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Effects of complex organohalogen contaminant mixtures on thyroid hormone homeostasis in selected arctic marine mammals

Thesis for the degree of Philosophiae Doctor

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Trondheim, October 2011

Gro Dehli Villanger

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Paper II Gro D. Villanger, Christian Lydersen, Kit M. Kovacs, Elisabeth Lie, Janneche U. Skaare, and Bjørn M. Jenssen. 2011. Disruptive effects of persistent organohalogen contaminants on thyroid function in white whales (*Delphinapterus leucas*) from Svalbard. Science of the Total Environment 409; 2511-2524.[2]

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Contributions

[1] Paper I was initiated by GDV and BMJ in collaboration with RD and CS, sample collection was organised by RD, CS and MK, the analytical work was performed by RRF, GDV, CS, MK, RJL and DCGM, and statistical analysis and writing were done by GDV with comments from the other authors.

[2] Paper II was initiated by GDV in collaboration with BMJ, sample collection was done by CL, KMK and GDV, analytical work was performed by EL and GDV, and statistical analysis and writing were done by GDV with comments from the other authors.

[3] Paper III was initiated by GDV, and planned by GDV and KMG in collaboration with BMJ, CL and KMK, sample collection was done by BMJ, CL and KMK, analytical work was done by KMG, MK and EL, and data analysis and writing were done by KMG and GDV in collaboration with BMJ and with comments from the other authors.

[4] Paper IV was initiated and planned by GDV in collaboration with BMJ, KMG, CL and KMK, sample collection was done by BMJ, CL and KMK, analytical work was done by KMG, MK and EL, and data analysis and writing were done by GDV in collaboration with BMJ, EGS and KMG and with comments from the other authors.

Summary

Arctic marine mammals are exposed to and accumulate high levels of complex mixtures of organohalogen contaminants (OHCs), such as polychlorinated biphenyls (PCBs), pesticides (e.g. dichlorodiphenyltrichloroethane related compounds [DDTs] and chlordanes [CHLs]) and polybrominated diphenylethers (PBDEs). Many OHCs and their metabolites (e.g. hydroxylated [OH]-PCBs and OH-PBDEs) have the ability to disturb the thyroid hormone (TH) system at multiple target points. Also, it is increasingly acknowledged that environmental OHC mixtures may act on the TH system in combination, resulting in additive and even synergistic effects. This causes concern for the impact of OHC mixtures on TH homeostasis and possible health impairments in wildlife, especially during the sensitive early developmental stages of foetuses and neonates. In the current thesis, the multivariate relationships between individual contaminants in the accumulated OHC mixture and circulating TH levels and ratios were investigated in three different species of arctic marine mammals from the European Arctic: polar bears (*Ursus maritimus*) from East Greenland, white (beluga) whales (*Delphinapterus leucas*) from Svalbard (Norway), and hooded seal (*Cystophora cristata*) mother-pup pairs from the West Ice, East of Greenland. The findings in the present thesis indicate that complex OHC mixtures are affecting the TH system in these three species, and that some OHCs may be acting in combination. The applied multivariate modelling identified specific combinations of chemicals that influenced TH status in the different species and age/sex groups. Both negative and positive associations between THs and individual OHCs were shown, however the specific TH parameters that were affected and the directions of the relationships varied among species and subgroups. Some contaminants appeared to disrupt TH status across species and age/sex groups: PBDEs (particularly PBDE-99 and -100), CHLs (e.g. *cis*-chlordane, oxychlordane), *ortho*-PCBs (e.g. PCB-52), PCB-118, hexachlorobenzene (HCB), α- and β-hexachlorocyclyhexane (HCH), and *p,p'*-DDT, *p,p'*-DDE and *p,p'*-DDD. This is consistent with the thyroid disruptive potential of these chemicals demonstrated in experimental *in vivo* and *in vitro* studies, and similar to results previously reported in other wildlife species and in human studies. The present thesis also showed that it is imperative to consider possible confounding factors such as age, sex, sampling date (season), lipid content, morphometric data, and physiological and reproductive status when assessing the impact of OHC mixtures on TH homeostasis. The results of the present thesis also suggest that newborn and young marine mammals are vulnerable to TH disruption by maternally transferred OHCs (e.g. α- and β-HCH, OH-PCBs, HCB, TCB, PBDE-99, PCB-118 -52, *p,p'*-DDE, *cis*-nonachlor, and *cis*-chlordane), which have been shown to affect TH balance and neurodevelopment in human newborns and children. This causes concern for possible health-related effects in later life stages of the young arctic marine mammals investigated in the present thesis, and for their future adaptability to climate changes rapidly taking place in the Arctic.

Introduction

The homeostasis (balance) of endocrine systems (e.g. sex hormones, thyroid hormones, glucocorticoids) is essential to prevent disorders or physiological malfunctions in organisms (Lintelmann et al., 2003). Although hormone systems are flexible in regards to their feedback systems, they are sensitive to disturbances, and it has been shown that many chemicals present in the environment have the ability to disrupt the natural balance of hormone systems (Lintelmann et al., 2003; Goodhead and Tyler, 2009). Disruption of endocrine homeostasis during sensitive periods of foetal and neonatal development may result in irreversible developmental impairments of organs and vital, physiological functions (Colborn et al., 1993; Lintelmann et al., 2003). Within ecotoxicology, endocrine disruption can be defined as "a hormonal imbalance initiated by exposure to a pollutant (i.e. endocrine disrupting chemical [EDC]) and leading to alterations in development, growth, and/or reproduction in an organism or its progeny" (Goodhead and Tyler, 2009). EDCs are composed of many different types of chemicals present in the environment, including natural plant steroids, man-made pharmaceuticals, pesticides and industrial chemicals (Goodhead and Tyler, 2009).

Environmental contaminants in the Arctic

Arctic wildlife are exposed to complex mixtures of organohalogen contaminants (OHCs), such as polychlorinated biphenyls (PCBs), polychlorinated diphenylethers (PBDEs) and organochlorine pesticides (OCPs; e.g. dichlorodiphenyltrichloroethane related compounds [DDTs]), which are thought to have endocrine disruptive abilities. Chronic, low-level exposure to EDCs may negatively affect development, behaviour, fertility and survival in wildlife species (Colborn et al., 1993; Crisp et al., 1998; Rolland, 2000; Lintelmann et al., 2003; Letcher et al., 2010). It has also been hypothesized that EDCs may reduce an individual's ability to adapt to the climate-related changes in the Arctic (Jenssen, 2006). Thus, there is a concern that life-long, low-level exposure to EDCs, such as OHCs, together with global climate changes may reduce the health and survival of Arctic wildlife and ultimately affect populations and ecosystems (Jenssen, 2006; Noyes et al., 2009; Letcher et al., 2010; UNEP/AMAP, 2011).

OHCs are man-made chemicals which are generally resistant to physical and biological degradation (i.e. persistent). They are used as industrial chemicals or pesticides, or released as bi-products of industrial processes or incineration (AMAP, 2004; see Appendix 1). Common characteristics (persistence, toxicity, and potential for long-range transport and bioaccumlation) define many OHCs as persistent organic pollutants (POPs) which are globally banned or

restricted under the United Nation's Stockholm convention on persistent organic pollutants (Stockholm Convention, 2009; see Appendix 1).

Although, OHC levels in abiota and biota are generally highest in areas close to points of release, OHCs are subjected to long-range transport from industrial and agricultural areas in lower latitudes to the remote Arctic areas mostly trough atmospheric transport, as well as ocean currents and rivers (AMAP, 1998, 2004; Hung et al., 2010). When entering the arctic marine food webs, the OHCs will bioaccumulate in organisms where these chemicals can be stored in lipid tissue or bound to proteins, leading to biomagnification up food webs. This may lead to high and potential toxic levels in upper trophic predators, such as seals, whales, polar bears (*Ursus maritimus*), birds, and humans consuming traditional, local diets (Thomann, 1989; Sørmo et al., 2006; AMAP, 2009; Letcher et al., 2010).

In vertebrates, OHCs may be biotransformed by xenobiotic-metabolising phase I (e.g. the cytochrome P450 [CYP] isozymes), and conjugating phase II (e.g. uridine diphosphoglucuronosyl transferase [UDPGTs]) and sulfontransferases [SULTs] enzymes), leading to excretion through bile or urine (Letcher et al., 2000). However, phase I (CYP) metabolism can also lead to the formation of metabolites that are more persistent and toxic than their parent compounds. Examples include hydroxylated (OH)-PCBs, OH-PBDEs and pentachlorophenol (PCP) formed by CYP-facilitated biotransformation of PCBs, PBDEs and hexachlorobenzene (HCB), respectively (van Ommen et al., 1985; Letcher et al., 2000; Hakk and Letcher, 2003). These phenolic metabolites have the ability to bind to proteins in blood or other tissues, and are in this way retained instead of excreted (Bergman et al., 1994; Malmberg et al., 2004; Gebbink et al., 2008a). As many lipophilic OHCs, the phenolic OHC metabolites are also shown to have endocrine disruptive activities (Lans et al., 1993; Boas et al., 2006; Legler, 2008).

Biomonitoring of arctic biota have shown a relatively high presence of legacy OHC contaminants (e.g. PCBs, DDTs, and OCPs). Although the legacy contaminants show a slowly decreasing trend due to bans or restrictions in use, the more recent monitored OHCs in the Arctic are adding to the concern. Examples of such "novel" contaminants are PBDEs and their new replacement brominated flame retardants (BFRs) such as hexabromocyclododecane (HBCD) isomers and pentabromoethylenbenzene (PBEB), as well as perfluorated compounds (PFCs) (de Wit et al., 2010; Letcher et al., 2010). Furthermore, climate changes are predicted to alter patterns and levels of OHC accumulation and biomagnification in Arctic ecosystems, as well as toxicokinetics - and dynamics of these contaminants in organisms (Noyes et al., 2009; UNEP/AMAP, 2011).

Even though several OHCs (e.g PCBs and DDTs) are suggested to negatively affect immune, reproductive and endocrine systems in wildlife inhabiting the more polluted waters in lower latitudes (Helle et al., 1976; Aguilar and Borell, 1994; De Guise et al., 1995; Nyman et al., 2003), the knowledge of potential toxicological effects of OHCs in arctic wildlife is still scarce (Letcher et al., 2010). Health risk of arctic wildlife is often assessed using "threshold levels" (no observed effect levels) from experimental or semi-field studies, or extrapolating from OHC-associated health effects reported in wildlife from more polluted waters (AMAP, 1998, 2004; Letcher et al., 2010). Studies of OHC-associated biological effects in polar bears and glaucous gull (*Larus hyperboreus*), as well as indigenous human populations consuming local diets, suggest that OHCs and their metabolites are at levels where biological systems might be affected and toxicological effects and impairments of health could occur (AMAP, 2009; Letcher et al., 2010; Sonne, 2010).

Endocrine disruption of the thyroid hormone system

The hypothalamic-pituitary-thyroid (HPT) axis is an important target of many OHCs (Brouwer et al., 1998; Howdeshell, 2002; Jugan et al., 2010). The role of the HPT axis in vertebrates is to produce and regulate circulating levels of the thyroid hormones (THs) 3,5,3',5'-tetraiodothyronine (thyroxine, T4) and 3,5,3'-triiodothyronine (T3) at homeostatic levels in regards to the physiological requirements of the organism (McNabb, 1992; see Fig. 1). T4 is produced by the thyroid gland in much larger quantities than T3. Extra-thyroidal deiodination of T4 is thought to be the largest source of the more biological active hormone, T3 (McNabb, 1992). Thyroidal TH production and secretion is induced by the pituitary thyroid stimulating hormone (TSH) (McNabb, 1992; Hadley, 1996).

In mammals, THs are transported in blood mainly bound to the circulating proteins transthyretin (TTR), albumin and thyroxine binding globulin (TBG) (McNabb, 1992, Hadley, 1996, Zoeller et al., 2007a). To evaluate TH status, it is important to considered the proportion of T3 and T4 in blood that are bound by circulating proteins, which are thought to function as storage and supply of THs to target tissues, and the free (i.e. unbound) T3 and T4 fractions (FT3 and FT4) that are available for cellular uptake (McNabb, 1992; St Aubin, 2001). Circulating levels of FT4 and FT3 are also responsible for the negative feedback (inhibition) on pituitary release of TSH (McNabb, 1992; Zoeller et al., 2007a).

THs exert their effects indirectly though binding to the nuclear thyroid hormone receptors TRα and TR β, and thus controlling expression of multiple genes. Also, it is thought that THs exert nongenomic (direct) effects on target tissues, e.g. by increasing cellular uptake of glucose and

alter mitochondrial function (McNabb, 1992; Zoeller et al., 2007a). THs are involved in the regulation of metabolism, thermoregulation, function of adipose tissue, and are important for keeping the general physiological homeostasis (Bernal and Refetoff, 1977; McNabb, 1992; Silva, 2006). Furthermore, THs are necessary for somatic growth and development in vertebrates, and they are key hormones for the development of brain and nervous system in the foetus, neonate and juvenile (Santisteban and Bernal, 2005; Ahmed et al., 2008). In addition, THs are also important for proper development and function of gonads, circulating levels of sex hormones and reproduction (Cooke et al., 2004; Zoeller et al., 2007a).

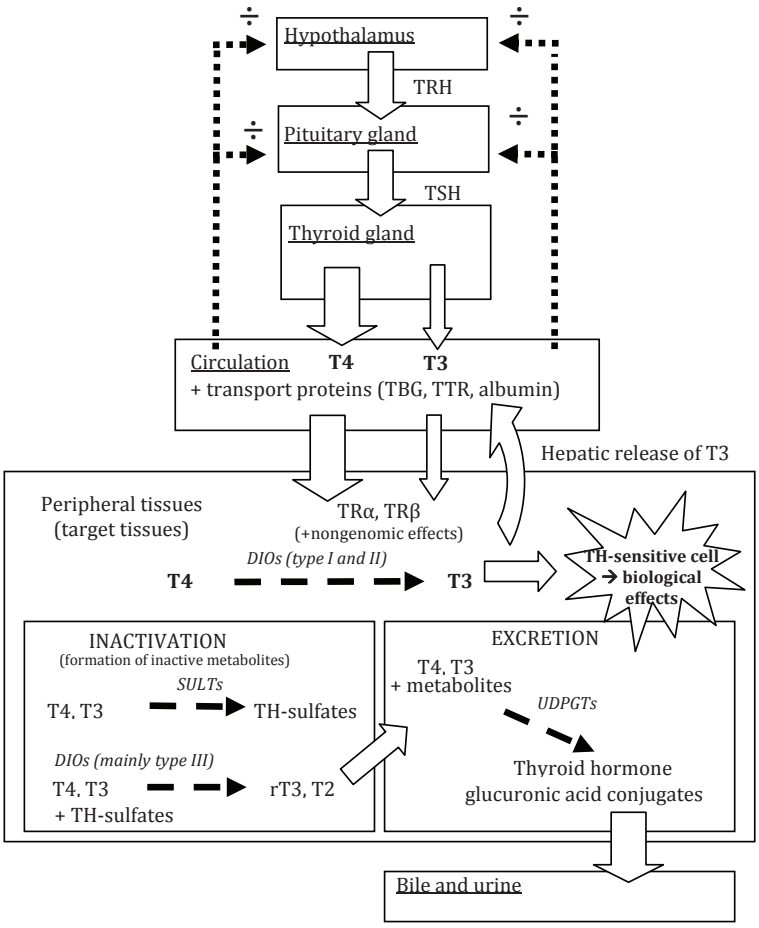

Figure 1. Regulation of the thyroid hormone system in mammals (modified from Sørmo, 2005).

TH=thyroid hormone, TSH=thyroid stimulating hormone, TRH=thyrotropin releasing hormone, TR=thyroid hormone receptor, T2=diiodothyronine, T3=triiodothyronine, rT3=reverse T3, T4=tetraiodothyronine (thyroxine), TTR=transthyretin, TBG=thyroxine binding globulin, SULTs=sulfotransferases, UDPGTs= uridine diphosphoglucuronosyl transferase, DIOs=deiodinases

OHCs and their metabolites can disrupt the HPT axis through multiple modes of action and thus alter the regulation and the natural TH balance. This disruption is often based on the structural resemblance of these contaminants with T3 or T4. The OHCs can act directly on the thyroid gland, interfere with negative feedback mechanisms that regulate TH production and secretion, bind to TH-transport proteins in blood (TTR, TBG and albumin) and hence displace THs from the blood, bind to TRs in target tissues, interfere with enzyme systems (iodinases, deiodinases [DIOs], CYP enzymes, UDPGTs, and SULTs) and thus affect production, transport, conversion of T4 to T3, biotransformation and excretion of THs, or interfere with the biological effects of THs (Lans et al., 1993; Brouwer et al., 1998; Schuur et al., 1998; Zhou et al., 2001; Kato et al., 2004; Boas et al., 2006; Langer et al., 2007; Zoeller, 2007; Cao et al., 2010).

The thyroid disruptive effects of OHCs and their metabolites have been documented in experimental *in vitro* and *in vivo* studies (Brouwer et al., 1989; Lans et al., 1993; De Swart et al., 1995; Meerts et al., 2000; Zhou et al., 2001; Arena et al., 2003; Hamers et al., 2006; Kuriyama et al., 2007; Kirkegaard et al., 2011). In humans and wildlife, including several studies of seals from temperate and sub-arctic waters, studies have shown associative relationships between OHCs and THs, which indicates that TH status is affected by these contaminants (e.g. Jenssen et al., 1995; Brouwer et al., 1998; Chiba et al., 2001; Hall et al., 2003; Ribas-Fito et al., 2003; Sørmo et al., 2005; Hall and Thomas, 2007; Langer et al., 2008; Routti et al., 2008; Bloom et al., 2009; Roze et al., 2009; Routti et al., 2010a). Only a few studies have focused on arctic wildlife. Studies of polar bears, glaucous gulls and ringed seals (*Phoca hispida*) from the arctic indicate that arctic top-predators may be at risk for potential TH disruptive effects of OHCs (Sandau, 2000; Skaare et al., 2001; Braathen et al., 2004; Verreault et al., 2004; Routti et al., 2010a).

Given the pleiotropic functions and the requirements of THs during development, TH disruption could negatively impact an individual's health in many ways. In human infants, maternal hypothyroidism has been associated with permanent neurocognitive deficits (Haddow et al., 1999; Pop et al., 1999, 2003). It has been suggested that neurodevelopmental effects (e.g. cognitive dysfunctions, lowered IQ, slowed mental development, behaviour changes, and reduced motor skills) in children born by mothers with high levels of OHCs are mediated through the ability of these contaminants to disrupt TH homeostasis at sensitive periods of TH-dependent brain development *in utero* and during postnatal juvenile stages (Porterfield, 2000; Howdeshell, 2002; Branchi et al., 2003; Zoeller and Crofton, 2005; Nakajima et al., 2006).

Neurocognitive deficits in young mammalian wildlife can reduce their ability to learn, and thus affect their ability to find and hunt prey, change behaviour (e.g. mating) and ultimately affect

reproduction and survival. Furthermore, disturbances of the TH system can also reduce an individual's ability to thermoregulate and to adjust metabolic rate in relation to external factors such as temperature, ice-cover, food availability (fasting), migratory needs or other shifts in energy requirements. Also sexual development and fertility may be affected through OHC-induced TH disruption (Cooke et al., 2004; Kuriyama et al., 2005).

Complex mixture effects

Wildlife, as well as humans, are exposed to and accumulate complex mixtures of "known" and "unknown" environmental chemicals. These mixtures have substantial potential for combined effects (Alexander, 2008). Additive effects of individual chemicals with similar mechanisms or responses are likely to occur by dose addition (similar action) or response addition (dissimilar action), respectively. Interactive effects (antagonism, synergism or potentiation) caused by specific combinations of chemicals are also possible (Koppe et al., 2006; Alexander, 2008). The biological effects of contaminant mixtures will likely depend on the relative contribution of each chemical in the mixture, the single chemical's potency to cause effects (alone or in combination) and the exposed organism's susceptibility (tolerance) to single or combinations of contaminants (Konemann and Pieters, 1996; Koppe et al., 2006; Alexander, 2008).

Even though all environmental exposures are complex mixtures, this complexity is seldom considered. The results of *in vivo* and *in vitro* studies of acute, high dose exposures to single contaminants or simple mixtures, that are often used to assess potential health effects in wildlife and humans, are not readily extrapolated to the multi-generational, low-dose and life-long exposures to "natural" contaminant mixtures. In fact, the cumulative low-dose effects of OHCs on thyroid or other endocrine systems could be more toxic than a single high exposure to a single compound (Koppe et al., 2006). Many OHCs and their metabolites may act through one or several target-points in the HPT axis, often with overlapping mechanisms, which raises the likelihood for combined effects. The potential for combined effects of OHC mixtures on thyroid homeostasis has also been demonstrated in rodent experimental studies with more environmental relevant doses, showing additive and even synergistic effects (Hallgren and Darnerud, 2002; Wade et al., 2002; Crofton et al., 2005; Gauger et al., 2007).

How to evaluate the toxic effects of multiple contaminants in wildlife is a major challenge in ecotoxicology. Studies of potential thyroid disruptive effects of OHCs in wildlife have to date mostly assessed the univariate associations (correlations or regressions) between hormone levels and the sums of tissue residue levels of major OHC groups (e.g. ΣPCB and ΣDDT), although

some studies have used multivariate statistical analysis (Skaare et al., 2001; Lundstedt-Enkel et al., 2005; Routti et al., 2010a). The summed levels of OHC groups give only an "average" of the potential TH disruption, but do take into account the toxic potentials of the individual chemicals. Identifying particular chemicals in mixtures that appear important in influencing TH homeostasis could provide more knowledge about modes of action and potential for combined effects.

Investigating the effects of individual compounds in contaminant mixtures is difficult with respect to data-handling and statistical analysis. However, many of these problems can be solved by using multivariate data analysis (Eide et al., 2001; Feron and Groten, 2002). With multivariate statistics, such as principal component analyses (PCA) and projections to latent structures by means of partial least squares (PLS – Eriksson et al., 2006), all data will be analysed simultaneously. This greatly reduces the risk of Type I and Type II errors, which is a problem when doing repeated number univariate analyses with multiple variables (Sokal and Rohlf, 1981; Norman and Streiner, 2000; Eriksson et al., 2006).

PCA is a multivariate method that can be used to explain the relationships between large numbers of X-variables. PCA will reduce the dimensionality of the data set by projecting the data to a smaller number of uncorrelated variables (latent variables), named principal components (Eriksson et al., 2006). As an extension of PCA, PLS uses and combines features from PCA and multiple linear regression. This multivariate regression method will simultaneously model the relationships between the predictor (X)-variables and the response (Y)-variables and thus identify the most important X-variables explaining the variation of the Y-variables (Wold et al., 2001; Umetrics, 2008). Thus, PLS is a useful method to describe toxic action as a function of multiple chemicals (Eide et al., 2001; Feron and Groten, 2002).

Other problems regarding toxicological data obtained by field studies are also overcome with PCA and PLS since these methods do not require normal distributions, are less sensitive to outliers and extreme values (i.e. noisy datasets), and can deal with datasets consisting of a lower number of observations (individuals) than variables and strongly correlated data (i.e. multi-colinearity) (Wold et al., 1984; Eriksson et al., 2006; Umetrics, 2008).

OHC exposure and biological effects in arctic marine mammals

Arctic marine mammals, such as seals, whales and polar bears are long-lived predators feeding high in the arctic, marine food web, and are known to accumulate considerable amounts of lipid-soluble OHCs in their large subcutaneous lipid-stores (G. Andersen et al., 2001; Sørmo et al., 2006; Letcher et al., 2010). The lipids stores are used for thermoregulation and as energy reserves in periods of greater energy needs (e.g. low food availability, migrations, and lactation). Thus, when stored lipids are liberated in these periods of increased energy requirements, the contaminants will be liberated into the blood (Polischuk et al., 1995; Lydersen et al., 2002), enabling transport to and toxic effects in target organs, as well as biotransformation with possible excretion or production of toxic metabolites. Marine mammals have the enzymatic ability to biotransform OHCs (Letcher et al., 1996; McKinney et al., 2004; Wolkers et al., 2009), and phenolic OHC metabolites (e.g. OH-PCBs and PBDEs) have been detected in polar bears, whales and seals (McKinney et al., 2006a,b; Gebbink et al., 2008b; Routti et al., 2009; Nomiyama et al., 2010). Generally, the polar bear has a higher ability to metabolise and excrete OHCs than cetaceans and pinnipeds, and cetaceans have a lower ability to biotransform OHCs than pinnipeds. Because of differences in the CYP enzyme profiles and activities, the total OHC mixture profile will be species-specific (Letcher et al., 1996; McKinney et al., 2004; Wolkers et al., 2009).

Female marine mammals, like other mammals, transfer contaminants to their offspring via placenta and milk. Lactation transfer of lipid-soluble OHCs from mother to offspring is particularly efficient in marine mammals because of the lipid-rich nature of the milk (Addison and Brodie, 1987; Lie et al., 2000; Wolkers et al., 2006a). The high OHC exposure of marine mammal offspring is of great concern because the developing mammals have a reduced ability to metabolise and excrete contaminants and are generally more susceptible to toxic effects compared to adults (Milsap and Jusko, 1994; Grandjean and Landrigan, 2006; Wolkers et al., 2009). Furthermore, it is not established whether foetuses or young animals themselves are capable of regulating perturbations to their internal endocrine environment (McNabb, 1992; Lintelmann et al., 2003). Thus, young marine mammals may be particularly vulnerable to EDCs and subsequent health-related impairments.

The endocrine disruptive abilities of OHCs raises serious concerns for health and survival of arctic marine mammals, such as polar bears, white whales (beluga whale, *Delphinapterus leucas*) and hooded seals (*Cystophora cristata*) inhabiting the Svalbard (Norway) and East Greenland regions. These areas are considered “hot spots” for contamination in the Arctic (Figure 2) and OHC levels in marine biota, including polar bears and seals, are generally found to be higher

when compared to other arctic areas where OHCs levels have been measured (de Wit et al., 2010; Letcher et al., 2010). Previous studies suggest that high OHC exposure causes health-related impairments in polar bears from East Greenland and Svalbard, such as reduced bone mineral density, histopathological lesions in kidney, liver and possibly thyroid gland, reduced immune response, decreased cub survival, and impaired development of sexual organs (Derocher et al., 2003; Lie et al., 2004; Sonne et al., 2004; Lie et al., 2005; Sonne et al., 2005, 2006a,b, 2007, 2011), which might be linked to endocrine disruptive actions of OHCs (Ropstad et al., 2006; Letcher et al., 2010; Sonne, 2010). Indeed, studies have found associations between OHCs and sex hormones, cortisol and THs in polar bears from Svalbard (Skaare et al., 2001; Haave et al., 2003; Oskam et al., 2003; Braathen et al., 2004; Oskam et al., 2004). However, no studies have investigated the potential OHC-associated effects on TH status or other hormone systems in East Greenland polar bears.

Previous studies have shown that OHC levels in white whales from Svalbard are among the highest reported for wildlife in the European Arctic and at the high range when compared to white whales from the North American Arctic (G. Andersen et al., 2001; Wolkers et al., 2004; G. Andersen et al., 2006; Wolkers et al., 2006b). Still, there is a lack of knowledge on the endocrine or other potential toxic effects of OHCs in arctic subpopulations of white whales. The especially high levels of lipid-soluble OHCs and other contaminants (e.g. polycyclic aromatic hydrocarbons) previously reported in beluga whales from the southern end of this species range in the more polluted St Lawrence River Estuary, have been associated with negative effects on individual and population health (De Guise et al., 1995; Martineau et al., 2003). Differences in exposure patterns and levels complicate extrapolation from these previously reported effects in stranded St Lawrence beluga whales to negative effects on health in arctic subpopulations (Letcher et al., 2010). But the past findings from the St Lawrence population indicate that white whales are vulnerable to effects of organic contaminants present in the marine ecosystem that they inhabit.

In the ice-breeding hooded seal, milk transfer of OHCs is extremely efficient with > 60% milk lipid content and a nursing period lasting only 3-4 days (Bowen et al., 1985; Kovacs and Lavigne, 1992; Espeland et al., 1997; Lydersen et al., 1997; Lydersen and Kovacs, 1999; Wolkers et al., 2006a). Also, hooded seals have a high prenatal investment (Kovacs and Lavigne, 1992; Lydersen et al., 1997), which also favours placental transfer of OHCs to the foetus. Previous studies of hooded seals have demonstrated high OHCs levels in adults, pups and milk, as well as substantial maternal transfer of lipophilic OHCs to pups (Espeland et al., 1997; Hobbs et al., 2002; Wolkers et al., 2006a). Thus, the hooded seal could be a good mammalian model for

studying trans-generation effects of maternally transferred contaminants on thyroid homeostasis.

Polar bears, white whales and hooded seals have a strong ice-association constituting an important part of their life-cycle and biology. This makes them especially vulnerable to global climate changes rapidly taking place in the Arctic (IWC, 1997; Kovacs and Lydersen, 2008; IUCN, 2009; Kovacs et al., 2010). Hooded seals and polar bears are red listed as vulnerable by the International Union for Conservation of Nature (IUCN) because of decreasing population trends (IUCN, 2011). The main reason for the red listing of the hooded seal is the large reduction of the West Ice breeding stock in the past 40-60 years due to hunting (Kovacs, 2008; Kovacs and Lydersen, 2008). All commercial hunting was stopped in 2007 for the West Ice stock, but the continued decreasing population trend and reduced pup production rate are of concern (Kovacs, 2008). Contaminants might also be a part of the problem, in addition to previous overhunting and climate changes (Kovacs, 2008). The white whale is listed as near threatened by IUCN (IUCN, 2011). In Svalbard, white whales have been protected by legislation since the 1960s (Gjertz and Wiig, 1994), but the status of this previously hunted subpopulation is unknown (Lydersen et al., 2001). There is a concern that the combined effects of multiple environmental stressors in the Arctic, such as climate change, contamination, acidification, radioactivity, changes in biodiversity, over-exploitation of resources (e.g. fisheries, hunting), oil and gas activities, and increased shipping may impact these species at the population level (Jenssen, 2006; IUCN, 2009; Kovacs et al., 2010; UNEP/AMAP, 2011).

All these major changes occurring in the Arctic are ecological stressors that require adaptation and acclimatisation. Species living at the edge of their physiological tolerance, such as in the polar regions, may have a limited capacity to acclimate and could therefore be extra vulnerable to the complex interactions between climate change and contaminants and other ecological stressors (Noyes et al., 2009; UNEP/AMAP, 2011). Endocrine systems, including THs, have important functions in arctic marine mammals with respect to the adaptability to environmental changes (Jenssen, 2006). The biological response to EDCs, even at relatively low exposures, could alter the homeostasis and thus the functioning of endocrine systems. Sub-optimal functioning of endocrine systems may not produce adequate responses to adapt to these predicted ice-habitat changes or to other changes in the Arctic (Jenssen, 2006; Letcher et al., 2010; UNEP/AMAP, 2011).

Objectives

The main objective of this thesis was to examine the multivariate influences of complex OHC mixtures on TH status in three arctic marine mammal species (hooded seal, white whale and polar bear). This was done in order to elucidate specific chemicals responsible for possible toxic effects on TH homeostasis and to improve our understanding of mechanisms and sensitivity of different life stages and species to the effects of complex contaminant mixtures.

To meet this objective, the following investigations were performed:

1. Investigate multivariate relationships between circulating TH levels or ratios and concentrations of individual contaminants in the OHC mixtures of polar bears (Paper I), white whales (Paper II) and hooded seal mother-pup pairs (Papers III and IV) inhabiting the Svalbard and East Greenland regions, using multivariate statistics (PLS and PCA).

2. Evaluate the usefulness of multivariate statistical analysis as a tool in ecotoxicology to study the effects of complex contaminant mixtures (Papers I, II, III, and IV).

3. Analyse how biological variables (morphometric parameters, lipid content, season, age, sex, and physiological and reproductive status) may confound investigations of effects of OHCs on TH levels or ratios (Papers I, II, III, and IV).

4. Evaluate the potential for combined effects of specific OHCs on TH homeostasis based on the results of multivariate PLS modelling (Papers I, II, and IV).

5. Evaluate species similarities and differences with respect to PLS modelled effects of OHC mixtures on TH homeostasis (Paper I, II, and IV).

6. Investigate maternal-offspring integrated exposure to complex OHC mixtures, including metabolites from biotransformation, and possible effects on TH homeostasis (Papers I, III, and IV).

Materials and methods

Field sampling

White whales: Blubber biopsies and blood samples, which were subsequently spun to prepare plasma samples, were taken from live-captured white whales at Spitsbergen, Svalbard in July to October in 1996 to 2001 (Fig. 2; Fig. 3; Paper II).

Hooded seals: In March 2008, lactating hooded seal mothers and their suckling, newborn pups (1-4 days old) were live-captured in the breeding area "West Ice" between East Greenland and Jan Mayen (Norway). Blood samples were obtained from both mother and pup, and spun to prepare plasma and serum (Fig. 2; Fig. 4; Papers III and IV).

Polar bears: Samples of whole blood and adipose tissue from polar bears were obtained by trained, local subsistence hunters in the Scoresby Sound area of central East-Greenland during the period of January to October in 1999 to 2001 (Fig. 2; Fig. 5; Paper I).

Ethical considerations

Sampling of polar bears in East Greenland was performed in collaboration with and by local hunters during their traditional, local hunt in the Scoresby Sound area. Polar bear hunting is regulated by quotas amounting to a sustainable 1-2% of the estimated population of 2000-3000 individuals in East Greenland (IUCN, 2006; www.pbsg.npolar.no). All handling procedures for white whales and hooded seals were done in accordance with, and under permits from, the Norwegian National Animal Research Authority. Capturing and handling of white whales at Spitsbergen was also done under permits from the Governor at Svalbard.

Analysis of contaminants

In adipose tissue of polar bears (Paper I), concentrations of PCBs and OCPs were measured at the former Letcher Labs at the Great Lakes Institute for Environmental Research (GLIER) and the University of Windsor (Ontario, Canada). PBDEs were analysed at Environment Canada (Burlington, Canada). In short, the analytical procedures included extraction of adipose tissue, clean-up and subsequent quantification by gas chromatography with micro electron capture detection (GC-μECD) for PCBs and OCPs and electron capture negative ion (low resolution) mass spectrometry (ECNI-MS) for PBDEs. The analytical methods are described more in detail elsewhere (Dietz et al., 2004; Sandala et al., 2004; Dietz et al., 2007).

Analysis of OHCs in white whale blubber biopsies and analysis of OHCs and phenolic OHCs (e.g. OH-PCBs) in hooded seal plasma (merged plasma and serum) were performed at the Laboratory of Environmental Toxicology at the Norwegian School of Veterinary Science, Oslo, Norway. Briefly, the analysis included sample extraction, clean-up, and separation and quantification using GC-μECD (PCBs and OCPs[5]) or GC-MS (BFRs, chlorinated bornanes [CHBs] and phenolic OHCs). Analytical procedures followed previously described methods (Brevik, 1978; G. Andersen et al., 2001, 2006; Sørmo et al., 2006) and were performed with some modifications in these studies (Papers II, III, and IV). In the white whale study (Paper II), previously reported OHC results of two whales sampled in 1996/97 were included (G. Andersen et al., 2001, 2006).

All contaminants analysed in this study are listed in Appendix 1 with full names, abbreviations and molecular structures.

Lipid content of polar bear adipose samples, beluga whale blubber biopsies and hooded seal plasma was determined gravimetrically and expressed as percent of total sample weight (Papers I, II, III, and IV). The concentrations of the lipophilic OHCs analysed in polar bear and white whale adipose/blubber samples were expressed on a lipid weight (l.w.) basis (Papers I and II). Due to analysis of the more water soluble, phenolic OHCs (i.e. OH-PCBs) in hooded seal plasma, all contaminant levels were expressed on a wet weight (w.w.) basis (Papers III and IV).

Analysis of thyroid hormones

Analysis of circulating thyroid hormone levels in all studies were performed at the Department of Biology, Norwegian University of Science and Technology (NTNU, Trondheim, Norway) using radioimmunoassay (RIA). In white whales and hooded seals concentrations of total and free levels of T3 and T4 (TT3, FT3, TT4 and FT4, respectively) in plasma were analysed using commercially available solid-phase ^{125}I RIA kits developed for humans (Papers II, III, and IV). The concentration ratios were also calculated in these studies (TT4:FT4, TT3:FT3, TT4:TT3, and FT4:FT3). TSH levels in white whale plasma were measured using solid-phase ^{125}I immunoradiometric (IRMA) kit developed for canines (Paper II).

Before analysing TH levels in whole blood of polar bears using commercial RIA kits as described above, an extraction step with ethanol was employed prior to RIA analysis (Paper I). Only the

[5] Except chlorinated bornanes (CHBs)

levels of TT3 and TT4 were quantifiable (Paper I), because the extraction liberates protein bound hormone in addition to the free fraction (McMaster et al., 1992).

A more detailed description of analytical procedures and validation for the TH analysis can be found in the individual papers (Papers I, II, III, and IV) and the references therein.

Statistical analysis

The multivariate influence of individual contaminants on TH parameters were investigated using the multivariate statistical tests PCA (Paper III) or PLS regression modelling (Papers I, II, and IV). The available biological variables of each study (e.g. lipid content of samples, sampling date [i.e. season], age, sex, morphometric data, and reproductive and physiological status) were included as covariates as well. Multivariate testing was performed separately based on age and sex, as well as reproductive status, when allowed by sample size (Papers I, III and IV). Further testing of the main findings from PCA and PLS, in addition to investigations of age/sex group differences and correlations between OHCs, THs and biological variables, were performed using univariate statistics (Papers I, II, III, and IV).

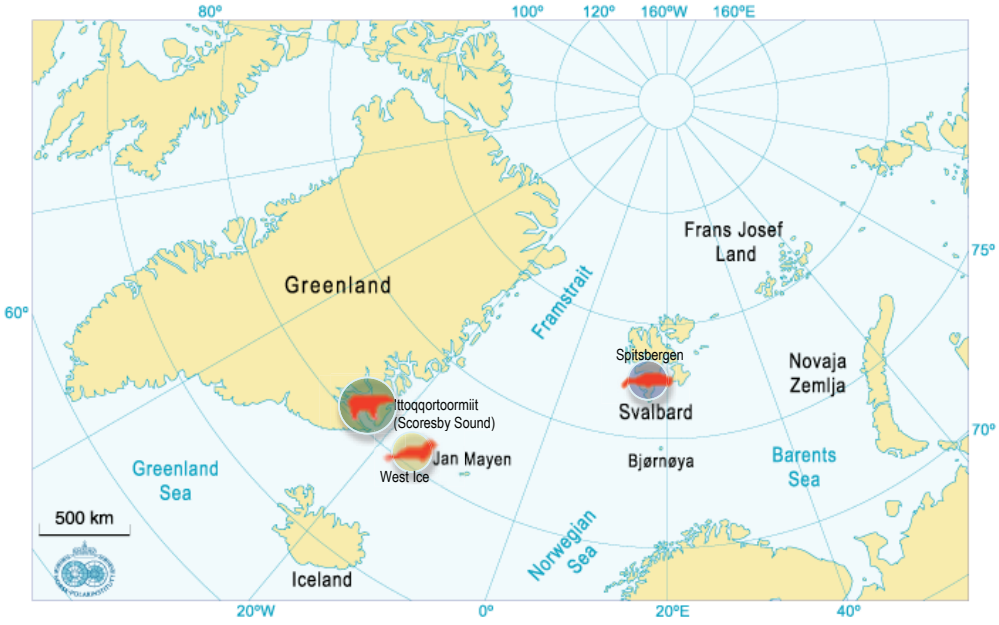

Figure 2. Study areas of investigated species (Map: Norwegian Polar Institute)

Polar bears (*Ursus maritimus*), Scoresby Sound area, East Greenland (1999-2001)

White whales (beluga whales, *Delphinapterus leucas*), Spitsbergen, Svalbard (1996-2001)

Hooded seals (*Cystophora cristata*), West Ice (2008)

Figure 3. Sampling of live-captured white whales (*Delphinapterus leucas*) at Spitsbergen, Svalbard (Norway) in 1997. The whales were caught using nets and held in shallow water near shore while sampling and measuring were conducted. Blood and blubber were sampled and analysed for thyroid hormones and contaminants, respectively. (Photos: Gro D. Villanger)

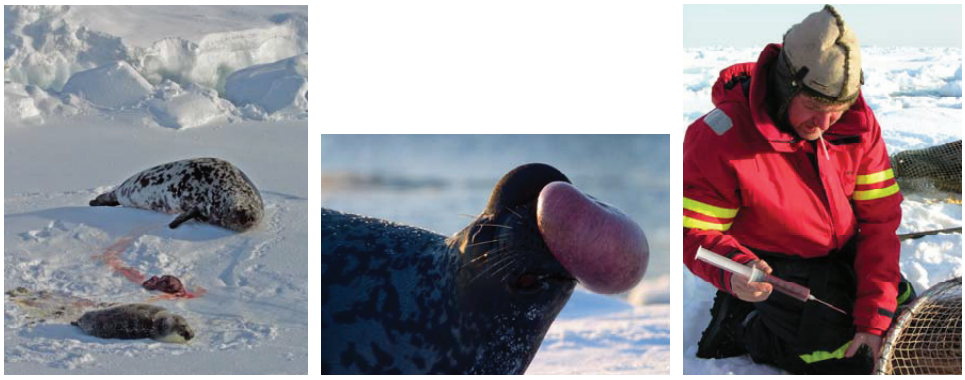

Figure 4. A hooded seal (*Cystophora cristata*) female with her newborn pup laying on the "West Ice" drift-ice between Jan Mayen (Norway) and East Greenland. The placenta in the middle illustrates the short time since parturition (left). A courting hooded seal male ready to mate the female as soon as her pup is weaned (centre). Blood was sampled from hooded seal mothers and their newborn pups in March 2008 for analyses of contaminants and thyroid hormones (right). (Photos: Bjørn M. Jenssen)

Figure 5. Sampling of polar bears (*Ursus maritimus*) in the Scoresby Sound area in East Greenland during the traditional subsistence hunt. The measurements performed included the polar bear's girth and length. Postmortem sampling of adipose tissue, organs and blood have since 1983 been performed by local hunters and scientists in collaboration with Aarhus University (Department of Bioscience) and the Greenland Institute of Natural Resources. A wide range of international studies have used these samples to study contaminant levels and biological effects in polar bears and the present thesis have analysed the effects of contaminants on thyroid hormone levels in bears sampled between 1999 and 2001. (Photos: Robert J. Letcher (left) and Steen Andersen)

Summary of papers

Paper I

OHC levels in East Greenland polar bears are among the highest reported in arctic wildlife, and similar to the levels measured in polar bears from Svalbard (Letcher et al., 2010) where previous studies have reported OHC-associated effects on TH homeostasis (Skaare et al., 2001; Braathen et al., 2004). The objective of the current study was to investigate the multivariate relationships between circulating TH levels and OHC mixtures in polar bears. The study included 62 polar bears of all ages and both sexes sampled during subsidence hunt in the Scoresby Sound area in East Greenland from 1999 to 2001.

TT3 and TT4 levels were analysed by RIA in extracted whole blood samples. Concentrations of 48 individual (or co-eluted) OHCs were analysed in subcutaneous adipose tissue. The relative large samples size in this study enabled subdivisions of results based on age, sex and reproductive status: subadults (SubA, n=23), adult males (AdM, n=17), adult females without cubs (AdF_S, n=15) and adult females with cubs (AdF_N, n=7). The multivariate regression method, PLS, was used to model the influence of contaminants and biological factors (X-variables; morphometric data [length, girth and estimated weight], age, sex, lipid content, and sampling date) on circulating TT3 or TT4 levels (Y-variables).

The PLS models for AdM were not significant, perhaps indicating that THs in males are less influenced by OHCs than in females, as previously suggested in a study of polar bears from Svalbard (Braathen et al., 2004). The PLS models for SubA, AdF_S and AdF_N showed acceptable validation and revealed specific combinations of OHCs that were important in explaining circulating TT3 or TT4 levels. For instance, DDTs had a positive influence on TT3 in AdF_S, and a group of 17 higher chlorinated *ortho*-PCBs had a positive influence on TT4 in AdF_N.

