Naturhistoriska riksmuseet

Metals and organic contaminants in eagle owl (*Bubo bubo*) and Eurasian lynx (*Lynx lynx*) from different parts of Sweden

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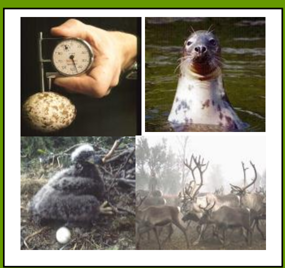

Content

Sammanfattning

Den här rapporten är skriven på uppdrag av Naturvårdsverket (Överenskommelse 221-1121). Syftet har varit att kartlägga geografiska skillnader i halter av metaller inklusive kvicksilver, spårämnen och ett antal organiska ämnen i lodjur (*Lynx lynx*) och berguv (*Bubu bubo*). Avsikten är att ta reda på om lodjur respektive berguv vore lämpliga att införa som predatorarter i en terrest miljöövervakning. Båda dessa arter tillhör Statens vilt vilket innebär att om de skjuts (legalt eller illegalt), dödas i en olyckshändelse eller hittas döda, ska tas om hand och skickas till Naturhistoriska riksmuseet (NRM) eller till Statens Veterinärmedicinska Anstalt (SVA)

25 berguvar fördelade på 5 län (områden) och 25 lodjur fördelade på 5 län (områden) valdes ut. Syftet var att få en så bra täckning som möjligt över landet. Eftersom studien var beroende av tillgång på befintligt material från miljöprovbanken begränsades urvalet till områden där det fanns ett någorlunda enhetligt material att tillgå.

För berguv valdes landskapen Norrbotten (län BD), Dalarna (län W), Södermanland (län D), Östergötland (län E) och Småland (län F,G och H). För lodjur valdes landskapen Jämtland (län Z), Ångermanland (län Y), Hälsingland (län X), Dalarna (län W) och området Bergslagen (län S, T och U).

För berguv valdes djur av båda könen medan enbart hanar av lodjur användes.

Av praktiska skäl är åldrarna varierande när det gäller berguv. Dessa är mycket svåra att åldersbestämma om de inte ringmärkts som boungar. Även beträffande lodjur varierar åldrarna något men ligger i de flesta fall mellan 2 och 4 år. För c:a hälften av berguvarna och en tredjedel av lodjuren saknades uppgifter om ålder.

Tio metaller (As, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Zn) analyserades i lever.

Ett antal PCB (28,52,101, 118, 138, 153, 180), och klorerade pesticider inklusive DDT analyserades i muskel.

Ett antal bromerade flamskyddsmedel (PBDE), tetrabromobisphenolA (TBBPA), decabromobiphenyl (DeBB) och hexabromcyclododecan (HBCD) analyserades i muskel.

Femton perflorerade substanser (PFAS) analyserades i lever.

Även de stabila isotoperna N^{15} och C^{13} har analyserats i muskel[1].

Lodjur hade högre kadmiumhalt (0,127µg/g ww) än berguv (0,042 µg/g ww). Median av samtliga analyserade prover. Kvicksilverhalten hos berguv (0,621 mg/g ww) var högre än

[1] Pga försening av analyserna kommer dessa att redovisas separat

hos lodjur (0,09 µg/g ww). En geografisk skillnad i halter av både kadmium och kvicksilver hittades hos lodjur men inte hos berguv. I inget fall var halterna så höga att en miljöbelastning kunde misstänkas. Blyhalterna var generellt låga hos både berguv (0,022 µg/g ww) och lo (0,017 µg/g ww) och för 11 av lodjursproverna och 8 av berguvsproverna var halterna <LOQ. Inget fall av hög blybelastning hittades hos berguv vilket tidigare hittats hos andra rovfåglar, bl a havsörn vilket tyder på att berguv inte är lika utsatt för blyförgiftning via födan.

Berguv hade generellt högre halter av samtliga analyserade organiska substanser. För ΣPCB var halterna hos berguv (9440 ng/g lw) betydligt högre än hos lodjur (150 ng/g lw) och fler PCB konjener hittades hos berguv.
En nord sydlig geografisk gradient hittades i belastning hos berguv med högre halter längre söderut.
Ingen sådan gradient hittades hos lodjur. Tvärtom hade i vissa fall lodjuren från de nordligaste länen (Z, Y) en tendens till högre belastning.

För berguv fanns en geografisk gradient i belastning även för bromerade ämnen med (icke signifikant) högre halter hos berguvar från Småland. Ingen av de analyserade bromerade substanserna hittades i halter >LOQ hos lodjur.

Även för perfluorerade ämnen (PFAS) var halterna högre hos berguv än hos lodjur med undantag av PFOA och PFNA som var högre i lodjur. PFOS var den dominerande substansen både hos berguv (74,5 ng/g ww) och hos lodjur (17,4 ng/g ww). För PFAS ämnena fanns inte någon tydlig geografisk gradient varken hos lodjur eller berguv. De högsta halterna hittades i en berguv från Norrbotten (ΣPFAS =914 ng/g ww).
Även för vissa PFAS substanser fanns en tendens till högre halter i lodjur från de två nordligaste länen.
Både berguv och lodjur är möjliga att använda i en terrest miljöövervakning om man tar hänsyn till vissa fakta.
Tillgången på material kan variera både geografiskt och över tid.
Framförallt kan tillgången på berguv variera både geografiskt och tidsmässigt. De djur som kommer till NRM är sådana som hittats döda, eller förolyckats i olyckor t ex genom kollision med kraftledningar eller genom kollisioner med bilar och tåg. Det förutsätter att det finns någon som hittar dem och att de inte är i för dåligt skick när de hittas. Därför kommer det av

naturliga skäl in mer material från mer befolkade områden vilket kan ha betydelse för halterna av miljögifter i uvarna.
För lodjur är tillgången bättre eftersom en begränsad lodjursjakt är tillåten i vissa delar av landet på villkor att djuren skickas till SVA för undersökning.
För båda dessa arter varierar halterna kraftigt mellan olika individer vilket gör att det är svårt att använda sig av poolade prover.

Summary

The present study has been carried out on mandate and in cooperation with the Swedish Environmental Protection Agency (SEPA) according to agreement 221-1121. The aim has been to obtain knowledge of geographical differences in levels of certain environmental contaminants in Eurasian lynx (*Lynx lynx*) and eagle owl (*Bubo bubo*). The aim has also been to evaluate the possibility to use the two predator species in the monitoring of contaminants in terrestrial biota.
Both of these species belongs to the State Game Act which states that they, if shot (legally or illegally), killed by accident or found dead should be taken care of and sent to the Swedish Museum of Natural History (SMNH) or the Swedish Veterinary Agency (SVA).
25 eagle owls from 5 different counties and 15 lynx from 5 different counties were chosen. The goal was to get a good geographical coverage of Sweden. As this study was dependent on material available from the Environmental Specimen Bank the choice was limited to areas were a reasonably good material was available.
For eagle owls the province of Norrbotten (BD), the province of Dalarna (W), the province of Södermanland (D), the province of Östergötland (E) and the province of Småland (counties F, G and H) was chosen.
For lynx the province of Jämtland (Z), the province of Ångermanland (Y), the province of Hälsingland (X), the province of Dalarna (W) and the area called Bergslagen (counties S, T and U) was chosen.
For eagle owls, individuals of both sexes were used while only males of lynx were chosen. The ages varied for eagle owls and no age was known for about half of the individuals. Most of the lynx was 2-4 years old but for ten individuals no age was known. All of the animals used appeared healthy and in good condition.
Ten metals and elements including cadmium, mercury and lead were analysed in liver.
Seven PCB conjeners (PCB28, 52, 101, 118, 138, 153, 180) and a number of chlorinated pesticides including DDT was analysed in muscle.

A number of brominated flame retardants (PBDE), tetrabromobisphenolA (TBBPA), decabromobiphenyl (DeBB) and hexabromocyclododecan (HBCD) was analysed in muscle. Fiftheen perfluorinated compounds (PFAS) were analysed in liver.
Stable isotopes N^{15} and C^{13} was analysed in muscle[2].

Lynx had higher levels of cadmium (0,127 µg/g ww) compared to eagle owls (0,042 µg/g ww) while mercury levels were higher in eagle owls (0,621 µg/g ww) compared to lynx 0,09 µg/g ww). A geographical difference could be detected in lynx but not for eagle owls. The levels were low and below levels were environmental exposure could be suspected.
Lead levels were low in both lynx (0,017 µg/g ww) and eagle owl (0,022 µg/g ww) and 11 of the lynx samples and 8 of the eagle owl samples were <LOQ. No case of elevated lead levels could be found in eagle owls as has earlier been found in other raptors. This indicates that eagle owls are not as exposed to lead intoxication through their food.

Eagle owls had generally considerably higher levels of most organic contaminants compared to lynx.
ΣPCB was substantially higher in eagle owls (9440 ng/l lw) compared to lynx (150 ng/g lw) and more PCB conjeners were also found in eagle owls.
A north –south gradient could be detected in eagle owls with higher levels in the southern parts of the country. No such gradient was found for lynx. On the contrary, the lynx from the two northernmost provinces (Z, Y) had a tendency towards higher levels.
For eagle owls a geographical gradient with higher levels further south could be detected also for brominated substances. None of the analysed brominated substances were found >LOQ in lynx.

Also for perfluorinated substances (PFAS) the levels were higher in eagle owls but there were a few exceptions. PFOA and PFNA were higher in eagle owls. PFOS was the dominating substance in both eagle owls (74,5 ng/g ww) and lynx (17,4 ng/g ww). No obvious geographical gradient could be detected in either lynx or eagle owls. The highest levels were found in an eagle owl from the province of Norrbotten (NB). In lynx, there was a tendency towards higher levels for some of the analysed PFAS in the two northernmost provinces.

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[2] Will be shown in an appendix to this report

The conclusion from the present study is that both lynx and eagle owls could be as predator species in terrestrial monitoring of environmental contaminants if some facts are taken into consideration.

Availability of material can differ considerably both geographically and over time.

Especially for eagle owls, this could be a problem. Animals that are brought to the SMNH are those that are found dead, usually close to electric wires, roads and railroads. This access to material is dependent on that they are actually found and that they are found in relatively good condition. There are probably more eagle owls found dead in the vicinity of populated areas which could be of importance for the levels of contaminants found in the birds

For lynx this is less of a problem as a hunt on lynx is allowed in certain areas on condition that the animal is sent to SVA for examination. This could be a good opportunity to get reliable samples of lynx.

There is a large variation between individuals for both eagle owls and lynx.

Aim

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

The aim was to investigate the possibility of introducing predator species in the monitoring of environmental contaminants in terrestrial biota.

Organisation

The material used (eagle owl and lynx) were collected through the Swedish “State Game” legislation (see below). Preparation and storage were performed by staff at the Swedish Museum of Natural History (SMNH). Chemical analyses of metals, chlorinated, and brominated compounds were performed by ALS Scandinavia AB. Perfluorinated compounds were analysed by ITM Department of Applied Environmental Science, Stockholm University. Stable isotopes were analysed by Aquatic Ecology, Department of Biology, Lund University. Funding (221 1121) were provided by SEPA.

Introduction

Comments on the choice of samples

The animals used in the present study represent levels of environmental contaminant around the year 2000. The reason that not more recent samples were used is that the main purpose of this study was to clarify the possibility to utilize these two top predators in terrestrial environmental monitoring. No top predators have earlier been used in the terrestrial monitoring of contaminants in biota. To be able to evaluate the results it was important that the material was as homogenous as possible regarding age, sex and nutritional status. It was also important to get enough material from the chosen areas to be able to draw some conclusions on individual variations and to make some statistical evaluation of the results. As these species are protected it is not possible to actively collect samples but we have to rely on samples from animals that are found dead or, as with the Eurasian lynx, shot in the legal hunt. A restricted hunt on lynx is allowed and earlier, tissue material from these animals was saved in the Swedish Environmental Specimen Bank (SESB). After the year 2000, only tissues from lynx that are illegally killed (illegal hunt), killed with special permission (skyddsjakt), accidently killed (traffic, trains), or found dead without any known cause of dead are preserved in SESB. Because of this there was a fairly good material available on lynx from the year 2000 but after that year the tissue material that was available was more scarce and of less quality. The restricted hunt on lynx that are allowed (licensjakt) could be a good way of acquiring good material for analysis of environmental contaminants as all animals from the hunt are taken care of and are examined at the Swedish Veterinary Institute. Unfortunately, very restricted amount of tissues has been saved from these animals after the year 2000 and these samples are not saved in the SESB. To get a fairly reliable and homogenous material from approximately the same time span of both lynx and eagle owls it was decided to use the material from around year 2000 although this would not give an up to date picture of the levels of contaminants in these animals.

The use of traumatically killed animals and animals found dead without known cause of dead might introduce a bias in the results as these animals might not be representative to the whole population. However, the animals used in the present study was chosen to be as homogenous as possible in sex and age and only animals that were presumed to be healthy and appeared to have been in good condition was used.

Materials and methods

Eurasian lynx

The Eurasian lynx (*Lynx lynx*) is Europe's largest wild cat. The Eurasian lynx belongs to the Felidae family. The Swedish population is about 1500 animals. Lynx can be found, although it is rare in all parts of the country (except on Gotland and Öland) but it is most abundant in central Sweden.

It feeds on roe deer, hare, small mammals, and forest birds. In northern parts of Sweden reindeer can be a large part of the diet.

Lynx are usually solitary animals (females live with their young of the year) and their home lands are large (100-2500 km^2)

A normal life span for lynx is 12-14 years. In lynx, age can be determined by counting growth layers in teeth.

Lynx is a protected species in Sweden and animals that are accidently killed or found dead belongs to the state ("State Game"). Although it is a protected species, a limited hunt on lynx is allowed.

Lynx in this study

Only males were used in the present study. Age was determined for 15 lynx and these were between 1 and 6 years of age. The median (min-max) weight for the lynx in the present study was 20 (14-25 kg).

All lynx except one was shot in legal hunt. One lynx was killed by a car.

Cause of death for lynx used in the present study is shown in Table 1. Biological data is shown in Table 2.

Table 1. Cause of death for lynx used in the present study

96% (24)	legal hunt
4% (1)	traffic

Table 2. Age, sex, weight and fat (%) in analysed muscle samples of Eurasia lynx used in the present study.

County	age	sex	weight (kg)	fat (%)
Z	2	M	18	1,7
Z	2	M	17	4,1
Z	2	M	21	3
Z	2	M	17,5	1
Z	2	M	14	1
Y	-	M	18,5	1,6
Y	6	M	20	1,9
Y	-	M	16	1,5
Y	-	M	20	1,3
Y	4	M	20	4,5
X	2	M	23	0,85
X	-	M	23	2,2
X	-	M	20	2,5
X	-	M	22	2,9
X	3	M	19,7	1,2
W	-	M	20	2,5
W	-	M	23	1,8
W	2	M	23,5	1,7
W	4	M	21,5	2,3
W	2	M	21,6	2,5
STU	2	M	19	2,7
STU	1	M	25	1,6
STU	2	M	21	2,2
STU	-	M	23	1,4
STU	-	M	22	1,3

Eagle owl

The eagle owl (*Bubo bubo*) is Europe's largest owl species. It is present in large parts of Europe and Asia. Eagle owls are found in all parts of Sweden except the northern mountain areas and inhabit the same type of biotope as the lynx, large forests with steep cliffs but it is also found in coastal areas and archipelagos with rocky islands. It can also be found in cities and industrial areas where it forages on refuse tips.

The eagle owl has earlier been a common bird but during the last century the population decreased. This was due both to a legal as well as illegal hunt and plundering of nests. From the middle of the last century increasing levels of environmental pollutants also played a role in diminishing the population of eagle owls in Sweden. The population declined almost to extinction and several breeding projects started in order to try to reestablish the population. In Sweden, the population has now been estimated to be approximately 500 breeding pairs.

Eagle owls are stationary and once it has established a territory (4-6 km^2), it usually stays there for the rest of its life. A normal life span for a wild eagle owl is about 20 years. Many eagle owls are ringed as nestlings, which makes it possible to identify single individuals and know both the age and the place of birth of a bird found dead.

