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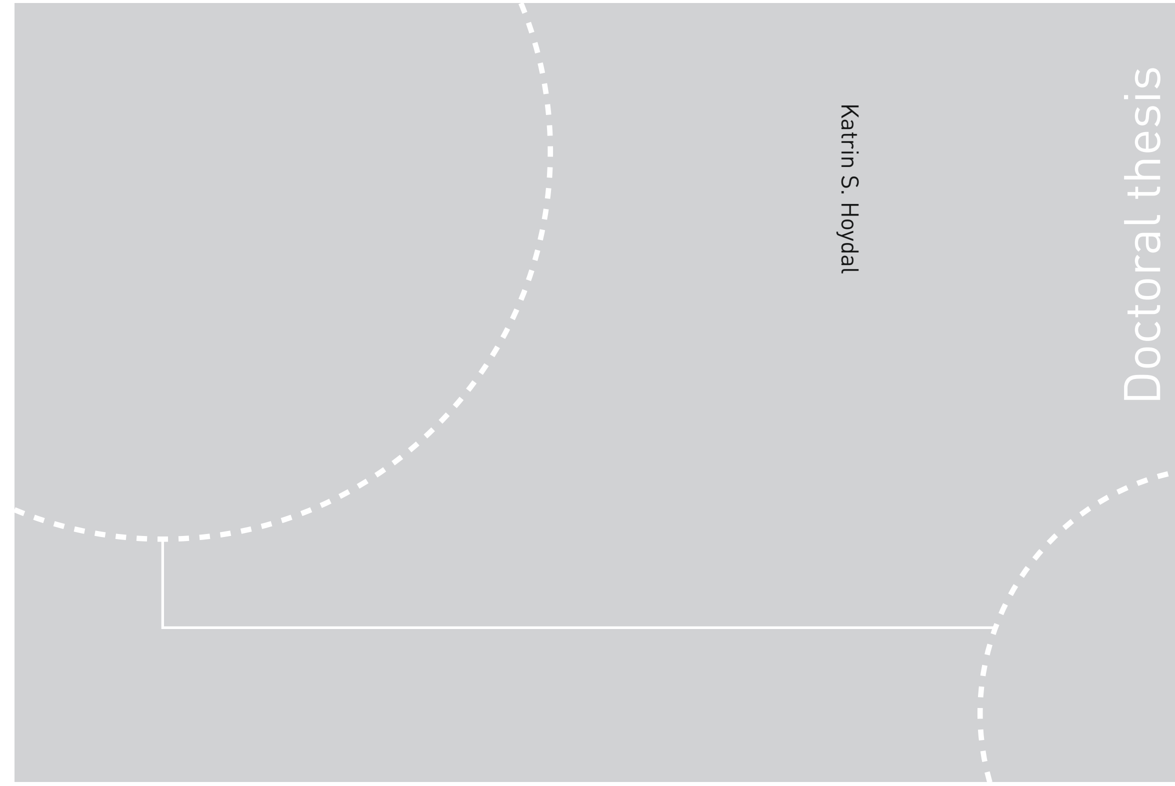

Katrin S. Hoydal

Doctoral theses at NTNU, 2017:279

NTNU
Norwegian University of Science and Technology
Thesis for the Degree of
Philosophiae Doctor
Faculty of Natural Sciences
Department of Biology

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Trondheim, October 2017

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I want to dedicate this thesis to my beloved father Kjartan Hoydal, who passed away in May of 2016.

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Abstract

The former large production and usage of persistent organic pollutants (POPs) has led to pollution of these compounds in natural environments throughout the world. Although the production and use of many POPs has been restricted for decades, pilot whales (*Globicephala melas*) in the Northeastern part of the Atlantic Ocean have high body concentrations of POPs, such as polychlorinated biphenyls (PCBs), organochlorinated pesticides (OCPs) and polybrominated diphenyl ethers (PBDEs). These compounds and their biological biotransformation products, i.e. metabolites, have been linked to serious health effects in mammals, including effects on the reproductive system, due to disruption of endocrine systems in the body. The aim of this thesis was to analyse levels of POPs and their metabolites in pilot whales from Faroese waters and to investigate the possible effects of the POP exposure on steroid hormones, thyroid hormones, and vitamin A, E and D, which have been suggested as sensitive biomarkers for endocrine disruptive effects. In addition, the ability of the whales to biotransform the POPs was investigated. The possible effects of the POPs on the biomarkers were studied by analysing correlative relationships between the POP concentrations and the biomarkers, and furthermore, the POP concentrations were compared to toxic reference values (TRVs) which have been suggested for toxic effects in marine mammals.

POP concentrations were analysed in liver and plasma of whales of different sexes and age classes. The results showed that PCBs and the OCP metabolite DDE were the dominating compounds in the whales. The adult females had significantly lower POP concentrations than juveniles of both sexes and adult males, most likely due to the maternal transfer of POPs to the offspring during gestation and lactation. The relative concentrations of the different POPs also differed between adult females, sub-adults and calves and indicated that highly halogenated compounds with high lipid solubility (Log K_{ow}) and high molecular weight are less easily transferred to the offspring than the compounds with lower molecular weight that are less halogenated and less lipid soluble. Very low concentrations of metabolites, such as hydroxylated PCBs and PBDEs, were detected, and OH-CB107/OH'-CB108 was the only metabolite detected in a significant number of individuals.

The POP concentrations in the pilot whales exceeded some of the TRVs, that have been suggested for toxicological effects in marine mammals, including effects on vitamin A and E and effects on calf survival and population growth.

The biotransformation ability of the whales was studied by analysing hepatic mRNA transcripts, protein expression and/or catalytic activity of phase I and phase II enzymes. The analyses of phase I enzymes showed that the pilot whales expressed enzymes of the CYP1, 2 and 3 families and that the expressions were related to the POP exposure. Catalytic activity of the CYP1A enzymes (EROD activity) was positively correlated to POP concentrations. The most important compounds explaining the relationship between the POPs and the EROD activity were the PCBs of the metabolizing group IV, PBDEs, cis-chlordane and HCB. The mRNA expression did not correlate with the POP concentrations in the whales. Phase II biotransformation was investigated by analysing the catalytic activity of the transferases UDPGT and GST. The catalytic activity of both phase I and II enzymes were, however low, which was in accordance with the low POP metabolite concentrations found in the present pilot whales, indicating that pilot whales have a low capacity related to hepatic biotransformation of POPs.

The biomarkers for endocrine disrupting effects did generally not show high correlative relationships with the POP concentrations, but differed with age and sex of the whales, indicating that that these are important confounding factors. Nevertheless, particularly in females, positive correlations were found between a few of the more recalcitrant POPs and single steroids hormones. The thyroid hormones correlated positively with the POPs, but since the thyroid concentrations were generally higher in juveniles than in adults and most of the adult whales were females which had significantly lower POP concentrations than the juveniles, this correlation could be explained as an age effect. However, within the age groups HCB was positively correlated with thyroid hormones in both adult females and juveniles. Positive correlations between thyroid hormones and a few compounds were also detected in the calves (0-2 years of age). For vitamin A negative correlations were found between hepatic concentrations of PBDEs and hepatic retinyl palmitate concentrations in adult females. The circulating vitamin E concentration was positively correlated to the POP exposure in the juveniles, indicating a response to oxidative stress induced by the POPs. These correlations indicated that the POP compounds may have some disturbing effects on the hormone and vitamin homeostasis in the pilot whales. However, no conclusions can be made on the toxicological, organismal and ecological relevant implications of these correlations between POPs and the biomarkers.

It is concluded, that although the pilot whales were exposed to relatively high concentrations of POPs, and the concentrations exceeded some of the toxic reference levels suggested for effects on marine mammals, the POPs did not seem to have clear overall negative effects on the analysed biomarkers. This indicates that the POPs in the Faroe Island population of pilot whales may be below threshold levels for negative effects on steroid and thyroid hormones and vitamin A levels. This could possibly be related to the apparent low biotransformation of POPs in pilot whales and thus their low concentrations of metabolites, such as OH-PCBs and OH-PBDE, which often have been linked to the toxic effects of POPs.

List of papers

Paper I: Hoydal, K.S., Letcher, R.J., Blair, D.A.D., Dam, M., Lockyer, C., Jenssen, B.M., 2015. Legacy and emerging organic pollutants in liver and plasma of long-finned pilot whales (*Globicephala melas*) from waters surrounding the Faroe Islands. *Science of the Total Environment* 520, 270–285.

Paper II: Hoydal, K.S., Jenssen, B.M., Letcher, R.J., Dam, M., Arukwe, A. Hepatic Phase I and II Biotransformation Responses and Contaminant Exposure in Long-Finned Pilot Whale from the Northeastern Atlantic. **Submitted**

Paper III: Hoydal, K.S., Styrishave, B., Ciesielski, T.M., Letcher, R.J., Dam, M., Jenssen, B.M. Steroid Hormones and Persistent Organic Pollutants in plasma from Northeastern Atlantic Pilot whales. **Submitted**

Paper IV: Hoydal, K.S., Ciesielski, T.M., Borrell, A., Wasik, A., Letcher, R.J., Dam, M., Jenssen, B.M., 2016. Relationships between concentrations of selected organohalogen contaminants and thyroid hormones and vitamins A, E and D in Faroese pilot whales. *Environmental Research* 148, 386–400.

Paper V: Hoydal, K.S., Jenssen, B.M., Ciesielski, T.M., Letcher, R.J., Dam, M., Arukwe, A. Changes in CYP26 expression and vitamin A levels in relation to contaminant levels in Faroese pilot whales. **Manuscript**

Declaration of contributions

KH wrote the manuscripts with contribution form co-authors. The project was planned and initiated by **KH** with help from MD, BMJ and AA. Samples were collected and initial sample preparation was done by **KH** and MD. **KH** contributed significantly in sample analyses in papers II-V. RJL/DB carried out the POP analyses (paper I). **KH** performed thyroid hormone analysis, vitamin analyses (A and E) and enzyme analyses at NTNU in collaboration with BMJ, TMC and AA (Papers II, IV, V), and steroid hormones at the University of Copenhagen in collaboration with BS (Paper III). Analyses of vitamin D and additional analyses of vitamin A and E were performed by AW at Gdansk University of Technology and by **KH** in collaboration with AB at University of Barcelona, and the data were treated by **KH** (Paper IV). **KH** conducted the statistical analyses in all papers in collaboration with TMC (Paper III, IV, V) and the other co-authors (Paper I, II). CL performed the age determination of the pilot whales.

1 Introduction

During the last four decades of the last century, it became evident that high concentrations of many persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs) and several organochlorinated pesticides (OCPs), caused harmful health related effects in humans, and that populations of wildlife species also were affected (Colborn et al., 1993). With respect to effects on marine mammal populations, the dramatic population decline in the populations of Wadden Sea common seals (*Phoca vitulina*) and of Baltic ringed seals (*Phoca hispida*) and grey seals (*Halichoerus grypus*) were linked to high body burdens of PCBs and the resultant negative effects on their reproduction abilities (Helle, 1976; Helle et al., 1976; Reijnders, 1986). Similarly, the failure of a St. Lawrence River beluga (*Delphinapterus leucas*) population to recover after a large decline was linked to effects on reproductive ability due to high levels of POPs (De Guise et al., 1995). As a result of the documentation of the harmful effects of POPs on humans and wildlife, an international political process was initiated to restrict the production, use and release of POPs, and in 2004 the Stockholm Convention on POPs (www.pops.int) was ratified. The convention is imposing global regulation for several POPs amounting to full bans, on the production and use of 12 POPs, sometimes reffered to as ”the dirty dozen”. These are aldrin, chlordane, dichlorodiphenyltrichloroethane (DDT), dieldrin, endrin, heptachlor, hexachlorobenzene (HCB), Mirex, toxaphene, PCB, polychlorinated dibenzo-*p*-dioxin (PCDD) and polychlorinated dibenzofuran (PCDF). According to the Stockholm Convention, POPs are defined as substances that are persistent, toxic, bioaccumulative, biomagnify through the food web, and are prone to distribution throughout the environment due to long-range transport. POPs are organohaloganted (Cl, Br, and F) man-made compounds (OHCs). Since then several additional POPs have been included in the convention, such as certain polybrominated diphenylethers (PBDEs). The production and use of several of the POPs was however, restricted with national regulations before the ratification of the Stockholm POP convention, and most of these compounds have not been produced since the 1970ties or 80ties (Jones and de Voogt, 1999). POPs that have been banned or regulated are sometimes referred to as “legacy” POPs, because the present day contamination largely is a “legacy” from releases in the past (Rigét et al., 2010).

Due to their restricted use, levels of these legacy POPs have declined in biota in most ecosystems (Dellinger et al., 2014; Dietz et al., 2013; Rigét et al., 2010). Nevertheless, although concentrations in top predators in many ecosystems have declined, levels of legacy POPs, such as PCBs are still very high and may be above threshold levels for population health effects. For instance it has been reported that PCB concentrations in polar bears (*Ursus maritimus*) are still significantly higher than modelled threshold levels for immune, reproductive and carcinogenic effects (Dietz et al., 2015) and that internal concentrations of POPs appear to affect sub-population sizes of polar bears (Nuijten et al., 2016). Furthermore, there are strong indications that high levels of POPs in killer whales (*Orcinus orca*) and harbour porpoises (*Phocoena phocoena*) in the NE-Atlantic, may be responsible for loss of reproductive success and calf recruitment, eventually resulting in population decline (Jepson et al., 2016; Murphy et al., 2015). Thus, there are still concerns that the levels of POPs that marine mammals currently experience may lead to health effects and resultant population level effects.

1.1 Background

The former large production and usage of POPs such as PCBs, OCPs and PBDEs has led to pollution of these compounds in environments throughout the world, including remote areas such as the Arctic (AMAP, 2004; Letcher et al., 2010). The OHCs are persistent, bioaccumulate in organisms and

biomagnify through the food webs (Borgå et al., 2004), and animals foraging in high trophic positions such as marine mammals are highly exposed (Letcher et al., 2010). Physiological and life trait characteristics such as long life span, late reproduction, and a thick subcutaneous lipid layer (blubber), generally leads to accumulation of high concentrations of lipophilic POPs in marine mammals, including cetaceans (Letcher et al., 2010).

Compared to other mammals, toothed whales (odontocetes) are known to have low ability to metabolize POPs and thus accumulate high concentrations in their tissues (Boon et al., 1997; Tanabe et al., 1988). The resultant high levels of these lipophilic contaminants in the tissues raise concern for the health of the animals, as these contaminants are known to interfere with several biological systems with serious effects on the individual as a result (Colborn et al., 1993). High levels of POPs have been associated with effects on body functions such as reproduction, immune function, growth and development in marine mammals (e.g. Helle 1976; Van Loveren et al. 2000; Reddy et al. 2001; Jepson et al. 2005). Continuous high contaminant loads in the tissues of the animals can thus have severe effects also at the population level, as previously indicated for Baltic seals (Helle, 1976; Helle et al., 1976; Reijnders, 1986), belugas (De Guise et al., 1995), killer whales and harbour porpoises (Jepson et al., 2016; Murphy et al., 2015) and polar bears (Nuijten et al., 2016).

1.2 Pilot whales

Long-finned pilot whales (*Globicephala melas*) are medium sized toothed whales of the dolphin family. Their distribution is in sub-polar and temperate zones in the Northern Atlantic and the Southern hemisphere (Figure 1). These subpopulations are geographically separated and are sometimes referred to as separate subspecies with the southern form known as *G. m. edwardi* and the northern form as *G. m. melas* (Klinowska, 1991). These two populations of long-finned pilot whales are, however, the same species, and to be distinguished from the related species of short-finned pilot whales (*Globicephala macrorhynchus*) which inhabit the warmer temperate and tropical areas not inhabited by the long-finned pilot whales, and with very little overlap in distribution between the two species (Klinowska, 1991; Rice, 1998). The term pilot whales in the text will subsequently refer to the long-finned pilot whales.

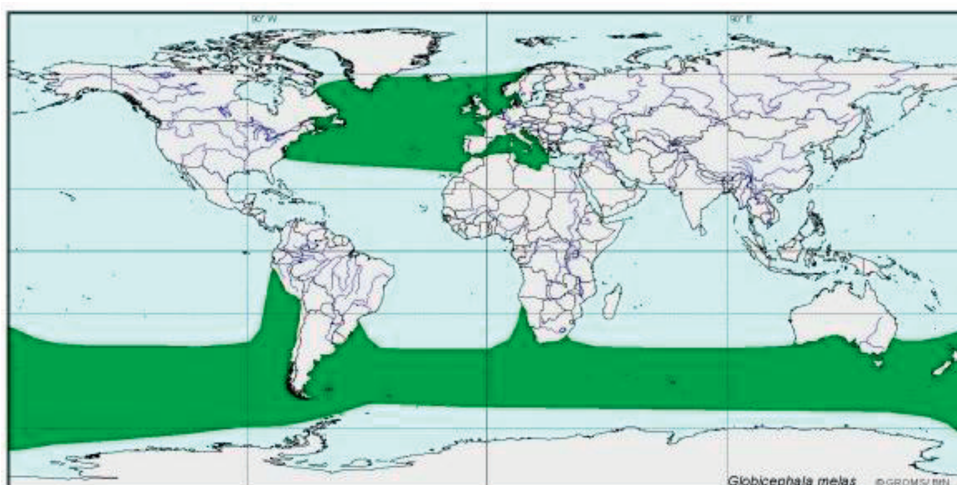

Figure 1 Distribution range for Long-finned pilot whales (Source: The Global Register of Migratory Species, www.groms.de)

Although there is a lack of evidence for the existence of separate sub-populations of pilot whales in the North–Atlantic, slight morphological differences between pilot whales from the western and Eastern North Atlantic (Bloch and Lastein, 1993) and genetic analyses (Fullard et al., 2000), support the division into a Western and an Eastern sub-population (ICES, 1991).

In the latest stock size estimate of pilot whales covering the whole North-East (NE) Atlantic area which was conducted in 1989, the stock size was estimated to be 778 000 whales (Buckland et al., 1993; NAMMCO, 1997). On the Faroe Islands this stock has been hunted and been part of the Faroese traditional diet for centuries (Bloch et al., 1993; Joensen, 2009, 1976). The whale hunt in the Faroe Islands is opportunistic and occurs year round with the main season from June to October (Zachariassen, 1993). The average annual take of around 1000 whales or less corresponds to an exploitation rate of 0.1% of the North-eastern population and has been considered sustainable (NAMMCO, 1997). Still, the IUCN Red List of Threatened Species defines the pilot whale to be data deficient, and the trend in the population is unknown (Taylor et al., 2008).