Some contaminants seemed important in all three groups, including PBDE-99, -100, -153, PCB-52, -118, *cis*-nonachlor, *trans*-nonachlor, tri –and pentachlorobenzene (TCB and QCB), and both negative and positive relationships with THs were found. Furthermore, the PLS models for SubA and AdF_N showed some similarities with respect to the most important OHC determinants for TT3 levels: PBDE-99, -100, TCB, PCB-52, -118 and α-hexachlorocyclyhexane (HCH). In addition, biological factors such as age, sex (in SubA), morphometric data, lipid content of adipose tissue and sampling date also influenced TH levels. When the influence of biological variables was removed by partial correlation analysis, the most important contaminants from the PLS models were still significantly associated with TH levels.

The nature of the relationships between OHCs and THs appears to be complex, probably reflecting multiple and overlapping target points on the HPT axis, perhaps resulting in additive and even synergistic responses (potentiation). Although statistical relationships do not represent the full cause-effect relationships, the results of the present study add to the "weight-of-evidence" that complex OHC mixtures might be interfering with TH homeostasis in polar bears.

Paper II

Previous studies of white whales from Svalbard show that OHC levels are relatively high in comparison with other marine mammals from this area, such as polar bears and seals (G. Andersen et al., 2001; Wolkers et al., 2004; G. Andersen et al., 2006; Wolkers et al., 2006b; Letcher et al., 2010). Associations between OHCs and THs, as well as other health variables, have been reported in polar bears from Svalbard and East Greenland (Skaare et al., 2001; Braathen et al., 2004; Ropstad et al., 2006; Letcher et al., 2010; Sonne, 2010; Paper I). The objective of this study was to investigate the multivariate relationships between OHC concentrations and levels of THs and TSH in white whales.

Blubber biopsies and blood were sampled from 12 live-caught white whales (6 adults [5 males and 1 female] and 6 subadults [4 males and 2 females]) in coastal waters of Spitsbergen, Svalbard, from 1996 to 2001. Blubber was analysed for 56 OHCs, and plasma was analysed for levels of THs (TT4, FT4, TT3, and FT3) and TSH using RIA.

The OHC levels in the present white whales were in accordance with previously reported levels in this subpopulation. The results confirmed that levels are high compared to other marine mammals from the same area and in the higher range compared to white whale stocks from the North American Arctic. The somewhat lower TH levels in white whales from Svalbard compared to previously measured levels in white whales from the Canadian Arctic (as reviewed in St Aubin, 2001) might be explained by differences in OHC levels. However, due to inter-study variations in biological factors (e.g. age, sex, season, and moulting status) of the sampled whales as well as analytical differences, this was not possible to determine.

Due to low samples sizes, subadults and adult males were grouped together in the PLS model. The model revealed that the known or suspected thyroid disruptive contaminants PBDE-28, -47, -99, -100, and -154, HCB, and PCB-105 were negatively correlated with circulating levels of FT4, FT3, and TT4. Most of these negative relationships were also confirmed using partial

correlations controlling for body length (as a proxy of age). The positive correlations of TT4, FT4 and FT3 with HBCD, α-HCH, CHB-40 and CHB-62 revealed by the PLS model were not confirmed by partial correlations.

Although the sample sizes were low and statistical models do no prove biological cause-effect relationships, this study suggests negative influences of specific OHCs, particularly some specific PBDE congeners (PBDE-28, -47, -99, -100, and -154), on TH levels in white whales. The impact this might have on individual and population health is unknown. Because THs are important for the opportunistic life-strategy and adaptability of white whales, disturbances of the TH system could reduce their capability to adjust to environmental changes, such as global warming.

Paper III

The reproductive strategy of hooded seals, with high prenatal investment and an intensive lactation period, may expose them to high doses of thyroid disruptive contaminants during gestation and lactation, coinciding with critical stages of foetal and neonatal development. Because of their structural resemblance to the natural THs, OH-PCBs are though to be very potent disruptors of TH homeostasis by potentially different mechanisms (Rickenbacher et al., 1989; Lans et al., 1993; Schuur et al., 1998). Although, maternal transfer of OH-PCBs and retention of these compounds in blood have been shown in other mammalian species (Meerts et al., 2002; Guvenius et al., 2003; Berg et al., 2010), little is known about maternal transfer and offspring exposure to OH-PCBs in seals. Thus, the objective of this study was to measure blood levels of OH-PCBs and investigate their association with circulating concentrations of THs in nursing hooded seal females and their newborn, suckling pups.

Blood was sampled from live-captured hooded seal mother-pup pairs (14 mothers and 14 pups) in 2008 in the West Ice, East of Greenland. Plasma was analysed for THs (TT4, FT4, TT3, and FT3), OH-PCBs, and lipid content. Body mass (kg) of mothers and pups and estimated pup age (1-4 days) were recorded. Lipid content (%) of pup plasma was two-fold higher than in plasma of their mothers. Sum OH-PCB levels (ΣOH-PCBs: 4-OH-CB107, 3'-OH-CB138, 4-OH-CB146, 4'-OH-CB172, 4-OH-CB187) were two times higher in mothers than in their pups. The pattern of OH-PCB congeners was similar in pups and mothers. Levels of individual OH-PCBs in both mothers and pups increased with pup age (i.e. time into the nursing period). The results imply that OH-PCBs found in pups are transferred from their mothers during gestation and that the transfer also continues after parturition via milk.

In pups, PCA showed that 4-OH-CB107 and 3'-OH-CB138 were negatively associated with FT4:FT3 and TT3:FT3 ratios, respectively. These relationships were confirmed by partial correlation analysis correcting for pup age. PCA also suggested that 4'-OH-CB172 and 4-OHCB187 were negatively associated with TT3 levels in mothers. However, this was not confirmed by correlation analyses. The study indicates that hooded seal pups perhaps are more sensitive compared to adults with respect to effects of OH-PCBs on TH homeostasis.

Paper IV

Foetal and neonatal development in mammals appears to be especially vulnerable to potential TH disruptive effects of OHC mixtures transferred from their mothers via placenta and milk (Brouwer et al., 1998; Darras, 2008). Since many lipophilic (i.e. lipid-soluble) OHCs and phenolic OHCs may be thyroid disruptive (Meerts et al., 2002; Boas et al., 2006), the objective of this study was to examine the concentrations of these contaminants in plasma of hooded seal mothers and pups, including previously reported OH-PCBs (Paper III), and to identify the most potent contaminants influencing circulating TH homeostasis.

The levels of OHCs (lipophilic and phenolic) were analysed in plasma from hooded seal mothers and their newborn pups sampled in 2008 in the West Ice. Pups had 1.1-2.5 times higher plasma levels of lipid-soluble OHCs compared to the mothers, even when considering the two times higher plasma lipid content in pups compared to mothers. The effects of all the detected single contaminants, including OH-PCBs, on TH levels and concentration ratios were modelled separately for mothers and pups, using PLS.

In both mothers and pups, significant PLS models were found for the response variable Y=TT3:FT3, which was influenced by α- and β-HCH, low-medium chlorinated *ortho*-PCBs, as well as chlordanes (CHLs) and DDTs in both groups. In pups, TT3:FT3 ratios were negatively influenced by several OH-PCBs, particularly by 3'-OH-CB138, but also by 4-OH-CB146, 4-OH-CB107 and 4-OH-CB187. This high influence of several OH-PCBs on TT3:FT3 ratios were not found in mothers. Furthermore, β-HCH was identified to have a particularly strong, positive influence on TT3:FT3 ratios in pups. HCB and PCB-118 had a stronger (positive) influence on TT3:FT3 ratios in pups than in mothers. These results are in accordance with findings in human newborns and children (Otake et al., 2007; Alvarez-Pedrerol et al., 2008a,b). In mothers, DDTs had a stronger, positive influence on TT3:FT3 ratios than in pups. Also, PLS modelling showed that the known TH-disruptive contaminants PBDE-99 and -100, as well as 4-OH-CB107, were negatively associated with TT4:FT4 ratios in pups. This was not found for the mothers.

Even though the samples were obtained during a limited time interval (within 3-4 days) after parturition and under comparative conditions in the two groups, biological factors, such as plasma lipid content and pup age (i.e. time into the nursing period) still had a high influence on TT3:FT3 ratios in both pups and mothers. This is probably related to the massive physiological changes occurring during the intensive 3-4 days of lactation, both for the fasting and lactating mothers as well as for the suckling and rapidly growing pups. Also, sex was an important factor explaining TT4:FT4 ratios in pups. Even though several biological factors influenced THs, the most important relationships between specific contaminants and TH ratios from the PLS models were confirmed by univariate statistics with biological variables as covariates.

This study identified particular contaminants that appear to disturb the homeostasis between circulating levels of protein-bound and free T3 and T4 in hooded seals. The results suggest that maternally transferred contaminants interfere with the TH balance in hooded seal pups during sensitive early development periods. This may cause a potential for serious hormone-related impairments on individual health that may become evident in later life stages.

Table 1. The statistical modelled responses of circulating thyroid hormone (TH) levels or ratios in relation to concentrations of organohalogen contaminants (OHCs) measured in subcutaneous adipose tissue (AD) or plasma (PL) of the arctic marine mammals investigated in the current thesis. **Bold type** indicates contaminants that were important in explaining TH levels/ratios within a species. **Bald and underlined type** indicates contaminants that were also important in explaining TH levels/ratios across species.

Species, area	n	Tissue	Lipid %	OHC levels (ng/g lipid weight)	TT3 (nmol/l)	TT4 (nmol/l)	FT3 (pmol/l)	FT4 (pmol/l)	TSH (ng/ml)	Major relationships of individual OHCs vs. THs indicated by PLS modelling, with the two most important biological factors *(shown in brackets)*
			Mean ± SD	Mean (range)	Ranges of mean					
Polar bears (*Ursus maritimus*), East Greenland Jan-Oct 1999-2001 (Paper I)	62	AD	88 ±14	ΣPCBs: 6673 (897-20407) ΣDDTs: 412 (74-1151) ΣCHLs: 1887 (354-8132) ΣHCHs: 196 (13.9-818) HCB:78.2 (2.40 -331) ΣPBDEs: 70.0 (17.0-229)	0.55-0.85[a]	6.74-18.4[a]	n.a.	n.a.	n.a.	Subadults (SubA, n=23): **PBDE-100**, **-153**, **PCB-52**, **-118**: ↓TT3 (*Capture day, sex*) PCB-64/71, -31/28, **PBDE-99**, TCB, **α-HCH**, ***cis*-nonachlor**: ↑TT3 (*sex*) **PCB-52**, -170/190, -194, **PBDE-99**: ↓TT4 **TCB, QCB**, PCB-129/178, **PBDE-100**, **-47**, **β-HCH**: ↑TT4 (*Body mass, length*) Adult females with cubs (AdF_N, n=7)) **α-HCH**, **PCB-52**, PCB-101/84, **oxychlordane**, ***p,p'*-DDE**: ↓TT3 (*Body mass, girth*) **PBDE-99**, **-100**, **TCB, QCB**, **PCB-118**, -66/95, -31/28, ***cis*-nonachlor**, ***trans*-nonachlor**: ↑TT3 **Σ_{17}*ortho*-PCBs**, ***cis*-chlordane**, heptachlor epoxide, ***trans*-nonachlor**, **oxychlordane**, **QCB**: ↑TT4 (*length*) Adult females without cubs (AdF_S, n=15) **PBDE-153**, PCB-94 and trans-chlordane: ↓TT3 (*age*) ***p,p'*-DDD**, ***p,p'*-DDT**, ***p,p'*-DDE**, **QCB**, ***cis*-nonachlor**: ↑TT3 (*capture day)* **PBDE-153**, **TCB** and PCB-60: ↑TT4 (*girth*) PCB-182/187 and **β-HCH**: ↓TT4 (*lipid content)* **Adult males** (n=17): no relationships found
White whales (*Delphinapterus leucas*), Svalbard, July-Oct 1996-2001 (Paper II)	11[b]	AD	43 ±27	ΣPCBs= 3369 (631-6210) ΣDDTs: 2965 (308-5805) ΣCHLs: 1670 (398-2989) ΣHCHs: 82.5 (39.9-145) HCB: 370 (66.8-498) ΣPBDEs: 87.9 (22.7-137) HBCD: 42.0 (5.48-237) ΣCHBs:2256 (210-12756)	1.71-1.84	79.1-98.4	0.22-0.89	8.16-12.6	0.043-0.088	**Adult males (n=5) & Subadults (n=6) [b]:** **PBDE**-28, **-47**, **-99**, **-100**, **-154**, **HCB**, PCB-105: ↓FT4, ↓TT4 &↓FT3 CHB-62, CHB-40, **α-HCH**, HBCD: ↑FT4, ↑TT4 and ↑FT3

Species, area	n	Tissue	Lipid %	OHC levels (ng/g lipid weight)	TT3 (nmol/l)	TT4 (nmol/l)	FT3 (pmol/l)	FT4 (pmol/l)	TSH (ng/ml)	Major relationships of individual OHCs vs. THs indicated by PLS modelling, with the two most important biological factors *(shown in brackets)*
			Mean ± SD	Mean (range)	Ranges of mean					
Hooded seals (*Cystophora cristata*) West Ice, March 2008 Adult females (F) and their newborn pups (P)	14	PL	F: 0.7± 0.2	F: ΣPCBs: 561 (256-1100) ΣDDTs: 259 (96.4-395) ΣCHLs: 115 (60-212) ΣHCHs: 7.19 (2.45-13.2) HCB: 12.7 (7.72-16.9) ΣPBDEs: 11.6 (5.13-18.7) ΣOH-PCBs:1.395 (0.339-2.042) [c]	F: 0.76±0.15	F: 15.8±3.26	F: 0.53±0.26	F: 3.67±1.23	n.a.	Females (n=14): **α-HCH**, PCB-141, and **β-HCH**, PCB-149, ***cis*-chlordane**, **PCB-52**, **3'-OH-CB138**, PCB-110, and **PBDE-154**: ↓TT3:FT3 ratio (*pup age)* ***p,p'*-DDE, *p,p'*-DDT**, and PCB-138: ↑TT3:FT3 ratio (*lipid content*)
(Papers III and IV)	14	PL	P: 1.5± 0.7	P: ΣPCBs: 855 (274-1610) ΣDDTs: 495 (163-854) ΣCHLs: 183 (81.2-289) ΣHCHs: 8.97 (4.96-22.2) HCB: 13.5 (8.58-21.9) ΣPBDEs: 23.7 (4.65-47.1) ΣOH-PCBs: 0.683 (0.141-1.059) [c]	P: 2.30±0.48	P: 77.5±29.4	P: 1.91±0.92	P: 25.2±15.3	n.a.	Pups (n=14): **3'-OH-CB138**, 4-OH-CB146, 4-OH-CB107 and 4-OH-CB187: ↓TT3:FT3 ratio (*pup age)* **β-HCH, α-HCH, HCB,** in addition **to *p,p'*-DDD**, ***p,p'*-DDT, *p,p'*-DDE, oxychlordane**, ***cis*-chlordane**, **PCB-118**, and several low-medium Cls PCBs (e.g. **PCB-52**, -99, -101, **-141**, -137, and **-149**.: ↑TT3:FT3 ratio (*lipid content*) **PBDE-99**, 4-OH-CB107, **PBDE-100**, PCB-189, -206, -209, and **PBDE-154**: ↑TT4:FT4 (*sex, pup age*) PCB-74: ↓TT4:FT4 (*sex*)

TT3 = Total Triiodothyronine (T3). TT4 = Total Tetraiodothyronine (T4, thyroxine). FT3=Free T3. FT4= Free T4. TSH=Thyroid stimulating hormone.
n.a. = not analysed
[a] Measured in whole blood
[b] Only juvenile males and females (SubA) and adult males (AdM) were included. A single adult females was not included in the statistical analysis or in the present table due to small sample size (n=1).
[c] Presented as ng/g wet weight

Discussion

In the present thesis the levels of OHCs and their multivariate influences on TH levels and ratios were investigated in three species of arctic marine mammals belonging to three different orders of class Mammalia: order Pinnipedia (hooded seal), order Cetacea (white whale) and order Carnivora (polar bear). This enabled a comparative evaluation of how complex OHC mixtures might influence TH homeostasis in these species (Papers I, II, III, and IV). Also, in the polar bear study (Paper I), and particularly in the studies of hooded seal mother-pup pairs (Papers III and IV) it was also possible to investigate more closely how maternal transferred OHCs may affect TH homeostasis in offspring.

Some considerations regarding study designs and methodologies

Study designs and species

Both advantages and disadvantages can be identified with respect to the choices of study designs and species in the present thesis. In the perspective of studying effects of EDCs in arctic wildlife species, it was an advantage that all three species feed high in the arctic marine food web. Polar bears prey mainly on seals, and white whales and hooded seals mainly prey on fish (Stirling and Archibald, 1977; Heide-Jørgensen and Teilmann, 1994; Dahl et al., 2000; Haug et al., 2007; Thiemann et al., 2008). All the three species can accumulate high and potential toxic levels of OHCs. They also have enzymatic ability to biotransform OHCs and produce potential toxic metabolites (Letcher et al., 1996; McKinney et al., 2004; Wolkers et al., 2009).

In both polar bears and white whales, health impairments have been suggested to be linked to environmental contaminants, such as OHCs (Martineau et al., 2003; Sonne, 2010). TH disruption has previously been associated with OHC exposure in polar bears from Svalbard (Skaare et al., 2001; Braathen et al., 2004). Subpopulations (or stocks) of all three species in the present thesis inhabit areas that are among the most polluted in the Arctic; the Svalbard and East Greenland regions (Fig. 2; as reviewed in Letcher et al., 2010). Hence, it was likely that relationships between OHC mixtures and THs would exist in the sampled animals of the present thesis.

Since all species in the present thesis are mammals, the results from the multivariate modelling could be compared to results reported in many experimental *in vivo* studies on rodents or other mammalian models, as well as to the numerous human studies that have investigated potential effect of OHCs on thyroid homeostasis, including trans-generational effects and developmental impairments in offspring.

There are, however, some disadvantages that need to be addressed. Differences in diets and thus trophic positions, xenobiotic-metabolising enzymes (e.g. CYP enzymes) and biology (e.g. TH physiology, reproduction) between polar bears, white whales and hooded seals, may result in species-specific toxicokinetics and toxicodynamics. Even though this is an interesting aspect, these factors also complicate species comparisons.

For practical, economical and logistical reasons with respect to field sampling and analysis, the designs of the studies in the present thesis vary in regards to time of sampling (year and season), matrixes sampled, analytical methods, sample sizes, and the age and sex distributions, as well as physiological and reproductive status of the sampled animals (Papers I, II, III, and IV). Furthermore, hormone and contaminant analyses included different numbers of TH parameters and individual OHC chemicals, respectively. The sample sizes were quite low for white whales from Svalbard and hooded seal mother-pup pairs from the West Ice (Papers II, III and IV), as is common for studies of non-hunted populations of marine mammals. Variations in sample sizes resulted in somewhat different data-handling and statistical analysis in the studies (Papers I, II, III, and IV). Thus, comparisons of TH and OHC levels across studies and species, as well OHC-associated TH responses, must be done with caution, keeping in mind factors (e.g. age, sex, physiological and reproductive status, sampling dates, sampled matrix, and analytical methods) that can influence the results.

Material and methodologies

Measured levels of thyroid hormones

Identical RIA methods were employed for TH analysis of all the studied species (Papers I, II, III, and IV). Since THs are highly conserved in vertebrate species, commercial RIA kits developed for humans is commonly used in analysing TH levels in marine mammals with acceptable results (Greenwood and Barlow, 1979; Ortiz et al., 2001; St Aubin, 2001; Braathen et al., 2004; Sørmo et al., 2005). In hooded seals and white whales, plasma levels of TT3, FT3, TT4 and FT4 were quantified (Papers II, III, and IV). Due to an extraction step prior to TH analysis with RIA, it was only possible to measure total TH levels (i.e. TT3 and TT4) and not the free fractions (FT3 and FT4) in polar bear whole blood (Paper I).

In the current thesis, TSH levels were measured only in the white whales (Paper II). TSH has generally been difficult to measure in marine mammals, at least by using RIA kits developed for human TSH (St Aubin, 2001). The use of canine IRMA-kit for TSH analyses showed good results in quantifying TSH levels in white whale plasma (Paper II). It might be that the amino acid

sequence of TSH in white whales resembles canine TSH more than human TSH. Hence, this method should be investigated further with regards to measuring circulating TSH levels in marine mammals.

For all three species in the current thesis, there is a general lack of baseline TH and TSH data from free-ranging and non-contaminated individuals. Such baseline or reference values are generally needed to clinically evaluate the TH status of an individual (Demers, 2008). Even though it was not possible to clinically evaluate hypo- or hyperthyroidism of the sampled individuals, circulating TH levels appeared to be within the ranges previously reported in polar bears, white whales and seal pups and adult females (Papers I, II, and III and references therein).

Body condition (i.e. energetic status: amount of stored fat relative to body size) was not determined for the sampled animals in the present thesis; though the included morphometric data and lipid content could be an indication of this parameter (Hall et al., 2001; Cattet et al., 2002; Thiemann et al., 2006). Prolonged fasting (i.e. loss of condition) can alter TH levels in organisms (McNabb, 1992; Ortiz et al., 2001; Hall et al., 2003). Also stress during capturing and handling can affect TH levels through increased cortisol production. However, it is shown in marine mammals, such as white whales, that handling stress affects TH levels only 4-6 hours after the capture (St Aubin and Geraci, 1988, 1992; St Aubin, 2001). Total chase and handling time was shorter in the sampled animals in the current thesis.

When comparatively assessing TH levels (Table 1), it is apparent that white whales as a species seem to have higher circulating levels of TT3, FT4 and especially of TT4 than polar bears and hooded seals (adult females). However, the circulating TT3 and TT4 levels in polar bears might be slightly underestimated due to less than 100% extraction efficiency of whole blood (Paper I). The high TH levels in the present white whales are, however, in accordance with previously recorded high TH levels in this species (St Aubin and Geraci, 1988, 1989a; St Aubin, 2001). The reason for this is yet to be discovered, but it might be related to the opportunistic life-strategy of the species, and perhaps also the need for a large TH buffer to meet the increased TH demands during the yearly epidermal moult (St Aubin, 1990, 2001).

The higher TH levels in the newborn hooded seal pups (Table 1; Paper III) compared to their mothers and to the other sampled white whale and polar bear individuals in the present thesis, coincide with the elevated TH levels commonly reported in neonate phocid seals and other mammalian neonates. This is probably related to the greater thermoregulatory and anabolic needs of neonates (Stokkan et al., 1995; Haulena et al., 1998; Woldstad and Jenssen, 1999).

As expected, TH levels seem to be species-specific, as well as dependent on age (McNabb, 1992; St Aubin, 2001; Zoeller et al., 2007a). It is suggested that the "set-point", which the HPT axis is regulated around, is in large part determined by genetics (Zoeller et al., 2007a), and thus varies between species and individuals. The "set-points" of the species in the present thesis could reflect different evolutionary strategies with regards to adaptations of their biology to their arctic habitats. This may affect inter-species susceptibility and responses of the TH system to potential endocrine effects of environmental contaminants.

Measured levels of organohalogen contaminants

OHC levels in white whales and polar bears were measured in blubber (Papers I and II), whereas in hooded seals OHCs were determined in plasma (Papers III and IV). Generally, the patterns and levels of OHCs can differ somewhat between blood and blubber (Lydersen et al., 2002; Sørmo et al., 2003; Wolkers et al., 2006a) and thus confound comparisons between these tissues. Also, lipophilic OHCs stored in adipose tissue are less available for toxic actions than if they were present in blood or various target organs. However, there will be some degree of partitioning of OHCs between lipids in all body compartments, including blood (Boon et al., 1994; Tanabe et al., 1994; Bernhoft et al., 1997; Polischuk et al., 2002; Sørmo et al., 2003). OHC levels in blood reflect the current situation and are therefore more prone to variations, e.g. due to recent meals, body condition, reproduction and other factors (see Lydersen et al., 2002; Wolkers et al., 2006a). In contrast, OHC levels in adipose tissue (e.g. blubber) is a result of life-long accumulation and are therefore more representative of the total body burden and thus suitable for assessing long-term contaminant exposure and effects (Aguilar, 1985; Wolkers et al., 2006a). Hence, contaminant levels in adipose/blubber tissue was relevant for assessing effects on TH status from long-term exposure to OHCs in white whales and polar bears (Papers I and II).

For hooded seals, it was considered more correct to analyse OHC levels in blood for investigations of potential effects of the ongoing maternal transfer of lipophilic and phenolic (i.e more water-soluble) OHCs (Papers III and IV). Additionally, measuring both OHC and TH levels in blood could shed some light on possible mechanisms of OHC interference with THs in blood, such as binding to transport proteins (e.g TTR and TBG). Many phenolic OHCs are shown to have binding affinity to these transport proteins (Brouwer et al., 1988; Vanraaij et al., 1991; Lans et al., 1993, 1994; Meerts et al., 2000; Sandau, 2000; Cao et al., 2010).

The use of standard biopsy punch for two white whales (Paper II) resulted in shorter biopsies that included mostly the outer blubber layer, and with some connective tissue. This resulted in a lower lipid% of these samples. Studies of baleen whales have shown higher OHC levels in the

outer compared to the inner blubber layer (Aguilar and Borrell, 1991), and this may have biased the OHC results slightly upwards in Paper II. Nevertheless, toothed whales have been shown to have thinner, less structured blubber column (Aguilar, 1985). This could suggest a more homogenous distribution of OHCs. Hence, inclusion of these two samples consisting of mostly outer blubber layer was considered appropriate when OHC levels were adjusted to lipid content (Paper II; see also discussion in G. Andersen et al., 2001).

For polar bears, white whales and hooded seals the most dominant OHCs were ΣPCBs, ΣDDTs and ΣCHLs, while ΣHCHs, HCB and ΣPBDEs appeared to have minor influence on the total OHC load in all three species (Table 1). ΣCHBs was also a major contaminant group in white whales (Paper II; Table 1), but these contaminants were not measured in polar bears or hooded seals (Papers I, III and IV). OH-PCBs were reported in plasma of hooded seal mothers and pups (Paper III). Novel contaminants such as HBCD, pentabromotoluene (PBT), PBEB, dibromopropyl tribromophenylether (DPTE), hexabromobenzene (HBB), bis-tribromophenoxyethane (BTBPE), and deca- and nonaBDEs as well as the phenolic OH-PBDEs, PCP, and tribromophenol (TBP) were not detected in hooded seal plasma (Paper IV). Except for the reported levels of HBCD in white whale blubber (Paper II), neither these novel contaminants nor OH-PCBs were analysed in polar bears or white whales (Papers I and II).

Apart from the higher levels of ΣPCBs and ΣCHLs in polar bears compared to white whales, the white whales have the highest levels of OHCs among the investigated species (Table 1). These inter-species patterns in OHC levels is probably best explained by factors such as geographical distribution, diet including trophic feeding level, and species-specific metabolic capacity to biotransform and excrete OHCs. In polar bears, the high and specific ability to metabolise many OHC probably explains the lower concentrations of most of these compounds compared to white whales, despite its higher trophic position in the arctic marine food web (Letcher et al., 1995; Letcher et al., 1998; Sandala et al., 2004). Even though the plasma OHC levels were adjusted to lipid content in Table 1, the levels in hooded seals were generally lower than in white whale and polar bear adipose tissue. This is probably due to generally lower OHC levels in blood compared to adipose tissue, even when levels are normalised to lipid content (Sørmo et al., 2003; Wolkers et al., 2006a). Nevertheless, cetaceans, including white whales, have been found to have a lower OHC metabolizing ability compared to seals (White et al., 1994; Goksøyr, 1995; McKinney et al., 2004, 2006a). This could also explain the lower OHC levels in hooded seals compared to white whales despite the similar or somewhat higher trophic feeding position of hooded seals relative to beluga whales (Lesage et al., 2001).

Multivariate effects of contaminants on thyroid homeostasis

Multivariate modelling

Several studies of marine mammals, including polar bears and seals, have reported possible disruption of TH homeostasis by PCBs or other OHCs, measured in lipid tissue or in blood (e.g. Jenssen et al., 1994; Hall et al., 2003; Braathen et al., 2004; Sørmo et al., 2005). In the present thesis, the multivariate methods PCA or PLS were applied. PLS is a relatively novel statistical method within wildlife ecotoxicology, although it has been used in some wildlife studies (e.g. Skaare et al., 2001; Lundstedt-Enkel et al., 2005; Murvoll et al., 2006).

Model improvements were performed (see Papers I, II, and IV) by using assigned parameters (explained variance of the X-matrix [R^2X], goodness of fit [R^2Y], goodness of prediction [Q^2], and permutation analysis) to evaluate the quality and validity of the models (Eriksson et al., 2006; Umetrics, 2008). Since the amount of "noise" will be larger in datasets derived from biological field sampling as compared to designed experimental studies, we used criteria for acceptable models suggested by Lundstedt et al. (1998): $R^2Y > 0.7$ and $Q^2 > 0.4$. Also, the permutation analyses had to be within criteria by Umetrics (2008) in order to deem the models acceptable. The resulting PLS models in the present thesis showed good validation (Papers I, II, and IV). This confirms that the results are not coincidental but are reproducible within the datasets.

In Paper III, the data did not produce valid PLS models for hooded seal mothers or pups (results not presented), and thus PCA was used to investigate associations between THs and OH-PCBs. Only the most important PCA results for pups were affirmed with univariate correlations. When the complexity of the contaminant data was increased by also including lipophilic OHCs in Paper IV, the PLS models for both hooded seal pups and mothers became valid. The modelled results indicated that the balance between circulating levels of protein-bound and free THs were affected by OHCs in both mothers and pups (Paper IV). Possible explanations are that several of the included OHCs may disrupt TH homeostasis, and that these compounds may act in combination. This emphasises the importance of including the "whole" mixture of individual OHCs that animals accumulate when investigating possible effects on TH status.

The relationships between individual or combinations of OHCs and THs inferred by PCA or PLS models in the present thesis were further verified by univariate statistics with the most important biological variables as covariates (Papers I, II, III, and IV). The modelled relationships between specific OHCs and TH parameters were also supported by previous findings in wildlife and humans, and *in vivo* and *in vitro* experimental studies (Brouwer et al., 1998; Rolland, 2000;

Boas et al., 2006; Zoeller et al., 2007b; Darnerud, 2008; Jugan et al., 2010). Hence, applying PLS to complex OHC mixtures measured in biological samples appear to be valuable for elucidating which compounds (alone or in combination) that are most important in affecting TH levels and ratios. It is, however, important to acknowledge that statistical modelled associations do not represent toxicological cause-effect relationships *per se*. Still, by cautiously evaluating the results from Paper I to IV, keeping the differences and limitations of the study designs and methodologies in mind, specific contaminants that seemed to be disrupting TH homeostasis within and across species, sexes and life stages could be identified.

Influence of biological factors

Sex-dependent differences in toxic responses may occur due to dissimilar exposure, variations in toxicokinetics and toxicodynamics caused by sex hormones or genetic differences (e.g. gene expression). These sex-dependent differences start during early foetal stages and last until old ages, but are often overlooked in toxicological studies (Mugford and Kedderis, 1998; Gochfeld, 2007; Vahter et al., 2007). In East Greenland polar bears, the relatively large sample size enabled an investigation of TH responses in different groups based on age and sex, as well as reproductive status (Paper I). Due to non-significant PLS models for AdM polar bears, TH levels in AdM seemed less influenced by OHCs than in AdF (Paper I). This is in accordance with a study of polar bears from Svalbard (Braathen et al., 2004) and a human study (Abdelouahab et al., 2008).

Sex was also an important variable influencing TT4:FT4 ratios in hooded seal pups (Paper IV) and TT3 levels in SubA polar bears (Paper I). In white whales (Paper II), the small samples sizes did not enable separated PLS analyses for the sexes. Sex-dependent TH responses associated with OHC exposures were also found in humans, and have been demonstrated in experimental rodent studies (Gochfeld, 2007; Vahter et al., 2007; Abdelouahab et al., 2008). As pointed out by Abdelouahab et al. (2008), sex should not just be a controlling factor in statistical analyses, but TH responses should ideally be investigated separately for the sexes, as was done for adult polar bears in the current thesis (Paper I) .

One important aspect of how sex may influence the effects of xenobiotics is sex-specific differences in biotransformation ability (e.g. enzyme activities/levels), affecting retention time and formation of different metabolites, and thus causing different toxic effects in males and females (Mugford and Kedderis, 1998). In polar bears, a higher bioaccumulation of CHLs in females than in males is probably explained a by lower biotransformation ability for this contaminant group in females (Letcher et al., 1996; Norstrom et al., 1998; Polischuk et al., 2002).

Furthermore, the nonachlors and their metabolite, oxychlordane, were the most persistent and toxic CHL compounds in experimental rat (*Rattus norvegicus*) studies, especially in females, and TH disruptive effects of *trans*-nonachlor was indicated (Bondy et al., 2000, 2004). This might explain why CHLs, such as *cis*-nonachlor, *trans*-nonachlor and oxychlordane, were important in explaining TT3 levels in AdF_N and AdF_S polar bears (Paper I; Table 1) and why *cis*-chlordane was important for TT3:FT3 ratios in hooded seal females (Paper IV; Table 1). A higher accumulation of CHLs in females compared to males also means that more of these toxic compounds are available for transfer to offspring via placenta and milk. Indeed, the CHLs were important in explaining T3 levels and ratios in SubA polar bears and hooded seal pups, respectively (Paper I and IV; Table 1). These results indicate that sex-dependent biotransformation and accumulation in females can affect trans-generational transfer of OHCs and thus exposure and potential effects on TH homeostasis in their offspring.

Hooded seal mothers and pups showed a concurrent pattern with respect to the contaminants influencing TT3:FT3 ratios. HCHs (α- and β-HCH) were among most prominent OHCs that influenced TT3:FT3 ratios in both mothers and pups (Paper IV; Table 1). Also α-HCH was important in explaining TT3 levels in AdF_N and SubA polar bears (Paper I). In hooded seal pups, particularly β-HCH was found to influence TT3:FT3 ratio (Paper IV). Also, the OH-PCBs were more important in explaining TT3:FT3 ratio in pups than in mothers (Papers III and IV). These findings are in accordance with studies of human newborns (Sandau et al., 2002; Ribas-Fito et al., 2003; Otake et al., 2007; Alvarez-Pedrerol et al., 2008a) and foetal and neonate rodents in experimental studies (Sinjari and Darnerud, 1998; Meerts et al., 2002). Differences in TH responses to OHCs in adult and young animals might be explained by differences in toxicokinetics and toxicodynamics, as well as differences in susceptibility to TH disruptive effects of OHCs. Why some particular compounds, such as β-HCH and OH-PCBs, seem to especially affect the TH system of young mammals warrants further studies.

In addition to age and sex, other biological variables were important in explaining TH levels and ratios in the investigated species, such as plasma lipid content, pup age and body mass in hooded seals (Papers III and IV), and capture date, morphometric data and adipose lipid content in polar bears (Paper I). The influence of season (capture date) on TH homeostasis in polar bears is probably related to the circannual TH rhythm that also can be modified by factors such as temperature, light, fasting (due to fluctuations in ice-cover and prey availability), migration, and reproductive status (Leatherland and Ronald, 1981; McNabb, 1992; Cattet, 2000). Furthermore, differences in the PLS models for AdF_N and AdF_S polar bears indicate that reproductive status is important for the TH response to contaminants (Paper I; Table 1). And the opposite

relationships between the same compounds (e.g. β-HCH, α-HCH, PCB-52, and -149) and TT3:FT3 ratios in hooded seal mothers and pups is possibly caused by the physiological differences and thus different toxicokinetics of OHCs in the milk producing females compared to their rapidly growing, suckling pups (Paper IV). For beluga whales, it was not possible to investigate the effect of season on TH levels (Paper II). In seals and white whales, the yearly fur and epidermal moult, respectively, are associated with elevated TH levels (St Aubin and Geraci, 1989b; St Aubin, 1990; Boily, 1996; Routti et al., 2010b). However, none of the sampled hooded seals or white whales were moulting (Papers II, III, and IV), except for one white whale that was in the process of moulting but did not show elevated TH levels (Paper II). In conclusion, the results herein indicate that biological factors such as time of sampling (i.e. season), physiological and reproductive status, as well as age and sex of the sampled individuals influence TH levels and the responses of the TH system to contaminants.

Species comparisons

In AdF and SubA polar bears and in hooded seal pups, the dioxin-like PCB-118 seemed to affect TH homeostasis (Paper I; Table 1). Several studies report a distinctive behaviour of this contaminant in relation to associative effects on TH homeostasis (e.g. Braathen et al., 2004; Sørmo et al., 2005; Alvarez-Pedrerol et al., 2008b). PCB-118 has the ability to induce CYP IA through binding to the aryl hydrocarbon receptor (AhR) (Safe, 1990; van den Berg et al., 2006). PCB-118 (and PCB-105) can be biotransformed to the phenolic metabolite 4-OH-CB107 (Letcher et al., 2000; Sjodin et al., 2000). This metabolite has the ability to bind to human and rat TTR, a major carrier-protein in the blood of these species, and displace T4. Binding of OH-PCBs or other phenolic compounds to TTR or other TH transport proteins is often used to mechanistically explain lowered circulating T4 levels in rats, humans and other species, including polar bears (Brouwer et al., 1988, 1990; Lans et al., 1993; Lans et al., 1994; Sandau et al., 2000; Cao et al., 2010; Gutleb et al., 2010; Ucan-Marin et al., 2010). In a study by Gauger et al. (2007), exposure of pregnant rats with a mixture of six PCB congeners (including PCB-105 and -118) reduced TH levels in rat dams, and also activated TRs *in vivo* and *in vitro*. Gauger et al. (2007) suggested that induction of CYP 1A by the AhR agonist PCB-126 in the mixture resulted in the formation of a metabolite (most probably 4-OH-CB107) from PCB-118 and -105, which in addition to lower T4 levels in blood could activate TRs in target tissues. In the present thesis, PCB-105 had a negative influence on FT3, FT4 and TT4 levels in white whales (Paper II; Table 1). Hence, the PLS modelled effects of PCB-118 (Papers I and IV) and PCB-105 (Paper II) could indirectly reflect the actions of their metabolite, 4-OH-CB107, perhaps with mixed antagonistic/agonistic responses as indicated by Gauger et al. (2007). The presence of TTR in polar bear blood has been verified as well as high plasma levels of OH-PCBs (Sandau, 2000; Sandau et al., 2000; Gutleb et al., 2010;

Ucan-Marin et al., 2010). This indicates that binding of 4-OH-CB107 to TTR is a likely mechanism for affecting TH homeostasis in polar bears. In hooded seals, 4-OH-CB107 was the most dominating OH-PCB in plasma of both mothers and pups (Paper III). This metabolite has previously been reported in white whales (McKinney et al., 2006b). For seals and white whales, however, the existence and importance of TTR as a TH-carrier in blood is uncertain, and TBG is probably a more important TH-binding protein in cetaceans (St Aubin, 2001). Thus, the importance of TTR-binding by OH-PCBs or similar compounds as a thyroid disrupting mode of action is more uncertain for seals and whales.

Chlorobenzenes (CBzs) were important in explaining TH levels in all three species of the present thesis (Papers I, II, and IV; Table 1). HCB influenced TT3:FT3 ratios in hooded seal pups and affected levels of FT3, FT4 and TT4 in white whales (Papers II and IV; Table 1). In polar bears two additional CBzs, TCB and QCB, were analysed and shown to influence TH levels in SubA and AdF, mostly with a positive impact on TT3 or TT4 levels (Paper I; Table 1). TCB and HCB may induce UDPGTs and increase biliary TH excretion. In addition, the phenolic metabolite of HCB, PCP, can potentially bind to TTR and thus displace T4. Both mechanisms can lead to lowered levels of circulating THs (Vanraaij et al., 1991; Vanraaij et al., 1993a, b). PCP has previously been reported in blood and blubber of East Greenland polar bears (Gebbink et al., 2008a). PCP was not detected in hooded seals (Paper IV), while PCP was not analysed in white whales (Paper II). The fact that CBzs influenced TH status in three species of arctic marine mammals shows their importance with regards to potential thyroid disruption in arctic wildlife.

The high influence of many PBDEs (PBDE-28, -47, -99, -100, and -154) on TH levels in white whales despite their relative low importance in the OHC mixture accumulated in their blubber could indicate high species-sensitivity for these compounds (Paper II; Table 1). PBDEs, particularly PBDE-99 and -100, were also important determinants for TT3 and TT4 levels in AdF and SubA polar bears and for TT4:FT4 ratios in hooded seal pups (Papers I and IV). PBDEs were also minor contributors to the total OHC load in polar bears and hooded seal pups. The results are in accordance with *in vivo* experimental studies, and findings in human and other wildlife studies (Zhou et al., 2001; Hallgren and Darnerud, 2002; Darnerud, 2003; Hall et al., 2003; Hall and Thomas, 2007; Herbstman et al., 2008; Dallaire et al., 2009; Routti et al., 2010a). Thus, the PLS models appear to correctly elucidate certain PBDEs, such as PBDE-99 and -100, as highly potent TH disruptors. The potential mechanisms for TH disruption of PBDEs and their OH-metabolites can be several, and may include induction of UDPGTs, or binding to TTR or TBG (Zhou et al., 2001; Hamers et al., 2006; Cao et al., 2010). Furthermore, the low proportion of the PBDEs in the OHC mixtures of the investigated arctic marine mammals indicates that the

identified TH disruptive PBDEs could be acting on the HPT axis in combination with other contaminants. Indeed, additive and even synergistic effects on TH homoeostasis were demonstrated in an experimental study exposing rats to mixtures of PBDEs, PCBs and chlorinated paraffins (Hallgren and Darnerud, 2002).

Ortho-PCBs, particularly PCB-52, and the pesticides CHLs and DDTs appeared to have a more important role in influencing TH homeostasis in hooded seals and polar bears than in white whales (Papers I, II and IV). In seals, *ortho*-PCBs and DDTs have demonstrated *in vitro* induction of constitutive gene expression of androstone receptor (CAR) (Sakai et al., 2009). CAR and the related pregnane X receptor (PXR) are known to be responsible for the activation of many different metabolising enzymes (e.g. CYP IIB, IIC and IIIA, SULTs, UDPGTs, and DIOs) that catalyze the breakdown of both endogenous (e.g. thyroid hormones) and exogenous (e.g. OHCs) compounds (Mortensen and Arukwe, 2006; Sakai et al., 2009; Routti et al., 2010a). Furthermore, since CAR regulates the induction of many of the CYP IIB enzymes that are highly inducible by phenobarbital (PB)-like contaminants (such as *ortho*-PCBs and OCPs), the existence of CYP IIB enzymes in both hooded seals and polar bears could explain the importance of these PB-like OHCs for TH levels in these species, perhaps acting through binding to CAR or PXR. Cetaceans, including white whales, are believed to have low or no constitutive CYP IIB-like enzyme activity (White et al., 2000; McKinney et al., 2004) and thus probably have low expression of CAR and PXR. Therefore, hypothetically this could explain the apparent lesser importance of *ortho*-PCBs, CHLs and DDTs on modulation of TH status in white whales compared to hooded seals and polar bears. This shows that species-specific CYP profiles might cause OHCs to act on the HPT axis via different modes of action. Further investigations into potential contaminant binding to CAR and PXR and subsequent effects on OHC and TH metabolism, might shed some light on species-specific mechanisms of TH disruption.

Complex mixture effects in relation to the health of Arctic marine mammals

The PLS models of the present thesis show that OHC mixtures produce small, variable and complex effects on TH levels or ratios in the investigated species and subgroups. This is probably related to the complicated nature of the TH system and that the OHCs may be affecting the HPT axis through multiple modes of action. The OHCs might also be acting in combination and have paradoxical effects on the TH system in the investigated marine mammals, as have been shown in previous experimental studies with OHC mixtures (Hallgren and Darnerud, 2002; Wade et al., 2002; Crofton et al., 2005; Gauger et al., 2007). It is also very likely that some modes of action are species-specific (e.g. due to different CYP profiles or TH-binding proteins), as

indicated in the present thesis. Although the particular OHC mixtures (i.e. content and relative amount of different chemicals) are important when assessing the impact of OHCs on TH homeostasis, some contaminants seemed to generally affect TH homeostasis across species and subgroups in the present thesis: PBDEs (particularly PBDE-99 and -100), CHLs (e.g. *cis*-chlordane, oxychlordane), *ortho*-PCBs (e.g. PCB-52), PCB-118, HCB, α- and β-HCH, and *p,p'*-DDT, *p,p'*-DDE and *p,p'*-DDE (Table 1). This is consistent with findings in other wildlife and in human studies, as well as in experimental *in vivo* and *in vitro* studies (Brouwer et al., 1998; Rolland, 2000; Boas et al., 2006; Zoeller et al., 2007b; Darnerud, 2008; Jugan et al., 2010).

