

Mercury and organic contaminant together with stable isotopes  $^{15}\text{N}$  and  $^{13}\text{C}$  in eggs of tawny owl (*Strix aluco*) and common kestrel (*Falco tinnunculus*).

Sakrapport  
Överenskommelse 2220-14-004

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## Sammanfattning

Den här rapporten är skriven på uppdrag av Naturvårdsverket (Överenskommelse 2220-14-004). Syftet har varit att undersöka halter av miljögifter i okläckbara ägg/rötägg från två rovfåglar som är sorkpredatorer, en stationär art - kattuggla (*Strix aluco*) och en migrerande art – tornfalk (*Falco tinnunculus*). Det har även varit att undersöka om insamlingsmetoden via licensierade ringmärkare kan fungera för insamlingen av rötägg för miljögiftövervakning.

En förfrågan gick via Ringmärkningscentralen (RC) på Naturhistoriska riksmuseet (NRM), ut till samtliga ringmärkare som ringmärker kattuggla och/eller tornfalk. Sexton stycken anmälde intresse för att delta och tio av dessa skickade in ägg under sommaren 2014.

Totalt kom det in 14 ägg från kattuggla och 86 ägg från tornfalk. Den geografiska spridningen när det gällde kattuggla var Skåne, Småland/Öland, Västergötland och Hälsingland.

De 86 tornfalkäggen kom från Skåne, Västmanland, Dalarna, Hälsingland, Västerbottens kustland och södra Lappland (inland).

Tio ägg av kattuggla och 40 från tornfalk analyserades på kvicksilver (totalkvicksilver) och fjorton perfluorerade substanser (PFPeA, PFBS, PFHxA, PFHpA, PFHxS, PFOS, PFDS, PFOA, PFNA, PFDA, PFUnDA, PFDODA, PFTrDA, PFTDA). Dessutom analyserades ägginnehållet på stabila isotoper av kväve ( $^{15}\text{N}$ ) och kol ( $^{13}\text{C}$ ). Ytterligare ett ägg, dvs 11 kattuggleägg och 41 tornfalkägg analyserades på pesticider (HCB,  $\alpha$ -, $\beta$ -, $\gamma$ -HCH, pp-DDE, pp-DDD, pp-DDT), sju PCBer (CB-28, CB-52, CB-101, CB-118, CB-(138+163) CB-153, CB-180), fem PBDEer (BDE-47, BDE-99, BDE-100, BDE-153, BDE-154) samt HBCD.

Halten, median (min-max) av kvicksilver var 72, 4 (19,3-150) ng/g ww i ägg från kattuggla och 64,3 (7,8 -230) ng/ g ww i ägg från tornfalk. Skillnaden var inte signifikant. Ingen signifikant geografisk skillnad i halter av kvicksilver hittades i äggen från varken kattuggla eller tornfalk.

HCB hittades i alla analyserade ägg av både kattuggla och tornfalk. Halterna var högre i tornfalk jämfört med kattuggla men skillnaden var inte signifikant.  $\beta$ -HCH hittades i drygt 80% av kattuggleäggen och i 95% av tornfalkäggen. För  $\beta$ -HCH var halterna signifikant högre i tornfalk.  $\alpha$ -HCH och  $\gamma$ -HCH (Lindan) hittades inte i något ägg. pp-DDE hittades i samtliga analyserade ägg av både kattuggla och tornfalk. Halterna var signifikant högre i kattuggla. pp-DDT hittades i drygt 70% av äggen från både tornfalk och kattuggla och halterna var högre i kattuggla men skillnaden var inte signifikant. En geografisk skillnad i halter av HCB och  $\beta$ -HCH hittades hos kattuggla med högre halter i ägg från Skåne jämfört med ägg från Småland och Västergötland. Ingen motsvarande geografisk skillnad för dessa

ämnen hittades i tornfalkägg. Även pp-DDE var högre i kattuggleägg från Skåne medan ingen motsvarande geografisk skillnad hittades för tornfalk.

CB-118, (138+163), 153 och 180 hittades i samtliga analyserade ägg från kattuggla och CB-118, 153 och (138+163) hittades i samtliga analyserade ägg från tornfalk. CB-180 hittades i alla tornfalksägg utom ett. Halterna för dessa PCBer var signifikant lägre i tornfalk. CB-52 och 101 hittades inte i något tornfalksägg men fanns i 18 respektive 54% av kattuggleäggen. Även för CB-118, 153, 138+163 och 180 var halterna högre i kattuggleägg från Skåne. Samma tendens kunde ses i tornfalksäggen.

BDE-47, BDE-99, BDE-100 and BDE-153 hittades i alla analyserade ägg av kattuggla medan BDE-153 var den enda bromerade substansen som hittades i samtliga tornfalkägg. Halterna var signifikant högre i kattuggla för BDE-47, BDE-99 och BDE-110 men inte för BDE-153. HBCD hittades i 7 (64%) kattuggleägg och i endast ett tornfalkägg. Även för bromerade substanser tenderade halterna att vara högre i de skånska äggen, dock inte signifikant i något fall. Det fanns en tydlig skillnad i kongenermönster mellan kattuggla och tornfalk där BDE-153 dominerade i tornfalk medan BDE-99 var mest förekommande hos kattuggla. BDE-47 saknades nästan helt hos tornfalk.

Sex perfluorerade ämnen PFOS, PFNA, PFDA, PFUnDA, PFDoDA och PFTrDA hittades i samtliga analyserade ägg från kattuggla medan PFOS var den enda perfluorerade substansen som hittades i samtliga ägg från tornfalk. PFNA, PFUnDA och PFTRDA hittades i mer än 80% av tornfalksäggen.  $\Sigma$ PFAAs var signifikant högre i ägg från kattuggla jämfört med i ägg från tornfalk. Även för ett par perfluorerade substanser (PFOS, PFDA) fanns tendenser till högre halter i ägg från Skåne hos kattuggla. Hos tornfalk fanns tendenser till högre halter av PFOS i ägg från Skåne.

Värdet för den stabila isotopen  $\delta^{15}\text{N}$  låg mellan 5,44 och 9,4 hos kattuggla och mellan 3,44 och 8,43 hos tornfalk och det fanns en tydlig syd nordlig gradient med sjunkande värden från söder till norr för båda arterna. Hos tornfalk fann det också stora skillnader (upp mot 6‰) mellan ägg från samma område. Värdet av  $\delta^{13}\text{C}$  var -28,14 - -27,85 hos kattuggla och -29,2 - -28,1 hos tornfalk. Inga geografiska skillnader kunde hittas för någongdera arten.

Sannolikt ger halterna i ägg från kattuggla, som är en stannfågel en bättre bild av gifter i miljön där fåglarna häckar. De flesta tornfalkar flyttar till södra eller sydvästra Europa under vintermånaderna och det går inte att utesluta att åtminstone en del av miljögiftbelastningen i äggen kommer från falkarnas övervintringsområden. Tornfalk har en bättre geografisk spridning jämfört med kattuggla och under 2014 var det relativt lätt att få in tornfalksägg från i princip hela landet. Det område som saknas för tornfalk är sydsverige, söder om Mälardalen

undantaget Skåne. För kattuggla å andra sidan saknas ägg norr om Mälardalen, undantaget ett ägg från Hälsingland. Det var också en stor skillnad i antalet ägg som kom in från tornfalk (86 st) respektive kattuggla (14 st). En orsak kan vara att 2014 var ett år med dålig reproduktion hos kattuggla vilket flera ringmärkare påpekade.

Metoden att få in rötägg via licensierade ringmärkare bygger på ett stort mått av frivillighet och personligt engagemang från dessa. Engagemanget finns och alla som deltog ska ha ett stort tack.

## Summary

The present study has been carried out on mandate and in cooperation with the Swedish Environmental Protection Agency (SEPA) according to agreement 2220-14-004.

The aim has been to introduce a predator species into the monitoring of environmental contaminants in the terrestrial environment. Unhatchable/ addled eggs of raptors have long been used in environmental research and monitoring of contaminants. The Swedish Museum of Natural History (SMNH) houses the Bird Ringing Centre that issues certificates for bird ringing all over Sweden. Bird ringers visit nests to ring fledglings and in connection with that, they also count the number of fledglings and the number of unhatched eggs. Unhatched eggs are then considered as unhatchable. In 2014, a number of bird ringers specialized in tawny owl (*Strix aluco*) and common kestrel (*Falco tinnunculus*) were contacted and asked to participate by sending unhatched eggs of these two species to the SMNH.

In all, 14 tawny owl and 86 common kestrel eggs were sent to the SMNH. The tawny owl eggs came from the counties of Skåne, Småland, Västergötland and Hälsingland. The common kestrel eggs came from Skåne, Västergötland, Dalarna, Hälsingland, Västerbotten and southern Lappland.

Ten eggs of tawny owl and 40 eggs of common kestrel were chosen for analyses of mercury (total mercury), fourteen perfluorinated compounds (PDPeA, PFBS, PFHxA, PFHpA, PFHxS, PFOS, PFDS, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTTrDA, PFTDA) and also for stable isotopes N<sup>15</sup> and C<sup>13</sup>. Eleven eggs of tawny owl and 41 eggs of common kestrel, i.e. an additional egg from each species, were analysed for chlorinated pesticides (HCB,  $\alpha$ -HCH,  $\beta$ -HCH,  $\gamma$ -HCH, pp-DDE, pp-DDD, pp-DDT), seven PCBs (CB-28, CB-52, CB-101, CB-118, CB-153, CB-(138+163), CB-180), and a number of brominated compounds (BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, HBCD),

Mercury levels (median; min-max) were 71,4 (19,3-150) ng/g ww in eggs from tawny owl and 64,3 (7,8-230) ng/g ww in eggs from common kestrel. The difference was not significant. No geographical differences in mercury levels were found for either tawny owl or kestrel. HCB was found in all of the analysed eggs from both species. Levels were somewhat higher in kestrels but the difference was not significant.  $\beta$ -HCH was found in 80% and 95% of the eggs from tawny owl and common kestrel respectively. For  $\beta$ -HCH, the levels were significantly higher in common kestrel.  $\alpha$ - and  $\gamma$ -HCH (lindan) was not found in any of the analysed eggs. pp-DDE was found in all of the analysed eggs. The levels were significantly higher in tawny owls compared with common kestrel. pp-DDT was found in approximately 70% of the eggs of both species. A geographical difference, with significantly higher levels in eggs from Skåne, was found in tawny owl but not in common kestrel.

CB-118, CB-(138+163), CB-153 and CB-180 were found in all eggs of tawny owl and CB-118, CB-(138+163), and CB-153 were found in all eggs of common kestrel. CB-180 was found in all but one of the common kestrel eggs. The levels of these PCBs were significantly higher in tawny owl. CB-52 and CB-101 were found in 18 and 54% of the eggs from tawny owl but in none of the eggs from common kestrel. There was a tendency towards higher levels of PCBs in eggs from Skåne for both tawny owl and common kestrel. For some congeners this was significant.

BDE-47, BDE-99, BDE-100 and BDE-153 were found in all eggs from tawny owl while BDE-153 was the only brominated compound that was found in all eggs from common kestrel. BDE-47, BDE-99 and BDE-100 but not BDE-153 were significantly higher in tawny owl. BDE-154 was not possible to quantify.

HBCD was found in 7 (64%) of the eggs from tawny owl and in one egg from common kestrel. There was a substantial difference in congener pattern between tawny owl and common kestrel with BDE-153 being the dominant congener in common kestrel while BDE-99 was more abundant in tawny owl. BDE-47 was almost absent in the eggs from common kestrel.

Six perfluorinated substances PFOS, PFNA, PFDA, PFUnDA, PFDoDA and PFTrDA were found in all eggs of tawny owl. PFOS was the only perfluorinated substance that was found in all of the common kestrel eggs while PFNA, PFUnDA and PFTrDA were found in more than 80% of the common kestrel eggs.  $\Sigma$ PFAAs were significantly higher in tawny owls. For some of the perfluorinated substances (PFOS, PFDA in tawny owl and PFOS in common kestrel) there was a tendency towards higher levels in eggs from the southernmost part of Sweden (Skåne).

The value of the stable isotope  $\delta^{15}\text{N}$  was between 5,44 and 9,4 in tawny owl and between 3,44 and 8,43 in common kestrel and there was a clear difference in south- north direction with the highest values in the south for both species. There was also a large individual variation in  $\delta^{15}\text{N}$  values in eggs from the same area for common kestrel. The value of  $\delta^{13}\text{C}$  was -28,14 - -27,85 for tawny owl and -29,2 - -28,1 for common kestrel and no geographical differences were found for either species.

The conclusion from the present study is that tawny owls generally had somewhat higher levels of contaminants than common kestrel. The contaminant level in eggs from southern Sweden (Skåne) was generally higher both in tawny owl and common kestrel. The large difference in congener pattern between kestrels and owls could be an indication of differences in metabolic capacity between the species. The larger variation in  $\delta^{15}\text{N}$  values in common kestrel could indicate that kestrels have a larger variation in diet but there can also be other explanations.

Tawny owls probably give a better picture of the contaminant level in the breeding area as they live their life within a fixed territory. Most common kestrels migrate to southern and southwestern Europe during the winter months and it cannot be ruled out that some of the burden of contaminants detected in the eggs originate from the kestrels' wintering grounds. The geographical distribution of common kestrel is larger than for tawny owls and at least in 2014 it was relatively easy to get common kestrel eggs from larger parts of Sweden, with the exception of the area between Mälardalen and Skåne. For tawny owls, on the other hand, eggs from the area north of Mälardalen were not collected. There is also a large difference in the number of eggs that could be collected from common kestrel (86) compared with tawny owl (14). A reason for this could be that tawny owls had a low reproduction in 2014, as some of the bird ringers pointed out.

Collecting addled/unhatchable eggs with the help of licensed bird ringers is largely dependent on voluntary work and personal interest and all of those who participated are greatly acknowledged.

## **Aim**

This work was carried out on request by and in cooperation with the Swedish Environmental Protection Agency (SEPA).

The aim was to investigate the possibility of using unhatchable/addled eggs of two vole-predators, tawny owl and common kestrel, in the monitoring of environmental contaminants in terrestrial biota.