Pilot whales in the NE Atlantic live in schools of mostly 10 to 200 individuals, but the schools may include more than 1000 individuals (Bloch et al., 1993; Joensen, 1976). The schools generally consist of related females with offspring of both sexes and all ages. Genetic analyses indicate that the males in a school are rarely the fathers to the calves in that school, but the mating seem to occur when schools meet (Amos et al., 1991). The sex distribution in pilot whale schools in the NE Atlantic is skewed towards more females which constitute 60% (49-70%) of the stock (Bloch et al., 1993). The mean population structure of the schools has been found to be 46% immatures of both sexes, 12% mature males, the rest being adult females, hereof 12% pregnant females and 24% lactating females (Bloch et al., 1993). The oldest pilot whales recorded are 46 and 59 years for males and females, respectively, and the females also have a higher mean age than males (Bloch et al., 1993). Females reach reproductive age when they are 7-9 years and around 375 cm (Martin and Rothery, 1993), whereas males are 14-16 years and 475-505 cm when they reach reproductive maturity (Desportes et al., 1993). The gestation time is approximately 12 months and the calf has a mean size of 177 cm at birth (Bloch et al., 1993). The mean age of the calf at the start of weaning is 6.5 months (Desportes and Mouritsen, 1993), but the lactation generally proceeds until the calves are 1.5-3.5 years old (Martin and Rothery, 1993).

There are no obvious migration patterns for pilot whales but they seem to follow the movements of their prey which is preferently squid (*Todarodes sagittatus* and *Gonatus sp*) but also fish such as greater Argentine (*Argentina silus*) and blue whiting (*Micromesistius poutassou*) (Desportes and Mouritsen, 1993).

1.3 POPs: PCBs, OCPs, and PBDEs

Persistent organic pollutants include a wide range of man-made chemicals produced for several purposes and as industrial by-products (Figure 2). PCBs are industrial chemicals used for several purposes such as heat transfer fluid, organic diluents, plasticizers, fire retardants, paint additives and dielectric fluids for capacitors and transformers since the 1930ies (Safe, 1984, 1994). The chemical structure of PCBs is a biphenyl with a varying number of chlorine attached. There are 209 possible congeners, which are found in different concentrations in the technical products. The congeners have different toxicodynamics and persistency in the environment and biological systems and the effects of PCB exposure are thus related to the concentrations of the different congeners. The

toxicity of PCB is, at least in part, related to the binding to the Aryl hydrocarbon receptor (AhR) in the cells (Safe, 1994; Van den Berg et al., 2006). The affinity of PCB congeners to the AhR is related to their structure and thus congeners with no or less than two chlorine atoms in the *ortho* position are stable in a planar structure and can bind to the receptor (Safe, 1994).

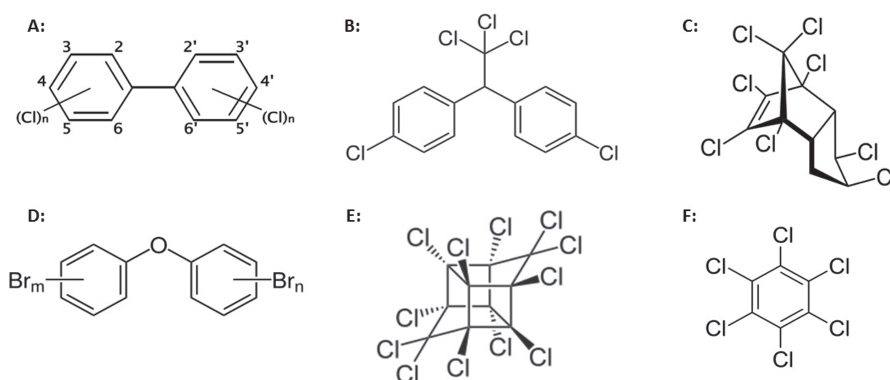

Figure 2 Examples of POP compounds. A: PCB, B: DDT, C: cis-chlordane, D: PBDE, E: Mirex, F: HCB

Organochlorine pesticides are a more diverse group of chlorinated compounds used as pesticides from the 30-50ties until 70-80ties when the use of most chlorinated pesticides were restricted in most developed countries (Turnbull, 1996). OCPs include chlordanes, mirex, HCB and DDT. DDT was extensively used until the 70ties and is still used in developing countries to fight malaria and typhus. In the Stockholm Convention DDT is listed in Annex B implying that its production and use should be restricted, but may be used by convention parties to combat vector borne diseases in accordance with defined criteria. DDT exists as the sum of various *ortho-para* (*o,p*) and *para-para* (*p,p*) isomers and metabolites of DDT, dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyldichloroethane (DDD). HCB is a fully chlorinated aromatic hydrocarbon and has been a widely used fungicide. Chlordane is a mixture of related chemicals such as cis- and trans-chlordane, cis- and trans-nonachlor, heptachlor and oxychlordane. Mirex is a fully chlorinated organic compound based on two linked five-member carbon rings. It has been used as an insecticide against fire ants since 1962 and its use was ceased in 1978 (www.inchem.org).

PBDEs have been used as flame retardants and were added to various products such as plastics, textiles, and electronics. The chemical structure is a diphenyl ether with varying numbers of bromine attached (as hydrogens are substituted with bromine). As with PCBs there are 209 congeners possible and there are three technical mixtures PentaBDE, OctaBDE and DecaBDE, named from the mean bromine content per molecule (Darnerud, 2003). PBDEs can leak out of the product since they are not chemically bound. PBDEs have been used since the 60ies but the usage of some PBDEs has been regulated in Europe since 2004 and the production was stopped in the USA in 2005 (www.epa.gov). PBDEs have later been included in the Stockholm POP convention (www.pop.int). The congeners have different stability and the environmentally most important congeners are BDE-28, -47, -99, -100, -153, -183 and 209 (Hakk and Letcher, 2003).

1.3.1 Metabolism of POPs

In addition to dietary intake, the concentrations of contaminants in tissues of animals is dependent on the ability of the animal to eliminate the compounds. However, due to their lipophilic properties, POPs are not easily excreted in mammals. The organism may, however, metabolize POPs, although to a varying degree depending on the chemical structure of the compound and the species-dependent ability to metabolize POPs (Boon et al., 1997; Tanabe et al., 1987). POPs can induce the synthesis of xenobiotic-metabolizing phase I and conjugating phase II enzymes which act in the process of making the compounds more water soluble and thus excretable from the organism (Hakk and Letcher, 2003).

The cytochrome P450 (CYP) monoxygenase system is the major enzyme system involved in the phase I metabolism of xenobiotics in mammals. The cytochrome P450 system consists of several enzyme families, some of which exhibit substrate induced genomal regulation of their enzyme activity following exposure of the animal to specific substrates or chemicals (Parke, 1990). The most important CYP gene families relevant for metabolization of POPs are CYP1A, CYP2B and CYP3A (Cederbaum, 2015; Goksøyr, 1995; Hakk and Letcher, 2003). The CYP1A forms are induced by planar molecules like dioxins, PAHs and non- or mono-*ortho* chlorinated PCBs by binding to the AhR. The CYP2B and CYP3A forms are induced by non-planar compounds like di- to tetra-*ortho*-PCBs and DDT (Hakk and Letcher, 2003). CYP3A enzymes are involved in the metabolism of steroids but also of foreign compounds like PCBs (McKinney et al., 2004). Other CYP enzymes are involved in endogenous processes such as steroid synthesis (Browne et al., 2006). Glutathione S-transferase (GST) and Uridine 5'-diphospho-glucuronosyltransferase (UDPGT) are important conjugating enzymes in xenobiotic metabolism (Jancova et al., 2010).

PCBs can be metabolized by insertion of an OH-group in the *meta* or *para* position of the PCB molecule catalysed by CYP enzymes either directly in *meta*-position or alternatively by catalyzing the formation of arene oxide intermediates in *meta-para* or *ortho-meta* position of the PCB congener (Letcher et al., 2000). The arene oxides may be further metabolized to OH-PCBs by epoxide hydroxylase or to methylsulfonyl metabolites ($MeSO_2$-PCBs) via the mercapturic acid pathway involving for instance GST. OH-PCBs may be further biotransformed and then eliminated by UDPGT involved in glucuronidation of OH-PCBs (Letcher et al., 2000).

PBDEs can, like the PCBs, be metabolized by CYP mediated oxidation with production of OH-metabolites (OH-PBDEs) and further by conjugations such as glucuronidation or sulfation, but can also be biodegraded by debromination (Hakk and Letcher, 2003).

DDT can undergo slow biodegradation through reductive dehydrodechlorination and dechlorination to form DDE and DDD, respectively, and then be further degraded to other metabolites (ATSDR, 2002). Further metabolism of DDE is slow and DDE is very persistent and even more persistent than DDT (ATSDR, 2002). DDTs (DDT, DDE and DDD) induce the CYP2B and 3A enzyme subfamilies (Nims et al., 1998). Oxidation products of the CYP metabolism of DDTs, especially DDE can be further metabolized, leading to methylsulfonyl metabolites such as $MeSO_2$-DDE (ATSDR, 2002).

In rodents, HCB has been shown to be metabolised via glutathion conjugation eventually to a sulphur containing derivative PCBT (Pentachlorobenzenethiol) (To-Figueras et al., 1997) and to a smaller degree to PCP (pentachlorophenol) via CYP3A (Den Besten et al., 1993). CYP2B and CYP3A enzymes are thought to be involved in the metabolism of chlordane and heptachlor in mammalian

systems (Kania-Korwel and Lehmler, 2013). Analyses indicate that Mirex is not metabolised in animals, but gut bacteria may be able to metabolise Mirex (Waters et al., 1977; WHO, 1990).

The metabolites produced by phase I and II metabolism, e.g. OH-metabolites or $MeSO_2$-metabolites, are in some cases reactive and thus more toxic than the original compound (Letcher et al., 2000). Cetaceans are known to be poor metabolizers of POPs due low or lacking expression of CYPs, especially CYP2B (Boon et al., 1997; Tanabe et al., 1988). However, studies have shown that PCBs are metabolized by liver enzymes in pilot whales and belugas (White et al., 2000). The analyses have shown induction of CYP1A, and CYP3A, whereas the occurrence and function of CYP2B enzymes in cetaceans is less clear (McKinney et al., 2004; White et al., 2000).

1.4 Endocrine distrupting effects of POPs

Of the most concerning biological effects of POPs exposure, is their ability to interfere with the endocrine systems (Colborn et al., 1993). Some POPs have structural similarities with endogenous hormones, and are able to interact with hormone transport proteins or to disrupt hormone metabolism. They can thus mimic or in some cases block the effects of endogenous hormones (O'Connor and Chapin, 2003; Sørmo et al., 2003). Possible mechanisms for endocrine disruption involve alterations in receptor-mediated signalling and post-receptor activation and alterations in hormone synthesis, transport, storage, release and metabolism (O'Connor and Chapin, 2003). Some of the endocrine systems that appear to be particularly affected by endocrine disrupting chemicals are the reproductive steroid hormone system, the thyroid hormone system, as well as vitamin homeostasis such as vitamin A (retinoids), vitamin E (tochopherol) and vitamin D (Colborn et al., 1993; Letcher et al., 2010). Biomarkers are biological responses that can be related to an exposure to, or toxic effect of, an environmental chemical or chemicals (Peakall, 1994), and the analyses of these hormones and vitamins can be used as biomarkers for POP exposure and/or effects.

1.4.1 Steroid hormones

In mammals sex steroids are synthesized primarily in the gonad, placenta and adrenal gland and are essential for reproduction and important for sexual behaviour (Norris, 2006). Steroid hormones function in the maintenance of reproductive tissue and coordination of reproductive events. The production of sex hormones are regulated by the hypothalamus – pituitary – gonadal (HPG) axis (O'Connor and Chapin, 2003). Gonadotropin releasing hormone (GnRH), secreted from the hypothalamus, stimulates release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the anterior pituitary to the blood stream. FSH and LH stimulate the secretion of androgens and estrogens from the testes and ovaries. The processes are regulated by feed-back loops, where the androgen and estrogen compounds inhibit the release of LH and FSH from the pituitary gland and GnRH from the hypothalamus (O'Connor and Chapin, 2003).

Steroids are synthesised from cholesterol involving several enzymes, including members of the cytochrome P450 family (Figure 3). The first step is the conversion of cholesterol to pregnenolone (PRE) by CYP11A1. Androgens can then be produced by two different pathways (Conley and Bird, 1997); the Δ-4 pathway where PRE is converted to progesterone (PRO) by 3β hydroxysteroid dehydrogenase (3βHSD) and then to 17α hydroxyprogesterone and further to androstenedione (AN) by CYP17, or the Δ-5 pathway where PRE is converted to 17α hydroxypregnenolone and further to dehydroepiandrostenedione (DHEA) by CYP17. DHEA is then further converted to AN by 3βHSD or by 17β hydroxysteroid dehydrogenase (17βHSD) to androstenediol (AL) which then is converted to

testosterone (TS) by 3βHSD. Estrogens are synthesized from the androgens by CYP19 (aromatase). AN can be converted to estrone (E1), which further can be converted to estradiol (βE2) by 17βHSD, and TS can be converted to βE2. AN can also be converted to testosterone (TS) by 17βHSD. Which pathway is the dominating differs between species with the Δ-5 pathway dominating in humans, primates and sheep, whereas in pigs, rats and mice the AN synthesis occurs by either the Δ-4 or the Δ-5 pathway (Conley and Bird, 1997). There is a lack of information on the steroidogenesis pathways in wildlife mammals. However, the Δ-4 pathway seems to be the dominant pathway in polar bears (Gustavson et al., 2015b), whereas the dog, although relatively related, appears to be a Δ-5 species (Sonne et al., 2014). Whether species have a Δ-5 or a Δ-4 pathway may influence the effects of POPs on the reproductive hormone system, and is thus important to take into consideration when using results from laboratory models to understand effects in wildlife species.

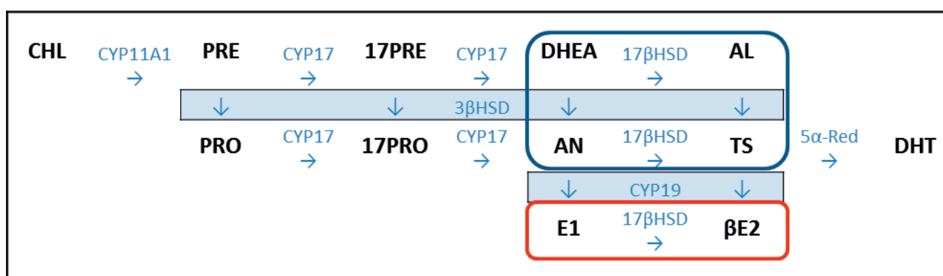

Figure 3 Diagram of the steroid synthesis pathways for synthesis of androgens (blue box) and estrogens (red box). *Abbrevations used*: CHL: Cholesterol, PRE: Pregnenolone, PRO: Progesterone, 17PRE: 17α-hydroxypregnenelone, 17PRO: 17α-hydroxyprogesterone, DHEA: dehydroepiandrostenedione, AN: Androstenedione, AL: Androstenediol, TS: Testosterone, DHT: Dihydrotestosterone, E1: Estrone, βE2: Estradiol, 3βHSD: 3β-hydroxysteroid dehydrogenase, 17βHSD: 17β-hydroxysteroid dehydrogenase, 5α-Red: 5α reductase. Adapted from Browne et al. (2006).

Thus by interfering with steroid hormone synthesis, transport, storage, release and metabolism or binding to steroid hormone receptors, POPs can disturb the reproductive hormone system, and the reproductive function of wildlife mammals (Colborn et al., 1993; O'Connor and Chapin, 2003). The developing foetus is uniquely sensitive to endocrine disruption and analyses using experimental animals have indicated that low dose exposure of PCB in utero and in the suckling period can influence reproductive functions (O'Connor and Chapin, 2003; Ropstad et al., 2006).

1.4.2 Thyroid hormones

Thyroid hormones (TH), thyroxine (T4) and triiodothyronine (T3), control metabolism, cell differentiation and growth and are essential for normal reproduction (Rolland, 2000), and POPs that affect thyroid hormones and the thyroid system may thus also affect the reproduction of exposed animals. The synthesis of THs is controlled through negative feedback by the hypothalamus-pituitary-thyroid (HPT) axis (Kirby, 1990) where thyrotropin releasing hormone (TRH) secreted from the hypothalamus triggers the release of thyroid stimulating hormone (TSH, thyrotropin) from the pituitary gland (St Aubin, 1987). TSH stimulates the production of the THs (T4 and T3) in the thyroid gland, which subsequently when released into the blood stream can inhibit the production of TSH from the pituitary (Kirby, 1990). T4 is transported in plasma to target tissues by binding to the thyroxine plasma transport protein (TTR), thyroxine binding globulin (TBG) or albumin. When delivered T4 is deiodinated by T4-monodeiodinase to T3, which is the active hormone. T3 binds to the thyroid hormone nuclear receptor (TR) and activates transcription (Gregory and Cyr, 2003).

Several mechanisms can be involved in the POP-mediated disruption of THs (Brouwer et al., 1998; Liu et al., 2014). OH-metabolites of POPs like PCBs or PBDEs can inhibit thyroxine (T4) binding to thyroxine plasma transport protein (TTR) by competing with T4 for TTR binding sites with the result of loss in T4 and retinol-RBP from the body (Brouwer et al., 1989a; Bytingsvik et al., 2013; Murk et al., 1998). POPs can also interact with thyroid gland function and morphology, leading to effects on the synthesis and secretion of T4 (Brouwer et al., 1998), including effects on deiodination enzymes (Gabrielsen et al., 2015). Also thyroid metabolism can be affected by POP exposure by induction of enzymes leading to increased glucuronidation of hepatic T4 and increased biliary secretion and elimination of T4 (Brouwer et al., 1998).

The T4-TTR complex is transported in the blood plasma in a complex with retinol bound to the retinol binding protein (RBP) (Rolland, 2000; Simms and Ross, 2000). Analyses in experimental animals have shown that POPs are able to interact with thyroid hormones and vitamin A (retinoids) in mammals (Brouwer et al., 1989a, 1989b). This interaction is mainly caused by hydroxy metabolites of POPs such as OH-PCBs or OH-PBDEs.