Given the importance of the specific OHC mixtures for the TH-related responses, great caution should be taken when extrapolating results of effects from more polluted wildlife populations to arctic populations, even within the same species. This is because the chemical mixtures that individuals from different regions or areas are exposed to often differ in composition. Also, due to species differences in TH responses to individual contaminants or mixtures, caution should be taken when extrapolating between different species from the same area. Caution should also be taken when assessing health effects of contaminants based on "threshold" levels from experimental studies. Such extrapolations does normally not consider the contaminant mixtures and levels that wildlife are exposed to, or the species-specific sensitivity to TH disruptive chemicals, including potential effects during sensitive periods of development, which also could affect individuals in subsequent juvenile and adult stages (Arena et al., 2003; Kuriyama et al., 2005, 2007; Roze et al., 2009; Herbstman et al., 2010; Kirkegaard et al., 2011).

Despite the many compensatory mechanisms of the TH system to maintain homeostasis, the diversity in how complex OHCs mixtures can affect the HPT axis does not produce physiological "normal" compensations (Zoeller et al., 2007a). It is suggested that exposure to low OHC levels may disrupt TH homeostasis, even without causing clinical changes in circulating TH levels (Haddow et al., 1999; Boas et al., 2006). Studies have also demonstrated that the individual range of TH levels is narrower than the population range (i.e. "reference-values"). Thus, even small disturbances of the TH system might place the TH levels of an individual outside its own reference–range and thereby causing increased risk of health impairments (S. Andersen et al., 2002, 2003; Zoeller et al., 2007a). Although there were no indications of hypo- or hyperthyroidism in the investigated species of the present thesis, the modelled positive and negative alterations of TH status by OHCs may still represent a health risk.

Many of the contaminants (e.g. α- and β-HCH, OH-PCBs, HCB, TCB, PBDE-99, PCB-118 -52, *p,p'*-DDE, *cis*-nonachlor, and *cis*-chlordane) that influenced TH balance in the mother and offspring

groups of hooded seals and polar bears (Papers I and IV) have in human studies been shown to influence TH balance in mothers, newborns and children, and have been associated with impaired neurodevelopment in children (Nagayama et al., 1998; Ribas-Fito et al., 2003; Takser et al., 2005; Nagayama et al., 2007; Alvarez-Pedrerol et al., 2008a,b; Carrizo et al., 2008; Herbstman et al., 2010). This could indicate that young mammals are at risk of neurocognitive effects from exposure to such contaminants, which can affect important traits such as behaviour, learning, and memory.

The timing of the contaminant exposure relative to the timing of the development of the TH system and brain, and thus the development mode of the species (i.e. altricial or precocial), is of importance in regards to contaminant-induced thyroid disruption and subsequent neurodevelopmental impairments (Howdeshell, 2002; Zoeller and Rovet, 2004). Polar bears fit the altricial development mode where offspring is born with a relatively undeveloped brain and TH system (McNabb, 1992; Ahmed et al., 2008). Its development continues postnatally and coincides with a substantial transfer of OHCs via milk, which for the first 3-4 months after parturition is solely produced from the fasting mother's lipid reserves (Arnould and Ramsay, 1994; Polischuk et al., 2002; Ahmed et al., 2008). Hooded seals and white whales can be considered as precocial species; where offspring reach a more advanced state of anatomical and physiological development before birth (McNabb, 1992; Ahmed et al., 2008). The most important and perhaps most sensitive period of brain and TH system development in these two species may be during gestation. This coincides with a high prenatal investment (Brodie, 1989; Kovacs and Lavigne, 1992; Heide-Jørgensen and Teilmann, 1994; Lydersen et al., 1997) and a possibly large prenatal transfer of potential thyroid disruptive contaminants via the placenta to the foetus. The prenatal development could theoretically represent a more sensitive period for TH-related effects of OHCs in white whales and hooded seals, while the postnatal period could be more critical for polar bears. However, there is a great need to increase the knowledge of sensitive development periods in relation to pre- and postnatal OHC exposure and possible effects on TH homeostasis and neurodevelopment in different species. In this context, the physiological development strategies (i.e. altricial versus precocial) should be considered.

In the present thesis, it was not possible to investigate if the PLS modelled responses of THs to OHC mixtures lead to adverse effects on individual health. However, many studies on East Greenland and Svalbard polar bears indicate that OHC exposure affect health-related indicators (e.g. reduced bone mineral density and histopathological lesions; as reviewed in Ropstad et al., 2006; Letcher et al., 2010; Sonne, 2010), which are suggested to be linked to disruption of hormone systems, among them the TH system (Skaare et al., 2001; Braathen et al., 2004).

Exposure studies with a "whole" mixture approach using "model-species" for polar bears (sledge dogs [*Canis familiaris*) and arctic fox [*Alopex lagopus*]) fed with natural contaminated minke whale (*Balaenoptera acutorostrata*) blubber, as well as exposure of goat (*Capra aegagrus hircus*) exposed to a few specific OHC compounds during foetal and neonatal stages, strongly support the assumed OHC-induced health impairments of polar bears from the European Arctic and that this may be linked to perturbations of endocrine systems (as reviewed in Ropstad et al., 2006; Sonne, 2010). The results in Paper I add to the "weight-of-evidence" that contaminants affect TH homeostasis, and thus possibly health in polar bears.

Fox (1991) suggested that demonstrating associations in more than one species or species population are strong indicators of causation in ecoepidemiology. Thus, the results from the studies of polar bears, white whales and hooded seals in the present thesis strongly suggest that long-term, low-dose exposure to OHC mixtures are interfering with the TH homeostasis in arctic marine mammals, and that maternal OHC transfer during foetal and neonatal stages is involved in TH disruption in offspring. This causes concern for possible health-related effects in later life stages of the young arctic marine mammals investigated in the present thesis. Endocrine effects of contaminants on TH homeostasis may also reduce individual and population adaptability to climate changes and other ecological stressors in the Arctic, which puts these species at risk for future population decline.

Conclusions and future perspectives

The conclusions of the present thesis and future recommendations are specified below in accordance to the investigative aims.

Conclusion 1. The results of the present thesis have given new insight into effects of complex contaminant mixtures on the TH system in arctic marine mammals. Paper I is the first study to report on TH disruptive effects in East Greenland polar bears. Paper II, and paper III and IV, are the first studies to report on disruptive effects of contaminants on THs in white whales and hooded seals, respectively. The findings in the present thesis are strong indicators that complex OHC mixtures are affecting the TH system in these three species of arctic marine mammals.

Conclusion 2. Despite the relatively low sample sizes, and other problems related to ecotoxicological field studies (e.g. known and unknown confounders), this study has shown that multivariate statistics, such as PLS, can be applied to "model" the effects of environmental OHC mixtures on THs in free-ranging wildlife species. Specific chemicals that appeared potent in disturbing TH homeostasis across the investigated species were revealed by the statistical models (e.g. PBDE-99, -100, oxychlordane, *cis*-chlordane, *ortho*-PCBs [e.g. PCB-52], PCB-118, HCB, α- and β-HCH, and *p,p'*-DDT, *p,p'*-DDE and *p,p'*-DDE). The modelled effects of these particular compounds are in accordance with previous reported results from experimental *in vivo* and *in vitro* studies, and well as human and wildlife studies.

Future perspectives. To follow up the current thesis, it might be possible to perform a meta-analysis of all species' PLS modelled results, together with known *in vivo* and *in vitro* data for TH-related toxicity, perhaps using cluster analysis (Feron and Groten, 2002). The resulting TH toxicity profiles can then be used to create predictive PLS models and assess the TH related effects of "similar" mixtures in these and other arctic species. Future studies should also focus on developing multi-response models incorporating several endocrine variables and model how contaminant-induced alterations of these parameters affect key health indicators.

Conclusion 3. This thesis also highlights the importance of including biological variables as confounders when investigating the relationships between contaminants and TH status. Age and sex were all over important confounders in the relationships between OHCs and THs.

Future perspectives. Future studies should evaluate subgroups based and age and sex separately in the statistical examination of relationships between OHC and THs. Other factors such as lipid content, season, morphometric data, and physiological and reproductive status should also be

accounted for in the data analysis. Ideally, future studies should try to reduce the number of confounding variables that could influence the interpretation of OHC-induced effects on TH homeostasis.

Conclusion 4. Many of the contaminants that were associated with alterations in TH levels or ratios were among the least dominant chemicals in the measured OHC mixtures in polar bears, white whales or hooded seals (e.g. PBDE-99 and -100, PCB-52, *cis*-chlordane, α- and β-HCH). This strongly indicates that these contaminants have a high potency to disrupt TH homeostasis. Also, it is likely that these chemicals are acting in combination with other chemicals in the OHCs mixture resulting in additive and perhaps even synergistic effects. This implicates that the relative contribution of each chemical in the mixture is very important for their total effect on TH homeostasis.

Future perspectives. In future studies, more effort should be put into assessing hormone disruptive effects of "natural" contaminant mixtures in wildlife, human and experimental studies, and especially during early development stages. Extrapolation from experimental studies to arctic wildlife should be done with great caution, as should comparisons with effects observed in wildlife populations inhabiting more polluted waters. In addition, studies on TH disruption in wildlife should account for the complexity of environmental contamination and include more contaminants that could affect TH homeostasis (e.g. PFCs, mercury, "new" brominated flame retardants, and other emerging contaminants). Since levels and patterns of OHCs in arctic biota are changing, studies should investigate TH-related effects of the present contaminant mixture in arctic wildlife.

Conclusion 5. Despite differences in study designs and methodologies, multivariate modelling of the effects of individual OHCs on THs in the present thesis revealed some species-specific trends. Some OHC compounds appeared generally important as determinants for TH homeostasis in the investigated species (see Conclusion 2); however, the specific TH parameters and the direction of the relationships with individual OHCs varied among the species, as well as between age/sex groups. These variations are probably related to species and age/sex-specific OHC profiles. Furthermore, differences in TH regulation and in "set-points" and other characteristics of the HPT axis (e.g TH-transport proteins) will likely account for species differences in sensitivities and thus responses of the TH physiology to the various modes of action by individual OHC chemicals.

Future perspective. To increase the understanding of potential effects of contaminants on the TH system and thus the health of arctic wildlife, it is imperative to gain more species-specific knowledge of TH physiology at the point of production, transport, metabolism and regulation, and how environmental chemicals mechanistically can affect these target points. Furthermore, it is important to investigate how endpoints for TH action in different target tissues might be affected by OHCs, in order to link the altered TH status to individual health impairments. There is a paucity of knowledge on the regulation of TH homeostasis, including transport into and effects of THs in the developing brain, during foetal and neonatal life stages.

Conclusion 6. The multivariate modelled relationships between OHCs and TH identified specific chemicals that were potent in causing TH disruption in mother and offspring groups of hooded seals and polar bears (e.g. α- and β-HCH, OH-PCBs, HCB, TCB, PBDE-99, PCB-118 -52, *p,p'*-DDE, *cis*-nonachlor, and *cis*-chlordane), coinciding with human studies of mothers and newborns. Although hooded seal mothers and their newborn pups had common sets of chemicals that influenced TH homeostasis, pups appeared more susceptible to the effects of certain chemicals (e.g. OH-PCBs and β-HCH). Also, it was quite clear that in hooded seals, the OHC mixture of lipophilic and phenolic metabolites was important for the modelled effects on the TH system, suggesting that the contaminants act in combination. The hooded seal appeared to be a good model for investigating effects of OHCs during sensitive life stages of mammals, including humans.

Future perspectives. The field of ecotoxicology would greatly benefit from comparative studies of both human cohorts and wildlife populations. For instance, neurocognitive impairments by OHC-induced alterations of TH status during pre- and postnatal brain development are not possible to study in free-ranging wildlife. However, results from human studies are useful for interpreting the possible effects on neurodevelopment and thus wildlife health. On the other hand, investigations of OHC-related health effects in human cohorts or epidemiologic studies must statistically correct for many lifestyle-related and socioeconomic confounding factors, thus reducing statistical power with the risk of loosing important toxicological relationships. These confounders are not present in wildlife studies and could therefore give complementary data in order to achieve a better understanding of toxicological effects of contaminants on the TH system in all mammals.

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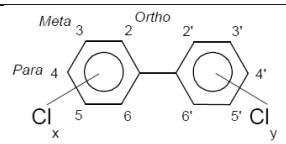
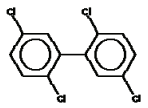
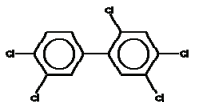
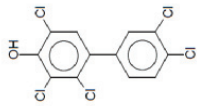
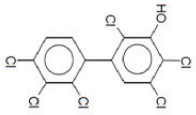
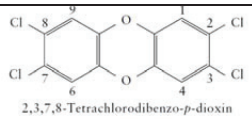
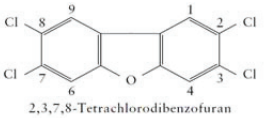
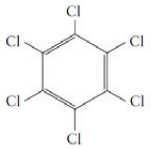
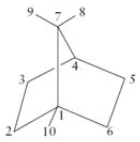
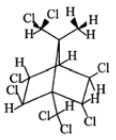
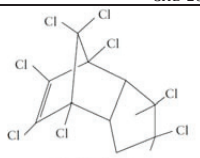
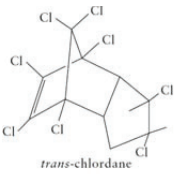
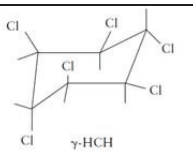
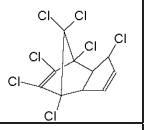
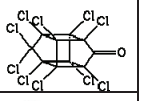
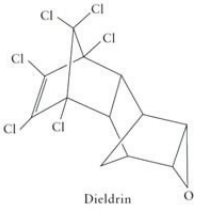
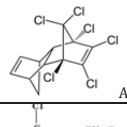
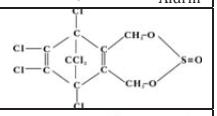
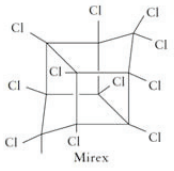
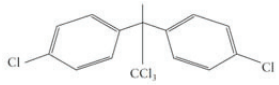
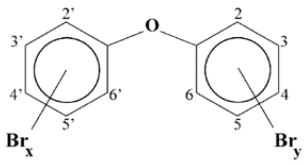
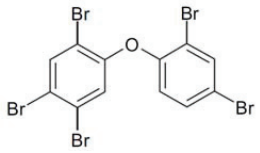
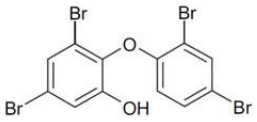
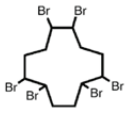
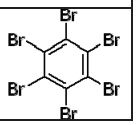
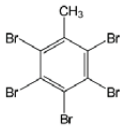
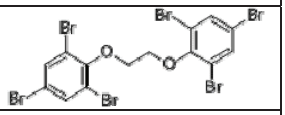
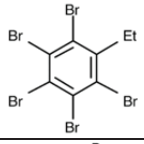
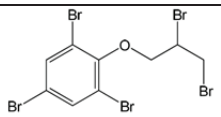
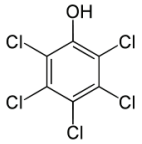
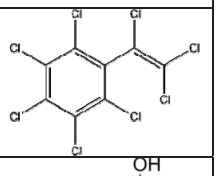
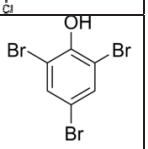
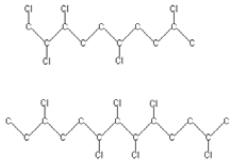
Appendix 1

A selection of organohalogen contaminants.

Chemical name	**Abbreviation**	**Regulation in the Stockholm Convention[a]** A. Banned/restricted B. Under consideration	
* Polychlorinated biphenyls[2,3]	PCBs (209 potential congeners numbered according to IUPAC[b])	A (All)	 General structure of PCB  PCB-52  PCB-118  4-OH-CB107  3'-OH-CB138
Polychlorinated dibenzo-p-dioxins/ polychlorinated dibenzofuranes[3]	PCDD/Fs	A. (All)	 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin  2,3,7,8-Tetrachlorodibenzofuran
*Chlorobenzenes[1, 2, 3]: hexachlorobenzene pentachlorbenzene trichlorobenzene	Cbzs; HCB QCB TCB	A. (HCB)	 HCB

*Chlorinated bornanes (Toxaphenes)[1]	CHBs Complex mixture consisting mainly of chlorinated bornanes. CHB congeners Parlar nos. 26, 40, 41, 44, 50 and 62 were analysed in the present thesis	A. (All)	 General structure of CHBs (most have 6-10 Cls)  CHB-26
*Chlordanes[1] (e.g. *cis*- og *trans*-chlordane, *cis* og *trans*-nonachlor, and oxychlordane)	CHLs Technical mixtures consist of at least 120 compounds	A. (All)	 *cis*-chlordane  *trans*-chlordane
*Hexachlorocyclohexane[1]	HCHs; α-, β and γ-HCH	A. (All)	 γ-HCH
Heptachlor[1]		A.	
Chlordecone[1]		A.	
*Dieldrin[1]		A.	 Dieldrin

Aldrin, Endrin[1]		A.	 Aldrin
Endosulfan[1]		B.	
*Mirex[1]		A.	 Mirex
*Dichloro-diphenyl-trichloroethane[1]	DDT (and related compounds/ metabolites - DDE and DDD)	A.	 *p, p'*-DDT
* Polychlorinated diphenylethers[2]	PBDEs (209 potential congeners numbered according to IUPAC[b])	A. (tetra- and penta-BDEs, and hexa- and hepta-BDEs)	 General structure of PBDE  PBDE-99  6-OH-BDE-47
*Hexabromo-cyclododecane[2]	HBCD (sum of α-, β- and γ-HBCD in the present thesis)	B.	
*Hexabromobenzene[2]	HBB	B.	

*Pentabromotoluene[2]	PBT		
*1,2-Bis(2,4,6-tribromophenoxy)ethane[2]	BTBPE		
*Pentabromo-ethylbenzene[2]	PBEB		
*2,3-dibromopropyl 2,4,6-tribromophenyl ether[2]	DPTE		
*Pentachlorophenol[1,2,c]	PCP		
*Octachlorostyrene[3]	OCP		
*2,4,6-tribromophenol[1,2,3]	TBP		
Short chained chlorinated paraffins[2]	SCCPs	B.	 C10H17Cl5 and C13H22Cl6
PFC[2]	Perfluorated compounds	A. Perfluorooctanesulphonic acid (PFOS)	 PFOS

Sources: [1] Pesticides, [2] Industrial chemicals, [3] By-products
*Compounds that were analysed in the present thesis.
[a] Stockholm Convention (2009). [b] IUPAC= International Union of Pure and Applied Chemistry. [c] PCP is also a metabolite formed during endogenous biotransformation of HCB (van Ommen et al., 1985)

Errata

Page 7, line 4: ".., and is has been shown.." has been corrected to ".., and it has been shown.."

Page 8, line 16: Bracket ")" has been inserted after "..sulfontransferases [SULTs] enzymes"

Page 14, line 5: "low food ability" has been corrected to "low food availability"

Page 15, line 5: Bracket ")" was removed from "… of sexual organs)"

Page 32, last sentence: "This is probably is related.." has been corrected to "This is probably related.."

Page 38, line 30: "inn" has been corrected to "in"

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Exposure to mixtures of organohalogen contaminants and associative interactions with thyroid hormones in East Greenland polar bears (*Ursus maritimus*)

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ABSTRACT

We investigated the multivariate relationships between adipose tissue residue levels of 48 individual organohalogen contaminants (OHCs) and circulating thyroid hormone (TH) levels in polar bears (*Ursus maritimus*) from East Greenland (1999–2001, n = 62), using projection to latent structure (PLS) regression for four groupings of polar bears; subadults (SubA), adult females with cubs (AdF_N), adult females without cubs (AdF_S) and adult males (AdM). In the resulting significant PLS models for SubA, AdF_N and AdF_S, some OHCs were especially important in explaining variations in circulating TH levels: polybrominated diphenylether (PBDE)-99, PBDE-100, PBDE-153, polychlorinated biphenyl (PCB)-52, PCB-118, *cis*-nonachlor, *trans*-nonachlor, trichlorobenzene (TCB) and pentachlorobenzene (QCB), and both negative and positive relationships with THs were found. In addition, the models revealed that DDTs had a positive influence on total 3,5,3′-triiodothyronine (TT3) in AdF_S, and that a group of 17 higher chlorinated *ortho*-PCBs had a positive influence on total 3,5,3′,5′-tetraiodothyronine (thyroxine, TT4) in AdF_N. TH levels in AdM seemed less influenced by OHCs because of non-significant PLS models. TH levels were also influenced by biological factors such as age, sex, body size, lipid content of adipose tissue and sampling date. When controlling for biological variables, the major relationships from the PLS models for SubA, AdF_N and AdF_S were found significant in partial correlations. The most important OHCs that influenced TH levels in the significant PLS models may potentially act through similar mechanisms on the hypothalamic–pituitary–thyroid (HPT) axis, suggesting that both combined effects by dose and response addition and perhaps synergistic potentiation may be a possibility in these polar bears. Statistical associations are not evidence per se of biological cause–effect relationships. Still, the results of the present study indicate that OHCs may affect circulating TH levels in East Greenland polar bears, adding to the "weight of evidence" suggesting that OHCs might interfere with thyroid homeostasis in polar bears.

1. Introduction

Chronic low-level exposure to endocrine disrupting chemicals (EDCs) may cause detrimental effects on development, behaviour, fertility and survival in wildlife species (Colborn et al., 1993; Crisp et al., 1998; Lintelmann et al., 2003). EDCs and global climate changes are among the currently important anthropogenic stressors to arctic wildlife and ecosystems. EDCs may affect an individual's ability to adapt to ongoing climate-related changes in the Arctic (Jenssen, 2006). Many anthropogenic organohalogen contaminants (OHCs), including polychlorinated biphenyls (PCBs), polybrominated diphenylethers (PBDEs), and organochlorine pesticides (OCPs) such as DDTs, have been shown to have endocrine disruptive activity (Colborn et al., 1993; Crisp et al., 1998; Goodhead and Tyler, 2009). Common characteristics define these compounds as persistent organic pollutants as they are subjected to long-range transport to the Arctic where marine food web biomagnification results in high levels in lipid-rich tissues of top predators, such as the polar bear (*Ursus maritimus*) (Letcher et al., 2009, 2010; Sørmo et al., 2006; Thomann, 1989).

Female polar bears, as with other mammals, transfer OHCs through the placenta to the foetus and through lipid-rich milk when lactating their cubs (Lie et al., 2000; Polischuk et al., 1995, 2002). The toxic properties of OHCs have raised serious concerns for health, reproduction, and survival in polar bears, especially in areas with high contamination loads such as the Svalbard and East Greenland regions

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(Letcher et al., 2010; Sonne, 2010). In these areas, OHCs have been associated with a range of detrimental effects in polar bears as reviewed in Letcher et al. (2010) and Sonne (2010); reduced immune response (Lie et al., 2004, 2005), disruption of endocrine and vitamin systems (Braathen et al., 2004; Haave et al., 2003; Oskam et al., 2003, 2004; Sandau, 2000; Skaare et al., 2001), reduced survival of cubs and adult females (Derocher et al., 2003), histopathological lesions in kidneys (Sonne et al., 2006a) and liver (Sonne et al., 2005), reduced size of sexual organs (Sonne et al., 2006b, 2007), and reduced bone mineral density (Sonne et al., 2004).

One important endocrine target of OHCs is the hypothalamic–pituitary–thyroid (HPT) axis (Brouwer et al., 1998; Howdeshell, 2002; Jugan et al., 2010), which serves to produce and regulate circulating levels of the thyroid hormones (THs) 3,5,3′,5′-tetraiodothyronine (thyroxine, T4) and 3,5,3′-triiodothyronine (T3). THs are produced by the thyroid gland in vertebrates, and are transported in blood, mainly bound to serum proteins like transthyretin (TTR), albumin and thyroxin binding globulin (TBG) in mammals (Hadley, 1996; McNabb, 1992; Zoeller et al., 2007a). TTR and albumin are present in polar bears, but their importance as TH transporters is not known (Gutleb et al., 2010; Sandau, 2000; Sandau et al., 2000). THs are essential for somatic growth and development in vertebrates, especially of the nervous system in the foetuses, in neonates and in juveniles. THs are also involved in the regulation of metabolism, thermoregulation, and reproduction, and in maintaining the general physiological homeostasis (Hadley, 1996; McNabb, 1992; Zoeller et al., 2007a). The thyroid disruptive effects of OHCs have been documented in experimental *in vitro* and *in vivo* studies, in human epidemiologic studies and in wildlife studies (Boas et al., 2006; Brouwer et al., 1998; Darnerud, 2008; Rolland, 2000; Zoeller et al., 2007b). In polar bears, several studies have reported inverse associations between OHCs and circulatory concentrations of THs (Braathen et al., 2004; Sandau, 2000; Skaare et al., 2001). The mechanisms of the TH disruptive effects of OHCs and their metabolites can be multiple, and often based on the structural resemblance of such compounds to T3 and T4. OHCs can act directly on the thyroid gland, interfere with the negative feedback mechanisms that regulate TH production and secretion, bind to TH-transport proteins in blood (e.g. TTR) thus displacing THs and influencing TH homeostasis in the blood, bind to and activate thyroid hormone receptors (TRs) in target tissues thus altering TH-mediated gene expression, interfere with enzyme systems [iodinases, deiodinases, cytochrome P450-dependent enzymes (CYP enzymes), uridin diphosphate glucoronyltransferases (UDPGTs), and sulfontransferanses (SULTs)] thus affecting production, conversion of T4 to T3, biotransformation and excretion of THs (Boas et al., 2006; Brouwer et al., 1998; Howdeshell, 2002; Langer et al., 2007; Zoeller, 2005).

THs are important for neurodevelopment in young animals and for proper development and function of gonads, and they influence circulating levels of sex hormones (Cooke et al., 2004; Zoeller et al., 2007a). In humans, thyroid disorders appear more frequently in women than in men, and female hypothyroidism can disturb menstruation, ovulation, and increase the rate of miscarriages (Chiovato et al., 1993; Cooke et al., 2004; Zoeller et al., 2007a). In a study of polar bears from Svalbard, females seemed to be more susceptible to thyroid disruptive effects of PCBs than males (Braathen et al., 2004). Maternal hypothyroidism can reduce transfer of THs from the mother to her foetus and cause permanent neurocognitive deficits in infants (Pop et al., 1999, 2003). In humans, even subclinical, maternal hypothyroidism has been associated with reduced neuropsychological performance in children (Haddow et al., 1999). It has been proposed that neurodevelopmental effects (e.g. cognitive dysfunctions, lowered IQ, slowed mental development, behaviour changes, reduced motor skills) in children born by mothers with high levels of OHCs are mediated trough the ability of these contaminants to disrupt TH homeostasis in sensitive periods of TH-dependent brain development *in utero* and postnatal juvenile stages (Branchi et al., 2003; Howdeshell, 2002; Nakajima et al., 2006; Porterfield, 2000; Zoeller and Crofton, 2005; Zoeller et al., 2007a). Some OHCs might also directly disturb TH signalling or alter neurotransmitter levels in the developing brain (Darras, 2008; Gauger et al., 2004; Porterfield, 2000).

Wildlife and humans are exposed to and accumulate complex mixtures of environmental contaminants. Thus, the effects of OHCs on the TH system is most likely dependent on the relative contribution of single TH disruptive compounds in such complex mixtures and their potency to affect the HPT axis, in addition to the exposed organism's susceptibility (tolerance) to single or combinations of these contaminants (Alexander, 2008; Konemann and Pieters, 1996; Koppe et al., 2006). Since many OHCs and their metabolites may act through one or several target-points in the HPT axis, often with overlapping mechanisms, it raises the likelihood of combined effects. In the total mixture of anthropogenic compounds that humans and wildlife are exposed to, additive effects of individual chemicals with similar mechanisms or responses are likely (dose or response addition). The cumulative low-dose effects of OHCs on thyroid or other endocrine systems could in fact be more toxic than one high exposure of a single compound (Koppe et al., 2006). Interactive effects (antagonism, synergism or potentiation) caused by certain combinations of chemicals are also likely (Alexander, 2008; Koppe et al., 2006). Studies on endocrine effects on wildlife has to date mostly assessed the associations between hormone levels and the sum of tissue residue levels of major OHC groups (e.g. ΣPCB, ΣDDT). Hence, the varying toxic potential of individual compounds or combinations is seldom considered.

The associations between TH and OHC levels reported in polar bears from Svalbard (Braathen et al., 2004; Skaare et al., 2001) suggest that relationships between THs and OHCs could exist in polar bears from East Greenland as well, since concentrations reported in East Greenland and Svalbard polar bears are equally high (Letcher et al., 2010; Sonne, 2010). The purpose of the present study was to investigate if TH levels in polar bears from East Greenland are affected by complex mixtures of OHCs that they accumulate. This was investigated by assessing relationships of individual OHCs measured in adipose tissue with circulating TH levels by using multivariate statistics. Also, confounding factors such as body size, age, sex, and sampling date was included as covariates in the analysis. The relationships elucidated by multivariate analysis was further explored by using univariate statistics.

2. Methods

2.1. Sampling procedures

Tissue sampling and biological measurements of individual polar bears were conducted *post mortem* by trained, local subsistence hunters in the Scoresby Sound area in central East Greenland (69°00′ N to 74°00′N) in January to October 1999–2001. Detailed descriptions of tissue sampling and biological measurements are given elsewhere (Dietz et al., 2004; Sandala et al., 2004). Briefly, the hunters recorded sex, body length and girth, date, geographical position, and if the females were solitary or had cubs. Blood samples were collected (lithium heparin — whole blood) and used for analyses of THs. Freezing caused the blood to completely haemolyse. For the analyses of OHCs, subcutaneous adipose tissue was collected from the chest region. Blood and adipose samples were frozen at −5 to −20 °C from the time of sampling, and then kept at −20 °C until analysis. The third incisor tooth (I_3) was used for age determination as described elsewhere (Dietz et al., 1991, 2004). Age was given in years and rounded to the nearest half year. The age data were first reported in Dietz et al. (2004). Body mass (BM) of the polar bears was calculated from the measured girth and length based on the equation given by Derocher and Wiig (2002).

2.2. Analyses of organohalogen contaminants

The analyses of PCBs and OCPs in subcutaneous adipose tissue were performed in the former Letcher Labs at the Great Lakes Institute for Environmental Research (GLIER) and the University of Windsor (Ontario, Canada) (Dietz et al., 2004; Sandala et al., 2004). PBDEs were analysed at Environment Canada (Burlington, Canada) (Dietz et al., 2007). Procedures involved extraction of adipose tissue, lipid determination, clean-up and subsequent quantification by gas chromatography with micro electron capture detection (GC-μECD) for PCBs and OCPs, and by electron capture negative ion (low resolution) mass spectrometry (ECNI-MS) for PBDEs. Lipid content of the adipose samples was determined gravimetrically using a fraction of the extract and expressed as % of total sample weight. The OHC concentrations were first reported in Dietz et al. (2004, 2007). The individual or co-eluted compounds included in the present study were: PCB-31/28, -42, -44, -49, -52, -60, -64/71, -66/95, -70, -74, -87, -97, -99, -101/84, -105, -110, -118, -128, -129/178, -138, -141, -146, -149, -151, -153, -158, -170/190, -171/202/156, -172, -174, -177, -179, -180, -182/187, -183, -194, -195, -200, -201, -203/196, and -206; PBDE-47, -99, -100, -153, and -155/126; α-, β-, and γ-hexachlorocyclohexane (HCH), hexachlorobenzene (HCB), trichlorobenzene (TCB), pentachlorobenzene (QCB), 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane) (*p,p′*-DDT), 1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene), (*p,p′*-DDE),1,1,dichloro-2,2-bis(4-chlorophenyl) ethane) (*p,p′*-DDD), oxychlordane, *cis*-chlordane, *trans*-chlordane, *cis*-nonachlor, *trans*-nonachlor, heptachlor epoxide, dieldrin and octachlorostyrene (OCS). All the OHC concentrations are given in nanograms per gram lipid weight (ng/g l.w.).

2.3. Analyses of thyroid hormones

Polar bear whole blood samples were analysed for total T3 (TT3) and total T4 (TT4) at the Department of Biology, Norwegian University of Science and Technology (NTNU, Trondheim, Norway) using radioimmunoassay (RIA). The preferred matrixes for the commercially available RIA-kits for TT3 and TT4 analyses are serum or plasma (Siemens, 2006a,b). Therefore, an extraction step was employed before the RIA analyses. Because the extraction liberates protein bound hormone in addition to the free fraction (McMaster et al., 1992), only circulating levels of TT3 and TT4 were quantifiable. The extraction method in this study was based on a previously described extraction method (Kobuke et al., 1987; Tagawa and Hirano, 1987) used with some modifications in the present study. Briefly, the blood samples were homogenised and then extracted three times using refrigerator cold ethanol. The final extracts were dried and dissolved in barbital buffer solution spiked with the highest standard solutions of TT3 or TT4 from commercial RIA kits (F-buffer$_{T3}$ and F-buffer$_{T4}$), to achieve better precision during quantification. Concentrations of TT3 and TT4 in the extracted blood samples were analysed using commercial RIA kits (Coat-A-Count Total T3 and Coat-A-Count Total T4, Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA) following the procedures described in the test protocols (Siemens, 2006a,b). Quantifications was done on a gamma counter (Cobra Auto-Gamma, Packard Instruments Company, Dowers Grove, IL, USA) with standard curves with six known standards run in duplicates (TT3: 0, 0.31, 0.77, 1.54, 3.07 and 9.22 nmol/L; TT4: 0, 12.9, 51.5, 129, 206 and 309 nmol/L). Generation of standard curves and calculations of TH levels in samples were performed by the gamma counter's software (Spectra Works Spectrum Analysis Software, Meriden, USA). Instrumental detection limit was given in each RIA run and ranged from 0.341 to 1.078 nmol/L for TT4 (n = 6) and from 0.022 to 0.046 nmol/L for TT3 (n = 5). The extract samples were run on RIA in triplicates, and outliers flagged by the gamma counter's software were omitted. The TT4 levels of 13 individuals were omitted because of non detectable results. The dilution during extraction procedures and the mean measured levels of TT3 and TT4 in F-buffer$_{T3}$ and F-buffer$_{T4}$ of each run were incorporated into the respective calculations of TT3 and TT4 levels (nmol/L) in the blood samples. The coefficient of variation (CV, %) for TT3 analyses of the blood samples were generally <30% and ranged from 0.1 to 39% when two samples with high CVs (87% and 141%) were omitted. For the TT4 analyses of blood samples,

Table 1
Circulating levels (nmol/L) of TT3 and TT4, and capture day, age, length, girth, and estimated body mass (BM) in polar bears (*Ursus maritimus*) from East Greenland 1999–2001 (n = 62). The subadult (SubA) group consists of young females (<5 years) and young males (<6 years). The adult males (AdM) are ≥6 years. The adult females are ≥5 years and subgrouped into solitary adult females (without cubs; AdF_S) and nursing adult females (with cubs; AdF_N). Mean and standard deviation (S.D.) are presented, as well as median, minimum (min.) and maximum (max.) levels.

		SubA	AdF_N	AdF_S	AdM
TT4 (nmol/L)	n	16	5	11	4
	Mean ± S.D.	13.6 ± 8.01[a]	8.07 ± 5.81	18.4 ± 12.4	6.74 ± 5.64
	Median (min.–max.)	14.5 (2.27–24.7)	8.34 (1.92–13.9)	15.9 (1.96–36.6)	5.42 (1.44–14.7)
TT3 (nmol/L)	n	20	7	15	17
	Mean ± S.D.	0.63 ± 0.29[a]	0.55 ± 0.27	0.66 ± 0.35	0.85 ± 0.41
	Median (min.–max.)	0.61 (0.18–1.27)	0.54 (0.17–0.91)	0.59 (0.35–1.64)	0.77 (0.38–2.21)
Capture day	n	23	7	13	17
	Mean ± S.D.	165 ± 100[a]	155 ± 98	133 ± 94	141 ± 99
	Median (min.–max.)	220 (8–279)	132 (44–258)	97 (5–262)	100 (24–267)
Age (years)	n	23	7	15	17
	Mean ± S.D.	3.5 ± 1.1	12 ± 4.6	12 ± 6.9	10 ± 6.6
	Median (min.–max.)	3.5 (0.5–5.0)	12 (5.5–19.0)	10 (5.0–23.0)	8.0 (6.0–28.0)
Length (cm)	n	22	7	13	16
	Mean ± S.D.	180 ± 29	210 ± 28[c]	183 ± 29	232 ± 19[b]
	Median (min.–max.)	179 (115–235)	218 (156–237)	194 (125–230)	238 (200–266)
Girth (cm)	n	22	7	14	16
	Mean ± S.D.	148 ± 30	171 ± 26	171 ± 32	181 ± 29[d]
	Median (min.–max.)	149 (101–208)	166 (138–204)	169 (126–232)	186 (128–240)
BM (kg)	n	22	7	13	16
	Mean ± S.D.	271 ± 121	425 ± 148	334 ± 108	538 ± 185[b]
	Median (min.–max.)	248 (101–563)	413 (261–667)	343 (190–502)	555 (233–970)

TT3 = total triiodothyronine, TT4 = total tetraiodothyronine (thyroxine).
Body mass (BM) was calculated based on measured body length and girth according to Derocher and Wiig (2002).
Capture day was calculated by converting the date of capture (day/month) into a numeric value between 1 and 365 days.
[a] No significant differences between SubA, AdF_N, and AdF_S. [b] AdM significantly different from SubA and AdF_S (Tukey's *post hoc* test, $p<0.01$). [c] AdF_N and SubA significant different (Tukey's *post hoc* test, $p<0.05$). [d] AdM significantly different from SubA (Tukey's *post hoc* test, $p<0.01$); AdF_N, AdF_S and AdM were not significantly different.

Table 2
Mean and standard deviation (S.D.) of groups sum (Σ) levels (ng/g l.w.) of major organohalogen contaminants (OHCs)[a] and lipid content (%) measured in subcutaneous adipose tissue of polar bears (*Ursus maritimus*) from East Greenland 1999–2001 (n = 62). The subadult (SubA) group consists of young females (<5 years) and young males (<6 years). The adult males (AdM) are ≥6 years. The adult females are ≥5 years and subgrouped into solitary adult females (without cubs; AdF_S) and nursing adult females (with cubs; AdF_N).

		Group			
		SubA (n = 23)	AdF_N (n = 7)	AdF_S (n = 15)	AdM (n = 17)
ΣPCBs	Mean	6566	6168	5742	7847
	S.D.	2981	2632	4479	3406
ΣDDTs	Mean	456	327	349	443
	S.D.	219	110	241	289
ΣPBDEs	Mean	67.9	63.0	77.5	69.1
	S.D.	25.0	18.4	51.6	47.0
ΣCHLs	Mean	2268	1582	2082	1327
	S.D.	1032	496	1825	620
ΣHCHs	Mean	192	139	188	233
	S.D.	69.8	31.5	187	168
ΣCBzs	Mean	156	91.7	102	82.9
	S.D.	86.9	83.2	89.2	41.9
Lipid content (%)	Mean	92.4	92.9	84.3	82.3
	S.D.	5.94	8.05	23.1	10.1

ΣPCBs includes individual or co-eluted PCB congener number PCB-31/28, -42, -44, -49, -52, -60, -64/71, -66/95, -70, -74, -87, -97, -99, -101/84, -105, -110, -118, -128, -129/178, -138, -141, -146, -149, -151, -153, -158, -170/190, -171/202/156, -172, -174, -177, -179, -180, -182/187, -183, -194, -195, -200, -201, -203/196 and -206.
ΣCHLs include oxychlordane, *cis*-chlordan, *trans*-chlordane, *cis*-nonachlor and *trans*-nonachlor, and heptachlor epoxide.
ΣDDTs include *p,p′*-DDT, *p,p′*-DDE and *p,p′*-DDD.
ΣHCHs include α-HCH, β-HCH and γ-HCH.
ΣCBzs include HCB, QCB and TCB.
ΣPBDEs include individual or co-eluted PBDE congener number PBDE-47, -99, -100, -153 and -155/126.
[a] The OHCs levels and lipid% were first reported in Dietz et al. (2004, 2007).

the CVs were generally <35% with a few exceptions of CVs between 35 and 93%. These were still accepted because of the low levels of extracted TT4 in these samples (<5 nmol/mL) which makes quantification during RIA more prone to variations. TT4 results from four polar bear samples with high CVs (60–105%) and TT4 levels (11–21 nmol/L) were omitted from the study.

To control the inter-assay variation during RIA-analyses we analysed standard reference material (SRM level 1, 2 and 3; Lyphochek® Immunoassay Plus Control, Bio-Rad Laboratories, LA, USA). For TT4, the inter-CV of SRM was 3.4–5.9% (n = 6). For TT3, the inter-CV of SRM was 3.6–4.9% (n = 5). This indicated good quality of the RIA procedures for both TT3 and TT4 analyses. Intra-assay variation was investigated by analysing SRM at the beginning and end, and sometimes in the middle of each RIA-run, and by use of repeated analyses of F-buffer$_{T3}$ and F-buffer$_{T4}$. For TT4, the SRM intra-CV was 3.3–13% (n = 5).

For F-buffer$_{T4}$ the intra-CV was 5.1–8.3% (n = 6). For TT3, SRM intra-CV ranged from 2.5 to 4.6% (n = 3/2) and for the F-buffer$_{T3}$ intra-CV ranged from 2.9 to 5.9% (n = 5).

To control the inter-extraction rounds, we used one polar bear whole blood sample and SRM as controls, which were subjected to extraction and subsequent RIA analyses. The inter-CV for TT4 extraction was 15% (n = 4) for the control blood sample and 6.6% (n = 5) for SRM, which were all found acceptable. The inter-CV for the TT3 extraction rounds was 12% (n = 3) for the control blood sample, which was also acceptable. All over, the precision of the TT3 and TT4 extraction and RIA-analyses was acceptable.

Recovery of TT4 was calculated from extracted and unextracted SRM analysed with RIA, and ranged from 87 to 101% (n = 4). No recovery data was available for TT3 extractions. SRM does not fully represent the whole blood matrix, but indicate acceptable quality of the extraction procedures in this study.

2.4. Data analyses

Four polar bears had TH levels that were defined as possible outliers or extreme values. For three of these individuals, TH levels were within the physiological range of polar bears (Braathen et al., 2004; Leatherland and Ronald, 1981; Sandau, 2000; Skaare et al., 2001). Thus, only the results from one of these four bears were removed from further statistical analyses (AdM, 16 yr: TT3 = 3.06 nmol/L; TT4 = 46.4 nmol/L). Also, one individual lacking OHC data was removed from the dataset. The remaining individual polar bears (n = 62) in this study were grouped according to age, sex (Rosing-Asvid et al., 2002) and reproductive status: Subadults (SubA, consisting of subadult females <5 yr and subadult males <6 yr, n = 23); Adult males (AdM, consisting of males ≥6 yr, n = 17), and nursing adult females (AdF_N, consisting of females ≥5 yr with cubs, n = 7) and solitary adult females (AdF_S, consisting of females ≥5 yr without cubs, n = 15). Because of different number of animals included in the TT3 and TT4 analytical results, as well as missing biological measurements (date of capture, girth, length) in some individuals, sample sizes vary accordingly. We did not have sufficient information to pair mothers with their cub(s). It was not possible to anatomically determine whether the AdF_S were in gestation, which may have influenced TH levels (McNabb, 1992).

The univariate statistical analyses were conducted using SPSS (Version 16, standard version, SPSS Inc., Chicago, IL, USA). The date of capture was computed into a numeric value between 1 and 365 days (capture day). The different years of sampling (1999–2001) were assumed not to influence the OHC data (see Dietz et al., 2004) or TH levels. Thus, OHC and TH data from different years were pooled. Normality of the data was tested using Kolmogorov–Smirnov test for n>50 (all polar bears) and Shapiro–Wilcoxon test for n<50 (age/sex groups). Levene's test of homogeneity was applied to test the variance among the age/sex groups. To achieve normal distributions and

Table 3
Results of PLS regression models between the response variable Y = TT3 with 55 (56 for SubA) predictor variables (X-variables); PCBs, PBDEs and OCPs in subcutaneous adipose tissue (ng/g l.w.) and biological data; age, sex (only in SubA), lipid content, BM, girth, length and capture day in polar bears (*Ursus maritimus*) from East Greenland 1999–2001. Final model was a result of step-wise optimising of the first model (see Section 2.4).