Eagle owls are active during the night and preys on small mammals up to the size of hares and rabbits, birds including gulls and crows, and raptors including other owl species.

Since 1950, eagle owl is a protected species and, like lynx, eagle owls accidently killed or found dead belong to “State Game”. All animals used in the present study came to the Swedish Museum of Natural History (SMNH) through the Swedish State Game Act.

Eagle owls in this study

To be able to know the age of eagle owls, they have to be ringed as nestlings. In this study 12 of the 25 eagle owls were ringed and thus could be aged. The age of these birds was between 0,5 and 14 years. For ten of these the place of birth was also recorded.

There were 11 males, 12 females and for two eagle owls the sex was unknown. The weight was known for 23 of the eagle owls, 8 males, 13 females and 2 of unknown gender. The median weight (min- max) was 2,3 (1,6-2,8) kg for males and 2,9 (2,3-3,3) kg for females. The cause of death for the eagle owls was electricity, i.e collisions with power lines (52 %) or collisions with cars or trains (24%). One eagle owl was found shot (shotgun) and for the rest, no known cause of death was registered.

Three of the eagle owls in the study were raised in breeding programs.

Cause of death is shown in table 3. Biological data are shown in table 4.

Table 3. Cause of death for eagle owls used in the present study.

52% (13)	electricity
24% (3+3)	traffic (train/car)
12% (3)	found dead – no cause of death available
4% (1)	shot (illegal hunt)
4% (1)	stuck in fence
4% (1)	no information

Table 4. Age, sex, weight and fat (%) in analysed muscle samples of eagle owls used in the present study.

County	age	sex	weight (kg)	fat (%)
BD	3	F	3,3	1,7
BD	4	M	2,8	2,2
BD	2	M	2,3	3,2
BD		M	1,9	0,78
BD	4	M		1,8
W		F	2,8	2,4
W	4	F	2,2	1,5
W	2	F	2,7	2,7
W	10	M	2,3	2,5
W		u	2,7	2,3
D		F	2,5	2,5
D	14	F	2,9	2,1
D		F	2,9	1
D	<1	M		1,8
D		u	2,4	1,6
E		F	3,1	1,9
E	1	M	2,2	1,9
E		M	1,6	1
E		M	2,5	2,1
E		M	2,8	2,9
FGH		F	2,6	1,9
FGH	6	F	3	2
FGH		F	2,9	3,4
FGH	1	M	2,4	1,5

Areas

The purpose of this study is to reveal the background burden of environmental contaminants in a mammal and a bird top predator in Sweden. Both the lynx and the eagle owl can be found in large parts of Sweden although both species are scarce.

The eagle owls used in the present study came from the provinces (the letter of the counties in brackets) of Småland (F, G, H) Östergötland (E), and Södermanland (D) in the south, Dalarna (W) in the central and Norrbotten (BD) in the northern part of Sweden. The lynx used in the present study came from the Västmanland/Värmland (S, T, U) area (Bergslagen), Dalarna (W) and Jämtland (Z) in the inland central parts and Hälsingland (X) and Ångermanland (Y) in the central coastal parts of Sweden.

Sampling and preparation

Muscle and liver samples of Eurasian lynx and eagle owl that has arrived to the SMNH through the State Game Act have been stored frozen (-25 C) in the Swedish Environmental Specimen Bank (SESB).

Liver and muscle samples from Eurasian lynx collected in 1999-2002 and eagle owls collected in 1998-2004 were used.
Livers were used for analysis of metal and perfluorinated compounds (PFAS).
Muscle was used for chlorinated and brominated compounds.
About 35-40 g of muscle tissue was prepared for analysis of chlorinated and brominated compounds
About 1 g of liver tissue was prepared for analysis of PFAS
About 5-8 g of liver tissue was prepared for analysis of metals.
Fat determination was done on the muscle samples.
Analysis of the stable isotopes N^{15} and C^{13} were performed on muscle tissue.

Results are presented on lipid weight basis (chlorinated and brominated compounds) and wet weight basis (metals and PFAS). When comparisons are made with data from the literature that are made on dry weight basis, a conversion factor of 4,6 have been used for liver data (Jager et al. 1996).

Limit of quantification (LOQ)

In the lab results the limit of quantification (LOQ) has been used. Values below LOQ have been assigned < in the lab reports. The LOQ denotes the lowest level of a substance where a reasonably accurate quantification is possible. For lipid soluble substances that are determined on lipid weight basis, LOQ is dependent on the lipid content of the sample. Thus, the LOQ for the analysis differs between samples. The expression “below LOQ” (or<LOQ) is used in the text to denote these cases but no actual numbers are given as it differs between samples. The span in LOQ for each analysis is given for eagle owls and lynx in table 5 (metals), table 6 (chlorinated compounds), table 7 (brominated compound) table 8 (perfluorinated compounds). When no LOQ is given in the tables, all of the analysed samples were above LOQ.

Analysed compounds

The compounds analysed in this study were "traditional" environmental contaminants i.e metals, chlorinated compounds (pesticides, PCB), brominated flame retardants (PBDE) and perfluorinated compounds. The purpose was to compare the levels in lynx and eagle owl with the contaminant level in the species that are included in the environmental monitoring programme on terrestrial biota earlier analysed.

Furthermore, analysis of the stable isotopes N^{15} and C^{13} was performed on muscle tissue. Analysis of the stable isotopes has been used in order to trace energy sources and evaluate trophic positions and in recent years these methods have been extensively used in ecological studies (Post 2002). There is however certain limitations in these methods and caution must be taken when interpreting the results. In the present study this method will be used only in a relative sense and no attempt will be made to draw conclusions on feeding habits of neither eagle owls nor lynx. Due to a delay in the analytic procedure, these results will be presented in a separate section at a later occation.

Metals

Ten metals were analysed in liver tissue (Table 5). Analysis of metals was performed by ALS Scandinavia (M-4).

Table 5. Metals and elements analysed in liver samples from eagle owl (*Bubo bubo*) and Eurasian lynx (*Lynx lynx*). LOQ (µg/g ww) is given for metals were at least one analysis was below LOQ.

	LOQ
As	0,03 - 0,04
Cd	
Co	
Cr	0,01 – 0,02
Cu	
Hg	
Mn	
Ni	0,01 - 0,02
Pb	0,01 - 0,02
Zn	

Chlorinated compounds

Chlorinated compounds and pesticides analysed (Table 6). Chlorinated compounds and pesticides were performed by ALS Scandinavia (OB-2 and OB-3a).

Table 6. Chlorinated compounds and LOQ (ng/g lipid weight) for the analysis in muscle tissue of eagle owls and Eurasian lynx.

		CAS-nr	LOQ[1]	LOQ[2]
polychlorinated biphenyl (PCB)		1336-36-6		
PCB 28			4,3-10	2,2-12
PCB 52			3,1-13	2,2-12
PCB 101			3,7-10	2,2-12
PCB 118				2,2-12
PCB 138				7,6-22
PCB 153				4,3
PCB 180				4,3
Pentachlorobenzene		608-93-5	31-130	22-120
Hexachlorobenzene	(HCB)	118-74-1	37-56	22-120
hexachlorocyclohexane	α-HCH	319-84-6	29-130	22-120
	β-HCH	319-85-7	31-130	22-120
	γ-HCH (lindan)	55963-79-6	29-130	22-120
aldrin		309-00-2	29-130	22-120
dieldrin		60-57-1	31-130	22-120
endrin		72-20-8	29-130	22-120
isodrin		465-73-6	29-130	22-120
telodrin		297-78-9	29-130	22-120
heptachlor		76-44-8	29-130	22-120
cis-heptachlorepoxid		28044-83-9	40-2700	22-120
trans-heptachlorepoxid			29-130	22-120
o,p-DDT		789-02-6	29-130	22-120
p,p-DDT		50-29-3	29-130	22-120
o,p-DDE		3424-82-6	29-130	22-120
p,p-DDE		72-55-9	31-130	22-120
o,p-DDD		53-19-0	29-130	22-120
p,p-DDD		72-54-8	56	22-120
alfa-endosulfan		959-98-9	29-130	22-120
hexachlorobutadien		87-68-3	29-130	22-120
hexachloroethan		67-72-1	29-130	22-120

[1]LOQ in eagle owl samples
[2]LOQ in lynx samples

Brominated compounds

Brominated compounds including brominated flame retardants analysed (Table 7). Of tetraBDEs, PBDE 47 was identified and quantified. Of pentaBDEs, PBDE 99 and PBDE 100 was identified and quantified. For hexa-, hepta-, okta-, and nonaBDE no single conjener were identified and quantified.

Analysis of brominated compounds was performed by ALS Scandinavia (OB-25).

Table 7. Brominated compounds and LOQ (ng/g lipid weight) for the analysis in muscle tissue of eagle owls and Eurasian lynx.

	CAS-nr	LOQ[1]	LOQ[2]
tetraBDE		64	11-59
PBDE 47	5436-43-1		1,1-5,9
pentaBDE		24-130	22-120
PBDE 99	60348-60-9	130	2,2-12
PBDE 100	189084-64-8	130	2,2-12
hexaBDE		37-130	22-120
heptaBDE		31-130	22-120
oktaBDE		63-260	44-240
nonaBDE		160-640	110-590
dekaBDE	1163-19-5	160-640	110-590
tetrabromobisphenol A (TBBPA)	79-94-7	160-640	110-590
decabromobiphenyl (DeBB)		13654-09-6	160-640
	110-590		
hexabromcyklododekan (HBCD)	3194-55-6	310-1300	220-1200

[1]LOQ in eagle owl samples

[2]LOQ in lynx samples

Perfluorinated compounds (PFAS)

Fifteen perfluorinated compounds (PFAS) was analysed in liver tissue of eagle owls and Eurasian lynx (Table 8).

Sample extraction and clean-up was based on the method by (Powley et al. 2005) with modifications for biota samples described by (Holmström et al. 2010). In short, 1 g of the homogenized liver was spiked with the mass-labeled internal standards. Extraction was performed twice with 5 mL acetonitrile in an ultrasonic bath. The combined extracts were concentrated to 1 mL and subjected to dispersive clean-up on graphitized carbon. The cleaned-up extract was added to aqueous ammonium acetate. Precipitation occurred and the extract was centrifuged before instrumental analysis of the clear supernatant. Aliquots of the final extracts were injected automatically on a high performance liquid chromatography system coupled to a tandem mass spectrometer, using an instrumental method described by (Vestergren et al. 2012). Compound separation was achieved on a C18 reversed phase column with a binary gradient of buffered (ammonium acetate) methanol and water. The mass spectrometer was operated in negative electrospray ionization mode. Quantification was performed in selected reaction monitoring chromatograms using the internal standard method.

The analyses on perfluorinated compounds were carried out by the Department of Applied Environmental Science (ITM), Stockholm University.

Table 8. Perfluorinated compounds (PFAS) and LOQ (ng/g ww) for the analysis in liver tissue of eagle owls and Eurasian lynx. Terminology and acronyms from (Buck et al. 2011).

		CAS-nr[1]	LOQ
Perfluorohexanoic acid (C_6)	PFHxA	307-24-4	0,388
Perfluoroheptanoic acid (C_7)	PFHpA	375-85-9	0,125
Perfluorooctanoic acid (C_8)	PFOA	335-67-1	0,049
Perfluorononanoic acid (C_9)	PFNA	375-95-1	0,187
Perfluorodecanoic acid (C_{10})	PFDA	335-76-2	
Perfluoroundecanoic acid (C_{11})	PFUnDA	2058-94-8	
Perfluorododecanoic acid (C_{12})	PFDoDA	307-55-1	0,08
Perfluorotridecanoic acid (C_{13})	PFTrDA	72629-94-8	
Perfluorotetradecanoic acid (C_{14})	PFTeDA	376-06-7	0,019
Perfluoropentadecanoic acid (C_{15})	PFPeDA	141074-63-7	0,026
Perfluorobutane sulfonic acid (C_4)	PFBS	375-73-5	0,035
Perfluorohexane sulfonic acid (C_6)	PFHxS	355-46-4	
Perfluorooctane sulfonic acid (C_8)	PFOS	1763-23-1	
Perfluorodecane sulfonic acid (C_{10})	PFDS	335-77-3	0,069
Perfluorooctane sulfone amid (C_8)	FOSA	754-91-6	

[1]Cas-nr for the acid form

Stable isotope analysis

Approximately 1 g of muscle tissue was dried at 60 C to constant weight. The dried muscle was pulverized and 200-600 mg of the dried, pulverized tissue was placed in plastic vials and kept in an air tight glass jar with drying material at room temperature until analysed. Between 250 and 450 µ were weighted in tin capsules (6x4 mm) and analysed for the stable isotopes N^{15} and C^{13}.

Analysis of stable isotopes was performed at Aquatic Ecology, Department of Biology, Lund University.

More information and results from this analysis will be provided in a supplement.

Statistics

Because of the small sample size and because much of the data was not normally distributed (left-scewed), a non-parametric Kruskal-Wallis test was used to detect differences in contaminant levels between different counties. The significance level was set to $p<0,5$. If statistical significance has been found in the Kruskal-Wallis ANOVA, no attempt has been made to further evaluate the data due to the small sample sizes.

Statistical software used was StatSoft, Inc. (2011). STATISTICA (data analysis software system), version 10. www.statsoft.com

Results

Lipid content

The lipid content of the analysed muscle samples was approximately the same (~ 2%) in eagle owls and lynx. (Table 2 and 4)

Metals and elements

Ten metals (As, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Zn) were analysed in liver tissue of eagle owl and lynx. Co, Cu, Mn, and Zn are essential elements. The median (min-max) for each province is shown in table 9 for eagle owls and in table 10 for lynx.

Toxic metals and elements

Cd and Hg were found in levels above LOQ in all samples. Pb were found in levels above LOQ in 17 (68%) of the eagle owl samples and in 14 (56%) of the lynx samples.

Cadmium levels were significantly higher in lynx (median=0,121; min-max= 0,02-0,30 mg/g ww) compared to in eagle owls (median=0,042; min-max=0,010-0,158 µg/g ww).

No geographical trend in cadmium levels could be detected for eagle owls (p=0,55). For lynx there was a significant different (p=0,045) between the different counties (Fig 1).

Mercury levels were higher in eagle owls (0,62; 0,054-1,68) compared to in lynx (0,09; 0,012-0,42).

No geographical trend in mercury level could be detected for eagle owls (p=0,62). For lynx, a significant difference could be detected between lynx from different counties (p=0,007) (Fig 2).

No difference was found in lead levels between eagle owl (0,022; <LOQ-0,076) and lynx 0,017; <LOQ-0,81). No geographical differences were found in either lynx or eagle owl. One lynx from the province of Hälsingland (X) and two from the provinces of Bergslagen (STU) had relatively high lead levels. As these three lynx were killed in hunt, one probable explanation to the higher lead levels was that the samples had been contaminated by the lead ammunition.

Arsenic (As) and nickel (Ni) are non-essential, toxic metals. Arsenic was found above LOQ in one of the lynx samples (0,152 µg/g ww), a 3 year old male from the province of Ångermanland (Y). None of the eagle owls had As levels above LOQ. Nickel was found in two of the eagle owl samples (0,0226-0,0366 µg/g ww) but not in any of the lynx samples.

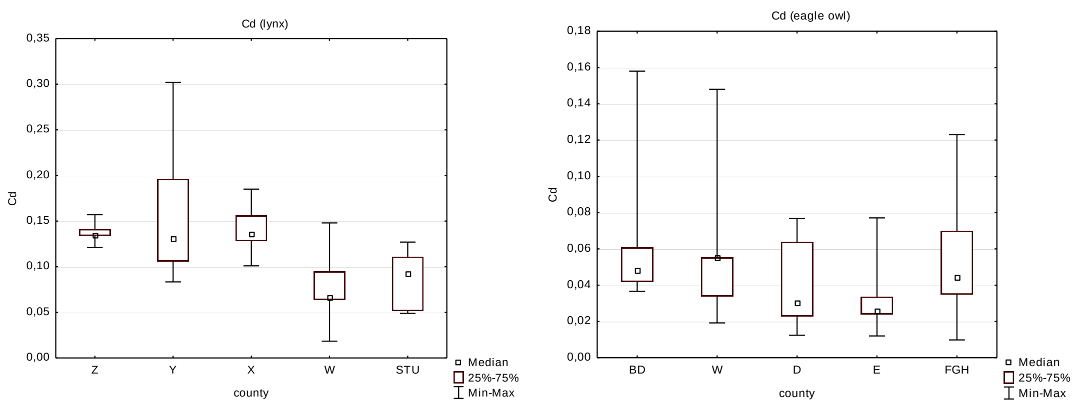

Fig 1. Cadmium levels in liver of lynx (p=0.045) and eagle owls (p=0,55) from different counties in Sweden.