## **Organisation**

The material used (addled eggs of tawny owls and common kestrel) were collected by bird ringers, licensed by the Bird Ringing Centre at the SMNH, with permission for collecting eggs from the SEPA. Preparation and storage were performed by staff at the Department of Environmental Monitoring and Research at SMNH. Chemical analyses of chlorinated and brominated compounds were performed by Department of Applied Environmental Science (ACES, Stockholm University). Perfluorinated compounds were analysed by MTM Research Centre, Örebro University. Total mercury was analysed by IVL Swedish Environmental Research Institute. Stable isotopes were analysed by MTM Research Centre Örebro University.

Funding (2220-14-004) were provided by SEPA.

## **Introduction**

Birds of prey are at the top of the food chain and consequently suitable for monitoring biomagnifying pollutants. Eggs that fail to hatch are especially useful for monitoring contaminants because their collection does not cause negative effects on the bird population. In Sweden, unhatchable eggs of white-tailed sea eagles, ospreys, and peregrine falcons, have been collected and analysed for contaminants (Odsjö 1971; Helander, Bignert et al. 2008; Nordlof, Helander et al. 2010; Johansson, Sellstrom et al. 2011; Nordlof, Helander et al. 2012; Nordlöf, Helander et al. 2012).

In this report we compare the levels of chlorinated pesticides, PCBs, brominated flame retardants, perfluorinated compounds and mercury in addled eggs of two birds of prey, tawny owl and common kestrel. Both these species are relatively common in Sweden and feed in a terrestrial food chain



## **Materials and methods**

### **Species**

#### **Tawny owl**

Tawny owl (*Strix aluco*) belongs to the Strigidae family. It is a resident bird and couples stay together in a well-established territory all their lives. It is a rather common owl in Sweden and is found both in populated areas (parks, church yards, gardens) as well as in forests and agricultural areas. Its main food is small rodents but it can also feed on birds, worms and insects. Data on the “normal” life time of tawny owls are scarce but a probable lifespan is 6-8 years for birds that survive their first year.

#### **Common kestrel**

Common kestrel (*Falco tinnunculus*) belongs to the Falconidae family. In Sweden, common kestrels are usually migratory and the winter is spent in western and southwestern Europe. The common kestrel is, as the name implies, common and in Sweden it is found all over the country. It feeds on small rodents but also on frogs and insects. As for tawny owls, it is hard to find data on “normal” life span for common kestrel but 4-5 years is a probable life span for a bird that survives its first year.

### **Collection**

Each year, young birds from a large number of species are banded by bird ringers with license from the Bird Ringing Center at SMNH. The number of banded birds and also unhatched eggs are reported each year. Bird ringers that marked tawny owls and common kestrels were contacted through the Bird Ringing Renter and were asked to collect the unhatched eggs that were found and send them to the SMNH. Unhatched eggs that were found at this time were eggs that would not hatch and their collection will not affect the bird population.

Ten bird ringers send a total of 100 eggs to the Department of Environmental Monitoring and Research at the SMNH.

The collectors were asked to store the eggs refrigerated until they were sent to the SMNH. Each egg was placed in a polyethene bag and in a separate plastic container to avoid cross contamination in case the egg was broken.

All eggs were collected in 2014.

## Geographical areas

The collection method used in this study was dependent on both the existence of breeding birds in the area, and that a licensed bird ringer was available to collect eggs.

Eggs of tawny owls were mostly available from the southern parts of Sweden (Skåne, Småland, Västergötland). There is also substantially less ringing of tawny owls compared to common kestrel. This resulted in a total of 14 eggs from tawny owls.

Eggs of common kestrel were available from more areas and a larger part of Sweden was covered. The majority of kestrel eggs came from central Sweden (Västmanland, Dalarna) and the northern coastal regions (Västerbotten). In all, 86 eggs of common kestrel were collected. The geographical distribution of all eggs collected in 2014 is shown in Table 1. The number of analysed eggs is shown in brackets.

Table 1. Geographical distribution of eggs from tawny owl (*Strix aluco*) and common kestrel (*Falco tinnunculus*) collected in 2014. Numbers in brackets refers to number of analysed eggs in this study.

	Tawny owl	Common kestrel
Skåne (SK)	4 (3+1 <sup>1</sup> )	4 (3)
Småland (inkl Öland)(SM)	5 (3)	
Västergötland (VG)	4 (4)	
Närke (N)		1
Västmanland (VM)		17 (10)
Dalarna (DA)		20 (12)
Hälsingland (HL)	1	9 (6)
Västerbotten (VB)		32 (7+1 <sup>1</sup> )
Lappland (LL)		3 (2)

<sup>1</sup>An additional egg of tawny owl and common kestrel were analysed for chlorinated and brominated compounds.

## Sampling and preparation

The eggs were placed in a refrigerator on arrival at the SMNH. The eggs were weighed and the length and breadth of the eggs were measured. The content of the eggs were removed, homogenized and frozen. The eggshell was dried to constant weight, after which it was weighed and the thickness of the eggshells was measured.

Ten eggs from tawny owls and forty eggs from common kestrel were selected for analysis of environmental contaminants and stable isotopes of carbon (<sup>13</sup>C) and nitrogen (<sup>15</sup>N).

The eggs were chosen to get a good representative of different geographical regions (Table 1). Primarily, eggs with no traces of an embryo were chosen for analyses, but eggs that contained

small embryos were also used. The condition of the egg was also taken into account and eggs that deemed to be in too bad a condition were not used.

### **Eggshell parameters**

An eggshell index (relative weight) was calculated according to Ratcliffe (1970).

Eggshell index = Eggshell weight (mg) / Length (mm) \* Breadth (mm)

### **Analysed compounds**

The compounds analysed in this study were mercury (total Hg), bromodiphenylethers (BDE-47, BDE-99, BDE-100, BDE-153, BDE-154), hexabromocyclododecane (HBCD) (table 2), polychlorinated biphenyls (CB-28, CB-52, CB-101, CB-118, CB-153, CB-(138+163), CB-180), hexachlorobenzene (HCB), hexachlorocyclohexane ( $\alpha$ -, $\beta$ -, $\gamma$ -HCH), *p,p'*-dichlorodiphenyltrichloroethane (pp-DDT), *p,p'*-dichlorodiphenyldichloroethylene (pp-DDE), *p,p'*-dichlorodiphenyldichloroethane (pp-DDD) (Table 3), ten perfluorinated carboxylic acids (6-14 carbon chain length), and four perfluorinated sulfonic acids (4-10 carbon chain length) (Table 4). Moreover, stable isotopes of carbon ( $^{13}\text{C}$ ) and nitrogen ( $^{15}\text{N}$ ) were analyzed. All compounds were analysed on homogenized egg content. Results are presented on wet weight basis for total Hg and perfluorinated compounds (PFAAs) and on lipid weight basis for chlorinated and brominated compounds.

### *Mercury*

Mercury was analysed by IVL (Swedish Environmental Research Institute). Total mercury was analysed with acid digestion followed by purge and trap on gold trap and cold vapour atomic fluorescence spectroscopy (CVAFS).

The LOQ was 0,1 ng/g and the LOD was 0,03 ng/g for THg. Only total mercury (THg) was analysed. According to (Ackerman, Herzog et al. 2013), a mean of 96% of the mercury analysed in eggs from 22 species was methylmercury (MeHg), indicating that the THg value is a reliable substitute for MeHg in eggs.

## Chlorinated and brominated compounds

### *Analysis of brominated flame retardants*

The samples of 10 g were extracted with a mixture of acetone/*n*-hexane and *n*-hexane/diethyl ether. The organic phase was liquid/liquid partitioned with a solution of sodium chloride/phosphoric acid. The aqueous phase was re-extracted with *n*-hexane and the combined organic phases were evaporated to dryness. The lipid content was determined gravimetrically. After treatment of the dissolved lipid extract with concentrated sulfuric acid (Jensen *et al.*, 1983), the samples were analysed by gas chromatograph/mass spectrometry (GC-MS) in electron capture ionization (ECNI) mode. A 30 m TG-5SilMS fused silica column (0.25 mm i.d., 0.25 µm film thickness) was used for the brominated analytes. Ammonia was used as the reaction gas. The mass fragments monitored were *m/z* 79 and 81 for all brominated compounds and *m/z* 237 and 239 for Dechlorane, used as internal standard (Sellström, Bignert *et al.* 2003).

### *Analysis of chlorinated substances*

The samples for the analysis of the chlorinated substances were extracted and cleaned-up in the same way as the brominated substances but analysed by a gas chromatograph equipped with an EC-detector. Two fused capillary columns of 60 m (0.25 mm i.d, 0.25 µm film thickness) were used in parallel, one TG-5MS and one DB-1701. Argon/Methane was used as make-up gas and CB 53 as internal standard (Eriksson, Häggberg *et al.* 1997).

The analyses were carried out by the Department of Applied Environmental Science (ACES), Stockholm University.

Table 2. Brominated compounds and LOQ (ng/g lipid weight) for analysis in addled eggs of tawny owl (*Strix aluco*) and common kestrel (*Falco tinnunculus*) collected from different provinces of Sweden in 2014.

	CAS-nr	LOQ <sup>1</sup>
BDE-47	5436-43-1	0,2
BDE-99	60348-60-9	0,2
BDE-100	189084-64-8	0,2
BDE-153	68631-49-2	0,2
BDE-154 <sup>2</sup>	207122-15-4	0,2
hexabromocyclododekane (HBCD)	3194-55-6	2

<sup>1</sup>General LOQ values, is depending on the lipid content of the samples.

<sup>2</sup>Coeluted with a hexabrombiphenyl (BB-153) and was not possible to quantify

Table 3. Chlorinated compounds and LOQ (ng/g lipid weight) for analysis in addled eggs of tawny owls (*Strix aluco*) and common kestrel (*Falco tinnunculus*) collected from different provinces of Sweden in 2014.

	CAS-nr	LOQ <sup>1</sup>
polychlorinated biphenyl (PCB)	1336-36-3	
PCB 28	7012-37-5	2
PCB 52	35693-99-3	2
PCB 101	35680-73-2	2
PCB 118	31508-00-6	2
PCB (138+163)		2
PCB 153	35065-27-1	2
PCB 180	35065-29-3	2
Hexachlorobenzene HCB	118-74-1	2
hexachlorocyclohexane $\alpha$ -HCH	319-84-6	2
hexachlorocyclohexane $\beta$ -HCH	319-85-7	2
hexachlorocyclohexane $\gamma$ -HCH (lindan)	55963-79-6	2
p,p-DDT	50-29-3	4
p,p-DDE	72-55-9	2
p,p-DDD	72-54-8	2

<sup>1</sup>General LOQ values, is depending on the lipid content of the samples

#### Perfluorinated compounds (PFAAs)

Samples were stored in plastic containers at  $-20^{\circ}\text{C}$  until analysis. Names and abbreviations of all PFAAs are listed in Appendix A together with information of internal standards and performance standards. Thawed egg samples were fortified with labeled standards and extracted using acetonitrile. After clean-up using dispersive carbon, analyses was performed on an Acquity UPLC TQS tandem mass spectrometer (Waters Corporation, Milford, USA) system by injecting 10  $\mu\text{L}$  onto a C18 ( $2.1 \times 100 \text{ mm}$ , 7  $\mu\text{m}$ ) analytical column using a water and methanol gradient with 2mM ammonium acetate. Analytes were analyzed on the MS/MS system run in electrospray ionization mode (ESI).

#### Quantification and quality assurance

Multiple reaction monitoring (MRM) was used and, when possible, two product ions were monitored for each compound (see Appendix A). Analytes were quantified using solvent calibration curves and isotope dilution. A minimum of five-point calibration curve was used. The internal standard closest in retention time was used for those compounds that did not have a corresponding labeled internal standard (PFBS, PFDS, PFPeA, PFHpA). The ratio between the two product ions in the samples were calculated and compared to an authentic standard, and did not exceed 50%. The performance standard was used to assess the recovery of the internal standard. Recoveries of internal standards for all egg samples are presented in Table 2 (Appendix A). An acceptable recovery limit of 50-120% was set. Recoveries between 20 and 50% were decided to be reported as reliable since authentic internal standards were used for quantification; those results are however marked in the result tables. Procedural blanks (water and low contaminated egg sample) were included in each extraction batch and treated the same way as the samples. Limit of detection (LOD) was calculated as mean concentration in blank samples with addition of three times the standard deviation. Quality

control samples (fish muscle and fortified egg) were included in each batch to assess reproducibility and accuracy.

Fourteen perfluorinated compounds (PFAAs) was analysed (Table 4)  
The analyses on PFAAs were carried out by the MTM Research Centre, School of Science and Technology, Örebro University

Table 4. Perfluorinated compounds (PFAAs) and LOQ (ng/g ww) for analysis in addled eggs of tawny owl and common kestrel. Terminology and acronyms from (Buck, Franklin et al. 2011).