Group	First PLS model	Final PLS model	Final PLS model — validation					
			PLS components	R^2X	R^2Y	Q^2	Permutation intercept x-axis (R^2Y) and y-axis (Q^2)	Regression coefficients with TT3
SubA n = 20	Significant (X = 56)	Significant (X = 48)	3	0.603	0.746	0.414	R^2Y: 0.701 Q^2: −0.240	Fig. 1A
AdF_N n = 7	Not significant (X = 55)	Significant (X = 49)	3	0.714	0.991	0.840	R^2Y: 0.927 Q^2: −0.279	Fig. 1B
AdF_S n = 15	Not significant (X = 55)	Significant (X = 42)	2	0.635	0.704	0.266	R^2Y: 0.575 Q^2: −0.103	Fig. 1C
AdM n = 17	Not significant (X = 55)	Not significant	–	–	–	–		–

Table 4
Results of PLS regression models between the response variable Y = TT4 with 55 (56 for SubA) predictor variables (X-variables); PCBs, PBDEs and OCPs in subcutaneous adipose tissue (ng/g l.w.) and biological data; age, sex (only in SubA), lipid content, BM, girth, length and capture day in polar bears (*Ursus maritimus*) from East Greenland 1999–2001. Final model was a result of step-wise optimising of the first model (see Section 2.4).

Sex/Age group	First PLS model	Final PLS model	Final PLS model — validation					
			PLS components	R^2X	R^2Y	Q^2	Permutation Intercept x-axis (R^2Y) and y-axis (Q^2)	Regression coefficients with TT4
SubA n = 16	Significant (X = 56)	Significant (X = 34)	2	0.680	0.724	0.573	R^2Y: 0.521 Q^2: −0.161	Fig. 2A
AdF_N n = 5	Significant (X = 55)	Significant (X = 46)	1	0.547	0.949	0.828	R^2Y: 0.761 Q^2: −0.113	Fig. 2B
AdF_S n = 11	Not significant (X = 55)	Significant (X = 21)	2	0.756	0.673	0.428	R^2Y: 0.513, Q^2: −0.078	Fig. 2C
AdM n = 4	Not significant (X = 55)	Not significant	–	–	–	–		–

homogeneity of variances among subgroups, TT3 and TT4, age and OHC data were log_{10}-transformed. One-way analyses of variance (ANOVA) with Tukey's *post hoc* test was used to investigate group differences in TH levels and biological data (BM, girth, length, capture day, lipid content). Pearson correlation (r) and partial correlation (r_p) was used to test bivariate relationships. Significance levels were set to $p \leq 0.05$, and p-values are two-tailed unless otherwise mentioned.

Multivariate analyses of individual OHC concentrations in adipose tissue, circulating levels of TT3 and TT4, as well as biological data were performed using the software SIMCA-P+ (Version 12.0, Umetrics AB, Umeå, Sweden). PLS, which stands for projections to latent structures by means of partial least squares (Eriksson et al., 2006), was applied to model the effects of OHCs and biological variables (predictor, X, variables) on TH levels (response, Y, variables). PLS is a multivariate regression analysis which in an unidirectional manner models the relationships between the X-variables and their simultaneous influence on the Y-variable(s) (SIMCA, 2008; Wold et al., 2001). PLS regression does not require normality distributions, is less sensitive to outliers and extreme values, and can deal with datasets consisting of a lower number of observations (individuals) than variables, as well as multicolinearity among the variables (Eriksson et al., 2006; SIMCA, 2008; Wold et al., 1984). Thus, we used the data in their original form (not log_{10}-transformed). A more detailed description of these multivariate methods can be found elsewhere (Eriksson et al., 2006; Wold et al., 2001).

TT3 and TT4 were not inter-correlated (Pearson correlation, r = −0.056, p = 0.757, n = 33). In fact when using both TT3 and TT4 as Y-variables in a preliminary PLS model (n = 62), they showed tendency to inversely relate to each other. Thus, using both TT3 and TT4 as Y-variables in the same PLS model could mask relationships between OHCs and THs. Furthermore, the response to thyroid disruptive compounds may be influenced by age, sex, and physiological/reproductive status of individuals (Abdelouahab et al., 2008; Gochfeld, 2007; Zoeller et al., 2007a). Hence, we developed eight different PLS models to investigate the effects of the individual OHCs included in this study (listed in Section 2.2) and biological variables (age, BM, girth, length, capture day, lipid content, TT3 or TT4 levels[1]) on TT3 or TT4 levels as separate Y-variables in models for each age/sex groups. Only individuals with TT3 or TT4 levels and OHCs data were included in the respective models. Variables that were missing in >50% of the observations were excluded; PCB-49, 44, 42, 70, 87, 110, 151, 149, 105, 141, 179, 158, 174, PBDE-155/126 and γ-HCH. Sex was included as an X-variable only in the SubA PLS models. All variables were centred and scaled (to variance 1), and significance level was set to 0.05 (SIMCA, 2008). The first (original) PLS model was step-wise optimised by removing X-variables with variable importance in the projection (VIP) values <0.5, as these have low or no importance in explaining the X-matrix or correlating with the Y-matrix (SIMCA, 2008). The X-variables with the lowest VIP values were then consecutively removed from the models. Each time an X-variable was removed and a new PLS model was created, the explained variation in the X-matrix (R^2X) and the explained variance (influence) of the X-matrix on the Y-variable (goodness of fit, R^2Y) was evaluated. Because of the risk of over-fitting the X-variables toward the Y-variable, we also evaluated the goodness of prediction (Q^2) obtained by cross-validation, permutation analyses (20 permutations) and observed-versus-predicted analyses for each step. The procedure stopped when optimal R^2X, R^2Y, Q^2 with acceptable permutation validation and observed-versus-predicted analyses were reached. In cases where the original PLS model were not significant, we imposed a model with two PLS components and removed variables with VIP values that were <0.5. If the new model still was not significant, the same stepwise procedure as described above was used until a significant and optimal model was reached. If a significant model was not achieved, it was defined as not significant.

[1] TT3 and TT4 levels were X-variables in PLS-models with Y = TT4 and Y = TT3, respectively.

3. Results

3.1. Thyroid hormones, biological data and contaminant levels

The circulating concentrations (nmol/L) of TT3 and TT4, and the biological data of the polar bears from East Greenland are shown in Table 1. There were no significant differences in TT3 or TT4 concentrations among the groups. There were no age differences among AdM, AdF_N and AdF_S. Girth, length and BM differed significantly among the four groups (ANOVA: F = 4.30, P = 0.009, df = 58; F = 14.11, p = 0.0001, df = 57; F = 11.52, p = 0.0001, df = 57; respectively). AdM had significantly larger girth as compared to SubA (Tukey's *post hoc* test; p = 0.007), but there were no differences in girth among AdM, AdF_N and AdF_S. AdM were significantly longer and had higher BM as compared to AdF_S (Tukey's *post hoc* test; p = 0.0001 and p = 0.002, respectively) and SubA (Tukey's *post hoc* test; p = 0.0001 and p = 0.0001, respectively). Furthermore, AdF_N were somewhat longer than SubA (Tukey's *post hoc* test; p = 0.047). No significant differences were found for capture day among the groups.

The summed (Σ) concentrations of major groups of OHCs included in this study and lipid content of adipose tissue are presented in Table 2. Investigating levels and patterns of OHCs is beyond the scope of this study. Dietz et al. (2004, 2007) reported OHC levels in SubA, AdM and AdF (nursing and solitary together) polar bears from East Greenland (1999–2001), which included the individuals in the present study. Dietz et al. (2004) found that AdM have the highest levels of ΣPCB, ΣChlorobenzenes (ΣCBzs), ΣDDTs and dieldrin as compared to AdF and SubA, whereas AdF had higher ΣHCHs as compared to AdM, and ΣChlordanes (ΣCHLs) were highest in AdF, followed by SubA and lowest in AdM. No differences in ΣPBDE levels were found among the polar bears groups (Dietz et al., 2007).

3.2. Modelled effects of contaminants and biological variables on thyroid hormone levels

The result of the best-fitted multivariate PLS models are presented in Table 3 (Y = TT3) and Table 4 (Y = TT4) with their respective number of PLS components, R^2X, R^2Y, Q^2, and permutation results (x and y intercepts). The PLS modelling gave significant models for TT3 and TT4 for SubA, AdF_N and AdF_S, whereas the AdM models were non-significant. The significant models had R^2X-values and Q^2-values close to or above criteria for good or acceptable models with biological field data; $R^2X > 0.7$ and $Q^2 > 0.4$ (Lundstedt et al., 1998). For the significant models we present column plots with regression coefficient (CoeffCS) values showing the correlation strength and direction of the regressions between the X-variables and TT3 (Fig. 1A, B and C) or TT4 (Fig. 2A, B and C).

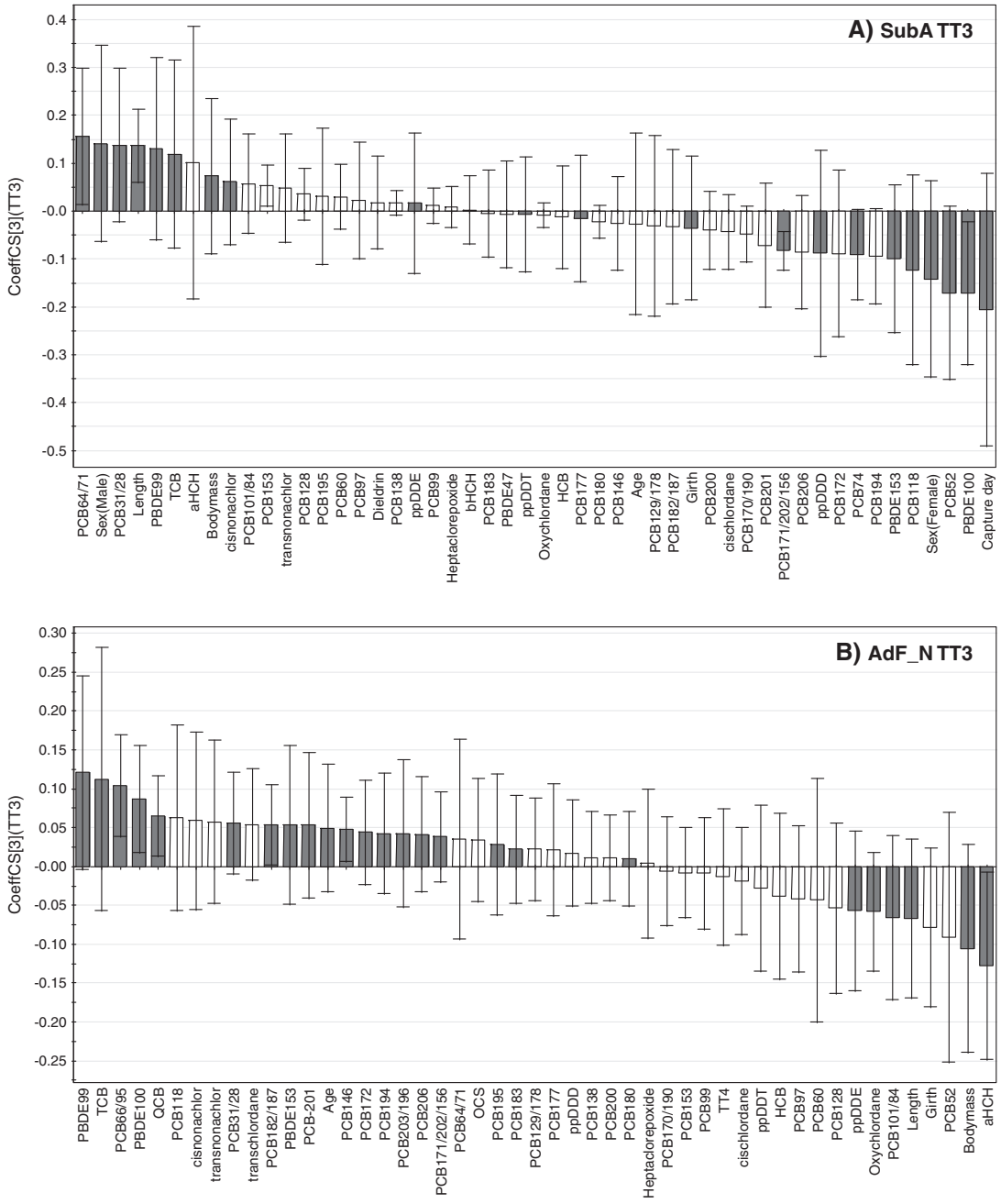

Fig. 1. Column plot of the final, significant PLS model for A) SubA, B) AdF_N and C) AdF_S polar bears (*Ursus maritimus*) from East Greenland 1999–2001 (n = 59), presenting the regression coefficient (CoeffCS) values between the response variable Y = TT3 and the predictor (X) variables organohalogen compounds and biological data that were included in the final model. The error bars show 95% confidence interval. The X-variables that are most important in explaining the Y-variable TT3 have the highest absolute CoeffCS values. The full bars present variables with VIP values ≥1, which indicate high importance in the PLS model (SIMCA, 2008). VIP and CoeffCS values are cumulative calculated from all extracted PLS components (SIMCA, 2008). For explanations and list of all the variables included in the original models, see Sections 2.2 and 2.4.

TT3 levels in SubA were influenced by biological variables (capture day, sex, length and BM; Fig. 1A). The contaminants with highest influence on TT3 levels were PBDE-100, PCB-52, PCB-118 and PBDE-153, which were negatively correlated with TT3 (Fig. 1A). The model also showed that PCB-64/71, PCB-31/28, PBDE-99, TCB, α-HCH and *cis*-nonachlor correlated positively with TT3. BM, length and girth were important biological factors in explaining TT4 levels in SubA (Fig. 2A). In the model, the

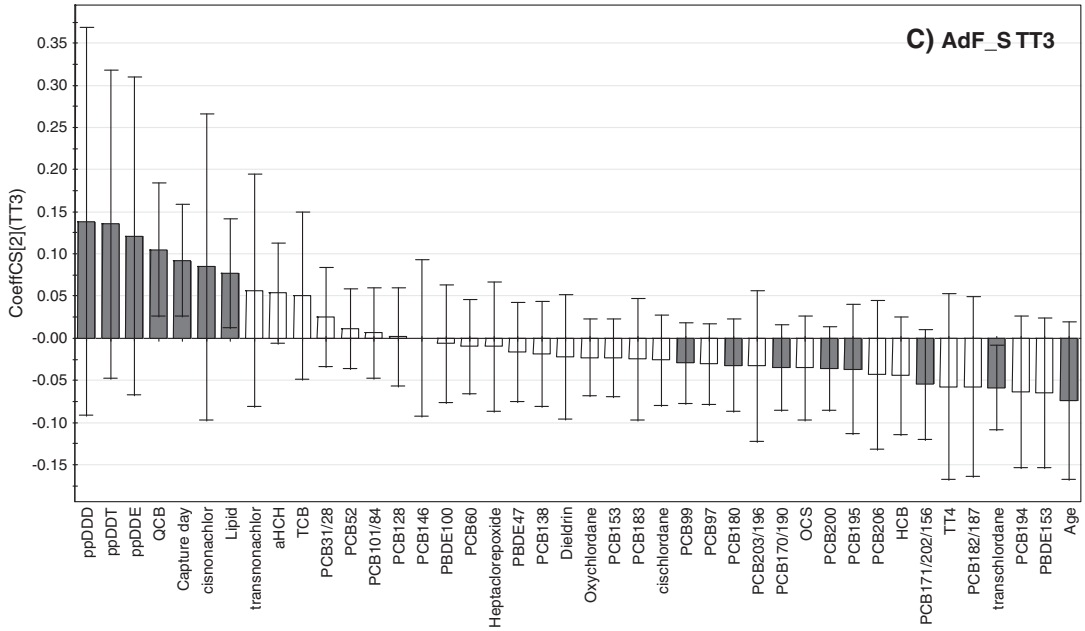

Fig. 1 (*continued*).

contaminants PCB-52, PBDE-99, PCB-170/190 and PCB-194 were negatively correlated with TT4 levels, whereas TCB, PCB-129/178, PBDE-100, QCB, PBDE-47 and β-HCH were positively correlated with TT4 in SubA (Fig. 2A).

In AdF_N, the most important contaminant variables for explaining TT3 levels in the model were α-HCH, PCB-52, PCB-101/84, oxychlordane and *p,p′*-DDE, which were negatively correlated with TT3. PBDE-99, TCB, PCB-66/95, PBDE-100, QCB, PCB-118, *cis*-nonachlor, *trans*-nonachlor and PCB-31/28 were positively correlated with TT3 (Fig. 1B). Also, BM, girth and length had a negative influence on TT3 levels in AdF_N (Fig. 1B). In the TT4 model for AdF_N, the most influential contaminants were a group of PCBs that were all positively correlated with TT4; PCB-183, -201, -153, -172, -203/196, -146, -138, -195, -180, -170/190, -177, -31/28, -182/187, -99, -129/178, -200 and -97 (17 individual or co-eluted PCB congeners; ΣPCB_{17}) in addition to *cis*-chlordane, heptachlor epoxide, *trans*-nonachlor, QCB and oxychlordane (Fig. 2B). Length was an important biological factor and positively correlated with TT4, but to a lesser degree than most contaminants listed above (Fig. 2B).

In AdF_S the most important contaminant variables explaining TT3 levels were *p,p′*-DDD, *p,p′*-DDT, *p,p′*-DDE, QCB, and *cis*-nonachlor, which were positively correlated, and PBDE-153, PCB-94 and *trans*-chlordane that were negatively correlated with TT3 (Fig. 1C). With respect to non-contaminant variables, TT3 in AdF_S was positively influenced by capture day and lipid content and negatively influenced by age (Fig. 1C). For TT4 in AdF_S, many variables had to be excluded in order for the model to become significant with an acceptable validation (Table 4). The most important biological determinants for TT4 were lipid content and girth (Fig. 2C). The most important contaminants in this model were PBDE-153, TCB and PCB-60, which were positively correlated with TT4 levels, and PCB-182/187 and β-HCH that were negatively correlated with TT4 (Fig. 2C).

The most important OHCs influencing TH levels in the SubA, AdF_N and AdF_S models were PBDE-99, PBDE-100, PBDE-153, TCB, QCB, *cis*-nonachlor, *trans*-nonachlor, PCB-52 and PCB-118, as well as a positive correlations between DDTs and TT3 levels for AdF_S and between ΣPCB_{17} and TT4 levels for AdF_N (Figs.1 and 2). Some of these relationships are presented as simple associations in scatter-plots together with Pearson correlation coefficients in Fig. 3A–H. All relationships in Fig. 3 showed the same direction as the CoeffCSs in the PLS models (Figs. 1 and 2) and half of them had significant Person correlation coefficients (Fig. 3A, D, E and F). Since biological variables also were important in explaining circulating TH levels in the PLS models for SubA, AdF_N and AdF_S (BM, length, girth, lipid content, capture day, and sex in SubA), they were considered confounding factors in the relationships presented in Fig. 3A–H. The influence of these biological variables was removed by partial correlation analyses (r_p) correcting for BM and capture day, thus controlling for most of the biological variables since BM correlated with age, length, and girth (Pearson correlation; $r>0.36$, $p<0.005$, $n=58/59$[2]), and capture day correlated with lipid content (Pearson correlation; $r=0.30$, $p=0.018$, $n=60$). This resulted in significant partial correlations ($p\leq0.05$, one-tailed, df = degrees of freedom) for all presented relationships in Fig. 3A–H: A ($r_p=-0.488$, $p=0.020$, df[3] = 16), B ($r_p=0.849$, $p=0.001$, df = 8), C ($r_p=0.871$, $p=0.027$, df = 3); E ($r_p=-0.829$, $p=0.041$, df = 3); F ($r_p=0.600$, $p=0.033$, df = 8), G ($r_p=-0.569$, $p=0.043$, df = 8) and H ($r_p=0.563$, $p=0.045$, df = 8). The relationship in Fig. 3D could not be tested by partial correlation due to low sample size.

4. Discussion

Levels of TT3 and TT4 in whole blood of polar bears from East Greenland (Table 1) were within ranges previously reported in plasma of polar bears from Svalbard and Canada (Braathen et al., 2004; Cattet, 2000; Leatherland and Ronald, 1981; Sandau, 2000; Skaare et al., 2001). Comparing TH levels between different studies is complicated due to differences in analytical methods, matrix, sampling year, and variances in age, sex, physiological, nutritional and reproductive status (McNabb, 1992; St Aubin, 2001). To clinically evaluate if measured TH levels indicate hypo- or hyperthyroidism, measurements of free levels of T3 and T4 as well as TSH levels are necessary (Demers, 2008). In the current study we measured TH levels in whole blood, and with the required extraction step it was not possible to analyse free T3 or T4. Nor did we analyse TSH levels. Thus, it was not feasible to clinically evaluate hypo- or hyperthyroidism in the investigated polar bears. Physiological studies have reported circulating TH levels in free-ranging Canadian polar bears (ranges of mean; TT3: 0.25–2.30 nmol/l, TT4: 7.72–66.9 nmol/l; Cattet, 2000; Leatherland and Ronald, 1981; as compiled in St Aubin, 2001). However, these are not TH values from non-contaminated polar bears and cannot be considered as reference levels. Still, the TH levels of the present East Greenland polar bears are within these above mentioned

[2] n = 59 for the variables girth and age, and n = 58 for length and BM.
[3] df = degrees of freedom.

physiological ranges (ranges of mean: TT3: 0.55–0.85 nmol/l, TT4: 6.74–18.4 nmol/l, see Table 5). The TH levels in the current study are similar or somewhat lower as compared to plasma TH levels of polar bears from Svalbard sampled in spring (March/April) analysed by the same method (Braathen et al., 2004; Table 5). This small discrepancy may be attributed to analysis of TH in whole blood with <100% extraction efficiency, as well as a higher influence of seasonal TH variations caused by a longer sampling period (January–October) in

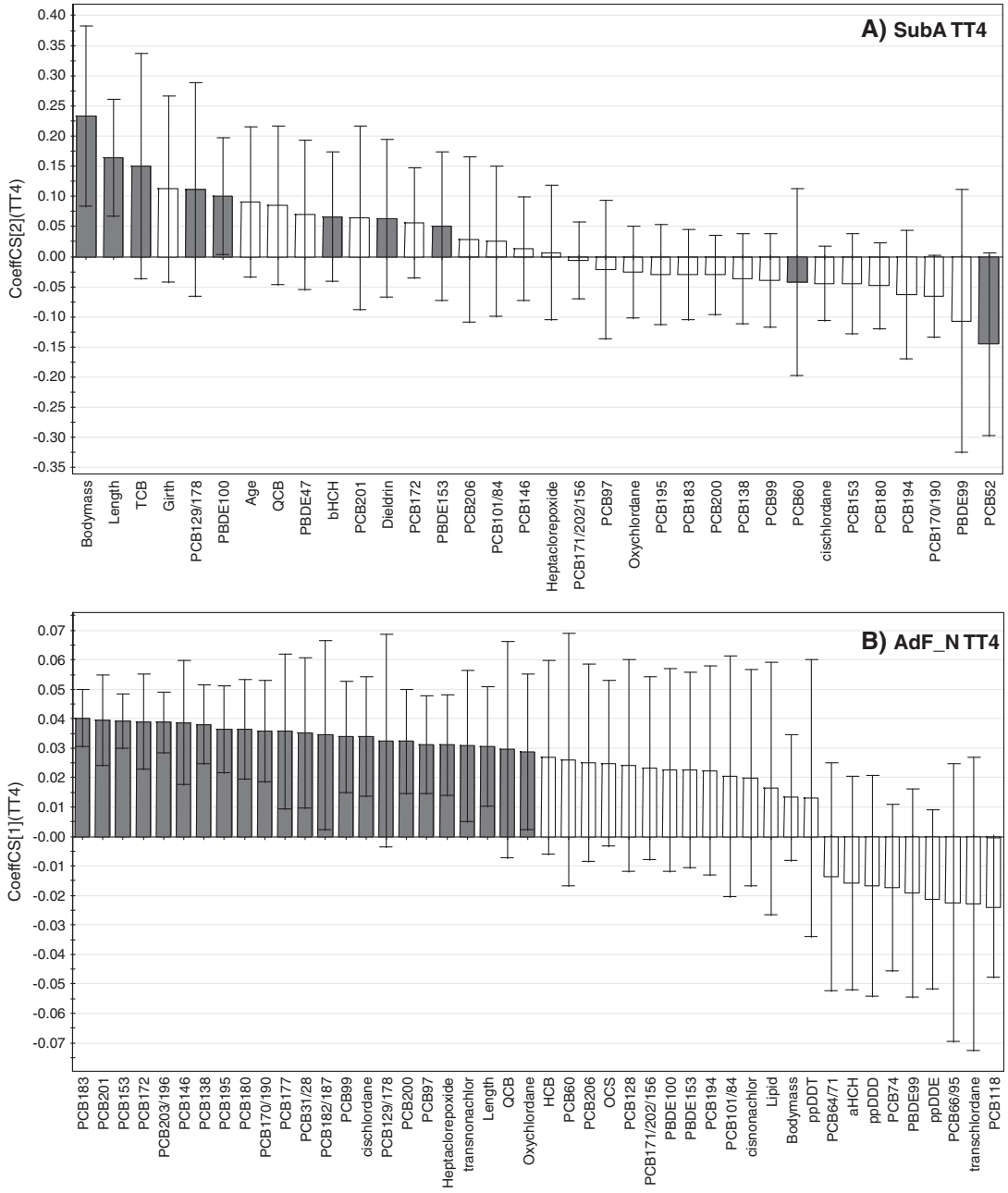

Fig. 2. Column plot of the final, significant PLS model for A) SubA, B) AdF_N and C) AdF_S polar bears (*Ursus maritimus*) from East Greenland 1999–2001 (n = 36), presenting the regression coefficient (CoeffCS) values between the response variable Y = TT4 and the predictor (X) variables organohalogen compounds and biological data that were included in the final model. The error bars show 95% confidence interval. The X-variables that are most important in explaining the Y-variable TT4 have the highest absolute CoeffCS values. The full bars present variables with VIP values ≥1, which indicate high importance in the PLS model (SIMCA, 2008). VIP and CoeffCS values are cumulative calculated from all extracted PLS components (SIMCA, 2008). For explanations and list of all the variables included in the original models, see Sections 2.2 and 2.4.

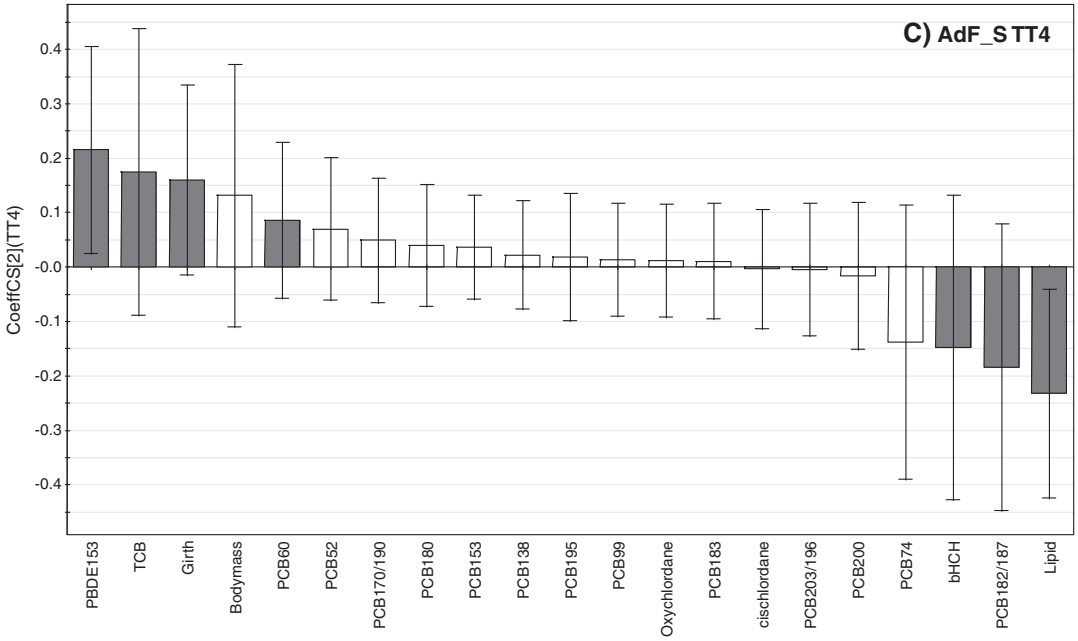

Fig. 2 (*continued*).

the present study. Capture day was an important biological variable influencing TT3 levels in SubA and AdF_S (Fig. 1A, C).

The non-significant PLS models for AdM suggest that the TH levels of AdM were not influenced by OHCs, although this cannot be ruled out. These results are in accordance with a previous study of polar bears from Svalbard where males seemed less influenced by PCBs compared to females (Braathen et al., 2004). Thyroid related diseases and hypothyroidism in humans are also more frequent in females than males (Chiovato et al., 1993; Zoeller et al., 2007a). Gender based differences in TH responses to OHC exposure have also been reported in a human study (Abdelouahab et al., 2008).

The PLS models for SubA, AdF_N and AdF_S were significant and met the criteria for good or acceptable models (Lundstedt et al., 1998; Tables 3 and 4). Biological factors, such as BM, age, length, girth and capture day, were important in explaining TH levels as well as the investigated contaminants, especially for SubA where also sex also had some influence. After removing the influence of most biological variables, many of the important relationships from the PLS models, presented as simple relationships in Fig. 3A–H, were significantly correlated in accordance with the multivariate relationships depicted by the PLS models. Thus, the PLS models indicate that TH levels in SubA and AdF are influenced by OHC levels. It is important to emphasise that this study presents statistical models on how complex OHC mixtures in adipose tissue explain circulating TH levels, thus not representing the full cause–effect relationships. Although we have no means of statistically testing inter-group differences of the modelled results, a quantitative assessment of the significant PLS models' CoeffCS value plots (Figs. 1 and 2) elucidated some interesting common features, as well as distinct combinations of OHCs influencing TH levels in these three groups of polar bears.

4.1. Adult females

The CHL compounds *cis*- and *trans*-nonachlor seemed important in influencing TT3 levels in AdF_N and also AdF_S in this study (Table 3; Fig. 1B and C). In polar bears, females probably have lower biotransformation abilities and higher bioaccumulation of CHLs as compared to males (Letcher et al., 1996; Norstrom et al., 1998; Polischuk et al., 2002). Dietz et al. (2004) found higher ΣCHL levels in AdF compared to SubA and AdM polar bears from East Greenland. Also in experimental studies, female rats accumulated higher CHL levels than males, especially of *trans*-nonachlor, and seemed more vulnerable to toxic effects. *trans*-Nonachlor influenced TH status, and gender differences in TH responses were reported (Bondy et al., 2000, 2004).

For AdF_S, the influence of OHCs on TT3 levels consisted mainly of positive correlations with *p,p′*-DDD, *p,p′*-DDT and *p,p′*-DDE (Table 3; Figs. 1C; 3F). These relationships were not found in the models for AdF_N or SubA. The positive relationships between DDTs and TT3 found in this study coincide with the findings of a positive relationship between *p,p′*-DDE and TT3 in a human study consisting of adult males and females (Langer et al., 2007). In Svalbard polar bears, a positive correlation between *p,p′*-DDE and TT4:FT4 ratio was found when corrected for age and sex (Skaare et al., 2001), but not for TT3 levels as in this study.

In AdF_N, the positive relationships between higher chlorinated *ortho*-PCBs (ΣPCB_{17}) and TT4 (Figs. 2B; 3D) are contrary to findings in human studies where *ortho*-PCBs were negatively correlated with T3 but not T4 (Abdelouahab et al., 2008; Hagmar et al., 2001; Takser et al., 2005). In polar bears from Svalbard, TT4 levels in adult females with cubs (yearlings or older) were negatively correlated with ΣPCB_5, consisting mainly of

Fig. 3. Scatter-plots of $\log_{10}$ thyroid hormone (TT3 or TT4, nmol/L) levels measured in blood in relation to $\log_{10}$ levels of individual OHCs measured in adipose tissue (ng/g l.w.) in East Greenland polar bears (1999–2001). The plots illustrate some of the simple relationships between TH and OHCs that were found important by the multivariate PLS models of SubA (A–B), AdF_N (C–E) and AdF_S (F–H). The dashed lines show the best fitted regression line through the data points illustrating the direction of the relationship in each plot. The Pearson correlation coefficient (r) are presented in the plots. * Significance at $p \leq 0.05$. All p-values are one-tailed because of expected outcome from the PLS models.

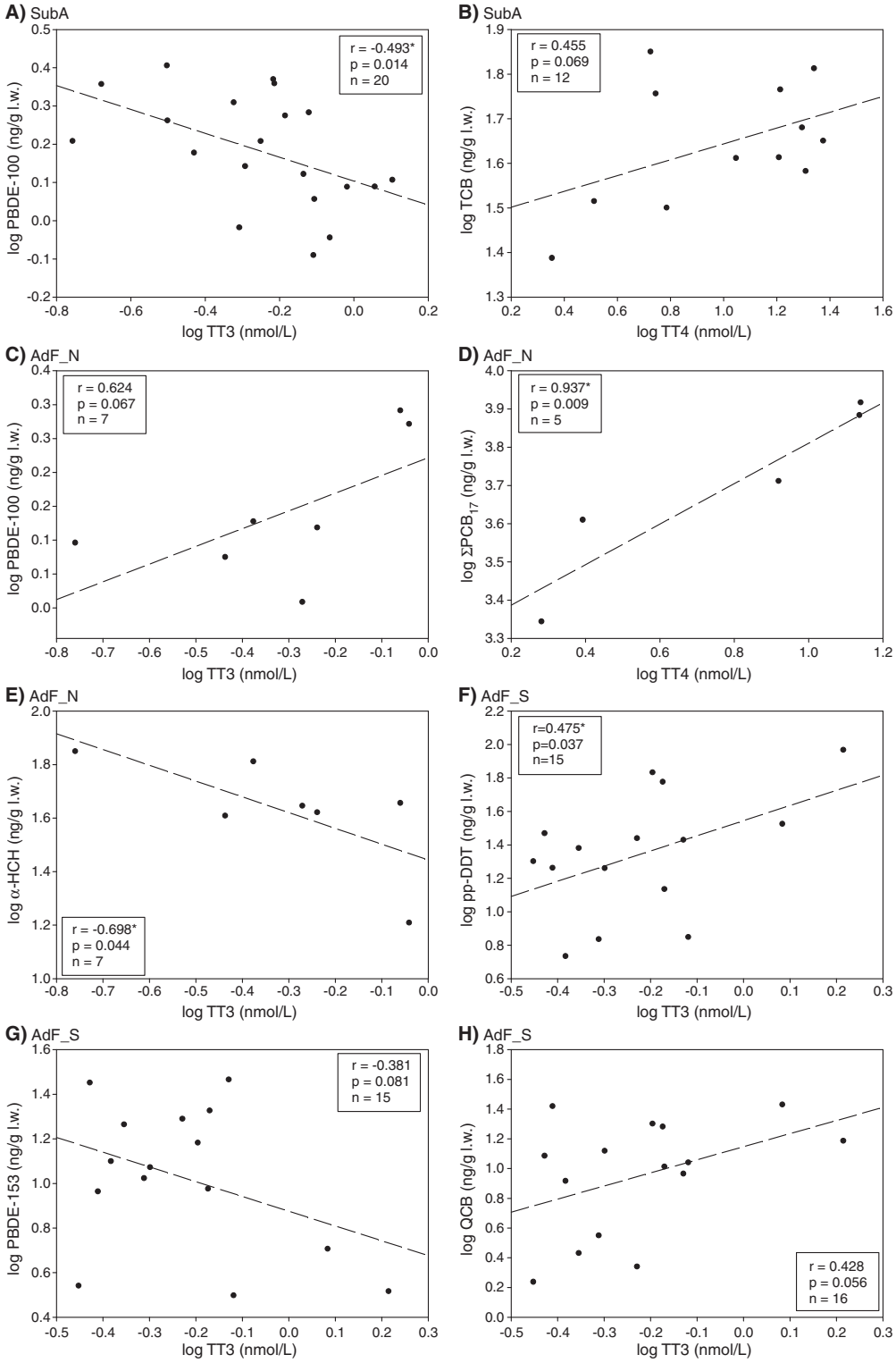
A) SubA
log PBDE-100 (ng/g l.w.)
log TT3 (nmol/L)
r = -0.493*
p = 0.014
n = 20
B) SubA
log TCB (ng/g l.w.)
log TT4 (nmol/L)
r = 0.455
p = 0.069
n = 12
C) AdF_N
log PBDE-100 (ng/g l.w.)
log TT3 (nmol/L)
r = 0.624
p = 0.067
n = 7
D) AdF_N
log ΣPCB17 (ng/g l.w.)
log TT4 (nmol/L)
r = 0.937*
p = 0.009
n = 5
E) AdF_N
log α-HCH (ng/g l.w.)
log TT3 (nmol/L)
r = -0.698*
p = 0.044
n = 7
F) AdF_S
log pp-DDT (ng/g l.w.)
log TT3 (nmol/L)
r=0.475*
p=0.037
n=15
G) AdF_S
log PBDE-153 (ng/g l.w.)
log TT3 (nmol/L)
r = -0.381
p = 0.081
n = 15
H) AdF_S
log QCB (ng/g l.w.)
log TT3 (nmol/L)
r = 0.428
p = 0.056
n = 16

Table 5
A selection of wildlife studies reporting relationships between plasma levels of thyroid hormones (TT3 and TT4) and concentrations of organohalogen contaminants (OHCs) measured in subcutaneous adipose tissue (AD) or plasma (PL), and the results from an exposure study of Greenland sledge dogs ("model-species" for polar bears). The presented data includes all ages and both sexes, unless otherwise mentioned.

Species, area	n	Tissue	Lipid %	OHC levels (ng/g l.w.)	TT3 (nmol/L)	TT4 (nmol/L)	Relationships OHCs vs. THs
				Mean (range)	Ranges of mean		
Polar bears, E. Greenland[1] (*Ursus maritimus*) 1999–2001 (Feb–Oct)	62	AD	88	ΣPCBs = 6673 (897–20407) ΣDDTs = 412 (74–1151) PCB-118 = 83.1 (16.02–154), *p,p′*-DDE = 366 (66.6–1050), HCB = 78.2 (2.40–331), Dieldrin = 186 (26.0–490), ΣPBDEs = 70.0 (17.0–229)	0.55–0.85[a]	6.74–18.4[a]	Subadults, adult females: PBDE-99, -100, -153, PCB-52, -118, *cis*-nonachlor, *trans* -nonachlor, TCB and QCB: ↑↓ TT3 and/or TT4. Adult females with cubs (AdF_N): ΣPCB_{17}: ↑TT4 Adult females without cubs (AdF_S): DDTs: ↑TT4
Polar bears, Svalbard[2] 1991–1994 (March–April)	79	PL	1.1[a]	ΣPCBs = 7110[b] (2010–27800) *p, p′*-DDE = 55[b] (<7–5280) PCB-118 = 39[b] (<10–169) HCB = 115[b] (27–765)	0.60–0.80	13.0–21.0	ΣPCBs, HCB: ↓TT4:FT4 *p, p′*-DDE: ↑TT4:TT3
Polar bears, Svalbard[3] 1997–1998 (March–April)	81	PL	1.1	Σ_5PCB = 7081[c] (1518–17272) PCB-118 = 26.4[c] (8.18–84.5)	1.23–1.58	13.5–18.5	♀: Σ_5PCBs (ww): ↓FT4, ↓FT3, ↓TT4:TT3, ↓TT4, ↑TT3:FT3 ♀PCB-118 (ww): ↑FT3, ↓TT3:FT3 ♂ Σ_5PCBs (ww): ↓FT3, ↑FT4:FT3 ♂ PCB-118 (ww): ↓FT4:FT3
Polar bears, Svalbard[4] 1998 (March)	33	PL	n.a.	ΣPCBs = 8323–20770[d] ΣDDTs = 78.2–96.4[d]	0.34–0.69[e]	0.39–3.88[e]	Both Svalbard and Resolute polar bears: ΣPCBs (and individual PCB-congeners): ↓TT4
Polar bears, Resolute Bay[4] (Canadian High Arctic) 1997 (March)	33	PL	n.a.	ΣPCBs = 2977–7217[d] ΣDDTs = 63.6–151[d]	0.08–0.19[e]	4.93–6.62[e]	
Greenland sledge dogs[5] (*Canis familiaris*) Exposed females (F) and pups (P)	10	AD	81	ΣPCBs = 2996 (936–6740) ΣDDTs = 167 (18–358) Dieldrin = 531 (13–948)	F:1.59 ± 0.12[f] P: 1.44 ± 0.04[f]	F:27.9 ± 1.65[f] P: 23.0 ± 1.16[f]	F: ↓FT4 (vs. control group) P: ↓TT3 (vs. control group) F + P: ΣDDTs: ↓thyroid gland weight, Dieldrin: ↑TT3
Harbour seals[6] (*Phoca vitulina*) U.K, 2003 (March–Oct)	60	AD	77–86[g]	ΣPCBs = 1300–8200[g] ΣDDTs = 220–830[g] ΣPBDEs = 97–630[g]	1.74 (1.53–1.94)[h]	90.6 (77.5 ± 103)[h]	PBDEs>PCBs>DDT: ↑TT3
Grey seal pups[7] (*Halichoerus grypus*) Norwegian coast (N) and Baltic Sea (B) 1998	35	AD	n.a.	B: ΣPCBs = 2625–26732[i] N: ΣPCBs = 665–2932[i] B: ΣDDTs = 871–8501[i] N: ΣDDTs = 306–1212[i]	B: 1.5–1.7 N: 1.9–2.2	B: 31.3–31.9 N:29.0–34.0	ΣPCBs and ΣDDTs: ↓TT 3PCB-118: ↓FT3

1 Current study. 2 Skaare et al., 2001. 3 Braathen et al., 2004. 4 Sandau, 2000; Fisk et al., 2005. 5 Sonne, 2010 ; Kirkegaard et al., 2011. 6 Hall and Thomas, 2007. 7 Sørmo et al., 2005.
TT3 = total triiodothyronine. TT4 = total tetraiodothyronine. n.a. = not available.
[a] Measured in whole blood.
[b] Median.
[c] Calculated mean of three group mean levels, and calculated from wet weight to lipid weight levels using mean lipid% of plasma.
[d] Calculated from wet weight to lipid weight levels assuming a mean plasma content of 1.1% (similar to Braathen et al., 2004 and Skaare et al., 2001).
[e] Cub levels were not included.
[f] Mean ± standard deviation.
[g] Ranges of mean; adult males and females.
[h] Mean (range); adult males and females.
[i] Range.

ortho-PCBs (Braathen et al., 2004). The above mentioned studies have analysed levels of PCBs and other OHCs in plasma and not in adipose tissue as in the present study, which complicate inter-study comparisons of OHC associated effects on TH homeostasis.

The differences in the PLS models of AdF_N and AdF_S may be linked to reproductive status, influencing both thyroid physiology and toxicokinetics of OHCs. Potential pregnancies within AdF_S could influence the TH levels (McNabb, 1992; Ramsay and Stirling, 1988). Specific milk transfer of contaminants could affect the remaining body burdens and patterns of OHCs in AdF_N compared to AdF_S (Bernhoft et al., 1997; Lie et al., 2000; Polischuk et al., 1995, 2002). A detailed investigation on OHC levels and patterns in polar bear groups is, however, beyond the scope of the present study.

4.2. Subadults

The most important OHC determinants for TT3 levels were quite similar for SubA and AdF_N in this study (e.g. PBDE-99, PBDE-100, TCB, PCB-52, PCB-118, α-HCH, see Fig. 1A and B). This could be a reflection of the interconnected exposure pattern of OHCs through placental and lactation transfer (Bernhoft et al., 1997; Lie et al., 2000; Polischuk et al., 1995, 2002), and interlaced TH disruptive mechanisms in females and their offspring (Brouwer et al., 1998). TCB, HCB and its major oxidative metabolite pentachlorophenol (PCP), have been found to be thyroid disruptive in experimental rat studies (Vanraaij et al., 1991, 1993a), in human studies of pregnant women (Chevrier et al., 2008a; Dallaire et al., 2009b; Sandau et al., 2002;

Takser et al., 2005) and in polar bears from Svalbard (corrected for age and sex) (Skaare et al., 2001). The importance of PCB-118 for TT3 levels in both SubA and AdF_N in this study was also supported by a study on polar bears from Svalbard (Braathen et al., 2004). In human studies of mothers and their infants, pre- and postnatal exposure to PCBs (e.g. PCB-118) and other OHCs (DDTs, HCBs, HCH), seemed to affect TH homeostasis, reducing circulating TH levels and perhaps contribute to congenital hypothyroidism (cretinism) in infants (Nagayama et al., 1998, 2005, 2007; Ribas-Fito et al., 2003). Effects of pre- and postnatal OHC exposures on TH homeostasis may also occur in later juvenile to adult stages, which is more comparable to the SubA polar bears in this study. Such delayed effects have been documented in juvenile rats (Arena et al., 2003), juvenile sledge dogs (Kirkegaard et al., 2011) and young human adults (Langer et al., 2007, 2008).