1.4.3 Vitamin A, E and D

Retinoids (Vitamin A and its metabolites) are lipophilic molecules essential for various physiological functions, including vision, growth, reproduction, immune function and cellular division and differentiation in mammals (Borrell et al., 2002; Debier and Larondelle, 2005; Rolland, 2000). Disruption of the levels of retinoids can thus potentially lead to impairment of these functions in mammals. Vitamin A is derived from the diet mostly as retinol esters (RE) and is sometimes referred to as “dietary hormones” (Simms and Ross, 2000). Retinol is the main circulating retinoid and the precursor of retinoic acid (RA), which is the biologically active metabolite of vitamin A (Thatcher and Isoherranen, 2009). The conversion of retinol via retinal to RA is catalysed via dehydrogenases and enzymes of the CYP450 family (Debier and Larondelle, 2005). The catabolism of retinoic acid may occur via hydroxylation by CYP26, a RA inducible CYP enzyme, but other CYP enzymes, such as CYP3A and CYP2C, which are known to be inducible by pollutants like PCB and other OHCs, are also involved in metabolism of retinoids (Ross and Zolfaghari, 2004; Thatcher and Isoherranen, 2009).

POPs and their metabolites can reduce uptake of dietary vitamin A, decrease liver vitamin A stores, disrupt circulatory transport to tissues and/or increase glomerular filtration and excretion of vitamin A metabolites (Simms and Ross, 2000). In marine mammals, retinoids are stored mainly in the liver, but also in other tissues like the blubber, mainly as retinyl esters (Borrell et al., 2002). Although there is great variation in the vitamin supply from the diet and in the liver or extrahepatic tissue stores, vitamin A levels remain constant in plasma apparently due to homeostatic regulation. Thus, body depletion of retinoids can better be evaluated through concentrations in depot tissues such as liver and blubber than in the blood (Borrell et al., 2002) and negative correlations have been found between POP concentrations and retinoid levels in blubber (Nyman et al., 2003; Tornero et al., 2005, 2004). However, negative relationships between contaminants and retinol levels in plasma have also been reported (Brouwer et al., 1989; Jenssen et al., 2003; Molde et al., 2013). Analyses in beluga from Arctic Canada found that PCB correlated negatively with vitamin A in liver, but positively in blubber and plasma (Desforges et al., 2013a).

Vitamin E refers to a group of tocopherols that function as chain breaking antioxidants preventing the propagation of free radical reactions (Brigelius-Flohe and Traber, 1999) with α-tocopherol as the

most active form in mammals. Vitamin E is supplied through the diet and α- and γ-tochopherols are the most common dietary tocopherols (Debier and Larondelle, 2005). Several POPs have properties of inducing free-radicals as a by-product of metabolic oxidation (Abdollahi et al., 2004; Fernie et al., 2005; Valavanidis et al., 2006). Positive correlations between POP concentrations and vitamin E in blubber and plasma have been reported in marine mammals and shark (Desforges et al., 2013a; Molde et al., 2013; Nyman et al., 2003; Routti et al., 2005) and vitamin E has been suggested as a biomarker for PCB and DDT exposure (Nyman et al., 2003). However, vitamin E in liver has shown negative correlations with POPs indicating depletion of the liver stores in relation to increased plasma levels (Desforges et al., 2013a).

Vitamin D_3 has several important roles in the organism, including mineral homeostasis, and is involved in calcium metabolism and bone mineralization together with other endocrine hormones. The predominant form of vitamin D is 25-hydroxyvitamin D_3 (25(OH)D3). Vitamin D can be produced by cutaneous exposure to ultraviolet-b light in terrestrial mammals, but fish eating marine mammals like pilot whales are able to satisfy their vitamin D_3 requirements from the diet (Kenny et al., 2004). Vitamin D has been shown to be influenced by body concentrations of OHCs in grey seals (*Halichoerus grypus*) (Routti et al., 2008).

1.5 POP exposure in pilot whales

In hunted pilot whales from the Faroe Islands, high concentrations of POPs like PCBs, PBDEs, DDTs, toxaphenes and other OCPs have been documented since the 70ies and have been continuously monitored since the mid 90ies (Borrell, 1993; Borrell and Aguilar, 1993; Dam, 2001; Dam and Bloch, 2000; Hoydal and Dam, 2009, 2005, 2003; Lindstrom et al., 1999).

In the late 1980ies the concentrations of PCBs and DDTs in pilot whales in Faroese waters were reported to be intermediate compared to pilot whales from other parts of the North Atlantic (Borrell and Aguilar, 1993). Faroese pilot whales were less polluted than pilot whales from the coast of the United States and France (Borrell and Aguilar, 1993; Taruski et al., 1975), but more polluted than pilot whales from Newfoundland, Canada (Muir et al., 1988). Since then few analyses have been reported of contaminant levels/concentrations in pilot whale in other areas than the Faroes waters. There are reports of analyses of POPs in blubber of one individual from the Irish Sea in 1987-89 (Troisi et al., 1998), in blubber and liver of pilot whales from the Cape Cod Bay (Gulf of Maine, NW Atlantic) from 1990-1996 (Weisbrod et al., 2001, 2000), and in blubber and liver, and for some individuals also kidney, brain and ovaries, of pilot whales stranded along the Massachusetts coast, US, in 1986 and 1990 (Tilbury et al., 1999). The concentrations from these studies are at the same level as in the pilot whales from Faroese waters around the same time (Dam, 2001; Dam and Bloch, 2000). Compared to other cetacean species living in the same area, pilot whales were found to have higher concentrations of PCB and DDT than harbour porpoise and similar concentrations as Atlantic white-sided dolphins (*Lagenorhynchus acutus*), all from Faroese waters (Borrell, 1993). The PCB concentrations pilot whales in Faroese waters in the 1990'ies were among the highest reported in mammals from the Arctic, however, surpassed by Alaskan killer whales (AMAP, 2004). The highest reported concentrations were similar to or exceeded those expected to be linked to reproductive failure in other cetaceans (Reddy et al., 2001). Also high concentrations of PBDEs in pilot whale blubber from the Faroe Islands have been reported (Rotander et al., 2012; van Bavel et al., 2001). Recent studies of pilot whales from the Mediterranean have shown higher levels than the most recent studies in the pilot whales from the Faroe Islands (Nielsen et al., 2014; Pinzone et al., 2015).

Much less information is available on levels of POPs in pilot whales from the Southern hemisphere. However, recent studies of PCBs in pilot whales from Tasmania (Weijs et al., 2013) show that concentrations of POPs are maximum on fifth to one tenth of the levels reported in the same species in Faroese waters.

As most mammals, the pilot whales are predominantly exposed to POPs through their diet (Aguilar et al., 1999). The contaminant pattern in organisms is determined by the relative concentrations in the prey and their ability to excrete and/or metabolize and eliminate the contaminants (Letcher et al., 2000). In addition to metabolization, reproducing females can eliminate lipophilic POPs to their developing foetus during gestation and to the offspring through their mother's milk (Borrell et al., 1995). The exposure of the calf during gestation is however, low compared to the exposure from milk during lactation. According to Borrell et al. (1995) in Faroese pilot whales 4-10% of the mothers body load can be transferred through gestation, whereas 60-100% can be transferred through lactation. This means that calves are highly exposed at an exceptionally vulnerable time. The exposure to POPs during gestation and suckling is dependent on the POP concentration in the mother. Since immature females accumulate POPs as a function of their age, primiparae females have relative high body burdens of POPs, and will transfer most of these contaminants to her offspring during her first pregnancy and subsequent lactation. Calves from first pregnancies are thus more exposed than calves from later pregnancies (Borrell et al., 1995).

The decreasing trends in POP concentrations in the environment due to ban of the use in most parts of the world since the 70ies has also been reported in pilot whales from the Faroe Islands (Hoydal and Dam, 2009; Nielsen et al., 2014). The decrease does, however, not seem to be as obvious in the adult females as in the juveniles and adult males (Nielsen et al., 2014). The non-decreasing POP trend in adult females compared to the decreasing trend in the other sex/age groups could indicate that the females are older before they become reproductively active or are overall less reproductively active, such as suggested in other cetaceans (Jepson et al., 2016; Murphy et al., 2015; Reddy et al., 2001).

1.6 Aim of the study

The overall aim of the present thesis was to investigate the levels of legacy POPs in Faroe Island pilot whales and determine to which extent the sex and age class of the whales influences the internal concentrations of these compounds and their possible endocrine effects. Correlative studies were performed to investigate possible effects on thyroid hormones, sex steroid hormones and the vitamins A, E and D. In addition, the aim was to study the ability of these whales to biotransform these compounds into metabolites, which also may have endocrine disruptive effects.

Concentrations of POPs (PCBs, OCPs and PBDEs), as well as relevant metabolites of these POPs, were analysed in liver and plasma of adult female and male and juvenile pilot whales. In addition, CYP enzymes involved in the metabolism of POPs were analysed in liver samples. Plasma concentrations of steroid hormones, thyroid hormones and vitamin D were analysed, and concentrations of vitamin A and E were analysed in both plasma and liver samples.

2 Methods

2.1 Sampling

Samples of whale blood and liver were collected during the traditional pilot whale hunt on the Faroe Islands in 2009, 2010 and 2011. Blood and liver sample pairs were sampled from a total of 27 whales, of which 14 were males and 13 females. Eleven whales of these were sampled in Sandagerði, Tórshavn on July 23, 2010, and 16 whales in Vestmanna on September 2, 2011. Liver samples from additionally 10 whales were sampled in 2009 and 2010; seven of these were sampled in Hvalvík on May 23, 2009, and three in Sandagerði, Tórshavn on July 2, 2010. See Figure 4 for the location of the sampling locations.

Figure 4 Map showing the Faroe Islands and the sample locations.

Blood samples were collected into clean heat treated (at 450°C for four hours) glass jars, containing heparin, and kept on ice until further sample preparation. The blood was centrifuged at 1500 *g* for five minutes and plasma was transferred into cryovials and frozen in liquid N_2. The samples were stored at -80°C until analysis. Liver samples were taken as soon as the whales had been transported from the beach onto the quay where the sampling was carried out. A piece of the distal part of the liver was wrapped in heat treated aluminium foil and frozen in liquid N_2 and later stored at -80°C until analysis.

The length of the individuals was measured (cm). For some of the whales the length was not measured and the length was instead estimated from the *skinn* value. *Skinn* is a measure unit for the mass of the whale used on the Faroe Islands when dividing the amount of meat and blubber among

the community and/or the hunters for human consumption (Bloch and Zachariassen, 1989). The lower jaw was sampled from twenty of the individuals, and the age was determined by counting growth layer groups formed annually in dentine and cement of teeth as described in Lockyer (1993). An overview of the biometrics and age of the sampled pilot whales is given in Table 1.

Table 1 Biological parameters of the individual pilot whales sampled. The length of the whales sampled in 2009 was not measured, but estimated from the ancient Faroe measuring unit "skinn" using regression analysis.

Date	ID	US ID	Length, cm	*Skinn**	Sex	Age, years	Age/sex group	Age group	Liver	Plasma
23.05.2009	230509-004	4	*457*[a]	10	M		Juvenile male	Juvenile >2y	x	
23.05.2009	230509-008	8	*574*[a]	16	M		Adult male	Adult	x	
23.05.2009	230509-029	29	*496*[a]	12	M		Adult male	Adult	x	
23.05.2009	230509-046	46	*263*[a]	11	F		Juvenile female	Juvenile >2y	x	
23.05.2009	230509-052	52	*594*[a]	17	M		Adult male	Adult	x	
23.05.2009	230509-057	57	*555*[a]	15	M		Adult male	Adult	x	
23.05.2009	230509-185	185	*672*[a]	21	M		Adult male	Adult	x	
02.07.2010	020710-003	3	405	10	F		Adult female	Adult	x	
02.07.2010	020710-007	7	330	5	F		Juvenile female	Juvenile >2y	x	
02.07.2010	020710-009	9	445	9	F		Adult female	Adult	x	
23.07.2010	230710-049	US 6	465	11	M	24	Adult male	Adult	x	x
23.07.2010	230710-085	US 7	435	9	F	25	Adult female	Adult	x	x
23.07.2010	230710-092	US 8	505	13	M		Adult male	Adult	x	x
23.07.2010	230710-063	US 9	205	2	M	<1	Juvenile male	Juvenile 0-2y	x	x
23.07.2010	230710-056	US 13	420	8	F	25	Adult female	Adult	x	x
23.07.2010	230710-060	US 14	450	10	F	22	Adult female	Adult	x	x
23.07.2010	230710-003	US 15	270	3	M	<1	Juvenile male	Juvenile 0-2y	x	x
23.07.2010	230710-032	US 17	300	3	F	2	Juvenile female	Juvenile 0-2y	x	x
23.07.2010	230710-106	US 18	460	10	F		Adult female	Adult	x	x
23.07.2010	230710-079	US 19	380	6	M		Juvenile male	Juvenile >2y	x	x
23.07.2010	230710-072	US 20	425	8	F		Adult female	Adult	x	x
02.09.2011	020911-049	US 21	309	3	M	2	Juvenile male	Juvenile 0-2y	x	x
02.09.2011	020911-066	US 22	439	9	M	13	Juvenile male	Juvenile >2y	x	x
02.09.2011	020911-046	US 23	390	6	F	10	Juvenile female	Juvenile >2y	x	x
02.09.2011	020911-069	US 24	438	8	M	11	Juvenile male	Juvenile >2y	x	x
02.09.2011	020911-032	US 25	349	4	M	4	Juvenile male	Juvenile >2y	x	x
02.09.2011	020911-075	US 26	199	1	M	<1	Juvenile male	Juvenile 0-2y	x	x
02.09.2011	020911-005	US 28	429	7	F		Adult female	Adult	x	x
02.09.2011	020911-058	US 29	322	4	F	3	Juvenile female	Juvenile >2y	x	x
02.09.2011	020911-053	US 30	356	5	M	2	Juvenile male	Juvenile 0-2y	x	x
02.09.2011	020911-018	US 40	379	6	F		Adult female	Adult	x	x
02.09.2011	020911-073	US 41	424	7	F	21	Adult female	Adult	x	x
02.09.2011	020911-025	US 43	262	2	M	1	Juvenile male	Juvenile 0-2y	x	x
02.09.2011	020911-019	US 44	424	7	F		Adult female	Adult	x	x
02.09.2011	020911-067	US 47	442	8	F	17	Adult female	Adult	x	x
02.09.2011	020911-003	US 48	434	8	M	11	Juvenile male	Juvenile >2y	x	x
02.09.2011	020911-040	US 49	162	2	M	<1	Juvenile male	Juvenile 0-2y	x	x

**Skinn* is a measure unit for the mass of the whale used on the Faroe Islands when dividing the amount of meat and blubber for human consumption. One *skinn* is corresponding to on average 34kg of blubber and 38kg of meat (see Bloch & Zachariassen, 1989).
[a]Length estimated from the *skinn* size.

2.2 Division into age/sex groups

The individuals were categorized into age and sex groups according to Desportes et al. (1993) and Martin and Rothery (1993), where females smaller than 375 cm and less than eight years of age and males smaller than 494 cm and less than 14 years of age were categorized as juveniles. The juvenile group was further categorized into two groups: Those that were less than two years old (calves: 0 - 2 years), and those that were older than two years old (sub-adults: > 2 years). This was done to distinguish between suckling juveniles that are still exposed to maternally transferred contaminants via suckling and those that are older and do not consume mothers milk. Lactation in Faroe Island

pilot whales has generally been shown to proceed until the calves are between 1.5 - 3.5 years old (Martin and Rothery, 1993). Thus, the calves (0 – 2 years) would be expected to receive a milk-transferred load of POPs from their mothers, whereas the sub-adults (> 2 years) would receive POP loads mostly from solid food.

2.3 Analysis of contaminants

The 27 plasma and liver sample pairs were analysed for 74 PCB congeners, 20 OCPs (chlorobenzenes, chlordanes, DDTs, Dieldrin, Mirex), 14 PBDE congeners, BFRs, 33 OH-PCBs, pentachlorophenol (PCP), 4-OH-heptachlorostyrene, 14 OH-PBDEs, 16 $MeSO_2$-PCBs and 3-$MeSO_2$-*p,p'*-DDE at the Organic Contaminants Research Laboratory/Letcher Labs at the National Wildlife Research Centre, Carleton University in Ottawa, Canada, using gas chromatography (GC) and quadrupole mass spectrometry (MS) as described in Paper I.

2.4 Analysis of phase I and phase II enzyme induction

The liver samples were analysed for CYP enzyme induction by analysing mRNA expression, protein expression and enzyme activity as described in detail in Paper II. In brief, cDNA was generated from isolated RNA from the liver and used for analysis of *cyp1a1*, *cyp3a29*, *cyp26a1*, *cyp26b1* and *AhR* mRNA transcripts using quantitative real-time PCR (Paper II). Microsomes were prepared from liver samples and CYP1A, 2B, 2E, 3A and CYP26 protein expression was analysed by immunochemical assays (Paper II). Western blot analyses were performed using polyclonal rabbit antibodies and monoclonal mouse antibodies (Mouse anti-rat CYP2B1/2, mouse anti-human CYP3A4, rabbit anti-human CYP2E1, rabbit anti-human CYP2B6, mouse anti-fish CYP1A, rabbit anti-human CYP26, rabbit anti-fish CYP3A and rabbit anti-fish CYP1A to detect the proteins. CYP3A was also semi-quantitatively analysed by the ELISA method using the rabbit anti-fish CYP3A antibody. Goat anti-rabbit/mouse horseradish peroxidase-conjugated secondary antibody was used for visualization in both analyses.

Liver CYP enzyme activities were measured by quantifying ethoxyresorufin-, benzyloxyresorufin-, methoxyresorufin- and pentoxyresorufin-O-deethylase (EROD, BROD, MROD and PROD, respectively) activities (Paper II). UDPGT activity towards p-nitrophenol (p-NP) metabolism was measured in microsomal samples as described by Andersson et al. (1985) and GST activity in hepatic cytosolic samples were measured using 1-chloro-2,4-dinitrobenzene (CNDB) as substrate as described by Habig et al. (1974). Total protein concentration was determined with the method of Bradford (1976), using bovine serum albumin (BSA) as standard.

2.5 Analysis of thyroid hormones

Plasma samples were analysed for of total thyroxine (TT4), total tri-iodothyronine (TT3), free thyroxine (FT4) and free tri-iodothyronine (FT3) at the Department of Biology, NTNU, using solid-phase 125I radioimmunoassay (Coat-A-Count, Diagnostic Products, Los Angeles, CA, USA) as described in Paper IV.

