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Environmental Toxicology and Chemistry
Submission date: May 2014
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Abstract

Persistent organic pollutants (POPs) have been reported to disrupt vertebrate endocrine systems in numerous wildlife, semi-field and laboratory studies, and thyroid homeostasis is among the systems reported to be susceptible for such perturbation. Thyroid hormones are important in a vast range of physiological processes, and a disruption of thyroid homeostasis might thus cause detrimental effects. Toxic effects exerted by POPs on the thyroid system has been reported in mammals and in birds at high trophic levels, but the knowledge of POPs toxicity in passerines is limited. The passerine species house sparrow (*Passer domesticus*) has to my knowledge never been investigated for thyroid disruption previous to the present study. The house sparrow has experienced a severe population decrease in Northern Europe since mid-1980s, and the reason for the decline is not completely elucidated. Increased knowledge regarding levels and toxic effects of POPs in house sparrows might contribute to further elucidation of the problem.

The aim of the present study was to investigate potential effects of POPs exposure on the thyroid system, herein represented by circulating free fractions of 3,3',5-triiodothyronine (FT3) and thyroxine (FT4), in house sparrows from Norway. An additional aim was to contribute to the existing documentation of POPs levels in passerines, specifically hepatic levels. The multivariate statistical analyses principal component analysis (PCA) and orthogonal projections to latent structures (O-PLS) were used to model the complexity of variables affecting FT3 and FT4 levels in the birds, including both biometric variables and contaminant levels as predictors. Additionally, bivariate correlations between contaminants and thyroid hormones were investigated with Spearman's rank correlation test. The study population was located on an agricultural island in Northern Norway, and sampling was conducted in February 2013.

Significant correlations between single POPs and thyroid hormones are reported in the present study. Although statistical correlations do not represent cause-effect relationship, these findings add further weight of evidence to the hypothesis of avian thyroid disruption caused by contaminant exposure in wildlife. The level of contamination in the investigated sparrows was in general low, but a few individuals had highly elevated levels of some polychlorinated biphenyl (PCB) congeners compared to the mean. PCBs and organochlorine pesticides (OCPs) were found at higher concentrations than polybrominated diphenyl ethers (PBDEs) in the sparrows, and the OCPs varied significantly between sexes. The level of one contaminant seemed to vary with age (PCB-52), and levels of three PBDE congeners (BDE-47, -99 and -100) varied significantly according to which farm the investigated birds was captured.

Samandrag

Stadig fleire studier rapporterar om endokrine forstyrringar i vertebrater som ei følgje av eksponering for persistente organiske miljøgifter. Dette gjeld både studier på frittlevande dyr og i laboratorieforsøk, og hormonsystemet tilknytta skjoldbruskkjertelen har vist seg å vere eitt av målsystema for slike forstyrringar. Skjoldbruskhormona er viktige i ei rekke fysiologiske prosessar i vertebrater, og ei forstyrring i nivåa av desse kan få svært alvorlige følgjer. Mange studier har dokumentert toksiske effekter av persistente organiske miljøgifter i pattedyr og i fuglearter høgt i næringskjeda, men det er særs begrensa kunnskap om toksisiteten av desse giftene i spurvefuglar. Toksisiteten til persistente organiske miljøgiftene på skjoldbruskkjertel-systemet til gråspurv (*Passer domesticus*) har etter kva eg veit ikkje blitt undersøkt tidlegare. Gråspurvpopulasjonane i Nord-Europa har minka dei siste tre tiåra, og årsaken bak er enno ikkje fullstendig kartlagt. Auka kunnskap om nivå og toksiske effekter av persistente organiske miljøgifter i gråspurven kan muligens hjelpe til med denne kartlegginga.

Hensikta med dette studiet var å undersøke eventuelle toksiske effekter av persistente organiske miljøgifter på skjoldbruskkjertelsystemet til gråspurv som lever i Noreg. Nivå av fritt 3,3',5-trijodtyronin (FT3) og fritt tyroksin (FT4) vart nytta som effektvariablar. Eit tilleggsmål var å bistå med dokumentasjon av nivå av miljøgifter i spurvelever. Principal component analysis (PCA) og orthogonal projections to latent structures (O-PLS) vart nytta for å forsøke å modellere korleis giftnivå og biometriske variablar påvirka nivået av FT3 og FT4 i spurvane. Det vart også utført korrelasjonsanalyser for å undersøke forholdet mellom giftstoffa og hormona nærmare. Fuglane vart fanga på ei øy i Nord-Noreg i februar, 2013.

I dette studiet vart det funne signifikante korrelasjonar mellom einskilde miljøgifter og skjoldbruskhormoner. Statistiske korrelasjonar beviser ikkje at det var kausalitet i det undersøkte forholdet, men funna i dette studiet bidreg likevel til dei eksisterande indikasjonane på at persistente organiske miljøgifter utøver toksiske effekt på hormonsystemet knytta til skjoldbruskkjertelen i frittlevande fuglar. Nivået av forureining i dei undersøkte gråspurvane var generelt lavt, men nokre få individ hadde svært høge nivå av nokre polyklorinerte bifenylar (PCBar) samanlikna med populasjonsgjennomsnittet. Nivået av PCBar og organoklorinerte pesticidar (OCPar) var høgare enn nivået av polybromerte difenyleterar (PBDEar). Dei undersøkte OCPane varierte signifikant mellom ho- og hannfuglar, ein PCB varierte med alder på fuglane (PCB-52), og nivåa av tre PBDE-forbindelsar (BDE-47, -99 og -100) varierte signifikant mellom fuglar frå ulike gardar.

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Trondheim, 11th of May 2014

Malene Vågen Dimmen

Acronyms

∑OCPs	Sum of individual organochlorine pesticides
∑PBDEs	Sum of individual polybrominated diphenyl ethers
∑PCBs	Sum of individual polychlorinated biphenyls
5'D-II	Type II thyroxine-5'-deiodinase
AhR	Aryl hydrocarbon receptor
ANOVA	Analysis of variance
BFR	Brominated flame retardant
BMR	Basal metabolic rate
CV%	Coefficient of variation
CV-ANOVA	Analysis of variance testing of cross-validated predictive residuals
CYP	Cytochrome P450 monooxygenase
DDT	Dichlorodiphenyltrichloroethane
FT3	Free 3,3',5-triiodothyronine
FT4	Free thyroxine
GC	Gas chromatography
H_2	Hydrogen gas
H_2SO_4	Sulfuric acid
HBCDD	Hexabromocyclododecane
HCB	Hexachlorobenzene
He	Helium
HPG	Hypothalamus-pituitary-gonadal axis
HPT	Hypothalamus-pituitary-thyroid axis
I.S.	Internal standard
IUCN	International Union for Conservation of Nature and Natural Resources
LOD	Limit of detection
LOQ	Limit of quantification
lw	Lipid weight
MS	Mass spectrometry
NMBU	The Norwegian University of Life Sciences
NTNU	The Norwegian University of Science and Technology

NVH	The Norwegian School of Veterinary Science
OC	Organochlorine
OCP	Organochlorine pesticide
OH-PCB	Hydroxylated PCB metabolite
O-PLS	Orthogonal projections to latent structures
p,p'-DDE	*p,p'*-dichlorodiphenyldichloroethylene
PBDE	Polybrominated diphenyl ether
PC	Principal component
PCA	Principal component analysis
PCB	Polychlorinated biphenyl
PLS	Projection to latent structures by means of partial least squares
POP	Persistent organic pollutant
RBP	Retinol binding protein
RIA	Radioimmunoassay
SD	Standard deviation
SRM	Standard reference material
T3	3,3',5-triiodothyronine
T4	Thyroxine
TH	Thyroid hormone
TRH	Thyrotropin-releasing hormone
TRα	Thyroid hormone receptor α
TRβ	Thyroid hormone receptor β
TSH	Thyroid-stimulating hormone
TT3	Total 3,3',5-triiodothyronine
TT4	Total thyroxine
TTR	Transthyretin
UDP-GT	Uridine diphosphate glucuronosyltransferase
UV	Unit variance
VIP	Variance importance in projection
WHO	World Health Organization
ww	Wet weight
μ-ECD	μ-electron capture detection

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1 Introduction

Persistent organic pollutants (POPs) are detected in wildlife all over the world and considered ubiquitous in the modern environment (Jones and de Voogt, 1999). These man-made synthetic contaminants exert various toxic effects on exposed organisms, including endocrine disruption (review in Tyler et al., 1998). Given the importance of endocrine systems for maintaining normal physiological function, a disruption of one such system may cause severe effects for the target organism's fitness. Such consequences of endocrine perturbation have been documented in wildlife species (Colborn et al., 1993; Crisp et al., 1998; Jimènez, 1997). One of the major targets for endocrine disruption is the hypothalamus-pituitary-thyroid (HPT) axis. The HPT axis regulates thyroid homeostasis in vertebrates, which several wildlife studies have documented to be susceptible towards POPs induced perturbation both in mammals (Bytingsvik et al., 2012; Villanger et al., 2011) and in birds (Mayne et al., 2005; Verreault et al., 2013; Verreault et al., 2004). In addition to wildlife studies, *in vivo*, *in vitro* and semi-field studies have found indications of POPs induced thyroid disruption in a range of species as well (review in Boas et al., 2006; Brouwer et al., 1989; review in Jenssen, 2006). The weight of evidence for thyroid disruption associated with POPs exposure gives reason for concern, especially for organisms living in high exposure areas. This includes house sparrow (*Passer domesticus*) populations living in close proximity to substantial human activities, e.g. close to large cities in Northern Europe.

1.1 Persistent organic pollutants

POPs constitute a group of organic compounds characterized by low water solubility and high lipid solubility, which give them the ability to bioaccumulate in fatty tissues. Bioaccumulative properties in combination with their persistent nature may lead to biomagnification from one trophic levels to the next, resulting in elevated levels in animals at high trophic levels (Routti et al., 2010). Several POPs are semi-volatile, which leads to their long-range atmospheric transportation before deposition occurs (Beyer et al., 2000; Wania and Mackay, 1995). Such transport combined with a resistance towards photolytic, biological and chemical degradation have resulted in the distribution of POPs, and subsequent accumulation in biota, far from the original sources, even as far as in the Arctic and the Antarctic areas (Corsolini et al., 2002; Verreault et al., 2004).

POPs can be divided in so called legacy POPs, which have a long history of use and release into the environment, and novel or emerging POPs, which are chemicals with a more recent history. A major group of legacy POPs are the polychlorinated biphenyls (PCBs). They were used for a range of industrial applications (e.g. in capacitors, transformers, hydraulic fluids, vacuum pumps, rubbers, inks, cutting oils, pesticide extenders, sealants and adhesives) from 1929 until the 1970s when they were phased out and eventually banned (Harrad, 2010). There are 209 PCB congeners, each characterized by the number of chlorine substituents (1-10) and the position of these substituents in the molecule. In addition to the PCBs, many organochlorine pesticides (OCPs) are categorized as legacy POPs, e.g. the insecticide dichlorodiphenyltrichloroethane (DDT) and the fungicide hexachlorobenzene (HCB). Before they were phased out in most countries during the 1970s and 1980s, DDT was used extensively for both agricultural purposes and as vector control (Li and Macdonald, 2005), and HCB for crop seed treatment, wood preservation, carbon anode treatment and other industrial applications (Bailey, 2001). DDT is still used for disease vector control in some developing countries as recommended by and under the guidance of the World Health Organization (WHO) (Stockholm Convention, 2008). DDT's major metabolite *p,p'*-DDE is recognized as highly toxic and possibly even more persistent than DDT in the environment (Ecobichon and Joy, 1993)

Many of the pesticides and industrial chemicals, including DDT, HCB and PCBs, which have been regulated and restricted in terms of production and use since the 1970s or 1980s, are still

found at relatively high concentrations in both biotic and abiotic components of the environment (Bouwman et al., 2013; Gioia et al., 2013; Harrad, 2010; Jimènez et al., 2005; Polder et al., 2008).

Polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCDD) are examples of newer and emerging POPs. Both are used as flame retardants; PBDEs in electronic devises (e.g. computers, TV sets), upholstery and carpets, HBCDD in textiles and in insulation boards in buildings (e.g. polystyrene foam) (Fromme et al., 2014). PBDEs and HBCDD are found both in humans (Darnerud et al., 2011) and in wildlife (Ciesielski et al., 2008; Lundstedt-Enkel et al., 2001).

PBDEs are available in commercial mixtures, i.e. the penta-mixture containing mainly tetra- and penta-BDEs, the octa-mixture containing mainly hexa- and hepta-BDEs, and the deca-BDE comprising predominantly deca-BDE (WHO, 1994). Use and import of penta- and octa-BDEs have been banned in Europe since 2004 (Directive EEC, 2003), and internationally since 2009 when they were included in Annex 1 of the Stockholm Convention on Persistent Organic Pollutants (Stockholm Convention, 2009). The use of deca-BDEs in electronic and electrical equipment has been banned in the European Union since 2008, but is unrestricted in other applications (DEFRA, 2010). HBCDD was listed under substances subject to authorisation in annex XIV of REACH in 2011 (European Commission), and in November 2013 an amendment was added to annex A in the Stockholm convention of 2001 including HBCDD to the POPs priority list. The amendment will enter into force in November 2014 (Stockholm Convention, 2013) .

Among the numerous toxic effects of POPs, endocrine disruption is a major one. Especially PCBs and PBDEs are documented to exert significant effects on vertebrate thyroid systems to such an extent that the levels of thyroid hormones (THs) are altered. The mechanisms by which these contaminants influence thyroid status are many; e.g. binding to transport proteins, alter the cellular uptake mechanisms, modify the metabolism of THs, or disturb at the receptor level (Boas et al., 2006; Cheek et al., 1999; Routti et al., 2010).

1.2 Thyroid hormones

THs play a crucial role in the control of metabolism, thermoregulation, reproduction, growth and development, as well as maintaining the general physiological homeostasis in vertebrates. They are especially important in the development of nervous system in fetuses, neonates and in juveniles (McNabb, 2007; Zoeller et al., 2007). Consequently, a contaminant-induced disturbance in thyroid homeostasis during early life stages may result in detrimental effects later in life (Derocher et al., 2003; Villanger et al., 2011). Tissue-specific differentiation in the skeletal system, the heart and body musculature of birds are all highly dependent on thyroid control, as is avian seasonal moulting and feather pigmentation. In addition, THs are believed to play a role in the development of photorefractoriness in avian females during egg laying, a process under which circulating thyroid levels can vary substantially (McNabb, 2007). Plasma THs are in general important for the initiation of gonadal development and other reproductive processes in vertebrates. Alterations in TH plasma concentrations associated with POPs exposure has been shown to cause reproduction failure in a semi-field study (Brouwer et al., 1989).

In birds, as in mammals, the pituitary is the predominant factor in thyroid gland regulation. This applies to both stimulatory and inhibitory hypothalamic regulation of the hypothalamic-pituitary-thyroid (HPT) axis, and for negative feedback on hypothalamic-pituitary function (McNabb, 2007). The avian hypothalamus stimulates the anterior pituitary by releasing thyrotropin-releasing hormone (TRH). As a response, the pituitary releases thyroid-stimulating hormone (TSH), which stimulates the thyroid gland to produce thyroxine (T4; Figure 1.1B). In birds, T4 is transported in the blood bound to the serum binding proteins albumin and transthyretin (TTR). T4 bound to these carrier proteins is distributed throughout the body and converted to the more potent and biologically active 3,3',5-triiodothyronine (T3; Figure 1.1A) by monodeiodinases in peripheral tissues (McNabb, 2007). As T4, T3 is transported in avian plasma bound to albumin and TTR. The unbound fraction of T4 and T3 in plasma is referred to as free T4 (FT4) and free T3 (FT3), respectively. The biological effects of THs are exerted through the activation of TH receptors (TRα and TRβ) present in the nuclei of most cells. This controls the expression of target genes (McNabb, 2007), while the clearance of THs is mediated by conjugating enzymes (Gereben et al., 2008).

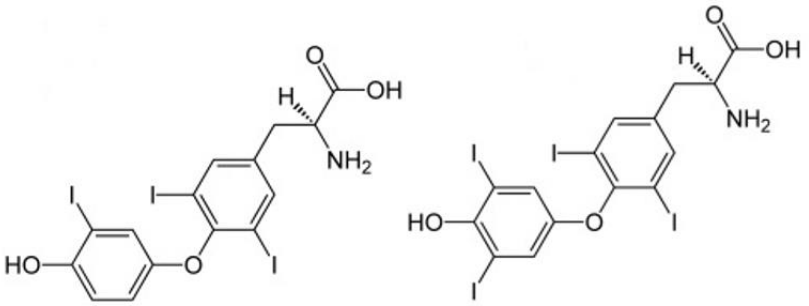


Figure 1.1 Molecular structure of thyroid hormones (THs); A) 3,3',5-triiodothyronine (T3) and B) thyroxine (T4).

In addition to thyroid homeostasis alterations caused by POPs exposure, many natural factors have the potential to affect vertebrate TH levels. This includes food availability, the content and composition of food stuffs consumed, iodine availability, temperature conditions, season, and reproductive condition (McNabb, 2007). Additionally, circulating levels of THs tend to vary according to a diurnal pattern in birds. Circulating T4 concentrations peak during the dark phase of the diurnal cycle, while T3 plasma concentrations peak during the light period due to a higher extrathyroidal T4 to T3 conversion (Cogburn and Freeman, 1987).

1.3 The house sparrow (*Passer domesticus*)

The house sparrow (*Passer domesticus*) is a small passerine bird which belongs to the family Passeridae, more commonly known as sparrows (Syvertsen, 2009). It has adapted to a life around humans and do not live far from human settlements. Typical habitats are cities, suburban regions and nearby farming activities in the countryside (Anderson, 2006). The nestling period for house sparrows is approximately 14 days, and both parents contribute with feeding during this time (Anderson, 2006). The most common food items for the offsprings are insects and spiders, collected by the parents in close proximity to the nest. Adults mainly eat small seeds and insects, but are also known to eat berries, grass, kitchen scraps or other food items acquired in the most opportune manner (Anderson, 2006). The house sparrow is highly sedentary (Summers-Smith, 1988), which is enhanced in insular populations. On an island, migration is even more seldom due to the discrete boundaries these habitats provide (Jensen et al., 2013). The house sparrow is the most widely distributed land bird species in the world with natural habitats in Europe, Asia and North Africa. It has also been introduced to the rest of the continents except from Antarctica (Anderson, 2006). In 2004, the population size was estimated to >540 000 000 individuals on a global scale (IUCN Red List of Threatened Species, 2012).

Despite the high global number of individuals, the house sparrow has experienced a severe decline in urban and rural areas throughout Northern Europe since mid-1980s (Anderson, 2006; Crick et al., 2002; Summers-Smith, 2003). The reason for the decline is not known, but several theories are proposed (review in Summers-Smith, 2003). Some of these theories include changes in agricultural practices alone (Hole et al., 2002) or in combination with increasing predation pressure (Anderson, 2006), lack of nest sites, changes in urban habitats (Chamberlain et al., 2007; Shaw et al., 2008), loss of food sources, diseases and pollution (Crick et al., 2002). In a research report discussing possible reasons for the decline, Crick et al. (2002) expressed a need for further research on pollution as a contributing factor. Although there are some reports on levels of POPs in house sparrows (Ciesielski et al., 2008; Kunisue et al., 2002), to my knowledge there are no studies that have investigated the toxic effects of POPs exposure as a possible contributing reason for the continually decreasing number of North-European house sparrows.

