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Possible Effects of Persistent Organic Pollutants on Steroid Hormone Homeostasis in House Sparrows (*Passer domesticus*) from Helgeland, Norway

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Abstract

Over the last decades, declines have occurred in the range and abundance of several passerines and farmland bird species and even though several possible causes have been suggested, the exposure to environmental contaminants and intensification of agriculture have received much attention. Several environmental contaminants, such as polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs) and polybrominated flame retardants (PBDEs) have been found to alter steroid hormones levels and may cause adverse effects on reproduction. Present decline in house sparrow numbers appears to be widespread in North-Western Europe since the 1970s. Among a variety of physical and chemical stressors, endocrine disruptive chemicals (EDCs) may contribute to the decline.

The aim of the present study was to determine levels of some selected PCBs (PBC-28, -52, -101, -118, -138, -153, -180), PBDEs (BDE-47, -99, -100, -153, -154, -209, hexabromocyclododecane [HCBD]) and OCPs (hexachlorobenzene [HCB], dichloro-diphenyl-dichloroethylene [*p,p'*-DDE]) and to investigate the possible effects of these contaminants on circulating levels of steroid hormones (estrone [E1], 17α-estradiol [αE2], 17β-estradiol [βE2], progesterone [PRO], pregnenolone [PRE], hydroxylated progesterone [OH-PRO], aldosterone [ALDO], androstenedione [AN], dehydroepiandrosterone [DHEA], dihydrotestosterone [DHT], testosterone [TS]) in adult house sparrows from Leka, Helgeland, Norway. Plasma samples were analysed for steroid hormones by gas chromatography tandem mass spectrometry (GC-MS/MS) and liver samples were analysed for environmental contaminants by gas chromatography-electron capture detection (GC-ECD) and gas chromatography mass spectrometry (GC-MS). It was hypothesized that POPs may have endocrine disrupting effects on the local house sparrow population and can thus interfere with the steroid hormone homeostasis. Multivariate data analysis (principal component analysis (PCA) and orthogonal projections to latent structures (O-PLS)), and bivariate correlation test (Spearman's rank correlation test) were applied to evaluate the effects of environmental contaminants and biometrical variables on steroid hormone levels.

Multivariate regression analysis indicated no strong relationships between contamination load and steroid hormone levels in adult female and male house sparrows, nor did biometrical variables seem to be very important in explaining the variation of the steroid hormones. However, bivariate correlations revealed negative relationships between PCB-118 and βE2 and *p,p'*-DDE, ∑OCPs and E1 among female house sparrows. Among male sparrows, bivariate correlations indicted positive relationships between DHT levels and PCB-118, BDE-100 and ∑PCBs. Furthermore, it is noteworthy to emphasize that statistical significant models were not found in the multivariate analysis and relationships indicated by bivariate correlations should be interpreted with caution. In addition, these statistical correlations do not represent direct cause-effect relationships and no definite conclusion can be made on possible disruption on estrogen and androgen levels.

Sammendrag

I løpet av de siste tiårene, har det blitt observert en betraktelig nedgang av flere spurvefugler og fuglearter som lever i habitater knyttet til jordbruk. Flere mulige årsaker har blitt foreslått og eksponering for miljøgifter og intensivering av jordbruket har fått mye oppmerksomhet. Flere miljøgifter, som polyklorerte bifenyler (PCB), organohalogenerte pesticider (OCP) og polybromerte difenyl etere (PBDE), er kjent for å forstyrre steroidhormoner og forårsake uønskede effekter på blant annet reproduksjon. Nedgangen i gråspurvpopulasjonen ser ut til å være utbredt i mesteparten av Nord-Vest-Europa siden 1970-tallet. Flere årsaker har blitt foreslått for å forklare nedgangen, herunder endringer i landbruksledelse, bruk av plantevernmidler og endokrinforstyrrende forbindelser.

Målet med dette studiet var å identifisere nivåer av noen utvalgte PCB (PBC - 28, -52, -101, -118, -138, -153, -180), PBDE (BDE - 47, -99, -100, -153, -154, -209, HCBD) og OCP (HCP, p, p'-DDE) samt undersøke mulige effekter av forurensninger på sirkulerende nivåer av steroidhormon (estron [E1], 17α-estradiol [αE2], 17β-estradiol [βE2], progensteron [PRO], pregnenolon [PRE], hydroksylert progesteron [OH-PRO], aldosteron [ALDO], androstenedion [AN], dehydroepiandrosteron [DHEA], dihydrotestosteron [DHT], testosteron [TS]) hos voksne gråspurv fra Leka, Helgeland, Norge. Plasmaprøver ble analysert for steroidhormoner ved bruk av gasskromatografi tandem massespektrometri (GC-MS/MS) og leverprøver for miljømessige forurensninger med gasskromatografi - elektron fangst deteksjon (GC- ECD) og gasskromatografi massespektrometri (GC-MS). Hypotesen var at miljøgifter kan ha hormonforstyrrende effekter på den lokale gråspurv populasjonen og kan dermed forstyrre steroidhormon homeostase. Multivariat dataanalyse (prinsipal komponent analyse (PCA) og ortogonale projeksjoner til latente strukturer (O - PLS)) og bivariat korrelasjonstest (Spearmans rank korrelasjon test) ble gjennomført for å evaluere effekten av miljøgifter og biometriske variabler på steroidhormonnivåer.

Multivariat regresjonsanalyse indikerte ingen sterke relasjoner mellom miljøgifter og steroidhormonnivåer i voksne gråspurv. I tillegg, viste resultatene til at biometriske variabler ikke var særlig viktige for å forklare variasjonen av steroidhormon. Bivariat korrelasjonsanalyser på sin side, avdekket negative sammenhenger mellom βE2 nivåer og PCB-118 samt mellom E1 nivåer og p, p'-DDE og ∑OCPs blant hunnlige gråspurv. Blant hannlige spurver, indikerte bivariat analyse positive relasjoner mellom DHT nivåer og PCB-118, BDE-100 og ∑PCB. Videre er det verdt å understreke at statistiske sammenhenger ikke ble identifisert i multivariat analyse og korrelasjoner indikert i bivariat analyse må derfor tolkes med forsiktighet. I tillegg er resultatet fra dette studiet kun basert på statistiske assosiasjoner og gjenspeiler ikke nødvendigvis biologiske årsak-virkningsforhold og ingen konkret konklusjon kan dermed stadfestes angående mulig endokrin forstyrrelse på steroid hormoner i gråspurv fra Helgeland, Norge.

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Trondheim

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Abbreviations

αE2	17α-estradiol
βE2	17β-estradiol
AhR	Aryl hydrocarbon receptor
AN	Androstenedione
AR	Androgen receptor
BMR	Basal metabolic rate
BFR	Brominated flame retardant
BDE	Brominated diphenyl ethers
CoeffCS	Regression coefficient
CV-ANOVA	Analysis of variance testing cross-validated predictive residuals
CYP	Cytochrome P450
CYP11A	Cholesterol side chain cleavage enzyme
CYP19	Aromatase
CBC	Common bird census
DCS	Derivatization control standard
DDE	Dichloro-diphenyl-dichloro-ethylene
DDT	Dichloro-diphenyl-trichloro-ethane
Deca-BDE	Decabromodiphenyl ether
DHEA	Dehydroepiandrosterone
DHT	Dihydrotestosterone
E1	Estrone
EDC	Endocrine disruptive chemical
ER	Estrogen receptor
GC-ECD	Gas chromatography electron capture detection
GC-MS	Gas chromatography mass spectrometry
GC-MS/MS	Gas chromatography tandem mass spectrometry
HBCD	Hexabromocyclododecane
HCB	Hexachlorobenzene
HPG	Hypothalamus-pituitary-gonadal axis
ICS	Instrumental control standard
IS	Internal standard
LOD	Limit of detection
MSTFA	N-methyl-N-trimethylsilyl-trifluoroacetamide

OCP	Organochlorine pesticide
Octa-BDE	Octabromodiphenyl ether
OHC	Organohalogenated contaminants
OH-PRO	Hydroxylated progesterone metabolite
O-PLS	Orthogonal projections to latent structures
PBDE	Polybrominated diphenyl ether
PC	Principal component
PCA	Principal component analysis
PCB	Polychlorinated biphenyl
PCDD	Polychlorinated dibenzo-p-dioxin
PCDF	Polychlorinated dibenzo-p-furan
Penta-BDE	Pentabromodiphenyl ether
PLS	Projections to latent structures by means of partial least squares
POP	Persistent organic pollutant
PRE	Pregnenolone
PRO	Progesterone
SPE	Solid phase extraction
SD	Standard deviation
T_4	Thyroxine
TMSI	N-trimethylsilylmidazole
TTR	Transthyretine (transporter protein for thyroid hormones)
TS	Testosterone
UV	Unit-variance
VIP	Variable important in projections

Contents

1.0 Introduction

For many decades, evidence of declining wildlife populations have been coupled to a number of different causes, including habitat loss, hunting and climate change (Ford et al., 2001, Giesy et al., 2003, Crick, 2004). The declines have also been related to the exposure of anthropogenic chemicals and it has been shown that environmental contamination have resulted in adverse effects on wildlife, even on population levels (Ratcliffe, 1967, Fry, 1995, Herzke et al., 2003, Jaspers et al., 2006). Substantial usage of organohalogenated contaminants (OHCs) started after the Second World War, but due to awareness about their toxic impact and persistence, many nations prohibited production and use in the 1970s and 1980s. However, due to continuous usage in several developing countries, these compounds are still found in relatively high concentrations in nature (Letcher et al., 2010). Many wild bird species are conspicuous in the surroundings, and decline in population sizes due to environmental pollutants are obvious indicators of environmental damage. Thus birds are used as bio-monitoring species in many scientific studies (Fry, 1995, Jaspers et al., 2007), including both terrestrial (Lincer, 1975, Luzardo et al., 2014) and marine environments (Bustnes et al., 2010, Corsolini et al., 2011). However, when investigating local contamination, passerine bird species have been shown to be useful as biomonitors because of their relative small territories and home ranges (Eens et al., 1999, Dauwe et al., 2003, Van den Steen et al., 2010b). Over the last decades, declines have occurred in the range and abundance of several passerines and farmland bird species, and even though the threats are many and varied, the intensification of agriculture and endocrine disruption has been suggested as a possible cause (Boatman et al., 2004).

1.1 Pesticides and persistent organic pollutants (POPs)

Persistent organic pollutants (POPs) are organic compounds that are ubiquitous in the environment and may cause adverse effects to both wildlife and human health (Tanabe, 2002). POPs have been detected in a wide range of animal groups, including passerine birds and in many cases in considerable concentrations (Dhananjayan et al., 2011, Bouwman et al., 2013). POPs are chemically stable and thus quite persistent in the environment. At the same time, they are highly toxic, have a strong bioaccumulative nature and hold the ability to biomagnify in the food-chain (Tanabe, 2002). Additionally, POPs tend to be distributed globally by long-range transport due to their persistence and volatile capacity, and are therefore found in vast distances from emission sites and in remote areas (Wania and MacKay, 1996, Tanabe, 2002). Among the most important classes of POPs are chlorinated and brominated aromatics; including polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins and furans (PCDD/Fs) and different organochlorine pesticides (OCPs: dichloro-diphenyl-trichloroethane [DDT] and its metabolites). Some are side-products from combustion or industrial synthesis, while others are deliberately synthesized for industrial use (Jones and De Voogt, 1999, Miniero and Iamiceli, 2008).

Polychlorinated biphenyls (PCBs) have been widely used in numerous applications since the 1930s, due to their desirable properties. Primarily, PCB was used as electrical insulating fluids in capacitors and transformers as well as hydraulic, heat transfer and lubrication fluids. Blended with other chemicals, PCBs where also used in products such as carbonless copy paper, adhesives, waxes, paint additives and plastics (Ribas-Fito et al., 2001, Erickson and Kaley II, 2011). Despite the fact that PCBs was banned in most countries in 1979 and global ban was implemented by the Stockholm Convention in 2001 (Porta and Zumeta, 2002), they continue to pose environmental risk due to their chemical properties and improper disposal practices (DeLeon et al., 2013). A total of 209 different congeners are theoretically possible and the degree and position of chlorination denotes their toxicity (McKinney and Waller, 1994). In general, highly chlorinated PCBs are more stable and less likely to metabolize and thus more toxic (DeLeon et al., 2013). Chlorination can happen either at the -*ortho*, -*meta* or -*para* position (Figure 1). Both coplanar (non-*ortho*) and mono-*ortho*-substituted PCBs are considered most toxic due to similar structure and chemical properties to dioxin (DeLeon et al., 2013). Adverse effects resulting from exposure to PCBs have been reported in a wide range of animal groups from invertebrates and reptiles to birds and mammals (Crain and Guillette Jr, 1998, Park et al., 2009, Verslycke et al., 2004, Jenssen et al., 2010). Furthermore, it has been shown that passerine bird species have detectable levels of PCBs (Van den Steen et al., 2009b). Even though few toxicological studies have been conducted in passerines, PCB exposure has caused reduced reproductive success in other bird species (Ratcliffe, 1967, Fernie et al., 2001, Arenal et al., 2004). Moreover, this might indicate that passerine bird species may also be receptive for adverse effects as a consequence of PCB exposure (McCarty and Secord, 2000, DeWitt et al., 2006) including endocrine disruption that is explained later in detail.

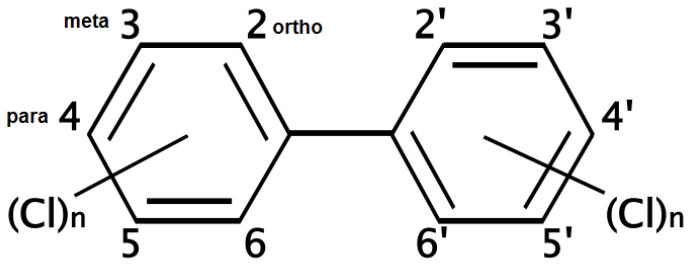

Figure 1: Molecular structure of polychlorinated biphenyls (PCBs*) (McKinney and Waller, 1994)*

Brominated flame retardants (BFRs) have been used in several consumer products for many decades to increase the fire resistance of the products and thus prevent the spread of fire (Alaee et al., 2003, Birnbaum and Staskal, 2004). Flame retardants are much used in plastics, textiles, electronic devices and many other materials (De Wit, 2002). There are five major classes of BFRs; brominated bisphenols, diphenyl ethers, cyclododecanes, phenols and phathalic acid derivatives where the first three classes represents the highest production volumes (Birnbaum and Staskal, 2004). One common type of BFRs is polybrominated diphenyl ethers (PBDEs) which were introduced as a flame retardant in the 1960s. The general structure of brominated diphenyl ethers has two phenol rings, each with varying

degree of hydrogen or bromine (Br) substitutions joined by an ether bond (Figure 2). PBDEs are additive flame retardants blended with polymers and leakage from the products into the environment are of great concern. With analogy to PCBs, the PBDEs have 209 different congeners depending on the number and position of bromine atoms (Wiseman et al., 2011, Alaee et al., 2003). There were produced three different commercial formulations of PBDE; pentabromodiphenyl ether (penta-BDE), octabromodiphenyl ether (octa-BDE) and decabromodiphenyl ether (deca-BDE). Due to concern of the adverse effects on wildlife and human health, penta- and octa-BDEs were included in the list of POPs being persistent, bioaccumulative and capable of long-range transport of the Stockholm Convention (Convention, 2009). However, there is still large-scale production of deca-BDEs resulting in continuous release into the environment (Wiseman et al., 2011). Among the most critical effects of PBDE exposure include developmental neurotoxicity and thyroid hormone disruption (Darnerud, 2003, Costa and Giordano, 2007). In addition, PBDEs have been reported in passerine bird species (Dauwe et al., 2006) making them an interesting study object of toxicological aspect due to the present decline of many passerine populations. This may indicate that passerines might be subject to adverse effects such as endocrine disruption as a consequence to PBDE exposure as documented for other bird species (Fernie et al., 2005, Voorspoels et al., 2006, Fernie et al., 2008, Van den Steen et al., 2010a).

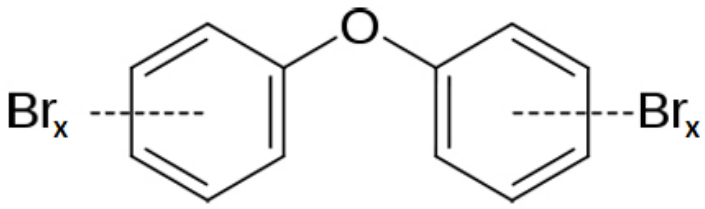

Figure 2: Molecular structure of polybrominated diphenyl ethers (PBDEs) *(Birnbaum and Staskal, 2004)*

Organochlorine pesticides (OCPs), such as hexachlorobenzene (HCB), aldrin, dieldrin, mirex and DDT, are volatile organic compounds designed to kill pests such as rodents, fungi, insects and weeds that undermine farming and gardening. Many OCPs possess properties that may be harmful for the environment; this include low water and high fat solubility which makes them able to persist in both the terrestrial and marine environment, bioaccumulate and bioconcentrate in tissues of invertebrates and vertebrates as well as in food-chains (Li and Macdonald, 2005, Mnif et al., 2011). Even though pesticides have proven to be harmful to the environment, there can also be health-beneficial aspects of pesticide usage; like controlling agricultural pests, plant diseases and livestock disease vectors as well as insuring food production for the world's population (Li and Macdonald, 2005, Mnif et al., 2011). Even though the use of OCPs have been banned in many industrial countries since the 1980s, many are still being used in undeveloped countries in Asia, Africa and Latin-America (Ali et al., 2014). Among the best known OCPs are DDT and its metabolites. Rachel Carson 's book *Silent Spring (1962)* identified the urban use of DDT as the cause of declining bird populations in the United States. The pesticide accumulated in non-target species, such as

earthworms, and thus transferring lethal doses of DDT to birds causing massive mortalities. Since the discovery of DDT in 1939, numerous pesticides (organochlorines, organophosphates and carbamates) have been developed and used worldwide with few guidelines and restrictions resulting in high concentrations in nature (Carson, 1962, Mnif et al., 2011). Occurrence of OCPs in wildlife has been stated in many publications both on birds of prey (Ludwig, 1996, Bustnes et al., 2003, Luzardo et al., 2014) as well as in passerines (Dauwe et al., 2003, Van den Steen et al., 2006, Eens et al., 2013). Even though passerines are less sensitive to OCP exposure than other birds, (Van den Steen et al., 2009b) the fact that the contaminants are detectable in birds lower on the food-chain is a cause for concern. Moreover, the indirect effect of OCPs through the food-chain, reducing important supply of insects during the nestling periods, might be a contributing factor for the globally declines of passerine populations (Boatman et al., 2004, Mitra et al., 2011) as well as the endocrine disrupting features of some OCPs (Tyler et al., 1998, Mattsson et al., 2011, Mnif et al., 2011).