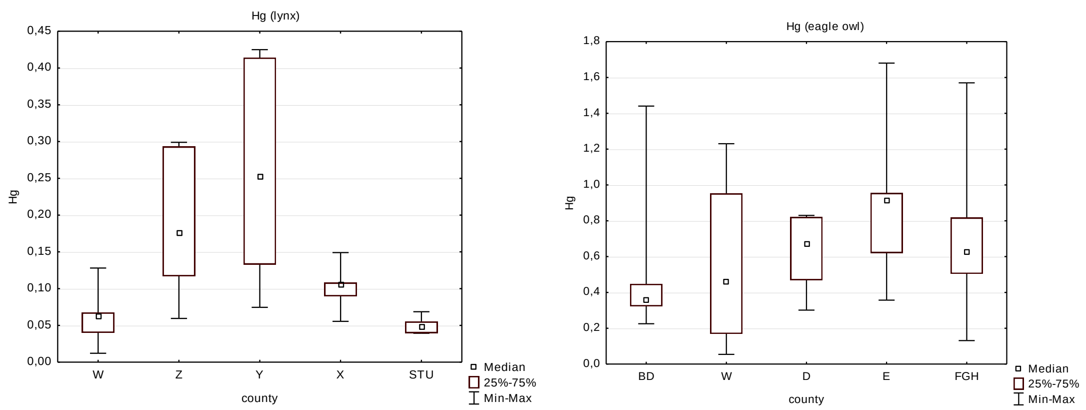

Fig 2. Mercury levels in liver of lynx (p=0,007) and eagle owls (p=0,62) from different counties in Sweden.

Essential metals and elements

No geographical differences were found in the level of cobalt (Co), copper (Cu), manganese (Mn) or zinc (Zn) for either lynx or eagle owl.

Chromium (Cr) was below LOQ in all but 4 of the lynx samples (0,015-0,0746 µg/g ww) and one of the eagle owl samples (0,0149 µg/g ww).

Comments on metals

Cadmium and mercury were found in in all samples of both eagle owl and lynx. Cadmium levels were higher in lynx while mercury levels were higher in eagle owl. Geographical differences were found for lynx but not for eagle owl. Lynx from the counties of Jämtland (Z) and Ångermanland (Y) had the highest levels of both cadmium and mercury.

In the present study, the highest Cd level found in an eagle owl was 0,158 µg/g ww found in a 4 year old male killed by electricity outside Luleå in the province of Norrbotten (BD). According to (Scheuhammer 1987), a concentration of < 3 µg/g dry weight (~ 0,7 µg/ g ww) of Cd in liver of wild freshwater ducks indicated that they had not been exposed to increased environmental exposure. According to these criteria, none of the eagle owls in the present study had Cd levels that indicated an increased environmental exposure.

The highest cadmium level in lynx was 0,303 µg/g ww found in a 3 year old male from the province of Ångermanland (Y).

Lead was found in detectable levels in 68% of the eagle owl samples and in 56% of the lynx samples. There were no difference in lead levels between lynx and eagle owl. Three of the lynx samples were probably contaminated by lead ammunition.

It has been shown that lead poisoning can be a significant cause of death in white tailed sea eagle (Helander et al. 2009). Lethal lead levels in white tailed sea eagle have been estimated to 20 µg/g dw in liver. Almost 14% of the white tailed sea eagles that were examined in the study by Helander et al (2009) had died of lead poisoning and 22% had elevated lead levels (> 6 µg/g dw) in either liver or kidney. In the present study the highest lead level found in the liver of an eagle owl was 0,076 µg/g ww. This is in accordance with previous studies were it has been found that owls usually are less likely to suffer from high lead levels (Pain et al. 1995).

In lynx, the median lead level was 0,0326 µg/g ww . The highest lead level was 0,81 µg/g ww and this was found in a lynx from the province of Hälsingland (X).

Mercury was found above LOQ in all of the analysed samples. In eagle owls, the median level was 0,621 µg/g ww. The highest mercury level found was 1,68 µg/g ww and this was found in an owl from the province of Östergötland (E). In all, four eagle owls had mercury levels above 1 µg/ g ww. Normal Hg concentrations in livers of birds living in non-polluted areas has been estimated to 1-10 µg/ g ww (Scheuhammer 1987). The higher levels are associated with fish eating birds. The levels found in eagle owls in the present study indicate no exposure to elevated environmental mercury levels.

In lynx, the median level was 0,0897 µg/g ww and the highest level was 0,425 µg/ g ww which was found in a lynx from the province of Ångermanland (Y).

Table 9. Metals (µg/g ww) in liver tissue of eagle owls (*Bubo bubo*) from different parts of Sweden. For data on LOQ see table x

	As	**Cd**	**Cr**	**Cd**	**Co**	**Cu**	**Hg**	**Mn**	**Ni**	**Pb**	**Zn**
BD											
N>LOQ	0	5	0	5	5	5	5	5	0	2	5
Median	<LOQ	0,0482	<LOQ	0,0482	0,0217	3,46	0,358	3,43	<LOQ	<LOQ	19,7
Min	<LOQ	0,0366	<LOQ	0,0366	0,0166	3,29	0,225	2,52	<LOQ	<LOQ	19,2
Max	<LOQ	0,158	<LOQ	0,158	0,0432	5,16	1,44	6,09	<LOQ	0,0632	33,9
W											
N>LOQ	0	5	1	5	5	5	5	5	1	5	5
Median	<LOQ	0,0551	<LOQ	0,0551	0,026	3,76	0,457	4,33	<LOQ	0,0223	29,7
Min	<LOQ	0,0192	<LOQ	0,0192	0,02	2,91	0,0542	2,59	<LOQ	0,0187	27
Max	<LOQ	0,148	0,0149	0,148	0,0316	4,15	1,23	5,59	0,0366	0,0721	37,8
D											
N>LOQ	0	5	0	5	5	5	5	5	0	3	5
Median	<LOQ	0,0301	<LOQ	0,0301	0,0344	3,56	0,669	3,62	<LOQ	0,0326	28,4
Min	<LOQ	0,0124	<LOQ	0,0124	0,0168	2,16	0,301	1,6	<LOQ	<LOQ	20,6
Max	<LOQ	0,0767	<LOQ	0,0767	0,0431	5,01	0,83	4,93	<LOQ	0,0764	46,2
E											
N>LOQ	0	5	0	5	5	5	5	5	1	3	5
Median	<LOQ	0,0253	<LOQ	0,0253	0,0308	4,12	0,916	3,53	<LOQ	0,0296	27,3
Min	<LOQ	0,012	<LOQ	0,012	0,0211	3,56	0,357	2,75	<LOQ	<LOQ	23,1
Max	<LOQ	0,0771	<LOQ	0,0771	0,0513	5,49	1,68	5,17	0,0226	0,0491	37,2
FGH											
N>LOQ	0	5	0	5	5	5	5	5	0	4	5
Median	<LOQ	0,0438	<LOQ	0,0438	0,0276	3,73	0,625	3,42	<LOQ	0,0272	32,5
Min	<LOQ	0,0098	<LOQ	0,0098	0,0232	2,62	0,131	2,7	<LOQ	<LOQ	18,8
Max	<LOQ	0,123	<LOQ	0,123	0,0681	7,24	1,57	5,81	<LOQ	0,0531	57,5

Table 10. Metals (µg/g ww) in liver tissue of Eurasian lynx (*Lynx lynx*) from different parts of Sweden. For information on LOQ see table x

	As	**Cd**	**Cr**	**Co**	**Cu**	**Hg**	**Mn**	**Ni**	**Pb**	**Zn**
Z										
N>LOQ	0	5	0	5	5	5	5	0	2	5
Median	<LOQ	0,134	<LOQ	0,0161	5,3	0,175	3,46	<LOQ	<LOQ	39,7
Min	<LOQ	0,121	<LOQ	0,0142	4,63	0,0595	2,54	<LOQ	<LOQ	35,8
Max	<LOQ	0,157	<LOQ	0,0186	6,47	0,299	4,54	<LOQ	0,062	50,5
Y										
N>LOQ	1	5	1	5	5	5	5	0	2	5
Median	<LOQ	0,131	<LOQ	0,016	5,31	0,253	3,33	<LOQ	<LOQ	38,9
Min	<LOQ	0,0834	<LOQ	0,0126	3,16	0,0746	2,17	<LOQ	<LOQ	24,4
Max	0,152	0,302	0,0296	0,0235	6,22	0,425	4,72	<LOQ	0,0243	65
X										
N>LOQ	0	5	0	5	5	5	5	0	4	5
Median	<LOQ	0,135	<LOQ	0,0175	5,2	0,106	3,2	<LOQ	0,0217	35,6
Min	<LOQ	0,101	<LOQ	0,0142	3,96	0,0555	2,82	<LOQ	<LOQ	32,2
Max	<LOQ	0,185	<LOQ	0,0218	7,46	0,149	3,91	<LOQ	0,81	41,1
W										
N>LOQ	0	5	1	5	5	5	5	0	3	5
Median	<LOQ	0,0655	<LOQ	0,0153	5,34	0,0618	2,62	<LOQ	0,0169	35,2
Min	<LOQ	0,0184	<LOQ	0,014	3,69	0,0121	2,3	<LOQ	<LOQ	32,6
Max	<LOQ	0,148	0,015	0,018	6,53	0,128	4,03	<LOQ	0,0802	56
STU										
N>LOQ	0	5	2	5	5	5	5	0	3	5
Median	<LOQ	0,0923	<LOQ	0,0169	5,21	0,0478	2,73	<LOQ	0,0791	30
Min	<LOQ	0,0489	<LOQ	0,0137	3,77	0,0393	2,13	<LOQ	<LOQ	25,7
Max	<LOQ	0,127	0,0746	0,0176	9,24	0,0686	3,95	<LOQ	0,227	49,6

Chlorinated compounds

Seven PCB conjeners were analysed (PCB 28, 52, 101, 118, 138, 153, 180). PCB 118, 138, 153, and 180 was found above LOQ in all of the eagle owl samples. PCB 118 was found in 16 (64%) of the lynx samples, PCB 138 and 180 was found in 23 (92%) of the lynx samples and PCB 153 was found in 24 (96%) of the lynx samples. PCB 28 and 101 was found above LOQ in 21 (84%) and 20 (80%) eagle owls samples but not in any lynx samples. PCB52 was found above LOQ in one of the analysed samples, an eagle owl from the province of Småland (FGH). Eagle owls had significantly higher levels of the analysed PCB conjeners (Fig 3). PCB levels in eagle owls are shown in table 11 and in lynx in table 12.
Dieldin, β-HCH and hexachlorobenzene (HCB) was detected in 18 (72%), 14 (56%) and 18 (72%) of the eagle owl samples. One lynx sample had detectable level of α-HCH (0, 34 µg/g ww) and β-HCH (0,097 µg/g ww). This lynx was from the province of Hälsingland (X). *p,p*-DDE was found in all but one of the eagle owl samples and in 5 (20%) of the lynx samples. *p,p*-DDD was found in 5(20%) eagle owl samples but not in any lynx samples.

Geographical differences

The level of ΣPCB for lynx and eagle owls from different counties is shown in fig 3. A significant difference was found for both eagle owls and lynx.

Eagle owl

The non-parametric Kruskal-Wallis ANOVA showed a significant difference in levels of PCB 118 (p=0,018), PCB 138 (p=0,036) and PCB 153 (p=0,022) but not PCB180 between eagle owls from the different counties (fig 4). Also for β-HCH (p=0,013), p,p-DDD (p=0,011), and p,p-DDE (p=0,034), a significant difference was detected between eagle owls from different counties. p,p-DDE was detected in all eagle owls except one that came from the county of Norrbotten (BD). Four of the five eagle owls with detectable levels of *p,p*-DDD was from the province of Småland (EFG). A tendency could be detected towards lower levels in eagle owls from the two northernmost counties Dalarna (W) and Norrbotten (BD).

Lynx

A significant difference was found between lynx from different counties for PCB153 (p=0,008) and PCB180 (p=0,007) (Fig 5). No north south gradient could be detected in lynx but lynx from Ångermanland (Y) had higher levels compared to lynx from other counties.

Pentachlorobenzene, γ-HCH (lindan), aldrin, isodrin, telodrin, cis-heptachlor, trans-heptachlor, α-endosulfan, hexachlorbutan, hexachloretan, o,p-DDT, p,p-DDT, o,p-DDD, and o,p-DDE was not found above LOQ in any of the analysed samples.

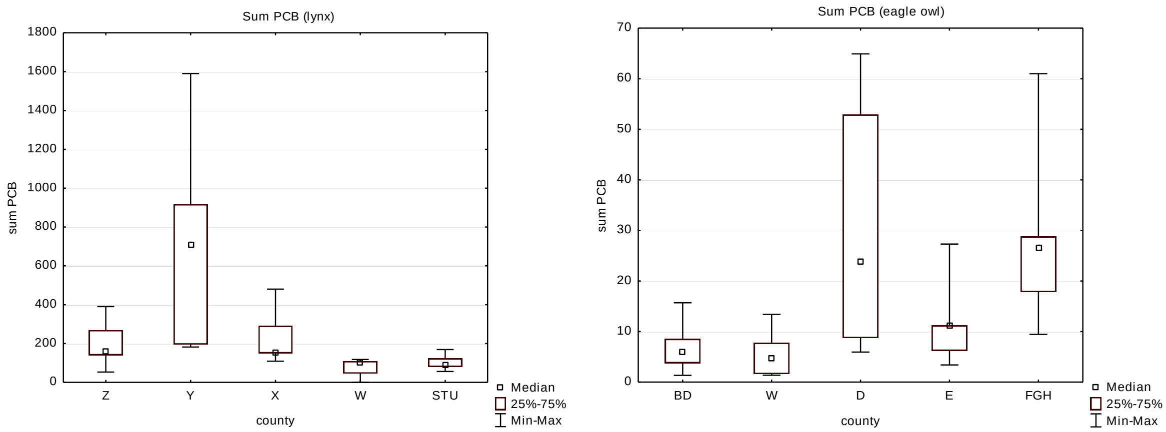

Fig 3. ΣPCB (ng/g lw) in lynx and eagle owl. p=0,03 (eagle owls) and p=0,008 (lynx) Kruskal-Wallis ANOVA.