The house sparrow has been added to the International Union for Conservation of Nature and Natural Resources (IUCN) Red list of Threatened species as a consequence of the abrupt decline recent years. According to the IUCN, the house sparrow does not decline globally in a sufficiently rapid manner, nor have a number of individuals below the threshold for reaching the "vulnerable" category. Additionally, it has such a vast range (about 95 native countries and 60 introduced countries) that it does not meet the range size criterion for this category. Consequently, it is placed in the "least vulnerable" category internationally (IUCN Red List of Threatened Species, 2012). However, in the UK and the Netherlands, the severity of house sparrow population decline is substantial, and it is placed on the Red List as a species of high conservation concern (Chamberlain et al., 2007).

1.4 Thyroid disruption in avian species

Numerous investigations on POPs induced thyroid disruption in birds at high trophic levels have been conducted, especially seabirds (Moccia et al., 1986; Nøst et al., 2012; Ucán-Marín et al., 2009, 2010; Verreault et al., 2007; 2013). Some studies have investigated such disruption in bird species on lower trophic levels as well, including passerines (Bouwman et al., 2013; Dauwe et al., 2006; Scollon et al., 2004; Van den Steen et al., 2009). A few studies have documented high levels of POPs in house sparrows, e.g. elevated levels of DDT in South African house sparrow eggs (Bouwman et al., 2013), and substantial hepatic concentrations of deca-BDE 209 in house sparrow populations from Norway (Ciesielski et al., 2008). However, to my knowledge, thyroid disruption associated with organic pollutants has never been investigated in this species previously. The Ciesielski et al. (2008) study populations were located in the same area in Northern Norway as the present study population. Consequently, the elevated levels of BDE-209 measured in their study adds value to the present investigation on levels and effects of POPs in house sparrows from this area. On a larger scale, further research on POPs exposure and the effects it might have on thyroid homeostasis in house sparrows might contribute to elucidating the reasons for the population decline this species is experiencing. Additionally, the general lack of investigations on POPs induced thyroid disruption in passerine birds makes any contribution to this issue valuable.

1.5 Aim of study

The main objective of the present study was to investigate the potential effects of legacy and emerging POPs on thyroid homeostasis in house sparrows from an island in Northern Norway. An additional aim was to provide information on levels of POPs in house sparrows that live in agricultural environments at northern latitudes. Based on previous studies on other species, I hypothesized that the thyroid homeostasis, reflected by plasma concentrations of FT3 and FT4, would be disrupted if the contaminant exposure was sufficiently high. Both female and male individuals were included in the study, and possible sex differences in contaminant concentrations, TH levels and response to contaminant exposure were investigated. Such sex differentiations might be plausible in the house sparrows due to previous reports of such differences in other wildlife species.

2 Materials and methods

2.1 Collection of data in the field

2.1.1 Study area

The fieldwork was conducted on the island Leka (Nord-Trøndelag county, Norway, 65°N, 11°E) in the period between the 4th and 15th of February 2013. Leka is characterized by a mixture of agricultural land, heathland and mountains. The house sparrows at this island generally live in small local populations (ranging from 2-39 individuals in 2013) in the vicinity of approximately 30 dairy farms, where they nest inside barns and silos. In February 2013, the total insular population was estimated at 137 individuals located at 12 farms.

2.1.2 Field procedures

Since 2002, all house sparrows at Leka have been captured in February each winter. Unmarked house sparrows were ringed with an individually numbered metal ring (supplied by Stavanger Museum, Norway) along with unique combinations of coloured plastic rings. This allowed for individual recognition of the birds in the field, which aids in the estimation of the population.

The birds were captured with mist nets in barns. Phenotypic traits and basal metabolic rate (BMR) were measured (described below), before they were released into a heated empty cow shed. Here they had *ad lib* access to food and water. This was the continuous sequence of action until all house sparrows on the island were captured. Accordingly, the birds spent between two and 11 days inside the cow shed before they were either released back to the farm they were captured, or sampled for POPs and hormone analyses as described below.

2.1.2.1 Measuring phenotypic traits and age determination

Bill length, bill depth and tarsus length were measured to the nearest 0.1 mm by slide calipers (Solberg and Ringsby, 1997). Wing length was measured using a ruler with an accuracy of 0.5 mm (Svensson, 1992). Body mass was measured with a 50-g Pesola spring balance to the nearest 0.1 g. For males, two types of badge measurements were performed. One was total badge size, which was the area covered with black feathers as well as feathers with a black base and light grey feather tips. The second was visible badge size. This was the area covered with black feathers without grey tips. For both badge measurements, the area was estimated using the regression equation developed by Møller (1987a;b): badge size (mm^2) = 166.7 + 0.45 [badge length (mm) x badge width (mm)]. Also, both measurements were adjusted by taking the square root in order to transform the values towards the same mean and variance as the other phenotypic traits.

In order to correct for measurement technique differences between field workers, each phenotypic measurement was adjusted to the corresponding measurement of T. H. Ringsby by regression techniques (Jensen et al., 2004).

Because the study population had been captured one time each year since 2002, birds captured unmarked were assumed to be recruits that hatched the previous year. This assumption was reasonable because a high proportion of the population (>90%) was captured and marked each winter. Given the high recapture rate, almost 100% of the adults were marked, which supports the assumption that unmarked individuals hatched previous year. For marked birds, age was estimated from registered data on first capture.

2.1.2.2 Measuring basal metabolic rate and selection

The BMR of all birds was measured based on estimating O_2-consumption rates using open flow-through respirometry. A Servomex Xentra, type 4100, two channel oxygen analyser and a Servomex two-channel analyser type 5400 (Servomex Controls, Crowborough, England) were used to measure oxygen concentration in the effluent air. Eight birds were measured simultaneously in separate black-enameled metabolic chambers (1.1 L) during the afternoon or night, i.e. during the birds' normal resting phase. The flow rate (500 mL/min) was adjusted using calibrated Bronkhorst High-Tech mass flow-meters (Ruurlo, the Netherlands). Furthermore, the ambient temperature in the climatic chamber containing the metabolic

chambers was kept at 25˚C which is within the thermoneutral zone for the house sparrow (Hudson and Kimzey, 1966).

After estimating individual whole body BMRs for the entire insular house sparrow population, the sparrows with a BMR above the population mean (76.5 mL/O_2 h for females and 78.5 mL/O_2 h for males) were selected for our project (n=49). This artificial selection procedure on BMR is part of a parallel project, investigating the evolutionary consequences of artificial selection on BMR across generations.

2.1.2.3 Sampling and storage procedures

Sampling of the sparrows took place on the 16th and 17th of February 2013. The birds were confined in individual cages during this time. They were collected one at the time and sacrificed by decapitation in an adjacent room. Blood was sampled during the sacrificial process and centrifuged in order to obtain blood fractioning. After the separation of plasma from other blood components, plasma was frozen in liquid nitrogen and kept for hormone analyses. Liver for chemical analysis, as well as gonads and brain, were extracted in a dissection procedure immediately post mortem and frozen in liquid nitrogen (gonads, brain and a fraction of the liver were stored for other projects). All treatment of the birds was according to standards of animal welfare and approved by the National Animal Research Authority (Oslo, Norway).

The plasma and liver samples were transported to the Norwegian University of Science and Technology (NTNU), Trondheim in liquid nitrogen. At NTNU they were transferred to a freezer (-80°C) where they were kept until final thyroid and POPs analysis.

2.2 Thyroid hormone analysis

House sparrow plasma samples (≤ 200 μL) were analysed for FT3 and FT4 plasma concentrations. The analysis was conducted with commercial radioimmunoassay (RIA) kits with coated tubes (Cout-A-Count Free T3 and Coat-A-Count Free T4, Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA) at the Department of Biology, NTNU, Trondheim in May 2013. Quantification was done on a gamma scintillation counter (Cobra Auto-Gamma, model 5003, Packard Instruments Company, Dowers Grove, IL, USA) with standard curves based on triplicates of seven known standards (FT3: 0, 0.91, 2.60, 5.84, 12.13, 35.33, 72.19 pmol L^{-1}, FT4: 0, 1.16, 6.95, 18.02, 34.75, 72.07, 168.60 pmol L^{-1}). Standard curves and TH levels were calculated with the gamma counter software (Spectra Works Spectrum Analysis Software, Meriden, USA).

2.2.1 The principle of Radioimmunoassay

The Coat-A-Count FT3 and FT4 procedures are solid-phase RIAs designed for the quantitative measurement of non-protein-bound T3 and T4 serum levels. The method is based on ^{125}I-labeled TH analogs competing with the corresponding THs in the sample for sites on TH specific antibodies. The antibodies are attached to the wall of tubes provided from the kit. The gamma counter measures the level of bound radiolabeled TH analogs, which is inversely related to the TH concentration in the sample. RIA is frequently used as an assessment tool of thyroid status and is acknowledged as a valid method for this purpose (Jaffe, 1978). It is suitable for extrapolation to birds (Davis et al., 2000; Verreault et al., 2007).

2.2.2 RIA procedure

Upon analysis, serum samples were thawed in room temperature and added to the coated tubes. The kits procedures were followed (Siemens, 2012a;b) with only an adjustment in the number of replicates per sample. The most common procedure is to analyse FT3 as duplicates and FT4 as triplicates. However, a limited sample volume inhibited a fulfillment of these conditions. For FT3, 48 samples were run; all of them as singles. For FT4, 45 samples were run; 10 as singles and the rest as duplicates. The kits had analytical sensitivities of 0.31 pmol L^{-1} for FT3 and 0.13 pmol L^{-1} for FT4.

2.2.2.1 Quality control

Standard reference material (SRM); human serum controls (Lyphochek® Immunoassay Plus Control level 1, 2 and 3, Biorad Laboratories, CA, USA) from the kit and the laboratory's own quality control, bovine (*Bos primigenius*) plasma samples, were run for both kits as quality insurance.

In order to investigate intra-assay variation, SRM was analysed at the beginning and end of the RIA-run for FT3, and at the beginning, in the middle and in the end for FT4. All coefficient of variation (CV%) values of the reference material were below 15% (1.0-13.8%) for FT4. A range limit of 15% is according to the laboratory's control routines and indicates an acceptable level of variation.

12 out of the 45 FT4 plasma samples were assigned a %CV value (range 0.9-131.7%). Three out of these 12 values were below the acceptable level of variation (15%), while the remaining 10 values ranged from 18.6 to 131.7%. 33 FT4 samples were not assigned a %CV value. For the FT4 duplicate samples, the lack of %CV values was a result of FT4 levels below the limit of detection (LOD). Consequently, no dose was calculated for the samples in question. For the FT4 samples run as singles, as well as all FT3 samples, %CV values were not possible to obtain due to the fact that they were run as singles.

Instrumental LOD were 0.075 pmol L^{-1} for FT3 and 0.0028 pmol L^{-1} for FT4. Blank samples were below the LOD. Results from 20 out of 48 individuals analysed for FT3 were non-detectable, and therefore omitted. For FT4, 18 out of 45 individuals were omitted on the same basis. In general, all levels of both FT3 and FT4 were low/close to the LOD (0.08-1.54 pmol L^{-1} for FT3 and 0.003-0.733 pmol L^{-1} for FT4, respectively). Low levels make quantification during RIA more prone to variations, which likely explain the high %CV values for some of the FT4 samples. Similar argumentation has been used previously in another study experiencing high %CV for TH levels measured with RIA (Villanger et al., 2011). The fact that FT4 was measured in duplicates (and singles) instead of triplicates may also have affected the %CV values. Based on the above argumentation, all FT4 samples with detectable results were included despite high %CV values. However, these results should be treated with caution.

2.3 Contaminant analysis

The liver samples were analysed for a selection of POPs at the Laboratory of Environmental Toxicology at the Norwegian University of Life Sciences (NMBU), previously Norwegian School of Veterinary Science (NVH) in Oslo. The laboratory is accredited by Norwegian Accreditation for measurements of organochlorines (OCs), brominated flame retardants (BFRs) and lipid content in biological matrices according to the requirements of the NS-EN/IEC 17025 (test 137). The analysed PCBs, BFRs and OCPs are listed in Table 2.1. Analyses were conducted during fall of 2013.

Table 2.1. The organochlorinated and brominated contaminants analysed in liver samples from house sparrows collected at Leka, Norway during winter 2013.

Group	Acronym	Analyte (IUPAC name)
OCPs	HCB	Hexachlorobenzene
	4,4'-DDE (*p,p'*)	*bis*-2,2-(4-chlorophenyl)-1,1-dichloroethylene
PCBs	PCB-28	2,4,4'-trichlorobiphenyl
	PCB-52	2,2',5,5'-tetrachlorobiphenyl
	PCB-101	2,2',4,5,5'-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
PBDEs	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'-hexabromobiphenyl ether
	BDE-154	2,2',4,4',5,6'-hexabromobiphenyl ether
	BDE-209	Decabromodiphenyl ether
HBCDD	HBCDD	Hexabromocyclododecane

2.3.1 Lipid extraction

The analytical method is based on principles of liquid/liquid extraction. Brevik (1978) was the first to describe the applied method, which was later modified by Polder et al. (2008).

Before use, all glass equipment was cleaned with a 1:1 mixture of acetone and cyclohexane. Liver samples were thawed for a short period in room temperature. They were manually homogenized with a scalpel, transferred to 80 mL centrifugation tubes and weighed to three decimals (0.278-0.672 g). A mixture of internal standards (I.S.) was added to the samples; PCB I.S. (PCB-29, -112 and -207, Ultra Scientific, RI, USA) and BFR I.S. (BDE-77, -119, -181 and -$^{13}C_{12}$-209, Cambridge isotope laboratories, Andover, MA, USA). 2 mL 6% NaCl and 10 mL distilled water in addition to acetone and cyclohexane (3:4) was added for the first extraction. Samples were sonicated for 2 min with an ultrasonic homogenizer (Cole Parmer CPX 750, Vernon Hills IL, USA) and centrifuged (Allegra X-12R Beckman Coulter, Fullerton, CA, USA). The supernatants were transferred to Zymark tubes. Further, a second round of extraction of the sample solution was performed with adding acetone and cyclohexane (1:2), sonicating for 1 min and centrifuging. The new supernatants were collected and added to their respective first supernatant. Further, they were concentrated by evaporation in a Zymark© evaporation system (Zymark TurboVap II, Zymark Corporation, Hopkinton, MA, USA) with a steady flow of nitrogen (purity: 99.6%; AGA AS, Oslo, Norway, pressure: 0.6 bar) at 40°C. The concentrated lipid extracts were quantitatively transferred to 5 mL volumetric flasks. The final volume was adjusted to 5 mL with cyclohexane.

2.3.2 Gravimetric lipid determination and acid clean up

A 1 mL aliquot of the sample was transferred to pre-weighed glass vials (8 mL) and placed in a sand bath (40°C) over night. After reaching room temperature, the vials were weighed once, dried with nitrogen gas for 10-15 min and weighed a second time. Since each paralleled weight measurement were within ± 0.0020 g for each glass vial, the weight was said to be constant. For calculation of percentage lipid content (Equation 1) the lowest measured weight for each vial was used.

The remaining 4 mL lipid extracts were cleaned with 4 mL sulfuric acid (H_2SO_4, purity: 97.5%; Fluka analytical, Sigma-Aldrich, St. Louis, USA). After centrifugation, the extracts were carefully transferred to clean tubes while checking for acid residues. A concentration of the

lipid-free extracts was performed by evaporation in a steady flow of nitrogen to a volume of 0.2 mL. Finally, the extracts were transferred to gas chromatography (GC) vials.

$$\% \text{ lipid} = \frac{(\text{weight vial w. fat} - \text{weight empty vial}) * \text{mL lipid extract}}{\text{weight initial sample} * \text{extracted mL lipid extract}} * 100 \qquad \text{(Equation 1)}$$

2.3.3 Gas chromatography analysis

All contaminants listed in Table 2.1 were quantified in the liver extracts using high resolution GC (Agilent 6890 Series GC system, Agilent Technologies, Santa Clara, CA, USA) equipped with an auto sampler (Agilent 7683 Series, Agilent Technologies) and configurated with additional quantification equipment. For OCs (OCPs and PCBs) the configurated quantifier was two ^{63}Ni μ-electron capture detectors (Agilent 6890 μ-ECD, Agilent Technology) coupled to the GC system, and for BFRs (PBDEs and HBCDD) it was a mass spectrometer (MS; Agilent 5973 Network Mass Selective Detector, Agilent Technologies).

2.3.3.1 OCs (OCPs and PCBs)

For separation and detection of OCs, 2 μL final sample solution was injected on a 1 m long pre-column. The pre-column was connected to a dual column system with columns of different polarity and selectivity (SPB-5 and SPB-1701) (60 m, 0.25 mm i.d., 0.25 μm film; Supelco, Bellefonte, PA, USA) in order to optimize the separation. Injector temperature was 270 ˚C and injector mode was pulsed splitless. Hydrogen (H_2) (purity 5.0, Hydro gas, Rjukan, Norway) was the carrier gas with a constant flow of 0.9 mL/min. Make up gas was 5% methane in Argon (Hydro gas, Rjukan, Norway). The temperature programme was as follows: 90 ˚C (2 min hold); 25 ˚C/min increase to 180 ˚C (2 min hold); 1.5 ˚C/min increase to 220 ˚C (2 min hold); and 2 ˚C/min increase to 275 ˚C (15 min hold). Total run time of 70 min. Detector temperature of 300 ˚C (Polder et al., 2008). Detection limits were determined as three times the noise level. LOD for individual compounds are presented in Table 2.2.

2.3.3.2 BFRs (PBDEs and HBCDD)

For separation and detection of BFRs, 1 μL final sample solution was injected on a DB-5MS column (30 m, film thickness 0.25 μm, inner diameter 0.25 mm, J & W Scientific, Agilent Technologies). Injector temperature was 250 ˚C and injector mode was pulsed splitless. Helium (He) (purity 6.0, AGA) was the carrier gas with a constant flow of 1.6 mL/min. The temperature program was as follows: 90 ˚C (2 min hold); 25 ˚C/min increase to 180 ˚C (1 min hold); 2.5 ˚C/min increase to 220 ˚C (5 min hold); and 20 ˚C/min increase to 320 ˚C (4 min hold). Total run time was 30.60 min and the quadrapole was 150 ˚C. Target ions used: BDE-28, -47, -77, -119, -100, -99, -154, -153, -181: m/z 71.0, HBCDD: m/z 79.0-81.0 (Sørmo et al., 2006). Detection limits for individual compounds were determined as three times the noise level, except for BDE-209. Due to procedural blank problems, the quantification limit (LOQ) for BDE-209 was set to mean of blank values plus two times standard deviation (SD). HBCDD was not detected at concentrations above LOD in any of the analysed samples (n=49), and therefore excluded from further statistical analyses. The LODs/LOQs of individual analytes are presented in Table 2.2.

Table 2.2. Limit of detection (LOD) and limit of quantification (LOQ)***** for all compounds investigated in house sparrow hepatic tissue using either GC-ECD (OCs) or GC-MS (BFRs).

Organochlorines (OCs)	LODs/LOQs*	Brominated flame retardants (BFRs)	LODs/LOQs*
HCB	0.075	BDE-28	0.125
p,p'-DDE	0.147	BDE-47	0.105
PCB-28	0.097	BDE-99	0.095
PCB-52	0.057	BDE-100	0.075
PCB-101	0.052	BDE-153	0.120
PCB-118	0.098	BDE-154	0.120
PCB-138	0.073	BDE-209	0.965*
PCB-153	0.099	HBCDD	1.130
PCB-180	0.064		

2.3.4 Quality control

In order to ensure the quality of the analysis and thereby the results, several quality parameters were measured according to the laboratory's routines. For each series of 15-17 samples, three blank samples were included to control contamination from air, solvents, equipment, and other background sources. The blank samples consisted of I.S. and solvents. One sample of the laboratory's own reference material (seal blubber) was included in each series in order to validate the reproducibility and repeatability of the analysis. Matrices of uncontaminated cattle muscle tissue were included in each series as well, with the intent to check recovery of contaminants. One sample was analysed as a blind sample and two others were spiked with target analytes. The relative recoveries (recoveries of analytes corrected for recovery of the I.S.) of the analytes were measured. For OCs, recovery ranged from 81 to 123% and for PBDE congeners 47-154 it ranged from 95 to 110%. These recoveries were within the acceptable range. The recovery for BDE-209 was 158% and for HBCDD it was 378%, both higher than the acceptable range. Because the quantification limit of BDE-209 was set to mean of blank values plus two times SD, results above this limit were regarded as acceptable and therefore not corrected for recovery. This was found an acceptable treatment of the results. The recovery for HBCDD was probably influenced by decomposition in the injector. However, as stated previously, HBCDD was not detected in any of the samples and thus excluded from further evaluation of results. Other quality parameters were found satisfactory.