Efforts were also made to analyse plasma concentrations of thyroid stimulating hormone (TSH) using a solid phase 125I immunoradiometric (IRMA) kit developed for canines (Coat-A-Count Canine TSH IRMA, Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA). However, the concentrations of TSH in the pilot whale plasma were lower than the lowest standard, although they were higher than the zero (NSB). Thus, unfortunately, these results had to be omitted from the study.

2.6 Analysis of vitamins in liver and plasma

The plasma samples were analysed for retinol (vitamin A), α-tocopherol (vitamin E) and 25(OH)D3 (vitamin D) by HPLC-MS-MS at Department of Analytical Chemistry, Gdańsk University of Technology, Poland. Liver samples were analysed for total vitamin A (total retinol) at the University in Barcelona, The Department of Animal Biology using reverse phase HPLC, and for different forms of vitamin A and E (retinol, retinyl palmitate, α-tocopherol and γ-tocopherol) by reverse-phase HPLC with fluorescence detection (PerkinElmer200 series, USA) at the Department of Biology, NTNU. The vitamin analyses are described in more detail in Paper IV.

2.7 Analysis of steroid hormones

The plasma samples were analysed for the most important steroid hormones, which included the progestagens, progesterone (PRO) and pregnenolone (PRE), the androgens androstenedione (AN), dehydroepiandrostenedione (DHEA), dihydrotestosterone (DHT) and testosterone (TS) and the estrogens estrone (E1), 17β-estradiol (βE2) and 17α-estradiol (αE2) using Solid Phase Extraction (SPE) according to the method described by Hansen et al. (2011). The analyses were conducted at the Toxicology Laboratory, Department of Pharmacy, University of Copenhagen, Denmark. A more detailed description of the steroid analyses is given in Paper III.

2.8 Statistical analyses

The statistical programs SPSS (IBM, version 21) and SIMCA-P+ (Umetrics, version 12.0, 2008) were used for the statistical analyses. Normal distributions of the data were analysed using Shapiro-Wilk normality test. Differences in contaminant concentrations between age/sex groups were analysed by a non-parametric Kruskall–Wallis 1-way ANOVA (all pairwise) test, since the contaminant data were not all normally distributed (Paper I). Differences between concentrations in liver and plasma were tested with pairwise t-test and correlations between contaminants in liver and plasma and between metabolites and precursor compounds were analysed with Spearman correlation (Paper I).

The relationships between contaminants and the analysed vitamins, hormones and phase I and II enzyme expressions were analysed with multivariate statistical analyses Principal component analysis (PCA) and/or Orthogonal projections to latent structures (OPLS) using SIMCA (Paper II - V). Biological parameters (age and length) were included in the analyses as covariates. PCA and PLS are methods that are able to deal with data consisting of a lower number of observations (individuals analysed) than variables, and with strongly correlated data (Eriksson et al., 2013). A detailed description of the methods is given in Eriksson et al. (2013) and Bylesjö et al. (2006).

Differences in the analysed biological variables between age/sex groups and the significance of relationships observed in the multivariate analyses were further analysed with univariate analyses (ANOVA, Pearson correlation or linear regression for normally distributed data and Kruskal-Wallis or Spearman correlation for non-normally distributed data) (Paper II – V). P-values < 0.05 were considered as significant.

3 Results and Discussion:

3.1 POP concentrations and maternal transfer

Pilot whales are exposed to a mixture of different groups of POPs reflecting the accumulation of the contaminants through the food web. In this study 175 different OHCs were analysed, and of these compounds 59 were detected in liver and 27 were detected in plasma of the pilot whales (Paper I). No recent reports of POPs in liver and plasma of pilot whales have been reported from other areas and populations. However, compared to recent analyses of contaminants in blubber of pilot whales, the concentrations in the Faroese pilot whales analysed in the present studies are intermediate, i.e. lower than in pilot whales from the Mediterranean but much higher than in pilot whales from the Southern hemisphere (Tasmania) (Nielsen et al., 2014; Pinzone et al., 2015; Weijs et al., 2013). The concentrations of POPs in pilot whale liver and plasma generally seemed to be higher than reported in these tissues in cetaceans from the Arctic (Canadian Arctic and Svalbard) and the North Sea (Kelly et al., 2008; McKinney et al., 2006; Weijs et al., 2010), but lower than in cetaceans from the Mediterranean, the Southern part of the Atlantic and US East coast and from the highly polluted St. Lawrence river (Carballo et al., 2008; Houde et al., 2009; Jiménez et al., 2000; McKinney et al., 2006; Montie et al., 2008).

In the present pilot whales, the main contaminant groups in both liver and plasma were PCBs and OCPs that contributed around 50% each, whereas the PBDE/BFRs only contributed to 1-3% of the total POP concentration. Of the single compounds analysed, *p*,*p*'-DDE was the compound found in highest concentrations constituting approximately 70% of the OCP concentration and around 30% of the total POP concentration in both liver and plasma. Of the PCBs, CB-153 and CB-138 showed the highest concentrations. These relative distributions of congeners and compound groups in liver and plasma of the pilot whales was generally comparable to the distribution previously reported in blubber of pilot whales (Nielsen et al., 2014), and to what has previously been reported in tissues of other toothed whale species (Bennett et al., 2009; Carballo et al., 2008; Desforges et al., 2013b; Fair et al., 2010; Hansen et al., 2004; McKinney et al., 2006; Weijs et al., 2009a).

In both liver and plasma, the POP concentrations were significantly lower in adult females as compared to in juveniles, both calves and sub-adults (Paper I). The concentrations in the juveniles were generally four to ten times higher than in adult females (Figure 5). Although several mechanisms can be interacting leading to these differences between age groups, such as differences in prey preferences and differences in ability to metabolize the compounds, the significantly lower POP concentrations in adult females than in the juveniles, can largely be explained by the transfer of these lipophilic POPs from the mother to offspring during gestation and to a larger extent during lactation (Borrell et al., 1995; Wells et al., 2005). In pilot whales from Faroe Islands, the transfer of organochlorines (PCB and DDT) to the offspring has been estimated to represent 60 - 100% of the mothers body load during lactation and 4 - 10% during gestation (Borrell et al., 1995). The lower levels in adult females than in juveniles is in general accordance with what has been reported in other cetaceans (Fair et al., 2010; Weijs et al., 2009b; Yordy et al., 2010). Although, in the present study, the POP concentration was analysed only in a few adult males, the concentrations in both the blood and livers of these were similar to that of the calves and sub-adults (Figure 5).

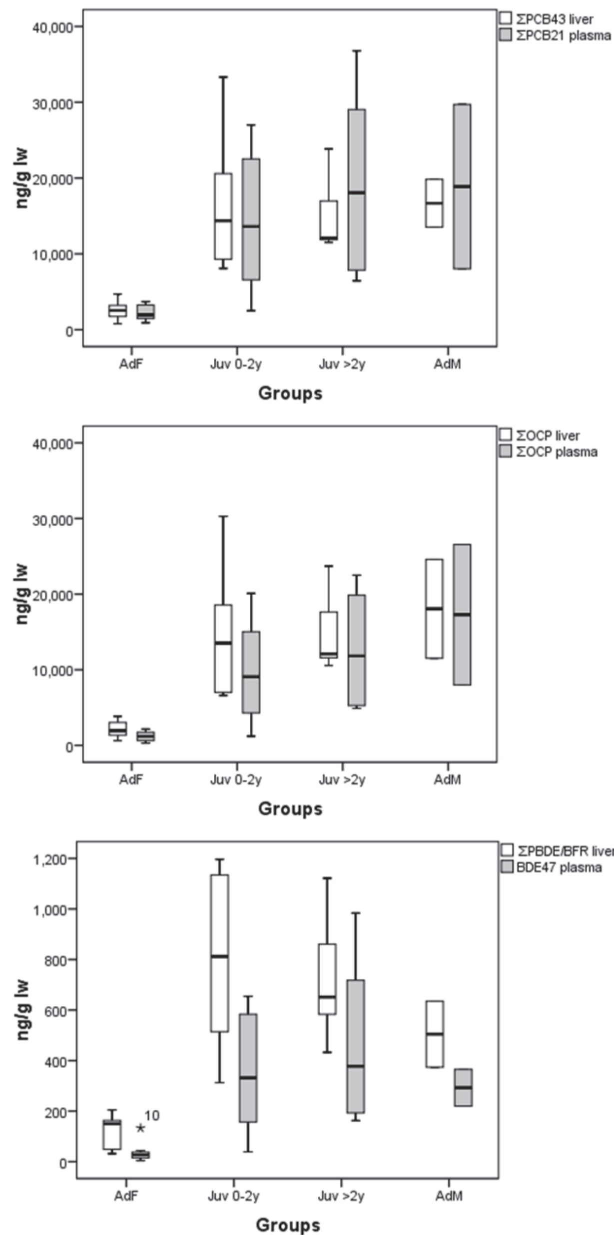

Figure 5 Box plots for concentrations of mean ΣPCB, ΣOC and ΣPBDE concentrations in ng/g lipid weight in liver and plasma of pilot whales divided into age and gender groups of adult females (AdF, n=10), juveniles 0 - 2 years old (0 - 2y, n=8), juveniles more than 2 years old (> 2y, n=7), and adult males (AdM, n=2). Figure from Paper I.

In Paper I it was shown that the relative concentrations of single POPs differed in adult females and the calves (0-2 years old), indicating that the maternal transfer of the various compounds occurred at different rates. Highly halogenated compounds with high molecular weight (MW) and higher lipid

solubility seemed to be transferred less easily than compounds with lower lipid solubility (Log K_{ow}), MW and halogenation degree. These parameters are inter-correlated and difficult to distinguish, but MW and log K_{ow} seemed to be the most important variables (Paper I). These findings are in accordance with analysis in bottlenose dolphins, which showed that compounds with Log $K_{ow} > 6.5$ were transferred to a much lesser degree to milk, whereas the transfer of compounds with Log $K_{ow} < 6.5$ appeared to be non-selective (Yordy, 2009). It should also be noted, that in beluga whales the transfer during gestation has shown the same dependency of the Log K_{ow} values of the compounds, with compounds with Log K_{ow} values < 6.5 being transferred to the foetus to a higher degree than the compounds with higher Log K_{ow} values (Desforges et al., 2012).

The present study shows that the adult females have significantly lower concentrations of POPs most likely due to the transfer of POPs to the offspring during gestation and lactation. Smaller, less halogenated and less lipid soluble compounds are transferred more easily, leading to differences in relative concentrations of the different POPs between adult females and juveniles.

3.2 CYP enzyme induction and POP metabolism

The toxicity of POPs is not always due to exposure to the parent POP compounds themselves, but in several cases it is the metabolites that are responsible for the toxic effect (Brouwer et al., 1998; Kester et al., 2000; Letcher et al., 2000). The metabolism of POPs in the exposed animal is thus important to consider when assessing the toxic effects of POPs (Letcher et al., 2000). The enzyme system responsible for most of the OHC metabolism is the cytochrome P450 system, in particular the CYP1, 2 and 3 families (Cederbaum, 2015; Goksøyr, 1995).

CYP1A, CYP2B, CYP2E and CYP3A protein expression and cyp1a1, cyp3a29, AhR, cyp26a1 and cyp26b1 mRNA expression were found in the pilot whale livers, although enzyme activities, measured by were very low (Paper II). Of the analysed enzyme activities only EROD activity, which reflects CYP1A activity (Burke et al., 1994), could be quantified. MROD, PROD and BROD could not be quantified due to low activity and high variability between the replicate samples. Although the EROD activity was low, it was positively correlated to hepatic OHC concentrations in the animals. The most important contaminants for induction of EROD activity were BDE-49, PCB-110, PCB-97, BDE-153, PCB-44, BDE-28, BDE-154, *cis*-chlordane, HCB, BB-153/BDE154 and BDE-100 (Figure 7, Paper II). Although EROD is a selective biomarker for CYP1A1 activity (Burke et al., 1994), the most important contaminants predicting the EROD activity were not mono-*ortho* or non-*ortho* (group III) PCBs with a planar configuration, which are the ligands of the AhR and thus involved in the induction of CYP1A1 (Boon et al., 1997, 1992; Stegeman and Hahn, 1994). Rather the contaminants important for EROD activity induction were PCBs with two *ortho*-chlorine atoms and vicinal H atoms in the *meta-para* position (group IV PCB), know to induce CYP2B enzymes (Boon et al., 1997), and PBDEs, HCB and cis-chlordane. PCBs that have been assigned toxic equivalent factors (TEF) values to calculate the total toxic equivalent value (TEQ) of the PCBs are mostly group III PCBs (Van den Berg et al., 2006). Using this approach, Paper II showed that EROD activity was also significantly positively correlated to TEQs (Figure 8) indicating that group III PCBs play a role in inducing CYP1A activity in pilot whales. Positive correlations between EROD activities and PCB TEQ have previously been reported in pilot whales from the Faroe Islands (Dam et al., 2010).

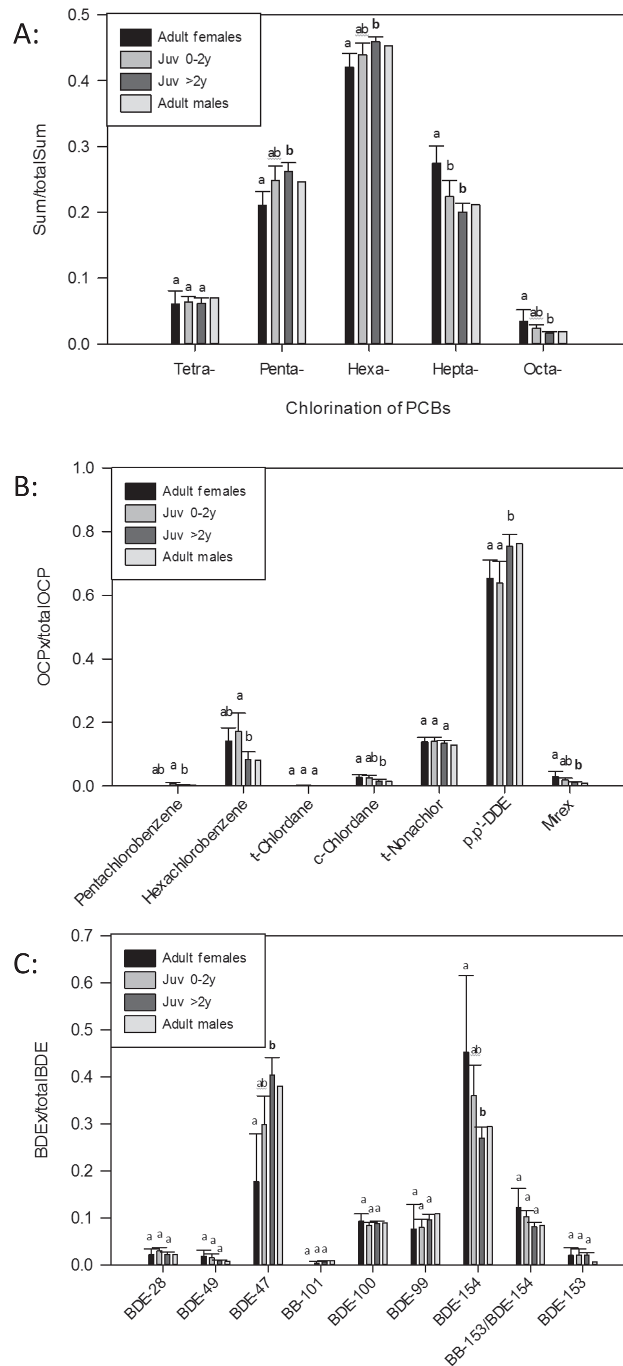

Figure 6 Relative distribution of A: tetra- to octa-clorinated PCB homolog group concentrations to total PCB concentration, B: individal PBDE concentration to total PBDE concentration, and C: individal OCP concentrations to total OCP concentrations in liver of different age/sex groups of pilot whales (mean + std.dev).The letters a and b indicate if there is significant difference or not between the groups (p=0.05, bold letters p=0.01). Figures from Paper I.

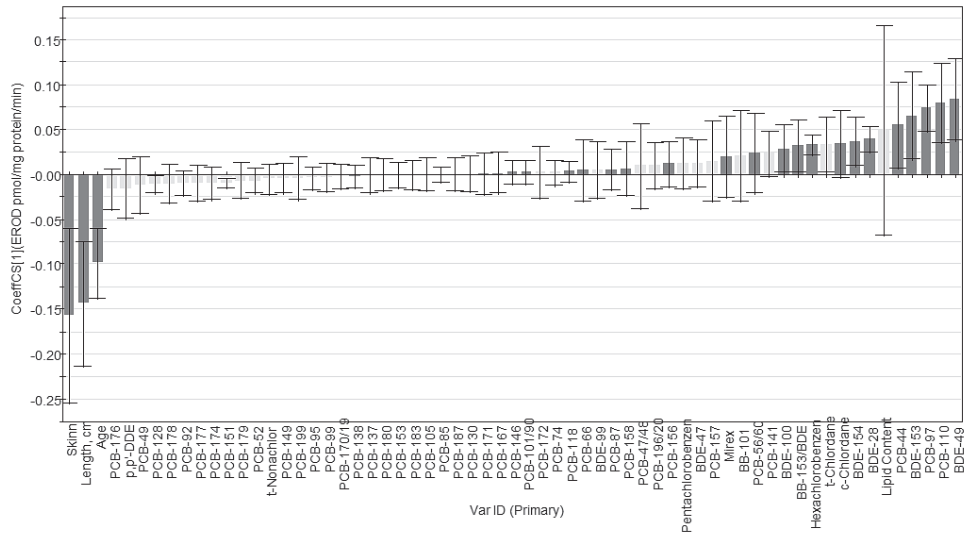

Figure 7 Regression coefficient plot of the OPLS model showing regression coefficient (CoeffCS) values of each variable indicating direction and strength of the relationship between individual X variables and the Y variable EROD. The dark grey bars present CoeffCS values of variables with VIP values > 1, which indicate high importance. Figure from Paper II.