1.2 House sparrow (Passer domesticus)

Until recently, bird species high in the food chain being subject to bioaccumulation, such as raptors, gulls and other fish-eating birds, have been the first choice as biomonitoring species of organochlorine contamination. These are typically wide-ranging species which are less suitable for investigation of contamination within small areas. Therefore, in order to monitor local contamination, non-migratory, sedentary passerine bird species may be more useful as biomonitors due to their small territories and small home-ranges (Eens et al., 1999, Dauwe et al., 2003, Van den Steen et al., 2010b). Growing numbers of studies have been conducted using passerines to investigate organochlorine contamination (Ormerod and Tyler, 1992, Jaspers et al., 2006, Van den Steen et al., 2009b) and they have also been successfully used as indicators for local heavy metal pollution (Eens et al., 1999, Dauwe et al., 2003).

The house sparrow (*Passer domesticus*) is a small semi-colonial passerine and one of the most abundant bird species globally. The sparrows are usually stationary after settlement, only migrating between 1-2 km, which makes it an convenient study object for environmental status and population ecology (Møller et al., 1996, Anderson, 2006). The house sparrow became a common species following the intensive mixed farming methods introduced in the eighteenth century and has one of the widest geographical distributions of all species (Anderson, 2006). In Norway the house sparrow is distributed throughout the whole country typically associated with human settlements in rural areas and nest close to farms and animal holdings. It is here, where the highest densities are found because spill food from stock farming and household provides a continuous source of food (Bjordal, 1981, Ringsby et al., 1999, Summers-Smith, 2005).

The adult male and female house sparrows are easily distinguished from each other; females are pale brown and grey whereas the males have brighter black, white and brown markings (Anderson, 2006). Males also develop a black badge during their first autumn moult in late September or early October. The size of the badge is related to social status and hence a signal

of dominance (Møller, 1988, Solberg and Ringsby, 1997). The badge is partially covered with grey feather tips after moult, but these are worn off due to preening and dust bathing making it bigger and darker and females are known to choose mates on the basis of male badges among other qualities (Møller et al., 1996, Møller, 1988). Female preference for mates with elaborated traits has often been explained on the basis that exaggerated secondary sexual traits may reflect individual male quality and females might gather direct and indirect benefits in mating with such males (Hamilton and Zuk, 1982, Gonzalez et al., 2001, Ringsby et al., 2009). It is believed that androgens, especially testosterone, directs the secondary sexual traits like the chest badge in house sparrows (Evans et al., 2000, Buchanan et al., 2001), however there have been contradicting reports on whether this is a direct relationship. Gonzalez et al. (2001) found a positive correlation between testosterone levels and size of the chest badge of house sparrows indicating that testoserone is important not only for dominance, but also for attracting female sparrows.

1.2.1 Present decline in the House sparrow population

Present decline in house sparrow abundance appears to be widespread in North-Western Europe since the 1970s. The British Trust of Ornithology conducted a survey of the house sparrow in Britain where they used the common bird census (CBC) giving indications off the species abundance. The abundance increased until the 1970s, but then decreased in the years after. By 1997, the density had decreased by about 60 % (Crick, 2002, De Laet and Summers-Smith, 2007). Many causes have been suggested to explain the decline, including changes in agricultural management, intensification of agriculture, the use of pesticides and possible endocrine disruption (Chamberlain et al., 2001). Even though pesticides have been suggested as a main concern for house sparrow population declines (Chamberlain et al., 2001), there are other aspects as well that needs to be considered, including reduced winter food supplies for the house sparrow due to the switch from spring sowing cereals to autumn. An increase in bird-proof storage at more modern farms can also reduce possible nesting sites for the sparrow in rural areas. Habitat loss, fragmentation as well as human influence might also contribute to the current decline (Hole et al., 2002, Giesy et al., 2003).

1.3 Endocrine disruption

Numerous chemicals in the environment have been associated with endocrine disrupting effects in exposed organisms and only a few toxicological reports exists on house sparrows (Ciesielski et al., 2008), though endocrine disruptive organochlorines have been detected in the species (Sanderson, 2006, Dhananjayan et al., 2011). These endocrine disrupting chemicals (EDCs) are exogenous substances that have the potential to alter normal function of the endocrine system due to structural similarities with endogenous hormones and thus interfere with reproduction, growth, development and maturation etc. (Sanderson, 2006, Swedenborg et al., 2009). The possible interferences of EDCs with the endocrine system involves several possible mechanisms; direct interactions with binding to receptors or indirect interactions as interfering with hormone concentrations or with signalling between different

components of the hypothalamus-pituitary-gonadal (HPG) axis (Lintelmann et al., 2003). Endocrine disruption has been widely studied in several animal groups, including adverse effects such as imposex in invertebrates (Hagger et al., 2006), abnormalities in reproductive organs of polar bears and reptiles (Guillette Jr et al., 1994, Sonne et al., 2006) and reproductive failure in birds (Hunt Jr, 1972, Kubiak et al., 1989). Furthermore, the most dramatic effect on avian reproductive performance is most definitely the result of eggshell thinning and dichloro-diphenyl-dichloroethylene (DDE), a degradation product of DDT (Ratcliffe, 1967, Blus et al., 1974, Wiemeyer et al., 1984, Lundholm, 1997).

The endocrine system in birds is generally very similar to that of all vertebrates, modulated by neurosecretory neurons in the hypothalamus; receiving input from external and internal stimuli which in turn regulates secretion of releasing hormones to the pituitary gland (Dawson, 2000). Subsequently the pituitary gland synthesizes and secretes the respective trophic peptide hormones that are transported by the circulation to target endocrine glands which in turn is stimulated to synthesize the active hormone. This gives EDCs the potential to disrupt several steps in the endocrine system (Dawson, 2000). Furthermore, there are several other aspects of avian physiology that makes them susceptible to endocrine disruption. Birds have high metabolic rates and on a weight-adjusted basis often have higher metabolism and food consumption than for instant mammals of similar size, increasing exposure to environmental contaminants (Damstra et al., 2002). In addition, migration, courtship, breeding and parental care are behaviours that require high energy consumptions and are often related to periods of starvation. During these periods, many birds mobilize stored lipids and thus raising the potential of increasing exposure to lipophilic compounds that are subsequently released (Damstra et al., 2002). Birds, as well as other animals, are dependent on an adequate interplay between the endocrine, nervous and immune system to obtain successful reproduction. The avian reproductive system is regulated by the HPG-axis which is involved in sexual differentiation and regulation of reproduction. Broadly, the hypothalamus stimulates the pituitary to synthesize and secrete luteinizing hormone (LH) and follicle-stimulating hormone (FSH) which are transported with the bloodstream to target endocrine glands, stimulating secretion of the active hormone which in turn regulates ovarian and testicular function (Ottinger and Bakst, 1995, Dawson, 2000). Research has shown that several POPs and pesticides may exhibit estrogenic and androgenic activities and thus influence reproduction and avian embryonic development (Giesy et al., 2003). Other studies have reported altered reproductive behaviour in ring-necked doves (*Streptopelia capicola*) fed mixtures of DDE and PCBs (McArthur et al., 1983). Correlative studies have also found negative associations between POPs/OCs and circulating levels of thyroid hormones in glaucous gulls (*Larus hyperboreus*), (Verreault et al., 2004) as well as in mammals such as the polar bear (*Ursus maritimus*), (Braathen et al., 2004). By disrupting the sex-steroid homeostasis, EDCs might impair reproduction, reproductive success and sexual differentiation in adult birds and developing chicks and thus contribute to population declines

1.3.1 Reproductive hormones and disruption of steroidogenesis

The biosynthesis of steroid hormones is called the steroidogenesis and includes synthesis of mineralocorticoids, glucocorticoids and sex steroid hormones (Sanderson and van den Berg, 2003). Steroid hormones are required for normal reproduction and homeostasis where steroidogenic enzymes (CYP-enzymes, hydroxylsteroid dehydrogenases and reductases) are responsible for the conversions to the different steroid hormones (Sanderson and van den Berg, 2003). In vertebrates, this involves a complex sequential pathway starting with the degradation of cholesterol to progestagens (pregnenolone [PRE], progesterone [PRO]) by side chain cleavage enzyme (CYP11A) and further conversions to androgens (testosterone [TS], androstenedione [AN], dihydrotestosterione [DHT], dehydroepiandrosterone [DHEA]) and finally estrogens (17β-estradiol [βE2], estrone [E1]). The last step from androgens to estrogens is converted by aromatase (CYP19), (Sohoni and Sumpter, 1998, Stocco et al., 2005). Vertebrate steroid hormones are mainly synthesized in gonads, adrenal glands, placenta, brain and adipose tissue and regulate a variety of different biological functions such as carbohydrate metabolism, salt and water balance, blood pressure, stress, reproductive function, fertility, development of secondary sex characteristics, growth, pregnancy, behaviour, breeding etc. (Stocco, 2001). In addition, the relative secretion of steroid hormones in birds is dependent on a variety of factors such as reproductive stage, breeding cycle, age, stress, environmental input, and the system has also been proven to be susceptible for EDCs (Pickering et al., 1987, Hau, 2001, Jenssen, 2006, Ellenberg et al., 2007, Ouyang et al., 2011). Such interactions may have detrimental effects and a cause for decreasing bird populations worldwide (Damstra et al., 2002).

1.3.1.1 Estrogens

PCBs and DDT have been reported to disrupt ovarian steroidogenesis in alligators (Guillette Jr et al., 1995) and porcine granulosa cells and thus alter ovarian function, cause estrogen deficiency or disrupt implantation, fertility and reproduction (Tiemann, 2008, Craig et al., 2011). Ovarian follicles synthesize estrogens in theca and granulosa cells stimulated by LH and FSH. Androgens produced in the theca cells are then converted to estrogens in the inner granulosa layer by various steroidogenic enzymes (Craig et al., 2011, Conley et al., 2012). Subsequently, estrogens elicit their effect by binding to estrogen receptors (ER) and induce transcription of target genes and cellular responses which is important in reproduction and other biological functions (Kuiper et al., 1997). Anti-estrogenic effects of environmental contaminants have been reported in many bird species (Lorenzen, 2001, Wiseman et al., 2011) and alterations in estrogen levels may have detrimental effects on reproduction. Anti-estrogenic actions include the inhibition of important enzymes in the steroidogenesis, such as aromatase responsible for the conversion from androgens to estrogens and thus decrease estrogen levels (Garcia-Reyero et al., 2006, Woodhouse and Cooke, 2004) and inhibiting the binding of estrogens to ER (Bonefeld-Jørgensen et al., 2001). In addition, ECDs may also bind to aryl hydrocarbon receptor (AhR) and thus increase expressions of enzymes capable of metabolizing estrogens and thus decrease estrogen levels in the exposed organism (Kharat and Saatcioglu, 1996)

1.3.1.2 Androgens

Androgens play a crucial role in male reproduction and the development of male reproductive tissue, development, secondary sex characteristics and spermatogenesis (Dohle et al., 2003). Testosterone is the primary androgen and the most abundant circulating steroid hormone in males and is synthesized by Leydig cells in the testis (Wilson, 1996, Shima et al., 2012). However, when in target organs TS might be metabolized to DHT, which is the more potent form of TS and both hormones elicit their effect through binding to the androgen receptor (AR) (Wilson, 1996). Low levels of TS/DHT and or AR may impair male development and reproduction and numerous contaminants have the potential to act as anti-androgens (Dohle et al., 2003) as reported in rats (Kuriyama et al., 2005) and in birds such as the Japanese quail (*Coturnix Japonica)* (Alexandre and Balthazart, 1986). Alterations in androgen levels may have detrimental effects on reproduction, reproductive behaviour and thus ultimately reduce fitness (Saino and Møller, 1995, Bayley et al., 2002). Anti-androgenic or agonistic androgenic actions that may alter androgen levels, include inhibition or induction of steroidogenic enzymes that may decrease or increase conversions to androgens respectively (Karpeta et al., 2011, Wang et al., 2011) and disruption of the binding of androgen to AR (Stoker et al., 2004). In addition, ECDs may also bind to AhR and thus increase expressions of enzymes capable of metabolizing androgens and decrease androgen levels in the exposed organism (Kharat and Saatcioglu, 1996).

1.4 Aim of study

The aim of the present study was to determine levels of some selected PCBs (PBC-28, -52, -101, -118, -138, -153, -180), PBDEs (BDE-47, -99, -100, -153, -154, -209, HCBD) and OCPs (HCP, *p,p'*-DDE), and investigate the possible effects of contaminants on circulating levels of steroid hormones in house sparrows from the island Leka, Helgeland, Norway. It was hypothesized that POPs may have endocrine disrupting effects on the local house sparrow population and thus might interfere with the steroid hormone homeostasis. Since little information is available on the endocrine disruptive aspects of contaminants on hormone homeostasis in passerines, the present study may therefore provide important information on the subject, and also give an indication whether contaminants contribute to the present decline in house sparrow populations.

2.0 Materials and methods

2.1 Field work

2.1.1 Study site and sampling

The sparrows used in the present study were collected on the island Leka (65°5'34"N, 11°42'18"E) on the coast of Helgeland, Norway during February and March 2013. The local house sparrow population live in close contact with human settlements and are often found inside farm buildings during the winter period where food is available (Saether et al., 1999). The house sparrow population estimate from Leka in 2013 included 137 individuals dispersed among 17 farms with varying degree of distance from each other. Typically, about 10-20 % of the recruits have dispersed from their natal area and settled in other farm populations before their first breeding season (Pärn et al., 2009, Pärn et al., 2012). Most of the dispersing individuals move short distances, and the proportion of long-distance dispersers decline with distance (Tufto et al., 2005). Adult birds were captured in field by mist nets, measured for biometry and ringed with individually number metal rings. Unique colour combinations were attached to the tarsi, which allowed for recognition of the birds in the field (Ringsby et al., 2009). Subsequently the captured sparrows were placed indoors in an isolated barn with access to fresh water and food (1 week) until approximately all birds on the island were captured. As a part of another ongoing, large-scale experimental study of basal metabolic rate (BMR) sparrows with high BMR were selected for toxicological analysis on Leka (BMR selection threshold: males > 78.53 mlO_2/h, females > 76.53 mlO_2/h)

2.1.2 Measuring biometric traits:

Body mass was measured using a 50 g Pesola spring balance (to the nearest 0.1 g). Tarsus length, bill length and bill depth where all measured to the nearest 0.1 mm using a Vernier slide calliper (Solberg and Ringsby, 1997). Wing length was measured using a ruler with an accuracy of 0.5 mm. Slide callipers were also used to measure length and width (to the nearest mm) of the black chest badge of adult males and two types of badge measurements were taken. The first was total badge size, which was the area covered with black feather and feathers with light bases and light grey feather tips (Ringsby et al., 2009). The second was visible badge size, which was the area covered with black feathers without light tips. Badge size was then calculated according to Møller (1987).

2.1.3. Sampling for toxicological analysis:

Both females (n = 22) and males (n = 25) were selected (total n = 47) for toxicological analysis based on measurement of BMR. The sparrows were killed by decapitation and blood was collected in heparinized tubes and subsequently centrifuged (3000 rpm, 10 minutes) to separate the plasma from the blood cells. The plasma (200 µl) was transferred to blood vials with a Pasteur pipette for steroid analysis and added 50 µl internal standard (IS: 50 µl of a 0.1

ng/µl stock solution). Subsequently tissue dissection was carried out to collect the liver which was weighed and wrapped in aluminium foil and all samples were marked, before stored in liquid nitrogen until further analysis.

2.2 Steroid hormone analysis

Plasma samples from all birds were analysed for steroid hormones. The steroid hormone analysis was conducted at the Department of Pharmaceuticals and Analytical Chemistry at the University of Copenhagen, Denmark. The plasma samples were analysed for eleven steroid hormones; pregnenolone (PRE), progesterone (PRO), hydroxylated progesterone (OH-PRO), androstenedione (AN), dehydroepiandrosterone (DHEA), dihydrotestosterione (DHT), testosterone (TS), aldosterone (ALDO), estrone (E1), 17α-estradiol (αE2), 17β-estradiol (βE2). Limit of detection (LOD) was determined visually due to a new gas chromatography (GC) -machine (Table 1). The procedure is based on the method originally described by Hansen et al. (2011) with some modifications, the procedure is illustrated in Figure 3.

All devices used in the analysis were cleaned thoroughly between every batch of samples. Plasma samples were stored at – 20 °C before analysis.

Table 1: Limit of detection (LOD) values for the steroid hormones analysed by GC-MS/MS at the Department of Pharmaceuticals and Analytical chemistry at the University of Copenhagen. Due to a new GC-MS/MS machine, LOD levels were only determined visually and in cases where detected levels were lower than the visually LOD, the detected value was set as LOD.