		CAS-nr <sup>1</sup>	LOQ
Perfluorohexanoic acid (C <sub>6</sub> )	PFH <sub>x</sub> A	307-24-4	<1
Perfluoroheptanoic acid (C <sub>7</sub> )	PFH <sub>p</sub> A	375-85-9	<0,2
Perfluorooctanoic acid (C <sub>8</sub> )	PFOA	335-67-1	<1,5
Perfluorononanoic acid (C <sub>9</sub> )	PFNA	375-95-1	<0,2
Perfluorodecanoic acid (C <sub>10</sub> )	PFDA	335-76-2	<0,5
Perfluoroundecanoic acid (C <sub>11</sub> )	PFUnDA	2058-94-8	<0,35
Perfluorododecanoic acid (C <sub>12</sub> )	PFDoDA	307-55-1	<0,4
Perfluorotridecanoic acid (C <sub>13</sub> )	PFT <sub>r</sub> DA	72629-94-8	<0,4
Perfluorotetradecanoic acid (C <sub>14</sub> )	PFTDA	376-06-7	<0,2
Perfluorobutane sulfonic acid (C <sub>4</sub> )	PFBS	375-73-5	<0,2
Perfluorohexane sulfonic acid (C <sub>6</sub> )	PFH <sub>x</sub> S	355-46-4	<0,2
Perfluorooctane sulfonic acid (C <sub>8</sub> )	PFOS <sup>2</sup>	1763-23-1	<0,2
Perfluorodecane sulfonic acid (C <sub>10</sub> )	PFDS	335-77-3	<0,1

<sup>1</sup>Cas-nr for the acid form

<sup>2</sup>n-PFOS

### Stable isotope analysis

The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  as well as the C and N contents of freeze dried samples (0.6 - 0.7 mg) were determined using an elemental analyser (model EuroEA3024; Eurovector, Milan, Italy) coupled on line to an Isoprime isotope-ratio mass spectrometer (GV Instruments, Manchester, UK). The results are expressed ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) in parts per thousand (‰) relative to their international standards Vienna Pee Dee Belemnite (V-PDB) and atmospheric N<sub>2</sub>, where  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N} = [(R_{\text{sample}} - R_{\text{standard}})/R_{\text{standard}}] \times 1000$  ‰, and R is the molar ratio of  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$ . The standard deviation of ten replicated analyses of the same egg sample was 0.08 ‰ for  $\delta^{13}\text{C}$  and 0.07 for  $\delta^{15}\text{N}$ .

Analysis of stable isotopes was performed at MTM Research Centre, School of Science and Technology, Örebro university

### Statistics

Non-parametric statistics were used. A Mann-Whitney U-test was used to test significant differences between the species. A Kruskal-Wallis ANOVA was used to detect differences in contaminant levels between different regions for each species. The significance level was set to  $p < 0,05$ . If statistical significance was found in the Kruskal-Wallis ANOVA, no attempt was

made to further evaluate the data statistically. Values <LOQ have been excluded from the statistical evaluation.

Statistical analysis was performed using StatSoft, Inc. (2011). STATISTICA (data analysis software system), version 10. [www.statsoft.com](http://www.statsoft.com)

## Results

### Lipid content

The median (min-max) lipid content of the analysed egg samples was 8,0 % (5,9-9,0) in tawny owl (n=11) and 6,3 % (4,6-8,4) in common kestrel (n=41).

### Eggshell parameters

Shell thickness and shell index for tawny owl and common kestrel are shown in Table 5

Table 5. Shell thickness and shell index in eggs from tawny owl (*Strix aluco*) and common kestrel (*Falco tinnunculus*) collected from different parts of Sweden in 2014. Median (min-max).

	tawny owl	common kestrel
shell thickness (mm)	0,3 (n=11) 0,25-0,39	0,26 (n=41) 0,21-0,31
shell index	1,61 (n=8) 1,34-1,85	1,32 (n=38) 1,03-1,55

### Mercury

The median (min-max) level of TotHg in all eggs of tawny owls (n=10) was 72 (19-150) ng/g ww. For common kestrel eggs (n=40), the corresponding values were 64 (7,8-230) ng/g ww.

Mercury was found > LOQ (0,1 µg/g ww) in all of the analysed samples.

No significant difference was found between eggs of tawny owls and kestrels.

#### Geographical differences

The TotHg levels in eggs from different geographical regions are shown in Fig 1 (and in Table 1, Appendix B). The highest level (150 ng/g ww) in tawny owl was found in an egg from the district of Nässjö in Småland and the lowest level (19 ng/g ww) was found in an egg from the district of Svedala in Skåne. The two highest TotHg levels in common kestrel (230 and 190 ng/g ww) were found in two eggs from the same locality in the district of Leksand in Dalarna. The lowest level (7,8 ng/g ww) was found in an egg from the district of Köping in

Västmanland. No significant differences were found in mercury levels between different regions for either tawny owls or kestrels

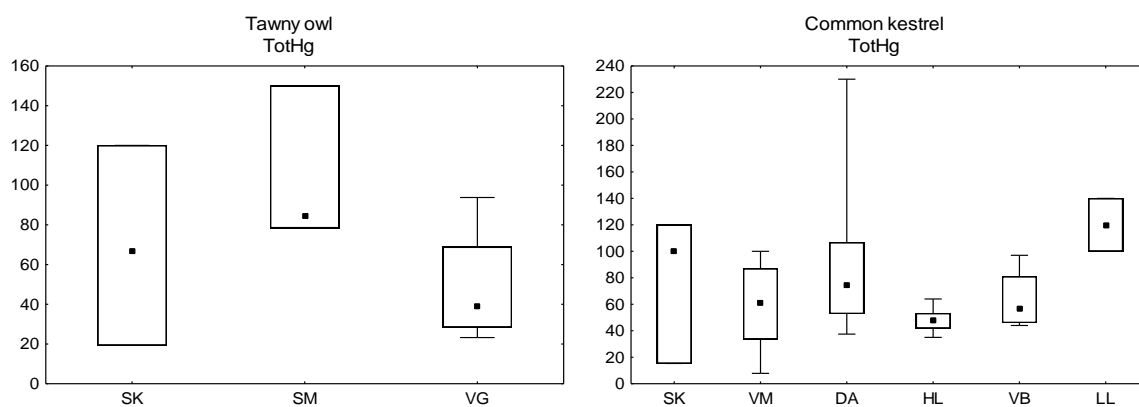


Figure 1. Mercury (ng/g ww) in addled eggs of tawny owl and common kestrel collected from different areas in Sweden in 2014.

## Brominated compounds

The median (min-max) concentration of brominated compounds in eggs of tawny owl and common kestrel are shown in Table 6.

BDE-153 was detected > LOQ in all eggs of both tawny owl and common kestrel. BDE-47, BDE-99, and BDE-153 were detected > LOQ in all eggs of tawny owl. For common kestrel BDE-47, BDE-99 and BDE-100 was detected in 17%, 88% and 66% of the eggs. BDE-154 was not possible to quantify (see materials and methods, Table 2).

HBCD was found in 64% of the eggs from tawny owl and in one egg from common kestrel. The levels of BDE-47, BDE-99, and BDE-100 as well as  $\Sigma$ PBDE was significantly higher in tawny owl ( $p < 0,05$ ) compared with common kestrel.

In tawny owls, the relative distribution between BDE-47, BDE-99 and BDE-153 were fairly equal while BDE-153 was the dominating congener in common kestrel (Fig 2).



Table 6. Brominated compounds (ng/g lw) analysed in addled eggs of tawny owl (*Strix aluco*) and common kestrel (*Falco tinnunculus*) collected in 2014. N refers to the number of eggs with levels >LOQ. For LOQ see table 2.

	tawny owl		common kestrel	
	median (min-max)	N	median (min-max)	N
BDE47 <sup>A</sup>	2,66 (0,518-9,02)	11	0,536 (0,033-2,50)	7
BDE99 <sup>A</sup>	5,89 (0,721-17,9)	11	0,415 (0,116-3,49)	36
BDE100 <sup>A</sup>	1,41 (0,381-5,77)	11	0,190 (0,007-1,52)	27
BDE153	2,63 (0,849-10,7)	11	2,34 (0,216-15,4)	41
HBCD	1,15 (0,930-3,68)	7	0,635	1
<b>sPBDE<sup>A</sup></b>	<b>12,6 (2,52-40,5)</b>	<b>11</b>	<b>3,13 (0,410-16,1)</b>	<b>41</b>

<sup>A</sup>significant difference between the species (p<0,05 Mann-Whitney U-test)

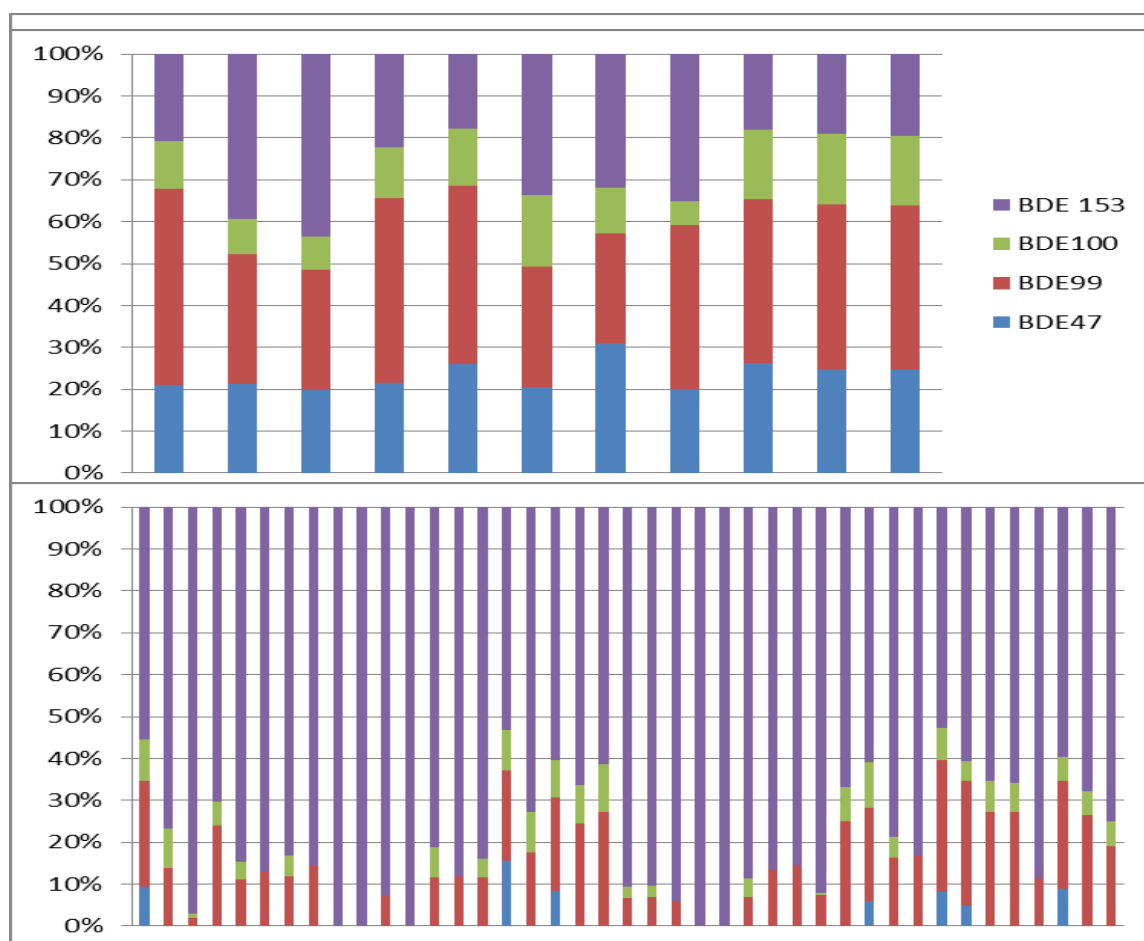


Figure 2. Relative distribution of BDE47, BDE99, BDE100, and BDE153 in addled eggs of tawny owl (above) and common kestrel (below) collected from different provinces in Sweden in 2014.

### Geographical difference

Geographical differences for  $\Sigma$ PBDE are shown in Fig 3, and for each congener separately in Fig 4 (Table 2 and 3, Appendix B). There was a tendency towards higher levels of brominated compounds in eggs from southern Sweden for both species but it was not significant.

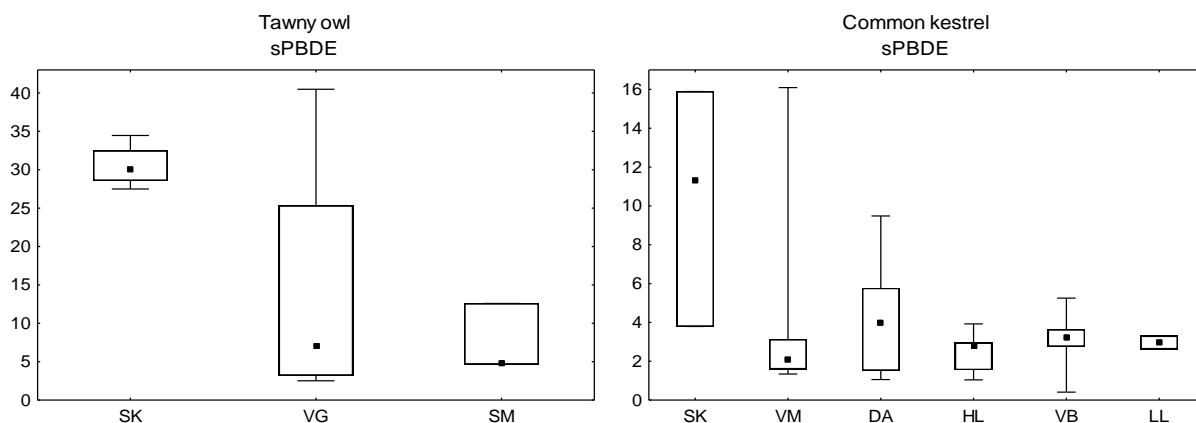


Figure 3.  $\Sigma$ PBDE (ng/g lw) in addled eggs of tawny owl and common kestrel collected from different areas in Sweden in 2014.

### Comments on brominated compounds

The congener pattern differed markedly between tawny owl and common kestrel.

BDE-99 had the highest median levels in tawny owl followed by BDE-47 and BDE-153. The same congener pattern was seen in a Norwegian study on brominated compounds in eggs from tawny owl (Bustnes, Yoccoz et al. 2007).

In common kestrel on the other hand, BDE-153 was by far the dominating congener followed by BDE-47 and BDE-99. BDE-153 was also the only congener found in all common kestrel eggs. In common kestrel, BDE-99 and 47 had approximately the same median value, 0,415 ng/g lw and 0,536 ng/g lw respectively. However, BDE-99 was found in 36 out of 41 eggs while BDE-47 was found in only 7 eggs. In eggs where BDE-47 was detected it constituted between 5 and 15% of the  $\Sigma$ PBDE and no geographical pattern could be detected. In tawny owl BDE-47 constituted between 20 and 30 % of the  $\Sigma$ PBDE.