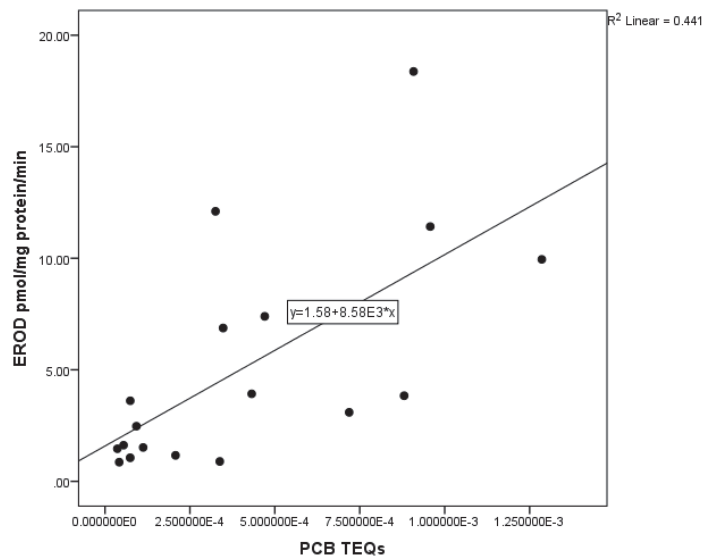

Figure 8 Correlation between PCB TEQs and EROD activity in pilot whale liver. TEQs were calculated from TEFs for PCB-105, -118, -156, -157, and -167 according to (Van den Berg et al., 2006). Figure from Paper II (supplemental data).

Compared to other mammals (including other marine mammals) and birds, cetaceans are known to be poor metabolizers of POPs due low or lacking expression of CYPs, especially CYP2B (Boon et al., 1997; Tanabe et al., 1988). However, several studies have shown induction of CYP enzyme expression in relation to POP exposure in cetaceans (White et al., 2000, 1994, Wilson et al., 2010, 2007). In beluga from the Canadian Arctic both CYP1A-mediated (EROD) and CYP3A-mediated (testosterone hydroxylase activity) enzyme activity have been measured, whereas the analyses indicated low CYP2B-type enzyme expression (McKinney et al., 2004). The EROD activities in the belugas were higher than found in the present pilot whales (Paper II), although the POP concentrations were lower (McKinney et al., 2006). This indicates a lower ability of the pilot whales to metabolize POPs compared to the beluga, as has previously been suggested (White et al., 2000). However, previous analyses in pilot whales from the Faroe Islands and NW-Atlantic area have shown higher EROD activities than in the present study (Dam et al., 2010; White et al., 2000). The differing results may have several causes, such as different analytical methods, different sex distribution within the sample pools, or seasonal differences related to the reproductive state of the animals (Paper II).

The EROD activity was significantly lower in adult females than in the other age groups. Similarly, CYP1A, CYP2B, CYP2E and CYP3A protein expression was detected in juvenile females and males and adult males but not in adult females. Since induction of CYP activity/expression is concentration dependent (Boon et al., 1997), this could be due to the low POP concentrations in adult females and indicating a lower induction of the CYPs (Paper II). The higher expressions of CYPs in young individuals is in contrast to what is generally reported in mammals, that young individuals have lower ability of CYP enzyme induction (Weijs et al., 2010; Wolkers et al., 2002).

Phase II enzyme activities (GST and UDPGT) were low in the pilot whales, but UDPGT activity was positively correlated to age and length (Paper II). The UDPGT activities were at the same level as reported in beluga from the Canadian Arctic (McKinney et al., 2004).

Expressions of *cyp1a1* and *cyp3a29* mRNA transcripts did not correlate significantly with POP concentrations in the present study, although cyp1a1 was significantly positively correlated with EROD and AhR expressions (Paper II). Studies of beluga whales from the Arctic Canada have shown similar correlations between AhR and Cyp1a1 mRNA transcripts in blubber, although these were also positively correlated with PCB concentrations (Noël et al., 2014). Also in killer whales from the Northeast Pacific Ocean, PCB-related increase in AhR mRNA expression, as well as other receptors, such as estrogen α receptor (ERα) and thyroid hormone α receptor (TRα), in blubber was found (Buckman et al., 2011).

The present studies showed that Faroe Island pilot whale calves, sub-adults and males had low, but detectable CYP expressions and EROD activity. In adult females, no significant CYP protein expression and low enzyme activity was detected. It is proposed that this difference between the groups is due to levels of POPs in adult females not being sufficiently high to induce expression or activities of CYP enzymes.

3.2.1 Metabolite concentrations and distribution

The low CYP enzyme activity (Paper II) was in accordance with the very low POP metabolite concentrations in the plasma pilot whales (Paper I).

Of the 33 OH-PCBs, 14 OH-PBDEs, 16 $MeSO_2$-PCBs, pentachlorophenol (PCP), 4-OH-heptachlorostyrene, and 3-$MeSO_2$-*p,p'*-DDEs, the only metabolite that was detected in plasma of more than 50% of the individuals was 4-OH-CB107/4'-OH-CB108 (coeluted). However, also 4'-OH-BDE17 was detected in plasma of 10 individuals and several $MeSO_2$-PCBs (3'- $MeSO_2$-CB49, 4'-$MeSO_2$-CB49, 4-$MeSO_2$-CB64, 3'-$MeSO_2$-CB101, 4'-$MeSO_2$-CB101, 4-$MeSO_2$-CB70) were detected in the livers of 7-12 individuals (Table 2). It should be noted that, although no or very low CYP expression or CYP activities were identified in the adult females, 4-OH-CB107/4'-OH-CB108, was present in the females in concentrations similar to that reported in the other groups (Table 2). This indicates that there may be CYP activity also in adult females, possibly during other parts of the year, or that dietary intake may contribute to exposure of this metabolite. The mRNA expression analysis also showed CYP enzyme expression in the adult females, although these were not detected in the protein and enzyme activity analyses (Paper II).

Since the concentrations of OH-metabolites were not lower in the females than in the juveniles, as was the case with all other POPs (Paper I), the maternal transfer of metabolites did not seem to be high. OH- metabolites such as 4-OH-CB107/4'-OH-CB108 are polar and are known to bind to plasma proteins (Letcher et al., 2000). This binding to transport proteins is thought to be one of the mechanisms of toxicity of POP metabolites and the affinity to plasma proteins could be the explanation for the less transfer of these compounds (Letcher et al., 2000). In polar bears and humans it has previously been shown that the maternal transfer of OH-PCBs via mothers milk is considerably lower than the transfer of the lipophilic PCBs (Bytingsvik et al., 2012; Fängström et al., 2005). The few detected $MeSO_2$-metabolites were, on the other hand, all found in liver of juvenile (both calves and sub-adults) and adult male pilot whales (Paper I).

Concentrations of $MeSO_2$-PCB metabolites have previously been reported in blubber of only one pilot whale from the Irish Sea, showing relative concentrations of $MeSO_2$-PCB metabolites to PCB of 0.05 (Troisi et al., 1998). Low levels of OH-metabolites and low metabolite/parent compound ratios have been reported for different species of cetaceans from different locations (Houde et al., 2009, 2006; Montie et al., 2008; Nomiyama et al., 2010; Ochiai et al., 2013). Compared to the mean ∑OH-PCB/∑PCB ratio in the pilot whales from the present study of 0.045 (range: 0.004 – 0.189), ratios in the range 0.001-0.0056 have been reported in several toothed whales from the Japanese coast (Ochiai et al., 2013) and of 0.02 and 0.68 in two different populations of bottlenose dolphins from USA (Houde et al., 2006; Montie et al., 2008).

The low concentrations of POP metabolites detected in the pilot whales are consistent with the identification of low CYP activities in this species (Paper II), as is found in other toothed whale species.

Table 2 Concentration of hydroxyl- (OH-) and methylsufonyl- ($MeSO_2$-) containing PCB and/or PBDE metabolites detected in the liver and plasma samples of 27 Faroese pilot whales in ng/g wet weight. Table from Paper I.

Matrix		n	mean±*std.dev.* ng/g ww	Min-max ng/g ww
Liver	3'-$MeSO_2$-CB49	10	2.65±*0.64*	(1.69-4.03)
	4'-$MeSO_2$-CB49	7	0.61±*0.19*	(0.42-1.00)
	4-$MeSO_2$-CB64	10	1.43±*0.44*	(1.03-2.40)
	3-$MeSO_2$-CB70	2	1.63±*0.14*	(1.53-1.73)
	3'-$MeSO_2$-CB101	12	1.88±*1.10*	(0.78-4.63)
	4-$MeSO_2$-CB70	8	1.39±*0.46*	(1.02-2.23)
	4'-$MeSO_2$-CB101	7	0.72±*0.33*	(0.52-1.39)
	4'-MeSO2-CB87	1	0.66	
Plasma	3-OH-CB118	1	0.46	
	4-OH-CB107 / 4'-OH-CB108	14	0.54±*0.11*	(0.39-0.71)
	4'-OH-BDE17	10	1.77±*1.40*	(1.04-5.68)
	6'-OH-BDE49	1	0.8	

3.3 Endocrine disruptive effects

3.3.1 Steroid hormones

In the analyses of circulating steroid hormones and their relationships with POPs only a few significant correlations were identified in the pilot whales (Paper III). All identified correlations between POPs and steroid hormones were positive. In adult females there were significant positive correlations between androgens (TS, AN) and E1 and PRE and some of the recalcitrant PCBs (CB-99, -138, -149, -153, -187) (Figure 9). In males βE2 was significantly positively correlated with 4-OH-CB107/4'-OH-CB108 (Figure 9). The positive correlations in adult females are in accordance with findings in an experimental study on sledge dog (*Canis familiaris*) bitches in which the concentrations of progestagens, androgens and estrogens were higher in the group exposed to POPs through their diet, compared to the control group fed a diet with low POP concentrations (Sonne et al., 2014). Although only very few significant correlations were found in the pilot whales, the positive correlations could be an indication of effects on the steroid homeostasis by the POPs. The mechanisms behind these positive correlations could involve POPs affecting the steroid synthesis or metabolism. In adult female polar bears positive correlations were found between PRO and ∑PCB, indicating inhibition of enzymes involved in the steps transforming PRO to E_2 and/or disruption of the feed-back mechanisms of the gonadotropins (LH, FSH) leading to increased synthesis (Haave et al., 2002; Ropstad et al., 2006). As a result the feed-back mechanisms of LH and FSH controlling the steroidogenesis could be disturbed by the POPs (Sonne et al., 2014).

Other analyses in female polar bears have shown negative relationship between OH-PCBs and PRE and AN (Gustavson et al., 2015a). In contrast to polar bears, pilot whales have very low concentrations of OH-PCBs (Paper I). The concentrations in adult female pilot whales are thus possibly below the threshold for negative effects on steroid hormones. Positive correlation between βE2 and 4-OH-CB107/4'-OH-CB108 was however, found in male pilot whales (Paper III). This could indicate inhibition of the estrogen metabolism in these individuals, since OH-PCBs have been shown to inhibit sulfotransferases involved in the metabolism of estrogens in human cells (Kester et al., 2000). Also the aromatase enzyme (CYP19) that catalyses the aromatization of androgens into estrogens, is a likely target for effects of the POP compounds (Li, 2007). CYP19 expression can be induced by coplanar PCBs (Li, 2007),and thus possibly those which are the precursors for 4-OH-CB107/4'-OH-CB108.

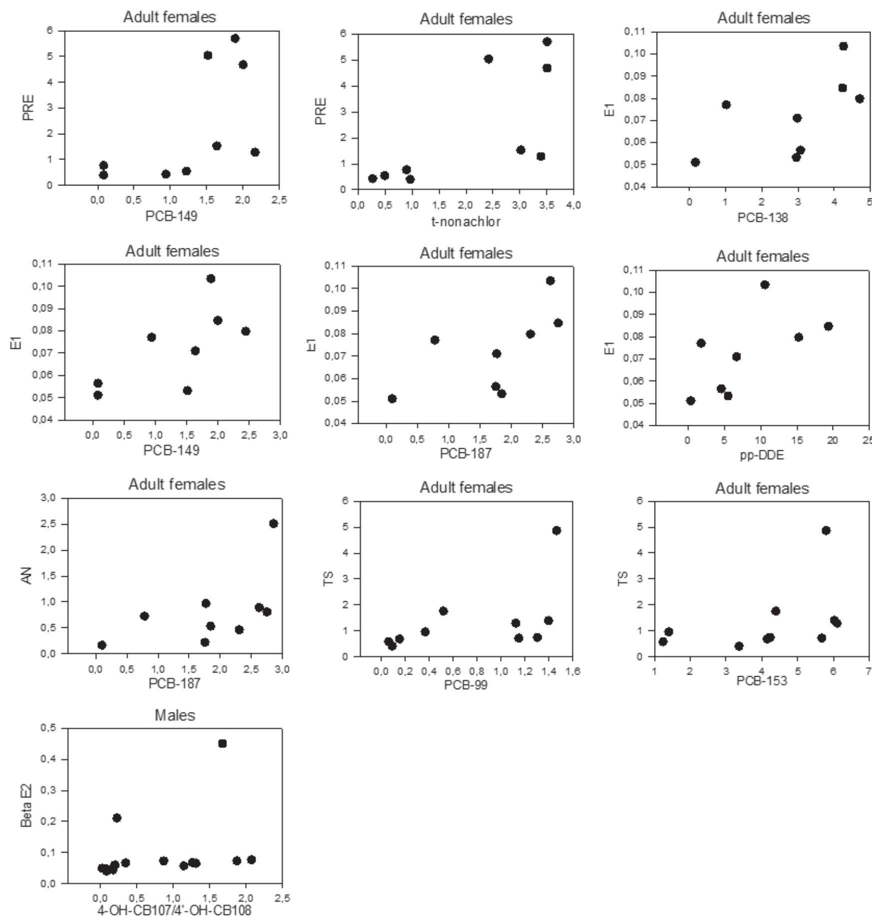

Figure 9 Significant (p<0.05) correlations between steroid hormones and single POP sin pilot whale plasma. Figure from Paper III.

Very few studies have been conducted on the topic of steroid disruption by POPs in cetaceans. However, negative correlation has been reported between TS and PCB in adult male Dall's porpoises with PCB concentrations at the same levels as in the pilot whales (Subramanian et al., 1987). These possible negative effects of POPs on TS concentrations have also been reported in polar bear males and male arctic foxes (Hallanger et al., 2012; Ropstad et al., 2006). These associations, which could be indicative of an effect, could however, not be studied in the pilot whales, since there were only two adult males analysed for steroids and POPs.

The present study shows that no negative correlations could be detected between steroid hormones and the POP exposure in the present pilot whales, but the positive correlations between some POPs and steroids in adult females could indicate effects on the steroid hormone homeostasis in pilot whales by POPs. How this affects the pilot whale physiology and reproduction is not known and should be analysed further.

3.3.2 Thyroid hormones

The relationship between thyroid hormones and POPs showed positive correlations, but this was influenced by the age/size of the animals (paper IV). The contaminant concentrations correlated positively with total and free T4 and T3, whereas the biological factors length and age correlated negatively with the thyroid hormones. This could be explained by the generally higher TH concentrations in juveniles than in adults and that the adults in this case were mostly adult females, which have several times lower POP concentrations than the juveniles. Higher TH levels in juveniles, as compared to in adults, is also found in other species, including humans (Fair et al., 2011; Flower et al., 2015; Gabrielsen et al., 2011; Hall et al., 1998; Kapelari et al., 2008; West et al., 2014), and can be explained by the higher need for these hormones, since the hormones regulate growth, development and other physiological parameters, that differ between juveniles and adults (Rolland, 2000).

When analysing the age groups separately the only significant correlations between thyroid hormones and POPs in both adult females and all juveniles were the positive correlation between HCB and TT3 (Figure 10), whereas a few single POPs were positively correlated with TH only in the calves (0 - 2 years of age). Although most other studies have reported negative associations between POPs and THs (Braathen et al., 2004; Debier et al., 2005; Schwacke et al., 2012; Tabuchi et al., 2006; Villanger et al., 2011b), the identified correlation may indicate that the POPs may affect the homeostasis of circulating THs in pilot whales. It should also be noted that significant positive correlations between some individual POPs and THs has been reported previously in marine mammals (Hall et al., 2003; Routti et al., 2010; Villanger et al., 2011a). Positive correlations can indicate stimulation of the TSH secretion from the pituitary and/or disturbance of the feedback from T4 and T3 on the TSH by POPs, as has been indicated in beluga whales with lower POP exposure than the pilot whales (Villanger et al., 2011b). Analysis of TSH was performed in the present pilot whales, but the concentrations were too low to be quantified.

The relative concentrations of FT3 compared to TT3 and FT4 (TT3:FT3 and FT4:FT3 ratios) were higher in juveniles than in adults and negatively correlated to POP concentrations (Paper IV). A suggested mechanism for TH disruption by POPs is binding of the contaminants to transport proteins for THs (Brouwer et al., 1998). The relative higher concentration of free T3 could thus indicate less binding of these hormones to transport proteins due to occupancy of the transport proteins by the POPs. However, this displacement is thought mostly to be associated with OH-metabolites of PCBs and PBDEs due to their structural similarity to THs (Brouwer et al., 1998; Liu et al., 2014). Since very low concentrations of OH-metabolites were found in the pilot whales this mechanism seems unlikely to explain the relative higher free T3 levels. Instead, it was proposed that this could be an age effect (Paper IV).

Thus, in the present study the TH concentrations did not seem to be negatively affected by the POPs, possibly due to the low concentrations of OH-metabolites in the pilot whale plasma. The positive correlations found between HCB and TT3, and between some of the POPs and THs in the calves, do however indicate that there are some interference between the POPs and TH regulation, at least in the youngest whales.

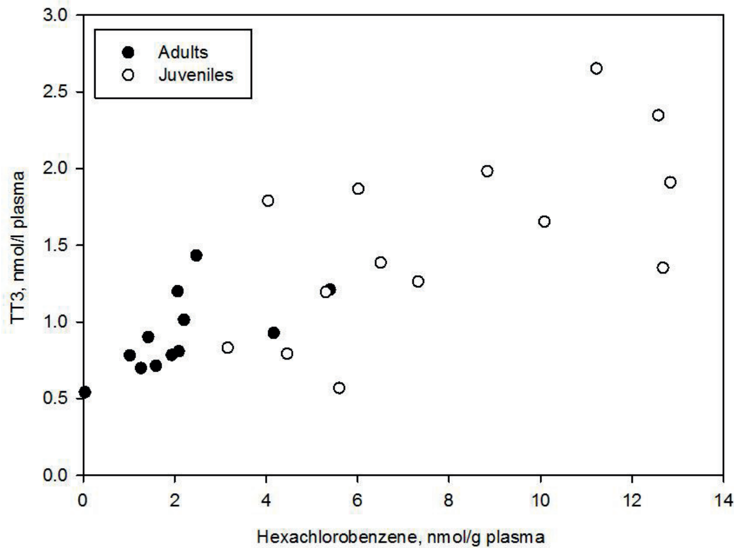

Figure 10 Correlation between total triiodothyronine (TT3) and hexachlorobenzene in pilot whale plasma.