2.3.5 Calculations

Calculations of GC data were performed using GC ChemStation (Version B.04.03, Agilent Technologies) and MSD ChemStation (Version E.02.01, Agilent Technologies).

2.4 Data analysis

Statistical analyses were conducted using SPSS Statistical Software (Version 21 for Windows, IBM, SPSS Inc., Chicago, IL) and SIMCA P+ (Version 12.0, Umetrics, Umeå, Sweden). Contaminants detected in < 60% of the samples were excluded from the statistical analyses: BDE-28, BDE-153, BDE-154, BDE-209 and HBCDD. Without these, a total of 31 variables were included for further analyses: 15 contaminant variables (HCB, *p,p'*-DDE, ∑OCPs, PCB-28, PCB-52, PCB-101, PCB-118, PCB-138, PCB-153, PCB-180, ∑PCBs, BDE-47, BDE-99, BDE-100, ∑PBDEs), 13 biometric variables (capture location, age, sex, μtarsi (mean right and left tarsi), beak height, beak length, μwing (mean right and left wing), mask, total badge, visible badge, weight, BMR, and liver lipid %), and three hormone variables (FT3, FT4 and the ratio between FT3 and FT4 (FT4:FT3)). All contaminant concentrations are given as ng/g wet weight (ww) and TH levels as pmol/L.

Missing values were replaced by randomly generated numbers between 0 and LOD for the contaminant and hormone variables included in further analysis. This applied to PCB-28 (LOD 0.097 ng/g, [1 individual]), PCB-52 (LOD 0.052 ng/g, [16 individuals]), PCB-101 (LOD 0.052, ng/g [9 individuals]), PCB-118 (LOD 0.098 ng/g, [13 individuals]), PCB-153 (LOD 0.099 ng/g, [1 individual]), PCB-180 (LOD 0.064 ng/g, [1 individual]), BDE-47 (LOD 0.105 ng/g, [11 individuals]), BDE-99 (LOD 0.095 ng/g, [4 individuals]), BDE-100 (LOD 0.075 ng/g, [14 individuals]), FT3 ([20 individuals]) and FT4 ([18 individuals]). Some of the biometric variables were lacking one value due to practical errors. The missing value was replaced by a random number between the lowest and the highest measurement for the variable in question. This applied to left tarsi (17.2-20.8 mm, [1 individual]), BMR (76.53-106.20 mL O_2/h, [1 individual]) and lipid % (0.46-5.68%, [1 individual]).

2.4.1 Multivariate data analysis

Multivariate analyses were performed using SIMCA P+ (version 12.0, Umetrics, Umeå, Sweden). All variables were mean-centered by subtracting variable mean to each observation in order to increase model interpretability, and scaled to unit-variance (UV) to allow comparison of variables with different SDs (Eriksson et al., 2006). Non-normally distributed variables were $\log_{10}$-transformed to approximate normality before analyses. The multivariate methods applied were principal component analysis (PCA) and orthogonal projections to latent

structures (O-PLS). These are designed to tolerate biological data with collinearity, noise, and a limited number of observations (Eriksson et al., 2006). PCA was used to explore the relationships among the variables and observations in a visualized manner, and O-PLS was used to model the effects of biometric characteristics and contaminant concentrations on hormone levels.

PCA is a multivariate method able to extract and display the systematic variation in a data matrix. The included variables are transformed to a dataset of uncorrelated variables orthogonally projected on each other. These transformed, uncorrelated variables are called principal components (PCs) and are significant if they have an eigenvalue > 1 (Eriksson et al., 2006). Validation of a PCA model is performed by evaluation of the validation parameters R^2X and Q^2. R^2X is a measure of explained variation; that is to what degree the model fits the data. Q^2 is a measure of the predicted variance; that is the predictability of the model (Eriksson et al., 2006). A good biological PCA model is characterized by a R^2X value > 0.5 and Q^2 value > 0.4 (Umetrics, 2008). Some observations were outside the Hotellings T^2 with a 95% confidence interval in the score plot, and thus explored as possible outliers.

The main principle of O-PLS is to divide the systematic variation in a set of predictor variables in two parts. One part models the correlations between each of the predictor (X) variable and Y, and one part models the variation in X that is unrelated to Y. Components correlated to Y are named predictive, and uncorrelated components are named orthogonal. In a single-Y model only one predictive component exists, but there is no limitation on the number of orthogonal components (Eriksson et al., 2006). Only single-Y O-PLS models were investigated in the present study, with FT3 and FT4 concentrations, and the FT4:FT3 ratio, as Y variables in separate models. Models were constructed for females and males separately. Nine biometric and 12 contaminant variables were included as X variables in each original female model, and 12 biometric and 12 contaminant variables were included as X variables in each original male model. The O-PLS models were tested by cross-validation in order to confirm reliability. The applied method was analysis of variance testing of cross-validated predictive residuals (CV-ANOVA), which tests the null hypothesis of equal residuals of two models fitted to the same dataset (Eriksson et al., 2008). The model was defined to be significant if it achieved a CV-ANOVA p-value ≤ 0.05. Each original model was optimized by step-wise removal of variables with variable importance in projection (VIP) values < 0.5, which is variables considered to have no/low importance in explaining Y in an O-PLS model. Variables with VIP values > 1 are considered to be the most important in explaining the variation in Y (Eriksson et al., 2008). If

the model had a CV-ANOVA $p > 0.05$ after removing variables with VIP < 0.5, a continued step-wise removal of variables with low VIP values were performed. If a model never achieved a p-value ≤ 0.05, it was defined as non-significant. Further, an assessment of the significant models' strength was conducted based on their validation parameters; R^2X (measure of explained variance in X-matrix), R^2Y (explained variance of Y by X matrix) and Q^2 (goodness of prediction) (Eriksson et al., 2006). For biological data, a good model is defined by an R^2Y value > 0.7 and a Q^2 value > 0.4 (Lundstedt et al., 1998).

An advantage with O-PLS compared to the conventional projection to latent structures by means of partial least squares (PLS) is the possibility of removing the non-correlated variation in X. This makes interpretation of the resulting PLS model easier, the detection limit for outliers is better, and the risk of over-fitting the model due to few components is reduced (Trygg and Wold, 2002).

2.4.2 Univariate and bivariate data analysis

All data variables were assessed for normality with Shapiro-Wilk's test for normality ($n < 50$) using SPSS Statistical Software (Version 21 for Windows, SPSS Inc., Chicago, IL). Logarithmic ($\log_{10}$ and ln) transformation was performed on the non-normally distributed variables in an attempt to achieve normal distribution. However, several of the variables were still non-normally distributed after transformation. Consequently, both parametric and non-parametric tests were performed. Independent samples student's t-test (further referred to as student's t-test) and Mann-Whitney U test were applied to test for variable differences between sex and age groups. One-way analysis of variances (ANOVA), Welch's ANOVA and Kruskal-Wallis one-way analysis of variance by ranks (further referred to as Kruskal-Wallis) were applied to test for variable differences between capture locations. The bivariate relationships among biometric variables, hormones and contaminants were investigated with bivariate Spearman's rank correlation test, especially focusing on the components included in significant O-PLS models. Significant correlations among biometric variables and between biometric and hormone variables will not be discussed in the present study, but are included in Appendix D. Significant correlations between hormones and contaminants are illustrated in scatter plots including Spearman's rank correlation coefficient (r_s) and probability level (p) and discussed further.

3 Results

3.1 Biometric characteristics

Table 3.1. Mean ± standard deviation (SD), median and range (min - max) of biometric variables of female and male house sparrows (*Passer domesticus*) sampled at Leka, Nord-Trøndelag in 2013.

	N[d]	Mean ± SD	Median	Min - Max
Females				
Age (years)	23	1.48 ± 1.25	1.00	1.00 – 7.00
Weight (g)	23	31.06 ± 1.35	31.10	28.70 – 34.10
Lipid %[a]	22	2.00 ± 0.90	2.04	0.46 – 3.85
BMR (mL O_2/h)	22	86.39 ± 6.68	87.24	76.53 – 106.20
µTarsi (mm)[b]	22	19.76 ± 0.65	19.81	18.50 – 21.25
µWing (mm)[c]	23	78.33 ± 1.54	78.04	75.80 – 82.93
Beak height (mm)	23	7.84 ± 0.21	7.81	7.48 – 8.31
Beak Length (mm)	23	13.27 ± 0.48	13.32	11.79 – 14.06
Males				
Age (years)	26	1.58 ± 1.12	1.00	1.00 – 5.00
Weight (g)	26	32.15 ± 1.62	32.05	29.20 – 35.90
Lipid %[a]	26	2.59 ± 1.14	2.45	1.13 – 5.68
BMR (mL O_2/h)	26	88.58 ± 6.33	86.62	79.15 – 101.76
µTarsi (mm)[b]	26	19.69 ± 0.72	19.78	17.27 – 20.43
µWing (mm)[c]	26	81.03 ± 1.21	81.05	78.76 – 82.71
Beak height (mm)	26	7.83 ± 0.20	7.83	7.45 – 8.15
Beak Length (mm)	26	13.28 ± 0.45	13.21	12.58 – 14.27
Mask (mm)	26	14.17 ± 1.14	14.30	11.40 – 16.70
Total badge (mm)	26	19.79 ± 1.30	19.81	16.84 – 22.83
Visible badge (mm)	26	14.35 ± 0.67	14.60	13.09 – 15.30

a Lipid percent in liver sample.

b The mean of right and left tarsi

c The mean of right and left wing

d Number of individuals measured for the trait in question.

Biometric measurements and estimated age of the 49 sampled house sparrows are summarized in Table 3.1. Individual biometrics are listed in Appendix A.

3.2 Thyroid hormones

Table 3.2 summarizes the results from thyroid analysis. FT3 was detected in 58.3% of the samples and FT4 in 60.0% of the samples.

Table 3.2. Mean ± standard deviation (SD), median and range (min - max) levels of FT3 and FT4, and the FT4:FT3 ratio in plasma from house sparrows (*Passer domesticus*) sampled at Leka, Nord-Trøndelag in 2013.

Thyroid variable	N[a]	Mean ± SD	Median	Min – Max
FT3 (pmol/L)	28	0.47 ± 0.44	0.30	0.08 – 1.54
FT4 (pmol/L)	27	0.17 ± 0.21	0.07	< 0.01 – 0.73
FT4:FT3	20	0.58 ± 0.60	0.44	0.04 – 2.57

a Number of samples with detectable concentrations/achievable ratio

3.3 Environmental contaminants

Table 3.3 summarizes the results from contaminant analysis. In total, 12 contaminants, including two OCPs, seven PCBs and three PBDEs, were detected in > 60% of the samples. In decreasing order, the contaminants measured at highest concentrations were PCB-153 > PCB-180 > *p,p'*-DDE > PCB-138 > BDE-99 > HCB > BDE-47 > PCB-118 > PCB-52 > BDE-100 > PCB-28 > PCB-101. Three individuals had extremely high levels of PCB-138, -153 and -180 compared to the other birds with a range from 9.1-15.0 ng/g, 12.8-32.3 ng/g, and 17.5-19.7 ng/g ww, respectively (mean without the extreme values: 1.0, 1.8, and 0.9 ng/g ww, respectively). These individual measurements are the reason for the high SDs for PCB-138, -153, -180 and ∑PCBs presented in Table 3.3.

Table 3.3. Mean ± standard deviation (SD), median and range (min - max) of contaminant concentrations measured in liver samples from female and male house sparrows (*Passer domesticus*) sampled at Leka, Nord-Trøndelag in 2013.

Analyte (ng/g ww)	N[d]	Mean ± SD	Median	Min - Max
Females				
HCB	23	0.80 ± 0.26	0.68	0.51 – 1.64
p,p'-DDE	23	1.43 ± 0.96	1.00	0.15 – 3.19
∑OCPs[a]	23	2.23 ± 1.10	2.00	0.74 – 4.22
PCB-28	22	0.15 ± 0.03	0.14	0.10 – 0.23
PCB-52	16	0.17 ± 0.06	0.19	0.06 – 0.25
PCB-101	19	0.15 ± 0.06	0.15	0.05 – 0.27
PCB-118	15	0.54 ± 0.48	0.37	0.12 – 2.07
PCB-138	23	1.50 ± 2.89	0.71	0.14 – 14.95
PCB-153	22	3.10 ± 6.04	1.43	0.32 – 29.43
PCB-180	22	1.80 ± 3.57	0.71	0.14 – 17.53
∑PCBs[b]	23	6.93 ± 12.63	3.91	0.26 – 64.45
BDE-47	18	0.36 ± 0.18	0.32	0.15 – 0.80
BDE-99	21	0.71 ± 0.37	0.71	0.16 – 1.65
BDE-100	17	0.14 ± 0.06	0.12	0.08 – 0.30
∑PBDEs[c]	21	1.14 ± 0.65	1.08	0.16 – 2.70
Males				
HCB	26	1.06 ± 0.51	0.96	0.46 – 2.92
p,p'-DDE	26	2.34 ± 1.45	2.17	0.36 – 5.74
∑OCPs[a]	26	3.40 ± 1.65	3.35	0.97 – 6.69
PCB-28	26	0.21 ± 0.19	0.16	0.10 – 1.14
PCB-52	17	0.24 ± 0.17	0.22	0.07 – 0.81
PCB-101	21	0.19 ± 0.10	0.18	0.07 – 0.49
PCB-118	21	0.59 ± 0.65	0.35	0.10 – 2.74
PCB-138	26	1.74 ± 2.74	0.92	0.17 – 12.26
PCB-153	26	3.76 ± 6.98	1.75	0.22 – 32.26
PCB-180	26	2.23 ± 4.94	0.75	0.07 – 19.67

Table. 3.3 continued.

∑PCBs[b]	26	8.72 ± 15.19	4.24	0.57 – 67.49
BDE-47	20	1.02 ± 2.20	0.27	0.11 – 10.33
BDE-99	24	1.55 ± 2.62	0.61	0.10 – 12.94
BDE-100	18	0.23 ± 0.18	0.15	0.04 – 0.59
∑PBDEs[c]	24	2.58 ± 4.78	0.91	0.10 – 23.81

a ∑OCPs include HCB and *p,p'*-DDE

b∑PCBs include the seven PCB congeners -28, -52, -101, -118, -138, -153 and -180.

c ∑PBDEs include the three PBDE congeners -47, -99 and -100.

d Number of samples with detectable concentrations.

3.4 Principal component analysis and further investigations of possible trends

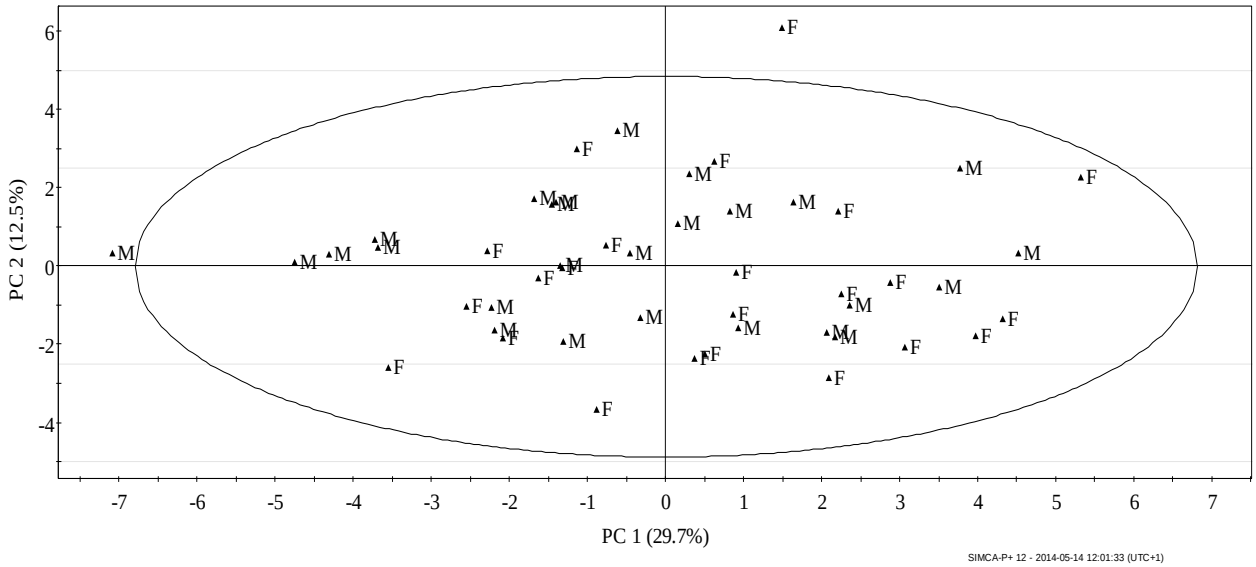
The PCA of all observations and 23 variables (9 biometric, 12 contaminant and two hormone variables) resulted in a PCA model with five significant components (eigenvalues > 1). The five components explained the following percentages of variation in the model: PC1: 29.7%, PC2: 12.5%, PC3: 10.4%, PC4: 7.8% and PC5: 6.4%. In Figure 3.1A, B and C, only PC1 and PC2 are graphed. The validation parameters for the PCA model were $R^2X = 0.668$ and $Q^2 = 0.116$. The R^2X value is above and the Q^2 value is below the values characterizing a good PCA model based on biological data ($R^2X > 0.5$ and $Q^2 > 0.4$ (Umetrics, 2008)). Two observations were outside the Hotellings T2 range (Figure 3.1A), and therefore treated as possible outliers. Further statistical analyses were conducted both with and without these observations. However, no improvement of test statistics were observed in the analyses excluding the observations in question. In addition, the biometric measurements, hormone levels and contaminant burden in each individual represent the spread in the score plot, resulting in a natural distribution throughout the plot according to these characteristics. Thus, the reason for the observations' placement outside the Hotellings T2 range ellipse (representing the 95% confidence interval) was identified as natural variation, and all observations were included in final models.