Steroid hormone	Visual LOD (ng/ml)
Progestagens	
Pregnenolone[a]	0.009
Progesterone	0.04
17-hydroxyprogesterone	0.01
Androgens	
Dehydroepiandrosterone	0.05
Androstenedione[a]	0.008
Testosterone	0.01
Dihydrotestosterone[a]	0.009
Estrogens	
Estrone	0.05
17α-estradiol	0.01
17β-estradiol	0.05
Mineralocorticoid	
Aldosterone	0.5

[a] LOD levels as the lowest detected concentrations

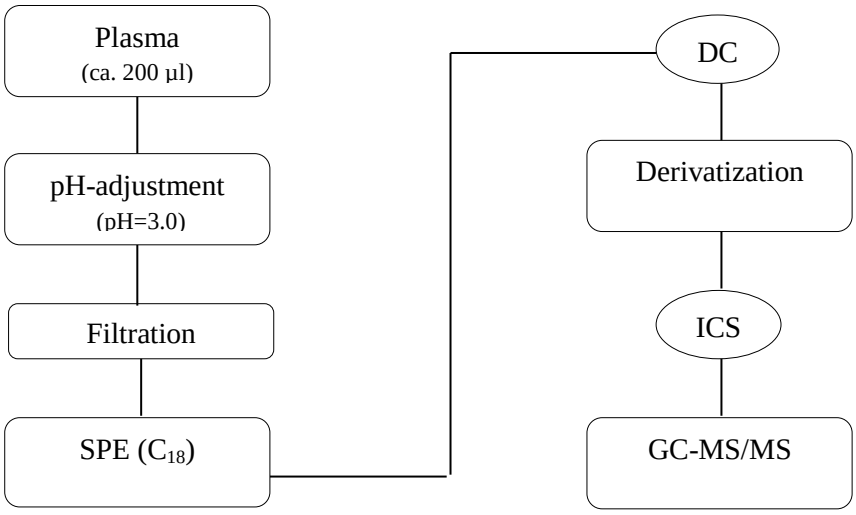

Figure 3: Overview of steps included in steroid hormone determination procedure as described in Hansen et al (2008) with some modifications. Plasma samples were spiked with internal standard (IS) during fieldwork and the clean-up steps were replaced with filtration of plasma prior to solid phase extraction (SPE). A derivatization control standard (DCS) was added followed by derivatization to increase volatility and thermal stability of the analytes. Finally, an instrumental control standard (ICS) was added followed by quantification on GC-MS/MS

Sample preparation

Plasma samples were filtrated using glass microfiber filters (Watman™ GF/C, 25 mm Circles, Cat No; 1822-025) and a home-made vacuum filtration devise to make sure the sample did not clot in the columns during solid phase extraction (SPE). Approximately 2 ml plasma was diluted with tap water (7 ml) and pH-adjusted (pH = 3) by dilution of sulphuric acid (1M H_2SO_4) in order to stabilize the steroid hormones. The filtration preparation procedure is illustrated in Figure 4.

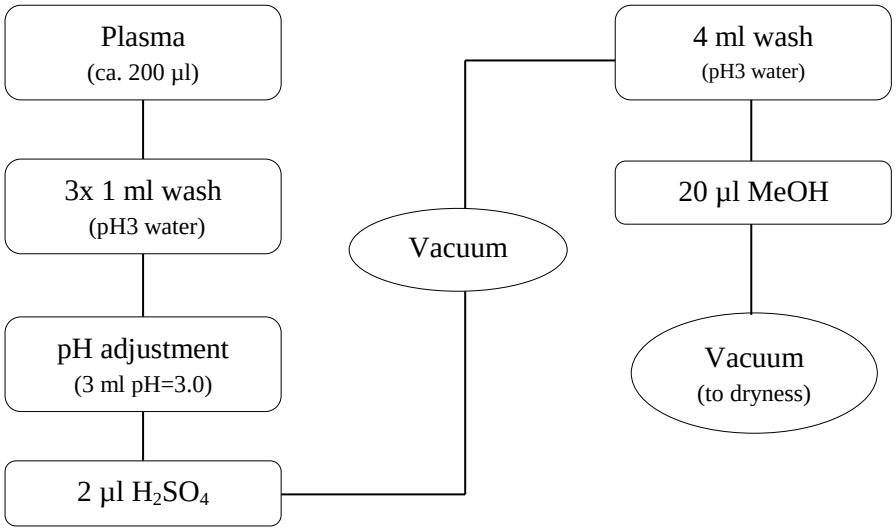

Figure 4: Procedure for filtration of plasma samples prior to solid phase extraction (SPE). The plasma was transferred to a 10 ml syringe and the container was washed with 7 ml, pH3 adjusted water to make sure all the plasma was transferred. The pH was further adjusted to 3.0 to stabilize the steroid hormones and vacuum was used to force the sample trough the filter. Subsequently methanol (MeOH) was added before the filter was sucked dry with vacuum. The filtration equipment was washed and sterilized with ethanol between every sample.

Solid phase extraction (SPE)

The steroid hormones were isolated by SPE and C18 columns (500 mg, 10mL reservoir, Varian Inc., California). Prior to isolation, the SPE columns were conditioned with heptane (5 ml), acetone (2 ml), methanol (5 ml) and pH adjusted tap water (pH=3, 7 ml). Subsequently, the filtrated samples were added and after isolation, the columns were air dried by suction (1h) using a vacuum manifold (IST VacMaster, Uppsala Sweden). Finally the analytes were eluted from the SPE columns with acetone (5 ml) and evaporated to dryness using a heat-block (60 °C) and nitrogen gas.

Derivatization

After evaporation to dryness, a derivatization control standard (DCS: 50 µl of a silylating reagent mixture of N-methyl-N-trietylsilyl-trifluoroacetamide (MSTFA), N-trimethylsilylmidazole (TMSI) and 1,4-dithioerythriol (DTE)) was added to the extracts containing the analytes followed by evaporation to dryness on heat-block (60 °C) and nitrogen gas. The vials were left in an oven (60 °C, 1h) and thereafter the mixture was evaporated to complete dryness using the heat-block (60 °C) and nitrogen gas. Finally, an instrumental control standard (ICS; esterone-3-methyl-ether MeE1 0.1 ng/µl in heptane) was added (200 µl) and samples were transferred to GC-vials for quantification by gas chromatography tandem mass-spectrometry (GC-MS/MS).

Quality control

Series of blanks were analysed with the rest of the samples as quality control. Also, known concentrations of heptane were analysed. In addition, the present method have previously been shown to determine steroid hormones in the low part per billion range with high selectively and low concentrations (Hansen et al., 2011). A derivatization quality control standard (DCS) and instrumental control standard (ICS) were added to insure the quality during the derivatization step and during instrumental quantification.

Quantification

The details of the quantification is as described in Hansen et al. (2011) with some modifications. The GC was a Bruker Scion™ GC with a Zebron-5HT Inferno (30m × 0.25mm, 0.25 µm, Phenomenex Inc., Torrance, CA, USA) column. The GC was connected to SCION TQ GC triple quadrupole (Bruker Daltonics, Bremen, Germany) equipped with an EI interface. For the hydroxy-steroids (OH-PRE and OH-PRO) not included in in the original method, the following ion transitions were used; OH-PRE: m/z 433.00 to m/z 253.10 (T) and m/z 343.20 (Q); OH-PRO: m/z 359.00 to m/z 145.10 (T) and m/z 269.20 (Q).

2.3 Persistent organic pollutants analysis

Liver samples from the sampled birds where analysed for environmental contaminants at the Norwegian School of Veterinary Science (NVH), laboratory of Environmental Toxicology, Oslo Norway. The laboratory is accredited by the Norwegian Accreditation for testing analysed chemicals in biological material according to the requirements of the NS-EN ISO/IEC 17025 (Test 137). The method used is based on the procedure originally described by Brevik and Bjerk (1978) and optimized by Polder et al. (2008).

Liver samples were analysed for seven PCBs (PCB-28, -52, -101- 118, -138, -153, -180), six BFRs (BDE -47, -99, -100, -153, -154, -209) and OCPs (HCB and pp`-DDE). All of the contaminants analysed are listed in Table 2 and LOD levels in Table 3.

All glassware used in the analysis was cleaned with 1:1 mixture of cyclohexane and acetone prior to use.

Table 2: Overview of persistent organic pollutants (POPs) and the congeners analysed in liver samples from house sparrows from the island Leka, Helgeland Norway.

Polychlorinated biphenyls	**Brominated flame retardants**	**Chlorinated pesticides**
PCB congeners	*BDE[a] congeners and HBCD*	
-28	-47	HCB[c]
-52	-99	pp`-DDE
-101	-100	
-118	-153	
	-154	
-138	-209	
-153	HBCD[b]	
-180		

[a] BDE: brominated diphenyl ethers
[b] Hexabromocyclododecane
[c] Hexachlorobenzene

Table 3: Limit of detection (LOD) values for contaminants analysed at the laboratory of Environmental Toxicology at the School of Veterinary Science, Oslo. Values are given as ng/g ww.

Contaminant	LOD
PCBs	
PCB-28	0.097
PCB-52	0.057
PCB-101	0.052
PCB-118	0.098
PCB-138	0.073
PCB-153	0.099
PBC-180	0.064
OCPs	
HCB	0.075
p,p'-DDE	0.147
PBDEs	
BDE-28	0.125
BDE-47	0.105
BDE-99	0.095
BDE-100	0.075
BDE-153	0.120
BDE-154	0.120
BDE-209[a]	0.965
HBCD	1.130

[a] For BDE-209 the LOD was set to as the mean of blanks (n=9)+2*SD

Quality control:

For each series of 17 samples; 3 blank samples, one blind and two recovery samples were analysed. The blank samples consisted of solvents and a mixture of internal standards (I.S.); PCB I.S. (PCB-29, -112 and -207, Ultra Scientific, RI USA) and BFR I.S. (BDE-77, -119, -181 and -13C12-209, Cambridge isotope laboratories, Andover, MA, USA. The blind and recovery samples consisted of spiked samples of cattle liver. Also, in-house reference of seal blubber (LRS) was used in the quality control.

Homogenization and extraction:

Sparrow livers (0.3-0.6 g) were cut into fine pieces using a scalpel and transferred to centrifuges tubes (80 ml) and weighed. Internal standards (IS) were added to the samples according to which analytes being analysed (PCB I.S [PCB-29, -112 and -207, Ultra Scientific, RI, USA] and BFR I.S. [BDE-77, -119, -181 and -13C12-209], Cambridge isotope laboratories, Andover, MA, USA]). After homogenization and addition of IS, the lipids and

the lipid soluble contaminants were extracted using liquid-liquid extraction. NaCl (6 %, 2 ml), grade 1 water (10 ml), acetone (15 ml) and cyclohexane (20 ml) were added to the centrifuge tubes followed by sonication by ultrasound probe (2 min), (Cole Parmer CPX 750, Vernon Hills IL, USA). After sonication the samples were centrifuged (10 min, 3000 rpm), (Allegra X-12R Beckman Coulter, Fullerton, CA, USA) and the supernatant was transferred to Zymark glass for evaporation by nitrogen gas (TurboVap II, Zymark Corporation, Hopkinton, MA, USA) to approximately 1 ml. The procedure was repeated for the remaining phase in the centrifuge tubes. The concentrated lipid extracts where then quantitatively transferred to volumetric flasks and adjusted to the respected volume (5 ml) with cyclohexane.

Gravimetric lipid determination

Aliquots of the concentrated lipid extract (1 ml) were used for gravimetric lipid determination and added to pre-weighed 8-drams glasses. The glasses were left on sand-bath (40 °C) overnight, weighed and evaporated to dryness on sand-bath with nitrogen gas before the glasses was weighed once more.

The lipid concentration was calculated according to the formula:

$$\frac{\text{(Weight of glass containing lipids - weight of empty glass)} \times \text{flask volume} * 100}{\text{weighted amount} \times \text{excised volume}}$$

Lipid clean-up:

Prior to quantification on GC, it is necessary to remove interfering substances like lipids from the extract. This was achieved by clean-up with sulphuric acid (H_2SO_4) of the remaining lipid extract; the extract was transferred to 10 mL test tubes and H_2SO_4 (96 %, 2 mL), (Fluka analytical, Sigma-Aldrich, St. Louis, USA) was added and stored at darkness for 1h. Finally, the test tubes were centrifuged (3000 rpm, 10 min) and the supernatant was transferred to GC vials for quantification by gas chromatography mass spectrometry (GC-MS) and gas chromatography electron capture detection (GC-ECD).

Quantification:

The details of the GC- quantification are as described in Polder et al. (2008) with some technical modifications of GC conditions. The constant flow of hydrogen carrier gas was increased to 1.2 mL/min and final holding time at 257 °C was increased to 21 min, changing the total run time to 72.6 min. The lowest level of detection (LOD) for individual compounds was set at three times the noise level, except for *p,p'*-DDT, PCB -28, - 52 and -101. Due to co-eluting compounds, the LOD of these compounds was set to ten times the noise level. Compounds not detected in the analysis, thereby being under LOD, were excluded from further statistical evaluations.

2.4 Statistical analysis:

2.4.1 Univariate data analysis

Statistical analysis was conducted using SPSS Statistical Software (Version 21 for Windows, IBM, SPSS Inc., Chicago, IL) and SIMCA P+ (Version 12.0, Umetrics, Umeå, Sweden). POPs and steroid hormones detected in less than 60 % of the individuals were excluded from the statistical analysis. These where: BDE-28, BDE-153, BDE-154, BDE-209, HBCD, ALDO, OH-PRO, αE2. For those variables detected in more than 60 % of the samples, potential values being under LOD were replaced by random numbers between 0 and LOD prior to analysis. These where: TS (LOD: 0.01 ng/ml, [1 male, 3 females]), PRE (LOD: 0.009 ng/ml, [1 male]), DHT (LOD: 0.009 ng/ml, [1 male]), DHEA (LOD: 0.05 ng/ml, [1 male]) βE2 (LOD: 0.05 ng/ml, [1 male]), AN (LOD: 0.008 ng/ml, [1 female]), ALDO (LOD: 0.5 ng/ml, [8 females]), PCB-28 (LOD: 0.097 ng/g ww, [1 female]), PCB-52 (LOD:0.057 ng/g ww, [9 males, 7 females]), PCB-101 (LOD: 0.052 ng/g ww, [5 males, 4 females]), PCB-118 (LOD: 0.098 ng/g ww, [4 males, 8 females]), PCB-153 (LOD: 0.099 ng/g ww, [1 female]), PCB-180 (LOD: 0.064 ng/g ww, [1 female]), BDE-47 (LOD: 0.105 ng/g ww, [6 males, 5 females]), BDE-99 (LOD: 0.095 ng/g ww, [2 males, 2 females]), BDE-100 (LOD: 0.075 ng/g ww, [8 males, 6 females]).

The data was also assessed for normality using Shapiro-Wilk test and homogeneity of variance in SPSS. Variables that were not normally distributed were transformed using natural log (ln) transformation and if normality was not obtained, non-parametric analysis was performed. Independent T-test and Mann-Whitney U test was applied to assess differences between sexes with a significance level $p<0.05$. Bivariate Spearman's rank correlation test was applied to assess possible correlations between steroid hormones, contaminants and biometrical variables with a significance level of $p<0.05$. Negative or positive correlations from the Spearman's rank correlation test were further illustrated by scatter plots.

2.4.2 Principal component analysis (PCA) and orthogonal projections to latent structures (OPLS):

Multivariate data analysis such as principal components analysis (PCA) and orthogonal projections to latent structures (O-PLS) was conducted using SIMA P+. PCA was conducted to explore the relationship between different POPs, steroid hormones and biometrical variables. A PCA uses orthogonal transformations to convert possibly correlated variables into a set of uncorrelated variables called principal components (PC). The PCs explain as much as possible of the variance in the dataset where the first component explains the mean load of the variance (Eriksson et al., 2006). Prior to PCA, all variables included were pre-treated by unit-variance (UV) scaling to obtain equal unit variance among the variables and mean-centering where the mean of variables are subtracted (Eriksson et al., 2006). The analyses were also performed on $\log_{10}$ transformed variables to assess normality. The model

performance was evaluated with respect to the goodness of fit (R^2X) and prediction (Q^2X) where values close to 1 indicates a model with good fit and prediction (Eriksson et al., 2006). In the present study, the PCs explained little of the variance in the data set (PC1: 25 %, PC2: 12 %) but was included to give an indication of relationships that was further evaluated in Spearman's rank correlation test in SPSS.

O-PLS was applied to explore the effects of contaminants and biometry (X-variables) and steroid hormone levels (Y-variable). O-PLS is an extension of partial least square (PLS) regression that separates the systematic variation on X into two parts; one that is correlated (predictive) to Y and one part that is uncorrelated (orthogonal) to Y resulting in a model with improved interpretability (Eriksson et al., 2013). Single Y O-PLS was performed with POPs and biometry as X-variables and one steroid hormone as Y-variable, generating nine models for each sex. To investigate which X-variables being the most important, regression coefficient (CoeffCS) plot and variable important in projection (VIP) plot was examined and the models were optimized by removing variables with VIP values<0.5 as they are considered to be less important to the models. The variables were excluded in a backwards manner, in which variables with low importance were deleted one-by-one until significance was obtained. If significance was not obtained, the model was considered as not significant. The significance test was performed by cross-validated predictive residuals (CV-ANOVA, $p<0.05$), (Eriksson et al., 2008). Subsequently, the performance of the models was evaluated by the goodness of the fit (R^2Y) and prediction (Q^2) in which a good quality model is indicated by R^2 values > 0.7 and Q^2 values > 0.4 (Lundstedt et al., 1998).

3.0 Results

3.1 Biometrical variables

Significant differences between females and males were observed for wing length and body mass (Mann-Whitney U test, $p<0.005$, n = 47) (Appendix E, Table E1) where males had significantly longer wings and higher body mass (Table 5). No other biometrical data were significantly different between the sexes.

Biometrical variables measured in both female and male house sparrows from Leka (n = 47) are listed in Table 4, and as separate in Table 5. Individual biometrical measurements are listed in Appendix B (Table B1 and B2). The average value of both right and left tarsus bone length, as well as average value of left and right wing length was used.