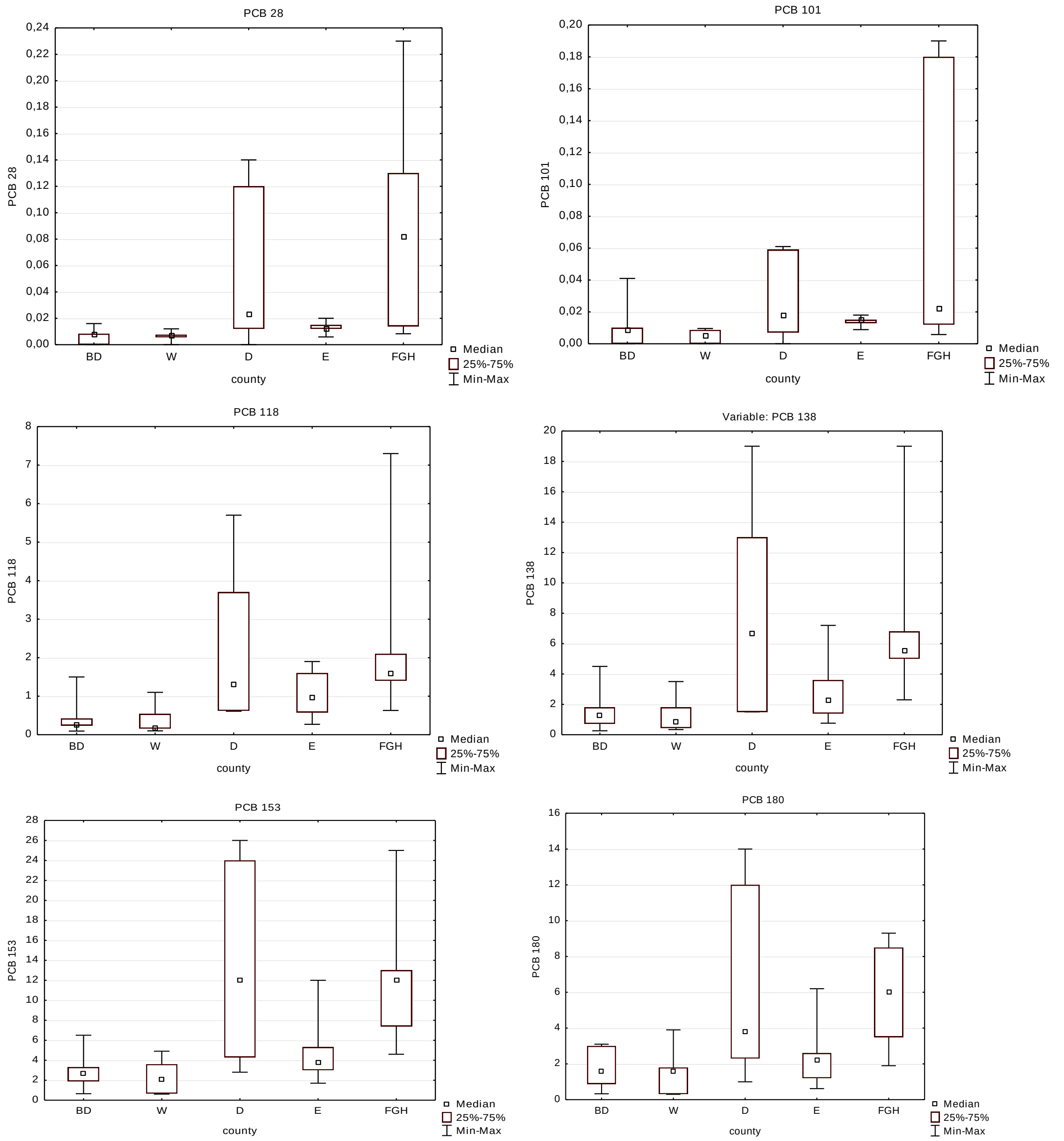

Fig 4. PCB 28, 101, 118, 138, 153 and 180 (ng/g lw) in muscle tissue of eagle owls from different parts of Sweden

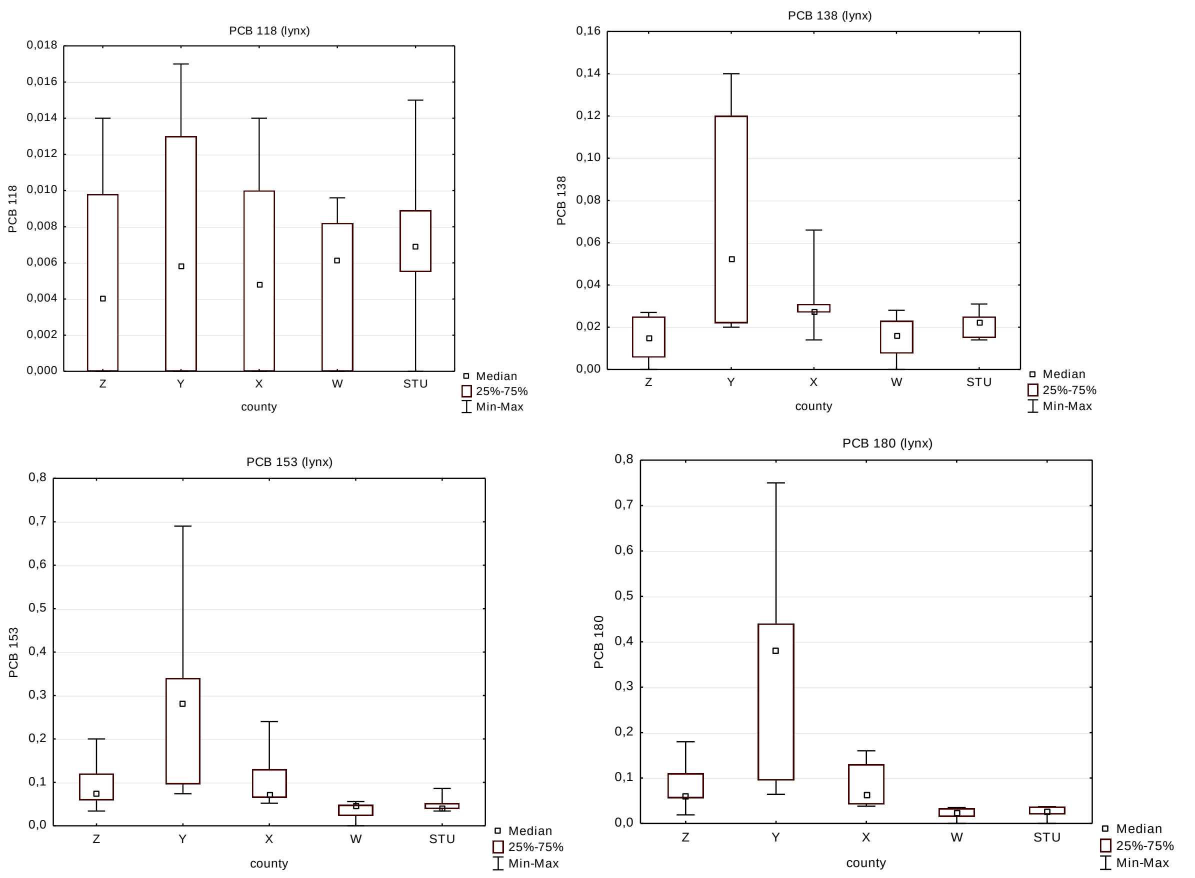

Fig 5. PCB 118, 138, 153 and 180 (ng/g lw) in muscle tissue of Eurasian lynx from different parts of Sweden.

Table 11. Seven PCBs (ng/g lw) in muscle tissue of eagle owl (*Bubo bubo*) from different parts of Sweden. For LOQs see table x.

	PCB 28	PCB 52	PCB 101	PCB 118	PCB 138	PCB 153	PCB 180	ΣPCB
BD								
N>LOQ	3	0	3	5	5	5	5	5
Median	7,8	<LOQ	8,4	250	1300	2700	1600	5860
Min	<LOQ	<LOQ	<LOQ	94	260	650	330	1330
Max	16	<LOQ	41	1500	4500	6500	3100	15700
W								
N>LOQ	4	0	3	5	5	5	5	5
Median	6,7	<LOQ	5,2	160	880	2100	1600	4750
Min	<LOQ	<LOQ	<LOQ	100	340	610	300	1360
Max	12	<LOQ	9,6	1100	3500	4900	3900	13400
D								
N>LOQ	4	0	4	5	5	5	5	5
Median	23	<LOQ	18	1300	6700	12000	3800	23900
Min	<LOQ	<LOQ	<LOQ	610	1500	2800	1000	5940
Max	140	<LOQ	61	5700	19000	26000	14000	64900
E								
N>LOQ	5	0	5	5	5	5	5	5
Median	12	<LOQ	15	980	2300	3800	2200	11200
Min	5,9	<LOQ	8,9	270	760	1700	620	3390
Max	20	<LOQ	18	1900	7200	12000	6200	27300
FGH								
N>LOQ	5	1	5	5	5	5	5	5
Median	82	<LOQ	22	1600	5500	12000	6000	26600
Min	8,3	<LOQ	5,8	630	2300	4600	1900	9440
Max	230	3,5	190	7300	19000	25000	9300	61000

Table 12. Chlorinated pesticides (ng/g lw) in muscle tissue of eagle owl (*Bubo bubo*) from different parts of Sweden. For LOQs see table x.

	HCB	α-HCH	β-HCH	γ-HCH	dieldrin	o,p'-DDT	p,p'-DDT	o,p'-DDD	p,p'-DDD	o,p'-DDE	p,p'-DDE	ΣDDT
BD												
N>LOQ	3	0	1	0	3	0	0	0	0	0	4	4
Median	50	<LOQ	<LOQ	<LOQ	140	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	730	730
Min	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Max	130	<LOQ	120	<LOQ	280	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	4200	4200
W												
N>LOQ	2	0	1	0	5	0	0	0	1	0	5	5
Median	<LOQ	<LOQ	<LOQ	<LOQ	41	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1800	1800
Min	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	650	650
Max	130	<LOQ	42	<LOQ	260	<LOQ	<LOQ	<LOQ	1000	<LOQ	7300	8300
D												
N>LOQ	4	0	4	0	5	0	0	0	0	0	5	5
Median	130	<LOQ	260	<LOQ	320	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	6400	6400
Min	<LOQ	<LOQ	<LOQ	<LOQ	67	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	2200	2200
Max	390	<LOQ	620	<LOQ	1800	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	41000	41000
E												
N>LOQ	4	0	3	0	2	0	0	0	0	0	5	5
Median	63	<LOQ	41	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	4600	4600
Min	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	2100	2100
Max	190	<LOQ	74	<LOQ	380	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	9000	9000
FGH												
N>LOQ	5	0	5	0	5	0	0	0	4	0	5	5
Median	290	<LOQ	130	<LOQ	1200	<LOQ	<LOQ	<LOQ	270	<LOQ	7000	7270
Min	58	<LOQ	68	<LOQ	180	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	4100	4100
Max	1100	<LOQ	1300	<LOQ	1500	<LOQ	<LOQ	<LOQ	730	<LOQ	23000	23730

Table 13. Seven PCBs (ng/g lw) in muscle tissue of Eurasian lynx (*Lynx lynx*) from different parts of Sweden. For LOQs see table x.

	PCB 28	**PCB 52**	**PCB 101**	**PCB 118**	**PCB 138**	**PCB 153**	**PCB 180**	**ΣPCB**
Z								
N>LOQ	0	0	0	3	4	5	5	5
Median	<LOQ	<LOQ	<LOQ	4	15	73	61	161
Min	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	34	19	53
Max	<LOQ	<LOQ	<LOQ	14	27	200	180	390
Y								
N>LOQ	0	0	0	3	5	5	5	5
Median	<LOQ	<LOQ	<LOQ	5,8	52	280	380	712
Min	<LOQ	<LOQ	<LOQ	<LOQ	20	74	64	182
Max	<LOQ	<LOQ	<LOQ	17	140	690	750	1590
X								
N>LOQ	0	0	0	3	5	5	5	5
Median	<LOQ	<LOQ	<LOQ	4,8	27	71	62	154
Min	<LOQ	<LOQ	<LOQ	<LOQ	14	52	38	109
Max	<LOQ	<LOQ	<LOQ	14	66	240	160	480
W								
N>LOQ	0	0	0	3	4	4	4	4
Median	<LOQ	<LOQ	<LOQ	6,1	16	46	23	105
Min	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Max	<LOQ	<LOQ	<LOQ	9,6	28	56	35	118
STU								
N>LOQ	0	0	0	4	5	5	4	5
Median	<LOQ	<LOQ	<LOQ	6,9	22	39	27	86,5
Min	<LOQ	<LOQ	<LOQ	<LOQ	14	34	<LOQ	56
Max	<LOQ	<LOQ	<LOQ	15	31	86	37	169

Table 14. Chlorinated pesticides (ng/g lw) in muscle tissue of Eurasian lynx (*Lynx lynx*)from different parts of Sweden. For LOQs see table x

	α-HCH	β-HCH	o,p'-DDT	p,p'-DDT	o,p'-DDD	p,p'-DDD	o,p'-DDE	p,p'-DDE	ΣDDT
Z									
N>LOQ	0	0	0	0	0	0	0	1	1
Median	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Min	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Max	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	41	41
Y									
N>LOQ	0	0	0	0	0	0	0	0	0
Median	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Min	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Max	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
X									
N>LOQ	1	1	0	0	0	0	0	2	2
Median	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Min	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Max	340	97	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	210	210
W									
N>LOQ	0	0	0	0	0	0	0	1	1
Median	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Min	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Max	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	72	72
STU									
N>LOQ	0	0	0	0	0	0	0	1	1
Median	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Min	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Max	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	41	41

Comments on chlorinated compounds.

Levels of PCB in eagle owls were considerably higher in eagle owls compared to lynx (fig 3). Furthermore, more PCB conjeners were found above LOQ in eagle owls compared to lynx. The geographical differences with a tendency towards higher levels in eagle owls from the more southern counties could not be detected in lynx. On the contrary, lynx from one of the northernmost counties, Ångermanland (Y) had a tendency towards higher levels of the analysed compounds. The individual variation was large for both eagle owls and lynx.

Most studies on contaminants in birds have been made on eggs and few studies are available were both eggs and muscle have been analysed from the same species at comparable times. A study on levels in eggs and muscle of guillemot (Uria aalge) showed a C_{egg}/C_{muscle} quotient between 0,94 (PCB180) and 1,94 (PCB 28) (Lundstedt-Enkel et al. 2006). In most cases the level in eggs were higher compared to levels in muscle.

The levels in muscle of eagle owls in the present study was considerably higher compared to levels in unhatched eggs of eagle owls in southeastern Spain (Gomez-Ramirez et al. 2012). The median level of ΣPCB (22 conjeners) was 544 ng/glw (84,8-34754) in unhatched eggs collected in southestern Spain in 2004-2009. This could be compared to the overall median of ΣPCB (7 conjeners) of 9440 (1330-64900) found in the present study.

Brominated compounds

Brominated compounds in eagle owls are shown in table 15. TetraBDE was found in 24 (96%) of the eagle owl samples. PentaBDE was found above LOQ in 22 (88%) of the eagle owl samples and hexa BDE was found in 19 (76%) of the eagle owl samples. HeptaBDE was found in six (24%) and okta BDE was found 2 (8%). Two eagle owls from the province of Småland (FGH) had detectable levels of heptaBDE (73 and 150 ng/g lw) and oktaBDE (150 and 500 ng/g lw), one of these also had detectable levels of nonaBDE (410 ng/g lw) and dekaBDE (880 ng/glw). This eagle owl, a female of unknown age also had the highest ΣPBDE (6060 ng/g lw). TBBA (430 ng/g lw) was found in one eagle owl, a 1,5 year old male from the province of Östergötland (E).

None of the anlaysed brominated compounds were found above LOQ in any of the lynx samples.