The present study identifies PBDEs as important variables influencing TH levels in all three groups, especially PBDE-99 and -100 had a high influence on TT3 levels in SubA and AdF_N. This is in accordance with the known thyroid disruptive effects of PBDEs, and the most toxic of these are considered to be the pentaBDE-99 and -100 (Darnerud, 2008; Darnerud et al., 2001; Hallgren et al., 2001). The importance of PBDEs for TH status identified herin, is supported by human studies that report negative associations between PBDEs and TH in adults (Dallaire et al., 2009a), pregnant women (Chevrier et al., 2008b) and newborn children (Herbstman et al., 2008). Rodent exposure studies with PBDE-mixtures, including PBDE-99 and -100, showed that prenatal maternal exposure caused reduced T4 levels in offspring and developmental effects such as impaired learning and memory in young offspring, changed behaviour (hyperactivity) at puberty and impaired female and male reproductive systems in adult offspring (Darnerud, 2003; Eriksson et al., 2001; Kuriyama et al., 2005, 2007; Talsness et al., 2005; Vos et al., 2003; Zhou et al., 2002).

The present study implicate that young polar bears could be vulnerable to thyroid disruption by several OHCs. This is of great concern because altered TH status in young mammals may be more detrimental than in adults, because of the importance of THs during growth and development, particularly with respect to the brain and the nervous system (Howdeshell, 2002; Zoeller and Crofton, 2005; Zoeller et al., 2007a). In young polar bears, neurodevelopmental deficits may reduce learning, the ability to find and hunt prey, change behaviour (e.g. mating) and ultimately affect reproduction and even survival. Indeed, Derocher et al. (2003) suggested that polar cub survival is lower in areas with high OHC loads, such as in Svalbard. Levels of OHCs are equally high in East Greenland and Svalbard polar bears (Letcher et al., 2010). Imbalance of the TH system in young polar bears could also reduce their ability to adjust metabolic rate to factors such as temperature, ice-cover, food availability (fasting), and energy requirements and more (Jenssen, 2006). Ultimately, OHC induced thyroid disturbances could have a large impact on juvenile polar bears from East Greenland and even reduce the population's ability to adjust to the global climate changes rapidly taking place in the Arctic.

4.3. Complex OHC mixtures — combination effects on TH homeostasis?

Some of the most conspicuous OHCs influencing TH levels in polar bears in the present study (discussed in Sections 4.1 and 4.2) were among the single compounds with the lowest concentrations (e.g. PBDE-99, PBDE-100, PCB-52, TCB, *p,p′*-DDD, QCB, α-HCH, *cis*-nonachlor). It is possible that in addition to being more potent thyroid disruptors, these compounds act on TH levels in combination with other OHCs accumulated in the polar bears. The potential for dose or response addition may be present because many HCHs, DDTs, PCBs, PBDEs and CHLs and their metabolites mediate their effects trough many of the same target sites on the HPT axis (Boas et al., 2006; Brouwer et al., 1998; Zoeller, 2007). For instance, several OHCs from the current study (HCB, TCB, PCBs and many PBDEs) induce UDPGTs, resulting in increased biliary excretion of T4 and lowering of circulating TH levels (Hallgren et al., 2001; Sacco and James, 2004; Vanraaij et al., 1993b; Zhou et al., 2002), which suggest dose addition (simple similar action). Dose addition could also occur between HCB, OCP, PBDEs, and/or their phenolic metabolites with a hypothetical binding affinity to polar bear TTR, thus displacing T4 and potentially lowering T4 levels in blood and other tissues (Brouwer et al., 1998; Chauhan et al., 2000; Gutleb et al., 2010; Hamers et al., 2006; Lans et al., 1994; Sandau et al., 2000). This may be plausible since phenolic metabolites (e.g. OH-PCBs, OH-PBDEs, PCP from HCB, and 4-OH-heptachlorostyrene from OCS) have been reported in polar bears from East Greenland (Gebbink et al., 2008a,b; Sandala et al., 2004). It is possible that some of the relationships in the PLS models indirectly reflect the actions of phenolic metabolites. Furthermore, *in vivo* studies showed that *ortho*-PCBs (e.g. PCB-52 in all groups, ΣPCB_{17} in AdF_N) reduced T4 levels possibly by interfering with negative-feedback responses of T4 in the pituitary gland (Khan and Hansen, 2003a,b; Khan et al., 2002). Acting together, these three above mentioned mechanisms may result in lowering of T4 levels by response addition (simple dissimilar action).

One probable interactive effect in the East Greenland polar bears could be potentiation through OHC mediated CYP1A enzyme induction increasing production of phenolic metabolites that through TTR-binding or other mechanisms could disrupt TH homeostasis (Brouwer et al., 1998; Hallgren and Darnerud, 2002; Hamers et al., 2006; Gutleb et al., 2010; Lans et al., 1994; Letcher et al., 1996; Sandau, 2000; Sandau et al., 2000; Ucan-Marin et al., 2010). Recent *in vivo* studies have demonstrated combined TH disruptive effects of OHC mixtures at environmental relevant doses (Crofton et al., 2005; Gauger et al., 2007; Hallgren and Darnerud, 2002; Wade et al., 2002), supporting the possibility of combined effects (dose/response addition and interactive effects) from the OHCs accumulated in polar bears from East Greenland.

Many experimental and wildlife studies report negative responses of environmental contaminants on TH homeostasis mostly based on negative relationships between OHC groups and TH levels (Braathen et al., 2004; Brouwer et al., 1989, 1998; Debier et al., 2005; Rolland, 2000; Skaare et al., 2001; Sørmo et al., 2005; Zoeller, 2005). Some wildlife studies on seals report mostly positive relationships between OHCs and TH levels or TH parameters (Hall and Thomas, 2007; Hall et al., 2003; Routti et al., 2010). In studies of relationships between OHC levels and TH status in polar bears from Svalbard, some positive relationships were also found (Braathen et al., 2004; Skaare et al., 2001). Although differences in matrixes, analytical methods, number of components included in group sums, sampling area, year and season, species, age, sex and physiological status of the sampled animals generally confound inter-study comparison of OHC levels, the OHC concentrations in adipose tissue of the present polar bears were within concentration ranges of many of these above referred studies reporting relationships between OHC and THs (Table 5).

In the polar bears from East Greenland, we identified both negative and positive relationships between THs and single OHCs. That different OHCs seem to cause both reductions and increases of TH levels within the same individuals might seem contradictory. However, the modelled TH responses in the present study reflect the simultaneous analyses of the influence of single chemicals in mixtures on TH levels, which may depend on their relative concentrations in the mixtures, their potential to act on the HPT axis by one or several mechanisms and their ability to act in combination with other compounds. Some contaminants might have paradoxical effects, acting as both thyroid antagonists and agonists. For example, PBDEs (e.g. PBDE-100) and OH-metabolites of PCBs (e.g. of PCB-118) can potentially displace T4 from TTR and reduce circulating T4 levels, while binding to TR and increasing TH related transcription and biological effects (Gauger et al., 2007; Kitamura et al., 2005, 2008; Zoeller, 2005, 2007). Furthermore, PCB and HCB metabolites may influence deiodination enzymes responsible for

conversion of T4 to T3 (Boas et al., 2006; Kato et al., 2004; Meerts et al., 2002; Wade et al., 2002), further adding to the complexity.

Combined and paradoxical effects of different OHCs and their metabolites may produce small, variable and unpredictable effects on TH levels, still with potential for detrimental effects on brain development in a manner that is disproportional to the measured changes in TH levels (Bansal et al., 2005; Gauger et al., 2007; Zoeller, 2005). In addition, some EDCs may show hormetic effects (Calabrese and Baldwin, 2003; Kefford et al., 2008), further complicating the prediction of endocrine responses of mixtures. Consequently, TH effect assessments should not be solely based on an evaluation of TH levels and correlative association with sums of contaminant groups. The results of the present study support the potential complex and dynamic actions of environmental OHC mixtures on TH homeostasis. More effort should be put into assessing endocrine effects of single or combinations of chemicals in complex mixtures in wildlife, human and experimental studies.

5. Conclusion

The results of the multivariate regression (PLS) modelling and validation by univariate statistics corrected for the influence of biological factors, indicate a linkage between TH levels and complex OHC mixtures accumulated in adipose tissue of East Greenland polar bears. The nature of these relationships appears to be complex, and are both positive and negative. Subadult and adult female polar bears may seem more susceptible to potential TH disruption as compared to adult males. In the models, specific combinations of individual OHCs seem to influence the TH levels. However, some compounds appear to be important for TH levels in the subadult and adult female groups: PBDE-99, PBDE-100, PBDE-153, TCB, QCB, PCB-118, PCB-52, *cis*-nonachlor and *trans*-nonachlor. Individual chemicals in the OHC mixtures detected in polar bear adipose tissue could potentially act in combination on the HPT axis and result in additive or even synergistic responses. Although statistical relationships are not evidence per se of biological cause-effect relationships, we suggest that the results of the present study add to the "weight-of-evidence" that OHCs may be interfering with TH homeostasis in polar bears.

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PAPER II

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Disruptive effects of persistent organohalogen contaminants on thyroid function in white whales (*Delphinapterus leucas*) from Svalbard

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ABSTRACT

We analysed levels of 56 organohalogen contaminants (OHCs) including brominated flame retardants, polychlorinated biphenyls (PCBs), and organochlorine pesticides in the blubber of white (beluga) whales (*Delphinapterus leucas*) from Svalbard, Norway (N = 12; 6 adults [5 males and 1 female] and 6 subadults [4 males and 2 females]) collected in 1996–2001. We also measured circulating levels of thyroid hormones (THs) and thyroid stimulating hormone (TSH) in the whales. The results confirm that OHC levels in these white whales are among the highest levels recorded in wildlife from Svalbard, and at the high end of the range when compared to white whales from the North American Arctic. A projection to latent structure (PLS) model (subadults and adult males grouped together) revealed that known or suspected thyroid disruptive contaminants (polybrominated diphenylether [PBDE]-28, -47, -99, -100, and -154, hexachlorobenzene [HCB], and PCB-105) were negatively correlated with circulating levels of total thyroxin (TT4), free T4 (FT4) and free triiodothyronine (FT3). Most of these negative relationships were also confirmed using partial correlations controlling for length (and thus age) of the whales. The positive correlations of TT4, FT4 and FT3 with hexabromocyclododecane (HBCD), α-hexachlorocyclohexane (α-HCH), chlorinated bornanes CHB-40 and CHB-62 revealed by the PLS model were not confirmed by partial correlations. TH levels in the present study appeared to be somewhat lower than levels measured in beluga whales from the Canadian Arctic. However, we were not able to determine if this was caused by different levels of OHCs, or differences in biological factors (e.g. age, sex, moulting status, and season) and analytical methods between the studies. Although the sample sizes were low and statistical models cannot depict the biological cause–effect relationships, this study suggests negative influences of specific OHCs, particularly PBDEs, on thyroid hormone levels in white whales. The impact this might have on individual and population health is unknown.

1. Introduction

The complex mixture of anthropogenic, long-range transported organohalogen contaminants (OHCs) that accumulates in arctic wildlife might disrupt endocrine systems and thus potentially affect development, behaviour, fertility and survival (Rolland, 2000; AMAP, 2002; Letcher et al., 2010). This is a cause for concern for arctic top-predators, especially in areas with high contaminant loads, such as the European Arctic (Letcher et al., 2010). Endocrine disrupting chemicals might also reduce an individual's ability to adapt to the ongoing climate-related changes in the Arctic (Jenssen, 2006).

One important target of endocrine disruptive chemicals is the thyroid hormone system. In vertebrates, triiodothyronine (T3) and tetraiodothyronine (thyroxine, T4) are produced by the thyroid gland. Production and secretion of these thyroid hormones (THs) are induced by thyroid stimulating hormone (TSH), which is released from the pituitary gland (McNabb, 1992). T4 is produced by the thyroid gland in much larger quantities than T3. Deiodination of T4 in extra-thyroidal tissues is the main source of the biological more active hormone, T3. Circulating T3 and T4 are responsible for negative feedback (inhibition) on pituitary release of TSH (McNabb, 1992; Hadley, 1996; Zoeller et al., 2007). THs are involved in regulation of temperature, metabolism, reproduction and growth, and are especially important for the growth and development of the nervous system in foetuses, neonates and juveniles (McNabb, 1992). Neuro-developmental effects (e.g. cognitive dysfunctions, behaviour changes, and reduced motor skills) have been observed in human and rodent offspring exposed to OHCs *in utero* and postnatally through lactation. These effects are thought to be mediated via the capacity of these contaminants to disrupt TH homeostasis in sensitive periods of TH-dependent brain development (Porterfield, 2000; Howdeshell, 2002; Branchi et al., 2003; Zoeller and Crofton, 2005;

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Nakajima et al., 2006; Zoeller et al., 2007). Neurodevelopmental deficits in young mammals can reduce their ability to learn (and thus affect their ability to find and hunt prey), change behaviour (e.g. mating) and ultimately affect reproduction and survival. Furthermore, disturbances of the TH system can also reduce an individual's ability to thermoregulate and to adjust metabolic rate in relation to external factors such as temperature, ice-cover, food availability (fasting), migratory needs or other shifts in energy requirements. Thyroid disruptive effects of polychlorinated biphenyls (PCBs) and other OHCs have been documented in experimental *in vivo* and *in vitro* studies, and similar findings have been reported in studies of Arctic wildlife and human populations (e.g. Braathen et al., 2004; Verreault et al., 2007; Dallaire et al., 2009; Villanger et al., 2011).

White whales (beluga whales, *Delphinapterus leucas*) are long-lived, arctic predators that feed high in the marine food chain. They have poor capacity to metabolise and excrete OHCs (Norstrom et al., 1992; Letcher et al., 2000), and are known to accumulate high levels of these contaminants in their large blubber lipid-reservoirs (Andersen et al., 2001, 2006; Wolkers et al., 2004, 2006; Letcher et al., 2010). Some of the most conspicuous effects associated with OHCs in wildlife have been reported for beluga whales from the southern end of this species' range in the St Lawrence River Estuary (Canada). Here, the high levels of OHCs and other contaminants (e.g. polyaromatic hydrocarbons) in past decades were coincident with high incidences of neoplasia and other lesions, immune dysfunction, abnormal sexual development, reduced reproduction and high mortality rates of this population (De Guise et al., 1995; Martineau et al., 2003). However, differences in exposure patterns and levels complicate extrapolation from previously reported effects in stranded St Lawrence beluga whales to potential negative effects on health in arctic populations (Letcher et al., 2010). But the past findings from the St Lawrence population demonstrate this species' susceptibility to effects of chemical contamination in the marine ecosystem that they inhabit.

Physiological studies of white whale stocks from the Canadian Arctic have reported some unique features in the TH system of this species, such as an unusually large thyroid gland, and high levels of T4 and reverse T3 (rT3) (St Aubin and Geraci, 1988, 1989; St Aubin, 2001). Though many unanswered questions regarding the function and regulation of the thyroid system in white whales remain, studies indicate that THs are important in this species. These hormones are thought to play a critical role in their opportunistic life-strategies (St Aubin and Geraci, 1988, 1989; St Aubin, 2001), and thus their adaptability to environmental changes, such as arctic climate changes. White whales are considered vulnerable to climate changes because of the predicted changes in their ice-habitats and they have repeatedly been recommended as a key species for monitoring change in the arctic environment (IWC, 1997; Kovacs and Lydersen, 2008; Kovacs et al., 2010).

Previous investigations of white whales from Svalbard show that OHC levels are relatively high in comparison with other marine mammals, such as polar bears (*Ursus maritimus*) and seals (Andersen et al., 2001, 2006; Wolkers et al., 2004, 2006). OHC-associated responses of THs and other potential detrimental health effects have been reported in polar bears from Svalbard and East Greenland (Skaare et al., 2001; Braathen et al., 2004; Letcher et al., 2010; Sonne, 2010; Villanger et al., 2011). But to date no studies have investigated the possible effects of OHCs on TH homeostasis in beluga whales from the European Arctic, or in populations elsewhere. A study of beluga whales from the St Lawrence Estuary and in Hudson Bay documented hyperplasia of the thyroid gland, but it was uncertain if this was linked to OHC exposure (Mikaelian et al., 2003). Hence, the objectives of this study were to: a) measure levels of OHCs accumulated in adipose (blubber) tissue of white whales from Svalbard, b) determine circulating concentrations of THs and TSH in the same whales, and c) assess the multivariate relationships between OHC concentrations and levels of THs and TSH.

2. Methods

2.1. Sampling

White whales (N = 12) were live-captured at Spitsbergen, Svalbard, Norway in July–October 1996–2001 (Table 1). Procedures for capturing and sampling are described in detail elsewhere (Andersen et al., 2001; Lydersen et al., 2001; Tryland et al., 2006). In brief, the white whales were captured in nets set from the beach. After detangling, the whales were kept in shallow water near the shore during sampling and measuring, which took about 20 min for each individual. White whales in Svalbard are protected by legislation (Gjertz and Wiig, 1994) and all handling was done under permits from the Norwegian Animal Research Authority and the Governor at Svalbard. The standard length was measured to nearest 5 cm, and sex was determined by examination of genitalia. The individual white whales were assigned to an age group (subadults or adults) based on body size and skin colour (Brodie, 1971; Sergeant, 1973, see Table 1). Samples consisting of skin and blubber were biopsied from a 20 × 20 cm area about 10 cm in front of the mid-dorsal ridge using custom made, hollow steel rods (diameter = 6 mm, length = 150 mm) or a gauge biopsy punch (diameter = 8 mm, Medizin Gmblt, Köln, Germany), see Table 1. Blubber biopsies were wrapped in aluminium foil and kept frozen at −20 °C until analyses. The skin was removed prior to OHC analyses. Blood was taken from the caudal vein (Venoject, Trumo Corporation, Leuven, Belgium) and plasma was prepared by centrifugation (1500 *g*, 15 min) and frozen at −20 °C until analyses of THs.

2.2. Analyses of organohalogen contaminants

Blubber samples of individual beluga whales were analysed for the following chlorinated and brominated contaminants: α-, β- and γ-hexachlorocyclohexane (HCH), hexachlorobenzene (HCB), oxychlordane, *trans*-chlordane, *cis*-chlordane, *trans*-nonachlor, *cis*-nonachlor (CHLs), [1,1-dichloro-2,2-bis(4-chlorophenyl) ethylene] (*p,p′*-DDE), [1,1-dichloro-2,2-bis(4-chlorophenyl) ethane] (*p,p′*-DDD), [1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane] (*p,p′*-DDT), Mirex, chlorinated bornane (toxaphene, CHB) congeners Parlar nos. 26, 40, 41, 44, 50 and 62, total hexabromocyclododecane (HBCD; sum of α-, β- and γ-HBCD), polybrominated diphenylether (PBDE) congeners IUPAC nos. 28, 47, 99, 100, 153, 154, and 183, and PCB congeners IUPAC nos. 28, 31, 47, 52, 56, 66, 74, 99, 101, 105, 110, 114, 118, 128, 137, 138, 141, 149, 151, 153, 156, 157, 170, 180, 183, 187, 189, 194, and 196.

Chemical analyses of OHCs were performed at the Laboratory of Environmental Toxicology at the Norwegian School of Veterinary Science, Oslo, Norway. The methods for extraction and sample clean-up are described in Brevik (1978) with modifications described in Andersen et al. (2001). Briefly, the method included homogenisation of pooled blubber samples of each whale (2–4 samples). Pooling of blubber samples was done in order to have enough tissue to perform contaminant analyses on individual whales. The blubber samples for each whale were obtained from the same area and mostly with the same sampling techniques (see Table 1). Water (Grade 1) was added to weighed samples to increase volume and thus optimise separation between solvent and water during the extraction. Internal standards were then added to the samples (analyses of PCBs, DDTs, HCHs, Mirex, HCB and CHLs: PCB-29, PCB-112, and PCB-207; analyses of CHBs: DETOX-409 [2-endo,3-exo,6-exo,8,9,10,10-Heptachlorobornane] and PCB-112; analyses of PBDEs and HBCD: PBDE-77, PBDE-119, PBDE-181, ^{13}C-PBDE-209). The samples were extracted twice with acetone and cyclohexane (2:3) using an ultrasonic homogeniser (4710 Series, Cole Parmer Instruments Co., Chicago, IL, USA). Lipid content of the samples, expressed as percent (%) of total sample weight, was determined gravimetrically. The extracts were cleaned (i.e. removal of lipids and proteins) using concentrated ultra-clean sulphuric acid

Table 1
Field data and blubber sampling methods of white whales from Svalbard.

Field code	Date	Location (position)	Age group	Sex	Blubber sampling device		OHC analyses	Thyroid horm. analyses
DLyear of catch/number					Metal rod	Biopsy punch	Blubber	Plasma
DL96/01	July 1996	Van Keulenfjorden (77° 26′ N, 16° 06′ E)	Adult	Male	x	x	1	2
DL97/06	August 1997	Van Mijenfjorden (77° 51′ N, 16°49′ E)	Adult	Male		x	1	2
DL99/06	August 1999	Storfjorden (78° 31′ N, 18° 55′ E)	Adult	Male	x		2	2
DL99/07	August 1999	Storfjorden (78° 31′ N, 18° 55′ E)	Adult	Male	x		2	2
DL99/08	August 1999	Storfjorden (78° 31′ N, 18° 55′ E)	Adult	Male	x		2	2
DL99/13	August 1999	Storfjorden (78° 31′ N, 18° 55′ E)	Subadult	Male	x		2	2
DL01/01	October 2001	Storfjorden (78° 31′ N, 18° 55′ E)	Subadult	Male	x		2	2
DL01/02	October 2001	Storfjorden (78° 31′ N, 18° 55′ E)	Subadult	Female	x		2	2
DL01/03	October 2001	Storfjorden (78° 31′ N, 18° 55′ E)	Subadult	Male	x		2	2
DL01/05	October 2001	Storfjorden (78° 31′ N, 18° 55′ E)	Adult	Female	x		2	2
DL01/07	October 2001	Storfjorden (78° 31′ N, 18° 55′ E)	Subadult	Female	x		2	2
DL01/08	October 2001	Storfjorden (78° 31′ N, 18° 55′ E)	Subadult	Male	x		2	2

1 Data from Andersen et al. (2001, 2006).
2 Analyses performed in the current study.

(H_2SO_4). The cleaned extracts were used in separation and detection of contaminants by gas chromatography (GC). PBDEs and HBCD were analysed by GC with mass spectrometry (MS) as described in Sørmo et al. (2006). The GC–MS was operated in negative chemical ionisation (NCI) mode with selected ion monitoring of PBDEs and HBCD at *m/z* 79/81. HBCD was also monitored at *m/z* 159.8. PCBs and organochlorine (OC) pesticides were analysed on a GC (Agilent 6890 Series, Agilent Technologies, PA, USA) equipped with an auto sampler (Agilent 7683 Series, Agilent technologies) and a dual column system (SPB-5 and SPB-1701, Supelco Inc., Bellefonte, PA, USA) coupled to two ^{63}Ni micro (μ) electron capture detectors (Agilent 6890 μECD, Agilent Technologies), following the specification and temperature programme described in Andersen et al. (2001). CHB analyses was done on a GC (Agilent 6890 Series, Agilent Technologies) with a 60 metre DB-5 MS capillary column (J&W Scientific, CA, USA) configured with an MS detector (Agilent 5973 Network, Agilent Technologies) which was operated in NCI mode with selected ion monitoring. The methods for silica fractionation prior to GC analyses, temperature programmes and other specification for GC-ECD/MS analyses of CHBs in this study followed previously described methods (Føreid et al., 2000; Andersen et al., 2006).

In the current study, we included the OHC results for two adult males captured in 1996 and 1997, previously reported in Andersen et al. (2001, 2006). These samples were analysed at the Laboratory of Environmental Toxicology using the same methods as in the present study and analysing the same compounds except for HBCD, PBDEs, Mirex, *trans*-chlordane, PCB-56, CHB-40, -41, and -44, which were not analysed for these the two individuals (Andersen et al., 2001, 2006). In order to have the same compounds summed (Σ) per OHC group in all white whales, PCB-56 was not included in ΣPCBs, *trans*-chlordane was not included in ΣCHLs and CHB-40, 41 and -44 were not included in ΣCHBs. Furthermore, the results of Mirex, HBCD and PBDEs presented in the current study are reported for only 10 individuals (i.e. they do not include levels from the two adult males from 1996/97).

The laboratory has been accredited since 1996 by Norwegian Accreditation (Kjeller, Norway) as a testing laboratory meeting the requirements of the Norwegian Standardisation–European Standardization–International Organisation for Standardisation International/International Electrotechnical Commission (NS-EN ISO/IEC) 17025 (Test 137). The laboratory has participated in several interlaboratory tests with approved results for the quantification of OC pesticides, CHBs, PCBs and brominated flame retardants (QUASIMEME Laboratory Performance studies and AMAP [Arctic Monitoring and Assessment Program] ring test for POPs in human serum). Quality assurance in this study included linear calibration curves with standard solutions of the analysed contaminants. Limit of detection (LOD) was defined as three times the average instrument signal (background noise) in the chromatograms of the sample extracts. In this study, LOD varied according to contaminant or contaminant group and ranged from 0.19 to 0.91 ng/g wet weight (w.w.) for PCBs, 0.10 to 0.29 ng/g w.w. for HCHs, 0.24 to 0.38 ng/g w.w. for CHLs, 0.21 to 0.48 ng/g w.w. for DDTs, 0.12 to 0.55 ng/g l.w. for CHBs, 0.19 ng/g l.w. for HCB, 0.72 for Mirex, 0.05 to 0.15 ng/g w.w. for PBDEs and 2.50 ng/g w.w. for HBCD. Quantifications were done within the linear ranges of the calibration curves ($R \geq 0.985$). Blanks containing solvents were analysed along with the samples to control for potential contamination during extraction procedures. Internal standards were used to correct for changes in OHC concentrations during analytical procedures. Because of inconsistent blank contamination of PBDE-183, these results should be considered with some caution. All samples were above LOD for all measured OHCs, except for three individuals where PCB-28, PCB-31 and PBDE-183 were below detection. These samples were assigned a value of half the LOD. Further quality assurance in this study included the use of analysed standard solutions to control drift during GC-analyses, and the use of seal blubber control samples to control reproducibility over time. The recoveries for spiked seal blubber samples ranged from 111 to 148% for PBDEs, 145% for HBCD, 102 to 113% for PCBs, 72 to 110% for CHBs, and 99 to 130% for DDTs. These standard procedures ensured adequate quality assurance and control, and also ensured that the accuracy, linearity, and sensitivity of the analyses were within the laboratory's accreditation requirements.

The quality assurance for the OHC analyses of these two individuals from 1996/97 is described in Andersen et al. (2001, 2006). For PCB-28, 31, 114, 189 and 194 levels were below LOD in these two individuals, and a value of half the LOD from Andersen et al. (2001) were assigned for data analyses in this study.

As described in Andersen et al. (2001, 2006), the different lengths of the blubber biopsies caused the lipid content to vary (3.47–95.8%) because short biopsies yield more connective tissue and less blubber. This was especially true for samples from two whales taken with gauge biopsy punches (Table 1) which included mostly the outer blubber layer. This might bias the OHC levels slightly upwards, as previously discussed in Andersen et al. (2001). This is based on a study conducted on baleen whales where OHC layering appears to occur in the blubber (Aguilar and Borrell, 1991). However, these results have been contradicted by a similar study by Gauthier et al., 1997. In addition, toothed whales have thinner, less structured blubber than baleen whales (Aguilar, 1985), which might suggest that they also have a more homogenous OHC distribution in their blubber column. Hence, we believe that the inclusion of the samples consisting mostly of the outer blubber is representative for the OHC blubber loads in these two beluga whales when adjusted to lipid content. Thus, the OHC results in this study were expressed as nanogram per gramme lipid weight (ng/g l.w.).

2.3. Analyses of thyroid hormones and TSH

Plasma samples of the 12 whales were analysed for THs and TSH at the Department of Biology, Norwegian University of Science and Technology (NTNU, Trondheim, Norway) using radioimmunoassay (RIA). Concentrations of total and free T3 and T4 (TT3, FT3, TT4 and FT4) were analysed using commercially available solid-phase ^{125}I RIA kits developed for humans (Coat-A-Count Total T3, Coat-A-Count Free T3, Coat-A-Count Total T4, and Coat-A-Count Free T4, Diagnostic Product Corporation, LA, CA, USA). The standard test protocols for these RIA kits were followed (DPC, 2005a,b,c,d). Because THs are highly conserved among vertebrate species, the RIA method developed for humans is commonly used in measuring marine mammals TH levels with acceptable results (Greenwood and Barlow, 1979; St Aubin, 2001; Braathen et al., 2004; Sørmo et al., 2005). However, since methods for measuring human TSH have been shown to be unsuccessful in detecting this hormone in beluga whales (St Aubin, 2001), we measured TSH levels in the present study using a solid-phase ^{125}I immunoradiometric (IRMA) kit developed for canines (Cout-A-Count Canine TSH IRMA, Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA). The procedures described in the test protocol were followed (Siemens, 2006).

Detection and quantification of THs and TSH, which are inversely related to bound radioactive antigen, were performed on a gamma counter (Cobra Auto-Gamma, Packard Instruments Company, Dowers Grove, IL, USA). The standard curves consisted of six–seven known standards (calibrators) run in duplicates. For TSH, FT3 and FT4 the calibrators were lot specific. Plasma samples of white whales, as well as control samples, were analysed in duplicates or triplicates. The coefficient of variation (CV) for duplicate/triplicate plasma samples ranged from 0.87 to 18.8% for TT4, FT4, TT3 and FT3, which was acceptable. For TSH, the sample CVs were mostly within acceptable ranges (4.28–17.9%), although two samples had higher CVs (27.5 and 37.4%, respectively). These were still accepted because of the low TSH concentrations in these two samples. Detection limits were as follows; TT4: 0.100 nmol/l, FT4: 0.027 pmol/l, TT3: 0.024 nmol/l, FT3: 0.107 pmol/l, and TSH: 0.040 ng/ml. All samples were above these detection limits.

Percent recovery, which was calculated from a white whale plasma sample spiked with TT3 and TT4 calibrators of the highest concentrations (calibrator:plasma = 1:9), was acceptable; TT4: 88.1% and TT3: 97.1%. Standard reference material (SRM Level 1, Biorad Lyphochek® Immunoassay Plus Control, Bio-Rad Laboratories, LA, USA), plasma samples of domesticated Arctic fox ("Blue fox" *Alopex lagopus*) and bovine (*Bos taurus*) plasma were used to control intra-assay variation. For TT3, the intra-assay CV for bovine plasma was 3.41% (n = 2). For FT3, intra-assay CV for bovine plasma was 5.72% (n = 2). Intra-assay CV for TT4 was 4.79% (n = 4) for bovine plasma and 11.0% (n = 2) for blue fox plasma. For FT4, intra-assay CV for bovine plasma was 1.38% (n = 3), and intra-assay CV for two blue fox control samples were 1.42% (n = 2) and 4.74% (n = 2), respectively. For TSH analyses, intra-assay CV was 6.94% (Blue fox plasma; n = 2) and 13.5% (SRM; n = 2).

Capture and handling stress can increase cortisol levels leading to a reduction in TH levels (St Aubin, 2001). This effect has been reported during capture of white whales, but only after 4–6 h (St Aubin and Geraci, 1988, 1992). The total chase and handling time in the current study was much shorter. Moreover, cortisol levels in white whales from Svalbard captured in 1996–1999 (including many of the individuals in the present study) did not seem to be affected by the stress of handling (Tryland et al., 2006).

2.4. Data analyses

Despite being caught at different geographical locations along the Spitsbergen coast, satellite tracking has shown that these white whales probably belong to the same population because of regular movements between the different sites of capture routinely occurs (Lydersen et al., 2001). The 6-year range over which sampling took place was assumed not to influence OHC levels in this study, as a minimum sampling period of 8–10 years is recommended in order to statistically elucidate temporal trends in OHC levels in biota (Henriksen et al., 2001). The 12 white whales included in the present study were grouped according to age and sex; Adult males (AdM, n = 5), Adult females (AdF, n = 1) consisting of only one mature female with a calf (the calf was not included in this study), and subadults (SubA, n = 6) consisting of juvenile males (n = 4) and juvenile females (n = 2). In the subadult sample, males and females were combined because there were no differences in TH levels or concentrations of major OHC groups detected between the sexes (Mann–Whitney U). The single AdF was not included in statistical analyses because of low sample size, and different OHC toxicokinetics compared with SubA and AdM due to placental and milk transfer of OHCs to its calf.

Statistical analyses were conducted using SPSS (Version 16, standard version, SPSS Inc., Chicago, IL, USA). Because of the small sample size in this study, non-parametric analyses were used. Mann–Whitney *U* test was used to investigate differences in levels of THs, TSH and OHCs between AdM and SubA. Spearman's rank correlation (r_s) was used to test the relationships between body length (as an indicator of age) and OHC concentrations and TH levels, as well as inter-correlation between TH variables (SubA and AdM, n = 11). Numerical values are presented as arithmetic means ± standard deviation (SD) or mean ranges in the text, and in addition as medians with minimum and maximum (range) values in the tables. Significance level was set to $p \leq 0.05$. The p-values are two-tailed unless otherwise specified.

Projections to latent structures by means of partial least squares (PLS — L. Eriksson et al., 2006) was applied to investigate the influence of individual OHC compounds and biological data (predictor [X]-variables) on TH and TSH levels (response [Y]-variables). PLS is a regression method that models the multivariate relationships between the X-variables and their simultaneous effect upon the Y-variable(s) in an unidirectional manner (Wold et al., 2001; SIMCA, 2008). For these analyses, the software Simca-P+ (Version 12.0, Umetrics AB, Umeå, Sweden) were used. Since factors such as age, sex and physiological/reproductive status can influence TH levels and responses, effect studies should ideally consider these as covariates or perhaps even perform separate statistical analyses based on these factors (Gochfeld, 2007; Zoeller et al., 2007; Abdelouahab et al., 2008). The samples sizes of the age/sex groups in the current study were too low to enable separate analyses. Also, since most subadults were caught in 2001 and all adult males were caught in 1996–1999 it was not appropriate to include the different years of sampling as an additional variable. Thus, the SubA and AdM samples were grouped together (n = 11) when performing PLS modelling of the effects of the 56 individual OHCs analysed in this study and the biological variable length (as a measure of age) on the Y-variables TT4, FT4, TT3, FT3, and TSH. PLS does not require normal distributions, is not sensitive to outliers, it can deal with multicolinarity among the variables and datasets with a lower number of individuals than variables (Wold et al., 1984; L. Eriksson et al., 2006; SIMCA, 2008). Therefore, PLS was considered an appropriate statistical method for analysing the influence of complex OHC mixtures (many Xs) on TH levels (several Ys), despite a low sample size and relatively large individual variation in OHC levels in this study. Since normality is not required for PLS regression, original data were used (i.e. not transformed). All variables were centred and scaled (to variance 1), and significance level was set to 0.05 (SIMCA, 2008). The PLS modelling was validated by the explained variation in the X-matrix (R^2X), explained variance of the Y-variables by the X-matrix (goodness of fit, R^2Y), goodness of prediction (Q^2) obtained by cross-validation, and permutation analyses (20 permutation). A more detailed description of PLS modelling can be found elsewhere (Wold et al., 2001; L. Eriksson et al., 2006; SIMCA, 2008).

3. Results

3.1. Contaminant levels and patterns

The concentrations of individual OHCs and the sums (Σ) of major OHCs groups detected in blubber tissue of white whales from Svalbard are presented in Table 2. The dominant OHC compounds were ΣPCBs, ΣDDTs and ΣCHLs, contributing 39–64%, 37–46% and 20–33%, respectively, to the total OHC load. ΣCHBs was also high (12–38%). ΣHCH, ΣPBDEs, HBCD, HCB, and Mirex were minor contributors to the total OHC load. The concentrations of ΣCHLs and ΣDDTs were significantly higher in AdM compared to SubA ($Z=-2.373$; $p=0.017$ and $Z=-2.191$; $p=0.030$, respectively, Table 2). No such relationships were found for ΣPCBs, ΣPBDEs, HCB, Mirex or HBCD (Table 2). Though not statistically explored, the single AdF in this study had concentrations of ΣPCBs, ΣDDTs, ΣHCHs, ΣCHLs and ΣPBDEs as well as HCB, Mirex and HBCD, that were similar or somewhat higher than in SubA, but lower than in AdM (Table 2).

The concentrations of ΣPCBs, ΣDDTs, ΣCHLs and Mirex were positively correlated with body length, and thus age, of the white whales (Fig. 1). There were no such significant correlations for ΣHCH, HCB, ΣPBDEs, HBCD or ΣCHBs.

The dominant PCB congeners were the penta- and hexa-CBs PCB-153, PCB-138, PCB-99, PCB-101, PCB-118 and PCB-149 (in decreasing order), see Fig. 2. The tetra-CB-52 was also a major contributor to ΣPCBs with concentrations comparable to that of PCB-99 and PCB-101. Together, these seven congeners constituted 68–71% of ΣPCBs found in white whales. The concentrations of PCB-99, -118, -149, -151, -156, -170, -183, -187, -194, and -196 were significantly higher in AdM than in SubA (Mann Whitney *U* test: $Z=-2.191$, $p=0.030$; $Z=-2.373$, $p=0.017$; $Z=-2.191$, $p=0.030$; $Z=-2.191$, $p=0.030$; $Z=-2.191$, $p=0.030$; $Z=-2.191$, $p=0.030$; $Z=-2.191$, $p=0.030$; $Z=2.191$, $p=0.030$; $Z=-2.739$, $p=0.004$; and $Z=-2.739$, $p=0.004$, respectively, Fig. 2). PCB levels in the AdF appear to be low compared to AdM but higher than in SubA (Fig. 2).

The most dominant PBDE congener in all three groups of white whales was PBDE-47 (71–78% of ΣPBDEs); followed by PBDE-100 (8–9% of ΣPBDEs) and PBDE-99 (4–11% of ΣPBDEs). The remaining PBDE congeners (PBDE-28, -153, -154 and -183) were only minor contributors

Table 2
Median, range, mean and standard deviation (SD) of individual and sum (Σ) concentrations (ng/g lipid weight, l.w.) of chlorinated and brominated organic contaminants measured in blubber tissue of white whales from Svalbard grouped into adult males (AdM), adult female (AdF), and subadults (SubA; juvenile males and females). Mann–Whitney *U* test was used to test for differences in OHCs levels between AdM and SubA.

	AdM (n = 5)		SubA (n = 6)		AdF (n = 1)
	Median (range)	Mean ± SD	Median (range)	Mean ± SD	
HCB	449 (358–476)	425 ± 50.8	328 (66.8–498)	323 ± 155	322
Mirex[a]	23.3 (20.3–28.7)	24.1 ± 4.25	12.6 (9.46–28.3)	15.7 ± 6.93	23.7
α-HCH	20.8 (16.9–40.0)	26.0 ± 9.73	18.0 (11.5–25.6)	18.3 ± 5.18	14.0
β-HCH	43.0 (32.5–97.0)	53.3 ± 26.9	26.5 (8.31–39.3)	25.5 ± 11.2	34.6
γ-HCH	22.2 (19.7–41.7)	26.4 ± 9.21	18.1 (9.43–33.3)	19.3 ± 8.06	16.0
ΣHCHs[b]	97.5 (70.5–145)	106 ± 36.3	57.7 (39.9–93.0)	63.2 ± 19.0	64.6
Oxychlordane	586 (474–667)	564 ± 83.0	329 (59.4–642)	320 ± 197	442
trans-chlordane	27.2 (24.5–56.9)	36.2 ± 18.0	21.4 (9.75–27.4)	20.5 ± 6.33	34.5
cis-chlordane	184 (36.4–345)	191 ± 110	137 (49.3–250)	141 ± 67.8	232
trans-nonachlor	1350* (773–1533)	1188 ± 320	610 (192–1010)	631 ± 278	1020
cis-nonachlor	187 (23.3–502)	226 ± 173	177 (97.0–210)	163 ± 47.9	241
ΣCHLs[c]	2160* (1620–2990)	2169 ± 573	1240 (398–2100)	1250 ± 571	1940
p,p′-DDE	3280* (1790–4480)	3205 ± 1162	1200 (149–3790)	1420 ± 1260	1900
p,p′-DDD	485* (296–680)	470 ± 147	243 (64.8–392)	227 ± 114	348
p,p′-DDT	553** (284–871)	611 ± 234	210 (93.9–416)	217 ± 112	409
ΣDDTs[d]	4320* (2370–5810)	4286 ± 1517	1580 (308–4600)	1870 ± 1480	2650
CHB-26	145 (20.4–5660)	1620 ± 2446	122 (5.92–194)	101 ± 75.3	19.3
CHB-40	3.81 (3.69–8.72)	5.41 ± 2.87	2.78 (2.44–5.22)	3.31 ± 1.13	3.83
CHB-41	33.2 (27.1–72.0)	44.1 ± 24.3	31.2 (18.1–41.5)	29.0 ± 8.81	34.0
CHB-44	23.2 (20.2–5780)	1939 ± 3321	19.7 (11.8–23.8)	19.2 ± 4.24	21.3
CHB-50	1020* (416–6950)	2694 ± 2932	407 (179–478)	368 ± 115	651
CHB-62	43.9* (16.0–150)	66.1 ± 56.9	15.1 (11.9–25.5)	16.5 ± 4.68	18.8
ΣCHBs[e]	1100 (551–12760)	4380 ± 5400	495 (210–664)	485 ± 167	690
ΣPCBs[f]	4150 (3380–6210)	4490 ± 1140	2310 (631–4990)	2440 ± 1450	3730
HBCD[a]	24.5 (15.7–44.2)	28.1 ± 14.58	12.6 (5.48–237)[g]	48.9 ± 92.4	5.93
PBDE-28	3.11 (1.99–4.61)	3.24 ± 1.31	2.68 (1.17–4.36)	2.59 ± 1.26	2.85
PBDE-47	72.8 (57.4–86.0)	72.1 ± 14.3	67.1 (14.8–86.5)	60.8 ± 28.4	93.8
PBDE-100	6.94 (6.43–7.79)	7.05 ± 0.688	7.86 (2.70–10.9)	7.55 ± 3.14	11.1
PBDE-99	3.30 (2.76–4.65)	3.57 ± 0.972	6.71 (0.935–29.4)	9.50 ± 10.2	6.68
PBDE-153	1.21 (0.593–9.47)	3.76 ± 4.96	0.888 (0.576–2.74)	1.22 ± 0.822	1.13
PBDE-154	1.89 (1.61–1.95)	1.82 ± 0.181	2.85 (0.737–4.08)	2.69 ± 1.24	3.92
PBDE-183	2.23 (0.331–3.85)	2.14 ± 1.76	0.740 (0.111–1.69)	0.75 ± 0.622	0.514
ΣPBDEs[a,h]	87.6 (77.6–116)	93.7 ± 19.8	89.4 (22.7–137)	85.1 ± 41.7	120
Lipid (%)	19.2 (3.47–95.8)	31.4 ± 36.7	54.8 (33.4–67.4)	53.3 ± 12.5	62.6

[a] Mirex, HBCD and PBDEs were not analysed for two AdM from 1996/97, therefore n = 3 for these compounds in the AdM group.
[b] ΣHCHs include α-HCH, β-HCH and γ-HCH.
[c] ΣCHLs include oxychlordane, *cis*-chlordane, *trans*-nonachlor and *cis*-nonachlor (*trans*-chlordane was not analysed in Andersen et al., 2001 and there for not included in ΣCHLs in the present study).
[d] ΣDDTs include *p,p′*-DDT, *p,p′*-DDE and *p,p′*-DDD.
[e] ΣCHBs include CHB-26, CHB-50 and CHB-62 (CHB-40, -41 and -44 were not analysed for two whales from 1996/1997 [Andersen et al., 2006] and therefore not included in ΣCHBs in the present study).
[f] ΣPCBs include the PCB congener numbers 28, 31, 47, 52, 66, 74, 99, 101, 105, 110, 114, 118, 182, 137, 138, 141, 149, 151, 153, 156, 157, 170, 180, 183, 187, 189, 194 and 196 (PCB-56 was not analysed for two whales from 1996/97 [Andersen et al., 2001] and therefore not included in ΣPCBs in the present study).
[g] The high variation (SD) is due to one individual's high HBCD level (SubA: 237 ng/g l.w.).
[h] ΣPBDEs include the PBDE congener number 28, 47, 99, 100, 153, 154 and 183.
* Significant: $p \leq 0.05$.
** Significant: $p \leq 0.01$.