Table 4: Mean ± standard deviation (SD), median and minimum and maximum values of biometrical variables measured in house sparrow (*Passer Domesticus*) (n = 47) collected at Leka, Helgeland Norway 2011. N denotes the number of individuals/observations

Variables	N	Mean ±SD	Median	Min-max
Age (years)	47	1.55±1.21	1	1-7
Body mass (g)*	47	31.68±1.64	31.70	28.70-35.90
Left tarsus bone length	47	19.68±0.67	19.69	17.21-20.81
Right tarsus bone length	47	19.73±0.70	19.80	17.33-21.25
Wing length (left)*	47	79.89±2.02	80.02	75.67-83.27
Wing length (right)*	47	79.73±1.89	79.97	75.92-82.92
Liver mass (g)	47	0.40±0.08	0.39	0.28-0.67
Lipid (% in liver)	47	2.31±1.10	2.31	0.46-5.68
BMR (mlO_2h)[b]	47	87.89±6.75	87.33	76.53-106.20

* Indicates significant differences between sexes ($p<0.05$)
[a] Phenotypic traits existing only for male house sparrows. Badge and beak category are ranged from 1-5 where
[b] Basal metabolic rate (BMR) calculated from the formula:

Table 5: Mean ± standard deviation (SD), median and minimum and maximum values of biometrical variables measured in 22 female and 25 male house sparrows (*Passer Domesticus*) collected at Leka, Helgeland Norway 2011. n denotes the number of individuals/observations

Variables	*Females*				*Males*			
	Mean ±SD	**Median**	**Min-max**	**n**	**Mean ±SD**	**Median**	**Min-max**	**n**
Age (years)	1.50±1.300	1	1.0-7.0	22	1.60±1.155	1	1.0-5.0	25
Body mass (g)*	31.1±1.423	31.2	28.7-34.1	22	32.18±1.680	32.1	29.2-35.9	25
Gonads (g)	0.02±0.017	0.02	0.001-0.090	22	0.09±0.136	0.042	0.005-0.559	
Tarsal bone	19.77±0.613	19.82	18.50-20.78	22	19.66±0.707	19.77	17.27-20.43	25
Wing length*	78.40±1.610	78.11	75.80-82.93	22	81.05±1.251	81.21	70.76-82.71	25
Beak height	7.85-0.225	7.810	7.48-8.31	22	7.84±0.204	7.85	7.45-8.15	25
Beak length	13.27±0.507	13.35	11.79-14.06	22	13.29±0.465	13.23	12.58-14.27	25
Liver mass (g)	0.41±0.087	0.396	0.291-0.672	22	0.38±0.065	0.389	0.278-0.581	25
Lipids (% in liver)	2.0±0.942	2.03	0.46-3.85	22	2.57±1.176	2.4	1.13-5.68	25
BMR (MlO_2h)[a]	86.55±6.954	87.24	76.53-106.2	22	89.07±6.483	87.77	79.15-101.76	25
Eye-mask[b]					14.11±1.145	14.3	11.40-16.70	25
Total badge[b]					19.79±1.351	19.83	16.84-22.83	25
Visible badge[b]					14.36±0.693	14.67	13.09-15.30	25
Badge category[b]					2.60±0.645	3	1.0-4.0	25
Beak category[b]					3.44±1.158	4	1.0-5.0	25

*Indicates significant differences between sexes ($p<0.05$)

[a] Basal metabolic rate (BMR)

[b] Phenotypic traits existing only for male house sparrows. Badge and beak category are ranged from 1-5 (Appendix B, Figure B1).

3.2 Environmental contaminants

The average contaminant groups found to have highest concentrations (ng/g ww) in the liver samples were, in decreasing order; $\sum_7$PCBs > $\sum_2$Pesticides > $\sum_3$PBDEs. The most prevalent congeners for $\sum_7$PCB were PCB-153 > PCB-180 > PCB-138 > PCB 118 > PCB-28 > PCB-101 > PCB-52. As for $\sum_2$Pesticides the most prevalent compound was *p,p'*-DDE > HCB and for $\sum_3$PBDEs; BDE-99 > BDE-47 > BDE-100 (Table 6, 7). The overall concentrations of the environmental contaminants were low. Individual concentrations of contaminants are listed in Appendix D (Table D1, D2, D3 and D4).

Among both females and males statistically significant correlations were observed between all the PCB congeners (Spearman, $p<0.005$), except for PCB-52, that did not correlate with any other PCBs. The PBDE congeners also correlated significantly with each other (Spearman, $p<0.05$), in both sexes. Statistically significant differences between females and males were observed for PCB-28, *p,p'*-DDE and HCB (independent t-test, $p<0.05$, Table 6). Statistical significance tests are listed in Appendix E (Table E4 and E2)

Table 6: Mean, standard deviation (SD), median and minimum and maximum values of concentrations (ng/g ww) of polychlorinated biphenyls (PCBs) and chlorinated pesticides (OCs) found in liver samples from 22 female and 25 male house sparrows collected at Leka, Helgeland Norway 2011. Only contaminants detected in ≥ 60 % of the samples were included and n denotes the number of individuals with detectable concentrations > LOD. All values in the table include random imputed numbers between 0-LOD.

PCBs and OCs	*Females*				*Males*			
	Mean ±SD	**Median**	**Min-max**	**n**	**Mean ±SD**	**Median**	**Min-max**	**n**
PCB-28*[a]	0.142±0.043	0.140	0.044-0.230	21	0.210±0.200	0.161	0.102-1.141	25
PCB-52[a]	0.128±0.081	0.130	0.025-0.246	16	0.166±0.172	0.105	0.032-0.812	16
PCB-101[a]	0.129±0.076	0.119	0.026-0.268	19	0.165±0.109	0.14	0.026-0.488	20
PCB-118[a]	0.295±0.283	0.234	0.029-0.900	15	0.505±0.645	0.34	0.039-2.737	22
PCB-138	0.893±0.726	0.704	0.135-3.211	22	1.796±2.841	1.018	0.165-12.262	25
PCB-153[a]	1.760±1.925	1.217	0.040-9.141	21	3.881±7.230	1.821	0.216-32.259	25
PCB-180[a]	1.007±1.286	0.562	0.028-5.944	21	2.302±5.131	0.773	0.065-19.665	25
HCB*[b]	0.794±0.277	0.682	0.507-1.640	22	1.081±0.517	0.968	0.465-2.924	25
p-p'-DDE*	1.380±0.972	0.988	0.154-3.194	22	2.415±1.450	2.252	0.598-5.738	25
$\sum_7$PCBs[c]	4.354±4.420	3.106	0.327-19.672		9.025±16.328	4.358	0.645-69.364	
$\sum_2$OCPs[d]	2.174±1.249	1.67	0.661-4.834		3.496±1.967	3.22	1.063-8.662	

*Indicates significant differences between sexes ($p<0.05$)

[a] Contaminants containing concentration values under detection limit of the analysis. Missing values were given a random number between 0 and limit of detection (LOD) and included in the statistics

[b] Hexachlorobenzene

[c] $\sum_7$PCBs include the congeners PCB-28, -52, -101, -118, -138, -153 and -180

[d] $\sum_2$OCPs include HCB and *p-p'*-DDE

Table 7: Mean, standard deviation (SD), median and minimum and maximum values for concentrations (ng/g ww) of polybrominated diphenyl ethers (PBDEs) found in liver samples from 22 female and 25 male house sparrows collected at Leka, Helgeland Norway, 2011. Only contaminants detected in ≥ 60 % of the samples are included, n denotes the number of individuals with detectable concentrations > LOD. All values in the table include random imputed numbers between 0-LOD.

PBDEs	*Females*				*Males*			
	Mean ±SD	**Median**	**Min-max**	**n**	**Mean ±SD**	**Median**	**Min-max**	**n**
BDE-47[a]	0.298±0.215	0.253	0.036-0.796	17	0.827±2.051	0.204	0.048-10.333	19
BDE-99[a]	0.661±0.424	0.70	0.035-1.654	20	1.478±2.643	0.518	0.063-12.938	23
BDE-100[a]	0.116±0.068	0.116	0.031-0.299	16	0.179±0.176	0.09	0.035-0.586	17
$\sum_3$PBDEs[b]	1.066±0.707	1.071	0.102-2.749		2.484±4.870	0.812	0.146-23.157	

[a] Contaminants containing concentrations under the detection limit of the analysis. Missing values were given a random number between 0 and limit of detection (LOD) and included in the statistics
[b] $\sum_3$PBDEs include the congeners BDE-47, -99 and -100

3.3 Steroid hormones

In the results from the steroid hormone analysis (Table 8), the sparrows were divided into groups in respect to gender. The highest steroid hormone concentration detected in females was TS (mean 0.930 ng/ml plasma), which also had high variability among individuals (range 0.004-3.446 ng/ml). Among the males, the highest steroid hormone concentrations detected were TS (mean 1.461 ng/ml plasma), and the variation among individuals were very high (range 0.002-7.160). Individual concentrations of steroids are listed in Appendix C (Table C1 and C2).

Statistical significant differences between females and males were only observed for PRO (Mann-Whitney U test, $p<0.033$, Table 8), Appendix E Table E3). Among females, statistically significant correlations between hormone levels were observed for AN and βE2 (Spearman, $r = 0.469$, $p = 0.028$). Among males, significant correlations between AN and E1 (Spearman, $r = 0.485$, $p = 0.014$) and TS and PRE (Spearman, $r = 0.483$, $p = 0.014$) were identified. No other significant correlations between steroid hormones were detected.

Table 8: Mean, standard deviation (SD), median and minimum and maximum values of concentrations (ng/ml plasma) of steroid hormones (progestagens, androgens, estrogens and mineralocorticoid) detected in plasma samples from 22 female and 25 male house sparrows collected at Leka, Helgeland Norway 2011. Only hormones detected in ≥ 60 % of the samples were included and n denotes the number of individuals with detectable concentrations > LOD. All values in the table include random imputed numbers between 0-LOD.

Steroid hormone	*Females*				*Males*			
	Mean ±SD	**Median**	**Min-max**	**n**	**Mean ±SD**	**Median**	**Min-max**	**n**
Progestagens								
PRE	0.220±0.312	0.121	0.015-1.370	22	0.228±0.466	0.097	0.009-2.317	25
PRO*	0.412±0.517	0.279	0.049-2.456	22	0.913±0.863	0.620	0.055-3.325	25
Androgens								
DHEA[a]	0.024±0.033	0.017	0.004-0.157	22	0.030±0.048	0.014	0.002-0.188	24
AN[a]	0.087±0.164	0.040	0.005-0.767	21	0.092±0.165	0.040	0.012-0.797	25
TS[a]	0.930±0.841	0.713	0.004-3.446	19	1.461±1-524	0.881	0.004-7.160	24
DHT[a]	0.161±0.091	0.188	0.028-0.307	22	0.118±0.097	0.065	0.002-0.346	24
Estrogens								
E1	0.525±0.320	0.217	0.093-6.391	22	0.193±0.104	0.155	0.091-0.480	25
βE2[a]	0.252±0.536	0.140	0.111-2.649	22	0.139±0.039	0.141	0.026-0.229	24
Mineralcor.								
ALDO[a]	0.029±0.047	0.004	0.001-0.172					

*Indicates significant differences between sexes ($p<0.05$)

[a] Individuals with steroid hormone concentrations under detection limit of the analysis; values were given a random number between 0 and limit of detection (LOD) and included in the statistics.

3.4 Principal component analysis (PCA)

The PCA analysis ($R^2X = 0.247$, $Q^2 = 0.16$) resulted in two significant principal components but it is noteworthy to point out that they only explained 25.0 % (PC1) and 11.3 % (PC2) of the variation in the data set (Figure 5). However, the score plot did show grouping between females and males, and because the analysis was conducted to see potential effects on contaminants on steroid hormones, further multivariate data analysis were performed separately for the two sexes. One outlier was observed among the males, being outside the Hotelling T2 range. This individual (8N72683) had in general higher levels of contaminants compared to the other individuals, and is thus not considered and outlier and was not removed.

The separate PCA-analysis for males resulted in two significant principal components ($R^2X = 0.253$, $Q^2 = 0.157$), explaining 25.2 % (PC1) and 12.2 % (PC2) of the variation in the data set (Figure 6). The loading plot indicated a clustering of PCB congeners with exceptions of PCB-52 being placed further along PC1. Variables oriented towards the centre of the plot indicated they were less important to the model. The variable eye mask was located opposite of the POPs indicating negative correlations with PCB-52, -138, -153 and -180. The steroid

hormones were not clustered together and were scattered around the loading plot. DHT was the only steroid hormone placed along PC1 indicating a positive correlation with POPs.

For females, the PCA analysis resulted in only one significant component ($R^2X = 0.253$, $Q^2 = 0.145$), and a second component was calculated to generate a plot (Figure 7). This resulted in no significant results from the second principal component (PC2). PC1 explained 25.6 % of the variation in the data set. The loading plots indicated a negative correlation between the steroids DHEA, βE2 and E1 and the PCBs. Variables oriented towards the centre of the plot indicate they are less important to the model.

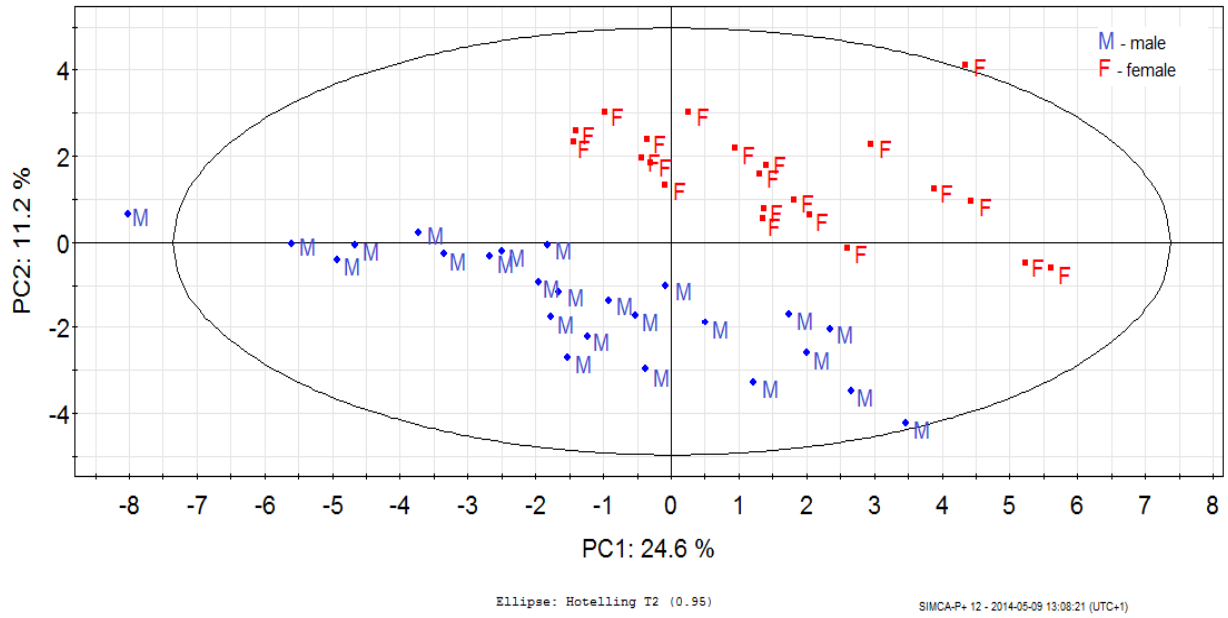

Figure 5: PCA score plot (PCA X&Y, y=gender) based on contaminants, biometry and circulating steroid hormones in male (n = 25) and female (n = 22) house sparrows from Leka, Helgeland Norway 2011.

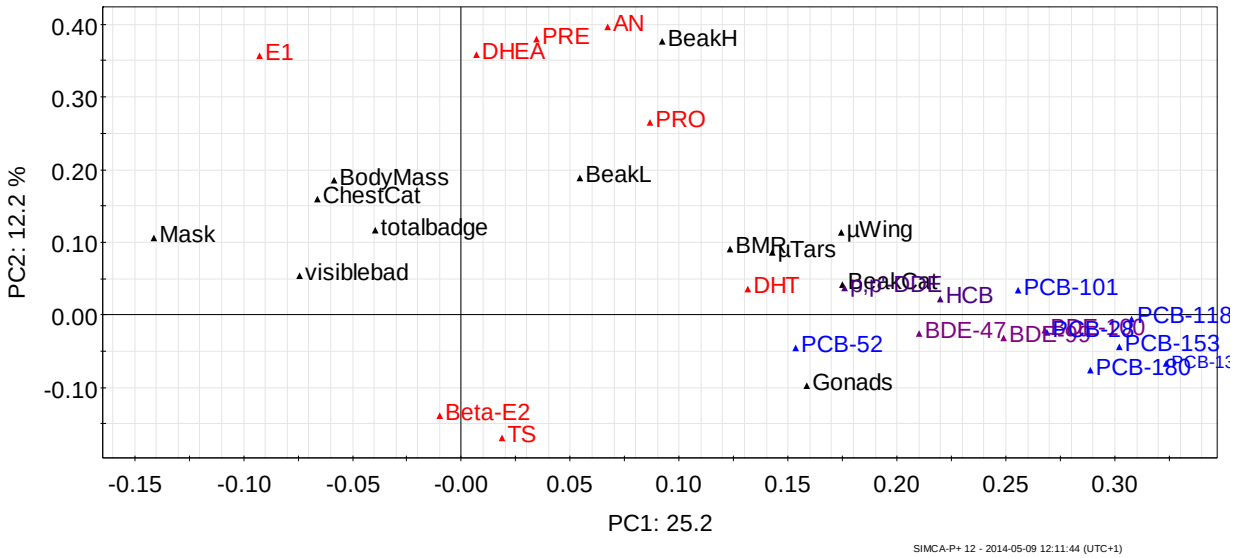

Figure 6: PCA loading plot based on contaminants, biometry and circulating steroid hormones in male (n = 25) house sparrows sampled at Leka, Helgeland Norway 2011

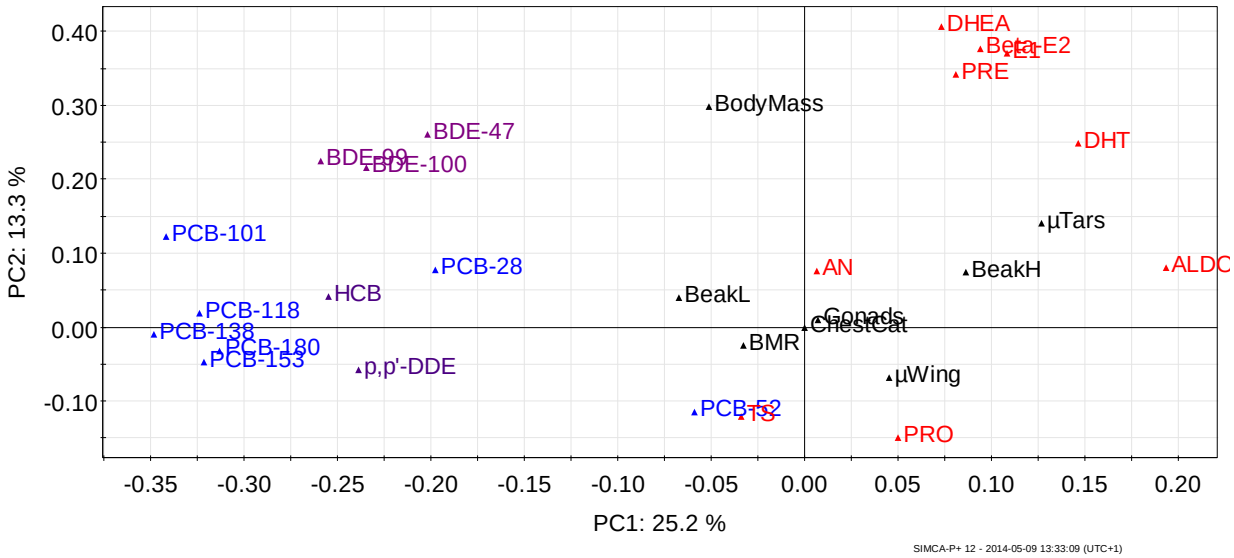

Figure 7: PCA loading plot based on contaminants, biometry and circulating steroid hormones in female (n = 22) house sparrows sampled at Leka, Helgeland Norway 2011

3.5 Orthogonal projections to latent structures (O-PLS)

To further investigate the correlations indicated in the PCA plot, single Y O-PLS was applied separately for males and females, to investigate possible relationships between contaminants and biometrical variables (x-variables) and the steroid hormones (y-variables). The analyses resulted in no significant models (CV-ANOVA, $p>0.005$), even after excluding x-variables considered not important for the model (VIP<0.5) and continuous excluding x-variables resulted in no significant models.