The congener pattern indicates a terrestrial food chain with lower values of BDE-47 and higher values of BDE-99 and 153. The absence of BDE-47 in most of the common kestrel eggs could indicate a biotransformation of this congener in kestrels, as has previously been reported in the American kestrel (*Falco sparverius*) (Fernie, Laird Shutt et al. 2006)

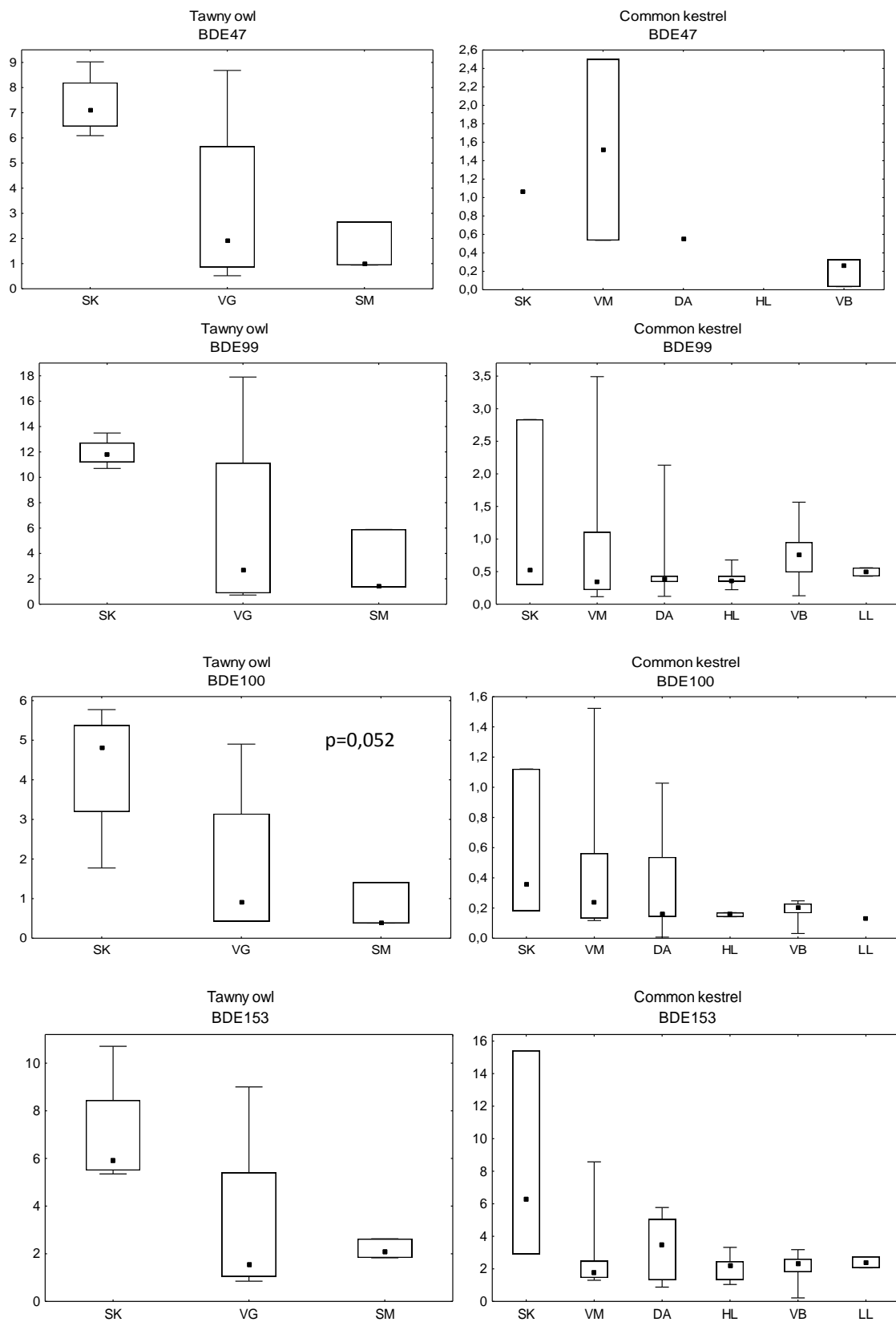


Figure 4. BDE-47, BDE-99, BDE-100 and BDE-153 in added eggs of tawny owl and common kestrel collected from different provinces of Sweden in 2014.

## Chlorinated compounds

The median (min-max) concentrations of chlorinated compounds in eggs of tawny owl and common kestrel are shown in Table 7.

### *Pesticides*

pp-DDE was found in all eggs of tawny owl (n=11) and common kestrel (n=41). HCB was found in all eggs from tawny owl and in 98% of the eggs from common kestrel.

$\beta$ -HCH, pp-DDT and pp-DDD were found >LOQ in 82%, 73% and 64% of the eggs from tawny owl and in 95 %, 71% and 32% of the eggs from common kestrel.

$\beta$ -HCH was significantly higher in common kestrel compared with tawny owl. HCB was also higher in common kestrel but this difference was not significant (Table 7).

pp-DDE, pp-DDD and  $\Sigma$ DDT were significantly higher in tawny owl (Table 7).

### *PCB*

CB-118, CB-153, and CB-(138+163) were found in all eggs from tawny owl (n=11) and common kestrel (n=41). CB-180 was also found in all the tawny owl eggs and in 98% of the common kestrel eggs. CB-28, CB-52 and CB-101 were found in 45%, 18% and 54% of the tawny owl eggs. CB-28 was found in 29% of the common kestrel eggs. CB-52 and CB-101 were not found >LOQ in any of the analysed eggs of common kestrel.

CB-118, CB-153, CB-(138+163) and CB-180 were significantly higher in eggs from tawny owls compared to in eggs from common kestrel (Table 7).

Table 7. Chlorinated compounds (ng/g lw) analysed in addled eggs of tawny owl (*Strix aluco*) and common kestrel (*Falco tinnunculus*) collected in 2014. N refers to the number of eggs with levels >LOQ. For LOQ see table 3.

	tawny owl		common kestrel	
	median (min-max)	N	median (min-max)	N
HCB	29,1 (9,75-66,5)	11	34,2 (20,7-1923)	40
$\beta$ -HCH <sup>A</sup>	3,36 (1,40-5,17)	9	6,10 (2,31-188)	39
pp-DDE <sup>A</sup>	476 (143-4846)	11	334 (18,5-2454)	41
pp-DDD <sup>A</sup>	10,7 (2,32-19,6)	7	1,97 (1,02-24,5)	13
pp-DDT	16,6 (2,53-25,0)	8	5,65 (1,74-21,8)	29
$\Sigma$ DDT <sup>A</sup>	<b>521 (143-4880)</b>	<b>11</b>	<b>338 (18,5-2473)</b>	<b>41</b>
CB-28	40,7 (1,58-57,1)	5	1,80 (1,44-4,62)	12
CB-52	5,03 (4,72-5,34)	2	<LOQ	
CB-101	3,64 (2,55-9,44)	6	<LOQ	
CB-118 <sup>A</sup>	27,9 (7,51-144)	11	16,0 (8,87-87,2)	41
CB-153 <sup>A</sup>	317 (81,1-1242)	11	157 (68,6-1312)	41
CB-(138+163) <sup>A</sup>	125 (30,5-639)	11	30,6 (14,3-339)	41
CB-180 <sup>A</sup>	147 (47,7-598)	11	92,0 (31,3-964)	40
$\Sigma$ PCB <sup>A</sup>	<b>594 (167-2886)</b>	<b>11</b>	<b>302 (123-2704)</b>	<b>41</b>

<sup>A</sup>significant difference between the species (p<0,05 Mann-Whitney U-test)

### Geographical differences

#### Pesticides

Geographical differences for HCB and  $\beta$ -HCH are shown in Fig 5 and Fig 6 (and in Tables 4 and 6 Appendix B)

There was a significant difference in HCB and  $\beta$ -HCH in eggs from tawny owl, but not in eggs from common kestrel, from different areas. In one common kestrel egg from Västerbotten (VB) there was an extreme outlier for HCB (1923 ng/g lw), and in another common kestrel egg, also from VB, there was an extreme outlier for  $\beta$ -HCH (188 ng/g lw). Both these eggs were from the coastal area in Västerbotten but from different counties.

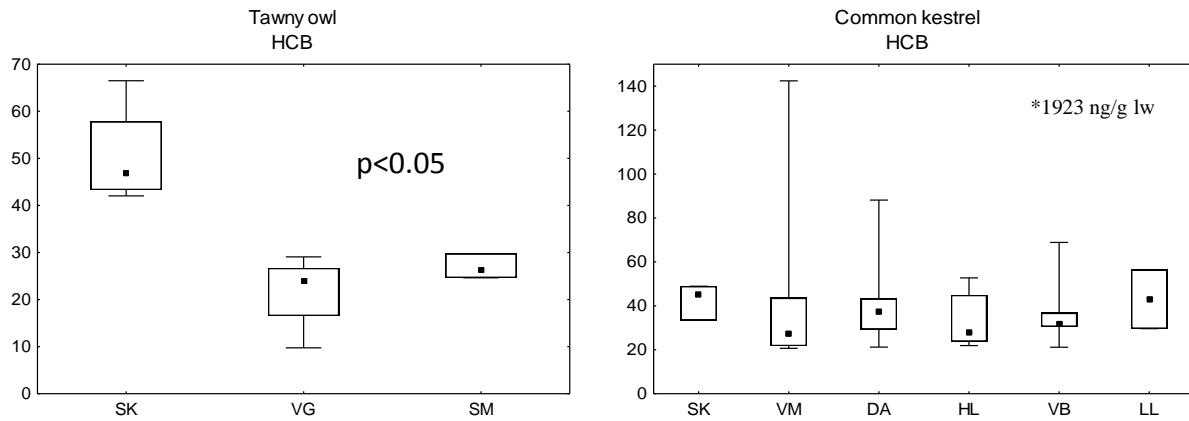


Figure 5. HCB (ng/g lw) in addled eggs of tawny owl and common kestrel from different provinces in Sweden collected in 2014. \*One outlier value (1923 ng/g lw) was found in a common kestrel egg from Västerbotten (VB)

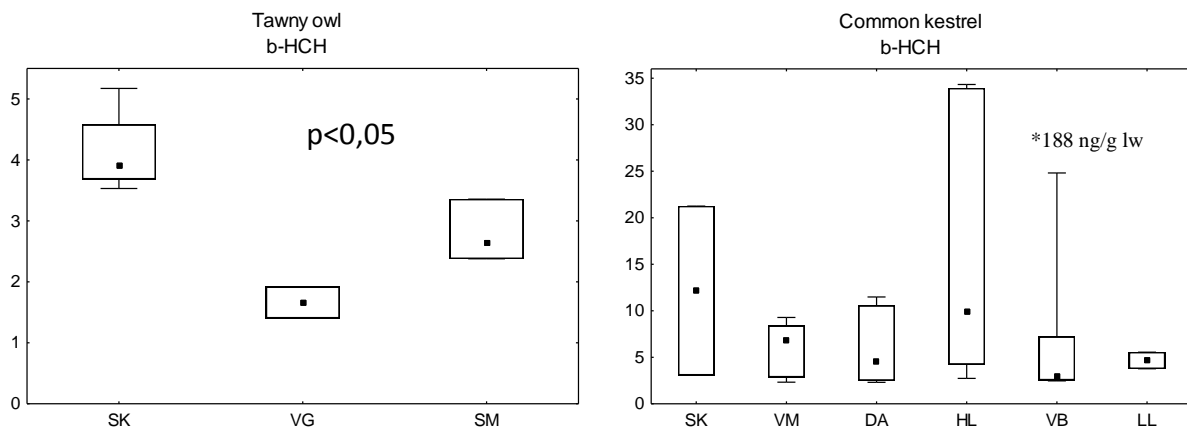


Figure 6.  $\beta$ -HCH (ng/g lw) in addled eggs of tawny owl and common kestrel from different provinces of Sweden collected in 2014. \*One outlier value (188 ng/g lw) was found in a common kestrel egg from Västerbotten (VB).

There was a significant difference in levels of pp-DDE in tawny owl eggs from different provinces, but not in eggs from common kestrel (Fig 7; Tables 5 and 7, Appendix B).

pp-DDT was detected in all tawny owl eggs (n=4) from Skåne and in all common kestrel eggs from Dalarna (n=10). pp-DDT was not detected in the two common kestrel eggs from southern Lappland.