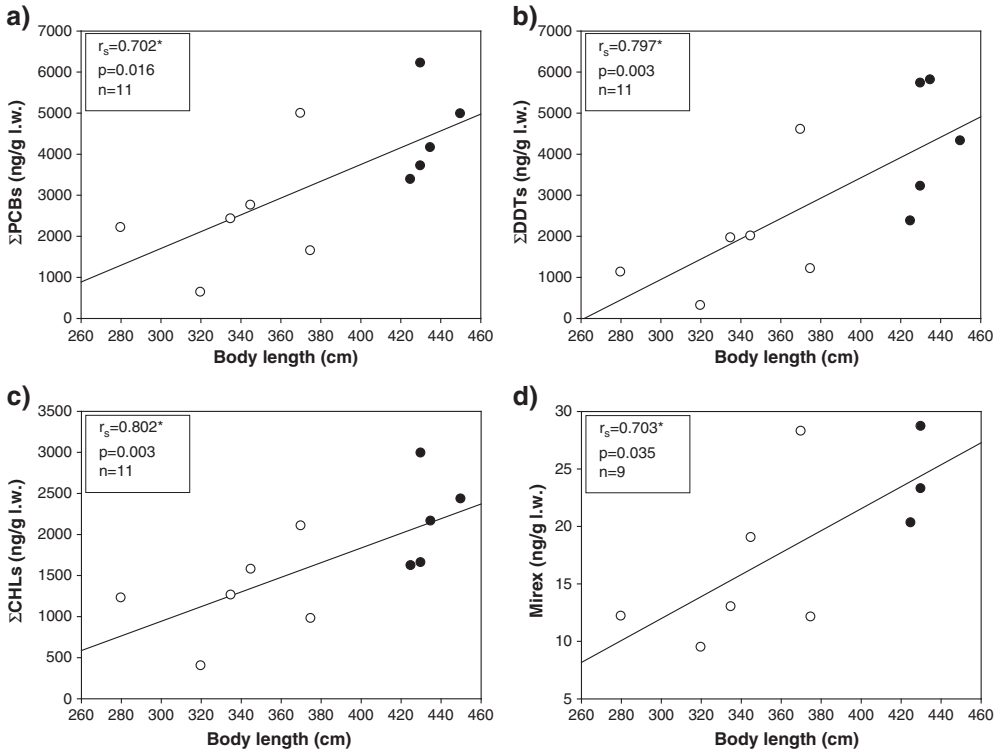

Fig. 1. a)–d). Body length vs major OHCs group sums or compounds in blubber of 11 white whales from Svalbard; subadults (SubA, n = 6) and adult males (AdM, n = 5). r_s = Spearman's rank correlation coefficient. p-values are two-tailed. * Significant; $p \leq 0.05$. ○ SubA ● AdM. n=9 for d) due to the lack of Mirex levels in 2 whales.

to ΣPBDEs. PBDE congener levels and patterns were comparable in SubA, AdM and AdF, and there were no statistical differences in PBDE levels between SubA and AdM (Table 2).

DDT patterns were similar in the three age/sex groups. The metabolite *p,p′*-DDE was the dominating DDT compound, accounting for 72–76% of ΣDDTs. The parent compound *p,p′*-DDT made up 12–15%

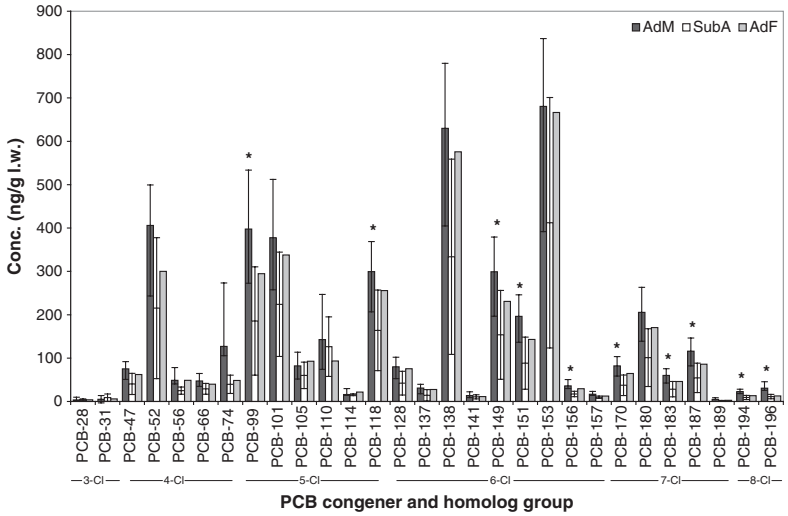

Fig. 2. Mean ± standard deviation of the concentration (ng/g l.w.) of 29 PCB congeners analysed in blubber tissue of 12 white whales from Svalbard. The results are divided according to age/sex groups; adult males (AdM, n = 5), subadults (SubA; juvenile males and females, n = 6) and adult female (AdF, n = 1). The PCB congeners are grouped according to degree of chlorination (homolog groups); 3-Cl, trichlorobiphenyls; 4-Cl, tetrachlorobiphenyls; 5-Cl, pentachlorobiphenyls; 6-Cl, hexachlorbiphenyls; 7-Cl, heptachlorobiphenyls; 8-Cl, octachlorobiphenyls. * Significant difference between AdM and SubA (Mann Whitney *U* test; $p \leq 0.05$).

and the metabolite *p,p′*-DDD made up 11–13% of ΣDDTs. The concentrations of *p,p′*-DDT, *p,p′*-DDE and *p,p′*-DDD were significantly higher in AdM as compared to SubA ($Z=-2.556$; $p=0.009$, $Z=-2.191$; $p=0.030$, and $Z=-2.373$; $p=0.017$, respectively, Table 2). In the AdF, *p,p′*-DDE concentration were intermediate, whereas *p,p′*-DDT and *p,p′*-DDD in the AdF appeared to be similar to AdM (Table 2).

β-HCH was the dominant HCH-isomer (40–53% of ΣHCHs), whereas γ-HCH constituted 25–31% and α-HCH constituted 22–29% of ΣHCHs in all three groups. In SubA, the HCH-pattern seemed less influenced by β-HCH and more influenced by α- and γ-HCH than in AdM and AdF (Table 2). There were no significant differences in levels of the HCH-isomers between AdM and SubA, and levels in the AdF seemed comparable (Table 2).

The patterns of CHL compounds in AdM, SubA and AdF were similar. The dominant CHL compounds were *trans*-nonachlor (49–53% of ΣCHLs) and oxychlordane (22–26% of ΣCHLs), followed by *cis*-chlordane (9–11% of ΣCHLs) and *cis*-nonachlor (10–13% of ΣCHLs), with only a minor influence from *trans*-chlordane. Even though ΣCHLs were significantly higher in AdM than in SubA, only *trans*-nonachlor was significantly higher in AdM as compared to SubA ($Z=-2.191$; $p=0.030$, Table 2). In the AdF, the concentrations of single CHLs seemed to be similar to AdM and were generally higher than in SubA (Table 2).

In AdM, the CHB pattern was dominated by CHB-50 (43% of total CHBs[1]), CHB-44 (30% of total CHBs) and CHB-26 (25% of total CHBs). In SubA and AdF, CHB-50 contributed even more to total CHBs with 68% and 86%, respectively. CHB-44 comprised only 3 and 4% of total CHBs in SubA and AdF, respectively, which was low compared to AdM. The large variation in CHB-44 levels in AdMs was mostly due to an outlier that had two magnitudes higher levels of CHB-44 than the other adult males. Only the concentrations of CHB-50 and CHB-62 were significantly higher in AdM than in SubA ($Z=-2.191$; $p=0.030$ and $Z=-2.191$, $p=0.030$, respectively, Table 2). The AdF appeared to have lower levels of CHB-50 and CHB-62 than AdM. CHB levels seemed comparable in all three groups otherwise (Table 2).

Table 3
Plasma concentrations of thyroid hormones (THs), thyroid stimulating hormone (TSH), TH ratios, and body length of white whales from Svalbard (n = 12) grouped into adult males (AdM), adult female (AdF), and subadults (SubA; juvenile males and females). Mean, standard deviation (SD), median, and range (Min.–Max.) are presented. There were no significant differences in TH levels or ratios between AdM and SubA (Mann–Whitney U).

		AdM (n = 5)	SubA (n = 6)	AdF (n = 1)
Length (cm)	Median	430	340	330
	Min.–Max.	425–450	280–375	
	Mean ± SD	434 ± 10	338 ± 35	
TT4 (nmol/l)	Median	98.1	90.3	79.1
	Min.–Max.	79.6–101	76.8–150	
	Mean ± SD	94.7 ± 8.58	98.4 ± 27.2	
FT4 (pmol/l)	Median	12.0	11.5	8.16
	Min.–Max.	10.1–15.7	9.36–21.0	
	Mean ± SD	12.4 ± 2.08	12.6 ± 4.24	
TT3 (nmol/l)	Median	1.38	1.86	1.84
	Min.–Max.	1.09–2.43	1.12–2.45	
	Mean ± SD	1.71 ± 0.64	1.77 ± 0.50	
FT3 (pmol/l)	Median	0.86	0.89	0.22
	Min.–Max.	0.33–0.96	0.38–1.53	
	Mean ± SD	0.75 ± 0.25	0.93 ± 0.54	
TSH (ng/ml)	Median	0.088	0.058	0.043
	Min.–Max.	0.046–0.254	0.047–0.065	
	Mean ± SD	0.110 ± 0.084	0.057 ± 0.007	
TT4:FT4	Median	7.91	8.02	9.69
	Min.–Max.	6.25–8.43	7.13–8.53	
	Mean ± SD	7.71 ± 0.87	7.89 ± 0.61	
TT3:FT3	Median	1.59	1.84	8.22
	Min.–Max.	1.15–7.37	1.25–6.07	
	Mean ± SD	2.95 ± 2.60	2.56 ± 1.83	
TT4:TT3	Median	71.4	63.2	43.1
	Min.–Max.	32.8–87.9	32.3–78.2	
	Mean ± SD	62.4 ± 23.5	58.8 ± 18.8	
FT4:FT3	Median	16.2	16.6	36.5
	Min.–Max.	12.1–30.5	7.92–25.0	
	Mean ± SD	18.3 ± 7.2	16.8 ± 7.30	

Conversion factors: μg/dl × 12.87 = nmol/l for TT4; ng/dl × 0.01536 = nmol/l for TT3; ng/dl × 12.87 = pmol/l for FT4; pg/ml × 1.536 = pmol/l for FT3.

3.2. Thyroid hormones and TSH

Plasma concentrations of TSH, THs (TT4, TT3, FT4 and FT3) and their ratios, as well as measured body lengths of the whales are presented in Table 3. There were no significant differences in levels of TSH, THs or TH ratios between AdM and SubA (Table 3). In the AdF, concentrations of TSH and THs as well as the TH ratios seemed to be generally similar to the other groups (Table 3).

TH levels and ratios were not correlated with length (SubA and AdM grouped together) ($|rs|<0.287$, $p>0.392$, $n=11$). However, TT4, FT4 and FT3 were positively inter-correlated (FT4 vs. FT3: $r_s=0.764$, $p=0.006$; FT4 vs. TT4: $r_s=0.891$, $p<0.0001$; TT4 vs. FT3: $r_s=0.691$, $p=0.019$; $n=11$ for all correlations), and TSH was negatively correlated with FT4 ($r_s=-0.627$, $p=0.037$, $n=11$).

3.3. Modelled effects of OHCs on thyroid hormones and TSH

The first PLS regression model consisted of 5 Y-variables (TSH, FT4, FT4, TT3 and FT3) and 57 X-variables (body length and the 56 single OHCs included in this study). This model was not significant even when two PLS components were imposed ($R^2X=0.707$, $R^2Y=0.453$, $Q^2=0.0594$). Improvement of the PLS model was done in a step-wise manner with evaluations of validation parameters and permutation analyses for each step. First, the Y-variables TSH and TT3 were removed from the model, since they had much lower R^2Y (≤0.214) and Q^2 (≤0.058) than the remaining Y-variables (TT4, FT4 and FT3; $R^2Y=0.593$–0.798 and $Q^2=0.179$–0.336). The loading plot now showed that the three remaining Y-variables were grouped together, meaning that they were positively correlated and responded in the same direction to the X-variables. Although still not significant, this step improved the model (two imposed PLS components; $R^2X=0.694$, $R^2Y=0.712$, $Q^2=0.309$), especially the Q^2 and R^2Y. The model was further improved by a successive removal of 40 X-variables with the lowest VIP values (VIP = variable importance for the projection; SIMCA, 2008), as they were the least important variables explaining the PLS model (SIMCA, 2008); PCB-28, PCB-196, *cis*-nonachlor, *cis*-chlordane, γ-HCH, PCB-141, length, PCB-74, CHB-41, PCB-189, PCB-66, PCB-180, PCB-153, PCB-52, CHB-26, *p,p′*-DDE, PCB-183, -128, -137, -187, -101, and -138, *trans*-chlordane, β-HCH, PCB-170, -47, CHB-50, *p,p′*-DDD, PCB-151, -156, -56, and -110, *p,p′*-DDT, PBDE-153, PCB-114 and -31, PBDE-183, CHB-44, PCB-157 and PCB-194. This resulted in a significant model with two components ($R^2X=0.752$, $R^2Y=0.744$, $Q^2=0.431$). R^2X and Q^2 were above the values that define a good or acceptable model using biological data; $R^2X>0.7$ and $Q^2>0.4$ (Lundstedt et al., 1998). The permutation analyses also confirmed the acceptable validity of the PLS model; FT4 intercepts: $R^2X=(0.0, 0.339)$, $Q^2=(0.0, -0.208)$; FT3 intercepts: $R^2X=(0.0, 0.297)$, $Q^2=(0.0, -0.221)$; TT4 intercepts: $R^2X=(0.0, 0.355)$, $Q^2=(0.0, -0.205)$. This best-fitted PLS model consisted of 17 X-variables. The loadings of the X-variables and thus their relations to each other and the Y-variables TT4, FT4 and FT3 are shown in Fig. 3a. Since TT4, FT4 and FT3 are inter-correlated and behave similarly in the PLS model, only the column plot of the regression coefficient

[1] Total CHBs include all analysed CHB-congeners in the present study; CHB-26, 40, 41, 44, 50 and 62 (CHB-40, 41 and 44 was not measure in 2 AdM from 1996/96) whereas ΣCHBs include CHB-26, -50 and -62 (n = 12).

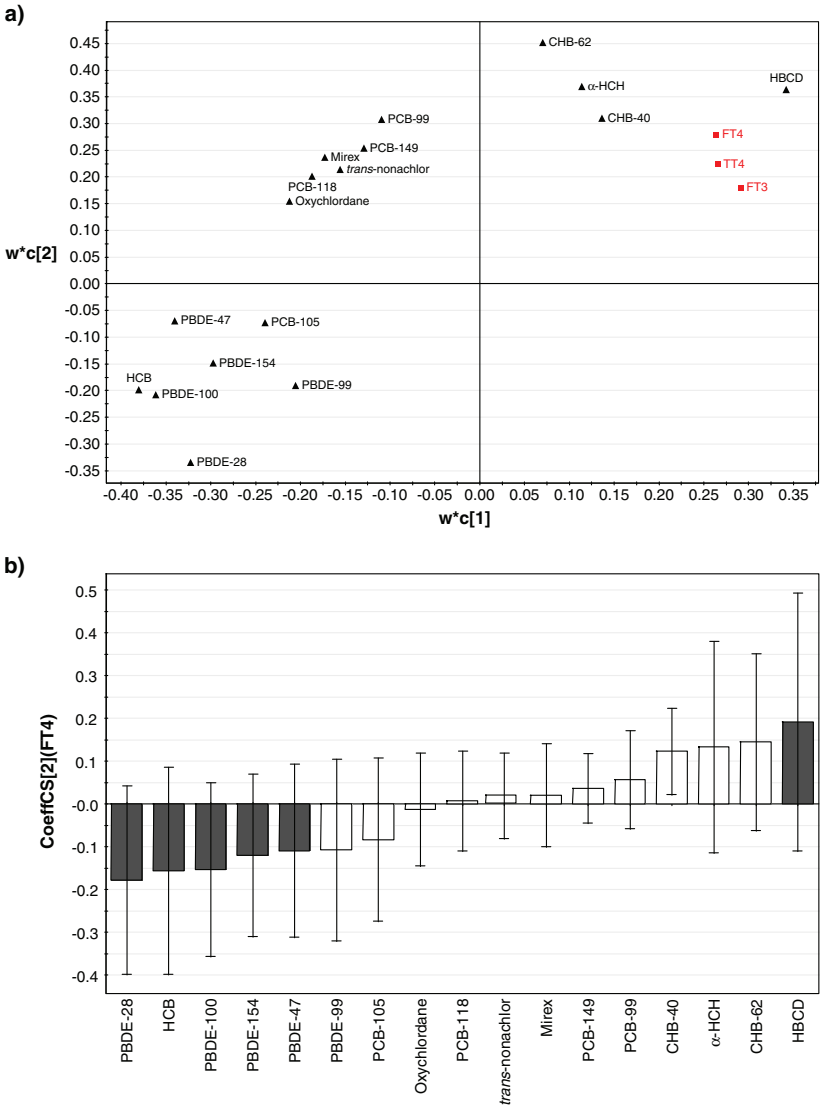

Fig. 3. Loading plot (a) of the significant and best-fitted PLS model between the response variables Y = FT4, TT4 and FT3 and 17 individual OHCs (X-variables) analysed in blubber tissue (ng/g l.w.) of 11 white whales (*Delphinapterus leucas*) from Svalbard (adult males and subadult males and females). The PLS model had two significant components and validation parameters $R^2X = 0.75$, $R^2Y = 0.74$ and a $Q^2 = 0.43$. ▲ X-variables ■ Y-variables The regression coefficient plot (b) of the PLS model showing regression coefficient (CoeffCS) values (open bars) of each variable that indicate the direction and strength of the relationships between individual X-variables and the Y-variable FT4. The error bars represent the 95% confidence intervals. The full bars present CoeffCS values of variables with VIP values ≥ 1, which indicate high importance on both the X- and Y-matrixes.

(CoeffCS) values for Y = FT4 (Fig. 3b), which had the highest R^2Y, were presented. The CoeffCS values in Fig. 3b (open bars) explain the correlative relationships (strength and direction) between single X-variables and Y. The full bars in Fig. 3b show the CoeffCS values of variables with VIP values ≥ 1, indicating high importance for the X- and Y-matrixes (SIMCA, 2008). R^2X, R^2Y, Q^2, VIP and CoeffCS values are cumulative calculated from all extracted PLS components (SIMCA, 2008). The most important variables explaining TT4, FT4 and FT3 levels in white whales from Svalbard were PBDE-28, HCB, PBDE-100, PBDE-154, PBDE-47, PBDE-99 and PCB-105 which were all negatively correlated with TT4, FT4 and FT3, and HBCD, CHB-62, α-HCH and CHB-40 that were positively correlated with TT4, FT4 and FT3 (Fig. 3a and b).

The relationships found in the best-fitted PLS model were further investigated with bivariate correlation tests between FT4 (also representing TT4 and FT3), using a partial correlation test on ranked data controlling for length (as a measure of age). Most of the important, negative relationships between OHCs (PBDE-28, HCB, PBDE-100, PBDE-154, PBDE-47, PBDE-99, and PCB-105) and FT4 from the PLS model were confirmed by significant partial correlations (see

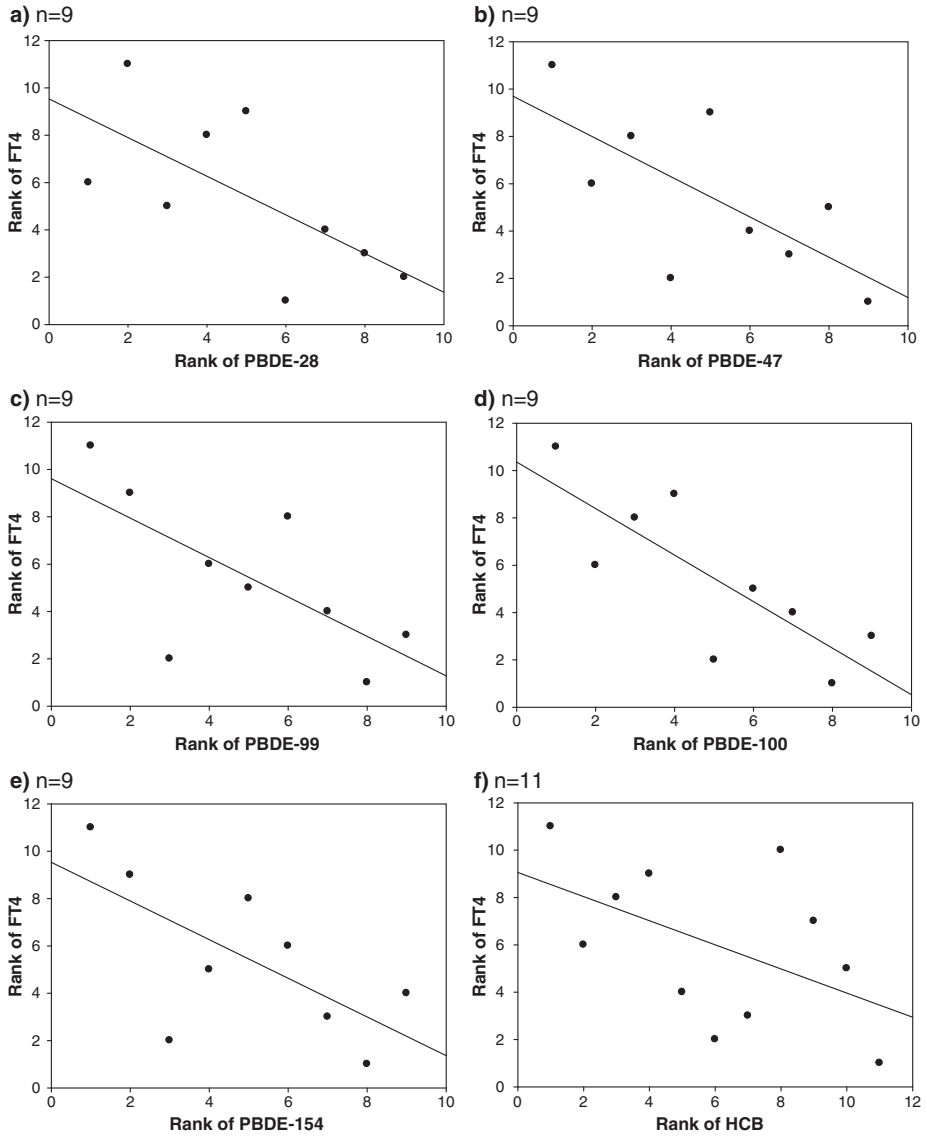

Fig. 4. Plasma levels of FT4 vs OHC concentrations measured in blubber of subadult (juvenile males and females) and adult male white whales from Svalbard (n = 11). All data are ranked. The lines show the best-fit regression line through the data points, illustrating the direction of the relationship in each plot. These scatter-plots show the most important relationships of the PLS model that were significantly correlated in partial correlations tests (r_p) controlling for body length (and thus age): a) FT4 vs PBDE-28: $r_p = -0.672^*$, $p = 0.034$, $df = 6$; b) FT4 vs PBDE-47: $r_p = -0.696^*$, $p = 0.028$, $df = 6$; c) FT4 vs PBDE-99: $r_p = -0.764^*$, $p = 0.014$, $df = 6$; d) FT4 vs PBDE-100: $r_p = -0.811^*$, $p = 0.007$, $df = 6$; e) FT4 vs PBDE-154: $r_p = -0.703^*$, $p = 0.026$, $df = 6$; f) FT4 vs HCB: $r_p = -0.765^*$, $p = 0.014$, $df = 6$. * Significance; $p \leq 0.05$. n = 9 for a)–e) due to the lack of contaminant levels in 2 whales. df = degrees of freedom. All p-values are one-tailed because of expected correlative relationships from the PLS model (see Fig. 3b).

Fig. 4a–f). The positive relationships found in the PLS model between OHCs (HBCD, CHB-62, α-HCH and CHB-40) and FT4 were not found to be significant in the partial correlations ($|rp| < 0.482$, $p > 0.113$, $n = 9$[2]/11).

[2] n = 9 for partial correlation of FT4 vs CHB-40.

4. Discussion

4.1. Contaminant levels and patterns

This study is the first to report Mirex levels in beluga whales from Svalbard (Table 2), which were within the ranges previously reported in blubber of Alaskan, Canadian and NW Greenland stocks (Stern et al., 1994; Krahn et al., 1999; AMAP, 2002; Hobbs et al., 2003), but 5–

9 times lower than reported in free-ranging beluga whales from the more polluted St Lawrence Estuary (Hobbs et al., 2003). This study is also the first to report HBCD levels in white whales in Svalbard (ranges of mean: 5.9–49 ng/g l.w.; see Table 2), which were higher than previously reported for white whales and narwhals from the western Canadian Arctic (both species: 2.0 ng/g l.w.; Tomy et al., 2008) and in white whales from the eastern Canadian Arctic (males and females, ranges of mean: 9.8–18 ng/g l.w.; de Wit et al., 2006). HBCD levels in white whales in Svalbard were similar to levels determined in ringed seals (20 ± 7.6 ng/g l.w.) and polar bears (12 ± 5.3 ng/g l.w.) within the Archipelago (Sørmo et al., 2006). de Wit et al. (2010) suggested that HBCD in biota might show similar geographical trends to OCs and PBDEs, with higher levels in the European Arctic compared to the North American Arctic. The results in the present study support this suggestion.

The levels of the remaining OHCs in the present study, including PCBs, DDTs, HCHs, CHLs, CHBs, PBDEs, and HCB are within the ranges previously reported in beluga whales from Svalbard (Andersen et al., 2001, 2006; Wolkers et al., 2004, 2006). Likewise, the congener/isomer patterns are similar to the previous literature from Svalbard (see citations above), except that this study found no indication of lower ΣPBDEs level in the single AdF compared to SubA an AdM, as was reported for two AdF in Wolkers et al. (2006). Comparisons of Svalbard beluga whales' OHC levels and patterns with beluga whale stocks in Canada and Alaska, and the more polluted St Lawrence Estuary and other marine mammals species in the Svalbard and Barents Sea region have been comprehensively discussed elsewhere (Andersen et al., 2001, 2006; Wolkers et al., 2004, 2006). In those studies, levels of CHBs, DDTs, PCBs and PBDEs in white whales from Svalbard were at the high end of the range when compared to white whale populations from the North American Arctic. OHC levels in Svalbard white whales were generally lower than in white whales from the St Lawrence Estuary. These earlier studies also reported that OHC levels in beluga whales were among the highest levels ever reported in wildlife from the Svalbard area. The results of the present study further support these findings.

Contrary to previous studies of white whales (Stern et al., 1994; Muir et al., 1996; Andersen et al., 2001), levels of ΣDDTs, ΣPCBs, ΣCHLs, and Mirex increased with age (Fig. 1a–d) in this study. This finding is in agreement with the general age-related accumulation of these persistent pollutants found in marine mammals, especially among males (Aguilar and Borrell, 1988; Tanabe et al., 1994). This trend is usually less clear in female marine mammals due to placental and lactational excretion of these contaminants (Martineau et al., 1987; Aguilar and Borrell, 1988; Muir et al., 1990; Tanabe et al., 1994). Although age-related increases in OHC levels of male white whales from Greenland and St Lawrence Estuary have not been apparent in some studies (Stern et al., 1994; Muir et al., 1996), positive correlations between age and OHC levels were demonstrated in Alaskan white whale stocks (Krahn et al., 1999) and in an older study of St Lawrence Estuary white whales (Martineau et al., 1987).

4.2. Thyroid hormones and TSH

In the present study, TT4, FT4 and FT3 were positively inter-correlated. This was probably due to their closely co-regulated concentrations in blood; FT3 and FT4 are in equilibrium with respective protein-bound fractions in blood (McNabb, 1992). TSH levels have not been reported from white whales, and only rarely from other cetacean species (St Aubin, 2001). Plasma levels of TSH in white whales (Table 3) were within the ranges reported in sledge dogs (Kirkegaard et al., 2011). The negative correlation between FT4 and TSH in the present study probably reflects the role of FT4 in inhibiting pituitary release of TSH, as a part of the negative feedback mechanism that regulates circulating T3 and T4 levels (McNabb, 1992; Hadley, 1996).

The levels of THs measured in plasma of white whales from Svalbard (Table 3) were similar or at the low end of the range previously reported in white whales from Hudson Bay and elsewhere in the Canadian Arctic (all ages and both sexes, mean or ranges of means); TT4: 103–247 nmol/l, FT4: 19.6 pmol/l, TT3: 0.91–2.72 nmol/l, and FT3: 2.58 pmol/l (St Aubin and Geraci, 1988, 1989; St Aubin et al., 2001; see Tables 2, 3 and 4 in St Aubin, 2001). It is important to consider factors that might have influenced TH levels in the sampled animals, such as age, sex, physiological and reproductive status, season, moulting status, stress and analytical methods (McNabb, 1992; St Aubin, 2001), when evaluating TH results and comparing with levels reported in other studies in order to assess toxic impact of OHCs.

The lack of relationships between THs and age or sex in the current study is similar to findings from a study of beluga whales from the Hudson Strait/Hudson Bay area in Canada (St Aubin and Geraci, 1989). However, in a larger compilation of TH measurements in beluga whales from Hudson Bay, Beaufort Sea and the Canadian High Arctic over a 15-year period, some age and sex-related differences in TT3 and TT4 levels were observed (St Aubin et al., 2001). Thus, the lack of influence of age and sex on TH levels in the present study might be due to the low sample size or different sampling periods.

St Aubin and Geraci (1989) reported seasonal variations in THs from white whales from the Hudson Strait/Hudson Bay area in Canada. Circulating TT3 and TT4 levels in the white whales were higher during their summer (late July–August) estuarine stay in the Hudson Bay than during spring (June to early July) and autumn (October–November) migrations. The summer increase in THs may be related to the moult, during which concurrent increase in epidermal cell growth and shredding of the outer layers of the epidermis takes place in the majority of the whales occupying these estuarine summer habitats in the Hudson Bay (St Aubin, 1990). Annual moulting of the skin is an event that occurs in all white whales, however it is not always as coordinated as described in Hudson Bay (St Aubin, 1990). The data in the present study were insufficient for investigation of seasonal variations in TH levels. Furthermore, all sampled white whales in the current study, except one whale captured in 1996, had already moulted. The white whale from 1996 was in the process of moulting, but did not have higher TH levels compared to the other whales in the study. In Svalbard, white whales do not have warm, estuarine areas as summer habitats to favour moulting, as they do in the Hudson Bay (St Aubin, 1990; Boily, 1995). The most probable reason for their summer and autumn habitat choices is feeding (Lydersen et al., 2001). Thus, in the present study, there is no reason to expect distinct variations in TH levels due to season or moulting status.

4.3. Effects of OHCs on TH levels in white whales

By using multivariate PLS regression, it was possible to model the unidirectional, statistical influences of single contaminants within the complex OHC mixtures measured in blubber on circulating TH levels in white whales. Even though PLS is a suitable statistical method for analysing contaminant and biological effect data, even with a lower number of individuals than variables (Wold et al., 1984; L. Eriksson et al., 2006; SIMCA, 2008), the low sample sizes in this study as well as variations in time of sampling, sampling techniques, and age and sex of the sampled animals require that interpretation of the results must be performed with caution. Still, the developed model showed good or acceptable validation and some trends of OHC associated impacts on TT4, FT4 and FT3 levels were revealed in the modelled results that warrant further consideration.

Among the most important relationships seen in the PLS models (Fig. 3a and b) were the negative influences of PBDE-28, -47, -99, -100, and -154, HCB and PCB-105 on levels of TT4, FT4 and FT3. These relationships, except for PCB-105, were also significant in partial

correlations controlling for length (and thus age; Fig. 4a–f). In a recent study of polar bears from East Greenland (Villanger et al., 2011) using similar PLS modelling techniques, PBDEs (especially PBDE-99 and -100) were the contaminants with the strongest influence on TH levels. Similar to white whales in the present study, the levels of PBDEs in polar bears were several magnitudes lower than the most dominant OHCs, such as PCBs and DDTs (Villanger et al., 2011). This suggests that these PBDE-congeners are disruptive to THs in arctic wildlife in a manner similar to effects that have been demonstrated in experimental *in vivo* studies, as well as findings in human and other wildlife studies (Hallgren et al., 2001; Zhou et al., 2001; Darnerud, 2003; Hall et al., 2003; Hall and Thomas, 2007; Herbstman et al., 2008; Dallaire et al., 2009; Routti et al., 2010). Thyroid disruptive abilities of HCB and PCB-105 and their major oxidative metabolites PCP and 4-OH-PCB-107, respectively, have also been shown in experimental studies using rodent models (Vanraaij et al., 1991, 1993a; Gauger et al., 2007), human studies (Sala et al., 2001; Sandau et al., 2002; Alvarez-Pedrerol et al., 2009; Dallaire et al., 2009) and in studies of some arctic wildlife (Sandau, 2000; Skaare et al., 2001; Verreault et al., 2004).

OHCs and their metabolites can disrupt TH levels or regulation of the hypothalamic-pituitary-thyroid (HPT) axis via several mechanisms and target-points; this disruption is often based on structural similarities with T3 or T4. OHCs can interfere with TH production, protein binding and transport in blood, enzymatic metabolism and excretion, or thyroid hormone receptor binding (Lans et al., 1994; Brouwer et al., 1998; Howdeshell, 2002; Zoeller, 2005; Boas et al., 2006; Hamers et al., 2006; Langer et al., 2007). Many PBDEs (e.g. PBDE-47, -99, and -100), PCBs (e.g. PCB-105 and -118) and their hydroxylated (OH)-metabolites, as well as HCB and its phenolic metabolite PCP, have the ability to bind to human and rat transthyretin (TTR), a major carrier-protein in the blood of these species, and displace T4. This is often used to mechanistically explain lowered T4 blood levels in these and other species (Brouwer et al., 1988, 1990; Lans et al., 1994; Meerts et al., 2000; Sandau et al., 2000; Hamers et al., 2006). However, in beluga whales, thyroxin-binding globulin (TBG) is the main TH carrier-protein in blood, and the existence of TTR has not been verified in this species (St Aubin and Geraci, 1989; St Aubin, 2001). Recent *in vitro* studies have demonstrated that OH-metabolites of PBDE-47, -49 and -99 are potent competitive binders with TBG (Marchesini et al., 2008; Cao et al., 2010). Although not measured in the present study, *in vitro* evidence of metabolic breakdown as well as measured tissue levels of OH-PBDEs (particularly metabolites of PBDE-47) have been demonstrated in beluga whales from the St Lawrence Estuary and the Canadian Arctic (McKinney et al., 2006a,b). Furthermore, PBDEs, HCB and PCBs can induce uridin diphosphate glucoronyltransferase (UDPGT), increasing biliary excretion of T4 and thus lower circulating TH levels (Vanraaij et al., 1993b; Hallgren et al., 2001; Zhou et al., 2002; McKinney et al., 2004). These above-mentioned mechanisms might explain the negative influence of PBDEs and other OHCs, on TH levels in the present study. It is also probable that the most important contaminants influencing TH levels in these beluga whales (e.g. PBDEs, PCBs, and HCB) work in combination with each other and other OHCs with the capacity to act through similar mechanisms or produce similar responses. Recent *in vivo* studies have shown that OHC mixtures at environmentally relevant doses can result in combined TH disruptive effects (Hallgren et al., 2001; Hallgren and Darnerud, 2002; Wade et al., 2002; Crofton et al., 2005; Gauger et al., 2007), implying that combination effects of OHCs on TH levels are probable in arctic top-predators, such as the beluga whale.

Some OHCs affected TT4, FT4 and FT3 levels in a positive manner in the white whales, particularly the brominated flame retardant HBCD, as well as α-HCH, CHB-40 and -62 (Fig. 3a and b). It is not unusual to find both positive and negative relationships between OHCs and TH in the same animals (Skaare et al., 2001; Hall et al., 2003; Braathen et al., 2004; Hall and Thomas, 2007; Routti et al., 2010). However, none of the positive relationships suggested by the PLS model in the present study were affirmed in bivariate correlation tests, perhaps indicating weaker relationships than for the negative ones. There is little knowledge about the endocrine disruptive effects of HBCD and CHBs, particularly in wildlife (Calciu et al., 1997; Darnerud, 2003). Some experimental studies have demonstrated that HBCD and CHBs affect the thyroid system negatively (P. Eriksson et al., 2006; van der Ven et al., 2006; Legler, 2008; Lilienthal et al., 2009). However, *in vitro* studies indicate that HBCD may act as a T3 agonist (Hamers et al., 2006; Schriks et al., 2006), which theoretically might support the positive relationship of HBCD with THs in the current study (Fig. 3a and b).

The negative relationships between OHCs and TH levels found in the present study are similar to findings in studies on polar bears from Svalbard (Braathen et al., 2004; Skaare et al., 2001) and from East Greenland (Villanger et al., 2011). These studies concluded that the high OHC loads might be affecting TH balance in polar bears. Except for PCBs, OHCs are higher in white whales than in polar bears in these two regions (Andersen et al., 2001, 2006; Wolkers et al., 2004, 2006; Verreault et al., 2005; Sørmo et al., 2006; Letcher et al., 2010). Sandau (2000) suggested that the lower levels of THs in polar bears from Svalbard compared to Resolute Bay (Canada) might be a consequence of higher levels of PCBs and OH-PCBs (in plasma) in Svalbard compared to Resolute Bay polar bears. Similarly, the levels of OHCs seem somewhat higher in Svalbard white whales compared to their Canadian Arctic counterparts, whereas TH levels seem somewhat lower. Nevertheless, observed differences in TH levels might be due to higher TH summer peaks in some Canadian stocks due to moulting (St Aubin and Geraci, 1989; St Aubin, 1990). These TH summer peaks are probably not found in white whales from Svalbard. When comparing the range of TT4 levels in the current study (79.1–150 nmol/l) with spring and autumn TT4 levels in Canadian arctic beluga whales (TT4: 57–155 nmol/l; St Aubin and Geraci, 1989), the levels in both areas are quite similar. Thus, it is not possible to conclude if the TH levels in white whales from Svalbard are lower than in the Canadian Arctic because of different OHC exposures. Still, the negative relationships between TH levels and single OHCs demonstrated through statistical modelling in the present study suggest that TH homeostasis in white whales might be influenced by OHCs. Even though statistical associations do not represent the full biological cause–effect relationships per se, these modelled responses might be linked to the thyroid disrupting potency of individual compounds, their ability to act in combination with other compounds, and their relative contribution in the complex mixture of contaminants accumulated in white whales. Since THs may have an especially important role in white whales' opportunistic life-strategy and adaptability, disturbance of the TH system could reduce their capability to adjust to environmental changes, including global warming.

5. Conclusion

The current study confirms that OHC levels in white whale blubber are among the highest levels recorded in wildlife from Svalbard, exceeding even the high levels recorded for polar bears for most OHC groups. Multivariate PLS regression revealed that known or suspected thyroid disruptive contaminants (PBDE-28, -47, -99, -100, and -154, HCB, and PCB-105) were negatively associated with circulating TT4, FT4 and FT3 levels. Most of these negative relationships were reconfirmed using partial correlations controlling for length (and thus age) of the white whales. PLS regression also found positive associations between HBCD, α-HCH, CHB-40 and -62 and TT4, FT4, and FT3 levels, though none of these relationships were supported by partial correlations. Although statistical models do not fully represent the biological cause–effect relationships, the negative relationships between PBDEs, HCB and PCB-105 and TH levels in beluga whales causes concern for possible OHC-induced reductions of TH levels in this population.

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Errata Paper II

Page 7, Table 3: All values (median, min., max, mean and SD) of TT3:FT3 and TT4:FT4 should have footnote [a] and is should be written below Table 3: [a] x 10^3

Page 7: “The permutation analyses also confirmed the acceptable validity of the PLS model; FT4 intercepts: R^2X=(0.0, 0.339), Q^2=(0.0,−0.208); FT3 intercepts: R^2X=(0.0, 0.297), Q^2=(0.0, −0.221); TT4 intercepts: R^2X=(0.0,0.355), Q^2=(0.0, −0.205)” should be changed to (changes indicated by **bold type**) “The permutation analyses also confirmed the acceptable validity of the PLS model; FT4 intercepts: $\mathbf{R^2Y}$=(0.0, 0.339), Q^2=(0.0,−0.208); FT3 intercepts: $\mathbf{R^2Y}$=(0.0, 0.297), Q^2=(0.0, −0.221); TT4 intercepts: $\mathbf{R^2Y}$=(0.0,0.355), Q^2=(0.0, −0.205).”

PAPER III

Levels and patterns of hydroxylated polychlorinated biphenyls (OH-PCBs) and their associations with thyroid hormones in hooded seal (*Cystophora cristata*) mother-pup pairs

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ABSTRACT

Blood (plasma/serum) samples from 14 adult female and their pups (1-4 days old) captured in the West Ice, east of Greenland were analysed for concentrations of total and free thyroxine and triiodothyronine (TT4, FT4, TT3, FT3), and hydroxylated polychlorinated biphenyls (OH-PCBs). The levels of all thyroid hormones (THs) were significantly higher in pups than in mothers. Sum OH-PCB levels (ΣOH-PCBs: 4-OH-CB107, 3'-OH-CB138, 4-OH-CB146, 4'-OH-CB172, 4-OH-CB187) were higher in mothers (1.40 ± 0.54 ng/g wet weight) as compared to pups (0.68 ng/g ± 0.28 ng/g wet weight). Plasma levels of TT4 and FT4 in mothers increased as function of pup age, as did levels of individual OH-PCBs in both mothers and pups. The patterns of OH-PCBs in the pups were similar to their mothers. We suggest that OH-PCBs found in pups are transferred from their mothers during gestation and that the transfer also continues after parturition via milk. Principal component analysis (PCA) showed that in pups, 4-OH-CB107 and 3'-OH-CB138 were negatively associated with FT4:FT3 and TT3:FT3 ratios, respectively. These relationships were confirmed by partial correlation analysis correcting for pup age. PCA suggested that 4'-OH-CB172 and 4-OH-CB187 were negatively associated with TT3 in mothers. However, this was not confirmed by correlation tests. The study indicates that young developing seals are more sensitive compared to adults with respect to TH-related effects of OH-PCBs.

KEY WORDS: ARCTIC; HYDROXYLATED PCBs; METABOLITES; SEALS; THYROID HORMONES.

1. Introduction

The industrial chemicals polychlorinated biphenyls (PCBs) are ubiquitous in the arctic environment, and their persistent and lipophilic properties cause them to bioaccumulate in organisms and biomagnify through food webs. The production and use of PCBs have been globally banned since 2004 (Stockholm Convention, 2009). However, due to historical large-scale global production, the persistence of the chemicals, continuing leakages from products containing them and from dumping sites, and long-range transport from lower latitudes, PCBs are still found in arctic biota and levels may still reach high concentrations in arctic top predators, such as seals (Breivik et al., 2002; Letcher et al., 2010).