3.3.3 Effects on vitamin concentration

In the pilot whales no significant relationship was found between retinol and POPs in plasma, and in liver only BDE-47 was negatively correlated to retinol. Although the vitamin concentrations in liver did not differ between the age groups (Paper IV), retinyl palmitate correlated negatively with BDE-28, -47, -49, and -153 in the adult female group. Analyses in beluga whales from the Arctic Canada have shown that PCB concentrations in blubber were negatively correlated to vitamin A in liver but positively correlated with vitamin A in plasma and blubber (Desforges et al., 2013a). Blubber PBDE concentrations were also positively correlated to vitamin A in the belugas but were less important (Desforges et al., 2013a). The POP concentrations in the beluga whales were much lower than in the pilot whales, indicating that either the belugas are more sensitive for the effects of POPs on vitamin A, that there are some confounding factors masking the effects in the pilot whales, or that the correlation in the belugas was reflecting other biological processes, where vitamin A or POPs were tagging along. The beluga study included a much larger number of individuals and most of the individuals were adult males (Desforges et al., 2013a). It is also important to note, that both POPs and vitamins are supplied to the whales with the diet (Aguilar et al., 1999; Borrell et al., 2002), and both are maternally transferred to the calves with the milk during lactation (Borrell et al., 1995; Debier and Larondelle, 2005). Correlations between POPs and vitamins can thus be influenced by this co-supply and -elimination.

POPs and their metabolites can interfere with retinoids by many different mechanisms, including the disruption of vitamin A metabolism (Borrell et al., 2002; Rolland, 2000; Simms and Ross, 2000). The CYP26 enzyme is involved in metabolism of retinoids by catalysing the oxidation of retinoic acid and CYP26 expression was therefore analysed in the pilot whale livers (Paper V). The CYP26 mRNA concentration was significantly negatively correlated to the POP concentration, which could indicate

a decrease in CYP26 expression with increasing POP concentrations. This has previously been reported in common frog (*Rana temporaria*), indicating an inhibitory effect of POPs on CYP26 activity (Leiva-Presa et al., 2006). Retinol concentrations in liver also correlated positively with the CYP26 expression (Paper V), but the retinol concentrations did not correlate with the POP concentrations (Paper IV). However, when excluding the females from the analyses, positive correlations were found between retinol in plasma and CYP26 expression in liver (Paper V). The results thus indicate that the vitamin A homeostasis could be influenced by contaminants due to downregulation of CYP26 gene expression, but that this did not affect the vitamin A storage in the liver. However, the effects of CYP26 could also be secondary due to changed levels of retinoids whether these were affected by the contaminant exposure or not (Paper V).

In juvenile pilot whale plasma α-tocopherol was positively correlated to POPs, indicating that the α-tocopherol functioned as a defence against oxidative stress induction by the POPs, but that the storages of tocopherol were not depleted as a result of the antioxidant defence, since the α-tocopherol concentration in liver was not correlated to the POP concentrations (Paper IV). This indicates that the higher vitamin E concentrations in the blood were sufficient for the higher requirement, presumably caused by POP exposure. In beluga from Arctic Canada positive correlations between PCBs and vitamin E in blubber, but negative correlations between PCBs and hepatic α-tocopherol were found (Desforges et al., 2013a). Positive correlations between vitamin E and POP exposure in plasma have previously been reported in ringed and grey seals (Nyman et al., 2003) and in Greenland shark (Molde et al., 2013), and were suggested to be a result of antioxidant defence from oxidative stress induced by the POPs. However, the vitamin E concentration in liver of the pilot whales increased with age and the concentration in adults was higher than in juveniles (Paper IV). The vitamin E is supplied through the diet, as are the POPs, and the positive correlations could alternatively be explained by that the young whales receive both POPs and vitamin E from the same source, which could possibly be the mother’s milk.

Vitamin D_3 (25(OHD3) in plasma did not correlate with POP concentrations when all individuals were pooled, but the vitamin D concentrations were positively correlated with 4-OH-CB107/4’-OH-CB108 and CB-172 in the calves (0-2 years of age) (Paper IV). The observed relationship between 25(OH)D3 and OHCs could possibly be due to inhibition of transformation of 25(OH)D3 to the 1,25$(OH)_2$D metabolite by 4-OH-CB107/4'-OH-CB108 and CB-172. Although negative effects of PCBs and DDT on vitamin D levels have been found in Baltic grey seals (Routti et al., 2008), very low concentrations of OH-CB107/4'-OH-CB108 were observed in the pilot whales and correlations were only found in the 0-2y age group consisting of very few individuals. It is thus likely, that this correlative relationship should not be regarded as an effect of OHCs on vitamin D homeostasis.

Effects of POPs on the vitamin levels in the present pilot whales were limited to positive correlations between Vitamin E and POPs in the calves without depletion of the hepatic vitamin E stores, and possibly a negative effect of PBDEs on liver vitamin A stores in adult females.

3.4 Effects of the POP exposure in pilot whales

To assess the influence of the contaminant exposure in the pilot whales is difficult, since it was not possible to compare the results with a control group of pilot whales, i.e. samples from a population

that is not exposed to POPs, or to markedly lower concentrations. Two approaches have been used for studying the effects of contaminants in wildlife (Fisk et al., 2005). One is to measure biological responses related to contaminant exposure often termed biomarkers, as has been done in the present study in Papers II - V. The other is to compare concentrations of the compound of interest to concentrations known to cause detrimental effects based on toxicodynamic laboratory studies, semi-field studies or from observations of affected animals in the wild. This latter concept uses so-called toxicity reference values (TRVs), which refer to contaminant concentration levels where toxic effects have been identified. Regarding the applicability of these two approaches in wildlife pollution effect assessments, it is important to be aware, that the associations between the biomarkers and the contaminant concentrations are not necessarily causal and may not reflect a relationship based on toxicity mechanisms. Most contaminants covariate and it is not always possible to differentiate between the effects of one of the contaminants on the biomarker from that of the others (Fisk et al., 2005). TRVs derived from animal model laboratory studies with short duration exposure at high concentrations does not reflect the wildlife situation where animals are exposed to lower concentrations of a mixture of different pollutants for a long time (often a lifetime) (Fisk et al., 2005). Furthermore, it is important to be aware that it is not always possible to extrapolate TRVs derived from analyses in one species to another, due to species differences in response and susceptibility, such as different abilities to express CYP enzymes and other genetic differences that governs their physiology and susceptibility.

3.4.1 Comparison of internal concentrations with toxicity reference values

In marine mammals, several TRVs for POPs have been suggested with different sensitivity depending on species, toxic compound and effects measured (Table 3). There are large differences between the TRVs that have been suggested for marine mammals, and these can often be related to the different methods for deriving these TRVs. The highest levels of the TRVs in Table 3 are based on the measured POP concentrations in individuals where effects have been detected (Helle, 1976; Kannan et al., 2000; Tornero et al., 2005), whereas the lowest TRVs are risk–based toxicity thresholds, below which effects are not to be expected, calculated from a 5% effect concentration (EC5) in the most sensitive species (Desforges et al., 2013a; Mos et al., 2010).

Several studies have indicated that high concentrations of POPs may impair reproduction in cetaceans (Jepson et al., 2016; Murphy et al., 2015; Reddy et al., 2001). Jepson et al. (2016) compared the PCB concentrations in different cetaceans species from European waters, applying a relatively low ∑PCB TRV of 9 mg/kg lipid weight (lw), suggested by Kannan et al. (2000), and a higher value of 41 mg/kg lw (equivalent to 77 mg/kg Clophen 50), suggested by Helle (1976). In the present study the ∑PCB concentrations in juvenile and adult male pilot whales exceeded the lower TRV of 9 mg/kg lw, but were below the TRV of 41 mg/kg lw (Table 1 in Paper I). Although the PCB concentrations in the present pilot whales were much lower than in most toothed whale populations reported by Jepson et al. (2016), in which reproductive effects were indicated, the ∑PCB concentration in juveniles (16 mg/kg lw) and adult males (17 mg/kg lw), but not the adult females (2.5 mg/kg lw), exceeded the estimated concentration threshold value of 10 mg/kg lw for effects on calf survival and population growth in bottlenose dolphins (Hall et al., 2006). Furthermore, a study of harbour porpoises with similar PCB concentrations as in the present pilot whales indicated that reproductive effects were present (Murphy et al., 2015), and PCB levels in some of the present pilot whales were also similar to those reported in bottlenose dolphins in which reproduction was assumed to be affected (Reddy et al., 2001). Thus, it is possible that although no apparent significant

negative relationships between the POPs and the reproductive steroid hormones were identified in the present study, the reproduction of pilot whales from Faroese waters may still be affected.

Analyses of bottlenose dolphins from Florida (Tornero et al., 2005) have suggested that the level of PCBs and DDTs are above 50 mg/kg lw and 40 mg/kg lw respectively in blubber, before effects are seen on the blubber retinoid level. Compared to these levels the POP concentrations in the present pilot whales were much lower (Table 1 in Paper I). However, a TRV of 1.6 mg/kg of PCBs was calculated for effects on vitamin A and E levels in beluga whales (Desforges et al., 2013a), and the POP concentrations in pilot whales exceeded this TRV. The pilot whales did, however, not show the same effects on vitamin A and E as the less polluted beluga whales, since negative correlation between retinol and POPs was only found for BDE-47 in all individuals and for most PBDEs in adult females (Paper IV). Furthermore, although vitamin E was positively correlated to POPs in the juveniles, this did not affect the storage of vitamin E in liver (Paper IV).

It should be noted, that most of these TRVs are referring to concentrations in blubber, but since the lipid normalized POP concentrations in liver and plasma were generally correlated (Paper I), and since studies have shown that lipid based concentrations in blubber and blood are highly correlated (Yordy et al., 2010), it is assumed, that the contaminant lipid based concentrations in one of these tissues can be used as an estimate for the other in such a crude estimate, as is possible from these TRVs.

The POP contamination in the analysed pilot whales is at a level that may induce a health risk for the whales, even though the concentrations are lower than those reported in many other toothed whale populations.

Table 3 Suggested threshold values for effects of POPs on marine mammals in the literature.

Species	Tissue	Biomarker	Compound	Treshold value	Reference
Ringed seals	Blubber	Reproductive failure	PCB	41 mg/kg lw	Helle, 1976
Seals, dolphins, otter and mink	Blood	Toxicological effects*	PCB	9 mg/kg lw	Kannan et al., 2000
Bottlenose dolphins	Blubber	Vitamin A	PCB DDT	50 mg/kg lw 40 mg/kg lw	Tornero et al., 2005
Bottlenose dolphins	Blubber	Population growth, calf survivorship	PCB	10 mg/kg lw	Hall et al., 2006
Juvenile Harbor seals	Blubber	Immunological and endocrine biomarker endpoints	PCB	1.3 mg/kg lw	Mos et al., 2010
Beluga whale**		Vitamin A and E	PCB	1.6 mg/kg lw	Desforges et al., 2013

*Hepatic vitamin A, thyroid hormone concentration, suppression of natural killer (NK) cell activity and proliferative response of lymphocytes to mitogens
**Integrated toxicity reference value

With respect to the biomarker approach for assessing effects of the POPs, the possible effects of the POPs were studied by analysing the correlative relationships between POPs and biomarker concentrations in individuals with differing internal POP concentrations within the sample-population of pilot whales. Although, the POP concentrations in the pilot whales exceeded some of the suggested TRVs, the TRV of 1.6 mg/kg lw of PCBs for affecting vitamin A and E (Desforges et al., 2013a), the TRV of 10 mg/kg lw for effects on calf survival and population growth in bottlenose dolphins (Hall et al., 2006) and the ∑PCB TRV of 9 mg/kg lw, for toxicological effects in marine

mammals (Kannan et al., 2000), very few correlations were found between the analysed biomarkers and POP concentrations (Paper II, III, IV).

The POP concentrations in the pilot whales, covered a relatively large range, but mostly the low concentrations were found in adult females, whereas the high concentrations were found in the juveniles and in the two adult males that were analysed for POPs (Paper I). Also, the results showed that the biomarker responses were confounded by age and sex differences. Furthermore, when analysing the age groups separately, it is possible that the low sample size or low range of contaminant concentrations between the individuals provided our analysis with too low statistical power to detect a significant relationship.

A weakness when analysing relationships between biomarkers and POPs is that it is not possible to conclude on that such associations between biomarkers and contaminant concentrations are causal. As shown in the present study (Paper IV, Fig. 4), most contaminants covariate more or less, and it is not always possible to differentiate between the effects of one of the contaminants from the others (Fisk et al., 2005). Although a large number of contaminants were analysed in the present study, there are still contaminant groups that are not included, such as perfluorinated compounds (PFCs), some of which (perfluorooctane sulfonic acid, PFOS) are also known to cause toxic effects (Berg et al., 2015; Grandjean et al., 2012).

When comparing the two approaches for assessing possible effects of POPs on Faroe Island pilot whales, the interpretation is ambiguous. The POP concentrations were higher than the lowest TRVs for effects on vitamin A and E, population growth and calf survival, but lower than the highest TRV for reproductive failure (Helle, 1976). Based on the TRV approach the concentrations of PCBs in Faroe Island pilot whales may thus suffer from physiological effects of PCBs, such as effects on the vitamin A and E levels, which eventually can lead to effects on the reproduction. However, on the other hand, very few of the molecular and physiological biomarkers that were analysed appeared to be affected by the POPs. This may indicate that POP concentrations in pilot whales from Faroese waters are below most threshold levels for these effects, except for possible effects on EROD and CYP26, and vitamin E in juveniles.

It should be noted that a temporal decline in the NE Atlantic pilot whale population has not been reported and pregnant females are frequently observed. Also the much lower POP concentrations found in adult females compared to the other age groups, can be seen as a strong indication towards that the females are reproducing and have offloaded a significant part of their POP body burden to their offspring. Together with the general lack of associations between POP concentrations and biomarker responses, it is suggested that the Faroe Island pilot whales are not affected by POPs in a way that significantly affects their endocrine and vitamin physiology or reproduction. However, it is still possible that the Faroe island pilot whales may suffer from other effects caused by POPs, such as immunological effects.

Although, most of the contaminants analysed in the present study are currently regulated, and generally show a decreasing trend in the environment (Dietz et al., 2013; Rigét et al., 2010), other emerging contaminants may represent an environmental risk. Internal concentrations of other emerging contaminants that currently are not regulated should be regularly monitored in Faroe Island pilot whales.

4 Conclusions

The present study showed that the studied pilot whales had relatively high internal concentrations of POPs, with PCBs and DDTs being the dominating compounds in liver and blood of the pilot whales. The concentrations of POPs were significantly lower in adult females than in juveniles and adult males, most likely due to the maternal transfer from mother to offspring during gestation and lactation. The relative concentrations of the different POP compounds also differed between adult females, calves and sub-adults and suggested that the transfer was determined by the lipid solubility and molecular size of the compounds.

The pilot whales expressed CYP enzymes of the CYP1, 2 and 3 families in their livers and the CYP1A enzyme activity, analysed by EROD activity, correlated positively with the POP exposure. The activities of the CYP enzymes and phase II enzymes were generally low, presumably leading to very low concentrations of OH- and $MeSO_2$-metabolites of PCBs. Low concentrations of OH-PCBs and $MeSO_2$-PCBs were indeed found in the pilot whales. The study thus shows that pilot whales have a low ability to biotransform POPs.

Although the POP concentrations in the pilot whales exceeded several toxic reference values suggested for marine mammals, the relatively high POP concentrations did not seem to have negative effect on the thyroid hormone and vitamin A levels or the steroid hormone level, although there were some indications on possible effects on steroid and thyroid hormone homeostasis, as inferred from co-variability. Since several of the POP-related effects previously found in marine mammals have been linked to OH- and $MeSO_2$-metabolites, the lack of clear effects in the present pilot whales, as indicated by the general lack of relationships among the pollutants and the biomarker responses, could be due to the very low concentrations of PCB-metabolites. This again could be due to their low ability to induce CYP enzyme expression and activity and their resultant low ability to biotransform POPs. The POP concentrations in the pilot whales were much lower than in several other studies in cetaceans, where effects on reproduction and calf survival has been suggested.

Both POP concentrations and the measured biomarkers were influenced by sex and age, showing the importance of including these parameters in the analyses of possible effects of POPs in marine mammals.

This study was based on a limited number of individuals from the same area sampled within a limited time and it was not possible to compare the results of the analyses with a less exposed control group. It is thus possible, that the POPs may cause effects that could not be detected with the methods used in this study.

The general lack of associations between POP concentrations and biomarker responses in this study however suggest that in the Faroe Island pilot whales, the POPs do not significantly affect their endocrine and vitamin physiology or reproduction.

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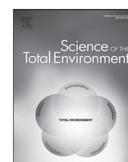

Legacy and emerging organic pollutants in liver and plasma of long-finned pilot whales (*Globicephala melas*) from waters surrounding the Faroe Islands

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HIGHLIGHTS

- POPs and metabolites were analysed in liver and plasma of pilot whales
- Lipid normalized POP concentrations were mostly similar in liver and plasma
- POP concentrations in adult females were lower than in juveniles
- Relative concentrations of POPs differed between age groups
- Concentrations of metabolites were low in both adult females and juveniles

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ABSTRACT

Concentrations of PCBs, organochlorine pesticides (OCPs), brominated flame retardants and a suite of relevant metabolites of these POPs, in all 175 different compounds, were determined in liver and plasma of traditionally hunted pilot whales ($n = 14$ males and $n = 13$ females of different age groups) from the Faroe Islands.
The main objectives of this study were to determine differences in the presence and concentrations of the compounds in the liver and plasma, how they depend on developmental stage (calves, sub adults, and adult females), and to assess maternal transfer of the compounds to suckling calves.
Generally, the lipid weight (lw) concentrations of quantified POPs in the liver and plasma of pilot whales were positively correlated, and lw concentrations of most POPs did not differ between these matrices. However, concentrations of some individual POPs differed significantly ($p < 0.05$) between plasma and liver; CB-153 ($p = 0.044$), CB-174 ($p = 0.027$) and BDE-47 ($p = 0.017$) were higher in plasma than in liver, whereas p,p′-DDE ($p = 0.004$) and HCB ($p < 0.001$) were higher in liver than in plasma.
POP concentrations differed between age/gender groups with lower levels in adult females than in juveniles. The relative distribution of compounds also differed between the age groups, due to the influence of the maternal transfer of the compounds. The results indicated that larger, more hydrophobic POPs were transferred to the offspring less efficiently than smaller or less lipid soluble compounds.
Very low levels of both OH- and/or $MeSO_2$-PCB and PBDE metabolites were found in all age groups, with no significant ($p > 0.05$) differences between the groups, strongly suggesting a very low metabolic capacity for their formation in pilot whales. The lack of difference in the metabolite concentrations between the age groups also indicates less maternal transfer of these contaminant groups compared to the precursor compounds.
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1. Introduction

Persistent organic pollutants (POPs), and in particular those that are halogenated, are widely distributed in the environment and bioaccumulate in marine food webs. Marine mammals occupy high trophic positions in their respective marine food webs, which along with

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physiological and life trait characteristics such as long life span, late reproduction, and a thick subcutaneous lipid layer (blubber), generally lead to accumulation of high concentrations of lipophilic POPs in marine mammals (Muir et al., 1988; Borrell, 1993; Troisi et al., 1998; Tilbury et al., 1999; Weisbrod et al., 2000, 2001; Tornero et al., 2005, 2006; Kajiwara et al., 2006; Fair et al., 2010; Letcher et al., 2010; Villanger et al., 2011).