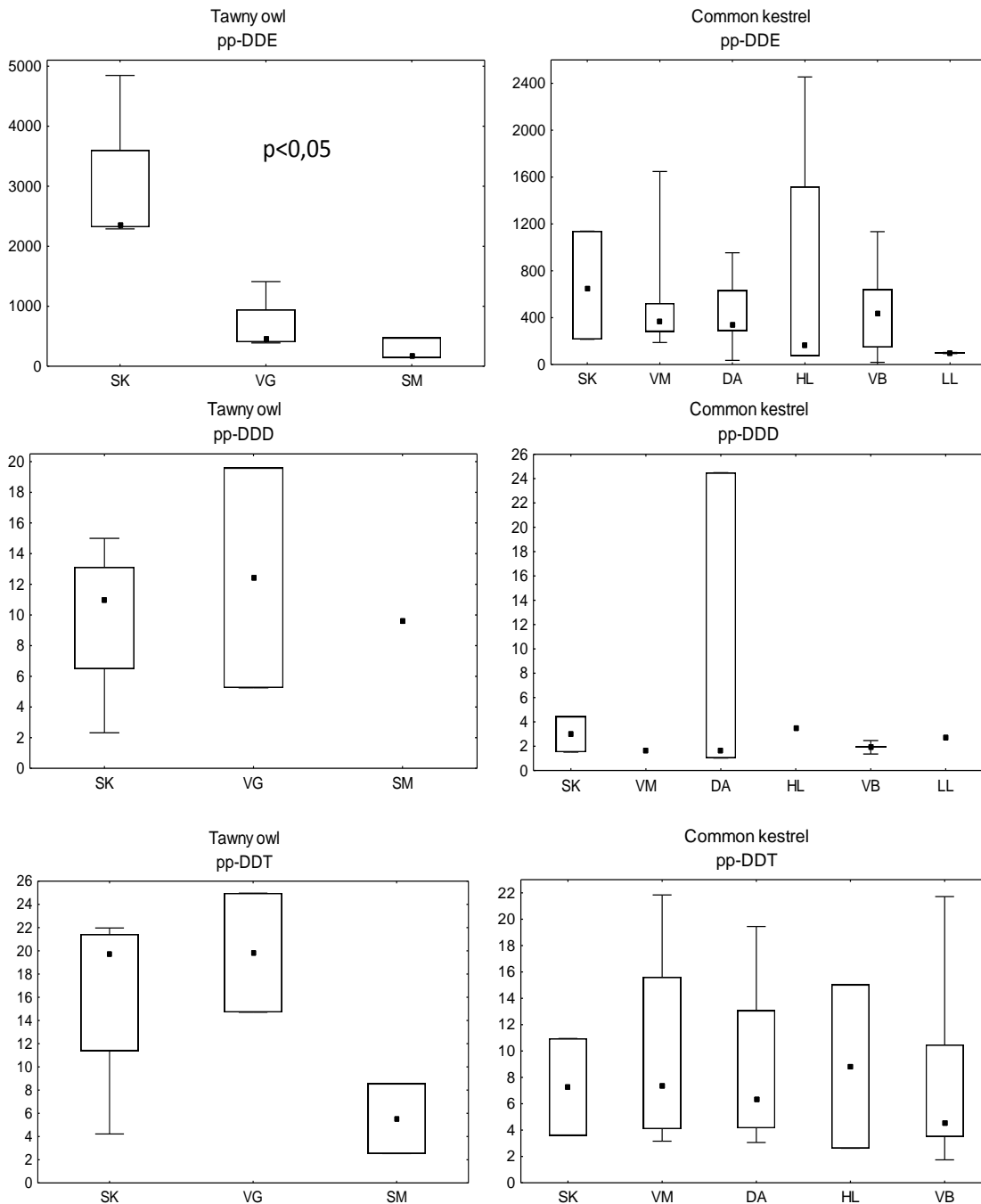


Figure 7. pp-DDE, pp-DDD and pp-DDT (ng/glw) in addled eggs of tawny owl and common kestrel collected from different provinces in Sweden in 2014. pp-DDT was not detected in the common kestrel eggs from southern Lappland.

### PCBs

In both tawny owl and common kestrel there was a tendency towards higher concentrations in eggs from the southernmost part of Sweden. The regional differences were significant for CB-118 in tawny owl and for CB-153 and CB-180 in common kestrel. In common kestrel the regional difference in  $\sum$ PCB was also significant. The median level of CB-28 was higher in

tawny owl egg compared to in common kestrel but this was mainly due to higher levels in three eggs, probably from the same nest (Fig 8; Table 5, Appendix B).

#### **Comment on pesticides and PCBs**

There was a geographical difference for most PCB concentrations, with higher levels in eggs from the southernmost part of Sweden (Skåne) for both tawny owl and common kestrel. For pesticides, on the other hand, median concentrations in common kestrel were approximately the same in eggs from all geographical areas - although the individual variation was large.

The levels found in tawny owls in the present study are lower compared with what was found in Norwegian tawny owl eggs collected in 2001-2004 near the city of Trondheim (Bustnes, Yoccoz et al. 2007). The PCB levels in common kestrel found in the present study are also considerably lower compared to what was found in eggs from common kestrel collected from around the city of Rome in 2005 (Dell'Omo, Costantini et al. 2008).



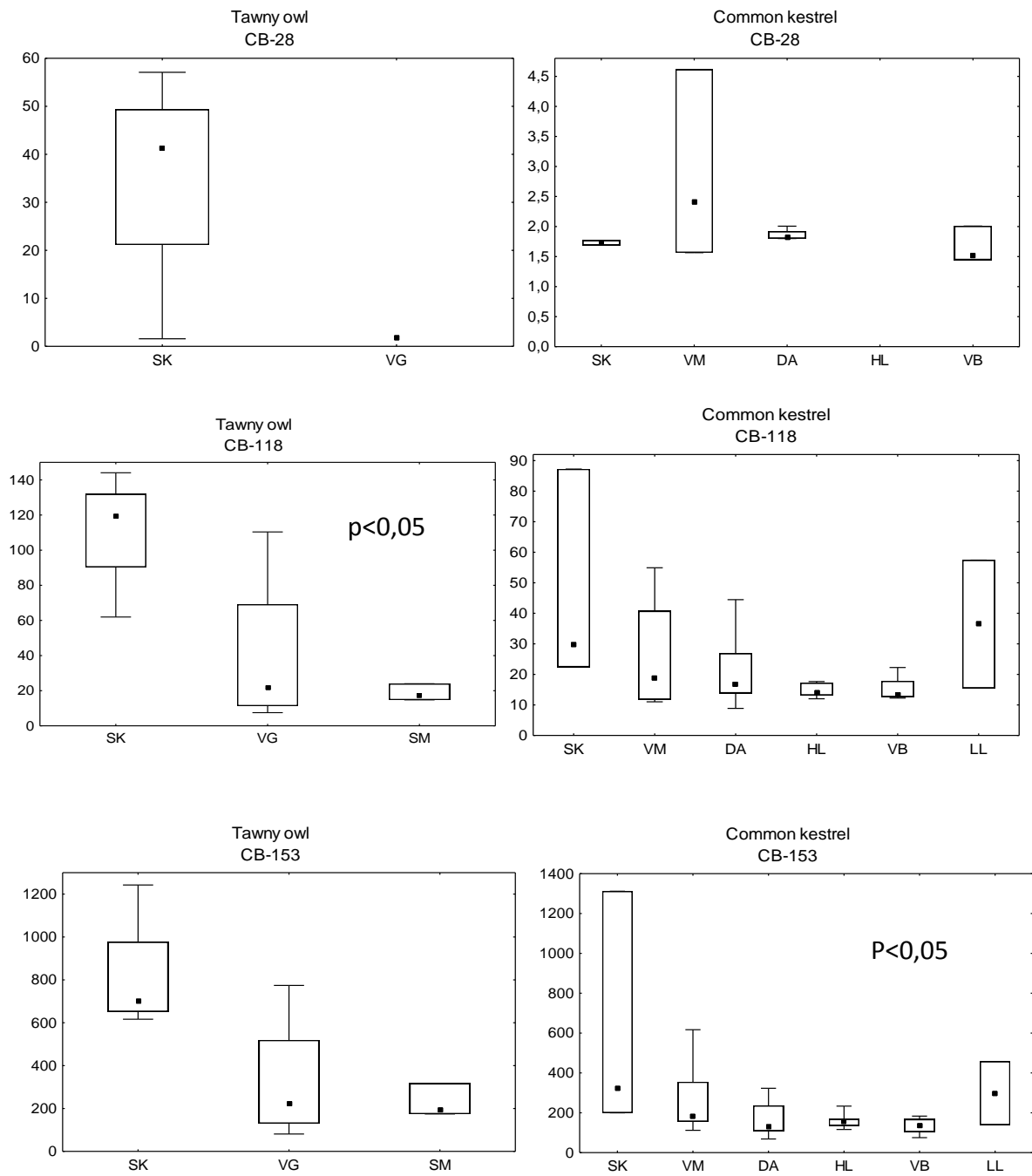


Figure 8. CB-28, CB-118, and CB-153 (ng/g lw) in addled eggs of tawny owl and common kestrel collected from different provinces of Sweden in 2014.

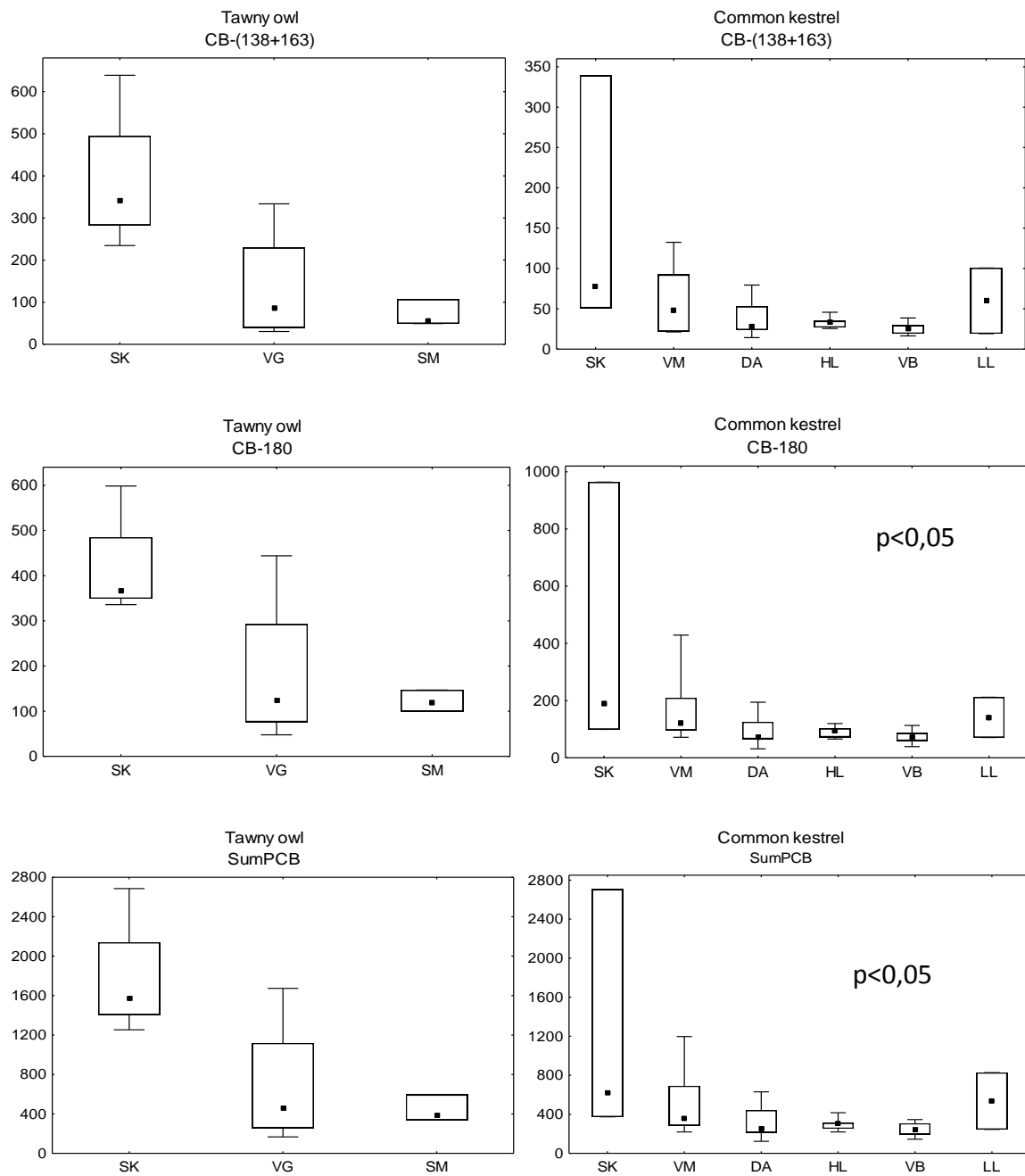


Figure 9. CB-(138+163), CB-180, and  $\sum$ PCB (ng/glw) in addled eggs of tawny owl and common kestrel collected from different provinces of Sweden in 2014.

## Perfluorinated compounds (PFAAs)

The median (min-max) of the different PFAAs in eggs from tawny owl and common kestrel are shown in Table 8. Data is missing from one common kestrel egg.

Six compounds (PFOS, PFNA, PFDA, PFUnDA, PFDODA, PFTrDA) were found >LOQ in all eggs from tawny owl. PFHxA and PFTDA were found in 9 of 10 (90%) analysed eggs from tawny owl. Both the linear and the branched isomer of PFOS was analysed but as almost all of the PFOS was in the linear form, only this isomer is accounted for (*n*-Perfluorooctane sulfonic acid, abbreviation L-PFOS).

PFOS was the only perfluorinated substance that was found >LOQ in all eggs of common kestrel. PFNA, PFUnDA, PFDODA and PFTrDA were found in at least 75% of the common kestrel eggs. PFHpA was only found in one egg, a common kestrel egg from Västmanland. PFOS was significantly higher in tawny owl eggs and PFNA was significantly higher in common kestrel eggs. The  $\sum$ PFAAs were approximately twice as high in tawny owl eggs 14,6 (7,56-43,1) compared with common kestrel eggs 7,60 (2,49-25,9).

The proportion of the different compounds to the total PFAAs is shown in Table 9.

PFOS was the most abundant compound in all eggs of both species. In tawny owl the proportion of PFOS was 28,2-81% and in common kestrel the proportion of PFOS was 23-91%.

### Geographical differences

$\sum$ PFAAs were higher in southern Sweden but the difference was not significant (Fig 10).

The level of PFOS was higher in eggs from the southernmost province (Skåne) for both tawny owl and common kestrel, and for tawny owl the regional difference was significant (Fig 11).

Also PFDA differed significantly between regions in tawny owl (Fig 11).

For common kestrel the regional differences were not significant for any perfluorinated compound (Fig 11). There was a considerable variation in the proportion PFOS between eggs from different regions. The proportion of PFOS in the present study was lower than what was found in tawny owl eggs from Norway, where it contributed up to ~83% of the  $\sum$ PFAAs (Ahrens, Herzke et al. 2011).