Geographical differences

A significant difference (p=0,048) due to area was found for tetraBDE (Fig 6). For none of the other brominated compounds, statistical significant difference was found between eagle owls from different parts of the country. However, the individual differences were large and the highest levels were found in some of the eagle owls from the province of Småland (EFG). In fig 7 the median values of ΣPBDE (tetraBDE, pentaBDE, hexaBDE, heptaBDE) for eagle owls from the different counties are shown. No statistical differences was found for ΣPBDE in eagle owls from different counties (p=0,077)

Table 15. Brominated compounds (ng/g lw) in eagle owl (*Bubo bubo*) from different parts of Sweden. For LOQs see table 7.

	tetraBDE	**pentaBDE**	**hexaBDE**	**heptaBDE**	**oktaBDE**	**nonaBDE**	**dekaBDE**	**TBBA**
BD								
N>LOQ	4	4	3	1	0	0	0	0
Median	50	64	91	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Min	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Max	280	230	200	65	<LOQ	<LOQ	<LOQ	<LOQ
W								
N>LOQ	5	4	4	0	0	0	0	0
Median	41	110	75	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Min	27	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Max	100	250	150	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D								
N>LOQ	5	5	4	0	0	0	0	0
Median	120	130	100	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Min	52	57	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Max	1400	810	380	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
E								
N>LOQ	5	4	3	0	0	0	0	1
Median	91	130	160	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Min	29	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Max	150	200	280	74	<LOQ	<LOQ	<LOQ	430
FGH								
N>LOQ	5	5	5	3	2	1	1	0
Median	320	490	230	71	<LOQ	<LOQ	<LOQ	<LOQ
Min	79	100	110	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Max	2000	1600	2600	350	500	410	880	<LOQ

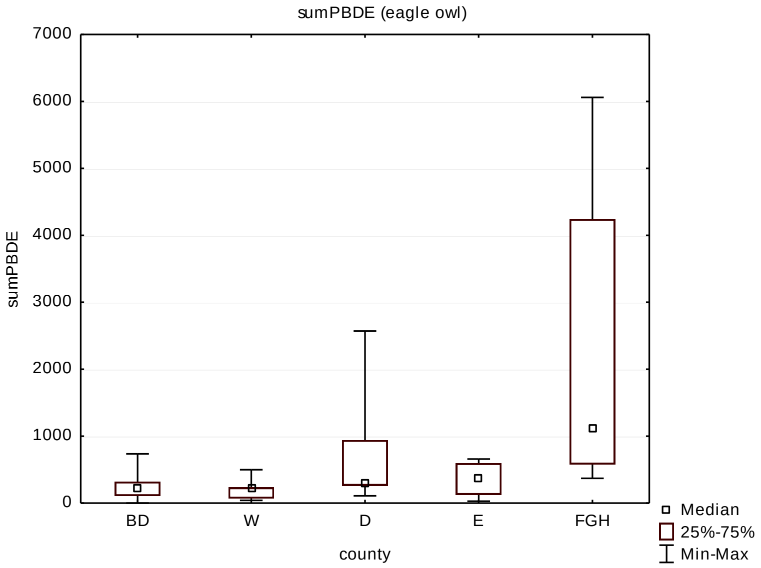

Fig 6. ΣPBDE (ng/g lw) (tetraBDE, pentaBDE, hexaBDE, heptaBDE) ng/g lw in eagle owls from five different counties in Sweden. p=0,077 (Kruskal Wallis ANOVA).

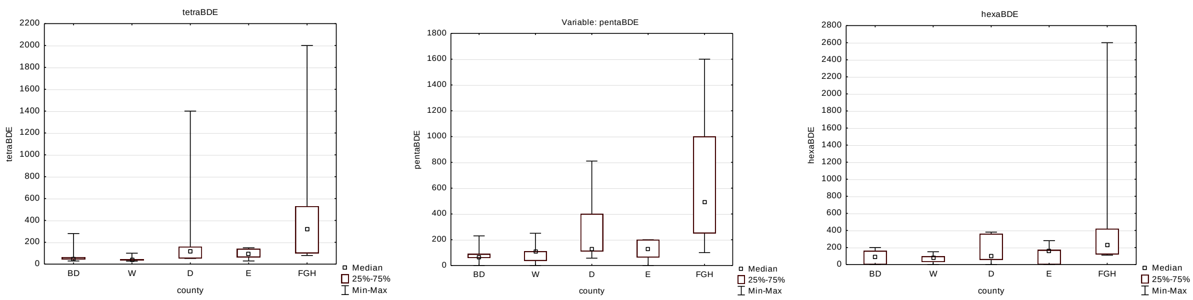

Fig 7. TetraBDE, pentaBDE and hexaBDE in muscle of eagle owls from different parts of Sweden.
Significant difference between different counties for tetraBDE p=0,048.

Comments on brominated compounds

PentaBDE was the most abundant of the PBDEs in eagle owls in that respect that it had the highest median values. Tetra BDE on the other hand was the most abundant in that respect that it was found in most samples. The individual variation was large but the eagle owls from Småland (FGH) appeared to have both higher levels and also more brominated substances compared to eagle owls further north. The median value for ΣPBDE for all eagle owls was 344 ng/g lw (<LOQ-6060). The median value of ΣPBDE for eagle owls from Småland was 1130 ng/g lw (370-6060). The higher levels of ΣPBDE in eagle owls from Småland were not statistically significant due to the large individual variation.

In a study on guillemot (*Uria aalge*), levels of organic contaminants was compared in muscle and eggs. For ΣPBDE the quotient C_{egg}/C_{muscle} was 0,96 indicating that the levels in eggs and muscle was comparable (Lundstedt-Enkel et al. 2006).
The levels found in muscle of eagle owls were 2-3 times lower compared to levels found in eggs of white tailed sea eagle (*Haliaeetus albicilla*) from different parts of Sweden (Nordlof et al. 2010).
The levels found in eagle owl muscle in the present study were higher compared to what was found unhatched eggs of eagle owls from southeastern Spain (Gomez-Ramirez et al. 2012). Gomez-Ramirez found a median of 19,6 ng/g lw (ND-457) in eggs collected between 2004 and 2009.
Other studies have found median ΣPBDE concentration of 110 ng/g lw in eggs of Belgian little owl (*Athene noctua*), 60 ng/g lw in liver of common kestrel (*Falco tinnunculus*), 1600 ng/glw in liver of barn owl (*Tyto alba*) and 3100 ng/g lw in liver of sparrowhawk (*Accipiter nisus*) (Chen & Hale 2010, Wu et al. 2012).

In the present study none of the analysed PBDEs were found in lynx. Very few studies have been made on brominated compounds in lynx. In a study from Norway, PBDEs were analysed in livers of lynx from different parts of Norway (Mariussen et al. 2008). In that study BDE47 (tetra BDE), BDE99 (pentaBDE), BDE 153, BDE 154 (hexaBDE) and BDE183 (heptaBDE) was found. The median value of ΣPBDE was 10 ng/g lw and the highest level found was 313 ng/g lw. According to that study BDE 153 was the dominating congener in lynx. Unfortunately, the LOQs in the present study (table 7) are very close to the levels found in lynx in the study by Maurissen et al (2008) which makes it difficult to compare. Another study from Norway found ΣPBDE of 237 (in 1997) and 19 (in 2007) ng/g lw in serum of female lynx (Polder et al. 2009).

Comparison with levels of chlorinated and brominated compounds in bank voles and earthworms

In table 16, the levels of the seven PCB conjeners analysed in eagle owls and lynx in the present study is compared to levels in bank voles and earthworms that was analysed in previous studies (Lind & Odsjo 2010, Lind 2011). In this table the median of all analysed samples is shown and no geographical comparison is made. As could be expected, the highest levels were found in eagle owls. All of the analysed PCB conjeners was found in eagle owls although PCB52 was found in only one sample. PCB28, PCB52, and PCB101 were not detected in lynx, bank voles and earthworms. PCB 118 was not detected in earthworms. The lowest levels of ΣPCB were found in bank voles.

DDE was found in all of the eagle owl samples and in all of the earthworm samples and in five of the lynx and two of the bank vole samples (Table 17). DDT was found in some bank vole and earthworm samples but not in any of the other samples. The levels in the earthworm samples from one sampling site in Västmanland (Bergslagen area) were surprisingly high (Lind 2011).

Brominated compounds were found in 24 out of 25 eagle owl samples but not above LOQ in any of the lynx samples. Brominated compounds were found in both bank voles and earthworms but only in a few samples (homogenates) and the levels were very low (Lind & Odsjo 2010, Lind 2011).

Table 16. PCBs (ng/g lw) in in individual samples of muscle tissue of eagle owl (*Bubo bubo*) and Eurasian lynx (*Lynx lynx*), in homogenates of muscle tissue of bank voles (*Myodes glareolus*) and in homogenates of whole earthworms (*Lumbricus* sp).

	PCB28	PCB52	PCB101	PCB118	PCB138	PCB153	PCB180	ΣPCB
Eagle owl(N=25)								
N>LOQ	25	1	25	25	25	25	25	25
Median	12	<LOQ	10	630	4300	2300	2300	9440
Min	3	<LOQ	3	94	610	260	300	1330
Max	230	3,5	190	7300	26000	19000	14000	64900
Lynx (N=25)								
N>LOQ	0	0	0	25	25	25	25	25
Median	<LOQ	<LOQ	<LOQ	7	65	23	42	150
Min	<LOQ	<LOQ	<LOQ	1	23	5,7	15	46
Max	<LOQ	<LOQ	<LOQ	17	690	140	750	1590
Bank vole(N=15)[1]								
N>LOQ	0	0	0	1	1	7	3	
Median	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Min	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Max	<LOQ	<LOQ	<LOQ	3	6	13	8	29
Earthworm (N=9)[2]								
N>LOQ	0	0	0	0	5	6	2	
Median	<LOQ	<LOQ	<LOQ	<LOQ	5	7	0	12,8
Min	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Max	<LOQ	<LOQ	<LOQ	<LOQ	29	47	9	85

[1] 15 homogenates (muscle samples of 10 individual bank voles in each homogenate)

[2] 9 homogenates of whole earthworms

Table 17 . Chlorinated pesticides (ng/g lw) analysed in individual samples of muscle tissue of eagle owl (*Bubo bubo*) and Eurasian lynx (*Lynx lynx*), in homogenates of muscle tissue of bank voles (*Myodes glareolus*) and in homogenates of whole earthworms (*Lumbricus* sp).

	HCB	α-HCH	β-HCH	γ-HCH lindan	DDT	DDD	DDE	ΣDDT
Eagle owl (N=25)								
N>LOQ	17	0	25	0	0	5	25	25
Median	30	<LOQ	68	<LOQ	<LOQ	<LOQ	4400	4500
Min	<LOQ	<LOQ	9	<LOQ	<LOQ	<LOQ	40	40
Max	1100	<LOQ	1300	<LOQ	<LOQ	1000	41000	41000
Eurasian lynx (N=25)								
N>LOQ	0	1	1	0	0	0	5	
Median	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Min	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Max	<LOQ	340	97	<LOQ	<LOQ	<LOQ	210	210
Bank vole (N=15)[1]								
N>LOQ	15	0	0	0	2	1	2	
Median	8,19	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Min	1,07	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Max	12,2	<LOQ	<LOQ	<LOQ	41	1,71	3,28	4,8
Earthworm								
(N=9)[2]	3	0	0	0	1	2	9	9
N>LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	82	82
Median	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	7,75	7,75
Min	10,2	<LOQ	<LOQ	<LOQ	5383	1321	4672	11376
Max								

[1] 15 homogenates (muscle samples of 10 individual bank voles in each homogenate)
[2] 9 homogenates of whole earthworms

Perfluorinated compounds (PFAS)

PFAS compounds in eagle owls from different provinces are shown in table 18 and in lynx in table 19. Nine of the analysed PFAS was found above LOQ in all[3] of the analysed samples of eagle owls and in eight of the lynx samples. There was however a slight difference in which of the PFAS that was found in eagle owl and lynx respectively. PFDA, PFUnDA, PFTrDA, PFOS, and FOSA were found in all of the analysed samples of both species. PFNA was found in all of the lynx samples and in 22 (88 %) of the eagle owl samples. PFDoDA was found in all of the eagle owl samples and in 22 (88%) lynx samples. PFTeDA was found in all of the eagle owl samples and in 23 (92 %) of the lynx samples. PFPeDA was found in all of the eagle owl samples and in 15 (60%) of the lynx samples.

There were significant differences in levels for all but one of the different PFAS substances between lynx and eagle owls.
The levels of PFAS were generally higher in eagle owls. However for PFOA and PFNA the level was higher in lynx (0,445 ng/g ww and 2,04 ng/g ww) compared to eagle owl (0,072 ng/g ww and 0,854 ng/g ww). The level of PFHxS did not differ significantly between lynx (0,498 ng/g ww) and eagle owls (0,810 ng/g ww).
PFBS was not found above LOQ in any of the eagle owl samples but in two of the lynx samples. Both of these were from the province of Ångermanland(Y). PFHpA was not found in any of the lynx samples but in three of the eagle owl samples. Two of these were from the province of Småland (FGH) and one from the province of Södermanland (D). PFHxA was not found above LOQ in any of the analysed samples.

Geographical differences

The ΣPFAS in eagle owls and lynx is shown in fig 8.
In eagle owls, significant difference (Kruskal-Wallis ANOVA) was found for PFDA (p=0,038) and PFUnA (p=0,016) between different geographical regions but no obvious north-south or east-west gradient could be detected (fig 9).
For lynx, a significant difference between geographical regions was found for PFUnA (p=0,021), PFDoA (p=0,012), PFTrDA (p=0,0096), PFTeDA (P=0,019), PFPeDA (P=0,035), and PFHxS (p=0,0105). There was a tendency towards higher levels in lynx from the county of Jämtland (Z) and Ångermanland (Y) while the lowest levels were found in lynx from the county of Hälsingland (X) (fig 10.)

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[3] Four of the eagle owl samples could not be quantified due to analytical problems

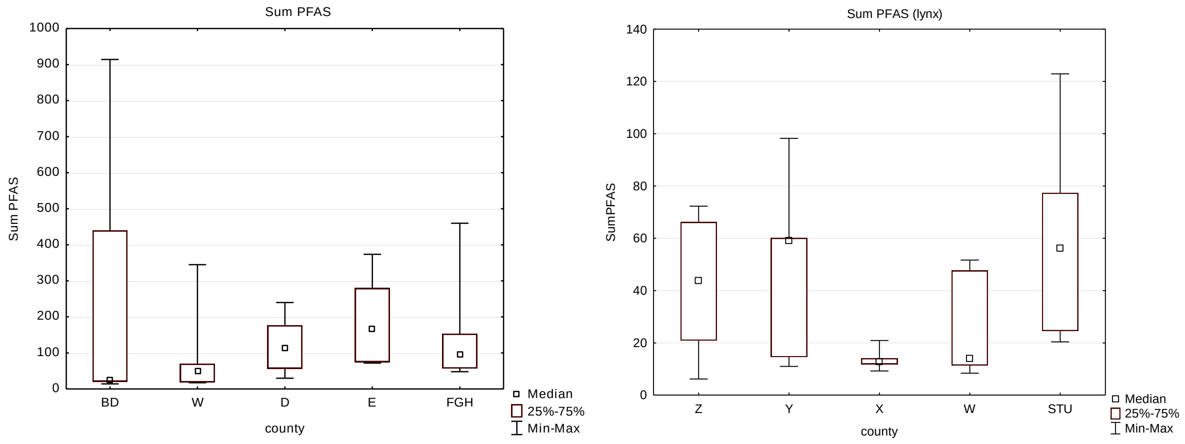

Fig 8. ΣPFAS (ng/g ww) in liver of eagle owls and lynx from different parts of Sweden

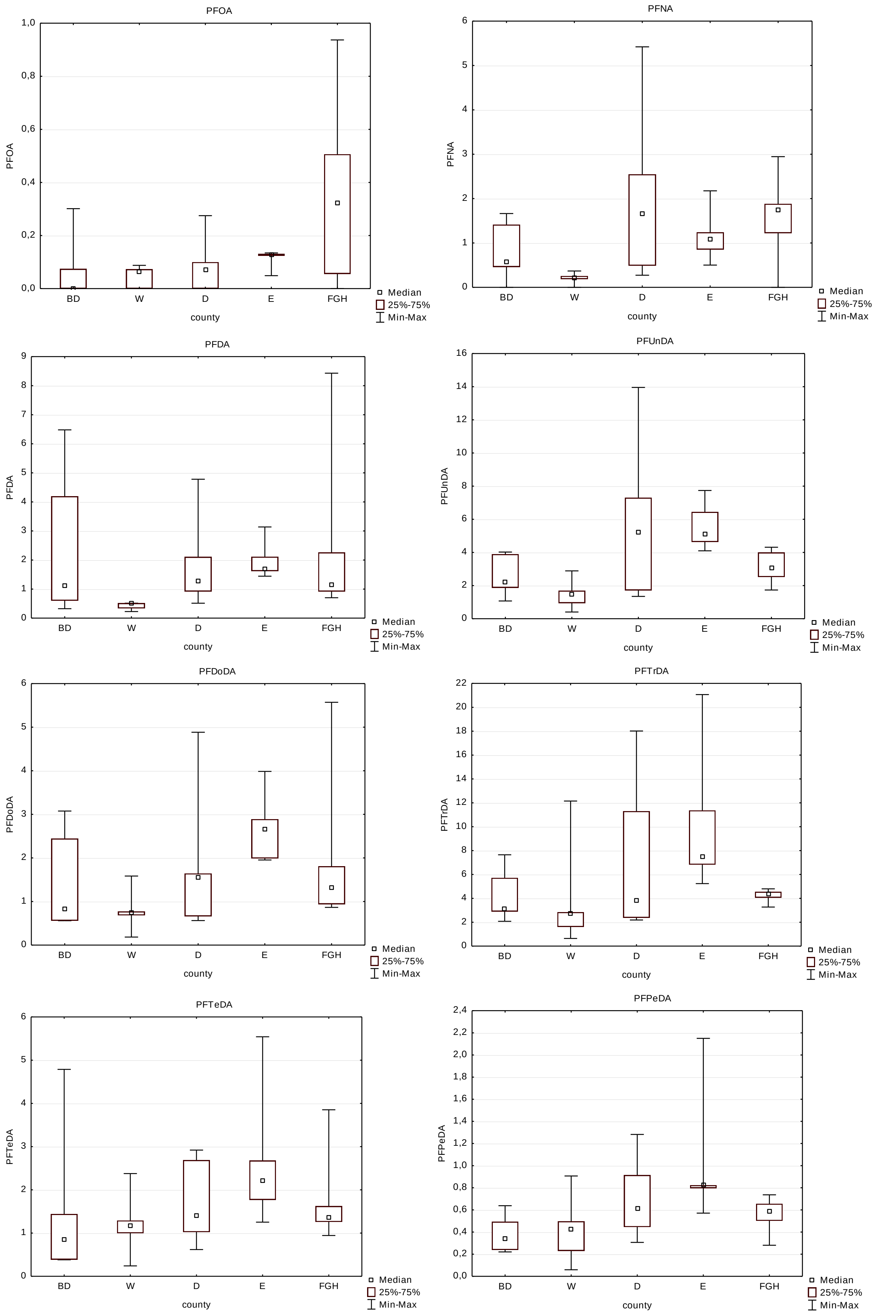

Fig 9. Perfluorinated substanses (ng/g ww) in liver of eagle owl from different parts of Sweden. Significant difference between different counties for PFDA p=0,038 and PFUnDA p=0,016.