A weak tendency of females and males clustering in opposite ends of PC1 are visible in Figure 3.1A. This trend was investigated further with parametric comparison of means and non-

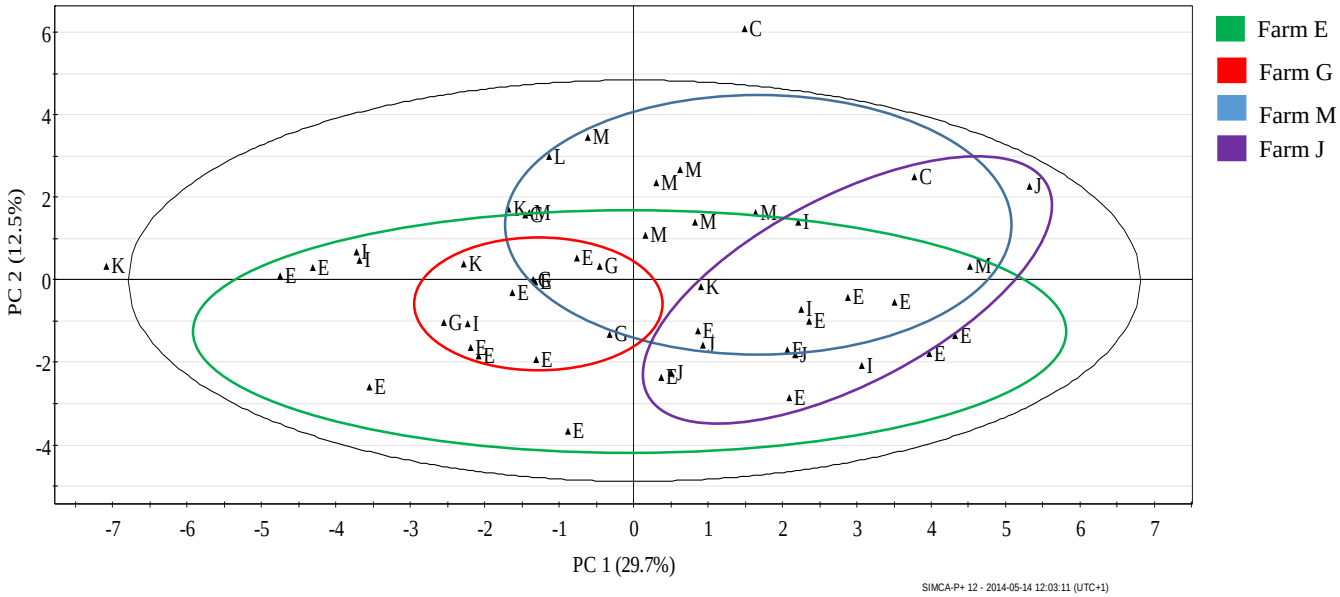
parametric comparison of distribution across groups. Parametric student's t-test confirmed significant sex differences in the normally distributed variables weight ($t = -2.465$, $p=0.017$), μWing ($t = -6.350$, $p < 0.001$) and *p,p'*-DDE ($t = -2.508$, $p=0.016$). Non-parametric Mann-Whitney U test confirmed a sex difference in non-normally distributed HCB levels ($U = 404.5$, $p=0.04$). All other variables were non-significant when tested for sex differences (student's t-test/ Mann-Whitney U test, $p > 0.05$). As some of the variables had significant sex variation, all further analyses were conducted with females and males as separate groups, improving accuracy of analyses and facilitating additional sex difference investigations.

Another trend observed in the PCA score plot (Figure 3.1B), was a distribution of observations according to capture location. To a certain degree, birds from the same farm seemed to cluster together. This was tested further and a significant variation across farms was confirmed with one-way ANOVA for weight ($F = 8.692$, $p < 0.001$), BDE-99 ($F = 5.136$, $p = 0.001$) and visible badge ($F = 3.754$, $p = 0.012$), and with Welch's ANOVA for BDE-100 ($F' = 22.546$, $p < 0.001$) and *p,p'*-DDE ($F' = 6.401$, $p = 0.005$). In addition, Kruskal-Wallis revealed a significant farm variation for BDE-47 ($H = 28.5$, $p < 0.001$), HCB ($H = 16.0$, $p = 0.025$) and PCB-52 ($H = 23.3$, $p = 0.002$).

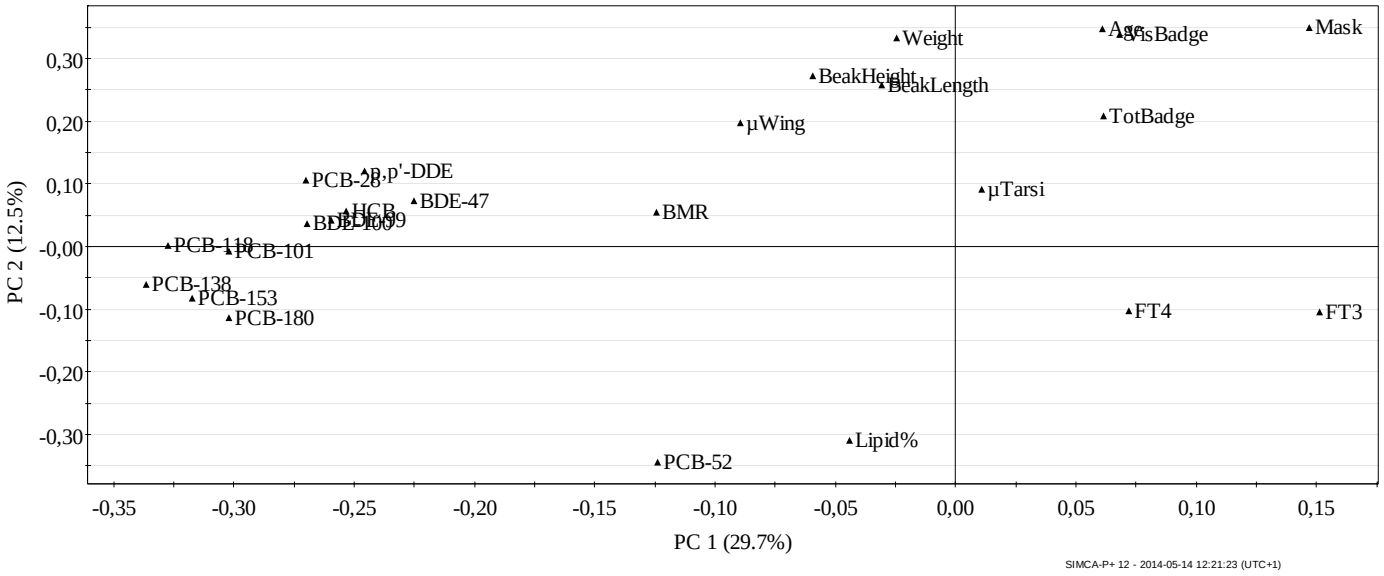
Figure 3.1C visualizes the relationships between the different variables in a PCA loading plot. All contaminant variables except for PCB-52 are clustered on the far left side around PC1. PCB-52 seems to be explained to a higher degree by PC2 than PC1, unlike the other contaminants, which mainly seem to be explained by PC1. FT3 is in the opposite end of PC1 as the contaminant cluster, while FT4 is positioned further towards the plot centre, but still relatively close to FT3 along the same axis. This indicates a negative relationship between the THs and the contaminants (except for PCB-52), with FT3 possibly having a stronger relationship to the contaminants than FT4 based on their position. FT3 and FT4 are placed in relatively close proximity to each other, indicating a potential correlation between these two hormone variables. The majority of biometric variables (not including lipid %, BMR and mask) are in rather close vicinity of each other around one end of PC2, indicating correlations among these variables. Age and PCB-52 are in directly opposite ends of PC2, and separated to a certain extent on the PC1-axis, indicating a negative relationship between these two variables. A significant Mann-Whitney U test confirmed age variation in PCB-52 levels ($U = 19.0$, $p < 0.001$), and further bivariate testing showed that the correlation between age and PCB-52 concentration was significantly negative (Spearman's rank correlation ($r_s = -0.677$, $p < 0.001$).


PC 2 (12.5%)
PC 1 (29.7%)
SIMCA-P+ 12 - 2014-05-14 12:01:33 (UTC+1)

A


Farm E
Farm G
Farm M
Farm J
PC 2 (12.5%)
PC 1 (29.7%)
SIMCA-P+ 12 - 2014-05-14 12:03:11 (UTC+1)

B



C

Figure 3.1. Principal component analysis (PCA) score plots (A, B) and loading plot (C) of the relationships among house sparrow observations (n=49) and variables (n=23; age, weight, BMR, µTarsi, µWing, mask, visBadge, totBadge, lipid%, FT3, FT4, three PBDEs, two chlorinated pesticides and seven PCBs. PC1 = 0.297, PC2 = 0.125, R^2X = 0.668, Q^2 = 0.116. In score plots (A, B), each symbol represent an individual. In (A) score plot, F denotes female and M denotes male individual. In (B) score plot, C, E, G, I, J, K, L and M denotes different capture locations, i.e. the farm in which the individual was captured. Coloured circles indicate clustering of individuals according to capture location. Individuals from farm E are circled with green, farm G with red, farm J with purple, and M with blue. Individuals from farm C, I, K and L did not cluster, and were thus not circled.

3.5 Orthogonal projections to latent structures and bivariate correlation tests

A further investigation of the associations observed in the PCA plots was conducted by O-PLS modelling. The final models presents interpretations of the relationship between a single Y variable (FT3, FT4 or FT4:FT3) and a set of predictor variables (biometric and contaminant variables). Predictor variables with a VIP value > 1 are considered important in explaining the variation in the Y-variable, while variables with a VIP value < 0.5 are assumed to have no/low importance in explaining the same variance. These low-importance variables were excluded step-wise in order to optimize the final model. Test statistics in the form of CV-ANOVA p-values and validation parameters (R^2X, R^2Y and Q^2) for final models are summarized in Table 3.4. Significant models were achieved for FT3 and FT4 in females, and FT3 in males (CV-ANOVA, $p < 0.05$). No significant model was achieved for FT4 in males, nor for FT4:FT3 in either of the sexes (CV-ANOVA, $p > 0.05$).

Table 4.1. Final orthogonal projections of latent structures (O-PLS) models obtained by optimizing the original model containing 23 predictive (X) variables: levels of OCPs, PCBs and PBDEs, and biometric variables of house sparrows (*Passer domesticus*) sampled at Leka, Nord-Trøndelag, in 2013. Optimization of the models was achieved by step-wise removal of X variables with VIP values < 0.5, or until the model was significant (CV-ANOVA, $p \leq 0.05$).

O-PLS model				Validation			
Y	Final model	X	Components (P + O)[a]	R^2X	R^2Y	Q^2	CV-ANOVA
Females							
FT3	sig.	11	1 + 0	0.320	0.441	0.271	0.042
FT4	sig.	14	1 + 0	0.213	0.532	0.300	0.028
FT4:FT3	n.s.[b]	-	-	-	-	-	-
Males							
FT3	sig.	3	1 + 0	0.660	0.351	0.287	0.021
FT4	n.s.[b]	-	-	-	-	-	-
FT4:FT3	n.s.[b]	-	-	-	-	-	-

a P = predictive components, O = orthogonal components

b n.s. = not significant

3.5.1 FT3 in females

The O-PLS model with female FT3 as Y-variable was statistically significant (CV-ANOVA, p = 0.042) after removing variables with VIP values < 0.5. The model had low validation power ($R^2X = 0.320$, $R^2Y = 0.441$, $Q^2 = 0.271$) compared to ideal validation. A good model based on biological data should have an R^2 value > 0.7 and a Q^2 value > 0.4 (Lundstedt et al., 1998). The final FT3 model included seven contaminants and four biometric variables (Figure 3.2). Four of the predictor variables had VIP values above 1: *p,p'*-DDE > PCB-28 > PCB-118 > weight. All contaminants included in the final model were negatively correlated to FT3, except for PCB-52, which was positively correlated (Figure 3.2).

Further bivariate correlation analysis did not confirm the indicated positive correlation between PCB-52 and FT3 in females (Spearman's rank correlation, p > 0.05). However, significant negative correlations were observed between FT3 and *p,p*'-DDE (Figure 3.3A, $r_s = -0.630$ p = 0.001), and between FT3 and PCB-28 (Figure 3.3B, $r_s = -0.499$, p = 0.015). The scatter plots in Figure 3.3 illustrates the significant correlations found between female FT3 and contaminants in Spearman's rank correlation test.

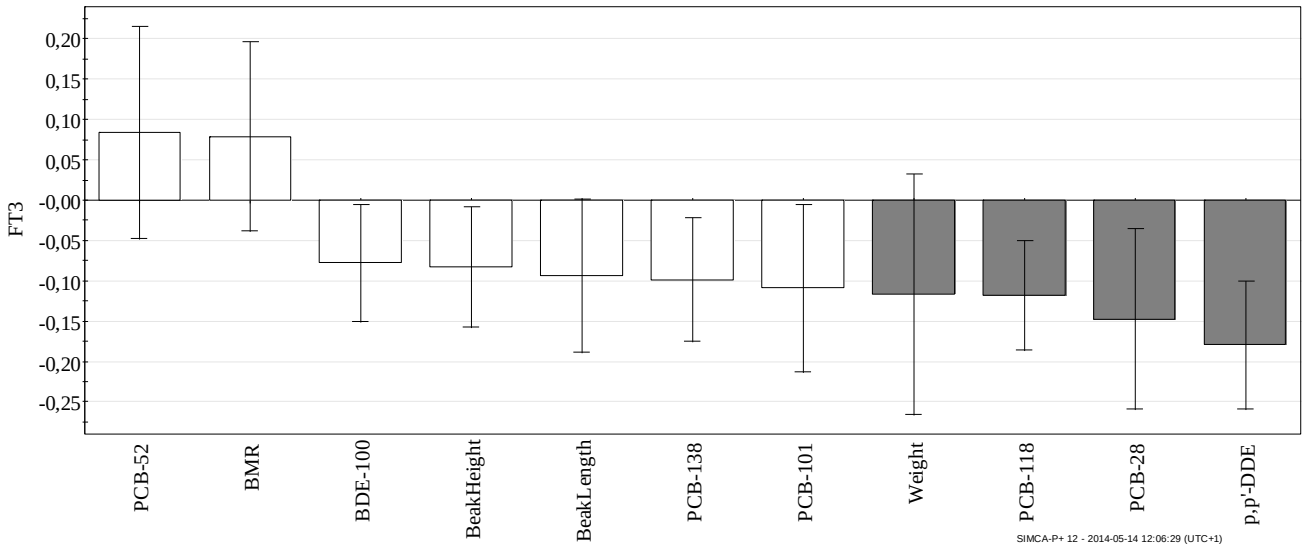
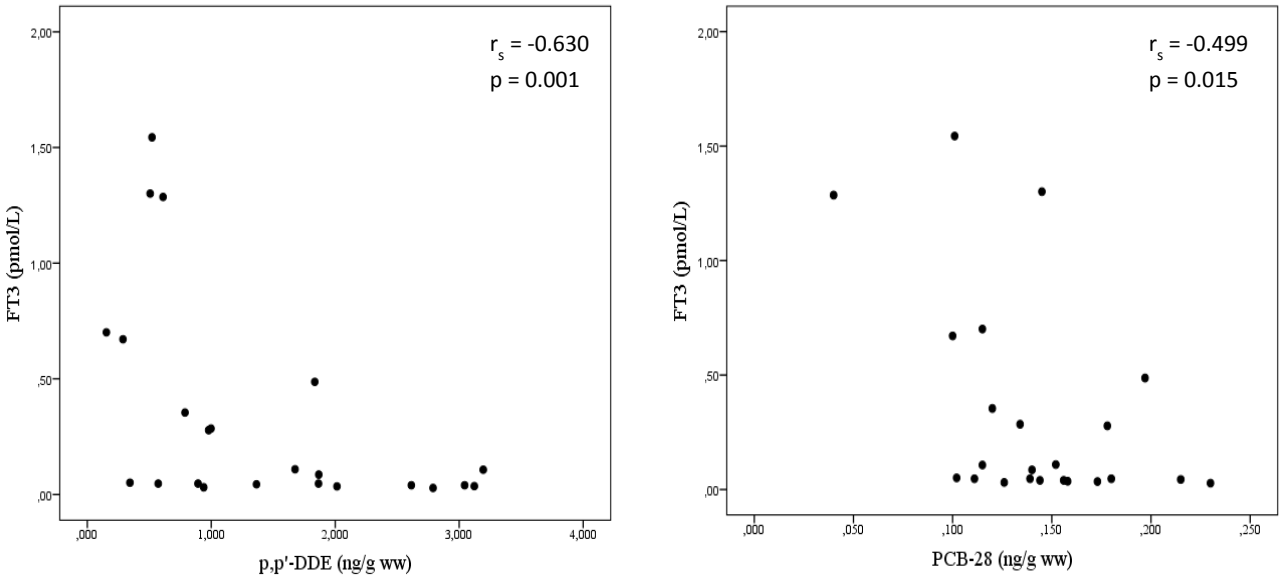


Figure 3.2. Orthogonal projections to latent structures (O-PLS) regression coefficient plot of final O-PLS model displaying the relationships between FT3 plasma levels (Y-variable) and a set of biometric and contaminant variables (predictor variables) in female house sparrow samples. Variables with VIP values < 0.5 are considered to have no/low importance for the variance in Y, and have thus been removed for optimization of the model. Closed column bars represent high-importance variables with a VIP value > 1. Negative coefficients indicate inverse relationships, and positive coefficient indicate positive relationships, between FT3 plasma levels and the predictor variables. The jacked-knife error bars reflects 95% confidence interval.



A B

Figure 3.3. Scatter plots showing the significant relationships between FT3 (pmol/L plasma) and contaminants (ng/g ww) in female house sparrows from Leka, Nord-Trøndelag. Significance is indicated by a p-value ≤ 0.05 in the bivariate correlation analysis; Spearman's rank correlation. r_s denotes Spearman's rank correlation coefficient. (A) FT3 vs p,p'-DDE: r_s = -0.630, p = 0.001; (B) FT3 vs PCB-28: r_s = -0.499, p = 0.015

3.5.2 FT3 in males

The O-PLS model for male FT3 was statistically significant (CV-ANOVA, p = 0.021) only when all predictive variables were removed except for three (Figure 3.4; totBadge, PCB-153 and PCB-180). Only totBadge had a VIP value > 1, and this was the only positively correlated variable in the model. Both PCB-153 and -180 was negatively associated with FT3 levels in male plasma. As with FT3 in females, the validation parameters for the final model (R^2X = 0.660, R^2Y = 0.351, Q^2 = 0.287) were not as strong as they ideally should be (R^2 > 0.7, Q^2 > 0.4; (Lundstedt et al., 1998)).

Further bivariate testing of the components in the final O-PLS model with Spearman's rank correlation test confirmed a significant negative correlation between FT3 and PCB-180 (r_s = -0.394, p = 0.047). No other correlations between FT3 and contaminants in male house sparrows were significant. Thus, the scatter plot in Figure 3.5 illustrates the single significant correlation found between male FT3 and contaminants in Spearman's rank correlation test.

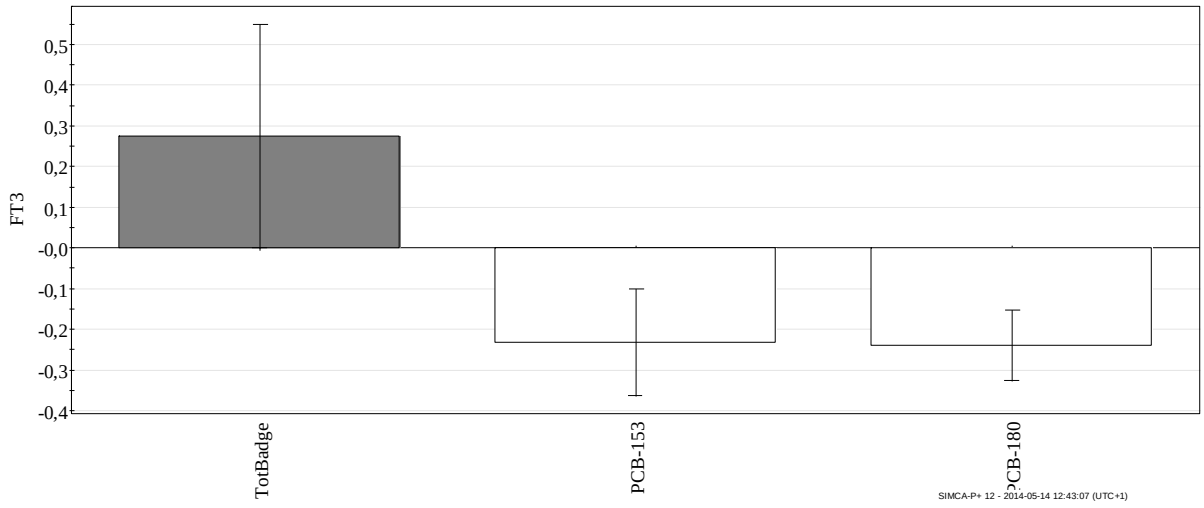


Figure 3.4. Orthogonal projections to latent structures (O-PLS) regression coefficient plot of final O-PLS model displaying the relationships between FT3 plasma levels (Y-variable) and a set of biometric and contaminant variables (predictor variables) in male house sparrow samples. Variables with VIP values < 0.5 are considered to have no/low importance for the variance in Y, and have thus been removed for optimization of the model. Closed column bars represent high-importance variables with a VIP value > 1. Negative coefficients indicate inverse relationships, and positive coefficient indicate positive relationships, between FT3 plasma levels and the predictor variables. The jacked-knife error bars reflects 95% confidence interval.