Table 8. Perfluorinated compounds (ng/g ww) in addled eggs of tawny owl (*Strix aluco*) and common kestrel (*Falco tinnunculus*) collected in 2014. N refers to the number of eggs with levels > LOQ. PFPeA, PFBS, PFDS, and PFOA were not found >LOQ in any of the analysed eggs. For LOQ see table 4.

	tawny owl median (min-max)	N	common kestrel median (min-max)	N
PFHxA	1,40 (1,30-2,30)	9	0,70 (0,57-0,38)	2
PFHpA	<LOQ		0,31	1
PFHxS	0,67 (0,38-0,96)	2	0,475 (0,47-0,48)	2
PFOS <sup>A</sup>	7,55 (3,20-34,0)	10	3,40 (1,10-20,0)	39
PFNA <sup>A</sup>	0,33 (0,17-0,67)	10	0,52(0,22-1,20)	34
PFDA	0,86 (0,47-2,10)	10	0,90 (0,42-1,80)	26
PFUnDA	1,30 (0,50-2,40)	10	1,40 (0,37-3,20)	36
PFDoDA	1,02 (0,48-2,40)	10	1,16 (0,47-3,80)	31
PFTTrDA	1,45 (0,63-2,80)	10	1,25 (0,65-3,00)	34
PFTDA	0,55 (0,27-2,60)	9	0,335 (0,20-1,50)	14
<b><math>\Sigma</math>PFAAs<sup>A</sup></b>	<b>14,6 (7,56-43,1)</b>		<b>7,60 (2,49-25,9)</b>	

<sup>A</sup>significant difference between species ( $p < 0,05$  Mann-Whitney U-test)

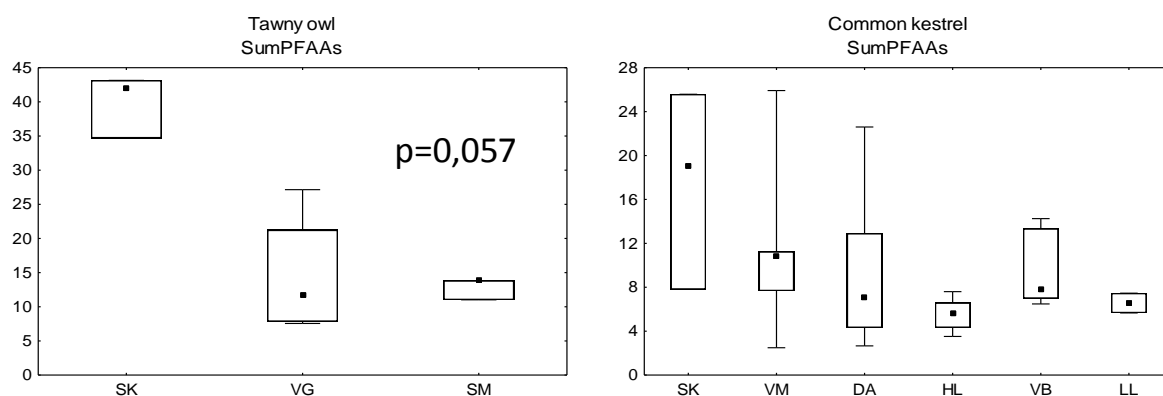


Figure 10.  $\Sigma$ PFAAs in addled eggs from tawny owl and common kestrel collected from different provinces in Sweden in 2014.

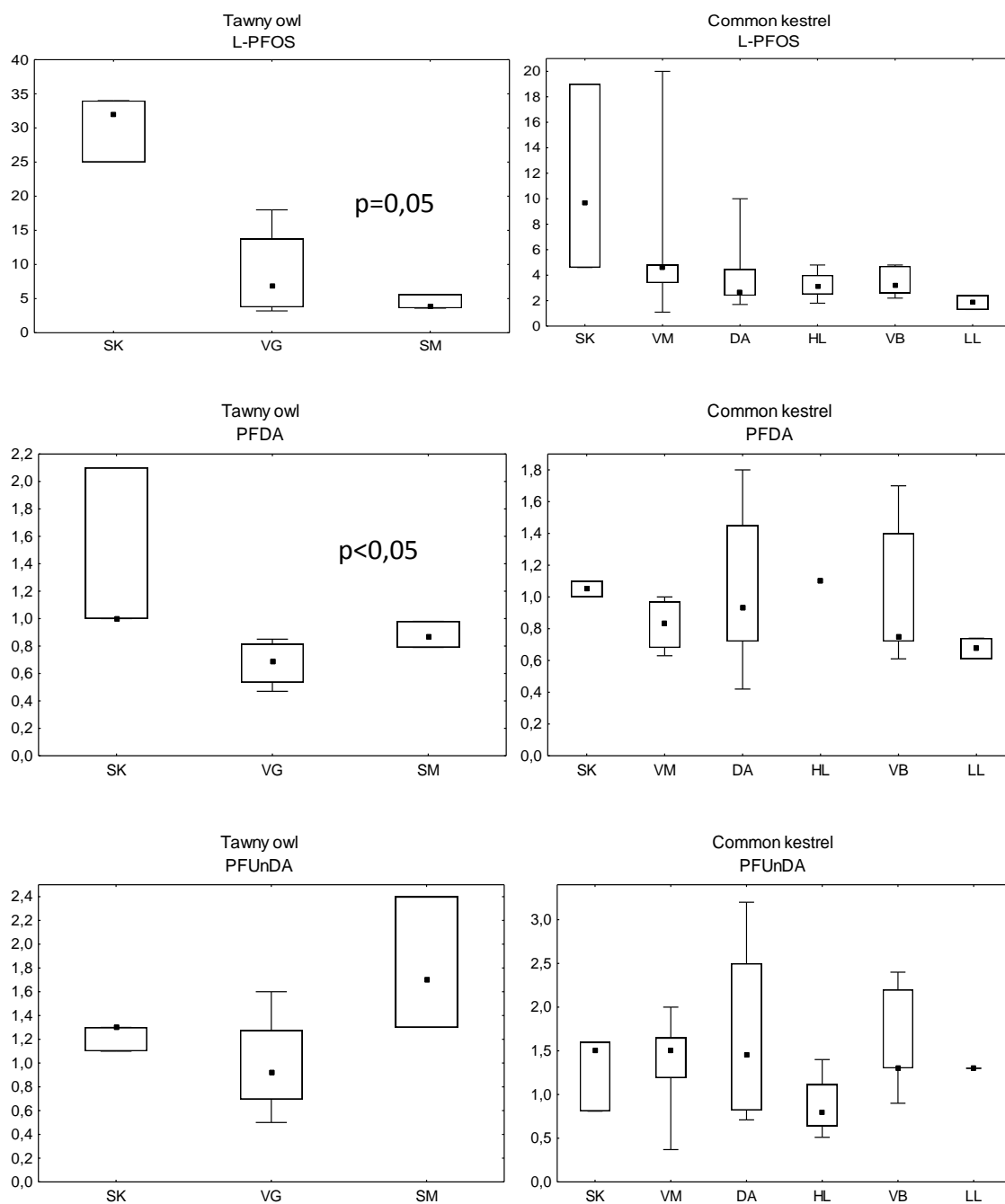


Figure 11. PFOS, PFDA, and PFUnDA (ng/g ww) in added eggs of tawny owl and common kestrel collected from different provinces of Sweden in 2014.

### **Comments on PFAAs**

PFAA levels found in terrestrial environments are normally much lower compared with what is found in aquatic environments and the results in the present study is in agreement with that. It is generally assumed that the level of PFAAs in the terrestrial environment is a result of airborne pollution. Aquatic environments acts as a sink for many PFAAs, and thus levels in aquatic environments are generally higher. On the other hand, it has been suggested that the level of PFAAs in the terrestrial environment is more prone to yearly variations as it is more dependent on short term emissions (Holmström, Johansson et al. 2010).

A time trend study on perfluorinated compounds in eggs from Swedish peregrine falcons found 47 ng/g ww PFOS in a pooled sample with five individuals in 2007 (Holmström, Johansson et al. 2010). A study on tawny owl eggs from central Norway found a geometric mean of 4,19 (1,93-11,0) ng/g ww in five eggs collected in 2009. This can be compared to 7,55 (3,20-34,0) and 3,40 (1,10-20,0) ng/g ww found in tawny owl and common kestrel eggs in the present study. The  $\Sigma$ PFAAs in the Norwegian study on tawny owl eggs was 7,06 ng/g ww. This is approximately half of the median value of  $\Sigma$ PFAAs found in tawny owl in the present study. On the other hand, the  $\Sigma$ PFAAs in tawny owls from Skåne was 42 ng/g ww which is close to what Holmström et al. (2010) found in peregrine falcon eggs.

In both these studies, the proportion of PFOS to the  $\Sigma$ PFAAs is high, ~83% (Ahrens, Herzke et al. 2011) and ~76% (Holmström, Johansson et al. 2010) compared to what was found in the present study (Table 9).

Table 9. Median percentage of different PFAAs in addled eggs of tawny owl (TO) and common kestrel (CK) collected in different provinces of Sweden in 2014. Number of valid n in brackets.

	PFHxA		PFHxS		PFOS		PFNA		PFDA		PFUnDA		PFDoDA		PFTrDA		PFTDA	
	TO	CK	TO	CK	TO	CK	TO	CK	TO	CK	TO	CK	TO	CK	TO	CK	TO	CK
<b>SK</b>	4 (3)	7 (1)	3 (1)	2 (2)	74 (3)	59 (3)	1 (3)	3 (3)	2 (3)	5 (2)	3 (3)	8 (3)	5 (3)	9 (3)	3 (3)	10 (3)	1 (3)	8 (1)
<b>VG</b>	8 (3)	-	1 (1)	-	57 (4)	-	2 (4)	-	5 (4)	-	6 (4)	-	6 (4)	-	9 (4)	-	4 (3)	-
<b>SM</b>	12 (3)	-	-	-	33 (3)	-	3 (3)	-	7 (3)	-	12 (3)	-	8 (3)	-	13 (3)	-	4 (3)	-
<b>VM</b>	-	-	-	-	-	44 (9)	-	4 (8)	-	7 (6)	-	13 (8)	-	11 (7)	-	19 (8)	-	5 (6)
<b>DA</b>	-	6 (1)	-	-	-	41 (12)	-	6 (8)	-	9 (12)	-	19 (9)	-	13 (9)	-	16 (8)	-	3 (2)
<b>HL</b>	-	-	-	-	-	61 (6)	-	13 (2)	-	14 (1)	-	16 (4)	-	11 (3)	-	18 (6)	-	-
<b>VB</b>	-	-	-	-	-	37 (7)	-	8 (7)	-	11 (7)	-	17 (7)	-	11 (7)	-	15 (7)	-	3 (4)
<b>LL</b>	-	-	-	-	-	28 (2)	-	8 (2)	-	10 (2)	-	20 (2)	-	11 (2)	-	22 (2)	-	3 (1)

## Stable isotopes

No significant difference was found in either  $\delta^{15}\text{N}$  or  $\delta^{13}\text{C}$  between the two species (Fig 12). The  $\delta^{13}\text{C}$  values indicate a terrestrial food chain for both tawny owl and common kestrel.

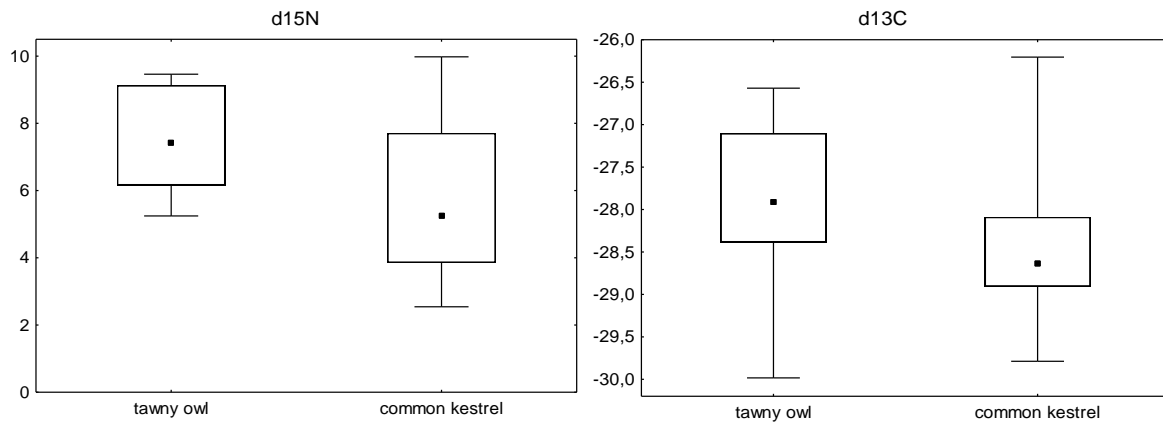


Figure 12.  $\delta^{15}\text{N}$  (left) and  $\delta^{13}\text{C}$  (right) in eggshells of tawny owl (n=10) and common kestrel (n=40) collected in Sweden in 2014.

## Geographical differences

A significant difference was found in  $\delta^{15}\text{N}$  between eggs from different provinces for both tawny owl and common kestrel. No such difference was found for  $\delta^{13}\text{C}$ .

### *Tawny owl*

$\delta^{15}\text{N}$  was highest in eggs from Skåne, 9,4 ‰ (9,13-9,46), and lowest in eggs from Småland, 5,44 ‰ (5,25-6,16). There was also a larger individual variation between eggs in Småland (0,913 ‰) compared to in eggs from Skåne and Västergötland (Table 10).

### *Common kestrel*

$\delta^{15}\text{N}$  was highest in common kestrel eggs from the province of Skåne, 8,4‰ (7,59-8,53). The lowest value for  $\delta^{15}\text{N}$ , 3,4‰ (2,54-5,64) was found in eggs from the province of Västerbotten. There was a considerable variation in  $\delta^{15}\text{N}$  both between eggs from different parts of the country but also between eggs from the same areas. There was a difference of 4-5‰ between eggs from the southern part of Sweden (Skåne) and the eggs from northernmost parts (Västerbotten and southern Lappland). The largest variation in  $\delta^{15}\text{N}$  between eggs from the same region was found in eggs from the province of Västmanland, where the difference between the highest and lowest  $\delta^{15}\text{N}$  value was 6,2‰ (Table 11).