3.6 Spearman's rank correlation coefficient test and Correlation plots

Due to no significant O-PLS models, all observations and variables were investigated with Spearman's rank coefficient test among males and females, and significant correlations ($p<0.005$) are listed in Appendix F (Table F1)

3.6.1 Females:

Significant negative correlations between single contaminants and steroid hormone levels, were observed between PCB-118 and βE2 levels ($r = -0.426$, $p = 0.048$) and *p,p`*-DDE and E1 ($r = -0.607$, $p = 0.003$) (Figure 8). The correlations were maintained even after removal of the potential outliers. Moreover, a negative relationship was also observed between E1 and ∑OCPs ($r = -0.533$ $p = 0.011$) (Table 8) and the negative relationship was maintained even after removing the potential outlier. In addition, some of the biometrical data also correlated significantly with steroid hormone levels; TS was negatively correlated with beak length ($r = -0.600$, $p = 0.003$) and PRE correlated positively with body mass ($r = 0.470$, $p = 0.027$). Significant correlation was almost obtained for DHEA and body mass, thus this association was only considered as a trend ($r = 0.413$, $p = 0.056$). In addition, PCB-52 was the only contaminant which correlated with age ($r = -0.704$, $p = 0.001$), indicating no age-related accumulation of the other contaminants.

3.6.2 Males

Significant positive correlations were observed between PCB-118 and DHT ($r = 0.408$, $p = 0.043$) and BDE-100 and DHT ($r = 0.400$, $p = 0.048$) (Figure 9). Moreover, a positive relationship was also observed between DHT and ∑PCBs ($r = 0.402$, $p = 0.046$) and the positive relationship was maintained even after removal of the potential outlier. In addition, some of the biometrical data also correlated with steroid hormones level: AN was positively correlated with beak length ($r = 0.548$, $p = 0.005$), TS was positively correlated with chest category ($r = 0.402$, $p =0.046$) and total badge size ($r = 0.434$, $p = 0.030$). In addition, PCB-52 was the only contaminant which correlated with age ($r = -0.633$, $p = 0.001$) indicating no age-related accumulation of the other contaminants.

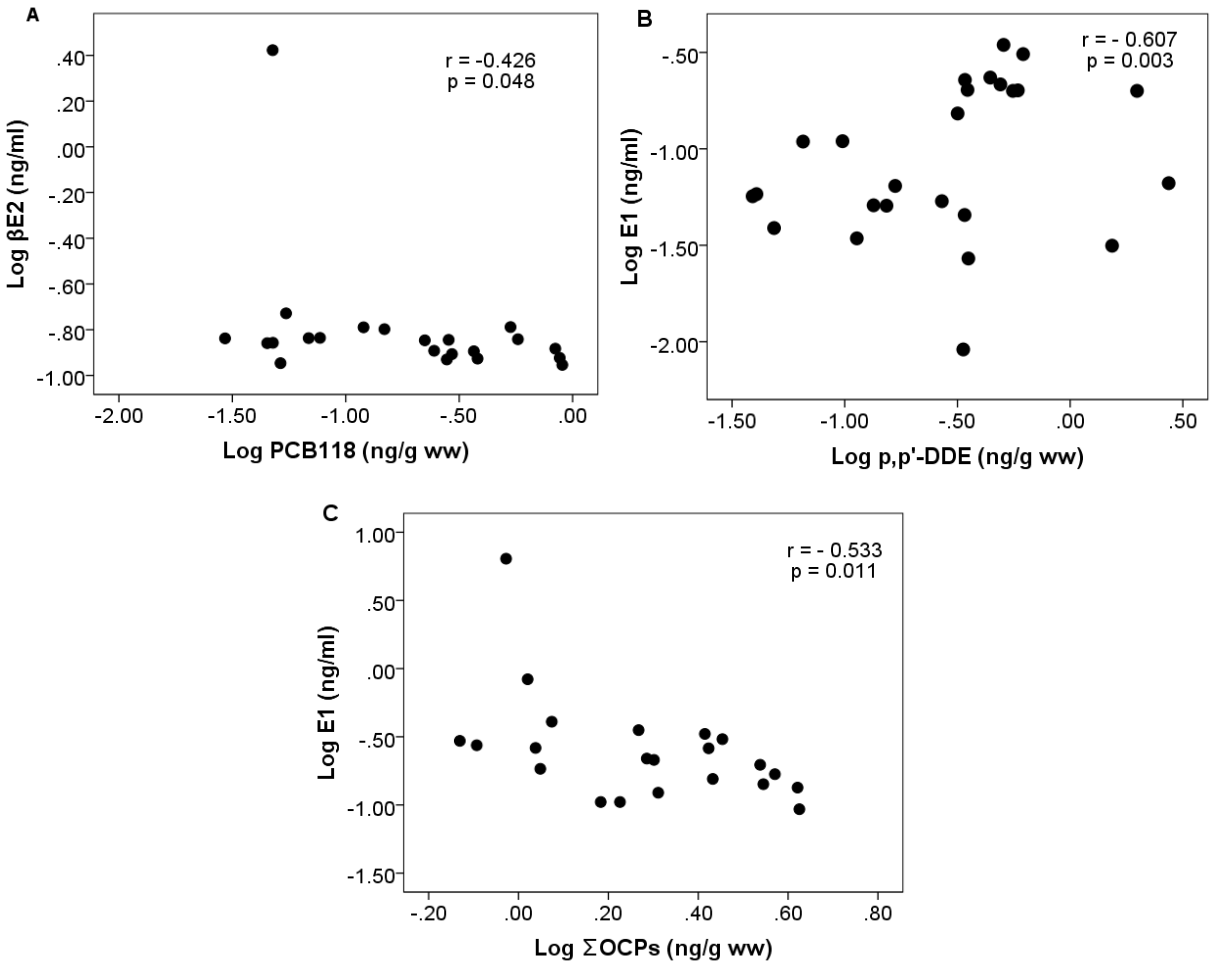

Figure 8: Plasma concentrations (ng/ml plasma) of βE2 vs PCB-118 and E1 vs *p,p'*-DDE and ∑OCPs in adult, female house sparrows from Leka, Helgeland, Norway. The scatter plots illustrates the correlations implied from the Spearman's rank correlation test of steroid hormones and POPs for n = 22 individuals. A) Significant negative correlation was observed for βE2 vs PCB-118 (r = - 0.426, p = 0.048). B) Significant negative correlation was observed for E1 vs *p,p'*-DDE (r = -0.607, p = 0.003). C) Significant negative correlations was observed for E1 vs. ∑OCPs (r = - 0533, p = 0.011)

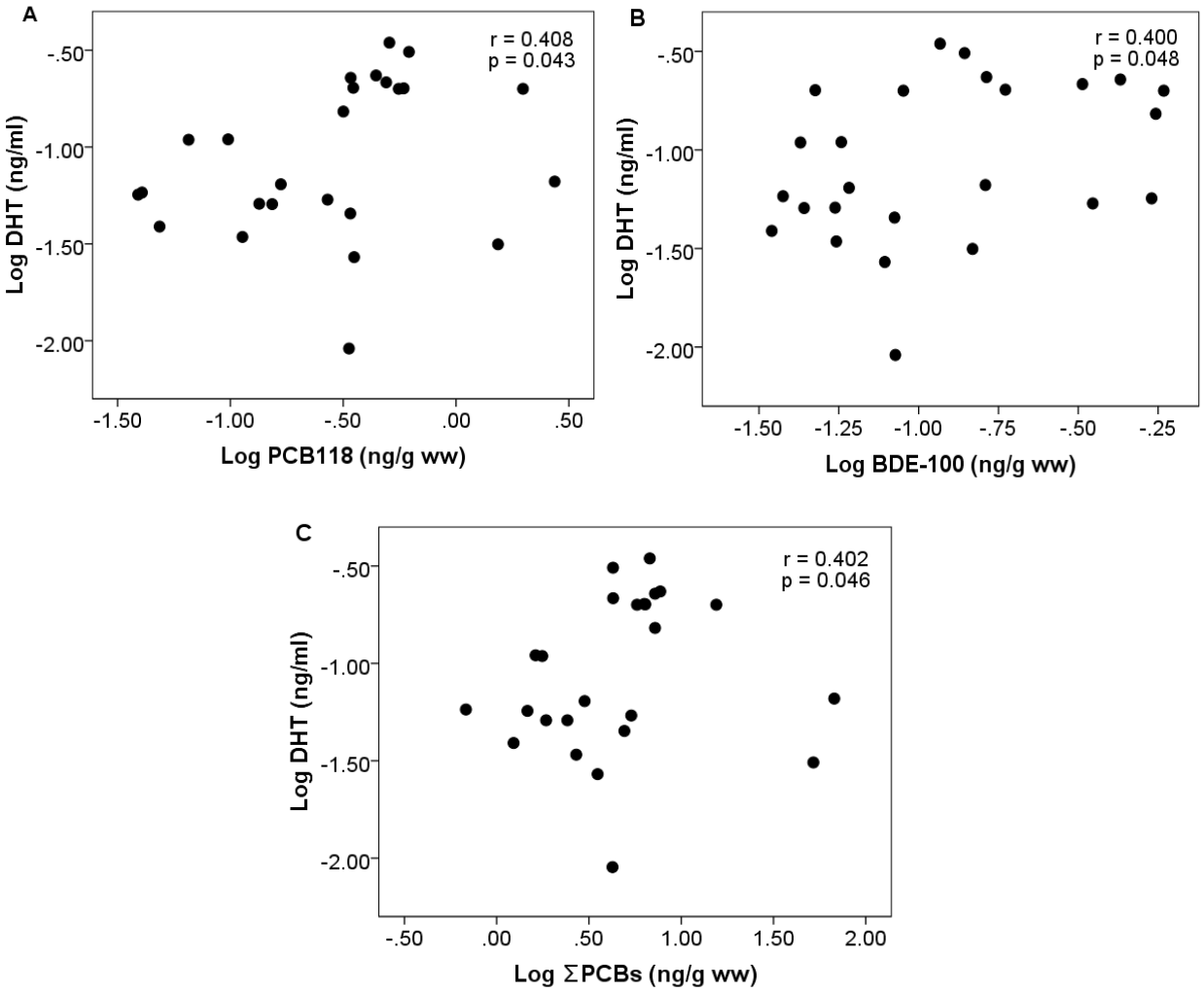

Figure 9: Plasma concentrations (ng/ml plasma) of DHT vs PCB-118, BDE-100 and ∑PCBs in adult, male house sparrows from Leka, Helgeland, Norway. Hormone and POPs levels were log transformed to obtain graphically understandable plots. The scatter plots illustrates the correlations implied from the Spearman's' rank correlation test of steroid hormones and POPs for n = 25 individuals. A) Significant, positive correlation was observed for DHT vs PCB-118 (r = 0.408, p = 0.043). B) Significant positive correlation was observed for DHT vs BDE-100 (r = 0.400, p = 0.048). C) Statistical positive correlations between DHT vs ∑PCBs (r = 0.402, p = 0.046)

4.0 Discussion

4.1 Levels of steroid hormones

Previous studies on steroid hormones in house sparrows have primarily focused on circulating levels of TS, PRO and βE2 and in some few cases DHT (Kempenaers et al., 2008, Partecke and Schwabl, 2008, Laucht et al., 2011). In the present study, levels of a total of nine steroid hormones where examined simultaneously and, is to my knowledge, the first to include wide ranges of steroid hormones and their precursors in house sparrows. Variation in patterns of circulating levels of reproductive hormones among species should be associated with mating system and needs to be considered when comparing steroid hormone levels among different species (Garamszegi et al., 2005). In addition, latitudinal distributions may be a potentially confounding variable that could affect the relationships between steroids and reproductive behaviour. Birds breeding in northern latitudes generally have, for instant, elevated plasma testosterone levels throughout the breeding season with a peak at the onset of breeding. This peak might probably reflect a shorter breeding season in the north and the socially unstable situation which birds have to cope (Moore et al., 2002, Garamszegi et al., 2005). Furthermore, when comparing hormone levels, there might be differences in the methods applied such as sample preparation and final determination techniques that may affect detection limits and possible interferences during analysis. It should also be noted that all house sparrows included in this study where collected during February and early March which is the pre-breeding period of this species in mid-northern Norway. However, if conditions are favourable during mild winters, the birds are known to start nest-building as well as egg-lying in late winter periods (Seel, 1968).

4.1.1 Androgens

In both female and male house sparrows the order of androgens according to concentrations was as following: TS>DHT>AN>DHEA. In the present study, the reported male TS levels (mean = 1.461 ng/ml) did not differ substantially from levels previously reported for house sparrows in New York during the winter period (mean = 0.9 ng/ml[1]) (Hegner and Wingfield, 1986) as well as levels reported in house sparrows from France (mean = 1.02 ng/ml), (Chastel et al., 2003) and great tit (*Parus major*), (mean = 1.5 ng/ml[1]), (Van Duyse et al., 2003). Seasonal differences in the testosterone levels have been reported in several bird species (Moore et al., 2002, Wikelski et al., 2003) and plasma testosterone levels is reported to peak during the onset of the breeding season which may vary among species. Moreover, large individual variations in TS levels as reported in the present study (range = 0.004-7.160 ng/ml) are expected due to normal individual differences. This is also in agreement with studies on individual fluctuations in male birds in general (Kempenaers et al., 2008). In addition to differences in reproductive status, the variation might also reflect differences in dominance status where some individuals may have higher TS levels due to more aggressive and

[1] Mean estimated from graphs in cited article

competitive behaviour than less dominant males (Møller, 1988). Also within-individual fluctuations of TS levels during day and night in male house sparrows (Laucht et al., 2011) and other bird species (Kempenaers et al., 2008) have been shown. This could explain, to some extend the variations in TS levels in the present study due to different sampling time of the sparrows in the field (morning/evening).

In female house sparrows, TS levels (mean = 0.930 ng/ml) were lower than in males, but this was not statistically significant (Mann-Whitney U test, $p>0.05$). The female TS levels in the present study was clearly higher than levels found in female house sparrows sampled during February in New York (mean = 0.300 ng/ml[1]) (Hegner and Wingfield, 1986). However, the annual reproductive cycle reported in that particular study refers strictly to the study population in New York, and may explain why females in the present study have higher TS levels. In addition, when comparing the female TS levels with levels presented by Ketterson et al. (2005) who compared data from the literature on different avian species, the levels reported herein do not differ substantially from the levels reported for pre-laying females[1]. High levels of TS in females have, in some birds species, also been associated with aggressive behaviour in periods of territorial establishment and defence of nest sites (Cristol and Johnsen, 1994, Geslin et al., 2004, Sandell, 2007) and such female aggression might explain the relative high TS levels in the present study. In addition, the fact that all birds were held in a barn for one week could have resulted in some variations of TS levels due to stress (Deviche et al., 2012).

AN was identified in more than 96 % in both males and females and levels in both sexes were low (male mean = 0.092 ng/ml, female mean = 0.088 ng/ml) compared to levels reported in Japanese quail (male mean = 1.9 ng/ml, female mean = 2.4 ng/ml) (Ramenofsky, 1984), but did not differ substantially from AN levels reported for pre-laying female blue-throats (*Luscinia svecica)*, (Pärn et al., 2008). AN is synthesized from two pathways: the Δ-4 pathway where PRO is synthesized to AN and the Δ-5 pathway where PRE is synthesized to DHEA and then AN in the steroidogenesis and the pathway is species-dependent (Conley et al., 2012). The higher levels of Δ-4 PRO than Δ-5 PRE and DEHA in house sparrows from the present study might indicate that AN is primary synthesized trough the Δ-4 pathway rather than the Δ-5 pathway in this species. However, this statement needs to be confirmed by further studies on steroid gene expressions, steroid precursor levels and enzyme affinities.

DHT was identified in 96 % of the males and circulating levels of DHT (mean 0.118 ng/ ml) did not differ substantially from levels reported in male house sparrows from New York during the winter period (mean = 0.2 ng/ml[1]), (Hegner and Wingfield, 1986). The same authors reported lower DHT levels in comparison to TS which is similar to the present study. However, a lower ratio between the two androgens was found in the present study compared to the New York sparrows (Hegner and Wingfield, 1986). Moreover, similar levels of DHT as reported herein were also found for pre-breeding male ringed doves (mean = 129.5 pg/ml), (Feder et al., 1977). In female house sparrows, DHT levels were identified in 100 % of the individuals and levels (mean = 0.161 ng/ml) did not differ substantially from levels reported

[1] Mean estimated from graph in cited article

for female house sparrows in New York (mean = 0.3 ng/ml[1]), (Hegner and Wingfield, 1986) or in pre-breeding female ring doves (mean =100 pg/ml[1]), (Feder et al., 1977). Both these studies reported DHT levels to be higher than TS levels, which is not in agreement with the present study. On the other hand, lower DHT (mean = 120 pg/ml) levels compared to levels of TS (mean = 318 pg/ml) were reported in western gulls (*Larus occidentalis*) from California (Wingfield et al., 1982). The differences in DHT and TS ratios might be explained by species differences in hormone levels throughout breeding and non-breeding periods as well as different periods of sampling.