Long-finned pilot whales (*Globicephala melas*) that inhabit the North-East Atlantic Ocean waters have been hunted as part of the Faroese traditional diet for centuries. In hunted pilot whales from the Faroe Islands, high concentrations of POPs like polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), dichlorodiphenyltrichloroethanes (DDTs), toxaphenes and other organochlorine pesticides (OCPs) have been documented (Borrell, 1993; Borrell and Aguilar, 1993; Lindstrom et al., 1999; Dam and Bloch, 2000; Dam, 2001; Hoydal and Dam, 2003, 2005, 2009; Nielsen et al., 2014). Although the concentrations of PCBs and DDTs in Faroese pilot whales have been reported as intermediate compared to pilot whales from other parts of the North Atlantic (Borrell and Aguilar, 1993), the concentrations in the 1990s were among the highest reported in mammals from the Arctic (AMAP, 2004). The highest reported concentrations were similar to or exceeded those expected to be linked to reproductive failure in other cetaceans (Reddy et al., 2001). The first reports of PBDEs in pilot whale blubber from the Faroe Islands were among the highest concentrations of PBDEs measured in biological samples (Lindstrom et al., 1999) at that time. PBDE determinations in blubber samples collected since then have not shown equally high concentrations to be common (van Bavel et al., 2001; Rotander et al., 2012a). Thus, concentrations of PBDEs in pilot whale blubber from the Faroe Islands appear to show a temporal decreasing trend (Rotander et al., 2012a; Nielsen et al., 2014).

POPs and their metabolites are known to interfere with several biological systems that can affect the health and fitness of exposed individual wildlife (Letcher et al., 2010). High levels of POPs have been associated with effects on reproduction, the immune system, and growth and development in marine mammals (Helle, 1976; Van Loveren et al., 2000; Reddy et al., 2001; Jepson et al., 2005). Thus, the high levels of contaminants in the tissues of pilot whales raise concerns for their health. It is also possible that continuous exposure to high POP loads in their tissues can cause subsequent effects at the population level.

Although very persistent, POPs can be eliminated by metabolism. In mammals the cytochrome P450 monoxygenases are the major enzyme class that catalyses the metabolism of xenobiotic, lipid soluble anthropogenic compounds, such as POPs. Although the presence and activities of several CYPs have been identified in different odontocete species (White et al., 1994; McKinney et al., 2004; Garrick et al., 2006; Fossi et al., 2008; Montie et al., 2008; Wilson et al., 2010; Godard-Codding et al., 2011), including pilot whales (Celander et al., 2000; White et al., 2000; Dam et al., 2010), the capacity of toothed whales to metabolize POPs is low compared to other mammalian species (Tanabe et al., 1988; Boon et al., 1992). As a consequence, relatively low levels of hydroxylated (OH) and methylsulfonyl ($MeSO_2$) metabolites of PCB and PBDE congeners (such as OH-PCBs and -PBDEs, and $MeSO_2$-PCBs and -DDEs) have been reported in odontocetes and the ratios between OH-PCB and PCB concentration are therefore also very low (McKinney et al., 2006; Houde et al., 2009; Montie et al., 2009; Weijs et al., 2009a; Nomiyama et al., 2010; Ochiai et al., 2013).

POPs are transferred from mother to offspring during gestation and lactation and a decrease in POP levels with age is often found in marine mammal females (Borrell et al., 1995; Sørmo et al., 2003; Vanden Berghe et al., 2010, 2012). In Faroe Island pilot whales, the transfer of organochlorines (OCs) to the offspring has been estimated to represent 60–100% of the mother's body load during lactation and only 4–10% during gestation (Borrell et al., 1995). Thus, suckling is the most important exposure route for OCs in young whales. Maternal transfer rates differ between different POP groups and specific compounds, where the difference has been linked to the lipophilicity, molecular size and chlorination of the compounds (Sørmo et al., 2003; Yordy, 2009; Bytingsvik et al., 2012). The more lipophilic chemicals are less transferable from the pregnant female to the foetus than less lipophilic chemicals (Borrell et al., 1995). Lower relative proportions of the higher chlorinated PCBs in young pilot whales compared to adults have been found in Faroese pilot whales, confirming a lower tendency of the most lipophilic compounds to be transferred to the offspring (Dam and Bloch, 2000).

It has also been reported that POP metabolites, such as OH-PCBs and OH-PBDEs, are transferred from the mother to the offspring in marine mammals, such as polar bears (*Ursus maritimus*) and seals, albeit to a much lower extent than for PCBs due to the more hydrophilic properties of these metabolites due to the hydroxyl group (Bytingsvik et al., 2012; Vanden Berghe et al., 2012). Since these metabolites have endocrine disrupting properties, there is particular concern about their harmful effects in young developing mammals, particularly with respect to thyroid disrupting effects (Bytingsvik et al., 2012, 2013; Simon et al., 2013). To our knowledge, there are no reports on the maternal transfer of these compounds in toothed whales. Information about levels in lactating calves of such whales would assist in risk assessment of POPs in these species.

The production and usage of PCBs and OCPs have been regulated globally since the 1970s, and the levels have been reported to have decreased in marine top predators that are known to have high capacities for metabolising POPs, such as polar bears (McKinney et al., 2010; Bytingsvik et al., 2012; Dietz et al., 2013). However, there is limited information on current levels and thus trends in marine mammals that have poor abilities to metabolize POPs, such as toothed whales. Thus, information on more recent levels of POPs in Faroe Island pilot whales will provide knowledge on temporal trends of POPs in a group of animals that is characterized by very low capacities to metabolize and excrete POPs.

Knowledge of the partitioning of contaminants between tissues is needed when assessing levels and effects of contaminants in animals. Blubber is the primary site of POP accumulation and in toothed whales more than 90% of the total body load of contaminants is found in the blubber (Yordy et al., 2010). Blood may however be a more accurate indicator of the bioavailable contaminant concentrations (Yordy et al., 2010). In other cetaceans it has been shown that the levels of PCBs are highly correlated in blubber and blood and that concentrations in blubber can be used to estimate the levels in blood and vice versa (Yordy et al., 2010). Levels of PCBs and PBDEs have also been found to be highly correlated between liver and blubber and the partitioning between the tissues is governed by the lipid content (Raach et al., 2011), although liver may be more representative of recent contamination of lipophilic compounds due to a slower partitioning of contaminants into blubber (Raach et al., 2011).

In the present study we determined the liver and plasma concentrations of PCBs (74 congeners), OCPs (20 compounds), brominated flame retardants (17 congeners/compounds) and relevant metabolites of some of these POPs: 33 OH-PCBs, 14 OH-PBDEs, 16 $MeSO_2$-PCBs, pentachlorophenol (PCP), 4-OH-heptachlorostyrene, and 3-$MeSO_2$-*p,p′*-DDE, in different age groups of pilot whales (0–2 year old calves, subadults, and adult females) from the Faroe Islands sampled in 2010 and 2011. The main objectives were to 1) determine differences in the presence and concentrations of the compounds in the liver and plasma; 2) determine how the presence and concentrations of POPs depend on developmental stage (0–2 year calves, subadults, and adult females); 3) assess maternal transfer of the compounds to lactating calves based on the differences in concentrations between adult females and calves. Based on the previously reported low concentrations of PCB and PBDE metabolites in toothed whales, we hypothesised the concentrations of these contaminant groups to be low in the adult females, even lower in calves and intermediate in the sub-adult pilot whales.

2. Materials and methods

2.1. Sampling

As shown in the regional map in Fig. S1, samples were collected during two pilot whale drive fisheries on the Faroe Islands in 2010 and 2011. Blood and liver were sampled from a total of 27 whales, of which 14 were males and 13 were females. Eleven whales were sampled in Sandagerði, Tórshavn on July 23, 2010, and 16 whales in Vestmanna on September 2, 2011. Blood samples were collected into clean heat treated (at 450 °C for 4 h) glass jars containing heparin and held on ice until further sample preparation. The blood was centrifuged at 1500 *g* for 5 min and plasma was transferred into cryovials and frozen in liquid N_2. The samples were stored at −80 °C until analysis. Liver samples were taken as soon as the whales had been transported from the beach onto the quay where the sampling could be carried out. A piece of the distal part of the liver was wrapped in heat treated aluminium foil and frozen in liquid N_2 and later stored at −80 °C until analysis.

The length of the individuals was measured (cm). The lower jaw was sampled from most of the individuals, and the age was determined by counting growth layer groups formed annually in dentine and cement of teeth as described in Lockyer (1993). An overview of the biometrics and age of the different groups of pilot whales are given in Table 1, whereas information on each of the individuals is given in the supplementary material (Table S2).

2.2. Division into age/sex groups

The individuals were divided into age and sex groups according to Desportes et al. (1993) and Martin and Rothery (1993), where females smaller than 375 cm and less than eight years of age and males smaller than 494 cm and less than 14 years of age were categorized as juveniles. According to this classification, the samples consisted of 10 adult females, 15 juveniles (of which only three were females) and two adult males. The juvenile group was further divided into two groups: Those that were less than two years old (calves: 0–2 years, $n = 8$), and those that were older than two years old (subadults: >2 years, $n = 7$). This was done to distinguish between suckling juveniles that are still exposed to milk transferred contaminants via suckling and those that are not. Lactation in Faroe Island pilot whales has generally been shown to proceed until the calves are between 1.5–3.5 years old (Martin and Rothery, 1993) and the splitting of the juvenile group at the age of two was based on earlier studies of the age of weaning in pilot whales from the Faroe Islands (Desportes and Mouritsen, 1993). In that study, around half of the examined two year olds had stomachs containing traces of milk and remains of solid food, whereas the other half had only remains of solid food and no traces of milk. At the age of three only around 25% had traces of milk in their stomachs (Desportes and Mouritsen, 1993). Thus, the calves (0–2 years) would be expected to receive a milk-transferred load of POPs from their mothers, whereas the subadults (>2 years) would receive POP loads mostly from their diet. Although we do not have known mother–calf pairs, concentrations of POPs in the adult female group compared to the levels in the youngest individuals of the juvenile group (0–2 years) can give information of the maternal transfer of contaminants and the sources of exposure.

Mother–offspring transfer rates have been shown to differ between different POP groups and specific compounds and the difference can be related to the physico-chemical properties of the compounds such as lipophilicity (log K_{ow}), size of the molecule (MW) and halogenation (Sørmo et al., 2003; Borrell and Aguilar, 2005; Yordy, 2009; Bytingsvik et al., 2012). By comparing log K_{ow}, MW and halogenation of the compounds with the concentrations in the juveniles 0–2 years relative to the concentrations in adult females, the importance of these properties in the maternal transfer in the pilot whales can be estimated.

2.3. Chemicals

^{13}C-labelled and unlabelled PCB, OC, PBDE and BFR standard solutions were purchased from Wellington Laboratories (Guelph, ON, Canada), Cambridge Isotope Laboratories (Andover, MA, USA) or Sigma-Aldrich (Oakville, ON, Canada) and were greater than 97% pure, as indicated by the manufacturer. All ^{13}C-labelled OH- and MeO-PCB standards were obtained from Wellington Laboratories (Guelph, ON, Canada). $MeSO_2$-PCB, OH-PBDE and MeO-PBDE standards were kindly provided by Å. Bergman (Stockholm University, Sweden). Solvents used for the extraction and clean up steps were purchased from Caledon Labs (Georgetown, ON, Canada). Diatomaceous earth and sodium sulphate were heated overnight at 600 °C in a muffle furnace prior to use (Gebbink et al., 2008a,b; Routti et al., 2008, 2009b).

2.4. Contaminant analysis

The plasma and liver samples were analysed for 74 PCB congeners (PCB-16/-32, -17, -18, -20/-33, -22, -28/-31, -41/-64, -42, -44, -47/-48, -49, -52, -56/-60, -66, -70/-76, -74, -85, -87, -90/-101, -92, -95, -97, -99, -105, -110, -114, -118, -128, -130, -137, -138, -141, -146, -149, -151, -153, -156, -157, -158, -167, -170/-190, -171, -172, -174, -176, -177, -178, -179, -180, -183, -187, -189, -194, -195, -196/-203, -199, -200, -201, -202, -205, -206, -207, -208 and -209), OCPs (1,2,4,5-tetrachlorobenzene, 1,2,3,4-tetrachlorobenzene, pentachlorobenzene, α-hexachlorocyclohexane, β-hexachlorocyclohexane, γ-hexachlorocyclohexane, hexachlorobenzene, octachlorostyrene, heptachlor epoxide, oxychlordane, *trans*-chlordane, *cis*-chlordane, *trans*-nonachlor, *p,p′*-DDE, Dieldrin, *p,p′*-DDD, *cis*-nonachlor, *p,p′*-DDT, Mirex, Photomirex), 14 PBDE congeners (BDE-17, -28, -47, -49, -66, -85, -99, -100, -138, -153, -154, -183, -190 and -209), BFRs (total-α-hexabromocyclododecane (HBCDD), brominated biphenyl-101 (BB-101) and -153 (BB-153)), 33 OH-PCBs (4′-OH-CB79, 4-OH-CB97, 4′-OH-CB101, 2-OH-CB114, 4-OH-CB107/4′-OH-CB108, 3-OH-CB118, 4′-OH-CB120, 4-OH-CB127, 4′-OH-CB130, 4-OH-CB134, 3′-OH-CB138, 4-OH-CB146, 4-OH-CB159, 4-OH-CB162, 4-OH-CB163, 4′-OH-CB172, 4′-OH-CB177, 4-OH-CB178, 3′-OH-CB180, 3′-OH-CB182, 3′-OH-CB183, 3′-OH-CB184, 4-OH-CB187, 4-OH-CB193, 4′-OH-CB198/4′-OH-CB203, 4′-OH-CB199, 4′-OH-CB200, 4′-OH-CB201, 4′-OH-CB202, 4′-OH-CB208, 4,4′-diOH-CB202), pentachlorophenol (PCP), 4-OH-heptachlorostyrene (4-OH-HpCS), 14 OH-PBDEs (6′-OH-BDE17, 4′-OH-BDE17, 6′-OH-BDE49, 2′-OH-BDE68, 6-OH-BDE47, 3-OH-BDE47, 5-OH-BDE47, 4′-OH-BDE49, 4-OH-BDE42, 6-OH-BDE90, 6-OH-BDE99, 2-OH-BDE123, 6-OH-BDE85, 6-OH-BDE137), 16 $MeSO_2$-PCBs (3-$MeSO_2$-CB52, 3′-$MeSO_2$-CB49, 4-$MeSO_2$-CB52, 4′-$MeSO_2$-CB49, 4-$MeSO_2$-CB64, 3-$MeSO_2$-CB70, 3′-$MeSO_2$-CB101, 4-$MeSO_2$-CB70, 4′-$MeSO_2$-CB101, 3-$MeSO_2$-CB110, 3-$MeSO_2$-CB149, 4-$MeSO_2$-CB110, 4′-$MeSO_2$-CB87, 3′-$MeSO_2$-CB132, 4′-$MeSO_2$-CB132, 4-$MeSO_2$-CB174) and 3-$MeSO_2$-*p,p′*-DDE.

The liver and plasma samples were analysed for POPs and metabolites by the Organic Contaminants Research Laboratory/Letcher Labs at the National Wildlife Research Centre, Carleton University in Ottawa, Canada. The method for PCB, PBDE and OCP determination in plasma and liver is previously described elsewhere with some modifications (McKinney et al., 2006; Gebbink et al., 2008a,b; Gabrielsen et al., 2015).

As described in detail elsewhere (McKinney et al., 2006; Gebbink et al., 2008a,b; Gabrielsen et al., 2015), the sample fractions were analysed for the various POPs using gas chromatography (GC) and quadrupole mass spectrometry (MS). The MS was set in electron-impact (EI) mode with selected ion monitoring (SIM) for PCB and OCP analysis and electron capture negative ionization (ECNI) mode with SIM for PBDE/BFR, and (derivatized) phenolic halogenated compound analysis. Details regarding analytical procedures are provided in the supplementary material.

Table 1
Concentration of persistent organic pollutants in pilot whale liver (ng/g lipid weight). Mean, standard deviation (SD) and median values are only given for compounds that were detected in more than 50% of the samples in the respective groups (see Materials and methods). An overview of the frequencies of detections of each compound, and the min–max concentrations are given in the Supplementary data (Table S4).