The toxic potential of PCBs is well known, though not completely understood at a mechanistic level. PCBs have been associated with reproduction impairment, pathological changes and endocrine disruption in seals inhabiting highly polluted waters (Helle et al., 1976; Reijnders, 1986; Routti et al., 2010; Sørmo et al., 2005). The thyroid hormone (TH) system is one of the endocrine systems that can be affected by PCBs (Colborn et al., 1993). Associations between levels of PCBs and reduced or altered circulating TH levels have been reported from human, laboratory and wildlife studies (Braathen et al., 2004; Hallgren et al., 2001; Koopman-Essebom et al., 1994;), including seals (Brouwer et al., 1989; Hall and Thomas, 2007; Sørmo et al., 2005; Tabuchi et al., 2006). In mammals, THs are involved in several important physiological functions, such as metabolism, growth, reproduction and normal brain maturation (McNabb, 1992; Zoeller et al., 2007). Contaminant induced alterations of levels or actions of THs can therefore have consequences for a wide array of physiological and developmental functions. Special concern has been raised regarding the potential effects from hydroxylated PCBs (OH-PCBs) on the TH system, due to their structural similarities to THs. The OH-PCBs are formed in the phase I biotransformation

process of PCBs, which is mediated by cytochrome P450 (CYP) monooxygenase isozymes, in order for organisms to produce more hydrophobic excretable metabolites (Letcher et al., 2000). The majority of the OH-PCBs formed in organisms are excreted unaltered or as conjugated entities formed as part of the phase II biotransformation processes. However, a few of these metabolites, usually those containing an OH-group in the *para*- or *meta*-position, are retained in the blood bound to proteins (Bergman et al., 1994; Letcher et al., 2000). Thus, they have the potential to exert toxic effects.

The hooded seal (*Cystophora cristata*) is an arctic seal that is characterised by having a very high prenatal energy investment in their pups compared with other pinnipeds. The pups have a thin subcutaneous layer of fat and weigh approximately 25 kg at birth (Kovacs and Lavigne, 1992; Lydersen et al., 1997). Their uniquely short nursing period lasts only for four days (Bowen et al., 1985), during which the pups consume approximately 10 L of extremely lipid rich milk (> 60 percent fat) daily (Lydersen and Kovacs, 1999; Lydersen et al., 1997). The nursing, fasting females mobilise lipids and concomitantly lipid-soluble contaminants, such as PCBs, from their blubber which is transferred to the blood and eventually to the milk (Addison and Brodie, 1987; Lydersen et al., 2002; Wolkers et al., 2006). Thus, this reproductive strategy favours placental and milk transfer of contaminants, such as PCBs, to the pre- and postnatal pups. Indeed, previous studies of hooded seals have reported high levels of PCBs and other persistent organic pollutants (POPs) in adults, pups and milk (Espeland et al., 1997; Hobbs et al., 2002; Wolkers et al., 2006), demonstrating the high lactational transfer of PCBs and other contaminants in hooded seals. Hepatic CYP enzyme activity (i.e. CYP1A and CYP3A) has also been reported in both hooded seal adults and pups (Wolkers et al., 2009). Thus, they have the ability to form OH-PCBs, which is consistent with the notion that OH-PCBs detected in adult seals are primarily a result of endogenous biotransformation of

dietary PCBs (Boon et al., 1997; Routti et al., 2008). In human and rodent laboratory studies, OH-PCBs are reported to be transferred directly to offspring via placental and lactational transfer (Guvenius et al., 2003; Meerts et al., 2002). However, the maternal transfer routes for OH-PCBs in seals are less well known.

In mammals, thyroid homeostasis is regulated by a negative feedback system involving the hypothalamus-pituitary-thyroid (HPT) axis (McNabb, 1992). There are several potential mechanisms by which OH-PCBs can disrupt the HTP axis, e.g. by interfering with the thyroid gland and its function, by transporting proteins in plasma, via metabolism and excretion of THs or through interaction with the TH receptor (TR) (Boas et al., 2006; Brouwer et al., 1998; Cheek et al., 1999; Gauger et al., 2007; Rickenbacher et al., 1989; Schuur et al., 1998, 1999). The majority of THs in mammals are transported in blood as 3,5,3',5'-tetraiodothyronine (thyroxine, T4) bound to the plasma proteins thyroxine-binding-globulin (TBG), transthyretin (TTR) and albumin. Only a small fraction of THs exist as free molecules. T4 is deiodinated to the more biologically active 3,3',5-triiodothyronine (T3) in peripheral tissues by deiodination enzymes (IDs) (McNabb, 1992). Several OH-PCBs have a high *in vitro* binding affinity for the transport protein TTR, some more than 10 times higher than the natural hormone T4 itself (Lans et al., 1993). The binding of OH-PCBs to TTR is believed to cause a displacement of T4, eventually leading to reduced levels of circulating T4 through metabolism and excretion (Brouwer and van den Berg, 1986). Placental TTR is also believed to be involved in the transport of maternal THs to the foetal circulation (Landers et al., 2009). This occurs both before and after the thyroid gland in the foetus starts to synthesise THs on its own (de Escobar et al., 2004), which in seals is thought to start 2-3 months before term (Harrison et al., 1962; Little, 1991). Thus, TTR represents a potential transport route for OH-PCBs to the foetus and could also mechanistically explain a linkage between contaminant-induced maternal and

foetal hypothyroidism (Brouwer et al., 1998). Other important function for THs are thermoregulation and the functioning of adipose tissue (Bernal and Refetoff, 1977; Silva, 2006). Thus, arctic marine mammals, which are dependent on blubber for insulation and an energy reserve during periods of fasting and milk production, could be especially vulnerable to a disruption of the TH system (Jenssen, 2006).

The hooded seal became Red Listed by the International Union for Conservation of Nature (IUCN) in 2008 due to the severe reduction of the West Ice population over the last 40-60 years. Commercial harvesting in combination with climate change are thought to be the major reasons for the decline (IUCN, 2011; Kovacs and Lydersen, 2008). Commercial hunting was stopped in 2007, but the population is continuing to decline and shows reduced pup production rates (Haug and Øigård, 2009; ICES, 2006). Concern has been raised regarding the impact from exposure to environmental contaminants. Levels of OH-PCBs and their potential effects on TH levels have not been investigated in hooded seals previously. There is reason to believe that their reproductive strategy may expose them to high levels of contaminants such as OH-PCBs during critical stages of their development. The potential effects from OH-PCBs and other contaminants on the TH system might result in population level effects, due to the variety of important physiological processes involving THs. Therefore, the aim of the present study was to investigate the relationships between concentrations of OH-PCBs and TH levels in mother-pup pairs of hooded seals from the West Ice.

2. Materials and methods

2.1 Sampling

Hooded seal mother-pup pairs (N = 14) were live-captured in the West Ice (approximately 73.30 °N, 14.50 °W) during March 2008. The sex of pups were noted and body masses (BM), measured to the nearest 1 kg, of both mothers and pups were determined. Pup ages were estimated based on their developmental state (Kovacs and Lavigne, 1992). Blood samples (50 mL) were collected from both mothers and pups from the intravertebral extradural vein. The blood samples were centrifuged and plasma and serum were transferred to cryogenic vials and stored frozen at – 20 °C during field work and then transferred to – 70 °C until analyses. All animal handling was performed in accordance with, and under permit by, the Norwegian National Animal Research Authority.

2.2. Contaminant analyses

The contaminant analyses were carried out at the Laboratory of Environmental Toxicology at the Norwegian School of Veterinary Sciences (NVH), Oslo, Norway. Plasma and serum samples from each individual were merged in order to increase the sample volumes and are henceforth referred to as plasma. The multicomponent method was used for OH-PCB extraction, clean-up and analysis, following the method first described by Brevik (1978) with modifications as described by Løken et al. (2006) and Berg et al. (2010). Briefly, internal standards (4'-OH-[$^{13}C_{12}$]-CB159 and 4-OH-[$^{13}C_{12}$]-CB-187), 2 mL 2 % sodium chloride (NaCl) and 10 mL 1 M sulphuric acid (H_2SO_4) were added to the samples (approximately 4 g) before extraction with acetone and cyclohexane (2:3) were performed. The lipid content (%) was determined gravimetrically. The removal of lipids and proteins was accomplished by treatment with concentrated H_2SO_4 (96 %). The samples were then separated into a neutral

and a phenolic fraction by extraction with 1 M potassium chloride (KOH) in 96 % ethanol: grade 1 water (1:1). The phenolic fraction was treated with drops of concentrated H_2SO_4 to a pH of 1-2 and then extracted to an organic phase using cyclohexane (3 x 5 mL). The OH-groups were then replaced with acetyl groups by derivatisation with acetic acid anhydride:pyridine (1:1).

The samples were analysed using a high resolution gas chromatograph (GC; Agilent 6890 Series, Agilent Technologies, Santa Clara, CA, USA) equipped with an autosampler (Agilent 7683 Series, Agilent Technologies) operated in pulsed splitless mode. The separation was performed on a DB-5MS 60 meter capillary column (0.25 mm inner diameter), 0.25 µm film thickness, J&W Scientific, CA, USA). The temperature program for OH-PCBs was 90 °C (2 min), 35 °C/min to 230 °C (3 min), 2 °C/min to 260 °C (5 min), 20 °C/min to 310 °C (4 min). Total run time was 34.5 min. The GC system was connected to a quadrupole mass spectrometer (Agilent 5973 Series, Agilent Technologies), and the OH-PCBs were detected using selective ion monitoring with negative chemical ionisation. Chromatographic data were treated using MSD Chemstation (E.02.00.493, Agilent Technologies). The quantities of OH-PCBs were derived from the peak area using linear calibration curves with minimum five calibration points created from analysed certified standard solutions. A total of 11 OH-PCBs were analysed:

4'-OH-CB106 (4-hydroxy-2',3,3',4',5'-pentachlorobiphenyl),

4-OH-CB107 (4-hydroxy-2,3,3',4',5–pentachlorobiphenyl),

4'-OH-CB108 (4-hydroxy-2',3,3',4',5–pentachlorobiphenyl),

3-OH-CB118 (3-hydroxy-2,3',4, 4',5–pentachlorobiphenyl),

4'-OH-CB130 (4-hydroxy-2,2',3,3',4',5–hexachlorobiphenyl),

3'-OH-CB138 (3-hydroxy-2,2',3',4,4',5–hexachlorobiphenyl),

4-OH-CB146 (4-hydroxy-2,2',3,4',5,5'-hexachlorobiphenyl),

4'-OH-CB159 (4-hydroxy-2',3,3',4',5,5'-hexachlorobiphenyl),

4'-OH-CB172 (4-hydroxy-2,2',3,3',4',5,5'-heptachlorobiphenyl),

3'-OH-CB180 (3-hydroxy-2,2',3',4,4',5,5'-heptachlorobiphenyl),

4-OH-CB187 (4-hydroxy-2,2',3,4',5,5',6-heptachlorobiphenyl).

The following target ions were used: 3-OH-CB118: m/z 310.0; 4-OH-CB107, 4'-OH-CB108, 4'-OH-CB106: m/z 384.0; 4-OH-CB146, 4'-OH-159: m/z 418.0; 3'-OH-CB138: m/z 346; 4'-OH-CB130: m/z 418.0; 3'-OH-CB180, 4'-OH-CB172, 4-OH-CB187: m/z 452.0; 4'-OH-^{13}C-CB159: m/z 430.0; 4-OH-^{13}C-CB187: m/z 464.0. All OH-PCB standards were from Wellington Laboratories Inc., Ontario, Canada. The plasma concentrations of OH-PCBs are given in ng/g wet weight (ww).

Quality assurance

The Laboratory of Environmental Toxicology at NVH is accredited for determination of POPs in biological matrices of animal origin according to the requirements of NS-EN ISO/IEC 17025, TEST 137. The determination of OH-PCBs is not an accredited method, but is validated by the same criteria. Standard procedures were used to ensure adequate quality assurance and control, and the accuracy, linearity, and sensitivity of the analyses were within the laboratory's accreditation requirements. Limit of detection (LOD) was defined as three times the average background noise in the chromatograms of the sample extracts and ranged from 0.004-0.020 ng/g ww for OH-PCBs. For each sample series a relative recovery was calculated from two samples of low-contaminated material (sheep blood) spiked with a standard containing all the analytes. The relative recovery rates were between 86-105 %. An analysed sample of the laboratory's internal reference material (seal blood) proved satisfactory reproducibility. The blanks (solvents) showed no OH-PCB contamination.

2.3 Thyroid hormone analysis

The analysis of total T4 (TT4), free T4 (FT4), total T3 (TT3) and free T3 (FT3) in the hooded seal plasma was conducted at the Department of Biology, Norwegian University of Science and Technology, Trondheim, Norway. The analysis was performed using commercially available solid-phase radioimmunoassay (RIA) kits (Coat-A-Count TT4, Coat-A-Count FT4, Coat-A-Count TT3, Coat-A-Count FT3, Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA). The procedures provided by the producer of the kits were followed (Siemens, 2006a, b, c, d). The bound radioactive antigen was quantified using a gamma counter (Cobra Auto-Gamma, Packard Instruments Company, Dowers Grove, IL, USA). The software for the gamma counter (Spectra Works Spectrum Analysis Software, Meriden, USA) generated calibration curves from calibrators analysed in duplicates and calculated the TH concentrations in the samples. The samples were analysed in duplicates (TT3 and FT3) or triplicates (TT4 and FT4). The means were used for statistical treatment of the results. The samples were analysed in two analytical runs, except for the FT3 analysis which involved only one analytical run.

Quality assurance

The reactivity of commercial RIA kits for seal THs has been reported previously (Ortiz et al., 2001). The instrumental detection limits were as follows: TT4: 0.92-0.97 nmol/L; FT4: 0.02-0.03 pmol/L; TT3: 0.01-0.05 nmol/L; FT3: 0.02 pmol/L. A standard reference material (Lyphochek® Immunoassay Plus Control, Levels 1, 2 and 3, BIO-RAD, CA, USA) and bovine plasma were used as quality controls. The limit for accepted variation for the control parameters was ≤ 15 %, and all intra- and inter-assay coefficients of variation (CV) were within this limit. For the samples, the CV range for FT4 was 0.1-11.6 %. For TT4 and TT3, 27 out of 28 samples had ranges of CV from 0.5-11.4 % and 0.6-14.8 %, respectively. One

sample from the TT4 analysis had a CV of 20.9 %, and one sample from the TT3 analysis had a CV of 37.5 %. These were also accepted. For FT3, 25 out of 28 samples had a CV range from 0.1-26.1 %. Three samples had high CVs due to low hormone concentrations in the samples which made the quantifications during RIA more prone to variation. The CV values were 31.6 %, 42.2 % and 99.9 %. However, these were also accepted due to the high sensitivity of the RIA method and the approved intra-assay control parameters.

2.4 Statistical analysis

The following OH-PCBs were detected in less than 60 % of the samples from mothers and pups and were therefore excluded from the statistical analyses: 3-OH-CB188, 4'-OH-CB106, 4'-OH-CB108, 4'-OH-CB130, 4'-OH-CB159, and 3'-OH-CB180. For the OH-PCBs that were included in the statistical analysis, values below the LOD were replaced with a random value between the LOD and zero. This was the case for 4-OH-CB146 (1 pup), 3'-OH-CB138 (3 mothers, 5 pups), 4'-OH-172 (2 pups) and 4-OH-CB187 (1 pup).

2.4.1 Univariate analysis

Univariate data analyses were performed using SPSS Statistics version 17.0 (SPSS Inc., Chicago, USA, 2008). All values are given as mean ± one standard deviation unless otherwise noted. A Shapiro Wilk test of normality revealed that the values were not normally distributed. Log transformation of the data did not result in normal distribution, so only non-parametric tests were applied. There were no significant differences between female (N = 8) and male (N = 6) pups with respect to TH levels or OH-PCBs levels (Mann-Whitney U test, $p > 0.14$) and thus, all pups are treated as a single sample group in the statistical analysis. Differences in the biometric, hormone and contaminant data between mothers and pups were

examined using Wilcoxon signed ranks test (2-tailed). Bivariate Spearman's rank correlations (r_s) or partial correlations (r_p) were performed. The level of significance was set at $p \leq 0.05$.

2.4.2 Multivariate data analysis

Principal component analysis (PCA) was performed to explore the relationships among the variables, using the software Simca-P+ version 12 (Umetrics AB, Umeå, Sweden, 2009). The PCA included the variables BM, lipid content of the blood (lipid %), estimated pup age, circulating levels of 4-OH-CB107, 3'-OH-CB138, 4-OH-CB146, 4'-OH-CB172, 4-OH-CB187, TT4, TT3, FT4, FT3, and the ratios TT4:FT4, TT3:FT3, TT4:TT3, FT4:FT3. The data were scaled to variance 1 and centred before analysis, and skewed variables were log transformed.

PCAs were performed separately for mothers and pups. Loading plots were used to interpret the data. A loading value close to zero indicates that the variable has little influence on the variation in the X variables (Wold et al., 1987). Loadings close to each other, i.e. they both have positive or negative value, are positively correlated, while loadings with opposite signs and separated diagonally across the plot are negatively correlated (Eriksson et al., 1992; Wold et al., 1987). The number of significant components was determioned by cross validation. Explained variation, R^2X, should be as close to 1 as possible and at least > 0.5 (Umetrics, 2008).

3. Results

BM of the mothers was 151 ± 28 kg (range 92-187 kg), while pups weighed 33 ± 8 kg (range 23-50 kg). Estimated pup age was 2.8 ± 0.8 (range 1.5-4.0 days); the BM of the pups was positively correlated with pup age ($r_s = 0.877$, $p < 0.001$). The lipid % in the blood was significantly higher in the pups (1.5 ± 0.68 %) compared to mothers (0.70 ± 0.15 %) (Wilcoxon signed ranks test, $p < 0.001$).

3.1 Thyroid hormones

The plasma concentrations of TT4, TT3, FT4, and FT3 were all significantly higher in pups compared to mothers (Fig. 1 and 2; Wilcoxon signed ranks test, TT4: $p < 0.001$; TT3: $p < 0.001$; FT4: $p < 0.001$; FT3: $p < 0.001$). In mothers, concentrations of TT4 and FT4 were positively correlated with pup age (TT4: $r_s = 0.615$, $p = 0.019$; FT4: $r_s = 0.548$, $p = 0.042$), while concentrations of TT3 and FT3 and TH ratios were not ($|r_s| < 0.480$, $p > 0.083$). Concentrations and ratios of THs in pups were not significantly correlated with age ($|r_s| < 0.489$, $p > 0.076$) or BM ($|r_s| < 0.513$, $p > 0.061$). TH concentrations and ratios in mothers were not significantly correlated with BM ($|r_s| < 0.394$, $p > 0.163$).

3.2 OH-PCBs

The concentrations of 4-OH-CB107, 4-OH-CB146, 4'-OH-CB172, and 4-OH-CB187 were higher in mothers than in pups (Fig. 3; Wilcoxon signed ranks test, 4-OH-CB107: $p < 0.001$; 4-OH-CB146: $p < 0.001$; 4'-OH-CB172: $p < 0.001$; 4-OH-CB187: $p < 0.001$). There was no significant difference between mothers and pups in the concentrations of 3'-OH-CB138 (Fig. 3; Wilcoxon signed ranks test, 3'-OH-CB138: $p = 1.000$). The concentrations of ΣOH-PCBs were approximately 2 times higher in mothers (1.4 ± 0.54 ng/g ww) than in pups (0.68 ± 0.28 ng/g ww).

The concentration of sum (Σ) OH-PCBs in pups was positively correlated with pup age (ΣOH-PCBs: $r_s = 0.562$, $p = 0.036$) and BM (Fig. 4; $r_s = 0.658$, $p = 0.011$). The concentration of ΣOH-PCBs in mothers was not correlated to their BM ($r_s = 0.359$, $p = 0.207$) or pup age ($r_s = 0.162$, $p = 0.580$). With respect to individual OH-PCBs, pup age was positively correlated with levels of 4-OH-CB146 in pups ($r_s = 0.656$, $p = 0.011$), and in both pups and mothers with 3'-OH-CB138 ($r_s = 0.743$, $p = 0.002$; $r_s = 0.610$, $p = 0.021$) and 4-OH-CB187 ($r_s = 0.560$, $p = 0.037$; $r_s = 0.713$, $p = 0.004$). In pups, all individual OH-PCBs were positively correlated with BM ($r_s \geq 0.616$, $p \leq 0.019$). In mothers, levels of 3'-OH-CB138 and 4'-OH-CB187 were positively correlated with BM ($r_s \geq 0.548$, $p \leq 0.042$).

The contribution of various OH-PCBs to the ΣOH-PCBs was similar in mothers and pups. The single predominat metabolite in both groups was 4-OH-CB107, which accounted for 83.7 % and 84.7 % of the ΣOH-PCBs in mothers and pups, respectively. It was followed by 4-OH-CB187, which made up 10.6 % and 8.7 % of the ΣOH-PCBs in mothers and pups, respectively. The remaining three detected metabolites were only minor contributors to the ΣOH-PCBs and combined they accounted for 5.7 % in mothers and 6.6 % in pups.

3.3 Associations between OH-PCBs and Thyroid Hormones

PCA for mothers resulted in five principal components (PCs) ($R^2X = 0.909$). The two dominatn PCs explained 34.4 % and 24.3 % of the variation in the dataset, respectively. The loading plot (Fig. 5) indicated negative associations between 4'-OH-172 and 4-OH-CB187, and TT3. Furthermore, 4-OH-CB107, 3'-OH-CB138 and 4-OH-CB146 seemed negatively associated with TT3:FT3. Moreover, 3'-OH-CB138 seemed to be positively associated with TT4. However, none of these relationships between the OH-PCBs and THs were significant when tested further with bivariate correlation analysis ($|r_s| < 0.371$, $p > 0.191$).

PCA for the pups resulted in two PCs where PC1 explained 37.5 % and PC2 explained 34.2 % of the variation in the dataset ($R^2X = 0.717$). The loading plot in (Fig. 6) showed a different pattern of relationships between OH-PCBs and TH variables in pups as compared to their mothers (Fig. 5). All the OH-PCBs were grouped together (Fig. 6) and all of the THs and TT4:TT3 seemed to be positively inter-correlated. The negative correlation between 4-OH-CB107 and FT4:FT3 indicated in the loading plot was confirmed by a bivariate correlation analysis (Fig. 7A, $r_s = -0.587$, $p = 0.027$), but no significant correlations were found between FT4:FT3 and the remaining OH-PCBs ($|r_s| < 0.481$, $p > 0.081$). The indicated negative association between the 3'-OH-CB138 and TT3:FT3 was confirmed by bivariate correlation analysis (Fig. 7B, $r_s = -0.609$, $p = 0.021$). However, the other OH-PCBs were not associated with TT3:FT3 ($|r_s| < 0.516$, $p > 0.059$).

Because the variables BM and pup age also influenced OH-PCB levels in this study, we performed partial correlation test (r_p) controlling for BM and pup age on the two significant relationships in Figures 7A and B. The analysis confirmed the significant correlations between 4-OH-CB107 and the FT4:FT3 ($r_p = 0.563$, $p = 0.028$, $df = 10$) and between 3'-OH-CB138 and the TT3:FT3 ($r_p = - 0.650$, $p = 0.011$, $df = 10$) in pups, supporting the relationships in Figures 7A and B.

4. Discussion

4.1 Levels of Thyroid Hormones

Levels of TT4 and TT3 in hooded seal pups in the present study were similar to levels reported for two 1 day old hooded seal pups (Stokkan et al., 1995), and within the range reported in other phocid seal neonates (Haulena et al., 1998; Leatherland and Ronald, 1979; Little, 1991; Woldstad and Jenssen, 1999). The TT4 levels in hooded seal pups were somewhat lower than levels reported for 1-2 day old grey seal (*Halichoerus grypus*) pups (Woldstad and Jenssen, 1999). TH levels in adult female hooded seals in this study were similar to TH levels reported in the first days of the lactating period in adult female harbour seals (*Phoca vitulina*) (Haulena et al., 1998). High plasma TH levels in newborn seal pups compared to older pups and adults has also been reported previously (Stokkan et al., 1995; Woldstad and Jenssen, 1999), and are in accordance with the general hyperthyroid physiology in neonate mammals (Cabello and Wrutniak, 1990; Erenberg et al., 1974; Parker et al., 1980; Wrutniak and Cabello, 1987). Hormone levels in seal pups usually drop to adult levels within 2-3 weeks after birth (Haulena et al., 1998; Little, 1991; Stokkan et al., 1995; Woldstad and Jenssen, 1999).

4.2 Levels of OH-PCBs

There are only a few studies that report OH-PCB levels in marine mammals. Levels of ΣOH-PCBs in female polar bears from East Greenland (Verreault et al., 2008) were more than 2 orders of magnitude higher than the ΣOH-PCBs measured in this study. This is probably due to the polar bears' higher position in the arctic marine food chain and their resulting higher levels of PCBs due to biomagnification (Derocher et al., 2002; Letcher et al., 2010), combined with their exceptional capacity to biotransform PCBs to OH-PCBs (Letcher et al., 1996). The plasma levels of ΣOH-PCBs in adult female hooded seals and pups in this study

were higher than levels reported in ringed seals (*Phoca hispida*) from Quebec, Canada (Sandau et al., 2000a) and Svalbard, Norway (Routti et al., 2008) but lower than levels reported in ringed seals from the Baltic Sea (Routti et al., 2008). These differences could be explained by a combination of geographical different exposure to parent PCBs, species specific diet preferences and biotransformation abilities as well as differences in physiological and reproductive status of the sampled individuals (Boon et al., 1997; Routti et al., 2008).

The dominating metabolite found in mothers and pups, 4-OH-CB107, was also the dominating metabolite reported in other seal studies (Bergman et al., 1994; Park et al., 2009a; Routti et al., 2008). The high dominance of 4-OH-CB107 across seal species from different geographic locations might reflect that biotransformation of PCBs in phocid seals are catalysed by the same CYP isozymes. In polar bears, 4-OH-CB187 is most commonly reported to be the dominating metabolite (Sandau, 2000; Verreault et al., 2008). Thus, the OH-PCB pattern in plasma of seals is somewhat different form the pattern reported in polar bears. Human populations feeding on traditional diets containing marine mammals are reported to have relatively high levels of OH-PCBs (Fängström et al., 2002; Sandau et al., 2000b). In human populations, similar to seals, 4-OH-CB107, 4-OH-CB187 and 4-OH-CB146 all appear to be major metabolites (Fängström et al., 2002; Park et al., 2008; Sandau, 2000; Sjödin et al., 2000). Thus, while still acknowledging physiological species-differences, the similar pattern of OH-PCBs in seals and humans suggests that seals could be good model organisms for studying TH-associated effects that are of relevance for humans.

4.3 Maternal transfer of OH-PCBs

Foetuses can receive OH-PCBs either directly via transplacental transport or indirectly via endogenous hydroxylation of PCBs transferred via the placenta. Several human as well as

rodent laboratory studies have reported that OH-PCBs are effectively transferred across the placenta (e.g. Guvenius et al., 2003; Meerts et al., 2002; Park et al., 2008). Although high levels of PCBs are reported in pinniped (Bacon et al., 1992; Debier et al., 2003) and human milk (Fängström et al., 2005), analysis of human milk indicates that only low amounts of OH-PCBs are subject to lactational transfer (Fängström et al., 2005; Guvenius et al., 2003). Levels of OH-PCBs in hooded seal pups in the present study constituted about 50 % of the maternal levels. The lower PCB:OH-PCB ratios in umbilical cord plasma compared to maternal plasma, as demonstrated in humans, could indicate a selective transport of OH-PCBs across the placenta relative to PCBs (Guvenius et al., 2003; Park et al., 2008; Soechitram et al., 2004). Part of this may be due to the potential transplacental transport route provided by TTR, for which OH-PCBs have high binding affinty (Lans et al., 1993, 1994). If most of the OH-PCBs in pups are transferred prenatally, seals may be exposed to relatively high levels of OH-PCBs at an even earlier and perhaps more sensitive stage in development than has been the general belief for contaminant exposure in phocid neonates. This is due to the fact that the quanitatively more important exposure route for more lipophilic contaminants, such as PCBs, occurs after birth via the lipid-rich milk, thus, placental transfer of contaminants has been regarded as less imporant (Addison and Brodie, 1987; Nakashima et al., 1997).

In the present study, the positive correlation between the levels of ΣOH-PCBs and pup age and BM (Fig. 4) of the pups indicates that some of these OH-PCBs were transferred after parturition via milk or due to some degree of endogenous biotransformation of PCBs in the pups themselves. Wolkers et al. (2009) detected hepatic CYP activity in neonate hooded seals. However, the levels were very low compared to the levels in adult males and females, especially when considering the high POP loads in the pups (Wolkers et al., 2006, 2009). Taking into consideration the similar OH-PCB pattern in mothers and pups in the present

study, we suggest that the OH-PCBs detected in pups are mainly a result of maternal transfer, but endogenous biotransformation of PCBs cannot be ruled out. Further studies are required to elucidate the quantitative contribution of each of these sources.

4.4 Associations between OH-PCBs and THs

The associations between OH-PCBs and THs in the present study, although limited, were statistically stronger for the hooded seal pups ($p > 0.011$) compared with their mothers ($p > 0.191$), with two negative correlations detected between individual OH-PCBs and TH ratios in pups (Fig. 6, Fig. 7). This might be due to differences in toxicokinetics or toxicodynamics of OH-PCBs in adult females versus pups, or differences in susceptibility to TH disruptive effects of OH-PCBs. Developing individuals are generally more sensitive to toxicants than adults (Grandjean and Landrigan, 2006). However, the lack of statistical significant relationships between plasma levels of OH-PCBs and THs in the adult females could be due to the fact that plasma levels of TT4 and FT4 in adult females increased during the nursing period, as did several OH-PCBs in pups and mothers. This may have confounded statistical analyses between OH-PCB and TH levels and ratios in the present study. Nevertheless, when correcting for the influence of pup age and BM, the negative correlations between 3'-OH-CB138 and TT3:FT3 ratio and between 4-OH-CB107 and FT4:FT3 ratios were still significant. Even though the present study suggests that OH-PCBs have a negative influence on THs in hooded seal pups, we must emphasise that statistical results based on field data cannot explain underlying biological cause-effect relationships, and hence interpretations must be made with caution.

The negative association between 4-OH-CB107 and FT4:FT3 in pups (Fig. 6, Fig. 7A) in the present study is, however, in accordance with rodent laboratory and human studies linking this metabolite to effects on the TH system (Meerts et al., 2002; Park et al., 2009b).

Prenatal exposure to 4-OH-CB107 in rats resulted in reduced TT4 and FT4 levels in foetal plasma as well as reduced levels of T4 in the foetal brain, and has also been associated with possible developmental effects on behaviour and neurodevelopment (Meerts et al., 2002, 2004). Gauger et al. (2007) demonstrated that a PCB mixture including among others the parent PCBs of 4-OH-CB107, namely PCB-105 and PCB-118, and the CYP 1A-inducing PCB-126 resulted in reduced TT4 levels *in vivo* in rat dams. In a human cohort study, 4-OH-CB107 measured in umbilical cord blood at parturition was negatively associated with the mental development in 16 month old children. The authors suggested that one explanation could be decreased TH concentrations in the brain (Park et al., 2009b).

3'-OH-CB138 was the metabolite that was detected in the lowest concentration in both mothers and pups. Still, a significant negative association was found between this metabolite and TT3:FT3 in pups. The metabolite has been detected in humans, seals and polar bears (Fängström et al., 2002; Guvenius et al., 2003; Park et al., 2008; Routti et al., 2008; Sandala et al., 2004; Sandau et al., 2000b). However, due to its low concentration compared to other OH-PCBs, few studies have been performed with respect to thyroid-related effects of this specific metabolite. Mechanistically, the two negative relationships between OH-PCBs and TH ratios in the hooded seals pups can be explained by binding of OH-PCBs to TTR. In fact, all the detected metabolites in hooded seal blood in the present study fulfil the criteria for high affinity towards TTR (Lans et al., 1993). OH-PCBs are reported to be an antagonist for human and rat TTR *in vivo*, and the TTR hypothesis is often used to mechanistically explain

reduced plasma levels of T4 in humans and rats, as well as other species (Brouwer et al., 1998; Brouwer and van den Berg, 1986; Darnerud et al., 1996). Although TTR is believed to exist in virtually all mammalian species (Larsson et al., 1985), its importance regarding TH transport in phocid seals is not known. Disruption of the TH system by OH-PCBs could also be mediated by mechanisms other than TTR binding. OH-PCBs are reported to inhibit ID type 1 activity *in vitro* (Rickenbacher et al., 1989), and inhibit sulfotransferases (SULTs) *in vitro* (Schuur et al., 1998, 1999). Both IDs and SULTs are important in regulating TH levels, being involved in activation and deactivation (IDs) and excretion of THs (SULTs) (Zoeller et al., 2007).

Other wildlife and human studies have also found relationships between PCBs and OH-PCBs and THs levels in neonates and juveniles. Sørmo et al. (2005) found that TT3 and FT3 were negatively correlated with ΣPCB in newly weaned grey seal pups. Effects on the TH system in foetuses and neonates after maternal exposure to PCBs and OH-PCBs have been demonstrated in rodent laboratory studies (Darnerud et al., 1996; Meerts et al., 2002; Sinjari and Darnerud, 1998) and in human studies (e.g. Koopman-Essebom et al., 1994; Otake et al., 2007; Sandau et al., 2002). THs are crucial for normal development of the brain, and hypothyroidism during foetal and neonatal life can lead to neurological defects in the developing brain in mammals (Brouwer et al., 1999; Santisteban and Bernal, 2005; Zoeller et al., 2007). Several studies have linked maternal PCB exposure to neurobehavioral effects in offspring in both human and experimental animals (reviewed in Mariussen and Fonnum, 2006). One suggested explanation for this relationship is that exposure to PCBs and metabolites during sensitive stages of foetal and neonatal development can disrupt TH homeostasis (Brouwer et al., 1998; Zoeller et al., 2002).

In the present study we have only assessed the effects of OH-PCBs on TH status in the hooded seals. It should be noted that other environmental contaminants, including PCBs, brominated flame retardants and organochlorine pesticides, also have been reported to disrupt the TH system and possibly act in combination affecting several mechanisms in the HPT axis (Hall and Thomas, 2007; Meerts et al., 2000). Multi-level interactions as well as the complex TH system with its feedback mechanisms make the study of TH disruption complicated and challenging. Statistically significant negative correlations between 4-OH-CB107 and FT4:FT3 and between 3'-OH-CB138 and TT3:FT3 in hooded seal pups suggest that there is some influence of these compounds on the TH system, while this was not detected in the adult female hooded seals. This indicates that newborn seals might be vulnerable to TH disruption by OH-PCBs to a greater extent than adult females.

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Figure captions

Figure 1: Concentrations (nmol/l) of TT4 and TT3 (A) and concentrations (pmol/l) of FT4 and FT3 (B) in plasma from 14 mother-pup pairs of hooded seal (*Cystophora cristata*) from the West Ice, 2008. The concentrations are displayed as box plots where the bottom represents the 25th percentile, the middle line the 50th percentile (median), and the top the 75th percentile. The whiskers represent the 10th and 90th percentile. The symbol • represents outliers (> 1.5 box length above/below the box). [a] Medians are significant different at $p < 0.001$ level (Wilcoxon signed ranks test (2-tailed)).

Figure 2: Ratios of thyroid hormones ([TT4:FT4], [TT3:FT3], [TT4:TT3], [FT4:FT3]) in plasma from 14 mother-pup pairs of hooded seal (*Cystophora cristata*) from the West Ice, 2008. The values are displayed as box plots where the bottom represents the 25th percentile, the middle line the 50th percentile (median), and the top the 75th percentile. The whiskers represent the 10th and 90th percentile. The symbol • represents outliers (> 1.5 box length above/below the box). [a] Medians are significant different at $p < 0.001$. [b] Medians are significant different at $p < 0.01$ (Wilcoxon signed ranks test (2-tailed)).

Figure 3: Concentrations (ng/g ww) of OH-PCBs in blood from 14 mother-pup pairs of hooded seal (*Cystophora cristata*) from the West Ice, 2008. The concentrations are displayed as box plots where the bottom represents the 25th percentile, the middle line the 50th percentile (median), and the top the 75th percentile. The whiskers represent the 10th and 90th percentile. The symbol • represents outliers (> 1.5 box length above/below the box). [a] Medians are significant different at $p < 0.001$. [b] Medians are significant different at $p < 0.01$ (Wilcoxon signed ranks test (2-tailed)).

Figure 4: The age of the pups (days) in relation to ΣOH-PCB concentrations (ng/g ww) in hooded seal (*Cystophora cristata*) neonates from the West Ice, 2008 (N = 14). r_s = Spearman's rank coefficient. The p-value is two-tailed.

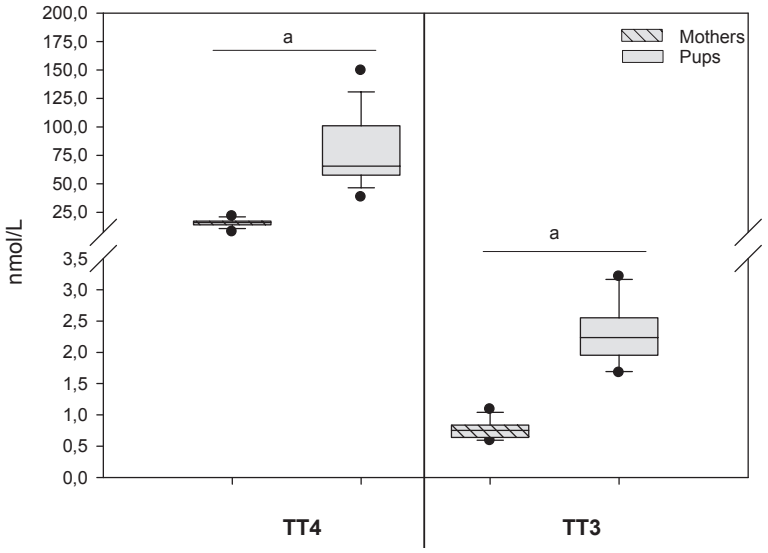
Figure 5: PCA loading plot with PC1 (34.4 %) and PC2 (24.3 %) showing the relationship between the variables in adult female hooded seals (*Cystophora cristata*) from the West Ice, 2008 (N = 14). The biological variables are marked in red, the OH-PCBs in blue, and the THs in green.

Figure 6: PCA loading plot with PC1 (37.5 %) and PC2 (34.2 %) showing the relationship between the variables in hooded seal pups (*Cystophora cristata*) from the West Ice, 2008 (N = 14). The biological variables are marked in red, the OH-PCBs in blue, and the THs in green.

Figure 7: Correlation of 4-OH-CB107 (ng/g ww) with FT4:FT3 ratio (A) and correlation of 3'-OH-CB138 (ng/g ww) with TT3:FT3 ratio (B) in hooded seal pups (*Cystophora cristata*) from the West Ice, 2008 (N = 14). r_s = Spearman's rank correlation coefficient. The p-values are 2-tailed.