Table 10. Stable isotopes of nitrogen ( $\delta^{15}\text{N}$ ) and carbon ( $\delta^{13}\text{C}$ ) in addled eggs of tawny owl (*Strix aluco*) collected from different provinces of Sweden in 2014. Median (min-max). A significant difference ( $p < 0,05$ , Kruskal Wallis ANOVA) between regions was found for  $\delta^{15}\text{N}$

	$\delta^{15}\text{N}^{\text{A}}$	$\delta^{13}\text{C}$
SK (n=3)	9,398 (9,13-9,46)	-27,85 (-27,95 - -27,43)
VG (n=4)	7,41 (7,15-7,62)	-28,14 (-29,98 - -26,999)
SM (n=3)	5,44 (5,25-6,16)	-27,98 (-28,38 - -26,57)

<sup>A</sup> $p < 0,05$

Table 11. Stable isotopes of nitrogen ( $\delta^{15}\text{N}$ ) and carbon ( $\delta^{13}\text{C}$ ) in addled eggs of common kestrel (*Falco tinnunculus*) collected from different provinces of Sweden in 2014. Median (min-max). A significant difference ( $p < 0,05$ , Kruskal Wallis ANOVA) between regions was found for  $\delta^{15}\text{N}$

	$\delta^{15}\text{N}^{\text{A}}$	$\delta^{13}\text{C}$
SK (n=3)	8,429 (7,59-8,53)	-29,201 (-29,79 - -27,33)
VM (n=10)	7,799 (3,71-9,98)	-28,77 (-29,78 - -27,49)
DA (n=12)	4,76 (3,598-7,345)	-28,096 (-29,53 - -26,204)
HL (n=6)	6,778 (3,415-8,36)	-28,67 (-29,739 - -27,28)
VB (n=7)	3,443 (2,54-5,64)	-28,18 (-29,07 - -26,70)
LL (n=2)	4,123 (3,23-5,01)	-28,476 (-28,81 - -28,14)

<sup>A</sup> $p < 0,05$

#### Comments on stable isotopes

It is difficult to draw any conclusions from the stable isotope analysis in the present study.

There are no baseline values and the eggs were in variable condition when arriving at SMNH, including some which were in rather bad condition and also broken. The large differences in  $\delta^{15}\text{N}$  between eggs from the same region in common kestrel might indicate a broader choice of prey for this species but there might also be other explanations.

## Concluding remarks

There were tendencies towards higher levels of many of the analysed compounds in eggs from the southern part of Sweden (Skåne). This tendency was found in both tawny owl and common kestrel. However, mercury did not display this pattern. No significant differences in mercury levels were found either between the species or between different regions.

In tawny owl, the highest levels of HCB,  $\beta$ -HCH,  $\Sigma$ DDT,  $\Sigma$ PCB and PFAAs were found in the same egg, an egg from Skåne. In common kestrel there was an extreme outlier for HCB (1923 ng/g lw) and one extreme outlier for  $\beta$ -HCH (188 ng/g lw). Both these values were 30-50 times higher than the median values for both common kestrel and tawny owl. However, these values were not found in the same egg, although both were from the same province (Västerbotten). This might be an indication that common kestrel are exposed to contaminants from different sources to a larger extent than tawny owl and the reason for this could be that common kestrel migrate during the winter. It could also be that kestrels have a broader choice of prey.

Furthermore, there was a large difference in the proportion of PFOS to the total PFAAs in both tawny owl and common kestrel. In tawny owl, the largest proportion of PFOS (74%) was found in eggs from Skåne and the lowest proportion (33%) was found in eggs from Småland. In common kestrel the largest proportion of PFOS (61%) was found in eggs from Hälsingland and the lowest proportion (28%) was found in the two eggs from southern Lapland. This is considerably less than what has previously been reported in eggs of terrestrial bird species (Holmström, Johansson et al. 2010; Ahrens, Herzke et al. 2011)

Changing patterns in the proportion of different perfluorinated compounds to the  $\Sigma$ PFAAs has been found in peregrine falcon eggs from different decades (1970s, 1980s, 1990s) with an increasing abundance of PFTrDA (Holmström, Johansson et al. 2010).

It is not unlikely that the reason that there were tendencies towards larger differences in levels of contaminants between different provinces for tawny owl compared to common kestrel is that the owls are resident. The common kestrel migrates to southern and southwestern Europe during the winter and therefore the contaminant levels in the eggs of these birds are probably not only reflecting the contaminant level where it is breeding but also where it is spending the winter. To complicate the picture, it is also possible that common kestrels breeding in the southern parts of Sweden do not migrate during the winter. In this case the contaminant level in the kestrels does reflect the contaminant level in the area where the kestrels are breeding. The median contaminant level in kestrel eggs from Skåne was generally higher compared to

the levels in eggs from the other provinces. However, individual variation was large and in some cases the maximum levels were found in eggs collected outside of Skåne.

The problem with using samples from migrating birds in environmental monitoring is that it is hard to know how much of the contaminant load comes from the breeding area and how much comes from the wintering area. A number of studies indicate that migration behavior influences the contaminant levels in birds (Henny, Ward et al. 1982; Springer, Walker et al. 1984; Tanabe, Senthilkumar et al. 1998). But there are also a number of other factors such as body condition and age (Wienburg and Shore 2004). A point of interest is how much the contaminant load in the egg is influenced by the contaminant load of the parent bird.

A study on non-migrating dippers (different *Cinclus* species) using stable isotopes of  $^{15}\text{N}$  and  $^{13}\text{C}$  indicated that the nutrients, and therefore the contaminant load, deposited in eggs were of recent dietary origin (Morrissey, Elliott et al. 2010). A study on endogenous versus dietary sources of PB-153 in eggs of ring doves (*Streptopelia risoria*), on the other hand, showed that endogenous maternal sources rather than dietary sources of contaminants dominated the deposition in eggs during yolk formation (Drouillard and Norstrom 2001).

A study on PCBs and chlorinated pesticides in eggs of common kestrel breeding in and around the city of Rome indicated that contaminant levels in the eggs were a good indicator of the local pollution in the breeding area as there were large differences between eggs from different breeding sites in and around Rome (Dell'Omo, Costantini et al. 2008). However, kestrel's living in this area, do not migrate and females remain within the breeding territory a long time prior to egg-laying.

Chlorinated and brominated contaminants are fat soluble, persistent and have a relatively long half-life in birds and it is reasonable to believe that the total body burden of the parent bird influences the contaminant level in the egg. Studies on body concentrations of different organochlorines (OC) and PCBs have shown that common kestrel males have higher concentrations compared to females probably because there is a net transfer of OCs to the eggs by females (Wienburg and Shore 2004; van Drooge, Mateo et al. 2008). A study on plasma levels of contaminants in the laying female of Arctic-breeding glaucous gulls (*Larus hyperboreus*) in relation to egg content revealed that maternal transfer to egg favored compounds with low  $K_{ow}$  (Verreault, Villa et al. 2006).

The perfluorinated compounds have a relatively short half-life in birds (Newsted, Beach et al. 2006) compared to the chlorinated and brominated compounds. This might indicate that levels of perfluorinated compounds in bird eggs more reflect levels in the diet at the time of egg-formation compared to the fat soluble organic compounds.

Obviously there are large species specific differences in how much of the contaminant load in eggs is derived endogenously from the laying female and the contaminant load in her diet during egg formation.

Eggs from a resident bird such as the tawny owl are most likely a better matrix for monitoring contaminants in the environment where it is breeding. The advantage of using common kestrel is that they are a common bird found almost all over Sweden and unhatchable/addled eggs are easy to collect in adequate number. The disadvantage is that the contaminant levels in the eggs probably do not only reflect the contaminant load in the environment where the kestrels breed. The contaminant load of common kestrel eggs probably more reflects the general contamination load of the population.

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## APPENDIX A

Name, abbreviation and multiple reaction monitoring (MRM) transitions for UPLC/MS/MS analysis of PFAAs. The quantification trace is indicated in bold.

Name	Abbreviation	MRM Transition
Perfluorobutane sulfonic acid	PFBS	298.9 > 79.96 <b>298.9 &gt; 98.9</b>
Perfluorohexane sulfonic acid	PFHxS	398.9 > 79.85 <b>398.9 &gt; 98.9</b>
<i>n</i> -Perfluorooctane sulfonic acid	L-PFOS	399.1 > 119.0 498.9 > 79.96 <b>498.9 &gt; 98.85</b>
Perfluorodecane sulfonic acid	PFDS	498.9 > 169.0 598.97 > 79.96 <b>598.97 &gt; 98.9</b>
Perfluoropentanoic acid	PFPeA	<b>263 &gt; 219</b>
Perfluorohexanoic acid	PFHxA	312.97 > 118.95 <b>312.97 &gt; 269</b>
Perfluoroheptanoic acid	PFHpA	362.97 > 168.97 <b>362.97 &gt; 319</b>
Perfluorooctanoic acid	PFOA	412.97 > 168.97 <b>412.97 &gt; 369</b>
Perfluorononanoic acid	PFNA	462.99 > 219 <b>462.99 &gt; 419</b>
Perfluorodecanoic acid	PFDA	512.97 > 219 <b>512.97 &gt; 469</b>
Perfluoroundecanoic acid	PFUnDA	562.97 > 269 <b>563.0 &gt; 51</b>
Perfluorododecanoic acid	PFDoDA	612.97 > 168.9 <b>612.97 &gt; 569.0</b>
Perfluorotridecanoic acid	PFTTrDA	662.90 > 168.96 <b>662.90 &gt; 619.0</b>
Perfluorotetradecanoic acid	PFTDA	712.9 > 168.97 <b>712.9 &gt; 669.0</b>
<sup>13</sup> C <sub>2</sub> - perfluorohexanoic acid	<sup>13</sup> C-PFHxA <sup>a</sup>	<b>314.97 &gt; 270</b>
<sup>13</sup> C <sub>4</sub> - perfluorooctanoic acid	<sup>13</sup> C-PFOA <sup>a</sup>	<b>416.97 &gt; 372</b>
<sup>13</sup> C <sub>5</sub> - perfluorononanoic acid	<sup>13</sup> C-PFNA <sup>a</sup>	<b>467.99 &gt; 423</b>
<sup>13</sup> C <sub>2</sub> - perfluorodecanoic acid	<sup>13</sup> C-PFDA <sup>a</sup>	<b>514.97 &gt; 470</b>
<sup>13</sup> C <sub>2</sub> - perfluoroundecanoic acid	<sup>13</sup> C-PFUnDA <sup>a</sup>	<b>564.97 &gt; 520</b>
<sup>13</sup> C <sub>2</sub> - perfluorododecanoic acid	<sup>13</sup> C-PFDoDA <sup>a</sup>	<b>614.97 &gt; 570</b>
<sup>18</sup> O <sub>2</sub> - perfluorohexane sulfonic acid	<sup>18</sup> O-PFHxS <sup>a</sup>	<b>402.9 &gt; 102.9</b>
<sup>13</sup> C <sub>4</sub> - perfluorooctane sulfonic acid	<sup>13</sup> C-PFOS <sup>a</sup>	<b>502.9 &gt; 98.85</b>
<sup>13</sup> C <sub>8</sub> - perfluorooctanoic acid	<sup>13</sup> C <sub>8</sub> -PFOA <sup>b</sup>	<b>421.1 &gt; 376.2</b>
<sup>13</sup> C <sub>8</sub> - perfluorooctane sulfonic acid	<sup>13</sup> C <sub>8</sub> -PFOS <sup>b</sup>	<b>506.9 &gt; 98.85</b>
<i>7H</i> -dodecafluoroheptanoic acid	<i>7H</i> -PFHpA <sup>b</sup>	<b>345 &gt; 281</b>

<sup>a</sup> Used as internal standard, i.e. is added before extraction

<sup>b</sup> Used as performance standard, i.e. added after extraction and before instrumental analysis

APPENDIX A

Table 2. Internal standard recoveries in eggs (n=45).

	C <sup>13</sup> PFHxA	O <sup>18</sup> PFHxS	C <sup>13</sup> PFOS	C <sup>13</sup> PFOA	C <sup>13</sup> PFNA	C <sup>13</sup> PFDA	C <sup>13</sup> PFUnDA	C <sup>13</sup> PFDoDA
Average %(min- max)	103 (29-167)	98 (47-177)	97 (30-177)	98 (31-170)	97 (44-144)	97 (46-143)	98 (47-142)	74 (28-165)
%RSD	27	24	24	24	23	24	24	42

Table 3. Accuracy (recovery) and reproducibility (day-to-day variation) of the method used determined by analyzing fortified egg samples (n=4) on four different occasions throughout the project (ng/g).

Sample ID	PFPeA	PFBuS	PFHxA	PFHpA	PFHxS	L-PFOS	PFDS	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTTrDA	PFTDA
Average	6.2	8.4	6.9	6.4	6.3	9.9	6.3	7.0	6.3	7.0	6.8	6.7	6.4	5.9
%RSD	13	19	8	11	13	7	9	13	9	10	14	11	8	13
%Recovery	96	136	105	97	101	109	101	105	96	103	100	97	95	85



## APPENDIX B

Table 1. Total mercury (ng/g ww) (median; min-max) in addled eggs of tawny owl (*Strix aluco*) and common kestrel (*Falco tinnunculus*) from different provinces in Sweden collected in 2014. N is number of samples >LOQ.