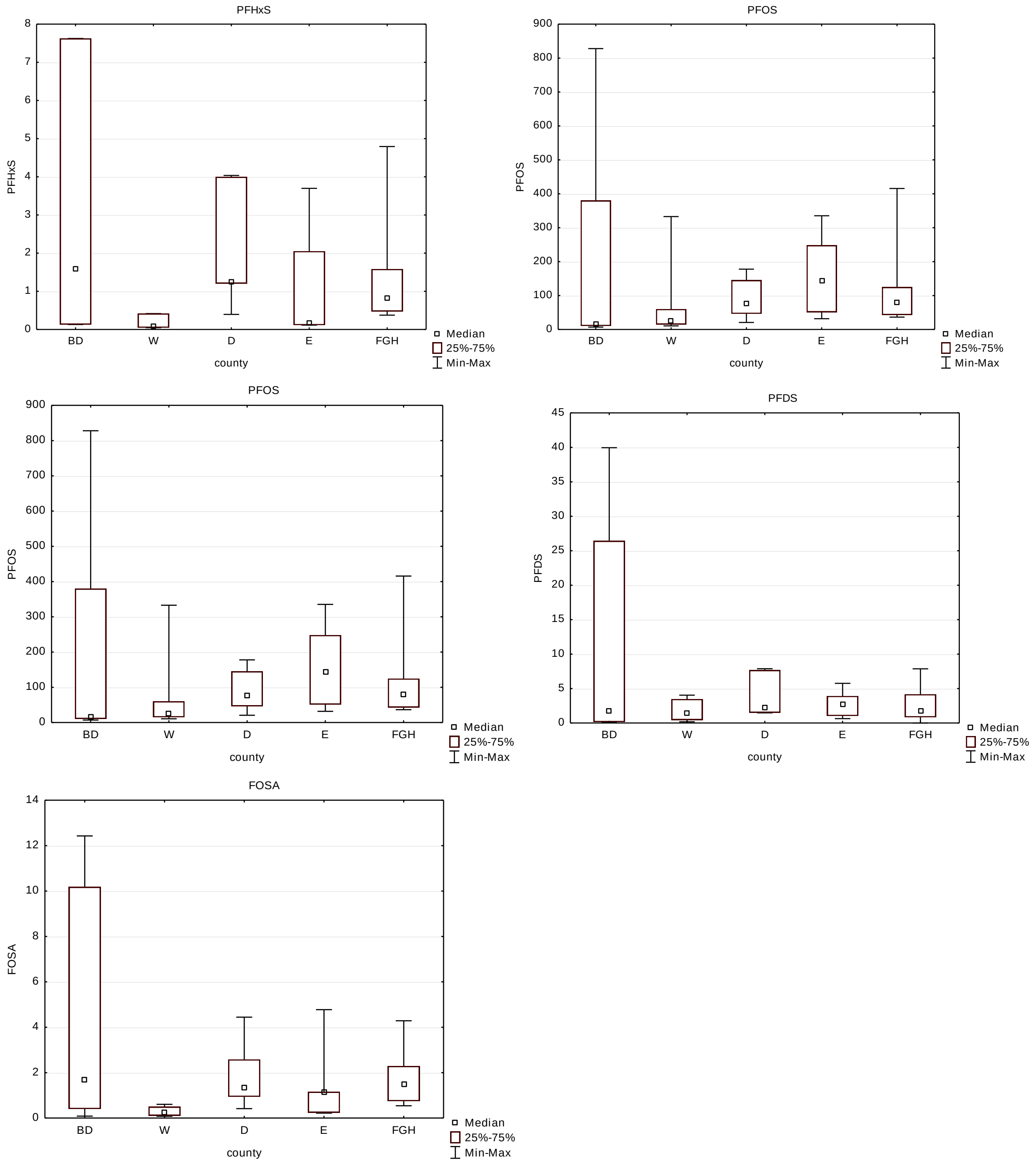

Fig 9 (forts). Perfluorinated substanses (ng/g ww) in liver of eagle owl from different parts of Sweden. Significant difference between different counties for PFHxS p=0,01.

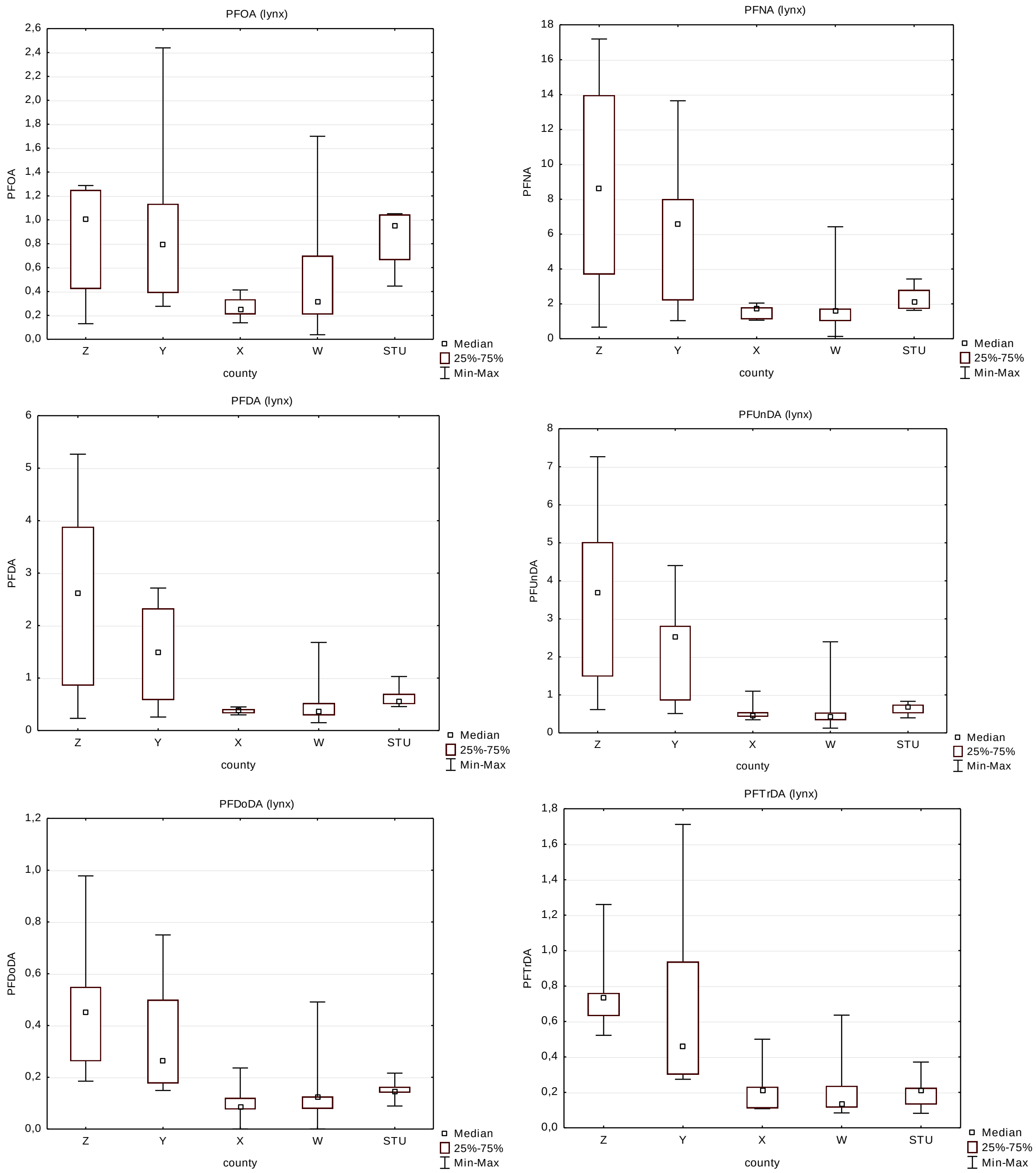

Fig 10. Perfluorinated substances in liver of Eurasian lynx from different parts of Sweden.
Significant differences between different counties for PFUnDA p=0,021, PFDoDa p=0,011, and PFTrDA p=0,0096.

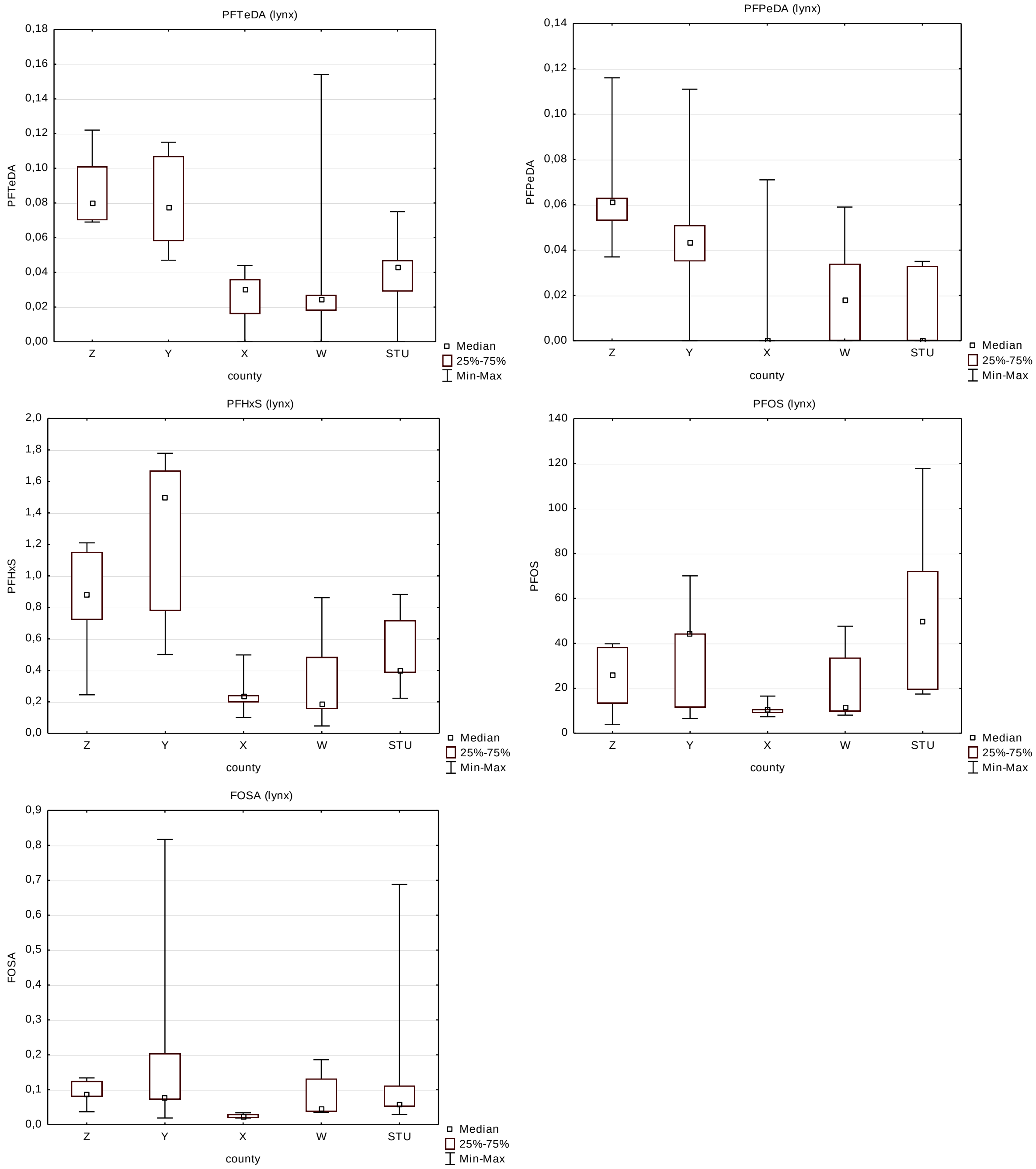

Fig 10 (forts). Perfluorinated substances in liver of Eurasian lynx from different parts of Sweden Significant differences between different counties for PFTeDA p=0,019, PFPeDA p=0,035 and PFHxS p=0,011.

Table 18. Perfluorinated substances (PFAS) (ng/g ww) in liver tissue of eagle owl (*Bubo bubo*) from different parts of Sweden. For LOQs see table x

	PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	PFTeDA	PFPeDA	PFHxS	PFOS	PFDS	FOSA	ΣPFAS
BD														
N>LOQ	0	2	4	5	5	5	5	5	5	3[1]	5	5	5	5
Median	<LOQ	<LOQ	0,577	1,11	2,20	0,840	3,16	0,851	0,342	1,59	15,8	1,71	1,70	24,2
Min	<LOQ	<LOQ	<LOQ	0,325	1,07	0,559	2,08	0,382	0,221	0,130	7,16	0,132	0,085	14,0
Max	<LOQ	0,301	1,66	6,48	4,02	3,08	7,65	4,79	0,638	7,62	828	40,0	12,4	914
W														
N>LOQ	0	3	4	5	5	5	5	5	5	3[1]	5	5	5	5
Median	<LOQ	0,064	0,204	0,498	1,47	0,752	2,73	1,17	0,427	0,100	26,3	1,49	0,246	48,6
Min	<LOQ	<LOQ	<LOQ	0,226	0,404	0,184	0,641	0,241	0,059	0,047	10,6	0,182	0,080	17,4
Max	<LOQ	0,088	0,368	0,515	2,89	1,58	12,1	2,38	0,906	0,416	333	4,04	0,604	345
D														
N>LOQ	1	3	5	5	5	5	5	5	5	5	5	5	5	5
Median	<LOQ	0,070	1,66	1,27	5,20	1,56	3,82	1,41	0,609	1,25	76,6	2,30	1,35	114
Min	<LOQ	<LOQ	0,273	0,513	1,35	0,563	2,18	0,619	0,307	0,395	20,7	1,47	0,416	29,9
Max	0,623	0,275	5,42	4,78	14,0	4,88	18,0	2,92	1,28	4,04	178	7,88	4,45	240
E														
N>LOQ	0	5	5	5	5	5	5	5	5	5	5	5	5	5
Median	<LOQ	0,127	1,09	1,69	5,13	2,66	7,49	2,22	0,822	0,162	142	2,69	1,14	165
Min	<LOQ	0,049	0,501	1,44	4,10	1,95	5,23	1,25	0,571	0,114	31,9	0,633	0,217	71,9
Max	<LOQ	0,134	2,18	3,14	7,74	3,99	21,1	5,54	2,15	3,70	335	5,75	4,77	374
FGH														
N>LOQ	2	4	4	5	5	5	5	5	5	5	5	4	5	5
Median	<LOQ	0,324	1,75	1,16	3,08	1,31	4,37	1,35	0,592	0,811	79,6	1,84	1,48	95,6
Min	<LOQ	<LOQ	<LOQ	0,701	1,73	0,866	3,27	0,944	0,280	0,375	36,5	<LOQ	0,542	47,9
Max	0,373	0,937	2,95	8,43	4,31	5,57	4,80	3,85	0,736	4,79	416	7,86	4,29	460

[1]missing data

Table 19. Perfluorinated substances (PFAS)(ng/g ww) in liver tissue of Eurasian lynx (*Lynx lynx*) from different parts of Sweden. For LOQs see table x.