DHEA was the steroid with the lowest concentrations in the male sparrows (mean = 0.030 ng/ml) and was lower than that reported in white-throated sparrows (*Zonotrichia albicollis*), (mean = 1.19 and 2.51 ng/ml), (Spinney et al., 2006). This may indicate that this particular androgen does not elicit its primary task during the present period of sampling. Female DHEA levels were also the lowest among androgens (mean = 0.024 ng/ml). These concentrations were also lower in comparison to levels found in white-throated sparrows (mean = 1.13 and 2.87 ng/ml) (Spinney et al., 2006) and zebra finches (*Taeniopygia guttata*), (mean = 1.2 ng/ml), (Soma et al., 2004). It is hypothesized that during the non-breeding seasons, circulating levels of DHEA might maintain winter aggression in all-round aggressive birds, when TS levels are low (Soma and Wingfield, 2001). This was illustrated in all-round aggressive song sparrows (*Melospiza melodia*), (Soma and Wingfield, 2001) where circulating levels of DHEA were higher compared to TS. However, since the levels of DHEA were low compared to TS in the present study, this may not be the case for house sparrows. In addition, low DHEA levels may also indicate that DHEA is not the primary pathway of conversions to more potent androgens or estrogens in the sparrows herein.

4.1.2 Estrogens

Not many publications are available on estrogen levels in male house sparrows so comparisons within the species are difficult. However, estradiol is being produced in male animals and humans as an active metabolic product of TS, converted by aromatase and is important in male reproduction (Hess, 2003). The role of estradiol in male reproduction includes regulation of spermatogenesis (Carreau et al., 2003). Concentrations in plasma of males are typically low, ranging 0.002-0.180 ng/ml depending on the species (Kelch et al., 1972, Hess, 2003). In the present study, βE2 was identified in 96 % of the males and the levels (mean = 0.139 ng/ml) were higher than reported for pre-breeding house sparrows from France (Chastel et al., 2003), but did not differ substantially from levels reported in male white-crowned sparrows (*Zonotrichia leucophrys*) from Canada, late in the breeding season (Charlier et al., 2011). The E1 levels in male house sparrows were detected in 100 % of the individuals and levels (mean = 0.193 ng/ml) did not differ substantially from βE2 detected in the same individuals from the present study (Table 8).

Female levels of βE2 (mean = 0.252 ng/ml) corresponded with levels found in female house sparrows from New York (0.3 ng/ml[1]), (Hegner and Wingfield, 1986) and pre-breeding house

[1] Mean estimated from graph in cited article

sparrows from France (mean = 0.41 ng/ml), (Chastel et al., 2003) as well as pre-laying European Starlings (*Sturnus vulgaris*), (mean = 201 pg/ml), (Williams et al., 2004). There was no large individual variation in βE2 levels, except for one female having very high βE2 concentrations compared to the others (2.649 ng/ml Appendix C, Table C1). Since βE2 secretion is dependent on reproductive state, all females in the present study seemed to be in the same stage of their reproductive cycle (except for one), which is expected since breeding usually is synchronized in birds (Emlen and Demong, 1975, Reed et al., 2006, Verhulst and Nilsson, 2008). To my knowledge, no studies have investigated the level of E1 in female house sparrows. In the present study E1 levels (mean = 0.525 ng/ml) did not differ substantially from rats (mean = 416 pg/ml) during the proestrus stage in ovulation (Shaikh, 1971). In addition, is has been reported that estrone sulphate is the main serum metabolite in rats (Cabot et al., 2007). However, since estrone-sulphate was not measured in the present study, it is not known if that is the case for house sparrows.

4.1.3 Progestagens

In the present study, PRO was the only steroid hormone which differed significantly between the sexes (Mann-Whitney U test, $p = 0.033$) showing that males had higher PRO levels than females. Little information is available on the levels of progestagens in passerines and only few comparisons are available. In comparison to other species, PRO levels in male sparrows (mean = 0.913 ng/ml) were lower than those reported in breeding male black kites (*Milvus migrans*), (mean pre-laying = 2.0 ng/ml, mean peak = 7.73 ng/ml) (Blas et al., 2010). The lower PRO levels reported herein can be explained by the fact that Blas et al. (2010) reported PRO levels in breeding individuals while PRO levels in the present study were from pre-breeding males. Furthermore, PRO levels did not differ substantially from PRO levels reported for male white crowned sparrows (mean = 0.8 ng/ml[1]), (Charlier et al., 2009). In female sparrows, PRO levels (mean = 0.412 ng/ml) were lower than reported in the control plasma of in female song sparrows used in Elekonich and Wingfield (2000) as well as for breeding female black kites (mean pre-laying = 4.0 ng/ml, mean peak = 7.52 ng/ml), (Blas et al., 2010), though differences in breeding-stages may again explain lower PRO levels reported herein. Some studies have reported no significant differences between breeding and non-breeding seasons (Heath et al., 2003) while others have found PRO to correlate with typical breeding behaviours such as parental care, pair-bonding (Blas et al., 2010, Smiley et al., 2012) and incubation (Stern and Lehrman, 1969, Davis et al., 1995). This illustrates the need for extended research to understand the role of PRO in avian species.

PRE levels in male (mean = 0.228 ng/ml) and female (mean = 0.220 ng/ml) sparrows in the present study were lower than that reported for Japanese quail (mean = 2.2 ng/ml[1]) (Tsutsui and Yamazaki, 1995). Even though time of sampling or breeding stage was not reported in that particular study, this could be a possible explanation for lower PRE levels reported herein. In addition, little information is available on plasma PRE levels in birds and comparisons are therefore scarce. However, more information is available on its neurosteroid activity and multiple reports have illustrated the presence of PRE in the avian brain (Tsutsui et al., 1999, Tsutsui, 2010, Schlinger and Remage-Healey, 2012).

4.1.4 Mineralocorticoid

ALDO was identified in 63 % of female house sparrows and levels (mean = 0.029 ng/ml) did not differ substantially from levels reported (10-20 pg/ml) in red wattlebirds (*Anthochaera carunculata*), (Goldstein and Bradshaw, 1998). In male house sparrows, ALDO was detected in less than 60 % of the individuals and was thus excluded. ALDO is known to regulate salt and water balance in terrestrial vertebrates as illustrated in McCormick and Bradshaw (2006).

4.2 Levels of contaminants

There are only a few publications devoted to the presence of contaminants in house sparrows, almost all of them concentrates on the levels in eggs (Bouwman et al., 2013). Even though collection of eggs is a less invasive approach to map the contamination load of the species, prevalence of POPs in eggs reflects only the contamination of the females (Gao et al., 2009). To my knowledge, this is the second study to investigate contamination levels of POPs in liver tissue of the house sparrow. In general, the contamination burden in both male and female house sparrows in the present study were low compared to studies of birds of prey (Echols et al., 2004, Arenal et al., 2004). However, the study site at which the present house sparrows were collected is considered to be low contaminated due to the fact that the island is located far from large industrial areas. It is thus more likely that pollutants mainly originate from long range transport rather than local sources. In addition, as the house sparrow belongs to a low/mid trophic level, concentrations are expected to be lower than for birds at higher trophic levels (Sagerup, 2009, Naso et al., 2003) due to different diets and accumulation rates. It is also important to emphasize that direct comparisons of concentrations from different tissue or different species can bring with some difficulties (Prestt et al., 1970). Seabirds and terrestrial passerine birds have different diet and feeding habitats that may affect the accumulation of contaminants. Migration could also contribute to a contaminant load that is not representative for non-migrate species. Moreover, physiological factors such as age, gender and condition as well as time and season of sampling, trophic level and metabolic capacity are factors that have shown to influence the contaminant concentration. Nevertheless, a comparison to previous studies may be informative in the present study due to little available literature on the contamination load in the house sparrow.

4.2.1 PCBs

In male and female house sparrows, the prevalence of detected contaminant groups were, in decreasing order; Σ_7PCBs>$\sum$2OCPs>Σ_3PBDEs. The distribution of contaminant groups to total contaminant burden differed slightly between females and males. The contribution of PCBs constituted in approximately 60 % of the total contaminant burden while OCPs and PBDEs constituted of 23 % and17 % respectively in males (Table 6 and 7). Among females the PCBs constituted nearly 60 % while OCP and PBDE constituted in 29 % and 14 % respectively (Table 6). In both sexes, the most abundant PCB congeners were PCB-153,

followed by PCB-180, -138, -118, -28, -51, -101. This order was similar to congener distribution found in great tit eggs and nestlings (Winter and Streit, 1992, Dauwe et al., 2003) as well as Caspian terns (*Hydroprogne caspia*) and great blue herons (*Ardea herodias*), (Mora, 1996). Furthermore, the predominance of higher chlorinated congeners found in the present study is also typical for species high in the food chain like polar bears and glaucous gulls (Gabrielsen et al., 1995, Kannan et al., 2005). This predominance is most likely due to the lipophilic and persistent nature of hepta-, hexa- and penta PCBs to facilitate absorption and accumulation. In addition, it is well known that higher chlorinated PCBs exhibit higher bioaccumulation in avian tissue than lower chlorinated congeners (Borgå et al., 2001, Naso et al., 2003) due to their molecular structure and chemical properties.

4.2.2 OCPs

Only two OCPs were analysed in the present study where *p,p'*-DDE was the most abundant which is consistent with a previous study of OCPs in great tit eggs (Van den Steen et al., 2009b) and a study of grassland passerines (Bartuszevige et al., 2002). DDE is the major breakdown product of DDT (Helander et al., 2002), but since levels of *p,p'*-DDT where not analysed in the present study, it is difficult to say whether the levels of *p,p'*-DDE indicate an old source of DDT. Males had also statistically significant higher levels of OCPs than females (independent t-test, $p<0.05$). This difference could be explained by males having slightly higher body mass compared to females (Mann-Whitney U test, $p = 0.032$). Bigger males usually have a higher food intake than smaller individuals and might increase body burdens of some contaminants. In addition, females have the ability to transfer some of their contamination load on to their eggs during breeding (Robinson et al., 2012) and might contribute to explain why males had higher body burdens of OCPs than females.

Levels of HCB in the present study (males: range = 0.465-2.924 ng/g ww, females: range = 0.507-1.640 ng/g ww) were similar to levels reported in the lower range in birds from different trophic levels in Italy (Naso et al., 2003). The fact that HCB is still detected in animal tissue despite EUs ban of HCB use can be explained by its persistence in the environment, as a by-product from production of various chlorine-containing chemicals and as an impurity in several pesticides (Bailey, 2001, Kim et al., 2009). The pesticide use at Leka is considered to be minor, but farmers do use herbicides during the spring turnover of the soil. Most of the farmers used herbicides with metsulfuron-methyl as the active substance which is a selective and systemic herbicide. Metsulfuron-methyl is considered neither persistent nor bioaccumulative and to pose little risk to wildlife (Tatum, 2004). In addition, OCP levels in the present study were well below levels associated with toxic effects on reproduction in the most sensitive avian species (Custer et al., 2003) and is expected due to little application of pesticides at Leka.

4.2.3 PBDEs

Few data exist on PBDE levels in passerine birds and in the present study, PBDEs were detected in low concentrations compared to other contaminant groups. PBDE levels have been

reported to be higher in urban than rural areas (Hale et al., 2006). Leka is located far from industrial sites and PBDE levels would thus be expected to be lower than other contaminant groups that are more prevalent in rural areas, such as OCPs. Highly brominated BDEs were not detected in the present study (BDE-209, -154, -153) while lower brominated congeners were more prevalent (BDE-47, -99, -100) in the sparrows and comparable with other studies on eggs of low trophic birds (Dauwe et al., 2009). However, this is in contrast to what was found in eggs of peregrine falcons (*Falco peregrinus*), in Sweden (Lindberg et al., 2004) where higher brominated BDEs were detected. Thus, the detection of the lower brominated BDEs (penta-BDE) in the present study might reflect the fact that the house sparrow is a bird low on the food chain compared to the peregrine falcon and differences in congener profiles could be expected. In both males and females, the most prevalent congeners were BDE -99, -47 and BDE-100 and similar with congener profiles found in great tit nestlings and eggs (Dauwe et al., 2006, Van den Steen et al., 2006, Van den Steen et al., 2009b). However, whether BDE-47 or BDE-99 was to be the most prevalent congener varied, which may be due to do feeding differences among the birds as well as slightly different profiles of egg compared to nestlings.

4.3 Effects of contaminants on steroid hormone levels

This is, to my knowledge the first study to examine the possible endocrine disruptive aspects of contaminants on circulating steroid hormone levels in house sparrows and only a few studies have investigated the toxicological relevance of POPs in passerines (Van den Steen et al., 2009a, Eng et al., 2014). Predatory bird species at the top of the food chain, such as glaucous gulls and peregrine falcons, are susceptible to bioaccumulation and have been the major study species when investigating the endocrine disrupting nature of POPs (Bosveld and van den Berg, 2002).

The reliability of multivariate O-PLS models were assessed by CV-ANOVA in the present study, however no significant models were obtained. Indications from the PCA plots may therefore be of less relevance and thus reflect the low explanation of the variation in the data set (PC1: 25 %, PC2: 12 %). Since no significant models were obtained from O-PLS, all variables were included in bivariate correlation test (Spearman's rank correlation test). Only statistically significant correlations included in the results are discussed. In addition, is important to emphasize that correlations represent only statistical associations and does not necessarily reflect biological cause-effects relationships.

4.3.1 Effects on estrogens

Negative correlations between contaminants (PCB-118, *p,p'*-DDE and ∑OCPs) and estrogens (βE2, E1) were observed among females. The negative relationship between βE2 levels and PCB-118 reported in the present study have also been reported in female polar bears from Svalbard (Haave et al., 2003), though only as a borderline significance. However, polar bears

are known to accumulate much higher levels of contaminants. In addition, polar bears are able to metabolize PCBs to OH-PCBs and might therefore be more susceptible to endocrine disruption (Bytingsvik et al., 2012). Negative associations between PCBs and estrogens levels were also found in PCB exposed rainbow trout (*Oncorhynchus mykiss*), (Sivarajah et al., 1978), where PCBs (Aroclor 1254) significantly decreased levels of estrogens. Furthermore, other authors (Bonefeld-Jørgensen et al., 2001) found that di-ortho and multiple chlorine substituted PCBs (hexa and penta PCBs) possessed antagonistic activities on ligand mediated ER functions through competitive binding. This indicates that higher chlorinated PCBs may lower estrogenic responses in exposed organisms (Bonefeld-Jørgensen et al., 2001). It has also been reported that di-ortho co-planar PCB congers can contribute to increased hormone catabolism through binding to AhR, inducing expression of enzymes, such as CYP1A1, that are involved in degradation of βE2 (Kharat and Saatcioglu, 1996) and thus decrease estradiol levels. A negative correlation between E1 and *p,p'*-DDE was also observed in the present study. DDE is a known estrogenic disruptor (Li et al., 2008) and is able to lower levels of estrogens in fish (Garcia-Reyero et al., 2006) where *p,p'*-DDE decreased the expression of aromatase (CYP19) which is responsible for the conversion from androgens to estrogens and (Garcia-Reyero et al., 2006). This is in agreement with several other studies that have reported PCB congeners and *p,p'*-DDE to inhibit aromatase activity *in vitro* (Raun Andersen et al., 2002, Woodhouse and Cooke, 2004, Benachour et al., 2007). Altered expression of genes involved in both hormone synthesis and metabolism of endogenous hormones might indicate that *p,p'*-DDE have multiple modes of actions (Garcia-Reyero et al., 2006). However, it is difficult to say whether this is the case in the present study, but illustrates possible mechanisms in which OCPs might elicit their anti-estrogenic properties.

Negative correlation was also observed for ∑OCPs and E1. OCPs were the contaminant group with the most significant correlations to estrogens in the present study which indicates that OCPs might asses a higher potential to disrupt estrogen levels in exposed organisms compared to the other contaminant groups. The mechanisms involved in anti-estrogenic actions of ∑OCPs are somewhat similar to compound specific mechanisms. However, effects of mixtures are more complex due to possible synergistic, additive or agonistic effects between the entities in the mixture (Koppe et al., 2006). It may also be difficult to determine whether the negative correlations observed in the present study is responsible for decreasing levels of circulating estrogens in the female sparrows. Especially when estrogen levels in the present study did not differ substantially from levels reported in other studies. Although there are only a limited number of studies on PCB toxicity in passerines, it has been reported that passerines are less sensitive to PCB exposure compared to other species, at least when considering hatching success (Ormerod et al., 2000, Custer et al., 2003, Van den Steen et al., 2009b). This may therefore, in combination with low levels of contaminants, indicate that house sparrows are less affected by endocrine disruption. Nevertheless, the potential of PCBs to disrupt estrogenic pathways should not be neglected.

4.3.2 *Effects on androgens*

Positive correlations between androgens (DHT) and contaminants (PCB-118, BDE-100 and ∑PCBs) were observed among males. While estrogenic agonists have been identified and

shown to interfere with endocrine function, few androgenic contaminants have been investigated (Sohoni and Sumpter, 1998). The positive relationship between DHT and BDE-100, PCB-118 and ∑PCBs found in the present study might indicate agonistic effects, however this needs to be verified by further investigations of enzyme and steroid gene expressions as well as studies on binding affinities to steroid receptors and or AhR. One possible androgenic action includes induction of 5α-reductase activity, responsible for converting TS to DHT (Ross et al., 1992). However, the higher levels of TS compared to DHT levels reported herein does not support this statement and one might assume that this was not the reason for the positive relationship observed. Anti-androgenic actions have also been reported to disrupt enzymes responsible for conversion to androgens. Finasteride has been found to reduce DHT levels in men with beginning prostatic hyperplasia by inhibiting 5α-reductase (McConnell et al., 1992). In contrast, other studies have reported negative associations of BDE congeners and DHT levels in male rats (Stoker et al., 2005), where specific BDE congeners were found to inhibit the binding of androgens to AR, contributing to the hypothesis that certain BDE-congeners are anti-androgenic rather than androgenic. The disadvantage of such *in vitro* experimental studies however, is that it might be challenging to extrapolate results to a whole body system. Nevertheless, the mechanisms behind anti-androgenic and possible androgenic capacities of certain contaminants are well known (Sonnenschein and Soto, 1998, Sanderson and van den Berg, 2003).