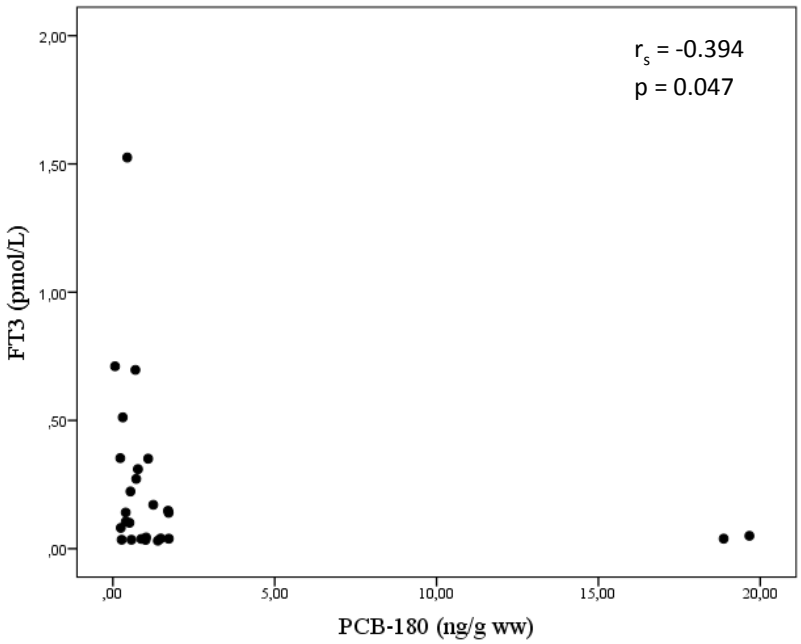


Figure 3.5. Scatter plot showing the only significant relationship between FT3 (pmol/L plasma) and contaminants (ng/g ww) in male house sparrows from Leka, Nord-Trøndelag. Significance is indicated by a p-value ≤ 0.05 in the bivariate correlation analysis; Spearman's rank correlation. r_s denotes Spearman's rank correlation coefficient. FT3 vs PCB-180: r_s = -0.394, p = 0.047.

3.5.3 FT4 in females

The O-PLS model for FT4 in females was significant (CV-ANOVA, p = 0.028) after removing variables with VIP values < 0.5. As with the two former models, the female FT4 model did not have a robust validity (R^2X = 0.213, R^2Y = 0.532, Q^2 = 0.300) compared to the characteristics of a good biological O-PLS model (R^2 > 0.7, Q^2 > 0.4; (Lundstedt et al., 1998)). The final model included seven contaminants (one OCP, four PCBs and two PBDEs) and seven biometric variables (Figure 3.6). Seven of the predictive variables had VIP values above 1 and were thus considered to be important for the model: BMR > PCB-28 > *p,p'*-DDE > BeakLength > BeakHeight > Weight > PCB-52. Out of these seven high-importance variables, two variables were positively correlated (BMR and PCB-52), and five were negatively correlated to FT4 (PCB-28, *p,p*'-DDE, BeakLength, BeakHeight, Weight).

Further bivariate testing with Spearman's rank correlation test confirmed a significant negative correlation between FT4 and *p,p*'-DDE (Figure 3.7A, r_s = -0.450 p = 0.031), and between FT4 and PCB-28 (Figure 3.7B, r_s = -0.439, p = 0.036). These significant relationships between FT4 and contaminants in female house sparrows are illustrated in the scatter plots in Figure 3.7.

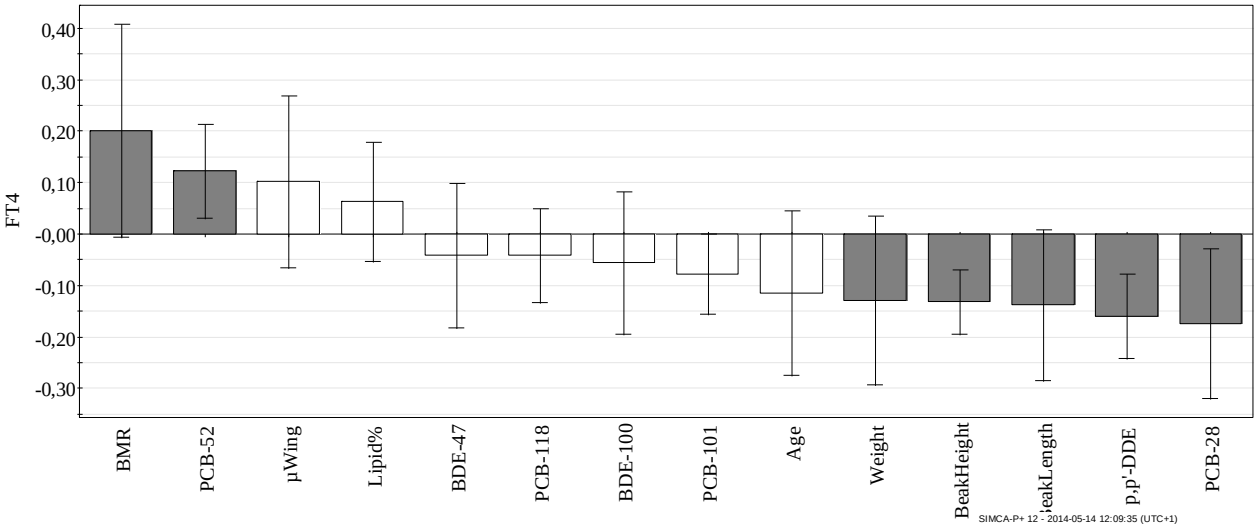
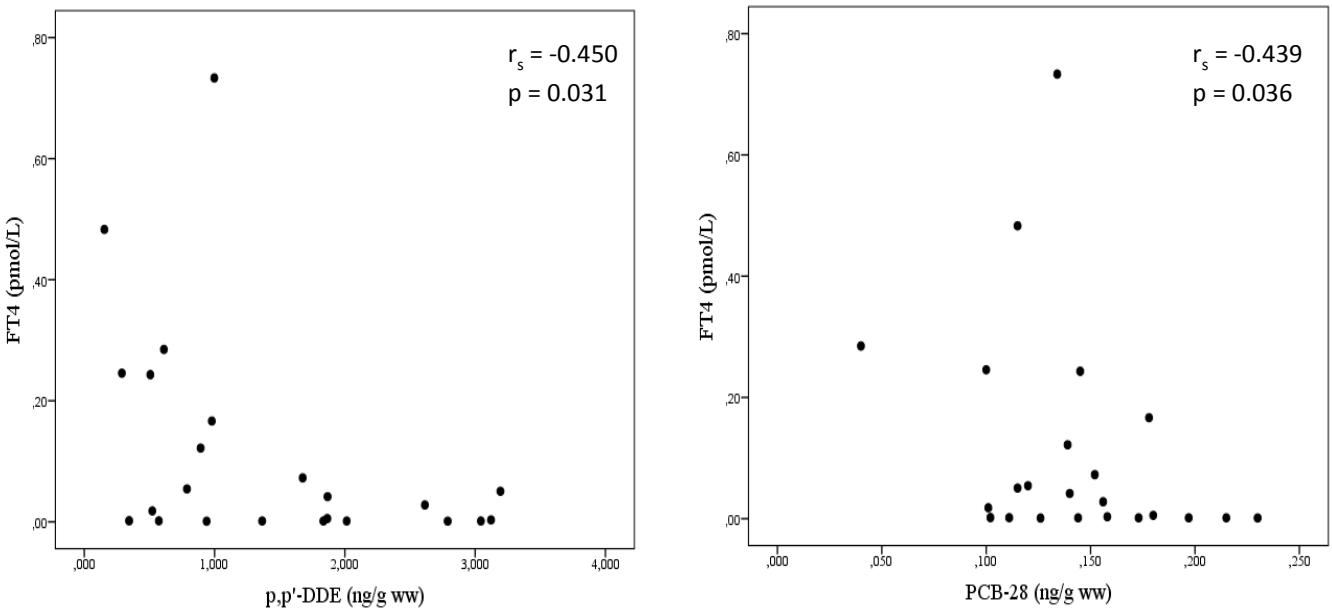


Figure 3.6. Orthogonal projections to latent structures (O-PLS) regression coefficient plot of final O-PLS model displaying the relationships between FT4 plasma levels (Y-variable) and a set of biometric and contaminant variables (predictor variables) in female house sparrow samples. Variables with VIP values < 0.5 are considered to have no/low importance for the variance in Y, and have thus been removed for optimization of the model. Closed column bars represent high-importance variables with a VIP value > 1. Negative coefficients indicate inverse relationships, and positive coefficient indicate positive relationships, between FT4 plasma levels and the predictor variables. The jacked-knife error bars reflects 95% confidence interval.



A B

Figure 3.7. Scatter plots showing the significant relationships between FT4 (pmol/L plasma) and contaminants (ng/g ww) in female house sparrows from Leka, Nord-Trøndelag. Significance is indicated by a p-value ≤ 0.05 in the bivariate correlation analysis; Spearman's rank correlation. r_s denotes Spearman's rank correlation coefficient. (A) FT4 vs p,p'-DDE: r_s = -0.450, p = 0.031; (B) FT4 vs PCB-28: r_s = -0.439, p = 0.036.

4 Discussion

4.1 Thyroid hormones

4.1.1 Levels of circulating FT3 and FT4

There is limited information on FT3 and FT4 plasma levels in passerine birds. Over the years, most studies have only analysed for the total levels of T3 and T4 (i.e. TT3 and TT4, respectively), and not separated between the free and bound fraction in sparrow plasma (Burger and Denver, 2002; Chastel et al., 2003; Reinert and Wilson, 1996; Smith, 1982; Wingfield et al., 2003). One passerine species with reported levels of circulating FT3 and FT4 is the Zebra finch (*Taeniopygia guttata*). Table 4.1 summarizes such plasma FT3 and FT4 concentrations measured in unexposed zebra finches included in recent PBDE exposure studies, and the levels measured in the present wildlife study. Comparison across species is necessary since there is a lack of information on levels of the free thyroid fractions in house sparrows. Species extrapolation is not ideal, but house sparrows and zebra finches are both small, passerine birds with seeds as main feed and they have both adapted to a life around humans. Such similar life strategies may produce a foundation for comparable endocrine systems. However, the fact that they have different breeding cycles, with house sparrows mainly breeding in April-August (Anderson, 2006) and zebra finches breeding when food availability peaks (Zann, 1996) may cause inaccuracy in a TH level comparison given the THs' role in reproductive processes. The reader should also bear in mind that the two species are adapted to very different environments. The zebra finches' natural habitat is Australia and parts of South East Asia, where ambient temperature is much higher than in Norway.

Table 4.1. Mean plasma concentrations (pmol/L) ± standard deviation (SD) of FT3 and FT4 in two species of passerine birds. Levels in females and males are presented as pooled values since none of the studies presented observed sex differences in TH levels (see section 4.3 herein, Eng et al. (2013), and Winter et al. (2013)).

Project	Experiment	Species	FT3 (pmol/L)	FT4 (pmol/L)
Present project	Wildlife	House sparrow (*Passer domesticus*)	0.47 ± 0.44	0.17 ± 0.21
Eng et al. (2013)	Laboratory	Zebra finch (*Taeniopygia guttata*)	2.58 ± 0.13	2.81 ± 0.5
Winter et al. (2013)	Laboratory	Zebra finch (*Taeniopygia guttata*)	4.86 ± 0.46	7.59 ± 0.10

The FT3 levels measured in zebra finches by Eng et al. (2013) and Winter et al. (2013) are 5.5 and 10.3 times higher than the levels measured herein, respectively. Species differences may be the main reason for this substantial variation, but the different natures of the experiments could also be important. The zebra finch studies were conducted in laboratories under controlled conditions with the control birds (in Table 4.1) being fed uncontaminated food. In a wildlife study, as the present one, food sources and activity of the birds are natural and uncontrolled. Consequently, the birds may be exposed to a variety of conditions affecting thyroid levels. Among other factors, food availability and temperature seem to be two of the most important factors in explaining variations in levels of circulating THs in birds (McNabb, 2007). Such factors are substantially easier to regulate in a controlled environment like a laboratory, than in a free-living population, and may be important in explaining the differences in thyroid levels observed in Table 4.1. In addition, age, diurnal patterns, iodine availability, season and reproductive conditions are important factors in explaining variations in avian thyroid levels (McNabb, 2007).

Another factor that may have influenced the house sparrow thyroid levels measured herein is toxicant exposure. In the present study, indications of THs being significantly affected by POPs were found. The observed significant relationships between the hormone levels and toxicant concentrations were all inverse (see Figure 3.3, 3.5 and 3.7), meaning that the level of POPs might have had a negative influence on the level of THs (FT3 and FT4 for females and FT3 for

males). Thus, the seemingly low levels of FT3 and FT4 in house sparrows compared to zebra finches in the present study may in part be caused by the presence of POPs (specifically *p,p'*-DDE and PCB-28 for females, and PCB-180 for males). However, this remains uncertain due to a lack of baseline FT3 and FT4 levels in house sparrows, and the observed variation in TH levels between house sparrows and zebra finches may simply be a result of species difference.

4.1.2 Relationship between FT3 and FT4 concentrations

The possible correlation between FT3 and FT4 indicated by the relatively close placement of these two variables along PC1 in the PCA loading plot (Figure 3.1C) was tested further with Spearman's rank correlation test. FT3 and FT4 levels were strongly correlated in females (r_s = 0.720, $p < 0.001$), but not in male house sparrows (r_s = 0.349, $p = 0.080$). Since T3 predominantly is produced by enzymatic deiodination of T4 in peripheral tissues, a correlation between the two hormones, as observed in females, was expected. However, the lack of a significant correlation between the THs in males was not surprising either. The close relationship between the hormones might not be evident in their free fractions since several factors (e.g. binding to carrier proteins, distribution to tissues, excretion) affect the ratio of free versus bound THs in the plasma, possibly masking the correlation when only free fractions are investigated. The total plasma levels of THs might have been significantly correlated herein even though the free fractions were not, but TT3 and TT4 were not investigate in the present study rendering such correlation analyses impossible to perform.

4.2 Levels of contaminants

Birds have an important role in the documentation of presence and effects of anthropogenic pollution with organic contaminants, and predatory bird species at high trophic levels are primarily chosen as sentinel species for such purposes (Ucán-Marín et al., 2009, 2010; 2007; 2013; Verreault et al., 2004). However recently, several studies have measured organohalogenated compounds in eggs of small passerine birds for biomonitoring purposes (Dauwe et al., 2003; 2006; 2007; Van den Steen et al., 2006; 2010a; 2010b; 2009). Despite the increasing number of toxicological exposure and effect studies on passerines, only a few have investigated hepatic levels of contaminants (Ciesielski et al., 2008; Jimènez et al., 2005). This complicates direct comparison of contaminant levels between the present and other studies. Inter-tissue comparisons are possible, but herein it will only be performed to give a general impression of the magnitude of contamination levels in the sparrows as compared to documented levels in other passerine studies. Concentrations based on lipid weight (lw) are used for comparisons between different tissues when possible in an attempt to remove variation in contaminant concentrations due to lipid content.

The contaminant levels measured herein were in general low, with a few individuals having high levels as interesting exceptions. Congener patterns for both PCBs and PBDEs were similar to those reported in other passerine studies (Chen et al., 2013; Dauwe et al., 2003; Eng et al., 2014), and concentration variations among farms were revealed for several contaminant congeners.

4.2.1 Polychlorinated biphenyls

PCBs was the dominant group of the measured contaminants with respect to mean concentrations (see Table 3.3). Statistical analyses revealed no significant sex variation for ∑PCBs (Mann-Whitney U test, $p > 0.5$). The level of individual PCB congeners detected in the sparrows were as follows (in decreasing order): PCB-153 > -180 > -138 > -118 > -52 > -28 > -101. This congener profile was identical in females and males, and were thus not sex dependent. The dominance of congener -153, -180 and -138 is in accordance with findings in eggs from European starlings (Eng et al., 2014), great tits (Dauwe et al., 2003; Van den Steen et al., 2006), and blue tits (Van den Steen et al., 2010b) from rural areas in Europe. The discussed congener profile reflects the congener composition of industrial mixtures (see section 4.2.1.2 herein).

4.2.1.1 Total PCB burden in sparrows

Jimenéz et al. (2005) investigated PCB concentrations in livers of house sparrows from Baja California Sur, Mexico. They reported a $\sum_{20}$ PCB concentration of 45 ng/g ww (it is unknown if this was the mean or the median concentration) in 25 sparrows living near small ranches and crop fields. This is several magnitudes higher than the levels documented herein (see Table 3.3). In fact, the authors claim that it is among the highest PCB concentrations recorded in species at low trophic levels. It is worth noticing that the number of PCB congeners included in the summed concentration is almost three times higher in the Jimenéz et al. study (2005) than in the present study (20 versus seven PCB congeners, respectively). The summed concentration of all PCB congeners might have been higher in the present study if a higher number of congeners was investigated (seven congeners investigated herein). Regardless of the number of congeners measured for, the Baja California Sur house sparrows would be expected to have elevated PCB levels compared to Leka house sparrows given the differences in sampling area. The Mexican sparrows were collected from the southern part of California Sur, not far from industrial activity and the city La Paz (ca. 200 000 inhabitants). In comparison, the island Leka has 556 inhabitants and is situated in an area with little industrial activity and more than 300 km to the closest large city (Trondheim; ca. 180 000 inhabitants). Except for the Jimenéz et al. (2005) study, investigations of hepatic POPs levels in house sparrows are scarce. However, other studies have investigated POPs burden in sparrows using other tissues than the liver (Table 4.1).

Table 4.1. Levels of ∑PCBs (ng/g lipid weight (lw)) in various tissues from birds in the genus Passer reported in selected studies. If available in the published work, number of congeners in the summed concentration is given in brackets.

Study	Sampling location	Sample year(s)	Sampling medium	∑PCBs (ng/g lw)	Species	N[a]
Present study	Leka, agricultural island	2013	Liver	F[b]: 346 [7] M[c]:336 [7]	House sparrow (*Passer domesticus*)	F[b]: 23 M[c]: 26
Kunisue et al. (2002)	Selenga delta (Lake Baikal)	1996	Whole body	1 000	House sparrow (*Passer domesticus*)	6
Senthilkumar et al. (2001)	South India, agricultural area	1997, 1998	Egg yolk	1 500	House sparrow (*Passer domesticus*)	3
Yu et al. (2014)	China, agricultural area	2009, 2011	Muscle tissue	44 [31]	Eurasian tree sparrow (*Passer montanus*)	8
Bouwman et al. (2013)	South Africa, area with small scale agriculture	2009, 2010	Egg	71[d] [13]	House sparrow (*Passer domesticus*)	2-3[e]

a Number of samples

b Female individuals

c Male individuals

d Mean of five sampling sites

e Variation in sample number across the five sampling sites

The Leka liver samples had approximately on third and one fifth of the PCB levels measured in whole body house sparrow samples from Lake Baikal (Kunisue et al., 2002) and egg yolk house sparrow samples from South India (Senthilkumar et al., 2001), respectively (Table 4.1). In contrast, the muscle tissue from Chinese Eurasian tree sparrows contained only about one eighth (Yu et al., 2014), and the South African house sparrow eggs only one fifth (Bouwman et al., 2013), of the total PCB burden measured in the liver samples from Leka house sparrows (Table 4.1). The explanation for the large differences in these studies is most likely a combination of variation in local contamination between sampling sites, year of sampling, and the nature of the tissues analysed. All sampling locations listed in Table 4.1 are relatively far from any obvious PCB source large enough to have a substantial impact on the results, but the presence of some regional differences in PCB exposure sources might have affected the measured concentrations. Either way, the choice of sample tissue is probably particularly defining for the reported levels presented in Table 4.1. Results are highly dependent on the characteristics of the tissue analysed, making inter-tissue comparisons of contaminant levels problematic (Jimènez et al., 2005).