	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	PFTeDA	PFPeDA	PFBS	PFHxS	PFOS	PFDS	FOSA	ΣPFC
Z														
N>LOQ	5	5	5	5	5	5	5	5	0	5	5	0	5	5
Median	1,00	8,59	2,62	3,70	0,451	0,733	0,080	0,061	<LOQ	0,880	25,9	<LOQ	0,085	43,8
Min	0,130	0,665	0,232	0,614	0,185	0,522	0,069	0,037	<LOQ	0,245	3,79	<LOQ	0,037	6,19
Max	1,29	17,2	5,27	7,26	0,978	1,26	0,122	0,116	<LOQ	1,21	39,8	<LOQ	0,134	72,3
Y														
N>LOQ	5	5	5	5	5	5	5	4	2	5	5	2	5	5
Median	0,789	6,60	1,48	2,54	0,263	0,459	0,077	0,043	<LOQ	1,50	44,2	<LOQ	0,076	59,2
Min	0,276	1,04	0,257	0,510	0,149	0,274	0,047	<LOQ	<LOQ	0,501	6,58	<LOQ	0,019	11,0
Max	2,44	13,6	2,72	4,40	0,750	1,71	0,115	0,111	0,051	1,78	70,0	0,124	0,817	98,2
X														
N>LOQ	5	5	5	5	4	5	4	1	0	5	5	0	5	5
Median	0,248	1,75	0,386	0,453	0,085	0,211	0,030	<LOQ	<LOQ	0,233	10,5	<LOQ	0,023	12,7
Min	0,138	1,06	0,298	0,345	<LOQ	0,108	<LOQ	<LOQ	<LOQ	0,100	7,33	<LOQ	0,019	9,27
Max	0,413	2,04	0,449	1,097	0,236	0,500	0,044	0,071	<LOQ	0,498	16,5	<LOQ	0,034	20,9
W														
N>LOQ	5	5	5	5	4	5	4	3	0	5	5	0	5	5
Median	0,310	1,61	0,371	0,439	0,122	0,136	0,024	0,018	<LOQ	0,183	11,4	<LOQ	0,046	14,1
Min	0,038	0,134	0,149	0,125	<LOQ	0,084	<LOQ	<LOQ	<LOQ	0,047	8,05	<LOQ	0,035	8,38
Max	1,70	6,42	1,68	2,40	0,491	0,636	0,154	0,059	<LOQ	0,862	47,6	<LOQ	0,186	51,7
STU														
N>LOQ	5	5	5	5	5	5	4	2	0	5	5	0	5	5
Median	0,947	2,10	0,549	0,684	0,146	0,210	0,043	<LOQ	<LOQ	0,394	49,4	<LOQ	0,057	56,2
Min	0,445	1,63	0,456	0,396	0,089	0,082	<LOQ	<LOQ	<LOQ	0,223	17,4	<LOQ	0,029	20,4
Max	1,05	3,43	1,03	0,830	0,216	0,371	0,075	0,035	<LOQ	0,88	118	<LOQ	0,688	123

Comments on perfluorinated compounds

Eagle owls generally had higher levels of most PFAS compared to lynx. The exception was PFOA and PFNA where the levels were higher in lynx. PFOS was the most abundant compound in both eagle owls and lynx.

In contrast to what was found for chlorinated and brominated compounds in eagle owls with a tendency towards higher levels further south, no geographical gradient could be detected and a significant difference between eagle owls from different counties was found only for PFDA and PFUnDA. The eagle owl with the highest levels of PFAS was from the county of Norrbotten (BD). In lynx there was a tendency towards higher levels for some PFAS in the county of Jämtland (X) and Ångermanland (Y).

Data on PFAS in terrestrial biota is scarce. No data comparable data on PFAS in lynx has been found. Neither has eagle owls been analysed for PFAS earlier.

In Norway, eggs of tawny owls (*Strix aluco*) has been analysed for PFAS (Ahrens et al. 2011). The levels were considerably lower compared to the levels in eagle owl liver in the present study.

In Sweden, PFAS have been analysed in eggs of peregrine falcon (*Falco peregrinus*) (Holmström et al. 2010). The mean concentration of PFOS in peregrine falcon eggs was 83 ng/g ww. PFAS has been more extensively analysed in marine biota. One study on PFAS in eggs and liver of guillemot (*Uria aalge*) found somewhat higher levels in liver tissue compared to in egg for PFNA, PFDA, PFUnDA, and PFDoDA, while the levels of PFOS were higher in eggs (Holmstrom & Berger 2008). PFOS was twice as high in adult guillemot livers (121 ng/g ww) compared to the levels in eagle owl livers in the present study (60 ng/g ww, median value for all analysed eagle owls).

Comparison with levels of PFAS in bank vole and earthworm.

In order to evaluate the levels of contaminants found in lynx and eagle owl in the present study the results were compared to what has earlier been found in other terrestrial matrices (Lind & Odsjo 2010, Lind 2011). In table 20, the levels of PFAS in individual samples of eagle owls and lynx from the present study are compared to the levels earlier found in bank voles (homogenate samples) and earthworms (homogenate samples). Levels of PFAS were generally higher in eagle owls and lynx compared to in earthworms and bank voles. However, there are certain differences in the distribution of different PFAS between these organisms.

Table 20. Median (min-max) ng/g ww of 15 PFAS analysed in individual samples of liver tissue of eagle owl (*Bubo bubo*) and Eurasian lynx (*Lynx lynx*), in homogenates of liver tissue of bank voles (*Myodes glareolus*) and in homogenates of whole earthworms (*Lumbricus* sp).

	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	PFTeDA	PFPeDA	PFBS	PFHxS	PFOS	PFDS	FOSA
Eagle owl															
N=25															
N>LOQ	0	3	17	22	25	25	25	25	25	25	0	21[1]	25	24	25
Median	<LOQ	<LOQ	0,073	0,854	1,16	3,08	1,56	4,37	1,35	0,571	<LOQ	0,811	60,1	1,84	0,937
Min	<LOQ	<LOQ	<LOQ	<LOQ	0,226	0,404	0,184	0,641	0,241	0,059	<LOQ	0,047	7,16	<LOQ	0,080
Max	<LOQ	0,623	0,937	5,42	8,43	14,0	5,57	21,1	5,54	2,15	<LOQ	7,62	828	40,0	12,4
Eurasian lynx															
N=25															
N>LOQ	0	0	25	25	25	25	22	25	23	15	2	25	25	4	25
Median	<LOQ	<LOQ	0,487	2,12	0,550	0,728	0,162	0,312	0,045	0,029	<LOQ	0,508	19,1	<LOQ	0,061
Min	<LOQ	<LOQ	0,055	0,195	0,198	0,182	<LOQ	0,090	<LOQ	<LOQ	<LOQ	0,069	4,80	<LOQ	0,017
Max	<LOQ	<LOQ	2,59	20,5	6,28	8,67	1,17	1,82	0,156	0,149	0,064	2,18	102	0,1327	0,867
Bank vole															
N=15[2]															
N>LOQ	0	0	0	12	11	15	13	13	0	0	0	15	15	0	0
Median	<LOQ	<LOQ	<LOQ	0,398	0,702	1,31	0,476	0,302	<LOQ	<LOQ	<LOQ	0,610	9,14	<LOQ	<LOQ
Min	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0,63	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0,396	2,01	<LOQ	<LOQ
Max	<LOQ	<LOQ	<LOQ	0,792	1,26	1,96	0,822	0,613	<LOQ	<LOQ	<LOQ	1,95	17,5	<LOQ	<LOQ
Earthworm															
N=9[2]															
N>LOQ[3]	0	1	3	6	0	4	4	9	6	7	0	0	9	0	0
Median	<LOQ	<LOQ	<LOQ	0,17	<LOQ	<LOQ	<LOQ	0,503	0,245	0,223	<LOQ	<LOQ	1,15	<LOQ	<LOQ
Min	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0,408	<LOQ	<LOQ	<LOQ	<LOQ	0,758	<LOQ	<LOQ
Max	<LOQ	0,404	0,463	0,293	<LOQ	0,407	0,383	1,02	0,379	0,356	<LOQ	<LOQ	3,69	<LOQ	<LOQ

[1]N=21 due to 4 missing data
[2] Bank vole samples consisted of homogenates with muscle samples of 10 individuals in each. Earthworm samples consisted of homogenates of whole earthworms.
[3]For PFHpA, PFOA, PFUnDA, PFDoDA, PFTeDA, PFPeDA some samples just below LOQ have been semi quantified and included.

Concluding remarks

Levels of toxic metals (Cd, Hg, Pb) were low in both lynx and eagle owls indicating no exposure to elevated levels. Eagle owls in the present study appeared not to be exposed to lead through their food as has been seen in other raptors, especially white tailed sea eagle.

Levels of ΣPCB were higher in eagle owls compared to lynx. The eagle owls in the present study had levels of PCB and chlorinated pesticides that indicated an exposure. Also brominated compounds were higher in eagle owls compared to lynx. No brominated compounds could be quantified in lynx.

The tendency of higher levels in eagle owls from the southern parts is in accordance with what could be expected. These eagle owls might have lived in closer vicinity to more populated areas and could have had a higher exposure by feeding on dumps.

The higher level of both chlorinated substances as well as many PFAS in lynx from the county of Ångermanland(Y) is harder to explain.

Both of these matrices could be suitable for monitoring environmental contaminants in a terrestrial predator. One advantage with using species from the Swedish State game Act is that when specimen are killed or found dead they are sent to the SMNH (or the Swedish Veterinary Agency SVA). This means that there is no need for special arrangements for collections.

There are however, certain aspects that should be kept in mind. Lynx have large homelands and the level in lynx reflect the contaminant levels over a very large area. In the present study male lynx of about 2 years of age was chosen (the actual age span was 1-6 and some of unknown age). Young male lynx also could wander for long distances in order to find a territory of their own. This should be taken into consideration when differences in contaminant levels between animals from different areas are compared. The legal hunt on lynx gives an opportunity to get good material from this animal.

Eagle owls also have reasonably large homelands but the levels in eagle owls probably reflect the levels of contaminants in the area where they are found to a larger extent than lynx. On the other hand, the access to material from eagle owls is dependent on that dead birds are found. They should also be found in reasonably good condition. Dead eagle owls are more likely found in the vicinity of urban areas and roads (railroads) which could affect the selection towards birds that have a higher load of contaminants.

Eagle owls are presently sampled at the SMNH. Lynx from the legal hunt that is the most valuable material for this purpose is at the moment not sampled at SMNH. A cooperation regarding the handling of this material between The National Veterinary Institute (SVA) that handles this material and SMNH should be possible to obtain.

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Appendix to Report 9:2012 from Swedish Museum of Natural History
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Metals and organic contaminants in eagle owl (*Bubo bubo*) and Eurasian lynx (*Lynx lynx*) from different parts of Sweden

Ylva Lind

Stable isotopes.

Sammanfattning

δ^{15}N och δ^{13}C har analyserats i muskelvävnad av lodjur (*Lynx lynx*) och berguv (*Bubo bubo*) från olika områden. Materialet som användes kom från Miljöprovbanken vid Naturhistoriska riksmuseet och var insamlat 1998-2004. Lodjur från Jämtland (Z), Ångermanland (Y), Hälsingland(X) Dalarna (W) och Västmanland/Värmland (STU). Berguv från Norrbotten (BD), Dalarna (W), Södermanland (D), Östergötland (E) och Småland (FGH). Fem individer/område analyserades.

Analyserna gjordes på Biologiska institutionen, Lunds universitet.

Medelvärdet (±sd) av δC^{13} och δN^{15} i berguv var -25,15 ±1,56 respektive 7,31±1,59. I lodjur var motsvarande medelvärden -24,98±1,06 och 5,79±0,859 .

δC^{13} och δN^{15} var signifikant positivt korrelerade i berguv men inte i lodjur.

För berguv fanns en geografisk skillnad i δN^{15} med signifikant lägre halt i de två nordliga områdena (BD, W) jämfört med de tre sydliga (D, E, FGH). Ingen motsvarande skillnad fanns i lodjur. För δC^{13} fanns ingen geografisk skillnad varken för berguv eller lodjur.

Inga tydliga samband fanns mellan δC^{13} och δN^{15} och halter av miljögifter. Undantaget PCB101 i berguv som visade ett positivt samband med δC^{13} och δN^{15}.

Introduction

The use of stable isotope analysis in ecology studies has been widely used study energy flow pathways of organic matter in food webs and to describe trophic levels in food webs.

The nitrogen isotope ^{15}N is used describe trophic levels in food webs and the theoretical background is that in nitrogen containing organic molecules, the heavier isotope ^{15}N is enriched as it passes through food webs.

There is generally a very small enrichment of ^{13}C between animals and their diets, thus $\delta^{13}C$ is not used to determine trophic level but rather the origin of carbon. From $\delta^{13}C$ values it can also be possible to distinguish between carbon of terrestrial origin and carbon of aquatic origin.

The ratio between the heavy and light isotope is expressed in parts per thousand (‰). For nitrogen the ratio is calculated as the ratio in the sample divided by the ratio in atmospheric N_2 as follows: $\delta^{15}N= \{[^{15}N/^{14}N_{sample}]/ [^{15}N/^{14}N_{air}]-1\}x1000‰$. Air has by definition $\delta^{15}N=0‰$.

For carbon the ratio between $^{13}C/^{12}C$ is calculated as $\delta^{13}C=\{[^{13}C/^{12}C_{sample}]/[^{13}C/^{12}C_{PDB}]-1\}x1000‰$. The standard for ^{13}C is Pee Dee Belemite (PDB).

The ratio between the light and heavy isotope differs between tissues as different tissues have different turnover rates. Thus different tissues can give information of diet for shorter or longer periods. In the present study, analysis of the stable isotopes N^{15} and C^{13} were performed on muscle tissue. Muscle tissue reflects what the predator has ingested during the last weeks-months.

Material and methods

Eurasian lynx (*Lynx lynx*) and eagle owl (*Bubo bubo*) from the Swedish Environmental Specimen Bank (SESB) was used. The material was collected in 1998-2004 and stored frozen (-25ºC). Five specimens from each of five different regions were used. Lynx came from Jämtland (Z), Ångermanland (X), Hälsingland (X), Dalarna (W) and Västmanland/Värmland(STU). Eagle owls came from Norrbotten (BD), Dalarna (W), Södermanland (D), Östergötland (E), and Småland (FGH).

A more thorough description can be found in NRM Report 9:2012

Stable isotope analysis

Approximately 1 g of muscle tissue was dried at 60 C to constant weight. The dried muscle was pulverized and 200-600 mg of the dried, pulverized tissue was placed in plastic vials and kept in an air tight glass jar with drying material at room temperature until analysed. Between 250 and 450 µ were weighted in tin capsules (6x4 mm). Samples were flash-combusted in a Flash 2000 elemental analyzer, and the isotopic ratios determined by a Delta V Plus isotope-ratio mass spectrometer connected to the elemental analyzer via the ConFlow IV interface (Thermo Scientific Inc., Bremen Germany). Isotopic ratios of the samples were calibrated against three different standard of known isotopic composition (six replicates of each). The analytical precision obtained for the standards was <0.12‰ for N and < 0.4‰ for C (1σ). The samples were analyzed for $^{12}C/^{13}C$ and $^{15}N/^{14}N$ ratios at the stable isotope facility at the Department of Biology, Lund University.