Weak positive correlations were observed between ∑PCBs and DHT. PCBs were the contamination group detected in the highest concentrations in the present study (Table 6) which might indicate that PCBs have higher potential to disturb androgen levels than the other contaminant groups. The body burden of contaminants among the sparrows herein are still low compared to other studies on birds at higher trophic levels, which might illustrate that the potential of endocrine disruption could be considered as minor in this case. The mechanisms of ∑PCBs involved in androgenic actions are somewhat similar to that of specific PCB congeners. However, effects resulting from exposure to mixtures are more complex due to possible synergistic, additive or agonistic effects between the entities in the mixture (Koppe et al., 2006). Furthermore, growing numbers of studies concerning endocrine disruption of PCBs are focusing on alteration of thyroid hormone functions (Tabuchi et al., 2006, Jugan et al., 2010). PCBs have been shown to structurally resemble thyroid hormones and to competitive bind to thyroid hormone transporter protein (TTR) and thus alter serum levels of thyroxine (T_4) (Ucán-Marín et al., 2009). The alteration in one endocrine system could most likely lead to alterations in another endocrine system (Gordon and Southren, 1977, Davis et al., 2011) and thus steroid hormones. However, it is difficult to say whether the positive correlations indicated between PCB-118, BDE-100 and ∑PCBs and DHT are increasing DHT levels in male house sparrows. The overall DHT levels did not differ substantially from levels reported for other house sparrows or other bird species which may indicate normal ranging levels of DHT. In addition, when comparing all steroid hormone levels (Table 8), DHT was detected amongst the lowest concentrations indicating low potential to alter DHT levels in the present study.

4.4 Correlations between steroid hormones, biometrical variables and POPs

The biometrical variables did not seem to be the most important in explaining the variation of the steroid hormones in bivariate correlations. However, a few interesting aspects were identified and will only be discussed briefly as this is considered to be outside the scope of this thesis.

Age did not correlate with contamination load (except for PCB -52) indicating lack of age-related accumulation in the present study. This could be explained by minor differences in the diet among age groups (Dauwe et al., 2003) where juveniles and adults primarily feed on same food sources. This has also been found in female great tit (Dauwe et al., 2003) where the diet differed only slightly among the age groups and thus no age-related differences in body burden was expected. In addition, many house sparrows in the present study were one year old individuals and only few were in the older age group (4-7 years) which might contribute to the lack of age related accumulation of any of the contaminants (except for PCB-52).

No relationship was found between gonad size and plasma TS levels in male house sparrows. The lack of a relationship may be explained by the fact that only a small portion of the testis is responsible for TS production (Leydig cells) (Wikelski et al., 2003). The largest tissue fraction of the gonads is seminiferous tubules which are responsible for spermatogenesis and thus may be responsible for gonad weight (Wikelski et al., 2003).

Positive correlation between TS and total badge size was observed among males and are in agreement with previous studies on house sparrows (Evans et al., 2000, Gonzalez et al., 2001). Such a positive correlation is expected as androgens are known to play a part in development of sexual secondary characteristics in birds (McGraw et al., 2006). Furthermore, it is also known that more dominant male house sparrows, having higher levels of TS, often molt into larger badges and thus be more successful in attracting female sparrows (Hamilton and Zuk, 1982, Møller, 1990). Female preference for elaborated traits illustrates the importance of maintaining a large badge. However, sexual signals are known to bear with some coast to be reliable for individual quality (Gonzalez et al., 2001). This illustrates that sparrows are not able to display high levels of TS without experiencing negative costs. High TS levels have been linked with immunosuppressive aspects and males with big badges illustrate their individual male quality by coping with the internal stress of maintaining a big badge (Buchanan et al., 2003). Females might thus, gather direct and indirect benefits by mating with such males. Males with higher TS and thus probably a bigger badge may be more successful in reproduction than less ornamented males. However, since the present study did not measure reproductive success it is difficult to determine whether big badged males are more successful than smaller badged males.

4.5 Future perspectives

Due to the limited knowledge on endocrine disruption on steroid hormone homeostasis in house sparrows, future studies should be conducted on different house sparrow populations from various locations to assess whether the results reported herein refers only to the

particular population on Leka. Both wildlife and experimental studies may also help to better understanding of the endocrinology of house sparrows. Furthermore, studies could also include investigations on urban populations versus rural populations to consider whether there are differences in contamination and possible endocrine disruption from a more industrialized area compared to rural area. Since birds are seasonal breeders further studies could also include investigations on whether the house sparrow is more susceptible for endocrine disruption during the breeding season compared to the non-breeding season. In order to increase the knowledge of endocrine disruption in house sparrows, a complete understanding of the steroidogenesis is preferable. This includes information on all aspects of steroid hormone biosynthesis, secretion, metabolism, transportation, enzyme activity and regulation. In addition, it would be interesting to investigate effects of POPs on gonadal, adrenal and brain steroid hormone levels. Since brain steroid hormones are mainly though to regulate behaviour, possible disruption may change important nesting and breeding behaviours.

5.0 Conclusion

The present study has provided information about steroid hormone levels and organic contamination load in house sparrows from Helgeland and the first study to investigate the possible effects of POPs on steroid hormone levels in this species. Although strong evidence of endocrine disruption were not observed in the current study, the identification of several potent EDCs in a bird species from a low trophic level might be of concern. Multivariate data analysis did not document any relationship between OHCs and steroid hormones. However some correlations between OHCs and steroid hormones were identified using bivariate correlation analysis. Spearman's rank correlation test indicated negative relationship between estrogens and PCB-118/*p,p'*-DDE and positive relationship between TS and ∑PBDEs among females and positive relationships between DHT and PCB-118s/BDE-100 and between AN and ∑PBDEs among males. These correlations may indicate possible endocrine disrupting effect of specific PCB and BDE congers and *p,p'*-DDE. However, it should be emphasized that some of these correlations were just below the significance level ($p<0.05$) and in combinations with the lack of significant multivariate models, the correlations presented herein should be interpreted with caution. Moreover, the correlations found represent only statistical associations, and may not necessarily reflect biological cause-effects relationships. Further studies are needed to verify the results presented herein. Due to low levels of contaminants detected and few significant correlations, the endocrine disrupting potential of POPs on circulating steroid hormones in the local sparrow population may be considered as minor, at least during the pre-breeding period. Since birds are seasonal breeders, future perspectives could maybe include assessment on seasonal changes of steroid hormone levels and contaminant load and thus investigate whether there are any differences between breeding and non-breeding periods. Due to levels of different contaminant groups and the fact that the house sparrow is a non-migratory, sedentary species living in close contact with human settlements, it might be useful as a biomonitoring species for local contamination.

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APPENDIX

A. Analysed contaminants

BDE-47	2,2’4,4’-tetrabromodiphenyl ether
BDE-99	2,2’4,4’,5-pentabromodiphenyl ether
BDE-100	2,2’4,4’,6-pentabromodiphenyl ether
BDE-153	2,2’4,4’,5,5-hexabromodiphenyl ether
BDE-154	2,2’,4,4’,5,6-hexabromodiphenyl ether
BDE-209	Decabromodiphenyl ether
HCB	Hexachlorocyclehexane
HBCD	Hexabromocyclododecane
PCB-28	2,4,4’-trichlorobiphenyl
PCB-52	2,2’,5,5’-tetrachlorobiphenyl
PCB-101	2,2’,4,4’,5-pentachlorobiphenyl
PCB-118	2,3’,4,4’,5-pentachlorobiphenyl
PCB-138	2,2’,3,4,4’,5’-hexachlorobiphenyl
PCB-153	2,2’,4,4’,5,5’-hexachlorobiphenyl
PCB-180	2,2’,3,4,4’,5,5’-heptachlorobiphenyl
p,p’-DDE	p,p’-dichlorodiphenyldichloroethylene

B. Biometrical variables

Abbreviations – biometrical measurements

ID	Number of individual metal ring
Location	Name of farm the bird was caught
Colour (L)	Colour combination, left tharsal bone
Colour (H)	Colour combination, right tharal bone
BeakCat	Beak category (blackness of beak) – males only
ChestCat	Chest category (blackness of chest badge) – males only
Tars (L)	Length of left tharsal bone
Tars (R)	Length of right tharsal bone
Wing (R)	Length of right wing
Wing (L)	Length of left wing
Mask	Length of the black eye-mask – males only
Total badge	Size of the potential chest badge, with light feather tips
Visual badge	Size of the visual chest badge, without light feather tips

Chest category determination

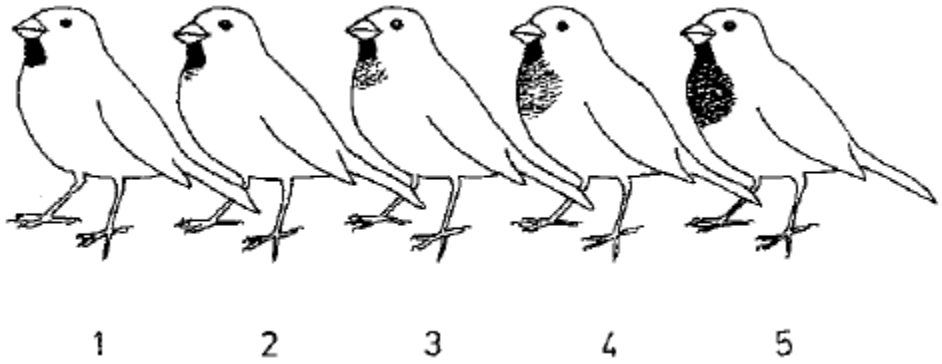

Figure B1: category determination of the chest badge in male house sparrows (Møller, 1987).

Table B1: Individual measures of biometrical traits among female house sparrows (n=22) samples at Leka, Helgeland Norway 2011

ID	Capture (date)	Location	Colour (L)	Colour (R)	Age (years)	ChestCat	Tars (R)	Tars (L)	Beak hight	Beak length	Wing (R)	Wing (L)	Body mass (g)	BMR (date)	BMR
8L48780	20 130 212	C	ME/RS	R/DB	7.00	7	19.84	19.84	8.31	13.84	78.20	78.16	33.10	20 130 213	78.56
8N05859	20 130 210	J	ME/BLY	RS/DB	2.00	7	20.44	20.35	8.03	13.42	78.01	77.79	32.10	20 130 211	76.95
8N06843	20 130 207	K	ME/BLY	Y/DG	2.00	7	20.36	20.29	7.69	12.39	78.55	78.44	31.45	20 130 207	
8N06862	20 130 208	E	ME/BLY	LG/W	2.00	7	19.71	19.74	7.73	13.47	82.92	82.95	30.00		
8N06880	20 130 206	L	ME/BLY	LB/LG	2.00	7	19.80	19.81	8.12	14.06	79.97	80.02	31.10	20 130 206	87.36
8N06881	20 130 212	I	ME/BLY	LI/LI	2.00	7	20.20	20.19	7.67	13.67	78.01	77.79	31.80	20 120 215	76.53
8N72674	20 130 205	M	Y/LB	ME/BLY	1.00	7	20.34	20.35	7.78	14.00	79.09	79.09	34.10	20 130 207	90.65
8N72681	20 130 207	K	DG/R	ME/BLY	1.00	7	20.76	20.81	7.93	13.44	79.59	79.96	32.50	20 130 207	88.99
8N72691	20 130 208	E	LG/O	ME/BLY	1.00	7	20.12	20.13	7.64	13.42	77.32	78.16	30.30	20 130 208	86.24
8N72695	20 130 208	E	DB/R	ME/BLY	1.00	7	19.80	19.80	7.52	13.26	77.22	77.04	28.70	20 130 209	77.91
8N73434	20 130 208	E	LB/O	ME/BLY	1.00	7	18.97	18.64	7.95	13.32	78.11	77.97	30.60	20 130 208	87.15
8N73436	20 130 208	E	LB/LG	ME/BLY	1.00	7	20.08	20.07	7.48	12.97	77.14	76.98	29.00	20 130 209	93.85
8N73439	20 130 208	E	LI/W	ME/BLY	1.00	7	20.03	20.07	7.82	13.29	78.20	78.16	30.00	20 130 208	90.07
8N73440	20 130 208	E	LI/R	ME/BLY	1.00	7	20.04	20.18	8.13	13.45	79.97	80.02	33.20	20 130 208	106.20
8N73441	20 130 208	E	LI/O	ME/BLY	1.00	7	21.25		7.73	13.38	77.48	77.70	29.50	20 130 208	89.50
8N73443	20 130 209	I	LI/DB	ME/BLY	1.00	7	19.00	18.97	7.57	13.10	77.66	77.97	31.00	20 130 210	88.14
8N73457	20 130 210	J	RS/R	ME/BLY	1.00	7	18.41	18.59	7.92	13.65	78.20	78.16	29.00	20 130 211	78.33
8N73467	20 130 211	G	R/RS	ME/BLY	1.00	7	19.68	19.64	7.81	13.31	78.01	77.79	31.50	20 130 211	84.12
8N73479	20 130 212	I	DB/DG	DG/ME	1.00	7	19.42	19.34	8.12	13.01	81.13	80.97	31.70	20 130 212	93.54
8N73482	20 130 214	E	DB/DG	RS/ME	1.00	7	19.13	19.18	7.65	11.79	75.92	75.67	30.70	20 120 214	81.14
8N73484	20 130 214	E	DB/LB	ME/LB	1.00	7	19.07	18.85	8.11	12.74	75.92	75.67	31.70	20 120 214	83.45
8N73485	20 130 214	E	DB/LB	ME/RS	1.00	7	19.27	19.28	7.91	13.12	78.20	78.16	31.35	20 130 214	87.33

Table B2: Individual measurements of biometrical traits among male house sparrows (n=25) samples at Leka, Helgeland Norway 2011

ID	Capture (date)	Location	Colour (L)	Colour (R)	Age (years)	BeakCat	ChestCat	Tars (R)	Tars (L)	Beak hight	Beak length	Wing (R)	Wing (L)	Mask	Total badge	Visible badge	Body mass (g)	BMR (date)	BMR
8L64499	2013-02-13	M	DG/ME	W/LB	5.00	2	3	20.10	19.80	7.81	12.69	82.18	82.03	13.60	21.37	13.32	32.60	20 130 213	83.62
8M31216	2013-02-12	C	ME/PB	LB/LG	4.00	5	3	20.24	20.24	7.92	13.78	82.62	82.80	14.50	19.32	15.20	33.70	20 130 213	79.15
8M31769	2013-02-13	C	ME/PB	R/Y	4.00	4	3	19.59	19.55	7.45	13.02	80.85	80.94	14.30	20.46	14.85	31.00	20 130 213	88.54
8M72594	2013-02-05	M	ME/RSW	LB/LI	3.00	4	4	20.29	20.24	8.12	13.78	82.05	83.27	16.70	22.83	15.25	32.40	20 130 207	94.52
8N05650	2013-02-07	K	W/LB	ME/BLY	2.00	5	3	20.42	20.36	7.86	12.77	82.48	82.48	14.95	20.90	15.30	32.00	20 130 207	83.16
8N06864	2013-02-06	M	ME/BLY	LG/O	2.00	3	3	19.77	19.79	8.15	13.44	80.85	80.94	15.00	19.79	14.81	34.00	20 130 206	93.72
8N06867	2013-02-05	M	ME/BLY	LG/LB	2.00	2	3	19.46	19.53	7.91	13.08	79.44	80.09	15.20	19.42	15.04	32.80	20 130 207	97.79
8N72672	2013-02-05	M	Y/LG	ME/BLY	1.00	1	3	18.96	18.92	8.10	12.95	78.78	79.29	15.10	18.10	14.80	35.90	20 130 207	83.18
8N72673	2013-02-05	M	Y/DB	ME/BLY	1.00	2	2	18.75	18.85	7.72	12.91	79.97	80.02	14.10	20.31	14.70	35.30	20 130 207	92.21
8N72677	2013-02-06	M	O/R	ME/BLY	1.00	2	2	17.33	17.21	7.87	13.85	81.74	81.87	15.30	19.21	14.36	32.50	20 130 206	86.94
8N72683	2013-02-07	K	DG/Y	ME/BLY	1.00	4	2	20.15	20.15	7.65	13.23	82.62	82.80	12.30	19.94	14.87	32.00	20 130 214	99.45
8N72690	2013-02-08	E	LG/Y	ME/BLY	1.00	5	2	19.50	19.48	7.79	13.39	79.97	80.02	12.20	17.47	13.34	30.90	20 130 208	82.23
8N72696	2013-02-08	E	DB/W	ME/BLY	1.00	5	3	20.24	20.26	7.61	12.94	82.48	82.48	13.95	20.04	14.76	30.50	20 130 209	95.36
8N73430	2013-02-08	E	DB/O	ME/BLY	1.00	4	2	20.32	20.32	8.14	13.42	80.75	81.68	14.60	16.84	14.53	32.00	20 130 209	94.56
8N73437	2013-02-08	E	LB/DB	ME/BLY	1.00	4	3	20.30	20.34	7.60	13.19	80.03	79.96	14.35	22.51	13.98	31.00	20 130 208	86.30
8N73438	2013-02-08	E	LI/LI	ME/BLY	1.00	4	3	19.44	19.50	7.66	12.74	79.44	80.09	15.00	19.79	14.67	29.60	20 130 208	84.60
8N73448	2013-02-09	I	O/LI	ME/BLY	1.00	4	1	19.65	19.66	7.87	13.33	81.51	81.49	13.30	19.83	13.09	32.60	20 130 210	80.75
8N73452	2013-02-10	J	LB/LI	ME/BLY	1.00	2	3	19.80	19.83	7.70	12.83	78.55	78.97	14.05	20.27	14.88	30.00		
8N73453	2013-02-10	J	DG/LI	ME/BLY	1.00	2	2	18.31	18.29	7.49	12.58	80.47	80.43	15.00	19.14	14.85	29.20	20 130 214	87.77
8N73465	2013-02-11	G	W/RS	ME/BLY	1.00	4	3	19.69	19.69	7.85	13.93	79.09	79.09	13.20	19.52	13.75	34.30	20 130 211	96.90
8N73468	2013-02-11	G	Y/RS	ME/BLY	1.00	3	2	19.21	19.27	7.73	14.27	81.74	81.87	14.30	19.38	14.19	32.10	20 130 211	91.41
8N73470	2013-02-11	G	LB/RS	ME/BLY	1.00	4	3	19.50	19.58	7.80	13.36	80.99	81.49	13.35	19.83	13.99	30.90	20 130 212	85.51
8N73477	2013-02-12	I	DB/DB	ME/W	1.00	3	2	19.75	19.80	8.07	13.66	80.85	81.87	13.80	19.84	13.52	33.80	20 130 212	84.90
8N73478	2013-02-12	I	DB/DB	RS/ME	1.00	4	3	20.41	20.45	8.09	14.08	80.85	80.94	13.20	20.77	13.75	32.40	20 130 212	101.76
8N73487	2013-02-14	E	DB/LG	DG/ME	1.00	4	2	20.42	20.31	8.05	13.04	82.18	83.09	11.40	18.00	13.19	31.10	20 130 214	80.21

C. Levels of steroid hormones

Table C1: Individual concentrations of steroid hormones (ng/ml) among female house sparrows (n=22) sampled at Leka, Helgeland Norway, 2011.