4.2.1.2 Individual PCB congeners and possible sources of PCB exposure

The only PCB congener detected in 100% of the samples was PCB-138. The rest were detected as follows: PCB-28, -153 and -180 (98%) > -101 (81.6%) > -118 (73.5%) > -52 (67.3%). PCB congener 153 was the individual compound with the highest mean concentration (3.45 ng/g ww) out of all contaminants detected, followed by PCB-180 (2.03 ng/g ww), and PCB-138 (1.63 ng/g ww) had the fourth highest measured level. Interestingly, the median concentrations compose another pattern. According to median values, PCB-153 (1.65 ng/g ww) had the third, PCB-138 (0.83 ng/g ww) the fifth, and PCB-180 (0.75 ng/g ww) the sixth highest concentration out of all contaminants analysed. The reason for such different results when comparing mean and medians is three individuals with exceptionally high levels of PCBs compared to the other birds. All of these three birds (two males and one female) were from the same farm (farm E), which indicate a local point source of these PCB congeners. In fact, all three congeners were major components of commercial PCB mixtures. PCB-138 and -153 were important constituents in mixtures known as Aroclors 1254, 1260 and 1262, and PCB-180 in Aroclors 1260 and 1262 (Frame, 1997). These mixtures were produced from approximately 1930-1979, and used for a range of applications (Harrad, 2010). If farm E was built before the use of such PCB mixtures were banned, it is possible that Aroclor residues from previous local usage are

the point sources of the high PCB-138, -153 and -180 levels in this farm. However, a total of 19 sparrows were captured in farm E, and only three of these had high PCB levels. The remaining 16 birds sampled from this farm had concentrations close to the population mean for PCB-138, -153 and -180, creating large individual differences for farm E.

When large variations in individual contaminant levels are present, age variation is an obvious possible explanation i.a. due to the bioaccumulative properties of POPs. An increase in contamination with age has been documented in wildlife studies (Muir et al., 1988; Ross et al., 2000). However, in the current case, age is probably not a critical factor since none of the PCB congeners discussed in this context (PCB-138, -153 and -180) differed significantly according to age (student's t-test; PCB-153: $p > 0.05$, Mann-Whitney U test; PCB-138 and -180: $p > 0.05$). Moreover, in farm E all sampled birds were yearlings. Such lack of age distribution excludes age as an explanational factor for the high intra-farm variation. Thus, I suggest that the most likely explanation for the discussed individual variation is individual diet preferences or feeding locations within the farm area. During winter, the house sparrows are dependent on food sources distributed in "patches" (Barnard, 1980), and if these separate food sources contain different levels of PCBs, this might explain the variation in individual contaminant burden.

Since the liver ratio of stable isotopes has been demonstrated to represent last week's diet (Hobson, 1993), the hepatic concentrations measured herein probably indicate a recent contamination of the individuals. Thus, the individuals with the relatively high levels of PCB-138, -153 and -180 were most likely exposed to the local PCB source quite recently. Given the fact that house sparrows tend to vary their feeding locations within a certain area (Barnard, 1980), the individual contaminant pattern may change considerably over time.

4.2.1.3 PCB-52

PCB-52 is a quite "anonymous" congener that has not been given much attention in bird exposure studies. However, in the present study this particular congener showed some interesting trends. Firstly, PCB-52 levels varied significantly across farms (Kruskal-Wallis, $p = 0.002$). As previously discussed with the PCB congeners -138, -153 and -180, the most natural reason for variation in contaminant exposure for the birds would probably be a variation in contaminant levels in feeding items. A significant age variation was the second interesting observation for this congener (Mann-Whitney U test, $p < 0.001$). The observed variation in PCB-52 levels both according to age and across farms, combined with different age distributions in different farms add complexity to the results. PCB-52 age and farm variation might have been intertwined. When inspecting the dataset (Appendix A and B) bearing in mind the above mentioned, an argument for age distribution affecting the observed farm variation is revealed; the individuals older than one year had non-detectable levels of PCB-52 independent of in which farm they were sampled. In farm E, I, J and K, all sampled yearlings had detectable PCB-52 concentrations, while the older individuals consistently did not. This reveals a clear connection between age and PCB-52 levels in the sampled birds. When comparing concentrations of this congener only in yearlings between farms (thus eliminating the age effect) no significant farm variation is observed for PCB-52 (Kruskal-Wallis test; $p > 0.05$). Thus, it seems like the observed PCB-52 variation across sample location is a direct result of the variation in age distribution between farms, rather than an actual difference in PCB-52 exposure at the sampling locations.

PCB-52 was the only contaminant, including all contaminant groups, with age variations. The relationship was inverse, indicating decreasing levels of PCB-52 with age. This was not expected, as persistent contaminants are known to accumulate in biota over time and thus increase by age (Muir et al., 1988; Ross et al., 2000). However, by examining the individual measurements (Appendix B) closer, there is reason for caution. Only one out of the 14 birds older than one year had a detectable level of PCB-52. The test statistics showing a significant age variation across age is purely based on the one individual and 13 randomly generated values between 0 and LOD (0.06 ng/g ww) for PCB-52. Thus, the observed age difference in PCB-52 levels is based on the assumption that the lack of PCB-52 detection in older birds is purely due to actual levels below LOD, and not e.g. methodical errors inhibiting detection of the contaminant. Since 32 out of 36 sampled yearlings had detectable levels (approximately 90%), and the single older individual with a detectable level had a PCB-52 concentration close to LOD

(0.07 ng/g ww), the assumption may be reality. However, the lack of actual measurements for the older individuals adds uncertainty to the results, making it difficult to conclude about PCB-52 decreasing in house sparrows with age.

4.2.2 Organochlorine pesticides

In the present study, levels of both OCPs analysed differed significantly between sexes (see below). ∑OCPs had a mean concentration of 2.23 ng/g (n=23) and 3.40 ng/g (n=26) ww in females and males, respectively. *p,p'*-DDE levels were 1.43 ng/g in females and 2.34 ng/g ww in males, while HCB had a mean level of 0.80 ng/g in females and 1.06 ng/g in males. These are low levels of DDE compared to other wildlife studies, e.g. on European starling eggs from an agricultural site in Canada (12 -174 ng/g ww; (Eng et al., 2014)) and house sparrow livers sampled in an agricultural site in Mexico (mean 3669 ng/g ww; (Jimènez et al., 2005)). The measured mean HCB level was somewhat higher herein than mean HCB concentrations measured in South Indian house sparrow egg yolks, when comparing lipid normalized concentrations; 40 ng/g lw herein versus 28 ng/g lw in the South Indian eggs (Senthilkumar et al., 2001).

Herein, ∑OCP levels were below ∑PCB levels, but higher than the levels of ∑PBDEs. Both pesticides investigated; HCB and *p,p'*-DDE were detected in 100% of the samples. According to median value, *p,p'*-DDE was the single compound detected at highest concentrations, including all contaminant groups, while HCB was number three on the same premises. When expressed as mean values, the OCPs had lower levels than some PCB congeners, but as previously discussed, this mean was highly affected by few individuals with extreme PCB levels compared to the average (see section 3.3 and 4.2.1.2). According to previous trends, OCPs are expected to be closer linked to rural and agricultural areas than PCBs or PBDEs (Eens et al., 2013; Sun et al., 2012; Van den Steen et al., 2008). This expectation was just partly fulfilled in the present study with some heavily chlorinated PCBs competing for the position as most prevalent contaminants in the sparrows.

p,p'-DDE and HCB both varied significantly between sexes (student's t-test, $p = 0.016$, and Mann-Whitney U test, $p = 0.040$, respectively) with males having higher levels of both pesticides compared to females. In addition, both pesticides varied significantly between the farms (*p,p'*-DDE; Welch's ANOVA, $p < 0.001$, HCB; Kruskal-Wallis, $p = 0.002$). Mean *p,p'*-DDE concentrations ranged from 0.91-3.20 ng/g ww between farms, and HCB concentrations

from 0.72-1.83 ng/g ww. This may indicate a variation in local pesticide exposure, which likely mirrors the variation in previous pesticide usage. However, further investigations of the dataset (Appendix A) revealed a sex ratio skewness across farms, which complicates the observed pesticide farm variation. The farms with the highest mean *p,p'*-DDE and HCB levels also had a sex ratio skewed towards males, which might indicate that the sex distribution was the source of OCP farm variation, and not local exposure variation. In order to test the validity of this hypothesis, additional statistical testing was conducted. Student's t-test revealed no significant relationship between percentage males and mean *p,p'*-DDE or HCB concentrations among farms ($p > 0.05$). Thus, the OCP variation across farms may in fact be a result of different *p,p'*-DDE and HCB exposure at the sample sites due to historical pesticide use, not just a result of skewed sex ratios. However, an observed positive relationship between male ratio and HCB and *p,p'*-DDE levels were observed, and may be an affecting factor despite the lack of significance.

4.2.3 Polybrominated diphenyl ethers

PBDEs is a ubiquitous group of organic pollutants found in birds all around the world. However, little is known of their concentrations or effects in free-living terrestrial passerines (Eng et al., 2014). In the present study, ∑PBDEs was the contaminant group measured at lowest levels. This is in accordance with a study investigating the same groups of contaminants as the present study (PBDEs, OCPs and PCBs) in lapwing (*Vanellus vanellus*), great tit (*Parus major*) and mediterranen gull (*Larus melanocephalus*) eggs from Belgium (Dauwe et al., 2009). The ∑PBDE levels in the house sparrows were low compared to existing documentation on European starling eggs from one agricultural site in Canada (Eng et al., 2014) and a range of sites across Canada (Chen et al., 2013), but approximately the same as the measured PBDE concentrations in eggs of great tits (Van den Steen et al., 2006). To my knowledge, PBDE levels in house sparrow liver tissue has only been investigated once prior to the present study. Ciesielski et al. (2008) reported elevated levels of deca-BDE congener 209 (mean: 172 ng/g lw, range: 4.46-1 710 ng/g lw) in liver samples collected from house sparrows living in the same area as the present study population. The hepatic levels of BDE-209 measured herein (mean: 72 ng/g lw, range: 27-172 ng/g lw) were low in comparison with the previous study, and only 27% of the samples had detectable concentrations of this contaminant herein. The substantial difference between the mean and the highest BDE-209 concentrations (172 ng/g versus 1 710 ng/g lw) in the Ciesielski et al. (2008) study might indicate that a few individuals with extreme

values were responsible for the wide range and relatively high mean for this contaminant. A lack of such high-level BDE-209 individuals in the present study could thus explain the different results regarding this contaminant in the present and the Ciesielski et al. (2008) study.

In the present study, three PBDE congeners were detected in > 60% of the house sparrow liver samples (in decreasing order): BDE-99 > -47 > -100. This is the same order of sequence documented in the above mentioned European starling studies in Canada (Chen et al., 2013; Eng et al., 2014), as well as in blue tit eggs from a range of European countries (Van den Steen et al., 2010b). In addition, BDE-99 has been identified as the most dominant PBDE congener followed by BDE-47 in studies on PBDE levels in great tit eggs (Dauwe et al., 2003; Van den Steen et al., 2006) and starling eggs from Europe and America (Eens et al., 2013). Thus, the observed PBDE congener pattern observed herein seems to be characteristic for passerine birds living in Europe and North America.

No significant correlations between individual PBDE congeners or ∑PBDEs and THs were discovered in the house sparrows (Spearman's rank correlation; $p > 0.05$). Eng et al. (Eng et al., 2013) and Van den Steen et al. (Van den Steen et al., 2010a) both performed PBDE exposure studies on passerine birds with environmentally relevant levels many magnitudes higher than the levels observed in the present study. No evident thyroid effects were observed in these studies (Eng et al., 2013; Van den Steen et al., 2010a). Based on this, the lack of thyroid disrupting effect exerted by PBDEs in the Leka house sparrows was as expected. Both Eng et al. (2013) and Van den Steen et al. (2010a) suggest that passerines in general seem to be less sensitive to PBDE exposure than other bird species.

Despite not having a significant effect on thyroid homeostasis, the distribution of PBDE burden in the birds showed some interesting trends. That is, there was a strong significant variation in levels of both BDE-47 (Kruskal-Wallis; $p < 0.001$), -99 (one-way ANOVA; $p = 0.001$) and -100 (Welch's ANOVA; $p < 0.001$) between sample locations. Considering PBDEs' ability to be long-range transported and rather efficiently removed from the atmosphere by precipitation (ter Schure et al., 2004), such transport is a major potential source of PBDE exposure. Individuals from farm I, K, L and M had high levels of BDE-47, -99 and -100 compared with farm C, E, G and J, which might indicate that the former four farms had been exposed to long-range transport of PBDEs to a greater extent than the latter four farms. However, Leka is a small island (110 km^2), and a local variation in PBDE levels due to differences in atmospheric precipitation is not very likely. Additionally, the four farms with the highest levels of PBDEs were scattered all over the island, eliminating the small possibility of local precipitation

producing elevated PBDE levels in a discrete area, and further reducing the possibility of long-range transport being the source of variation in PBDE contamination among farms. Thus, I suggest that local contamination of PBDEs might be the predominant exposure source in house sparrows at Leka. The congeners BDE-47 and -99 were the major congeners in the Penta-BDE commercial mixture (WHO, 1994) that was widely used before withdrawal from the European market in 2004 (Directive EEC, 2003). Documentation supports that congeners originating from these mixtures still are ubiquitous in the environment (Hites, 2004), and it is possible that there for some unknown reason are, or has been, differences in usage of such mixtures between the farms. Environmental or biotic debromination of higher brominated congeners may also have contributed to the total level of BDE-47 and -99 measured in the sparrows (Söderström et al., 2004; Van den Steen et al., 2007).

4.3 Contaminant effects on thyroid hormone levels

There is growing evidence that environmental chemicals can disrupt endocrine system, including the thyroid system. Numerous studies have revealed PCBs, dioxins, furans and flame retardants as thyroid disruptive at environmentally relevant concentrations (review in Boas et al., 2006). Among pesticides, the thyroid disrupting effects of DDT and HCB are the most studied (Boas et al., 2006). Compared to the numerous studies of thyroid disruption in mammals (Brouwer et al., 1989; Bytingsvik et al., 2012; Knott et al., 2011; Villanger et al., 2011), the number of avian studies is more restricted. However, in recent years, the number of avian thyroid disruption studies have steadily increased, and research have been conducted both in laboratories (Winter et al., 2013) and on wildlife bird species (Eng et al., 2014). The majority of avian toxicology studies have been on predatory birds at high trophic levels, predominantly on gulls (Verreault et al., 2007; Verreault et al., 2013; Verreault et al., 2004). The number of studies on contaminant-associated thyroid disruption in avian species at low or medium trophic levels are highly limited.

The house sparrows sampled in the present study were naturally exposed to a mixture of contaminants. Consequently, in addition to the bivariate analyses investigating the thyroid effect of single contaminants, O-PLS models were generated to investigate the combined effect of the analysed contaminants. The O-PLS models with FT3 and FT4 in females and FT3 in males as a Y-variable were significant (CV-ANOVA; p=0.042, 0.028, and 0.021, respectively). Thus, the set of predictor variables included in the model were theoretically able to explain the measured plasma levels of FT3 and FT4. However, it should be noted that the validation parameters were not acceptable for neither of the significant models (FT3 female: R^2X=0.320, R^2Y=0.441, Q^2=0.271; FT4 female: R^2X=0.213, R^2Y=0.532, Q^2=0.300, FT3 male: R^2X=0.660, R^2Y=0.351, Q^2=0.287) according to the criterias for a good biological model (Lundstedt et al., 1998). This means that neither the explained variation in Y or X, nor the predictability of the variation in Y, were satisfactory. Despite this, the models have been included and discussed herein. The reason for this is to give an overall view of the factors potentially affecting the thyroid levels in the house sparrows. However, the poor validation parameters indicate low model fitness, which require a high degree of caution when interpreting the O-PLS models.

All O-PLS models included both biometric variables and contaminant levels, so a direct causative link between thyroid and contaminant levels was not investigated with these analyses. In fact, a biometric variable was the single most important variable in explaining FT4 levels in

females and FT3 levels in males according to the final O-PLS models in the present study. For FT4 in females this variable was BMR and for FT3 in males it was total badge size. The high importance of biometric predictor variables in these O-PLS models indicated a close association between biometric characteristics and levels of FT3 and FT4, which was as expected due to the importance of THs in a wide range of physiological processes. However, close associations were also observed between contaminants and hormone levels, both in the O-PLS models and in bivariate correlation analyses. The observed significant correlations (see Figure 3.3, 3.5 and 3.7) were strong indications of actual relationships between the hormones and the contaminants in question. However, they are purely statistical associations and should not be considered as evidence of biological cause-effect relationships.

4.3.1 Observed associations between contaminants and FT3 in females

In the significant O-PLS model with female FT3 as the Y variable, 11 predictor variables were included. Seven of these were contaminants, and in decreasing order according to VIP value, these were: *p,p'*-DDE > PCB-28 > PCB-118 > PCB-101 > PCB-138 > PCB-52 > BDE-100. Three of these (*p,p'*-DDE, PCB-28 and PCB-118) had VIP values above 1 and were thus important for explaining Y. The coefficient plot (Figure 3.2) shows that all contaminants in the model had an inverse relationship with FT3, except for PCB-52. PCB-52 had a VIP value < 1 indicating that the variable was not among the most important for explaining Y. In addition, it had a large jacked-knife confidence interval crossing the X-axis, indicating a high uncertainty of its contribution to the variance in Y. A variable with a jacked-knife interval longer than the variable column in a O-PLS coefficient plot is considered non-significant in the model (Lundstedt et al., 1998). In addition, further testing did not reveal a significant correlation between PCB-52 and FT3 (Spearman's rank correlation; $p > 0.05$). Consequently, the effect of PCB-52 on FT3 in females will not be discussed further in this section. The only contaminants significantly correlated to female FT3 were *p,p'*-DDE (Figure 3.3A, p=0.001) and PCB-28 (Figure 3.3B, p=0.015). Both had a negative effect on the FT3 level according to the O-PLS model, and these negative associations with FT3 were confirmed in correlation analysis. A significant negative correlation between plasma T3 and PCB-28 levels has previously been observed in humans (Pelletier et al., 2002). Pelletier et al. (2002) also reported a significant negative correlation between plasma T3 and *p,p'*-DDT, which can be related to the observed correlation between FT3 and *p,p'*-DDE found herein.