Statistical analyses

Some of the data was not normally distributed and the sample size was small and because of this non-parametric tests were used. Kruskal-Wallis test was used to detect differences between three or more groups and Mann-Whitney U-test was used to detect differences between two groups.

The significance level was set to p<0,05. A principal component analysis (PCA) was performed to detect relationships between contaminants and δC^{13} and δN^{15} . Statistical software used was StatSoft, Inc. (2011). STATISTICA (data analysis software system), version 10. www.statsoft.com

Result

The mean (±sd) of the δC^{13} and δN^{15} in eagle owls was -25,15 ±1,56 and 7,31±1,59 respectively. In lynx the corresponding levels was -24,98±1,06 and 5,79±0,859 .
δC^{13} and δN^{15} was positively correlated in eagle owls (r=0,54; p=0,008) but not in lynx (r=-0,14; p=0,5).

Between species

There was a significant difference in δN^{15} between lynx and eagle owls (Mann-Whitney U-test, p<0,001). For δC^{13} no significant difference was found (p=0,147).

From one region, the county of Dalarna (W), both eagle owls and lynx were sampled. No significant difference was found for either $\delta^{15}N$ or $\delta^{13}C$ between eagle owls and lynx from the same geographical region (fig 1).

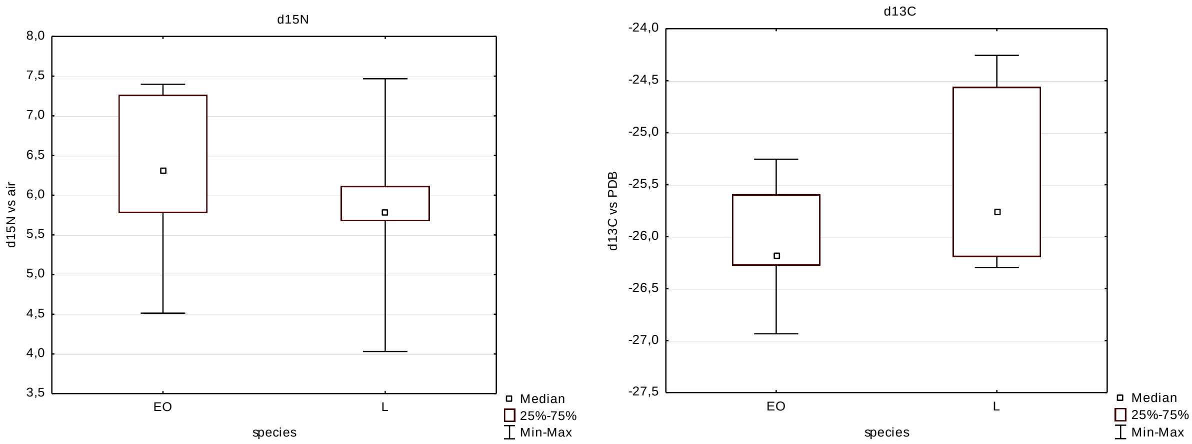


Figure 1. $\delta^{15}N$ (left) and $\delta^{13}C$ (right) in muscle of Eurasian lynx (L) and eagle owl (EO) from the county of Dalarna (W).

Between geographical areas

For eagle owls there was a significant difference in δN^{15} between animals from different counties. The two northernmost counties (BD and W) had lower δN^{15} compared to the three counties further south (Kruskal-Wallis $p<0,03$). For δC^{13} no significant difference was found (Fig 2). The difference in $\delta^{15}N$ between eagle owls from BD and W (north) and D, E and FGH (south) was 2‰.

For Eurasian lynx, no significant differences were found for either δN^{15} or δC^{13} between animals from different parts of Sweden (Fig 3).

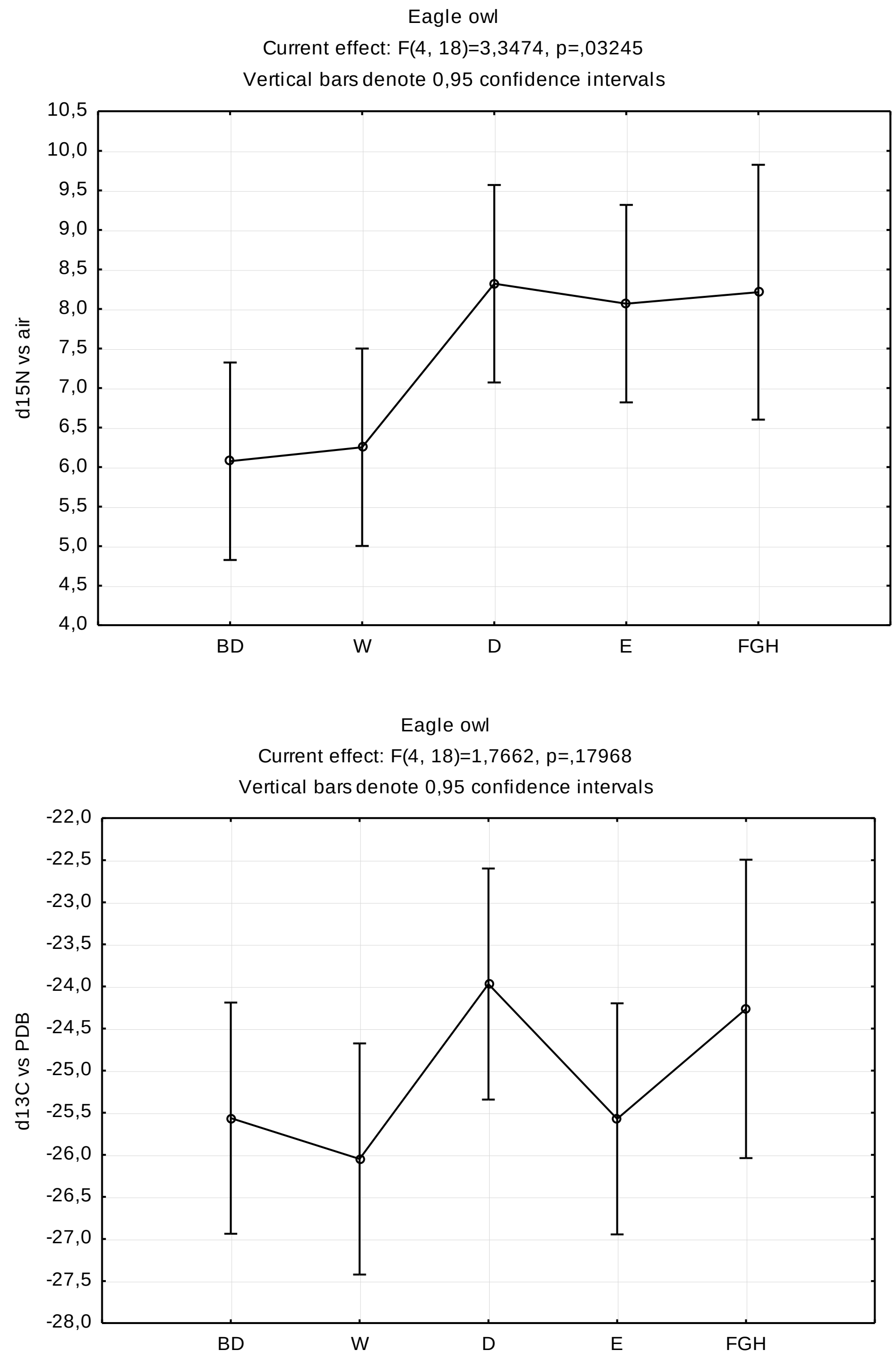
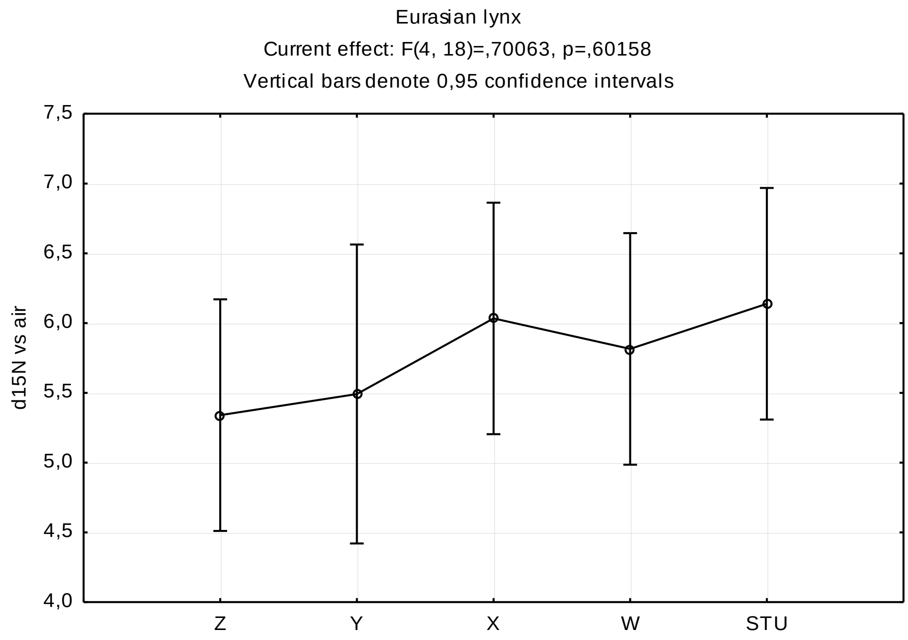


Figure 2. $\delta^{15}N$ (above) and $\delta^{13}C$ (below) in muscle of eagle owls from different parts of Sweden



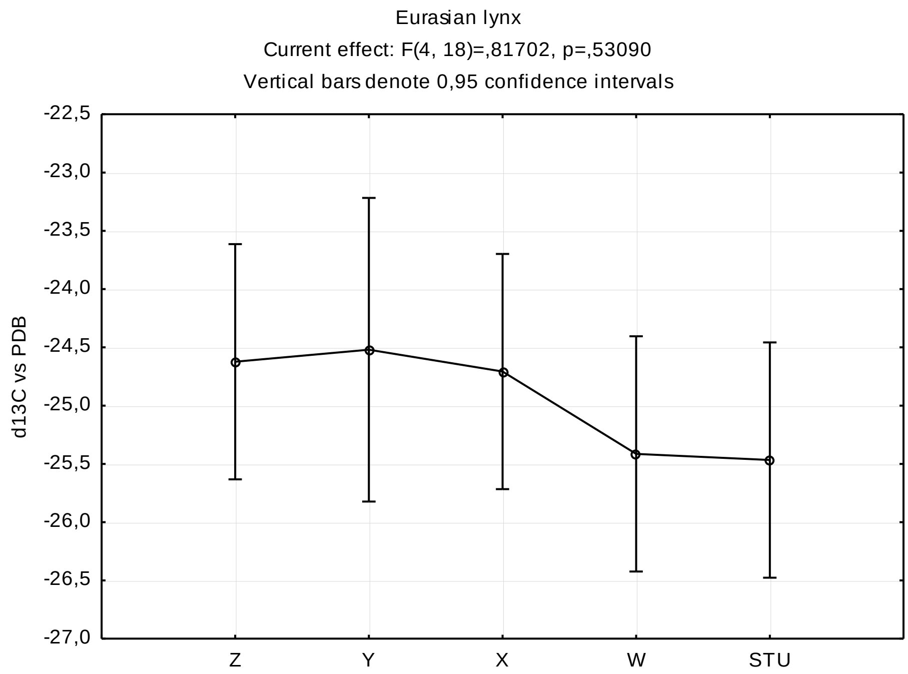


Figure 3. $\delta^{15}N$ (above) and $\delta^{13}C$ (below) in muscle of Eurasian lynx from different parts of Sweden.

Stable isotopes in relation to environmental contaminants

No general pattern was detected between levels of contaminants and levels of either δN^{15} or δC^{13}.

In eagle owls, an association between PCB101 and δC^{13} and δN^{15} was found (fig 4). In lynx none of the samples contained PCP101 >LOQ and no similar pattern were found for the other PCBs.

δC^{13} and δN^{15} appeared to be more associated with some PFAS in eagle owl while there appeared to be less of an association in lynx (fig 5).

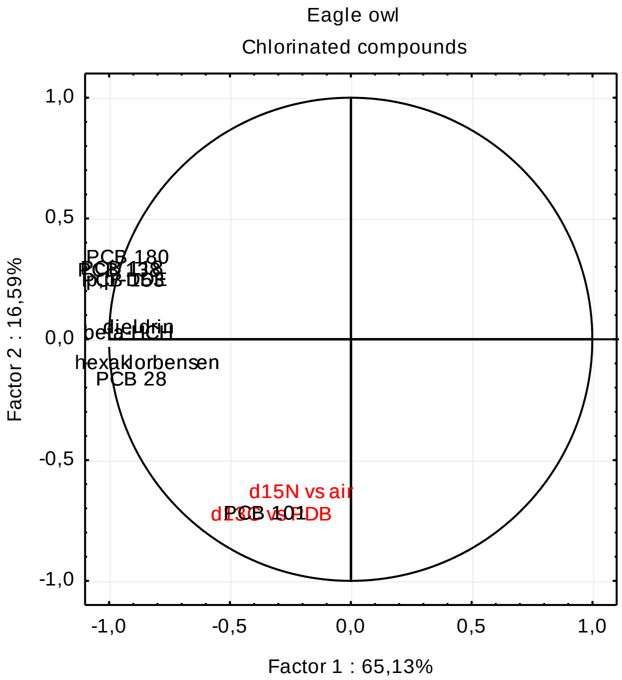


Figure 4. Principal component analysis (PCA) on chlorinated compounds and $\delta^{15}N$ and $\delta^{13}C$ in muscle of eagle owls from different geographical regions in Sweden.

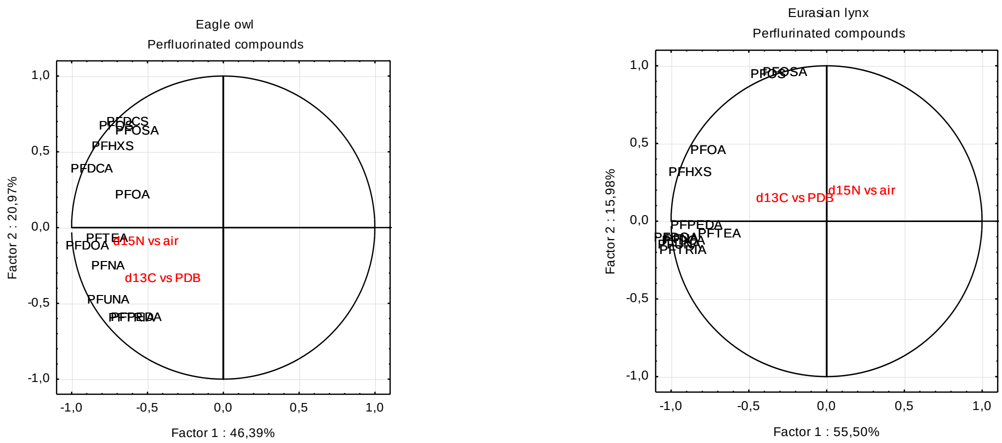


Figure 5. Principal component analysis (PCA) on perfluorinated compounds (PFAS) in liver in relation to $\delta^{15}N$ and $\delta^{13}C$ in muscle of eagle owl (left) and Eurasian lynx (right).

Discussion

$\delta^{15}N$ is generally said to reflect the trophic position while $\delta^{13}C$ is more associated with the origin of carbon.

In eagle owls $\delta^{15}N$ and $\delta^{13}C$ were significantly and positively correlated while no such correlation was found in lynx. There was also a significant difference in $\delta^{15}N$ between eagle owls from different geographical regions with owls from the three northernmost areas having higher $\delta^{15}N$. This was not detected in lynx.

No baseline values for either $\delta^{15}N$ or $\delta^{13}C$ was available in the present study and because of this no conclusions on differences in trophic levels for eagle owls in the northern and southern parts of Sweden can be made.

More studies on $\delta^{15}N$ or $\delta^{13}C$ in potential prey species have to be made in order to be able to draw conclusions on relationships between $\delta^{15}N$ and $\delta^{13}C$, food webs, trophic levels and levels of environmental contaminants in eagle owls and lynx.