87.66	17.37	76.95	7.23	28.00	8.79	119.72	7.12
PCB194	277.02	15.77	17.33	18.44	53.80	16.70	51.92	9.82	19.37	11.73	48.27	8.82
BDE28	0.21	12.37	0.05	13.10	0.05	5.30	0.05	11.77	0.07	24.16	0.20	13.96
BDE47	130.24	5.26	1.48	9.31	9.29	23.91	12.94	4.22	4.40	13.01	40.54	7.08
BDE100	92.86	7.65	0.72	6.08	10.32	12.60	6.16	7.37	2.99	6.33	16.99	7.97
BDE99	132.89	6.65	3.66	7.89	27.99	15.01	19.87	3.95	8.93	8.11	40.15	7.35
BDE154	21.06	14.22	0.40	21.16	3.13	15.03	2.48	10.32	1.06	12.32	3.48	8.12
BDE153	75.25	9.34	5.07	16.24	24.08	19.45	17.60	7.32	6.48	14.83	30.07	7.05
BDE209	2.19	29.07	0.35	13.10	0.38	19.13	0.74	10.77	0.45	64.10	9.36	25.74
HBCD	10.73	4.64	2.08	13.10	2.08	5.30	2.22	11.77	3.04	24.16	8.61	6.16
Mean		9.66		25.42		15.06		8.07		15.83		9.56