4.3.2 Observed associations between contaminants and FT3 in males

In the significant O-PLS model with FT3 in males as the Y variable, three predictor variables were included. In addition to total badge size, the contaminants PCB-180 and PCB-153 were included. Neither PCB-180 nor PCB-153 had a VIP value above 1, but both were only marginally below this limit of high model importance (PCB-180: 0.96 and PCB-153: 0.93). Both of these PCBs had inverse relationships with FT3 levels, but the relationship was only significant for PCB-180 (Spearman's rank correlation; $p=0.047$, see Figure 3.5). This O-PLS model had, in addition to the previously discussed weak validation parameters, a highly limited number of predictor variables included in the final model as well. Consequently, the model should not be given much attention. The major result for FT3 contaminant influence in males was thus the negative correlation with PCB-180. A negative correlation between THs and PCB-180 has previously been reported in polar bears (Skaare et al., 2001), but this was on plasma TT4/FT4 ratio and not FT3 levels. In weanling rats, deiodination of T4 to T3 was significantly reduced when exposed to low doses of Aroclor 1254 containing PCB-180 (Morse et al., 1996). A reduction in T3 production may lead to reduced levels of circulating T3 levels, which thus would make the Morse et al. (1996) study in accordance with the herein reported negative correlation between PCB-180 and FT3.

Routti et al. (2008) found a negative correlation between FT3 levels and hepatic levels of POPs in Baltic ringed seals sampled in the 1990s. However, results from a later study on the same seal population conducted during 2002-2007 revealed a positive correlation between FT3 plasma levels and hepatic contaminant levels (Routti et al., 2010). These contradictory FT3 responses show a variation in effects probably due to different contaminant concentrations, which indicate that FT3 in ringed seals may have a hormetic, or bell-shaped, response curve (Calabrese and Baldwin, 2003) to POPs exposure. Whether this is the case for house sparrows remains uncertain.

4.3.3 Observed associations between contaminants and FT4 in females

In the significant model with FT4 in females as the Y variable, 14 predictor variables were included. Seven of these were contaminants, and in decreasing order according to VIP value, they were: PCB-28 > *p,p'*-DDE > PCB-52 > PCB-101 > BDE-100 > PCB-118 > BDE-47. PCB-28, *p,p'*-DDE and PCB-52 had VIP values above 1 and were thus considered important for explaining Y. The coefficient plot (Figure 3.6) shows that the relationship between FT4 and PCB-52 was positive. However, this was not a significant relationship (Spearman's rank correlation; $p > 0.05$), contrary to the inverse relations between FT4 and PCB-28, and FT4 and *p,p'*-DDE ($p=0.031$ and $p=0.036$, respectively).

PCB-28 was one of the contaminants with a significantly negative effect on both FT3 and FT4 levels in female sparrows. This is in accordance with a laboratory study on rats, where PCB-28 decreased serum T4 concentrations in female individuals (Desaulniers et al., 1997). The thyroid effects in the Desaulniers et al. (1997) study were observed in rats fed food items that contained a PCB-28 concentration of 50 000 ppb. For comparison, the mean level of PCB-52 measured in the house sparrow livers herein was 0.18 ppm. In the present study, PCB-28 concentrations were the second lowest of all the contaminants that was detected in > 60% of the samples. Since this congener was significantly correlated to FT4 despite low levels, PCB-28 might be a particularly potent congener in disruption of circulating FT4 levels in house sparrows. The observation of *p,p'*-DDE correlating negatively with FT4 is possibly in accordance with a study by Scollon et al. (Scollon et al., 2004) on Gambel's white-crowned sparrow (*Zonotrichia leucophrys gambelli*). In that study, levels of circulating T4 were depressed in the birds exposed to high DDT exposure (Scollon et al., 2004), which by extension might be linked to DDT's major metabolite, *p,p'*-DDE and thus correspond with the *p,p'*-DDE effect on FT4 levels observed in the present study.

4.3.4 Suggested mechanisms of action

The investigated thyroid variables - circulating FT3 and FT4 levels - are among the major targets for POPs induced thyroid disruption. Disruption of circulating TH levels is especially relevant for hydroxylated metabolites of halogenated POPs, e.g. PCBs (i.e. OH-PCBs). The metabolism of PCBs is not well understood in wildlife avian species (Letcher et al., 2000), but the presence of OH-PCBs have been reported in free-living bird populations (Fängström et al., 2005; Helgason et al., 2010), and cytochrome P450 monooxygenases (CYPs) are believed to be the enzymes hydroxylating PCBs in birds as in mammals (Helgason et al., 2010). OH-PCBs with the hydroxyl group in either the *para*- or the *meta*- position of the biphenyl ring, adjacent to chlorine atoms on both sides, are compounds with a structural resemblance to the THs. These OH-PCBs compete with THs for interaction with plasma TH carrier proteins, and may thus alter the free versus bound fraction of T3 and T4 in plasma (Villanger et al., 2011). One theory suggests that when contaminants bind to a complex consisting of TTR and a retinol binding protein (RBP), a structural change happens that inhibits THs from binding to the complex (Brouwer et al., 1998). The hypothesis of PCBs and PCB metabolites binding competitively to TTR is well established in mammalian studies (Bytingsvik et al., 2013; Cheek et al., 1999; Gutleb et al., 2010; Lans et al., 1994; Van den Berg, 1990), and to some degree in avian studies (Ucán-Marín et al., 2009, 2010). The other major TH-transport protein present in birds; albumin, have only recently been investigated in relation to such contaminant binding. However, in a study by Ucán-Marín et al. (2010), the PCB metabolites 4-OH-CB107/108 and 4-OH-CB187 were observed to have a higher affinity to recombinant avian TTR and albumin than the THs. Thus, the hydroxylated metabolites of PCBs might have interferred with both TTR- and albumin-TH binding in the present study.

The described disturbance of TH-binding to plasma transport proteins alters the free versus bound fractions of THs, but does not directly reduce the level of THs in an animal. However, since the free fractions of THs are more prone to excretion than bound THs, circulating levels of THs might decrease if the free fractions of the hormones increase (Brouwer et al., 1998; Purkey et al., 2004). This is a possible explanation for the herein observed negative correlation between the free THs and PCB-28 concentrations in females, and between FT3 and PCB-180 levels in males. That is, if two assumptions are met. The first assumption is that the PCB congeners in question can be hydroxylated to metabolites able to compete with T3 and/or T4 for the binding to TTR and/or albumin. The second is that the severity of disruption is sufficient for decreasing the circulating levels of TT3 and/or TT4 in the birds, and thus causing a decrease

in the level of the free thyroid fractions. If not, the free fraction of the hormones should in theory increase as a consequence of inhibited binding to the transport proteins, producing a positive relationship between the PCBs and FT3 or FT4. In the present study, it was not possible to test if either of the two presented assumptions were met.

The fact that FT3 and FT4 were negatively correlated with the same contaminants in female house sparrows may indicate a mechanism of action affecting both circulating hormones. The discussed disruption by OH-PCBs binding to TH-transporting proteins is a suggestion for one such mechanism, since both THs bind to TTR and albumin in avian plasma. T3 is documented to inhabit a stronger affinity than T4 towards TTR and albumin in avian species (Chang et al., 1999; Ucán-Marín et al., 2010), and this might have affected how the potential competitive binding by contaminants affected T3 and T4 levels herein. To my knowledge, no studies have documented DDT or *p,p'*-DDE binding to neither TTR nor albumin. Thus, the discussed hypothesis is most likely not valid for the observed negative correlation between *p,p'*-DDE and the level of FT3 and FT4 in female house sparrows.

Another possible explanation for FT3 and FT4 being affected by the same contaminants in female house sparrows could be a decreased production of T3 due to a depression in T4 levels. T4 is the precursor for T3, and a decrease in T4 levels thus means fewer precursor molecules as substrates for the deiodination enzymes to complete T3 synthesis. Morse et al. (1993) demonstrated that a decrease in T4 levels in rats can be compensated by an increase in the activity of type II thyroxine-5'-deiodinase (5'D-II); the enzyme believed to be responsible for T4 conversion to T3 in brain tissue (Kaplan et al., 1983; Visser et al., 1982), and thus avoid decreased levels of T3. However, in a later study, Morse et al. (1996) demonstrated that this compensation might not salvage T3 levels if the reduction in T4 levels is substantial. It is not known whether it is possible to extrapolate the findings from the Morse et al. (1996) study on rats to the present avian study. However, regardless of the extrapolation validity, the theoretical possibility of T3 decreasing as an indirect effect of depressed T4 levels remains.

Contaminant-interaction with enzymes involved in various TH processes is another possible mechanism of action for the observed associations between POPs and THs herein. One such major mechanism in mammals is the induction of Phase II biotransformation enzymes in the liver. Contaminants, including PCBs, are known to increase the activity of uridine diphosphate glucuronosyltransferase (UDP-GT) which glucuronidates T4, and thus facilitates T4 excretion in bile (Barter and Klaassen, 1992; Bastomsky, 1974). This may be a possible explanation for the observed reduction in T4 levels linked to PCB exposure in the present study, and by

extension, perhaps T3 levels as well. However, the main hypothesis for PCBs increasing UDP-GT activity is through binding to the aryl hydrocarbon receptor (AhR) (Aueyeung et al., 2003; Craft et al., 2002; Schuur et al., 1997), and only co-planar (dioxin-like) PCBs are able to bind to the AhR. Neither of the two PCB congeners observed to exert thyroid effects in the present study (PCB-28 and -180) are co-planar, making AhR-binding as the initial mechanism for thyroid disruption herein doubtful. However, the possibility of hepatic biotransformation enzymes being induced through other mechanisms still remains. Some authors have also suggested that polar derivatives of PCBs can reduce deiodinase activities by binding directly to the enzymes (Rickenbacher et al., 1989), indirectly affecting the conversion of T4 to T3.

4.4 Sex differences

Significant sex variations were observed for weight, wing length, HCB and *p,p'*-DDE concentrations, with males having higher values than females for all four variables. Higher levels of environmental pollutants in male than in female birds has previously been documented in glaucous gulls from Bear Island (Verreault et al., 2004). The general reason for male birds being more contaminated than female birds might often be complex and include various physiological differences (e.g. metabolization capacity). However, for the contamination sex differences observed herein I propose an additional explanation based on feeding habits. The male house sparrows sampled were significantly heavier than the female sparrows (student's t-test; p=0.017), which would mean that they were eating more. The birds in the present study were most likely exposed to the detected contaminants mainly through foodstuffs since this is the major exposure route for terrestrial birds. A higher feeding rate would thus mean a higher level of contaminant exposure, which is the main hypothesis I suggest for explaining the observed sex difference in HCB and *p,p'*-DDE levels. However, Spearman's rank correlation test revealed no significant correlation between weight and the contaminant levels in either sex ($p > 0.05$) so the hypothesis remains unconfirmed.

In addition to the above presented variables (weight, wing length, HCB and *p,p'*-DDE), the significant correlations between THs and contaminants differed between sexes as well. Despite higher concentrations of both *p,p'*-DDE and PCB-28 in males, only females had significant negative relationships between these contaminants and the thyroid variables. In addition, both investigated thyroid variables were significantly correlated to contaminants in females (Spearman's rank correlation; FT3 and *p,p'*-DDE: $p = 0.001$; FT3 and PCB-28: $p = 0.015$; FT4

and *p,p'*-DDE: p = 0.031; FT4 and PCB-28: p = 0.036) while only FT3 had a significant contaminant correlation in males (FT3 and PCB-180: p = 0.047). Additionally, the FT3-PCB-180 correlation in males had the weakest significance value out of all five hormone-contaminant correlations observed in the present study. Together, this might indicate a higher sensitivity in female thyroid system towards pollutant exposure, either specific for *p,p'*-DDE and PCB-28 or in general, than in the house sparrow male thyroid system. Responses to toxic compounds are in general expected to have a higher degree of complexity in female vertebrates than in males, as the females go through several physiological processes the males do not. As an example, THs are believed to associate with estrogens in female vertebrates (Braathen et al., 2004; Skaare et al., 2001), possibly linking a disruption of the hypothalamus-pituitary-gonadal (HPG) axis with alterations in thyroid homeostasis.

The indications of sex differences in TH homeostasis susceptibility for POPs induced disruption observed herein are in accordance with studies on polar bears. One study reported a higher susceptibility for thyroid disruptive effects of PCBs in female polar bears from Svalbard, than males (Braathen et al., 2004). Another study reported a negative correlation between toxicants and T4 in solitary female polar bears (i.e. females without cubs) from Southern Beafort Sea, but no such correlations in males (Knott et al., 2011). Also, in East Greenland polar bears, a correlation between TH levels and OHCs in females was observed, while no such correlation was discovered in males (Villanger et al., 2011). Equivalent studies on avian species are limited, especially since many have investigated POPs levels in eggs, and thus only included female individuals (Van den Steen et al., 2010b; Van den Steen et al., 2009). However, a study on glaucous gulls actually discovered that the thyroid variables FT4:FT3 and TT4:TT3 correlated significantly with several organochlorine contaminants in males, while no significant correlations were discovered between thyroid variables and contaminants in females (Verreault et al., 2004). This indicate a higher susceptibility for thyroid disruption in male than in female glaucous gulls, which is the opposite of the polar bear studies and the findings in the present house sparrow study. Another study on tree swallows nesting in pesticide-sprayed apple orchards in Canada also reported correlations between pesticide exposure and thyroid levels in male chicks, but no such correlations in females (Bishop et al., 1998). The contradictory results in the discussed studies might be interpreted as indications of species differences in sex specific susceptibility in POPs induced thyroid disruption.

4.5 Evaluation of results

Hepatic toxicant levels have been suggested to be an indication of recent contamination (Hobson, 1993). Consequently, the documented level of POPs herein is most likely closer to a snapshot of the current state, rather than reflecting a permanent contamination status in the birds. The sampling was conducted during wintertime, while the major food (and thus contaminant) sources were seeds and concentrated cattle feed inside cowsheds. During spring and summer, the house sparrows feed outside the cowsheds to a higher extent, and are thus exposed to different contaminant sources. Therefore, sampling in another season might have produced different contaminant patterns and levels. However, the sample size in the present study (n=49) approximated about 36% of the total house sparrow population at Leka in 2013 (n=139). Such a relatively high sample percentage provides a credible image of the general contamination burden in the population at the time of sampling. An advantage of sampling during winter, as in the present study, is that potential effects of reproductive processes on the thyroid system during breeding season is avoided.

The sampled birds were selected based on BMR, which is reason for caution when investigating THs due to these hormones' important role in vertebrate metabolism (McNabb, 2007). A close relationship between THs and BMR is well documented (review in Kim, 2008), and in the present study BMR was the most important variable for explaining FT4 levels in female house sparrows according to O-PLS modelling (see Figure 3.6). Spearman's rank correlation test confirmed the close relationship between FT4 levels and BMR in females as significant ($p = 0.020$; Appendix D). All samples investigated herein came from individuals with a BMR above average for the total population. High BMR means high food ingestion and thus a high probability of contamination via food items. It is possible that samples from low- or mean-BMR individuals would have produced different results in the present study. Additionally, the stressful situation under which the samples were collected might have affected the presented results. All birds spent a certain amount of time (between two and 11 days) in a sealed up cow shed in between capture and sampling, and even though measures were taken for making their stay in captivity as comfortable and close to natural conditions as possible, the treatment is likely to have stressed the birds to some extent. During the two days of sampling, the birds were confined to individual cages, and during this time, some of the individuals seemed observably stressed. Influence of acute stress on the measured hormone and contaminant levels might have been avoided by a rather short handling time (approximately 1-3 min) from the birds were brought out of the cage and until they were sacrificed. However, both FT3, FT4 and

contaminant levels might have been influenced by long-term stress responses activated in the birds during their stay in captivity.

4.6 Future perspectives

Information on house sparrow thyroid disruption obtained in the present study is potentially valuable when estimating general limits/thresholds for effects induced by environmental contaminants. Additionally, the obtained knowledge of thyroid system response to POPs exposure is valuable information for the house sparrow as a species. The significant population decrease that this species has experienced the last decades makes such information especially relevant. Despite the many theories as to why the house sparrow populations are decreasing, few studies include exposure to POPs and their variety of documented toxic effects on vertebrates. To my knowledge, no study has investigated POPs induced thyroid disruption in this species previously. Here, we provide information on the issue, and the findings give cause for concern for the house sparrows' thyroid function. This is especially relevant for the many populations living in areas with significantly higher concentrations of POPs than the levels measured herein.

Both European starlings, great tits and blue tits are considered valuable model species for assessment of concentrations and effects of environmental contaminants in terrestrial birds (Dauwe et al., 2003; 2006; 2007; Van den Steen et al., 2006; 2010a; 2010b; 2009). Many of the qualities making these passerine species suited candidates for such purposes (e.g. sedentary, low to medium trophic levels, close to humans) are qualities that also apply to the house sparrow. In the present study, the results indicate that the house sparrow thyroid system is highly susceptible for toxicant induced alterations. Such sensitivity could be valuable for a sentinel species. In addition, the house sparrow is currently the most abundant and widespread terrestrial bird species in the world, providing a global relevance for house sparrow research. Based on this, I propose the house sparrow as another suited passerine candidate for biomonitoring purposes. In order to reduce the invasiveness, feathers or eggs might be a good substitute for liver samples with regards to prospective contaminant analyses.

5 Concluding remarks

The present thesis has provided further weight of evidence to the hypothesis stating that some organohalogenated contaminants in the group of POPs exert thyroid disrupting effects in avian species. Additionally, new information on toxicant burden in house sparrows resident in an agricultural environment at northern latitudes has been reported. Indications of altered TH plasma levels associated with POPs exposure have been presented in a wildlife population of house sparrows at low hepatic contaminant concentrations by the use of PCA, O-PLS and Spearman's rank correlation. It should be noted that all of the three significant O-PLS models obtained had low validation parameter values. Thus, the modelled relationships between hormones and contaminants in the O-PLS models do not provide sufficient grounds for final conclusions. However, some of the trends observed in the O-PLS was confirmed by significant bivariate correlations, including several associations between hormone levels and contaminant concentrations. Both legacy and emerging POPs were investigated, but only legacy compounds were found to affect circulating TH levels at a significant level. The legacy POPs; PCBs, *p,p'*-DDE and HCB, were in general found at higher levels than the novel compounds; PBDEs. Different contaminants were observed to affect the female thyroid system compared to the male thyroid system, and significant sex differences were observed for certain contaminant levels. Indication of age variation was only observed for one contaminant (PCB-52), while three PBDE congeners varied significantly between the capture locations (BDE-47, -99 and -100).

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Appendices

A Individual biometric measurements

Table A.1. Mean ± standard deviation (SD), median and range (min - max) of age and biometric variables of female and male house sparrows (*Passer domesticus*) sampled at Leka, Nord-Trøndelag in 2013. Lipid % refers to lipid percent in sparrow liver.