For LOQ see Material and methods

	Tawny owl	N	Common kestrel	N
Skåne (SK)	66,5 (19,4-120)	3	100 (15,3-120)	3
Västergötland (VG)	38,9 (23,2-93,8)	4		
Småland (SM)	84,6 (78,4-150)	3		
Västmanland (VM)			61,3 (7,8-100)	10
Dalarna (DA)			74,4 (37,5-230)	12
Hälsingland (HL)			48,2 (35,0-64,0)	6
Västerbotten (VB)			57,0 (44,0-97,0)	7
Lappland (LL)			120 (100-140)	2

APPENDIX B

Table 2. Median, minimum and maximum values of BDE-47, BDE-99, BDE-100, BDE-153,  $\Sigma$ PBDE, and HBCD (ng/g lw) in addled eggs of tawny owl (*Strix aluco*) from different provinces in Sweden collected in 2014. Number in brackets refers to number of eggs >LOQ. For LOQ see materials and Methods

	BDE-47	BDE-99	BDE-100	BDE-153	$\Sigma$ PBDE <sup>1</sup>	HBCD
Skåne (SK)	7,10 (4) 6,09-9,02	11,8 (4) 10,7-13,5	4,80 (4) 1,77-5,77	5,91 (4) 5,35-10,7	30,1 (4) 27,5-34,5	1,13 (4) 0,971-1,28
Västergötland (VG)	1,92 (4) 0,518-8,68	2,69 (4) 0,721-17,9	0,908 (4) 0,427-4,90	1,52 (4) 0,849-9,0	7,04 (4) 2,52-40,5	2,15 (2) 0,930-3,67
Småland (SM)	0,992 (3) 0,940-2,66	1,44 (3) 1,36-5,89	0,398 (3) 0,381-1,41	2,07 (3) 1,83-2,63	4,74 (3) 4,66-12,6	3,68 (1)

<sup>1</sup>sum of values >LOQ for BDE-47, BDE-99, BDE-100, BDE-153

APPENDIX B

Table 3. Median, minimum and maximum values of BDE-47, BDE-99, BDE-100, BDE-153,  $\Sigma$ PBDE, and HBCD (ng/g lw) in addled eggs of common kestrel (*Falco tinnunculus*) from different provinces in Sweden collected in 2014. Number in brackets refers to number of eggs >LOQ. For LOQ see materials and Methods

	BDE-47	BD-E99	BDE-100	BDE-153	$\Sigma$ PBDE <sup>1</sup>	HBCD
Skåne (SK)	1,06 (1)	0,523 (3) 0,299-2,83	0,359 (3) 0,182-1,12	6,27 (3) 2,91-15,4	11,3 (3) 3,79-15,9	0,635 (1)
Västmanland (VM)	1,52 (2) 0,536-2,50	0,345 (8) 0,116-3,49	0,237 (6) 0,117-1,52	1,74 (10) 1,30-8,58	2,07(10) 1,34-16,1	<LOQ
Dalarna (DA)	0,549 (1)	0,386 (10) 0,121-2,13	0,163 (7) 0,007-1,03	3,48 (12) 0,878-5,77	3,96 (12) 1,06-9,48	<LOQ
Hälsingland (HL)	<LOQ	0,353 (5) 0,222-0,679	0,163 (3) 0,142-0,170	2,17 (6) 1,04-3,32	2,76 (6) 1,04-3,92	<LOQ
Västerbotten (VB)	0,258 (3) 0,033-0,326	0,751 (8) 0,130-1,56	0,203 (7) 0,031-0,248	2,32 (8) 0,216-3,18	3,19 (8) 0,410-5,25	<LOQ
Lappland (LL)	<LOQ	0,494 (2) 0,430-0,559	0,129 (1)	2,4 (2) 2,06-2,74	2,96 (2) 2,62-3,30	<LOQ

<sup>1</sup>sum of values >LOQ for BDE-47, BDE-99, BDE-100, BDE-153

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Table 4. Median, minimum and maximum values of HCB,  $\beta$ -HCH, pp-DDE, pp-DDD, pp-DDT and  $\Sigma$ DDT (ng/g lw) in addled eggs of tawny owl (*Strix aluco*) from different provinces in Sweden collected in 2014. Number in brackets refers to number of eggs >LOQ. For LOQ see materials and Methods

	HCB	b-HCH	pp-DDE	pp-DDD	pp-DDT	$\Sigma$ DDT <sup>1</sup>
Skåne (SK)	46,9 (4) 42,0-66,5	3,91 (4) 3,53-5,17	2352 (4) 2288-4846	10,9 (4) 2,32-15,0	19,7 (4) 4,22-22,0	2373 (4) 2320-4880
Västergötland (VG)	23,8 (4) 9,75-29,1	1,66 (2) 1,40-1,92	448 (4) 388-1411	12,4 (2) 5,25-19,6	19,8 (2) 14,7-25,0	470 (4) 388-1431
Småland (SM)	26,5 (3) 24,6-29,8	2,64 (3) 2,38-3,36	163 (3) 143-476	9,62 (1)	5,55 (3) 2,53-8,56	165 (3) 143-494

<sup>1</sup>Sum of values >LOQ for pp-DDE, pp-DDD and pp-DDT

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Table 5. Median, minimum and maximum values of CB-28, CB-52, CB-101, CB-118, CB-153, CB-(138+163), CB-180 and  $\sum$ PCB (ng/g lw) in added eggs of tawny owl (*Strix aluco*) from different provinces in Sweden collected in 2014. Number in brackets refers to number of eggs >LOQ. For LOQ see materials and Methods

	CB-28	CB-52	CB-101	CB-118	CB-153	CB-(138+163)	CB-180	$\sum$ PCB <sup>1</sup>
Skåne (SK)	41,2 (4) 1,58-57,1	5,03 (3) 4,73-5,34	3,17 (4) 2,55-4,07	119 (4) 61,9-144	700 (4) 616-1242	341 (4) 234-639	367 (4) 336-598	1576 (4) 1253-2685
Västergötland (VG)	1,77 (1)	<LOQ	6,89 (2) 4,33-9,44	21,5 (4) 7,51-110	221 (4) 81,1-774	87,2 (4) 30,5-334	123 (4) 47,7-444	455 (4) 167-1673
Småland (SM)	<LOQ	<LOQ	<LOQ	17,5 (3) 14,8-24,0	193 (3) 174-317	56,6 (3) 49,3-106	120 (3) 100-147	387 (3) 388-594

<sup>1</sup>sum of values >LOQ for CB-28, CB-52, CB-101, CB-118, CB-153, CB-(138+163) and CB-180

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Table 6. Median, minimum and maximum values of HCB,  $\beta$ -HCH, pp-DDE, pp-DDD, pp-DDT and  $\Sigma$ DDT (ng/g lw) in addled eggs of common kestrel (*Falco tinnunculus*) from different provinces in Sweden collected in 2014. Number in brackets refers to number of eggs >LOQ. For LOQ see materials and Methods

	HCB	$\beta$ -HCH	pp-DDE	pp-DDD	pp-DDT	$\Sigma$ DDT <sup>1</sup>
Skåne (SK)	45,2 (3) 33,4-48,9	12,2 (2) 3,08-21,2	644 (3) 215-1138	2,98 (2) 1,51-4,45	7,27 (2) 3,59-10,9	649 (3) 219-1151
Västmanland (VM)	27,2 (9) 20,7-142	6,82 (10) 2,32-9,28	370 (10) 188-1648	1,62 (1)	7,33 (10) 3,15-21,8	383 (10) 198-1670
Dalarna (DA)	37,6 (12) 21,2-88,1	4,54 (11) 2,31-11,5	338 (12) 35,7-954	1,68 (3) 1,02-24,5	6,35 (9) 3,06-19,4	351 (12) 36,7-978
Hälsingland (HL)	27,9 (6) 21,9-52,7	9,91 (6) 2,73-34,3	166 (6) 74,6-2454	3,51 (1)	8,83 (2) 2,62-15,0	166 (6) 74,6-2473
Västerbotten (VB)	32,3 (8) 21,2-1923	4,09 (8) 2,46-188	437 (8) 18,4-11,3	1,97 (5) 1,36-2,46	4,58 (6) 1,74-21,7	451 (8) 18,5-1146
Lappland (LL)	43 (2) 29,6-56,4	4,66 (2) 3,77-5,55	98,3 (2) 93,7-103	2,74 (1)	<LOQ	99,7 (2) 93,7-106

<sup>1</sup>Sum of values >LOQ for pp-DDE, pp-DDD and pp-DDT

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Table 7. Median, minimum and maximum values of CB-28, CB-52, CB-101, CB-118, CB-153, CB-(138+163), CB-180 and  $\Sigma$ PCB (ng/g lw) in added eggs of common kestrel (*Falco tinnunculus*) from different provinces in Sweden collected in 2014. Number in brackets refers to number of eggs >LOQ. For LOQ see materials and Methods

	CB-28	CB-52	CB-101	CB-118	CB-153	CB-(138+163)	CB-180	$\Sigma$ PCB <sup>1</sup>
Skåne (SK)	1,73 (2) 1,69- 1,77	<LOQ	<LOQ	29,7 (3) 22,4-87,2	323 (3) 200-131	77,9 (3) 251,1-339	191 (3) 99,4-964	621 (3) 374-2704
Västmanland (VM)	2,4 (3) 1,56-4,62	<LOQ	<LOQ	18,7 (10) 11,0-54,9	183 (10) 112-617	48,5 (10) 21,3-132	121 (9) 71,8-429	360 (10) 219-1197
Dalarna (DA)	1,82 (4) 1,80-2,01	<LOQ	<LOQ	16,6 (12) 8,87-44,5	128 (12) 68,6-323	28,7 (12) 14,3-79,4	74 (12) 31,3-194	251 (12) 123-630
Hälsingland (HL)	<LOQ	<LOQ	<LOQ	13,9 (6) 12,0-17,6	158 (6) 116-233	32,9 (6) 25,5-45,7	94,5 (6) 65,7-120	303 (6) 219-416
Västerbotten (VB)	1,51 (3) 1,44-2,01	<LOQ	<LOQ	13,5 (8) 12,2-22,2	133 (8) 75,3-183	25,6 (8) 16,4-38,6	72,5 (8) 39,1-113	245 (8) 145-346
Lappland (LL)	<LOQ	<LOQ	<LOQ	36,5 (2) 15,5-57,4	298 (2) 139-458	59,9 (2) 19,2-101	141 (2) 70,9-212	536 (2) 245-827

<sup>1</sup>sum of values >LOQ for CB-28, CB-52, CB-101, CB-118, CB-153, CB-(138+163) and CB-180

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Table 8. Median, minimum and maximum values of PFHxA, PFHxS, PFOS, PFNA, PFDA, PFUnDA, PFDoDA, PFTTrDA, PFTDA, and  $\Sigma$ PFAs (ng/g ww) in addled eggs of tawny owl (*Strix aluco*) from different provinces in Sweden collected in 2014. Number in brackets refers to number of eggs >LOQ. For LOQ see materials and Methods

	PFHxA	PFHxS	PFOS	PFNA	PFDA	PFUnDA	PFDoDA	PFTTrDA	PFTDA	$\Sigma$ PFAs <sup>1</sup>
Skåne (SK)	1,50(3) 1,3-1,6	0,960 (1)	32,0 (3) 25-34	0,35 (3) 0,34-0,67	1,0 (3) 1,0-2,1	1,3 (3) 1,1-1,3	1,8 (3) 1,7-2,0	1,3 (3) 1,2-2,8	0,55 (3) 0,37-2,6	42,0 (3) 34,7-43,2
Västergötland (VG)	1,4 (3) 1,3-1,4	0,38 (1)	6,9 (4) 3,2-18	0,24 (4) 0,17-0,33	0,69 (4) 0,47-0,85	0,92 (4) 0,5-1,6	0,74 (4) 0,48-0,94	1,4 (4) 0,63-2,7	0,61 (3) 0,34-1,1	11,735 (4) 7,56-27,2
Småland (SM)	1,7 (3) 1,3-1,5	<LOQ	3,9 (3) 3,6-5,6	0,33 (3) 0,29-0,37	0,87 (3) 0,79-0,98	1,7 (3) 1,3-2,4	1,1 (3) 0,86-2,4	1,8 (3) 1,3-2,4	0,46 (3) 0,27-0,65	13,82 (3) 11,0-13,8

<sup>1</sup>Sum of values >LOQ for PFHxA, PFHxS, PFOS, PFNA, PFDA, PFUnDA, PFDoDA, PFTTrDA, PFTDA.



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Table 9. Median, minimum and maximum values of PFHxA, PFHxS, PFOS, PFNA, PFDA, PFUnDA, PFDoDA, PFTTrDA, PFTDA, and  $\Sigma$ PFAs (ng/g ww) in addled eggs of common kestrel (*Falco tinnunculus*) from different provinces in Sweden collected in 2014. Number in brackets refers to number of eggs >LOQ. For LOQ see materials and Methods

	PFHxA	PFHpA	PFHxS	PFOS	PFNA	PFDA	PFUnDA	PFDoDA	PFTTrDA	PFTDA	$\Sigma$ PFAs <sup>1</sup>
Skåne (SK)	0,57 (1)	<LOQ	0,475 (2) 0,47-0,48	9,7 (3) 4,6-19	0,53 (3) 0,34-0,74	1,05 (3) 1,0-1,1	1,5 (3) 0,81-1,6	1,16 (3) 0,7-1,9	1,5 (3) 0,79-2,5	1,5 (1)	19,1 (3) 7,81-25,6
Västmanland (VM)	<LOQ	0,31(1)	<LOQ	4,6 (9) 1,1-20	0,44 (8) 0,22-0,59	0,835 (6) 0,63-1	1,5 (8) 0,37-2	1,2 (7) 0,92-2	1,9 (8) 0,82-3	0,48 (6) 0,2-0,91	10,84 (9) 2,49-25,9
Dalarna (DA)	0,83 (1)	<LOQ	<LOQ	2,65 (12) 1,7-10	0,455 (12) 0,25-1	0,93 (8) 0,42-1,8	1,45 (12) 0,71-3,2	1,3 (9) 0,52-3,8	1,65 (8) 0,65-3	0,275 (2) 0,23-0,32	7,1 (12) 2,66-22,6
Hälsingland (HL)	<LOQ	<LOQ	<LOQ	3,1 (6) 1,8-4,8	0,945 (2) 0,89-1	1,1 (1) 0,51-1,4	0,8 (4) 0,47-0,59	0,55 (3) 0,84-1,2	0,95 (6)	nd	5,61 (6) 3,52-7,6
Västerbotten (VB)	<LOQ	<LOQ	<LOQ	3,2 (7) 2,2-4,8	0,56 (7) 0,48-1,2	0,75 (7) 0,61-1,7	1,3 (7) 0,9-2,4	0,88 (7) 0,55-1,6	1,2 (7) 0,94-2,5	0,335 (4) 0,27-0,35	7,76 (7) 6,48-14,2
Lapland (LL)	<LOQ	<LOQ	<LOQ	1,85 (2) 1,3-2,4	0,525 (2) 0,51-0,54	0,675 (2) 0,61-0,74	1,3 (2) 1,3-1,3	0,685 (2) 0,64-0,73	1,4 (2) 1,3-1,5	0,25 (1) 0,25	6,56 (2) 5,66-7,46

<sup>1</sup>Sum of values >LOQ for PFHxA, PFHpA, PFHxS, PFOS, PFNA, PFDA, PFUnDA, PFDoDA, PFTTrDA, PFTDA.