Figure 1

A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175,0
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125,0
100,0
75,0
50,0
25,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
nmol/L
a
a
Mothers
Pups
TT4
TT3

B

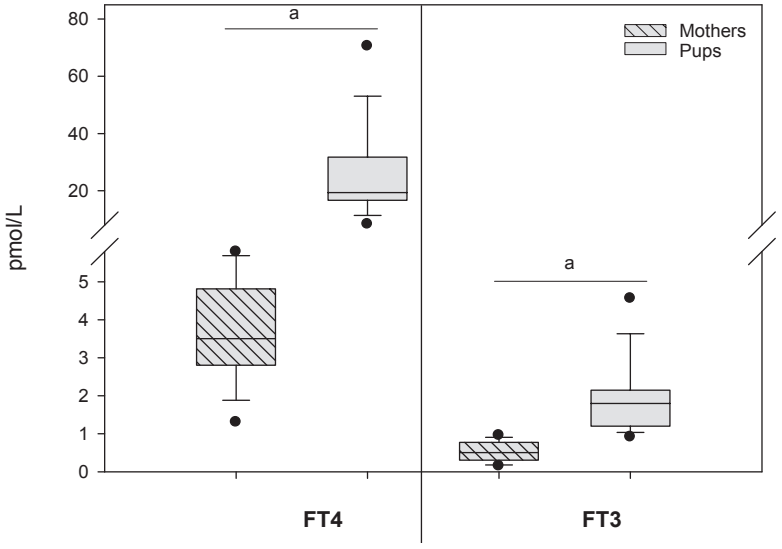

80
60
40
20
5
4
3
2
1
0
pmol/L
a
a
Mothers
Pups
FT4
FT3

Figure 2

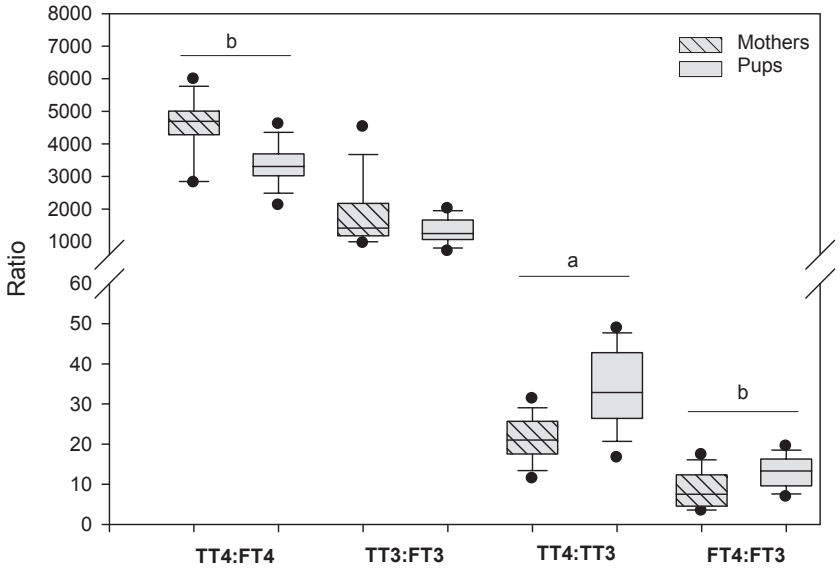

8000
7000
6000
5000
4000
3000
2000
1000
60
50
40
30
20
10
0
Ratio
b
a
b
Mothers
Pups
TT4:FT4
TT3:FT3
TT4:TT3
FT4:FT3

Figure 3

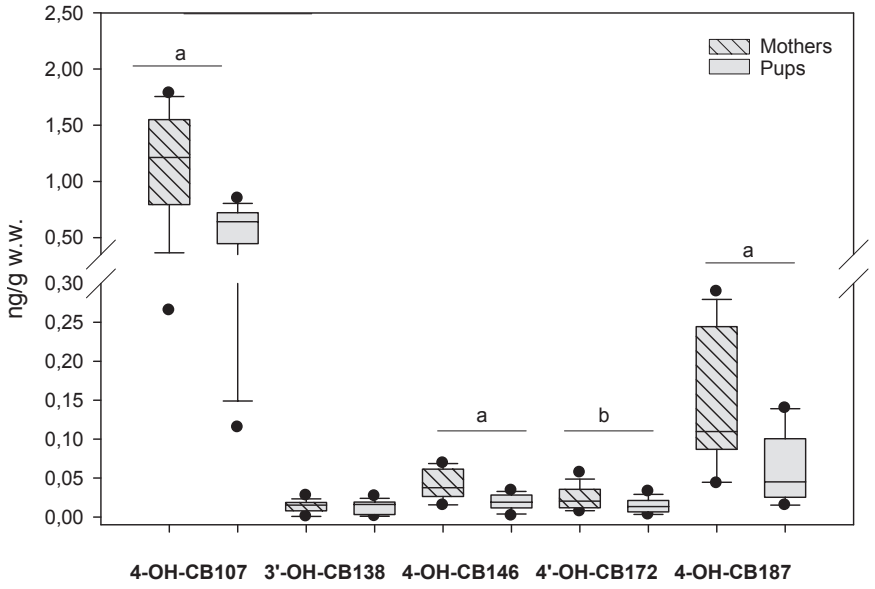

2,50
2,00
1,50
1,00
0,50
0,30
0,25
0,20
0,15
0,10
0,05
0,00
ng/g w.w.
Mothers
Pups
a
a
a
b
4-OH-CB107
3'-OH-CB138
4-OH-CB146
4'-OH-CB172
4-OH-CB187

Figure 4

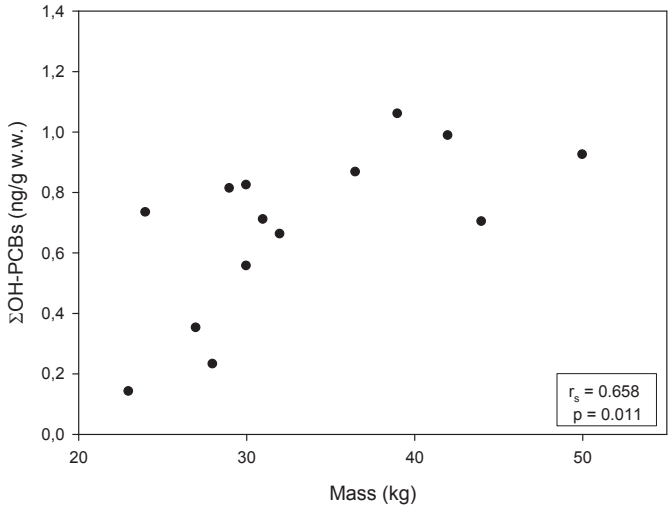


Figure 5

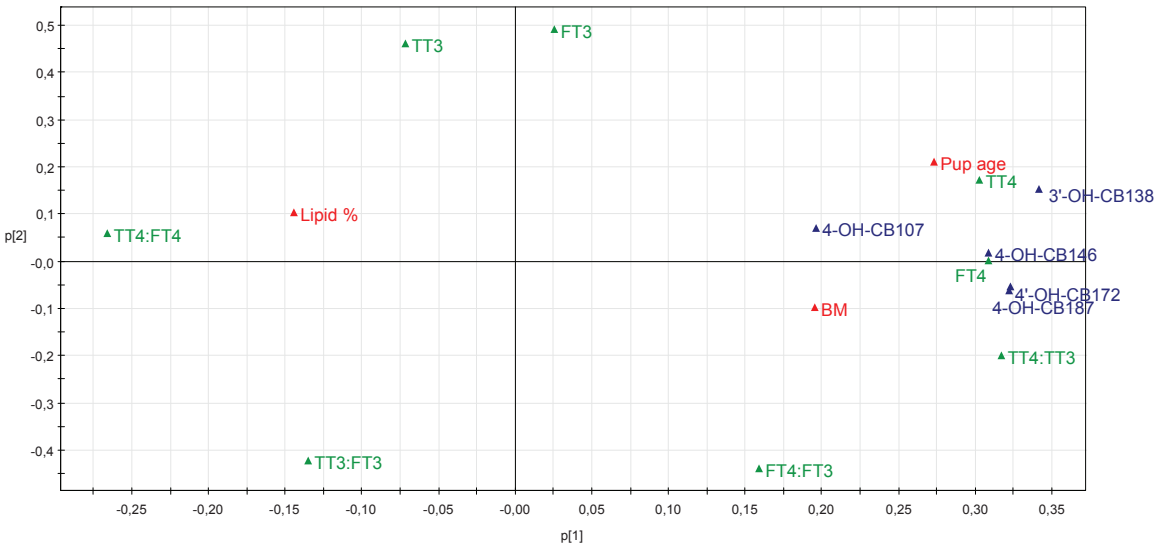
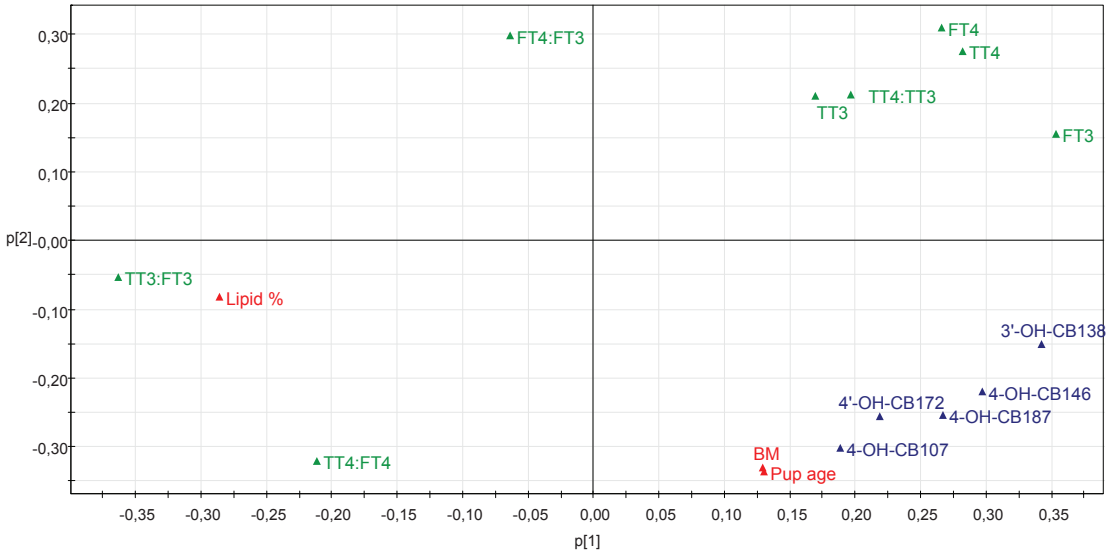


Figure 6


FT4:FT3
FT4
TT4
TT4:TT3
TT3
FT3
TT3:FT3
Lipid %
3'-OH-CB138
4-OH-CB146
4'-OH-CB172
4-OH-CB187
4-OH-CB107
BM
Pup age
TT4:FT4
p[2]
p[1]
0,30
0,20
0,10
-0,00
-0,10
-0,20
-0,30
-0,35
-0,30
-0,25
-0,20
-0,15
-0,10
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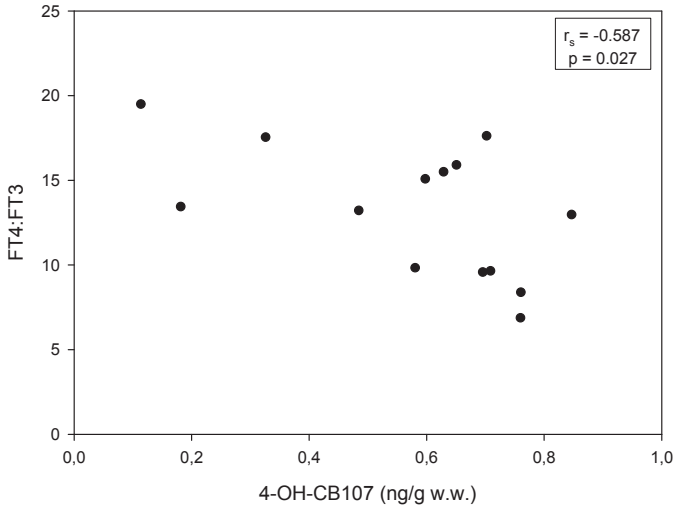
0,00
0,05
0,10
0,15
0,20
0,25
0,30
0,35

Figure 7

A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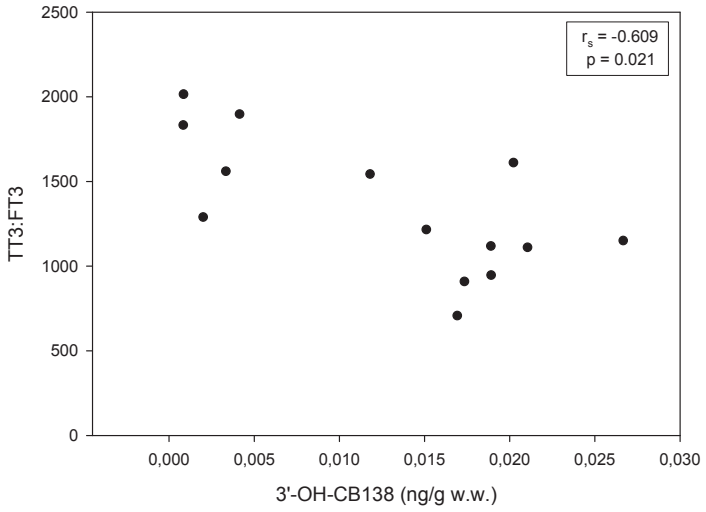
p = 0.027
FT4:FT3
25
20
15
10
5
0
0,0
0,2
0,4
0,6
0,8
1,0
4-OH-CB107 (ng/g w.w.)

B


r_s = -0.609
p = 0.021
TT3:FT3
2500
2000
1500
1000
500
0
0,000
0,005
0,010
0,015
0,020
0,025
0,030
3'-OH-CB138 (ng/g w.w.)

PAPER IV

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Doctoral theses in Biology
Norwegian University of Science and Technology
Department of Biology

Year	Name	Degree	Title
1974	Tor-Henning Iversen	Dr. philos Botany	The roles of statholiths, auxin transport, and auxin metabolism in root gravitropism
1978	Tore Slagsvold	Dr. philos Zoology	Breeding events of birds in relation to spring temperature and environmental phenology
1978	Egil Sakshaug	Dr.philos Botany	"The influence of environmental factors on the chemical composition of cultivated and natural populations of marine phytoplankton"
1980	Arnfinn Langeland	Dr. philos Zoology	Interaction between fish and zooplankton populations and their effects on the material utilization in a freshwater lake
1980	Helge Reinertsen	Dr. philos Botany	The effect of lake fertilization on the dynamics and stability of a limnetic ecosystem with special reference to the phytoplankton
1982	Gunn Mari Olsen	Dr. scient Botany	Gravitropism in roots of *Pisum sativum* and *Arabidopsis thaliana*
1982	Dag Dolmen	Dr. philos Zoology	Life aspects of two sympartic species of newts (*Triturus, Amphibia*) in Norway, with special emphasis on their ecological niche segregation
1984	Eivin Røskaft	Dr. philos Zoology	Sociobiological studies of the rook *Corvus frugilegus*
1984	Anne Margrethe Cameron	Dr. scient Botany	Effects of alcohol inhalation on levels of circulating testosterone, follicle stimulating hormone and luteinzing hormone in male mature rats
1984	Asbjørn Magne Nilsen	Dr. scient Botany	Alveolar macrophages from expectorates – Biological monitoring of workers exosed to occupational air pollution. An evaluation of the AM-test
1985	Jarle Mork	Dr. philos Zoology	Biochemical genetic studies in fish
1985	John Solem	Dr. philos Zoology	Taxonomy, distribution and ecology of caddisflies (*Trichoptera*) in the Dovrefjell mountains
1985	Randi E. Reinertsen	Dr. philos Zoology	Energy strategies in the cold: Metabolic and thermoregulatory adaptations in small northern birds
1986	Bernt-Erik Sæther	Dr. philos Zoology	Ecological and evolutionary basis for variation in reproductive traits of some vertebrates: A comparative approach
1986	Torleif Holthe	Dr. philos Zoology	Evolution, systematics, nomenclature, and zoogeography in the polychaete orders *Oweniimorpha* and *Terebellomorpha*, with special reference to the Arctic and Scandinavian fauna
1987	Helene Lampe	Dr. scient Zoology	The function of bird song in mate attraction and territorial defence, and the importance of song repertoires
1987	Olav Hogstad	Dr. philos Zoology	Winter survival strategies of the Willow tit *Parus montanus*
1987	Jarle Inge Holten	Dr. philos Botany	Autecological investigations along a coust-inland transect at Nord-Møre, Central Norway

1987	Rita Kumar	Dr. scient Botany	Somaclonal variation in plants regenerated from cell cultures of *Nicotiana sanderae* and *Chrysanthemum morifolium*
1987	Bjørn Åge Tømmerås	Dr. scient. Zoolog	Olfaction in bark beetle communities: Interspecific interactions in regulation of colonization density, predator - prey relationship and host attraction
1988	Hans Christian Pedersen	Dr. philos Zoology	Reproductive behaviour in willow ptarmigan with special emphasis on territoriality and parental care
1988	Tor G. Heggberget	Dr. philos Zoology	Reproduction in Atlantic Salmon (*Salmo salar*): Aspects of spawning, incubation, early life history and population structure
1988	Marianne V. Nielsen	Dr. scient Zoology	The effects of selected environmental factors on carbon allocation/growth of larval and juvenile mussels (*Mytilus edulis*)
1988	Ole Kristian Berg	Dr. scient Zoology	The formation of landlocked Atlantic salmon (*Salmo salar* L.)
1989	John W. Jensen	Dr. philos Zoology	Crustacean plankton and fish during the first decade of the manmade Nesjø reservoir, with special emphasis on the effects of gill nets and salmonid growth
1989	Helga J. Vivås	Dr. scient Zoology	Theoretical models of activity pattern and optimal foraging: Predictions for the Moose *Alces alces*
1989	Reidar Andersen	Dr. scient Zoology	Interactions between a generalist herbivore, the moose *Alces alces*, and its winter food resources: a study of behavioural variation
1989	Kurt Ingar Draget	Dr. scient Botany	Alginate gel media for plant tissue culture
1990	Bengt Finstad	Dr. scient Zoology	Osmotic and ionic regulation in Atlantic salmon, rainbow trout and Arctic charr: Effect of temperature, salinity and season
1990	Hege Johannesen	Dr. scient Zoology	Respiration and temperature regulation in birds with special emphasis on the oxygen extraction by the lung
1990	Åse Krøkje	Dr. scient Botany	The mutagenic load from air pollution at two work-places with PAH-exposure measured with Ames Salmonella/microsome test
1990	Arne Johan Jensen	Dr. philos Zoology	Effects of water temperature on early life history, juvenile growth and prespawning migrations of Atlantic salmion (*Salmo salar*) and brown trout (*Salmo trutta*): A summary of studies in Norwegian streams
1990	Tor Jørgen Almaas	Dr. scient Zoology	Pheromone reception in moths: Response characteristics of olfactory receptor neurons to intra- and interspecific chemical cues
1990	Magne Husby	Dr. scient Zoology	Breeding strategies in birds: Experiments with the Magpie *Pica pica*
1991	Tor Kvam	Dr. scient Zoology	Population biology of the European lynx (*Lynx lynx*) in Norway
1991	Jan Henning L'Abêe Lund	Dr. philos Zoology	Reproductive biology in freshwater fish, brown trout *Salmo trutta* and roach *Rutilus rutilus* in particular
1991	Asbjørn Moen	Dr. philos Botany	The plant cover of the boreal uplands of Central Norway. I. Vegetation ecology of Sølendet nature reserve; haymaking fens and birch woodlands
1991	Else Marie Løbersli	Dr. scient Botany	Soil acidification and metal uptake in plants
1991	Trond Nordtug	Dr. scient Zoology	Reflctometric studies of photomechanical adaptation in superposition eyes of arthropods
1991	Thyra Solem	Dr. scient Botany	Age, origin and development of blanket mires in Central Norway

1991	Odd Terje Sandlund	Dr. philos Zoology	The dynamics of habitat use in the salmonid genera *Coregonus* and *Salvelinus*: Ontogenic niche shifts and polymorphism
1991	Nina Jonsson	Dr. philos	Aspects of migration and spawning in salmonids
1991	Atle Bones	Dr. scient Botany	Compartmentation and molecular properties of thioglucoside glucohydrolase (myrosinase)
1992	Torgrim Breiehagen	Dr. scient Zoology	Mating behaviour and evolutionary aspects of the breeding system of two bird species: the Temminck's stint and the Pied flycatcher
1992	Anne Kjersti Bakken	Dr. scient Botany	The influence of photoperiod on nitrate assimilation and nitrogen status in timothy (*Phleum pratense* L.)
1992	Tycho Anker-Nilssen	Dr. scient Zoology	Food supply as a determinant of reproduction and population development in Norwegian Puffins *Fratercula arctica*
1992	Bjørn Munro Jenssen	Dr. philos Zoology	Thermoregulation in aquatic birds in air and water: With special emphasis on the effects of crude oil, chemically treated oil and cleaning on the thermal balance of ducks
1992	Arne Vollan Aarset	Dr. philos Zoology	The ecophysiology of under-ice fauna: Osmotic regulation, low temperature tolerance and metabolism in polar crustaceans.
1993	Geir Slupphaug	Dr. scient Botany	Regulation and expression of uracil-DNA glycosylase and O^6-methylguanine-DNA methyltransferase in mammalian cells
1993	Tor Fredrik Næsje	Dr. scient Zoology	Habitat shifts in coregonids.
1993	Yngvar Asbjørn Olsen	Dr. scient Zoology	Cortisol dynamics in Atlantic salmon, *Salmo salar* L.: Basal and stressor-induced variations in plasma levels ans some secondary effects.
1993	Bård Pedersen	Dr. scient Botany	Theoretical studies of life history evolution in modular and clonal organisms
1993	Ole Petter Thangstad	Dr. scient Botany	Molecular studies of myrosinase in Brassicaceae
1993	Thrine L. M. Heggberget	Dr. scient Zoology	Reproductive strategy and feeding ecology of the Eurasian otter *Lutra lutra*.
1993	Kjetil Bevanger	Dr. scient. Zoology	Avian interactions with utility structures, a biological approach.
1993	Kåre Haugan	Dr. scient Bothany	Mutations in the replication control gene trfA of the broad host-range plasmid RK2
1994	Peder Fiske	Dr. scient. Zoology	Sexual selection in the lekking great snipe (*Gallinago media*): Male mating success and female behaviour at the lek
1994	Kjell Inge Reitan	Dr. scient Botany	Nutritional effects of algae in first-feeding of marine fish larvae
1994	Nils Røv	Dr. scient Zoology	Breeding distribution, population status and regulation of breeding numbers in the northeast-Atlantic Great Cormorant *Phalacrocorax carbo carbo*
1994	Annette-Susanne Hoepfner	Dr. scient Botany	Tissue culture techniques in propagation and breeding of Red Raspberry (*Rubus idaeus* L.)
1994	Inga Elise Bruteig	Dr. scient Bothany	Distribution, ecology and biomonitoring studies of epiphytic lichens on conifers
1994	Geir Johnsen	Dr. scient Botany	Light harvesting and utilization in marine phytoplankton: Species-specific and photoadaptive responses
1994	Morten Bakken	Dr. scient Zoology	Infanticidal behaviour and reproductive performance in relation to competition capacity among farmed silver fox vixens, *Vulpes vulpes*

1994	Arne Moksnes	Dr. philos Zoology	Host adaptations towards brood parasitism by the Cockoo
1994	Solveig Bakken	Dr. scient Bothany	Growth and nitrogen status in the moss *Dicranum majus* Sm. as influenced by nitrogen supply
1994	Torbjørn Forseth	Dr. scient Zoology	Bioenergetics in ecological and life history studies of fishes.
1995	Olav Vadstein	Dr. philos Botany	The role of heterotrophic planktonic bacteria in the cycling of phosphorus in lakes: Phosphorus requirement, competitive ability and food web interactions
1995	Hanne Christensen	Dr. scient Zoology	Determinants of Otter *Lutra lutra* distribution in Norway: Effects of harvest, polychlorinated biphenyls (PCBs), human population density and competition with mink *Mustela vision*
1995	Svein Håkon Lorentsen	Dr. scient Zoology	Reproductive effort in the Antarctic Petrel *Thalassoica antarctica*; the effect of parental body size and condition
1995	Chris Jørgen Jensen	Dr. scient Zoology	The surface electromyographic (EMG) amplitude as an estimate of upper trapezius muscle activity
1995	Martha Kold Bakkevig	Dr. scient Zoology	The impact of clothing textiles and construction in a clothing system on thermoregulatory responses, sweat accumulation and heat transport
1995	Vidar Moen	Dr. scient Zoology	Distribution patterns and adaptations to light in newly introduced populations of *Mysis relicta* and constraints on Cladoceran and Char populations
1995	Hans Haavardsholm Blom	Dr. philos Bothany	A revision of the *Schistidium apocarpum* complex in Norway and Sweden
1996	Jorun Skjærmo	Dr. scient Botany	Microbial ecology of early stages of cultivated marine fish; inpact fish-bacterial interactions on growth and survival of larvae
1996	Ola Ugedal	Dr. scient Zoology	Radiocesium turnover in freshwater fishes
1996	Ingibjørg Einarsdottir	Dr. scient Zoology	Production of Atlantic salmon (*Salmo salar*) and Arctic charr (*Salvelinus alpinus*): A study of some physiological and immunological responses to rearing routines
1996	Christina M. S. Pereira	Dr. scient Zoology	Glucose metabolism in salmonids: Dietary effects and hormonal regulation
1996	Jan Fredrik Børseth	Dr. scient Zoology	The sodium energy gradients in muscle cells of *Mytilus edulis* and the effects of organic xenobiotics
1996	Gunnar Henriksen	Dr. scient Zoology	Status of Grey seal *Halichoerus grypus* and Harbour seal *Phoca vitulina* in the Barents sea region
1997	Gunvor Øie	Dr. scient Bothany	Eevalution of rotifer *Brachionus plicatilis* quality in early first feeding of turbot *Scophtalmus maximus* L. larvae
1997	Håkon Holien	Dr. scient Botany	Studies of lichens in spurce forest of Central Norway. Diversity, old growth species and the relationship to site and stand parameters
1997	Ole Reitan	Dr. scient. Zoology	Responses of birds to habitat disturbance due to damming
1997	Jon Arne Grøttum	Dr. scient. Zoology	Physiological effects of reduced water quality on fish in aquaculture
1997	Per Gustav Thingstad	Dr. scient. Zoology	Birds as indicators for studying natural and human-induced variations in the environment, with special emphasis on the suitability of the Pied Flycatcher
1997	Torgeir Nygård	Dr. scient Zoology	Temporal and spatial trends of pollutants in birds in Norway: Birds of prey and Willow Grouse used as Biomonitors

1997 Signe Nybø	Dr. scient. Zoology	Impacts of long-range transported air pollution on birds with particular reference to the dipper *Cinclus cinclus* in southern Norway
1997 Atle Wibe	Dr. scient. Zoology	Identification of conifer volatiles detected by receptor neurons in the pine weevil (*Hylobius abietis*), analysed by gas chromatography linked to electrophysiology and to mass spectrometry
1997 Rolv Lundheim	Dr. scient Zoology	Adaptive and incidental biological ice nucleators
1997 Arild Magne Landa	Dr. scient Zoology	Wolverines in Scandinavia: ecology, sheep depredation and conservation
1997 Kåre Magne Nielsen	Dr. scient Botany	An evolution of possible horizontal gene transfer from plants to sail bacteria by studies of natural transformation in *Acinetobacter calcoacetius*
1997 Jarle Tufto	Dr. scient Zoology	Gene flow and genetic drift in geographically structured populations: Ecological, population genetic, and statistical models
1997 Trygve Hesthagen	Dr. philos Zoology	Population responces of Arctic charr (*Salvelinus alpinus* (L.)) and brown trout (*Salmo trutta* L.) to acidification in Norwegian inland waters
1997 Trygve Sigholt	Dr. philos Zoology	Control of Parr-smolt transformation and seawater tolerance in farmed Atlantic Salmon (*Salmo salar*) Effects of photoperiod, temperature, gradual seawater acclimation, NaCl and betaine in the diet
1997 Jan Østnes	Dr. scient Zoology	Cold sensation in adult and neonate birds
1998 Seethaledsumy Visvalingam	Dr. scient Botany	Influence of environmental factors on myrosinases and myrosinase-binding proteins
1998 Thor Harald Ringsby	Dr. scient Zoology	Variation in space and time: The biology of a House sparrow metapopulation
1998 Erling Johan Solberg	Dr. scient. Zoology	Variation in population dynamics and life history in a Norwegian moose (*Alces alces*) population: consequences of harvesting in a variable environment
1998 Sigurd Mjøen Saastad	Dr. scient Botany	Species delimitation and phylogenetic relationships between the Sphagnum recurvum complex (Bryophyta): genetic variation and phenotypic plasticity
1998 Bjarte Mortensen	Dr. scient Botany	Metabolism of volatile organic chemicals (VOCs) in a head liver S9 vial equilibration system in vitro
1998 Gunnar Austrheim	Dr. scient Botany	Plant biodiversity and land use in subalpine grasslands. – A conservtaion biological approach
1998 Bente Gunnveig Berg	Dr. scient Zoology	Encoding of pheromone information in two related moth species
1999 Kristian Overskaug	Dr. scient Zoology	Behavioural and morphological characteristics in Northern Tawny Owls *Strix aluco*: An intra- and interspecific comparative approach
1999 Hans Kristen Stenøien	Dr. scient Bothany	Genetic studies of evolutionary processes in various populations of nonvascular plants (mosses, liverworts and hornworts)
1999 Trond Arnesen	Dr. scient Botany	Vegetation dynamics following trampling and burning in the outlying haylands at Sølendet, Central Norway
1999 Ingvar Stenberg	Dr. scient Zoology	Habitat selection, reproduction and survival in the White-backed Woodpecker *Dendrocopos leucotos*
1999 Stein Olle Johansen	Dr. scient Botany	A study of driftwood dispersal to the Nordic Seas by dendrochronology and wood anatomical analysis

1999	Trina Falck Galloway	Dr. scient Zoology	Muscle development and growth in early life stages of the Atlantic cod (*Gadus morhua* L.) and Halibut (*Hippoglossus hippoglossus* L.)
1999	Marianne Giæver	Dr. scient Zoology	Population genetic studies in three gadoid species: blue whiting (*Micromisistius poutassou*), haddock (*Melanogrammus aeglefinus*) and cod (*Gradus morhua*) in the North-East Atlantic
1999	Hans Martin Hanslin	Dr. scient Botany	The impact of environmental conditions of density dependent performance in the boreal forest bryophytes *Dicranum majus*, *Hylocomium splendens*, *Plagiochila asplenigides*, *Ptilium crista-castrensis* and *Rhytidiadelphus lokeus*
1999	Ingrid Bysveen Mjølnerød	Dr. scient Zoology	Aspects of population genetics, behaviour and performance of wild and farmed Atlantic salmon (*Salmo salar*) revealed by molecular genetic techniques
1999	Else Berit Skagen	Dr. scient Botany	The early regeneration process in protoplasts from *Brassica napus* hypocotyls cultivated under various g-forces
1999	Stein-Are Sæther	Dr. philos Zoology	Mate choice, competition for mates, and conflicts of interest in the Lekking Great Snipe
1999	Katrine Wangen Rustad	Dr. scient Zoology	Modulation of glutamatergic neurotransmission related to cognitive dysfunctions and Alzheimer's disease
1999	Per Terje Smiseth	Dr. scient Zoology	Social evolution in monogamous families: mate choice and conflicts over parental care in the Bluethroat (*Luscinia s. svecica*)
1999	Gunnbjørn Bremset	Dr. scient Zoology	Young Atlantic salmon (*Salmo salar* L.) and Brown trout (*Salmo trutta* L.) inhabiting the deep pool habitat, with special reference to their habitat use, habitat preferences and competitive interactions
1999	Frode Ødegaard	Dr. scient Zoology	Host spesificity as parameter in estimates of arhrophod species richness
1999	Sonja Andersen	Dr. scient Bothany	Expressional and functional analyses of human, secretory phospholipase A2
2000	Ingrid Salvesen	Dr. scient Botany	Microbial ecology in early stages of marine fish: Development and evaluation of methods for microbial management in intensive larviculture
2000	Ingar Jostein Øien	Dr. scient Zoology	The Cuckoo (*Cuculus canorus*) and its host: adaptions and counteradaptions in a coevolutionary arms race
2000	Pavlos Makridis	Dr. scient Botany	Methods for the microbial econtrol of live food used for the rearing of marine fish larvae
2000	Sigbjørn Stokke	Dr. scient Zoology	Sexual segregation in the African elephant (*Loxodonta africana*)
2000	Odd A. Gulseth	Dr. philos Zoology	Seawater tolerance, migratory behaviour and growth of Charr, (*Salvelinus alpinus*), with emphasis on the high Arctic Dieset charr on Spitsbergen, Svalbard
2000	Pål A. Olsvik	Dr. scient Zoology	Biochemical impacts of Cd, Cu and Zn on brown trout (*Salmo trutta*) in two mining-contaminated rivers in Central Norway
2000	Sigurd Einum	Dr. scient Zoology	Maternal effects in fish: Implications for the evolution of breeding time and egg size
2001	Jan Ove Evjemo	Dr. scient Zoology	Production and nutritional adaptation of the brine shrimp *Artemia* sp. as live food organism for larvae of marine cold water fish species
2001	Olga Hilmo	Dr. scient Botany	Lichen response to environmental changes in the managed boreal forset systems

2001	Ingebrigt Uglem	Dr. scient Zoology	Male dimorphism and reproductive biology in corkwing wrasse (*Symphodus melops* L.)
2001	Bård Gunnar Stokke	Dr. scient Zoology	Coevolutionary adaptations in avian brood parasites and their hosts
2002	Ronny Aanes	Dr. scient	Spatio-temporal dynamics in Svalbard reindeer (*Rangifer tarandus platyrhynchus*)
2002	Mariann Sandsund	Dr. scient Zoology	Exercise- and cold-induced asthma. Respiratory and thermoregulatory responses
2002	Dag-Inge Øien	Dr. scient Botany	Dynamics of plant communities and populations in boreal vegetation influenced by scything at Sølendet, Central Norway
2002	Frank Rosell	Dr. scient Zoology	The function of scent marking in beaver (*Castor fiber*)
2002	Janne Østvang	Dr. scient Botany	The Role and Regulation of Phospholipase A_2 in Monocytes During Atherosclerosis Development
2002	Terje Thun	Dr.philos Biology	Dendrochronological constructions of Norwegian conifer chronologies providing dating of historical material
2002	Birgit Hafjeld Borgen	Dr. scient Biology	Functional analysis of plant idioblasts (Myrosin cells) and their role in defense, development and growth
2002	Bård Øyvind Solberg	Dr. scient Biology	Effects of climatic change on the growth of dominating tree species along major environmental gradients
2002	Per Winge	Dr. scient Biology	The evolution of small GTP binding proteins in cellular organisms. Studies of RAC GTPases in *Arabidopsis thaliana* and the Ral GTPase from *Drosophila melanogaster*
2002	Henrik Jensen	Dr. scient Biology	Causes and consequenses of individual variation in fitness-related traits in house sparrows
2003	Jens Rohloff	Dr. philos Biology	Cultivation of herbs and medicinal plants in Norway – Essential oil production and quality control
2003	Åsa Maria O. Espmark Wibe	Dr. scient Biology	Behavioural effects of environmental pollution in threespine stickleback *Gasterosteus aculeatur* L.
2003	Dagmar Hagen	Dr. scient Biology	Assisted recovery of disturbed arctic and alpine vegetation – an integrated approach
2003	Bjørn Dahle	Dr. scient Biology	Reproductive strategies in Scandinavian brown bears
2003	Cyril Lebogang Taolo	Dr. scient Biology	Population ecology, seasonal movement and habitat use of the African buffalo (*Syncerus caffer*) in Chobe National Park, Botswana
2003	Marit Stranden	Dr.scient Biology	Olfactory receptor neurones specified for the same odorants in three related Heliothine species (*Helicoverpa armigera, Helicoverpa assulta* and *Heliothis virescens*)
2003	Kristian Hassel	Dr.scient Biology	Life history characteristics and genetic variation in an expanding species, *Pogonatum dentatum*
2003	David Alexander Rae	Dr.scient Biology	Plant- and invertebrate-community responses to species interaction and microclimatic gradients in alpine and Artic environments
2003	Åsa A Borg	Dr.scient Biology	Sex roles and reproductive behaviour in gobies and guppies: a female perspective
2003	Eldar Åsgard Bendiksen	Dr.scient Biology	Environmental effects on lipid nutrition of farmed Atlantic salmon (*Salmo Salar* L.) parr and smolt
2004	Torkild Bakken	Dr.scient Biology	A revision of Nereidinae (Polychaeta, Nereididae)
2004	Ingar Pareliussen	Dr.scient Biology	Natural and Experimental Tree Establishment in a Fragmented Forest, Ambohitantely Forest Reserve, Madagascar

2004	Tore Brembu	Dr.scient Biology	Genetic, molecular and functional studies of RAC GTPases and the WAVE-like regulatory protein complex in *Arabidopsis thaliana*
2004	Liv S. Nilsen	Dr.scient Biology	Coastal heath vegetation on central Norway; recent past, present state and future possibilities
2004	Hanne T. Skiri	Dr.scient Biology	Olfactory coding and olfactory learning of plant odours in heliothine moths. An anatomical, physiological and behavioural study of three related species (*Heliothis virescens, Helicoverpa armigera* and *Helicoverpa assulta*)
2004	Lene Østby	Dr.scient Biology	Cytochrome P4501A (CYP1A) induction and DNA adducts as biomarkers for organic pollution in the natural environment
2004	Emmanuel J. Gerreta	Dr. philos Biology	The Importance of Water Quality and Quantity in the Tropical Ecosystems, Tanzania
2004	Linda Dalen	Dr.scient Biology	Dynamics of Mountain Birch Treelines in the Scandes Mountain Chain, and Effects of Climate Warming
2004	Lisbeth Mehli	Dr.scient Biology	Polygalacturonase-inhibiting protein (PGIP) in cultivated strawberry (*Fragaria* x *ananassa*): characterisation and induction of the gene following fruit infection by *Botrytis cinerea*
2004	Børge Moe	Dr.scient Biology	Energy-Allocation in Avian Nestlings Facing Short-Term Food Shortage
2005	Matilde Skogen Chauton	Dr.scient Biology	Metabolic profiling and species discrimination from High-Resolution Magic Angle Spinning NMR analysis of whole-cell samples
2005	Sten Karlsson	Dr.scient Biology	Dynamics of Genetic Polymorphisms
2005	Terje Bongard	Dr.scient Biology	Life History strategies, mate choice, and parental investment among Norwegians over a 300-year period
2005	Tonette Røstelien	ph.d Biology	Functional characterisation of olfactory receptor neurone types in heliothine moths
2005	Erlend Kristiansen	Dr.scient Biology	Studies on antifreeze proteins
2005	Eugen G. Sørmo	Dr.scient Biology	Organochlorine pollutants in grey seal (*Halichoerus grypus*) pups and their impact on plasma thyrid hormone and vitamin A concentrations
2005	Christian Westad	Dr.scient Biology	Motor control of the upper trapezius
2005	Lasse Mork Olsen	ph.d Biology	Interactions between marine osmo- and phagotrophs in different physicochemical environments
2005	Åslaug Viken	ph.d Biology	Implications of mate choice for the management of small populations
2005	Ariaya Hymete Sahle Dingle	ph.d Biology	Investigation of the biological activities and chemical constituents of selected *Echinops* spp. growing in Ethiopia
2005	Anders Gravbrøt Finstad	ph.d Biology	Salmonid fishes in a changing climate: The winter challenge
2005	Shimane Washington Makabu	ph.d Biology	Interactions between woody plants, elephants and other browsers in the Chobe Riverfront, Botswana
2005	Kjartan Østbye	Dr.scient Biology	The European whitefish *Coregonus lavaretus* (L.) species complex: historical contingency and adaptive radiation

2006	Kari Mette Murvoll	ph.d Biology	Levels and effects of persistent organic pollutans (POPs) in seabirds Retinoids and α-tocopherol – potential biomakers of POPs in birds?
2006	Ivar Herfindal	Dr.scient Biology	Life history consequences of environmental variation along ecological gradients in northern ungulates
2006	Nils Egil Tokle	ph.d Biology	Are the ubiquitous marine copepods limited by food or predation? Experimental and field-based studies with main focus on *Calanus finmarchicus*
2006	Jan Ove Gjershaug	Dr.philos Biology	Taxonomy and conservation status of some booted eagles in south-east Asia
2006	Jon Kristian Skei	Dr.scient Biology	Conservation biology and acidification problems in the breeding habitat of amphibians in Norway
2006	Johanna Järnegren	ph.d Biology	Acesta Oophaga and Acesta Excavata – a study of hidden biodiversity
2006	Bjørn Henrik Hansen	ph.d Biology	Metal-mediated oxidative stress responses in brown trout (*Salmo trutta*) from mining contaminated rivers in Central Norway
2006	Vidar Grøtan	ph.d Biology	Temporal and spatial effects of climate fluctuations on population dynamics of vertebrates
2006	Jafari R Kideghesho	ph.d Biology	Wildlife conservation and local land use conflicts in western Serengeti, Corridor Tanzania
2006	Anna Maria Billing	ph.d Biology	Reproductive decisions in the sex role reversed pipefish *Syngnathus typhle*: when and how to invest in reproduction
2006	Henrik Pärn	ph.d Biology	Female ornaments and reproductive biology in the bluethroat
2006	Anders J. Fjellheim	ph.d Biology	Selection and administration of probiotic bacteria to marine fish larvae
2006	P. Andreas Svensson	ph.d Biology	Female coloration, egg carotenoids and reproductive success: gobies as a model system
2007	Sindre A. Pedersen	ph.d Biology	Metal binding proteins and antifreeze proteins in the beetle *Tenebrio molitor* - a study on possible competition for the semi-essential amino acid cysteine
2007	Kasper Hancke	ph.d Biology	Photosynthetic responses as a function of light and temperature: Field and laboratory studies on marine microalgae
2007	Tomas Holmern	ph.d Biology	Bushmeat hunting in the western Serengeti: Implications for community-based conservation
2007	Kari Jørgensen	ph.d Biology	Functional tracing of gustatory receptor neurons in the CNS and chemosensory learning in the moth *Heliothis virescens*
2007	Stig Ulland	ph.d Biology	Functional Characterisation of Olfactory Receptor Neurons in the Cabbage Moth, (*Mamestra brassicae* L.) (Lepidoptera, Noctuidae). Gas Chromatography Linked to Single Cell Recordings and Mass Spectrometry
2007	Snorre Henriksen	ph.d Biology	Spatial and temporal variation in herbivore resources at northern latitudes
2007	Roelof Frans May	ph.d Biology	Spatial Ecology of Wolverines in Scandinavia
2007	Vedasto Gabriel Ndibalema	ph.d Biology	Demographic variation, distribution and habitat use between wildebeest sub-populations in the Serengeti National Park, Tanzania

2007	Julius William Nyahongo	ph.d Biology	Depredation of Livestock by wild Carnivores and Illegal Utilization of Natural Resources by Humans in the Western Serengeti, Tanzania
2007	Shombe Ntaraluka Hassan	ph.d Biology	Effects of fire on large herbivores and their forage resources in Serengeti, Tanzania
2007	Per-Arvid Wold	ph.d Biology	Functional development and response to dietary treatment in larval Atlantic cod *(Gadus morhua* L.) Focus on formulated diets and early weaning
2007	Anne Skjetne Mortensen	ph.d Biology	Toxicogenomics of Aryl Hydrocarbon- and Estrogen Receptor Interactions in Fish: Mechanisms and Profiling of Gene Expression Patterns in Chemical Mixture Exposure Scenarios
2008	Brage Bremset Hansen	ph.d Biology	The Svalbard reindeer (*Rangifer tarandus platyrhynchus*) and its food base: plant-herbivore interactions in a high-arctic ecosystem
2008	Jiska van Dijk	ph.d Biology	Wolverine foraging strategies in a multiple-use landscape
2008	Flora John Magige	ph.d Biology	The ecology and behaviour of the Masai Ostrich (Struthio camelus massaicus) in the Serengeti Ecosystem, Tanzania
2008	Bernt Rønning	ph.d Biology	Sources of inter- and intra-individual variation in basal metabolic rate in the zebra finch, (*Taeniopygia guttata*)
2008	Sølvi Wehn	ph.d Biology	Biodiversity dynamics in semi-natural mountain landscapes. - A study of consequences of changed agricultural practices in Eastern Jotunheimen
2008	Trond Moxness Kortner	ph.d Biology	"The Role of Androgens on previtellogenic oocyte growth in Atlantic cod (*Gadus morhua*): Identification and patterns of differentially expressed genes in relation to Stereological Evaluations"
2008	Katarina Mariann Jørgensen	Dr.Scient Biology	The role of platelet activating factor in activation of growth arrested keratinocytes and re-epithelialisation
2008	Tommy Jørstad	ph.d Biology	Statistical Modelling of Gene Expression Data
2008	Anna Kusnierczyk	ph.d Bilogy	*Arabidopsis thaliana* Responses to Aphid Infestation
2008	Jussi Evertsen	ph.d Biology	Herbivore sacoglossans with photosynthetic chloroplasts
2008	John Eilif Hermansen	ph.d Biology	Mediating ecological interests between locals and globals by means of indicators. A study attributed to the asymmetry between stakeholders of tropical forest at Mt. Kilimanjaro, Tanzania
2008	Ragnhild Lyngved	ph.d Biology	Somatic embryogenesis in *Cyclamen persicum*. Biological investigations and educational aspects of cloning
2008	Line Elisabeth Sundt-Hansen	ph.d Biology	Cost of rapid growth in salmonid fishes
2008	Line Johansen	ph.d Biology	Exploring factors underlying fluctuations in white clover populations – clonal growth, population structure and spatial distribution
2009	Astrid Jullumstrø Feuerherm	ph.d Biology	Elucidation of molecular mechanisms for pro-inflammatory phospholipase A2 in chronic disease

2009	Pål Kvello	ph.d Biology	Neurons forming the network involved in gustatory coding and learning in the moth *Heliothis virescens:* Physiological and morphological characterisation, and integration into a standard brain atlas
2009	Trygve Devold Kjellsen	ph.d Biology	Extreme Frost Tolerance in Boreal Conifers
2009	Johan Reinert Vikan	ph.d Biology	Coevolutionary interactions between common cuckoos *Cuculus canorus* and *Fringilla* finches
2009	Zsolt Volent	ph.d Biology	Remote sensing of marine environment: Applied surveillance with focus on optical properties of phytoplankton, coloured organic matter and suspended matter
2009	Lester Rocha	ph.d Biology	Functional responses of perennial grasses to simulated grazing and resource availability
2009	Dennis Ikanda	ph.d Biology	Dimensions of a Human-lion conflict: Ecology of human predation and persecution of African lions (*Panthera leo*) in Tanzania
2010	Huy Quang Nguyen	ph.d Biology	Egg characteristics and development of larval digestive function of cobia (*Rachycentron canadum*) in response to dietary treatments -Focus on formulated diets
2010	Eli Kvingedal	ph.d Biology	Intraspecific competition in stream salmonids: the impact of environment and phenotype
2010	Sverre Lundemo	ph.d Biology	Molecular studies of genetic structuring and demography in *Arabidopsis* from Northern Europe
2010	Iddi Mihijai Mfunda	ph.d Biology	Wildlife Conservation and People's livelihoods: Lessons Learnt and Considerations for Improvements. Tha Case of Serengeti Ecosystem, Tanzania
2010	Anton Tinchov Antonov	ph.d Biology	Why do cuckoos lay strong-shelled eggs? Tests of the puncture resistance hypothesis
2010	Anders Lyngstad	ph.d Biology	Population Ecology of *Eriophorum latifolium*, a Clonal Species in Rich Fen Vegetation
2010	Hilde Færevik	ph.d Biology	Impact of protective clothing on thermal and cognitive responses
2010	Ingerid Brænne Arbo	ph.d Medical technology	Nutritional lifestyle changes – effects of dietary carbohydrate restriction in healthy obese and overweight humans
2010	Yngvild Vindenes	ph.d Biology	Stochastic modeling of finite populations with individual heterogeneity in vital parameters
2010	Hans-Richard Brattbakk	ph.d Medical technology	The effect of macronutrient composition, insulin stimulation, and genetic variation on leukocyte gene expression and possible health benefits
2011	Geir Hysing Bolstad	ph.d Biology	Evolution of Signals: Genetic Architecture, Natural Selection and Adaptive Accuracy
2011	Karen de Jong	ph.d Biology	Operational sex ratio and reproductive behaviour in the two-spotted goby (*Gobiusculus flavescens*)
2011	Ann-Iren Kittang	ph.d Biology	*Arabidopsis thaliana* L. adaptation mechanisms to microgravity through the EMCS MULTIGEN-2 experiment on the ISS:– The science of space experiment integration and adaptation to simulated microgravity
2011	Aline Magdalena Lee	ph.d Biology	Stochastic modeling of mating systems and their effect on population dynamics and genetics
2011	Christopher Gravningen Sørmo	ph.d Biology	Rho GTPases in Plants: Structural analysis of ROP GTPases; genetic and functional studies of MIRO GTPases in *Arabidopsis thaliana*

2011	Grethe Robertsen	ph.d Biology	Relative performance of salmonid phenotypes across environments and competitive intensities
2011	Line-Kristin Larsen	ph.d Biology	Life-history trait dynamics in experimental populations of guppy (*Poecilia reticulata*): the role of breeding regime and captive environment
2011	Maxim A. K. Teichert	ph.d Biology	Regulation in Atlantic salmon (*Salmo salar*): The interaction between habitat and density
2011	Torunn Beate Hancke	ph.d Biology	Use of Pulse Amplitude Modulated (PAM) Fluorescence and Bio-optics for Assessing Microalgal Photosynthesis and Physiology
2011	Sajeda Begum	ph.d Biology	Brood Parasitism in Asian Cuckoos: Different Aspects of Interactions between Cuckoos and their Hosts in Bangladesh
2011	Kari J. K. Attramadal	ph.d Biology	Water treatment as an approach to increase microbial control in the culture of cold water marine larvae
2011	Camilla Kalvatn Egset	ph.d Biology	The Evolvability of Static Allometry: A Case Study
2011	AHM Raihan Sarker	ph.d Biology	Conflict over the conservation of the Asian elephant (*Elephas maximus*) in Bangladesh