ID	Sex	Location (farm ID)	Age (years)	Weight (g)	BMR (mL 0_2/h)	BeakHeight (mm)	BeakLength (mm)	µTarsi (mm)	µWing (mm)	Mask (mm)	TotBadge (mm)	VisBadge (mm)	Lipid %
8L48780	F	C	7	33,10	78,56	8,31	13,84	19,84	78,18	-	-	-	0,46
8N05859	F	J	2	32,10	76,95	8,03	13,42	20,39	77,90	-	-	-	1,62
8N06843	F	K	2	31,45	-	7,69	12,39	20,32	78,49	-	-	-	1,70
8N06862	F	E	2	30,00	87,10	7,73	13,47	19,72	82,93	-	-	-	1,05
8N06880	F	L	2	31,10	87,36	8,12	14,06	19,81	79,99	-	-	-	0,79
8N06881	F	I	2	31,80	76,53	7,67	13,67	20,19	77,90	-	-	-	0,97
8N72674	F	M	1	34,10	90,65	7,78	14,00	20,35	79,09	-	-	-	1,08
8N72681	F	K	1	32,50	88,99	7,93	13,44	20,78	79,78	-	-	-	2,10
8N72691	F	E	1	30,30	86,24	7,64	13,42	20,13	77,74	-	-	-	2,38
8N72695	F	E	1	28,70	77,91	7,52	13,26	19,80	77,13	-	-	-	3,85
8N73433	F	E	1	30,00	87,37	7,72	13,11	18,93	76,84	-	-	-	2,09
8N73434	F	E	1	30,60	87,15	7,95	13,32	18,80	78,04	-	-	-	1,89
8N73436	F	E	1	29,00	93,85	7,48	12,97	20,08	77,06	-	-	-	2,03
8N73439	F	E	1	30,00	90,07	7,82	13,29	20,05	78,18	-	-	-	1,59
8N73440	F	E	1	33,20	106,20	8,13	13,45	20,11	79,99	-	-	-	2,16
8N73441	F	E	1	29,50	89,50	7,73	13,38	21,25	77,59	-	-	-	2,31
8N73443	F	I	1	31,00	88,14	7,57	13,10	18,98	77,82	-	-	-	2,16
8N73457	F	J	1	29,00	78,33	7,92	13,65	18,50	78,18	-	-	-	3,55
8N73467	F	G	1	31,50	84,12	7,81	13,31	19,66	77,90	-	-	-	2,05
8N73479	F	I	1	31,70	93,54	8,12	13,01	19,38	81,05	-	-	-	-
8N73482	F	E	1	30,70	81,14	7,65	11,79	19,15	75,80	-	-	-	3,32
8N73484	F	E	1	31,70	83,45	8,11	12,74	18,96	75,80	-	-	-	3,56
8N73485	F	E	1	31,35	87,33	7,91	13,12	19,27	78,18	-	-	-	1,38

Table A.1. continued.

ID	Sex	Location (farm ID)	Age (years)	Weight (g)	BMR (mL 0_2/h)	BeakHeight (mm)	BeakLength (mm)	µTarsi (mm)	µWing (mm)	Mask (mm)	TotBadge (mm)	VisBadge (mm)	Lipid %
8L64499	M	M	5	32,60	83,62	7,81	12,69	19,95	82,10	13,60	21,37	13,32	1,27
8M31216	M	C	4	33,70	79,15	7,92	13,78	20,24	82,71	14,50	19,32	15,20	2,54
8M31769	M	C	4	31,00	88,54	7,45	13,02	19,57	80,90	14,30	20,46	14,85	1,17
8M72594	M	M	3	32,40	94,52	8,12	13,78	20,27	82,66	16,70	22,83	15,25	4,32
8N05650	M	K	2	32,00	83,16	7,86	12,77	20,39	82,48	14,95	20,90	15,30	3,09
8N06864	M	M	2	34,00	93,72	8,15	13,44	19,78	80,90	15,00	19,79	14,81	3,17
8N06867	M	M	2	32,80	97,79	7,91	13,08	19,49	79,76	15,20	19,42	15,04	1,13
8N72672	M	M	1	35,90	83,18	8,10	12,95	18,94	79,04	15,10	18,10	14,80	1,15
8N72673	M	M	1	35,30	92,21	7,72	12,91	18,80	79,99	14,10	20,31	14,70	1,67
8N72677	M	M	1	32,50	86,94	7,87	13,85	17,27	81,80	15,30	19,21	14,36	1,64
8N72683	M	K	1	32,00	99,45	7,65	13,23	20,15	82,71	12,30	19,94	14,87	2,40
8N72689	M	E	1	31,30	82,78	7,68	12,96	20,37	80,45	15,70	19,61	14,00	3,19
8N72690	M	E	1	30,90	82,23	7,79	13,39	19,49	79,99	12,20	17,47	13,34	2,96
8N72696	M	E	1	30,50	95,36	7,61	12,94	20,25	82,48	13,95	20,04	14,76	1,80
8N73430	M	E	1	32,00	94,56	8,14	13,42	20,32	81,21	14,60	16,84	14,53	1,47
8N73437	M	E	1	31,00	86,30	7,60	13,19	20,32	80,00	14,35	22,51	13,98	1,60
8N73438	M	E	1	29,60	84,60	7,66	12,74	19,47	79,76	15,00	19,79	14,67	5,68
8N73448	M	I	1	32,60	80,75	7,87	13,33	19,65	81,50	13,30	19,83	13,09	3,93
8N73452	M	J	1	30,00	85,80	7,70	12,83	19,81	78,76	14,05	20,27	14,88	3,82
8N73453	M	J	1	29,20	87,77	7,49	12,58	18,30	80,45	15,00	19,14	14,85	3,19
8N73465	M	G	1	34,30	96,90	7,85	13,93	19,69	79,09	13,20	19,52	13,75	2,24
8N73468	M	G	1	32,10	91,41	7,73	14,27	19,24	81,80	14,30	19,38	14,19	1,96
8N73470	M	G	1	30,90	85,51	7,80	13,36	19,54	81,24	13,35	19,83	13,99	3,62
8N73477	M	I	1	33,80	84,90	8,07	13,66	19,78	81,36	13,80	19,84	13,52	2,02
8N73478	M	I	1	32,40	101,76	8,09	14,08	20,43	80,90	13,20	20,77	13,75	3,92
8N73487	M	E	1	31,10	80,21	8,05	13,04	20,36	82,63	11,40	18,00	13,19	2,50

B Individual contaminant levels on a wet weight basis

Table B.1 Mean ± standard deviation (SD), median and range (min - max) of contaminant concentrations measured in female and male house sparrows (*Passer domesticus*) sampled at Leka, Nord-Trøndelag in 2013.

ID	BDE-47	BDE-99	BDE-100	HCB	*p,p'*-DDE	PCB-28	PCB-52	PCB-101	PCB-118	PCB-138	PCB-153	PCB-180
8L48780	0,148	0,386	0,077	0,692	2,014	0,173	-	0,105	0,120	0,404	0,912	0,311
8N05859	-	-	-	0,585	0,939	0,126	-	-	-	0,135	-	-
8N06843	0,426	0,770	0,144	1,063	0,788	0,120	-	0,174	0,223	0,574	0,808	0,274
8N06862	-	0,189	-	0,507	0,611	-	-	-	0,148	1,146	2,395	1,697
8N06880	0,796	1,384	0,299	0,680	1,365	0,215	-	0,146	0,245	0,852	1,622	0,830
8N06881	0,465	0,817	0,139	0,594	0,344	0,102	-	0,125	-	0,317	0,508	0,314
8N72674	0,183	0,403	-	0,677	3,044	0,144	-	0,103	0,367	0,694	1,011	0,377
8N72681	0,773	1,654	0,272	1,640	1,867	0,140	0,189	0,252	0,574	1,477	1,692	0,781
8N72691	0,265	0,697	0,119	1,023	0,979	0,178	0,088	0,109	-	0,714	1,204	0,479
8N72695	0,216	0,551	0,110	0,614	0,572	0,111	0,225	0,063	-	0,354	0,959	0,310
8N73433	0,243	0,548	0,117	0,875	2,614	0,156	0,136	0,170	2,072	14,946	29,433	17,535
8N73434	0,320	0,842	0,137	0,661	2,789	0,230	0,113	0,168	0,532	1,503	2,199	1,330
8N73436	0,241	0,716	0,118	0,683	0,998	0,134	0,058	0,135	0,294	0,986	1,668	1,172
8N73439	-	-	-	0,540	0,507	0,145	0,204	-	-	0,164	0,319	0,143
8N73440	0,173	0,508	0,102	1,038	0,893	0,139	0,246	0,114	0,839	0,944	1,230	0,646
8N73441	-	0,222	-	0,569	0,522	0,101	0,201	-	-	0,239	0,422	0,220
8N73443	0,518	0,841	0,119	0,519	0,288	0,100	0,222	0,053	-	0,209	0,323	0,155
8N73457	-	0,158	-	0,986	3,194	0,115	0,229	0,207	0,284	0,654	2,387	0,785
8N73467	0,214	0,425	0,103	1,095	3,122	0,158	0,146	0,251	0,900	3,211	9,149	5,944
8N73479	0,405	0,706	0,114	0,586	0,154	0,115	0,171	0,068	-	0,426	0,599	0,360
8N73482	0,314	1,106	0,135	0,972	1,676	0,152	0,193	0,201	0,279	1,253	2,631	2,399
8N73484	0,381	1,057	0,158	1,008	1,835	0,197	0,199	0,268	0,877	1,999	4,147	2,080
8N73485	0,434	1,028	0,163	0,734	1,865	0,180	0,085	0,169	0,381	1,394	2,500	1,520
8L64499	-	0,292	-	1,063	2,997	0,151	-	0,113	0,586	1,248	3,161	1,089
8M31216	-	0,104	-	0,489	0,790	0,111	-	-	-	0,203	0,532	0,272
8M31769	0,193	0,700	0,117	0,988	4,996	0,255	-	0,230	0,506	1,389	3,313	1,025
8M72594	0,287	0,822	0,139	1,224	2,636	0,174	-	0,266	0,618	0,720	1,685	0,773
8N05650	0,954	2,313	0,326	1,709	1,437	0,205	-	0,249	0,490	1,226	1,518	0,542
8N06864	0,109	0,215	-	0,886	1,917	0,168	0,069	0,140	0,167	0,639	1,093	0,719
8N06867	10,333	12,938	0,537	0,903	1,348	0,127	-	-	-	0,388	0,539	0,305
8N72672	0,110	0,205	-	0,797	2,252	0,148	-	0,114	0,134	0,416	0,775	0,227

Table B.1. continued.

ID	BDE-47	BDE-99	BDE-100	HCB	*p,p'*-DDE	PCB-28	PCB-52	PCB-101	PCB-118	PCB-138	PCB-153	PCB-180
8N72677	0,203	0,373	0,090	1,244	5,450	0,267	-	0,098	0,557	1,041	2,749	1,009
8N72683	1,571	3,604	0,586	2,924	3,453	1,141	0,812	0,488	1,980	4,319	5,046	1,705
8N72689	0,134	0,404	0,085	0,602	0,364	0,118	0,228	0,069	-	0,349	0,616	0,398
8N72690	0,352	1,106	0,187	0,906	2,652	0,243	0,129	0,131	0,350	1,453	2,528	1,475
8N72696	0,299	0,917	0,162	0,841	5,738	0,269	0,072	0,225	2,737	12,262	32,259	19,665
8N73430	0,357	0,965	0,147	1,021	3,003	0,178	0,150	0,312	1,535	9,131	21,824	18,870
8N73437	-	0,192	0,043	0,571	0,679	0,112	0,077	-	-	0,371	0,660	0,442
8N73438	0,169	0,396	-	0,629	0,598	0,102	0,105	-	0,153	0,492	0,951	0,572
8N73448	1,216	2,486	0,351	0,862	1,326	0,191	0,254	0,229	0,270	1,351	1,821	1,247
8N73452	-	0,153	-	1,099	2,828	0,161	0,323	0,237	0,113	0,418	0,936	0,511
8N73453	-	-	-	1,082	1,162	0,122	0,224	0,180	0,098	0,275	0,483	0,242
8N73465	0,204	0,518	0,084	0,955	3,229	0,161	0,343	0,126	0,340	1,018	2,057	0,876
8N73468	0,217	0,326	0,078	0,968	3,592	0,152	0,189	0,179	0,354	0,829	1,427	0,394
8N73470	0,176	0,386	0,085	1,173	2,840	0,149	0,214	0,107	0,336	0,770	1,967	0,695
8N73477	1,947	3,388	0,428	1,903	1,226	0,226	0,313	0,287	0,341	1,934	2,715	1,388
8N73478	1,378	3,606	0,553	1,584	2,096	0,214	0,311	0,153	0,317	1,471	3,028	1,721
8N73487	0,246	0,815	0,163	0,465	1,157	0,103	0,215	0,095	0,442	1,380	3,744	1,725

C Individual contaminant levels on a lipid weight basis

Table C.1 Mean ± standard deviation (SD), median and range (min - max) of single contaminant concentrations (ng/g) measured in female and male house sparrows (*Passer domesticus*) sampled at Leka, Nord-Trøndelag in 2013. Concentrations are presented on a lipid weight basis.

ID	BDE-47	BDE-99	BDE-100	HCB	*p,p'*-DDE	PCB-28	PCB-52	PCB-101	PCB-118	PCB-138	PCB-153	PCB-180
8L48780	32	83	17	150	435	37	-	23	26	87	197	67
8N05859	-	-	-	36	58	8	-	-	-	8	-	-
8N06843	25	45	8	63	46	7	-	10	13	34	48	16
8N06862	-	18	-	48	58	-	-	-	14	109	228	162
8N06880	101	176	38	87	174	27	-	19	31	108	206	106
8N06881	48	84	14	61	35	11	-	13	-	33	52	32
8N72674	17	38	-	63	283	13	-	10	34	65	94	35
8N72681	37	79	13	78	89	7	9	12	27	70	81	37
8N72691	11	29	5	43	41	7	4	5	-	30	51	20
8N72695	6	14	3	16	15	3	6	2	-	9	25	8
8N73433	12	26	6	42	125	7	6	8	99	716	1409	839
8N73434	17	45	7	35	148	12	6	9	28	80	116	70
8N73436	12	35	6	34	49	7	3	7	14	49	82	58
8N73439	-	-	-	34	32	9	13	-	-	10	20	9
8N73440	8	24	5	48	41	6	11	5	39	44	57	30
8N73441	-	10	-	25	23	4	9	-	-	10	18	10
8N73443	24	39	6	24	13	5	10	2	-	10	15	7
8N73457	-	4	-	28	90	3	6	6	8	18	67	22
8N73467	10	21	5	53	152	8	7	12	44	156	446	290
8N73479	18	31	5	25	7	5	7	3	-	19	26	16
8N73482	9	33	4	29	51	5	6	6	8	38	79	72
8N73484	11	30	4	28	52	6	6	8	25	56	117	58
8N73485	31	75	12	53	135	13	6	12	28	101	181	110
8L64499	-	23	-	84	236	12	-	9	46	98	249	86
8M31216	-	4	-	19	31	4	-	-	-	8	21	11
8M31769	17	60	10	85	428	22	-	20	43	119	284	88
8M72594	7	19	3	28	61	4	-	6	14	17	39	18
8N05650	31	75	11	55	46	7	-	8	16	40	49	18
8N06864	3	7	-	28	61	5	2	4	5	20	34	23
8N06867	915	1145	48	80	119	11	-	-	-	34	48	27
8N72672	10	18	-	69	196	13	-	10	12	36	67	20

Table C.1. continued.

ID	BDE-47	BDE-99	BDE-100	HCB	*p,p'*-DDE	PCB-28	PCB-52	PCB-101	PCB-118	PCB-138	PCB-153	PCB-180
8N72677	12	23	5	76	332	16	-	6	34	63	167	61
8N72683	65	150	24	122	144	47	34	20	82	180	210	71
8N72689	4	13	3	19	11	4	7	2	-	11	19	12
8N72690	12	37	6	31	90	8	4	4	12	49	85	50
8N72696	17	51	9	47	319	15	4	12	152	682	1794	1093
8N73430	24	65	10	69	204	12	10	21	104	619	1480	1280
8N73437	-	12	3	36	42	7	5	-	-	23	41	28
8N73438	3	7	-	11	11	2	2	-	3	9	17	10
8N73448	31	63	9	22	34	5	6	6	7	34	46	32
8N73452	-	4	-	29	74	4	8	6	3	11	25	13
8N73453	-	-	-	34	36	4	7	6	3	9	15	8
8N73465	9	23	4	43	144	7	15	6	15	45	92	39
8N73468	11	17	4	49	183	8	10	9	18	42	73	20
8N73470	5	11	2	32	78	4	6	3	9	21	54	19
8N73477	97	168	21	94	61	11	16	14	17	96	135	69
8N73478	35	92	14	40	54	5	8	4	8	38	77	44
8N73487	10	33	7	19	46	4	9	4	18	55	150	69

D Bivariate correlations among biometric and thyroid variables

Table D.1 Bivariate correlations among biometric and hormone variables for female house sparrows (*Passer domesticus*) sampled at Leka, Nord-Trøndelag in 2013. The analysis applied is Spearman's rank correlation, and the significance level was $p \leq 0.05$. Significant correlations are presented as the relevant p-value, while non-significant correlations ($p > 0.05$) are indicated with the abbreviation n.s. The variables FT3 and FT4 are levels of FT3 and FT4 in

	FT3	Weight	BMR	BeakHeight	BeakLength	µTarsi	µWing	Lipid%	FT4	FT4:FT3	Location	Age
FT3		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	< 0.001	n.s.	n.s.	n.s.
Weight	n.s.		n.s.	0.005	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
BMR	n.s.	n.s.		n.s.	n.s.	n.s.	n.s.	n.s.	0.020	0.037	n.s.	0.036
BeakHeight	n.s.	0.005	n.s.		n.s.	n.s.	0.008	n.s.	n.s.	n.s.	n.s.	n.s.
BeakLength	n.s.	n.s.	n.s.	n.s.		n.s.	0.011	0.017	n.s.	n.s.	n.s.	n.s.
µTarsi	n.s.	n.s.	n.s.	n.s.	n.s.		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
µWing	n.s.	n.s.	n.s.	0.008	0.011	n.s.		0.017	n.s.	n.s.	n.s.	n.s.
Lipid%	n.s.	n.s.	n.s.	n.s.	0.017	n.s.	0.017		n.s.	n.s.	n.s.	< 0.001
FT4	< 0.001	n.s.	0.020	n.s.	n.s.	n.s.	n.s.	n.s.		< 0.001	n.s.	n.s.
FT4:FT3	n.s.	n.s.	0.037	n.s.	n.s.	n.s.	n.s.	n.s.	< 0.001		n.s.	n.s.
Location	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.		n.s.
Age	n.s.	n.s.	0.036	n.s.	n.s.	n.s.	n.s.	< 0.001	n.s.	n.s.	n.s.	

Table D.2 Bivariate correlations among biometric and hormone variables for male house sparrows (*Passer domesticus*) sampled at Leka, Nord-Trøndelag in 2013. The analysis applied is Spearman's rank correlation, and the significance level was $p \leq 0.05$. Significant correlations are presented as the relevant p-value, while non-significant correlations ($p > 0.05$) are indicated with the abbreviation n.s. The variables FT3 and FT4 are levels of FT3 and FT4 in plasma, Location represent in which farm the birds were captured, Lipid % refers to lipid content in the liver, and Mask, TotBadge and VisBadge are measurements of male feather ornamentations.

	FT3	Weight	BMR	BeakHeight	BeakLength	µTarsi	µWing	Mask	TotBadge	VisBadge	Lipid %	FT4	FT4:FT3	Location	Age
FT3		n.s	n.s	n.s	n.s	n.s	n.s	n.s	0.021	n.s	n.s	n.s	n.s	0.012	n.s
Weight	n.s		n.s	0.001	0.045	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	0.010	n.s
BMR	n.s	n.s		n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
BeakHeight	n.s	0.001	n.s		0.009	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
BeakLength	n.s	0.045	n.s	0.009		n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
µTarsi	n.s	n.s	n.s	n.s	n.s		0.044	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
µWing	n.s	n.s	n.s	n.s	n.s	0.044		n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
Mask	n.s	n.s	n.s	n.s	n.s	n.s	n.s		n.s	0.002	n.s	n.s	n.s	n.s	n.s
TotBadge	0.021	n.s	n.s	n.s	n.s	n.s	n.s	n.s		n.s	n.s	n.s	n.s	n.s	n.s
VisBadge	n.s	n.s	n.s	n.s	n.s	n.s	n.s	0.002	n.s		n.s	n.s	n.s	n.s	0.021
Lipid%	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s		n.s	n.s	n.s	n.s
FT4	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s		< 0.001	n.s	n.s
FT4: FT3	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	<0.001		n.s	n.s
Location	0.012	0.010	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s		n.s
Age	**n.s**	**n.s**	**n.s**	**n.s**	**n.s**	**n.s**	**n.s**	**n.s**	**n.s**	**0.021**	**n.s**	**n.s**	**n.s**	**n.s**	