ID	ALDO	αE2[a]	βE2	E1	DHT	TS	DHEA	AN	OH-PRO[a]	PRE	PRO
8L48780	0.005		0.162	0.155	0.307	0.98	0.027	0.036		0.226	0.412
8N05859	0.004		0.113	0.105	0.212	0.649	0.012	0.005*		0.136	0.239
8N06843	0.001*	0.132	0.142	0.354	0.072	0.637	0.004	0.045		0.015	0.33
8N06862	0.002*	0.132	0.159	0.184	0.028	0.741	0.012	0.061		0.08	0.321
8N06880	0.001*	0.131	0.128	0.123	0.049	0.284	0.004	0.03		0.017	0.355
8N06881	0.065		2.649	6.391	0.207	0.338	0.157	0.138	0.009	0.757	0.203
8N72674	0.037		0.128	0.168	0.195	0.004*	0.019	0.098	0.021	0.564	0.137
8N72681	0.008		0.144	0.142	0.158	0.836	0.019	0.029		0.102	0.049
8N72691	0.001*	0.175	0.187	0.214	0.068	0.68	0.017	0.062		0.03	1.163
8N72695	0.003		0.138	0.408	0.182	1.333	0.012	0.767		0.078	0.133
8N73434	0.002*	0.168	0.163	0.197	0.039	0.738	0.014	0.058		0.172	0.144
8N73436	0.001*		0.124	0.105	0.078	1.011	0.008	0.031		0.185	0.505
8N73439	0.172		0.139	0.835	0.17	3.466	0.019	0.021	0.059	0.066	0.217
8N73440	0.012		0.131	0.219	0.214	0.005*	0.068	0.017	0.023	1.37	0.318
8N73441	0.064		0.145	0.262	0.299	0.005*	0.02	0.013	0.009	0.158	0.1
8N73443	0.141		0.146	0.274	0.298	2.303	0.022	0.054	0.014	0.288	0.39
8N73457	0.002*	0.132	0.143	0.134	0.047	0.263	0.006	0.051		0.036	0.68
8N73467	0.001*	0.156	0.111	0.093	0.053	0.688	0.016	0.012		0.089	0.15
8N73479	0.027		0.146	0.295	0.212	1.54	0.009	0.074		0.139	2.456
8N73482	0.031		0.117	0.26	0.232	1.498	0.017	0.024		0.108	0.194
8N73484	0.058		0.119	0.304	0.255	2.08	0.018	0.008		0.134	0.108
8N73485	0.004		0.118	0.332	0.193	0.397	0.035	0.229	0.016	0.085	0.447

*****Concentrations being under limit of detection (LOD) in the analysis. Values indicated are random numbers between 0-LOD

[a] Excluded from results: steroid hormones detected in less than 60 % of the individuals

Table C2: Individual concentrations of steroid hormones (ng/ml) among male house sparrows (n=25) sampled at Leka, Helgeland Norway, 2011.

ID	ALDO[a]	αE2[a]	βE2	E1	DHEA	TS	DHT	AN	OH-PRO[a]	PRE	PRO
8L64499	0.010		0.150	0.18	0.013	0.543	0.201	0.014	0.973	0.068*	0.129
8M31216			0.160	0.408	0.025	0.567	0.039	0.196		0.150	1.285
8M31769	0.015		0.229	0.257	0.024	1.296	0.346	0.029	0.964	0.115	2.106
8M72594	0.011		0.143	0.139	0.070	2.275	0.31	0.072		2.317	0.262
8N05650	0.039		0.121	0.110	0.018	1.658	0.216	0.039		0.264	0.390
8N06864		0.191	0.181	0.171	0.007	1.024	0.064	0.036		0.106	2.287
8N06867		0.132	0.142	0.146	0.002	0.755	0.057	0.04		0.009	0.246
8N72672		0.129	0.087	0.379	0.022	1.267	0.051	0.056		0.097	0.962
8N72673			0.136	0.125	0.010	0.69	0.058	0.022		0.089	0.171
8N72677	0.006		0.128	0.124	0.027	0.544	0.200	0.025		0.067	0.976
8N72683			0.141	0.178	0.007	0.431	0.200	0.059		0.050	0.462
8N72690	0.009		0.130	0.168	0.014	0.686	0.202	0.019		0.077	1.339
8N72696			0.165	0.111	0.006	0.819	0.066	0.093		0.060	0.941
8N73430		0.128	0.120	0.091	0.009	0.819	0.031	0.012		0.081	1.009
8N73437		0.253	0.205	0.309	0.013	2.218	0.109	0.049		0.096	0.56
8N73438		0.129	0.125	0.119	0.009	3.212	0.051	0.016		0.077	0.326
8N73448		0.13	0.107	0.13	0.002	4.046	0.054	0.026		0.114	0.182
8N73452			0.147	0.164	0.028	0.943	0.034	0.017		0.155	0.68
8N73453		0.172	0.149	0.121	0.016	0.586	0.110	0.033		0.041	0.055
8N73465			0.119	0.127	0.006	0.798	0.045	0.096		0.088	2.321
8N73468		0.037	0.026*	0.480	0.188	0.004*	0.027*	0.319		0.665	1.492
8N73470		0.177	0.147	0.146	0.005	1.375	0.009	0.048		0.128	0.129
8N73477	0.011		0.122	0.183	0.021	1.348	0.228	0.102		0.159	0.265
8N73478	0.008		0.156	0.271	0.172	7.160	0.152	0.797	0.042	0.410	3.325
8N73487	0.015		0.131	0.191	0.002*	0.746	0.234	0.059		0.054	0.198

*****Concentrations being under limit of detection (LOD) in the analysis. Values indicated are random numbers between 0-LOD

[a] Excluded from results: steroid hormones detected in less than 60 % of the individuals

D. Levels of contaminants

Table D1. Individual concentrations of PCBs and OCPs (ng/g ww) in female house sparrows (n=22) sampled at Leka, Helgeland Norway 2011.

ID	PCB-28	PCB-52	PCB-101	PCB-118	PCB-138	PCB-153	PCB-180	HCB	*p,p'*-DDE
8L48780	0.173	0.037*	0.105	0.120	0.404	0.912	0.311	0.692	2.014
8N05859	0.126	0.039*	0.030*	0.052*	0.135	0.040*	0.028*	0.585	0.939
8N06843	0.120	0.035*	0.174	0.223	0.574	0.808	0.274	1.063	0.788
8N06862	0.044*	0.039*	0.031*	0.148	1.146	2.395	1.697	0.507	0.611
8N06880	0.215	0.040*	0.146	0.245	0.852	1.622	0.83	0.68	1.365
8N06881	0.102	0.025*	0.125	0.048	0.317	0.508	0.314	0.594	0.344
8N72674	0.144	0.036*	0.103	0.367	0.694	1.011	0.377	0.677	3.044
8N72681	0.14	0.189	0.252	0.574	1.477	1.692	0.781	1.64	1.867
8N72691	0.178	0.088	0.109	0.055	0.714	1.204	0.479	1.023	0.979
8N72695	0.111	0.225	0.063	0.045	0.354	0.959	0.31	0.614	0.572
8N73434	0.23	0.113	0.168	0.532	1.503	2.199	1.33	0.661	2.789
8N73436	0.134	0.058	0.135	0.294	0.986	1.668	1.172	0.683	0.998
8N73439	0.145	0.204	0.026*	0.048*	0.164	0.319	0.143	0.54	0.507
8N73440	0.139	0.246	0.114	0.839	0.944	1.23	0.646	1.038	0.893
8N73441	0.101	0.201	0.037*	0.029*	0.239	0.422	0.22	0.569	0.522
8N73443	0.1	0.222	0.053	0.069*	0.209	0.323	0.155	0.519	0.288
8N73457	0.115	0.229	0.207	0.284	0.654	2.387	0.785	0.986	3.194
8N73467	0.158	0.146	0.251	0.900	3.211	9.149	5.944	1.095	3.122
8N73479	0.115	0.171	0.068	0.077*	0.426	0.599	0.36	0.586	0.154
8N73482	0.152	0.193	0.201	0.279	1.253	2.631	2.399	0.972	1.676
8N73484	0.197	0.199	0.268	0.877	1.999	4.147	2.08	1.008	1.835
8N73485	0.18	0.085	0.169	0.381	1.394	2.5	1.52	0.734	1.865

***** Concentrations being under limit of detection (LOD) in the analysis. Values indicated are random numbers between 0-LOD.

Table D2: Individual concentrations of PCBs and OCPs (ng/g ww) in male house sparrows (n=25) sampled at Leka, Helgeland Norway 2011.

ID	PCB -28	PCB-52	PCB-101	PCB-118	PCB-138	PCB-153	PCB-180	HCB	*p,p'*-DDE
8L64499	0.151	0.039*	0.113	0.586	1.248	3.161	1.089	1.063	2.997
8M31216	0.111	0.035*	0.033*	0.049*	0.203	0.532	0.272	0.489	0.790
8M31769	0.255	0.038*	0.230	0.506	1.389	3.313	1.025	0.988	4.996
8M72594	0.174	0.032*	0.266	0.618	0.720	1.685	0.773	1.224	2.636
8N05650	0.205	0.043*	0.249	0.490	1.226	1.518	0.542	1.709	1.437
8N06864	0.168	0.069	0.140	0.167	0.639	1.093	0.719	0.886	1.917
8N06867	0.127	0.044*	0.026*	0.039*	0.388	0.539	0.305	0.903	1.348
8N72672	0.148	0.039*	0.114	0.134	0.416	0.775	0.227	0.797	2.252
8N72673	0.119	0.041*	0.034*	0.041*	0.165	0.216	0.065	0.727	0.975
8N72677	0.267	0.034*	0.098	0.557	1.041	2.749	1.009	1.244	5.450
8N72683	1.141	0.812	0.488	1.980	4.319	5.046	1.705	2.924	3.453
8N72690	0.243	0.129	0.131	0.350	1.453	2.528	1.475	0.906	2.652
8N72696	0.269	0.072	0.225	2.737	12.262	32.259	19.665	0.841	5.738
8N73430	0.178	0.150	0.312	1.535	9.131	21.824	18.870	1.021	3.003
8N73437	0.112	0.077	0.037*	0.065*	0.371	0.660	0.442	0.571	0.679
8N73438	0.102	0.105	0.041*	0.153	0.492	0.951	0.572	0.629	0.598
8N73448	0.191	0.254	0.229	0.270	1.351	1.821	1.247	0.862	1.326
8N73452	0.161	0.323	0.237	0.113	0.418	0.936	0.511	1.099	2.828
8N73453	0.122	0.224	0.180	0.098	0.275	0.483	0.242	1.082	1.162
8N73465	0.161	0.343	0.126	0.340	1.018	2.057	0.876	0.955	3.229
8N73468	0.152	0.189	0.179	0.354	0.829	1.427	0.394	0.968	3.592
8N73470	0.149	0.214	0.107	0.336	0.770	1.967	0.695	1.173	2.840
8N73477	0.226	0.313	0.287	0.341	1.934	2.715	1.388	1.903	1.226
8N73478	0.214	0.311	0.153	0.317	1.471	3.028	1.721	1.584	2.096
8N73487	0.103	0.215	0.095	0.442	1.380	3.744	1.725	0.465	1.157

*****Concentrations being under limit of detection (LOD) in the analysis. Values indicated are random numbers between 0-LOD.

Table D3: Individual concentrations of BFRs (ng/g ww) among female house sparrows (n=22) sampled at Leka, Helgeland Norway 2011.

ID	BDE-47	BDE-99	BDE-100	BDE-153[a]	BDE-154[a]	BDE-209[a]
8L48780	0.148	0.386	0.077	0.093		
8N05859	0.071*	0.052*	0.041*			1.218
8N06843	0.426	0.770	0.144			
8N06862	0.075*	0.189	0.031			
8N06880	0.796	1.384	0.299	0.252	0.116	1.085
8N06881	0.465	0.817	0.139			
8N72674	0.183	0.403	0.046	0.121		
8N72681	0.773	1.654	0.272	0.148		
8N72691	0.265	0.697	0.119			
8N72695	0.216	0.551	0.110			1.057
8N73434	0.320	0.842	0.137	0.120		
8N73436	0.241	0.716	0.118			
8N73439	0.043*	0.035*	0.038*			
8N73440	0.173	0.508	0.102			1.053
8N73441	0.050*	0.222	0.059*			
8N73443	0.518	0.841	0.119			
8N73457	0.036*	0.158	0.040*			
8N73467	0.214	0.425	0.103			2.478
8N73479	0.405	0.706	0.114			
8N73482	0.314	1.106	0.135	0.197		
8N73484	0.381	1.057	0.158	0.148		1.393
8N73485	0.434	1.028	0.163	0.150		

*****Concentrations being under limit of detection (LOD) in the analysis. Values indicated are random numbers between 0-LOD

[a] Excluded from results: BDE-congeners detected in less than 60 % of the individuals

Table D4: Individual concentrations of BFRs (ng/g ww) among male house sparrows (n=25) samples at Leka, Helgeland Norway 2011

ID	BDE-47	BDE-99	BDE-100	BDE-153[a]	BDE-154[a]	BDE-209[a]
8L64499	0.063*	0.292	0.047*			
8M31216	0.052*	0.104	0.035*			
8M31769	0.193	0.7	0.117	0.183		
8M72594	0.287	0.822	0.139			1.212
8N05650	0.954	2.313	0.326	0.228	0.121	1.27
8N06864	0.109	0.215	0.061*			
8N06867	10.333	12.938	0.537	2.288	0.274	
8N72672	0.110	0.205	0.055*			
8N72673	0.070*	0.063*	0.038*			
8N72677	0.203	0.373	0.090			
8N72683	1.571	3.604	0.586	0.33	0.231	
8N72690	0.352	1.106	0.187	0.156		
8N72696	0.299	0.917	0.162	0.144		1.367
8N73430	0.357	0.965	0.147	0.134		1.072
8N73437	0.052*	0.192	0.043			
8N73438	0.169	0.396	0.044*			
8N73448	1.216	2.486	0.351	0.183		1.573
8N73452	0.065*	0.153	0.055*			
8N73453	0.048*	0.071*	0.057*			
8N73465	0.204	0.518	0.084			
8N73468	0.217	0.326	0.078			
8N73470	0.176	0.386	0.085			
8N73477	1.947	3.388	0.428	0.214		
8N73478	1.378	3.606	0.553	0.426	0.154	
8N73487	0.246	0.815	0.163	0.152		

*****Concentrations being under limit of detection (LOD) in the analysis. Values indicated are random numbers between 0-LOD

[a] Excluded from results: BDE-congeners detected in less than 60 % of the individuals

E. Statistical differences between sexes

Table E1: Mann-Whitney U Test: statistical significant differences between males and females for biometrical variables wing length and body mass. Non-parametric test was used, since normality was not obtained

	Right wing length	Left wing length	Body mass (g)
Mann-Whitney U	60.000	50.000	174.500
Wilcoxon W	313.000	303.000	427.500
Z	-4.589	-4.804	-2.144
Asymp. Sig. (2-tailed)	.000	.000	.032

a. Grouping Variable: Female, male

Table E2. Mann-Whitney U Test: statistical significant differences between males and females for PCB-28. Non-parametric test was used, since normality was not obtained.

	PCB28
Mann-Whitney U	179.000
Wilcoxon W	432.000
Z	-2.047
Asymp. Sig. (2-tailed)	.041

a. Grouping Variable: Female, male

Table E3: Mann-Whitney U Test: statistical significant differences between males and females for PRO. Non-parametric test was used since normality was not obtained

	Progesterone
Mann-Whitney U	175.000
Wilcoxon W	428.000
Z	-2.132
Asymp. Sig. (2-tailed)	.033

a. Grouping Variable: Female, male

Table E4: Independent t-test: statistical significant differences between males and females for HCB, *p,p'*-DDE. Parametric test was used since normality was obtained by transformation. Both transformed and non-transformed data are shown.

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
HCB	Equal variances assumed	1.636	.207	-2.320	45	.025	-.286543	.123500	-.535285	-.037800
	Equal variances not assumed			-2.406	37.601	.021	-.286543	.119109	-.527751	-.045335
p,p'-DDE	Equal variances assumed	2.697	.108	-2.832	45	.007	-1.034700	.365329	-1.770511	-.298889
	Equal variances not assumed			-2.903	42.204	.006	-1.034700	.356398	-1.753837	-.315563
HCB (transformed)	Equal variances assumed	.159	.692	-2.514	45	.016	-.27088	.10775	-.48791	-.05386
	Equal variances not assumed			-2.558	44.104	.014	-.27088	.10588	-.48426	-.05751
*p,*p'-DDE (transformed)	Equal variances assumed	1.404	.242	-3.066	45	.004	-.66057	.21544	-1.09448	-.22665
	Equal variances not assumed			-3.015	39.216	.004	-.66057	.21910	-1.10365	-.21748

F. Correlations between variables

Table D1: Statistically significant correlations (Spearman's rank correlation test) between steroid hormones and contaminants, steroid hormones and biometry and contaminants and biometry for male (n=25) and female (n=22) house sparrows sampled at Leka, Helgeland Norway 2011.

Correlated variables	Sex	Correlation coefficient (r)	Significance level (p)
βE2 - PCB-118	Female	-0.426	0.048
E1 - *p,p'*-DDE	Female	-0.607	0.003
E1 - ∑OCPs	Female	-0.533	0.011
DHT - PCB-118	Male	0.408	0.043
DHT - ∑PCBs	Male	0.402	0.042
TS - beak length	Female	-0.600	0.003
PRE - body mass	Female	0.470	0.027
DHEA - body mass[a]	Female	0.413	0.056
AN - beak length	Male	0.548	0.005
TS - chest category	Male	0.402	0.046
TS - total badge	Male	0.434	0.030
PCB-52 - age	Female	-0.704	0.001
PCB-52 - age	Male	-0.633	0.001

[a] Border line significance between DHEA and body mass.