Lipid weight	Adult females			Juv 0–2 years			Juv >2 years			Adult males
	n = 10			n = 8			n = 7			n = 2
	Mean	Std. dev	Median	Mean	Std. dev	Median	Mean	Std. dev	Median	Mean
Length, cm	426.8	22.76	425	255.4	65.11	264	389.7	46.34	386	485
Age	22	3.32	22	0.9	0.99	0.5	8.7	4.13	10.5	
Lipid content (%)	1.7	0.31	1.6	1.5	0.24	1.4	1.5	0.21	1.5	1.24
CB-44				77.5	18.4	71.7 [b]	45.9	18.0	47.4 [b]	66.8
CB-47/48				103.0	60.4	84.4 [b]	93.6	16.1	85.0 [b]	107.4
CB-49	23.7	16.1	19.3 [a]	119.0	65.8	103.3 [b]	105.0	32.1	95.5 [b]	147.4
CB-52	41.8	20.6	43.7 [a]	390.4	303.8	315.0 [b]	364.0	87.8	323.7 [b]	465.8
CB-56/60				31.8	11.0	28.1 [b]	28.0	7.8	27.3 [b]	21.6
CB-66	16.7	12.7	13.1 [a]	153.3	59.9	137.6 [b]	129.6	33.6	129.3 [b]	139.8
CB-74	20.8	12.9	19.1 [a]	155.4	94.0	137.9 [b]	143.9	37.4	133.7 [b]	183.2
CB-85	19.6	10.0	21.0 [a]	152.9	90.4	136.1 [b]	154.3	43.5	133.1 [b]	149.4
CB-87	28.9	13.6	29.9 [a]	200.5	92.2	180.8 [b]	190.6	58.0	163.2 [b]	178.8
CB-92	23.8	13.3	25.0 [a]	208.0	151.5	178.1 [b]	203.7	60.3	170.9 [b]	234.3
CB-95	61.7	28.0	62.0 [a]	539.5	400.2	448.4 [b]	503.6	138.7	443.1 [b]	593.1
CB-97	9.86	6.08	8.64 [a]	57.3	12.3	55.7 [b]	32.3	16.2	35.2 [ab]	31.3
CB-99	87.2	41.2	89.1 [a]	689.8	491.1	589.1 [b]	669.7	210.3	553.5 [b]	756.4
CB-101/90	102.7	47.9	101.5 [a]	839.3	486.6	747.0 [b]	841.1	243.4	745.0 [b]	845.6
CB-105	43.9	22.4	46.0 [a]	350.3	213.2	321.1 [b]	339.1	106.9	282.7 [b]	312.4
CB-110	31.4	10.4	31.1 [a]	137.7	40.0	135.5 [b]	72.0	27.4	70.3 [ab]	72.3
CB-118	110.3	53.8	105.4 [a]	930.6	580.8	862.4 [b]	888.6	292.9	733.8 [b]	884.3
CB-128	52.7	28.8	52.1 [a]	352.8	211.6	306.3 [b]	328.7	116.4	268.0 [b]	407.8
CB-130	11.0	8.0	13.0 [a]	85.0	48.3	74.1 [b]	80.6	26.1	65.7 [b]	81.3
CB-137	19.7	16.3	15.8 [a]	153.5	91.1	132.3 [b]	158.0	64.3	128.4 [b]	203.3
CB-138	316.0	154.5	320.6 [a]	2086	1168	1829 [b]	2009	720.9	1597 [b]	2190
CB-141	14.8	10.4	16.2 [a]	85.1	16.7	81.7 [b]	62.6	28.4	54.9 [b]	62.6
CB-146	56.9	27.1	57.5 [a]	363.6	183.2	339.2 [b]	345.7	123.2	280.3 [b]	350.2
CB-149	137.0	65.6	138.5 [a]	1022	654.6	841.9 [b]	980	329.9	796.1 [b]	1150
CB-151	50.1	27.2	51.0 [a]	348.0	213.7	308.3 [b]	344.6	128.3	276.4 [b]	385.4
CB-153	378.3	191.8	385.2 [a]	2453	1317	2066 [b]	2345	851.6	1876 [b]	2498
CB-156	11.4	7.3	10.5 [a]	78.9	34.3	73.3 [b]	73.6	26.5	62.2 [b]	74.6
CB-157				30.7	15.6	27.6 [b]	26.2	10.8	20.9 [b]	21.7
CB-158	13.6	4.8	14.1 [a]	87.6	56.3	72.9 [b]	80.2	25.1	71.5 [b]	85.1
CB-167	9.5	7.4	7.75 [a]	86.9	46.9	68.8 [b]	76.5	29.6	67.4 [b]	82.5
CB-170/190	69.0	33.9	66.1 [a]	329.8	146.8	286.1 [b]	279.3	111.4	226.8 [b]	336.3
CB-171	16.1	8.0	13.6 [a]	90.1	44.6	75.0 [b]	79.7	28.9	66.5 [b]	94.3
CB-172	15.4	9.7	16.7 [a]	74.3	26.0	72.2 [b]	62.9	23.5	50.8 [b]	71.6
CB-174	47.7	26.1	47.1 [a]	289.0	161.5	219.2 [b]	263.2	107.7	205.5 [b]	347.2
CB-176				29.5	15.9	23.4 [b]	26.6	11.9	20.7 [b]	35.7
CB-177	33.9	20.1	34.9 [a]	191.4	91.6	158.1 [b]	161.8	54.9	132.3 [b]	193.4
CB-178	32.2	19.2	32.1 [a]	173.06	73.9	159.6 [b]	153.3	62.2	122.8 [b]	167.4
CB-179	22.0	10.1	22.8 [a]	118.26	59.7	99.4 [b]	109.0	43.9	87.0 [b]	138.0
CB-180	213.5	109.7	209.9 [a]	1003	429.6	878.2 [b]	863.5	350.7	689.5 [b]	999.4
CB-183	58.5	29.3	57.4 [a]	285.7	124.4	247.7 [b]	249.0	98.4	202.3 [b]	285.2
CB-187	194.3	94.3	189.8 [a]	935.1	389.8	840.5 [b]	799.5	319.4	641.8 [b]	908.3
CB-196/203	40.9	25.7	36.1 [a]	160.9	51.5	157.6 [b]	113.6	43.6	96.8 [ab]	138.5
CB-199	43.1	31.6	41.9 [a]	186.3	57.5	183.0 [b]	138.1	56.1	111.3 [ab]	180.0
Σ_{43}PCB	2523	1211	2533 [a]	16236	8617	14364 [b]	15016	5128	12077 [b]	16678
PeCB	9.2	8.09	6.88 [a]	78.6	17.0	74.0 [b]	35.6	24.4	34.3 [ab]	40.4
HCB	280.5	134.4	264.5 [a]	2088	580	1892 [b]	1206	472.5	947 [b]	1224
trans-Chlordane	4.85	4.06	3.55 [a]	21.7	7.92	18.6 [b]	18.2	7.57	16.0 [b]	18.8
cis-Chlordane	51.1	20.5	50.2 [a]	299.9	70.7	283.5 [b]	239.0	120.9	174.4 [b]	238.1
trans-Nonachlor	294.1	142.5	297.6 [a]	2082	1439	1856 [b]	2026	780.4	1619 [b]	2125
p,p′-DDE	1447	817	1227 [a]	9581	6242	8549 [b]	11280	3945	9231 [b]	14240
Mirex	59.1	36.9	62.0 [a]	230.2	76.9	215.7 [b]	168.0	72.89	133.1 [ab]	165.7
ΣOCP	2145	1106	1954 [a]	14381	8204	13515 [b]	14973	5170	12089 [b]	18052
BDE-28	2.56	2.09	1.54 [a]	23.4	10.2	22.2 [b]	17.0	7.91	14.3 [b]	11.4
BDE-49	2.27	1.59	2.71 [a]	10.7	4.34	10.4 [b]	6.30	2.44	6.77 [b]	4.30
BDE-47	25.9	21.1	28.9 [a]	254.3	143.8	253.2 [b]	287.3	79.7	272.9 [b]	192.5
BB-101				4.12	3.04	4.27 [b]	5.11	2.39	3.94 [b]	4.27
BDE-100	10.7	5.0	12.0 [a]	67.3	27.3	74.0 [b]	64.0	20.4	60.2 [b]	45.2
BDE-99	11.3	11.0	10.6 [a]	68.1	40.1	65.1 [b]	71.0	28.4	64.6 [b]	56.2
BDE-154	49.8	22.5	54.0 [a]	274.9	103.5	259.8 [b]	199.9	81.5	172.7 [b]	147.0
BB-153/BDE-154	14.9	8.0	18.0 [a]	81.3	33.1	82.8 [b]	61.0	27.0	50.0 [b]	41.4
BDE-153	3.04	2.83	3.06 [a]	19.4	12.1	24.0 [b]	15.9	8.72	13.4 [ab]	
ΣPBDE	105.5	57.1	128.5 [a]	718.1	315.0	725.7 [b]	661.4	224.9	599.2 [b]	460.3
ΣBFR	15.8	8.2	18.6 [a]	85.4	35.6	86.2 [b]	66.1	29.1	53.49 [b]	45.7
ΣPBDE/BFR	121.3	64.8	150.7 [a]	803.5	348.3	811.9 [b]	727.6	253.6	651.1 [b]	506.0

The letters a and b indicate if there is significant difference or not between the groups. No common letter indicates significant difference (Kruskall–Wallis (pairwise comparison) 95% significance level).

2.4.1. Lipid determination

The lipid content in liver samples was determined by drying a 1 mL aliquot (10% of the original sample extract) taken from the ASE (accelerated solvent extraction) extract (see Supplementary material) at 120 °C overnight. The difference in mass, when compared to one tenth of the original sample mass, was interpreted to be the lipid content of the liver sample. The lipid content in blood was determined by a sulfo-phospho-vanillin reaction using an olive oil-derived calibration curve ranging from 2 to 12 mg/mL (Frings et al., 1972; Gebbink et al., 2008b). The absorbance was measured at 540 nm, the maximum absorbance.

2.5. Statistical analysis

Only compounds and congeners that were detected in more than 50% of the individuals were included in the statistical analysis. Samples with concentrations below the method limits of quantification (MLOQs) or method limits of detection (MLODs) were set to a random value between 0 and the MLOD or MLOQ. The group of adult males consisted of only two individuals and the results on the adult males are thus not included in the statistical comparison between groups. The results on these two males are nevertheless presented in the tables and figures. The statistical programme SPSS was used for the statistical analyses. Normal distributions of the data were analysed using the Shapiro–Wilk normality test, and since not all of the data were normally distributed the difference between medians were analysed by a non-parametric Kruskall–Wallis 1-way ANOVA (all pairwise) test. Correlations between contaminants in the liver and plasma and correlations between metabolites and precursor compounds were analysed with Spearman correlation. Differences between concentrations in the liver and plasma were tested with pairwise t-test. The statistical level of significance was set to $p < 0.05$.

3. Results

Of the 175 contaminant compounds analysed for, 59 and 27 were detected in >50% of the pilot whale liver and plasma samples, respectively. The concentrations of all parent contaminant groups (ΣPCB, ΣOCP, ΣBFR) were significantly higher in the two groups of juvenile whales as compared to the adult females, both in the liver and in the plasma (Fig. 1). The only halogenated phenolic compound that was detected in >50% of the samples, was co-eluting 4-OH-CB107/4′-OH-CB108 in plasma. An overview of the lipid weight (lw) concentrations of the compounds detected in >50% of the liver and plasma samples are given in Tables 1 and 2, respectively. The wet weight (ww) concentrations of the compounds detected in >50% of the liver and plasma samples are given in the supplementary material Table S3 and for the compounds that were detected in <50% of the respective tissue samples, an overview is given in Tables S4 and S5 for ww and lw, respectively.

The higher number of compounds detected in the liver samples than in the plasma samples is probably due to the higher lipid levels in liver as compared to plasma. The lipid content in plasma was only approximately 20% that of the liver (range 8–49%). Thus, the low lipid levels in the plasma most likely caused some of the concentrations to be below the MLODs. There were no significant ($p > 0.05$) differences in the lipid concentrations in liver and plasma respectively, between the three groups (adult females, Juv 0–2 years, Juv >2 years). Within each of the groups, the hepatic ww concentrations of the compounds were approximately five times higher than in the plasma, reflecting the different lipid content in the two matrices (Table S3). There was no significant correlation between ww concentrations of OCs and lipid concentrations either in liver or in plasma, and the lipid content in the liver and in the plasma was not correlated, thus indicating the lesser importance of the lipid content in the concentrations of the measured contaminants. However, to facilitate comparison of concentration between

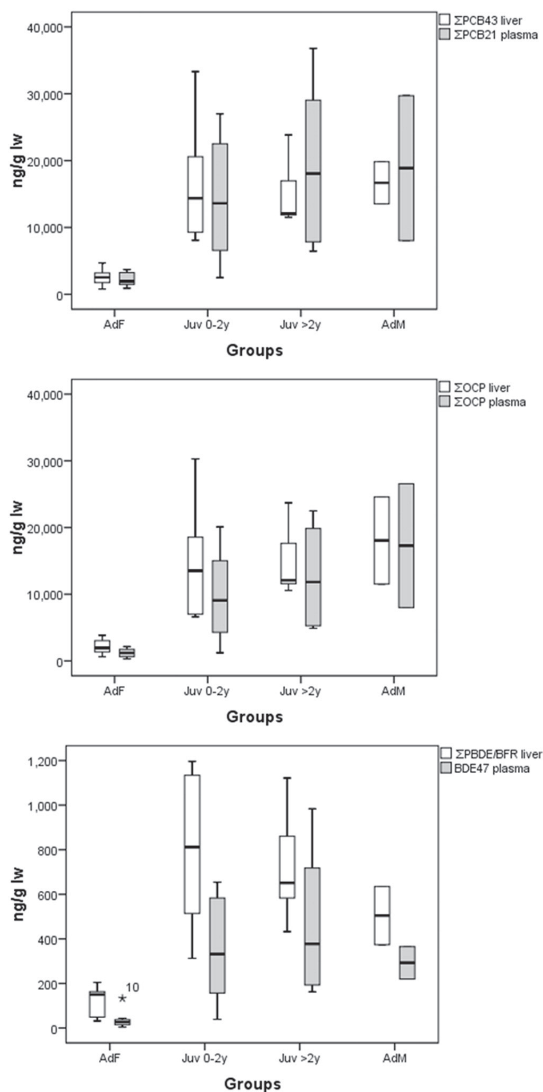

Fig. 1. Box plots for concentrations of mean ΣPCB, ΣOC and ΣPBDE concentrations in ng/g lw in the liver and plasma of pilot whales divided into age and gender groups of adult females (AdF), juveniles 0–2 years old (0–2 years), juveniles more than 2 years old (>2 years), and adult males (AdM). Note the different scale for the PBDE/BFR results and that only BDE-47 is shown for plasma.

tissues, the lipid normalized concentrations have been calculated and are used unless noted otherwise.

The PCBs and the OCPs constituted the main contaminant groups in both the liver and plasma, each contributing 47–53% and 45–51% respectively, of the total OC concentrations in liver and 51–64% and 35–47% respectively, in plasma. The BFR compounds contributed to only 1.5–2.5% and 1–3% of the ΣOC concentrations in liver and in plasma respectively.

With respect to the individual POPs, *p,p′*-DDE was the predominant compound in both the liver and plasma in all whale groups. *p,p′*-DDE constituted around 70% of ΣOCPs in both the liver and plasma and around 30% of the total OC concentration. Among the OCPs, the second highest levels were *trans*-nonachlor and hexachlorobenzene (HCB).

Table 2
Concentration of contaminants in pilot whale plasma (ng/g lipid weight). Mean, standard deviation (SD) and median values are only given for compounds that were detected in more than 50% of the samples in the respective groups (see Materials and methods). An overview of the frequencies of detections of each compound, and the min–max concentrations are given in the Supplementary data (Table S4).

Lipid weight	Adult females			Juv 0–2 years			Juv >2 years			Adult males
	n = 10			n = 8			n = 7			n = 2
	Mean	Std. dev	Median	Mean	Std. dev	Median	Mean	Std. dev	Median	Mean
Lipid content (%)	0.38	0.14	0.37	0.35	0.21	0.29	0.38	0.21	0.28	0.19
CB-52	42.70	28.6	41.74 [a]	386.4	312.9	360.9 [b]	460.9	243.5	466.3 [b]	571.2
CB-74				154.6	111.0	153.1 [b]	186.9	98.9	140.6 [b]	178.8
CB-87				184.2	128.7	127.9 [b]	285.4	187.8	290.8 [b]	186.8
CB-92				220.0	151.0	206.3 [b]	283.0	172.6	340.4 [b]	310.9
CB-95	50.05	42.07	43.52 [a]	511.6	393.0	445.6 [b]	604.8	337.6	529.2 [b]	752.2
CB-99	69.7	53.7	74.49 [a]	661.7	506.7	566.7 [b]	841.1	540.6	965.8 [b]	908.5
CB-101/90	111.9	70.7	119.7 [a]	932.7	618.8	884.6 [b]	1290	855.1	1443 [b]	1181
CB-105				354.1	251.4	327.3 [b]	432.7	300.7	410.7 [b]	436.8
CB-118	98.0	57.5	94.2 [a]	1025	703.8	969.0 [b]	1289	796.6	1140 [b]	1174
CB-128				358.5	224.3	341.7 [b]	495.5	355.9	514.4 [b]	429.2
CB-138	309.6	166.1	266.4 [a]	2078	1391	1945 [b]	2750	1791	2575 [b]	2752
CB-146				351.0	278.0	358.0 [b]	505.5	333.9	481.1 [b]	349.4
CB-149	142.3	90.4	134.5 [a]	1081	781.4	941.8 [b]	1402	854.2	1299 [b]	1644
CB-151	32.80	30.5	28.45 [a]	316.2	243.6	280.3 [b]	443.5	282.9	436.6 [b]	480.7
CB-153	426.5	208.6	359.8 [a]	2757	1830	2597 [b]	3709	2428	3293 [b]	3577
CB-170/190	55.39	47.5	31.38 [a]	335.5	207.6	307.3 [b]	449.7	310.1	436.6 [b]	367.6
CB-174				350.7	217.2	351.7 [b]	496.6	394.6	399.6 [b]	573.4
CB-179				93.70	67.98	83.62 [b]	137.5	94.4	125.8 [b]	139.9
CB-180	252.5	148.2	222.4 [a]	1099	690.8	1008 [b]	1545	1125	1291 [b]	1425
CB-183	53.40	27.49	58.53 [a]	271.1	191.9	273.3 [ab]	396.5	343.7	366.3 [b]	363.4
CB-187	198.5	99.4	184.9 [a]	840.0	496.0	826.4 [b]	1294	1026	1088 [b]	1069
ΣPCB 21	2187	1027	1957 [a]	14363	9527	13617 [b]	19298	12729	18065 [b]	18871
HCB	128.1	68.43	130.8 [a]	756.1	313.6	767.0 [b]	650.5	512.4	386.2 [b]	720.1
cis-Chlordane				240.8	113.5	223.9 [b]	292.9	261.1	196.1 [b]	271.6
trans-Nonachlor	270.3	186.3	264.5 [a]	1999	1445	1770 [b]	2683	1680	2624 [b]	2696
p,p′-DDE	769.1	556	711.8 [a]	6767	4921	6166 [b]	9157	5573	8659 [b]	13590
ΣOCP	1210	672	1188 [a]	9763	6674	9076 [b]	12784	7886	11823 [b]	17277
BDE-47	34.49	36.9	26.59 [a]	354.6	246.8	331.5 [b]	477.8	349.1	377.4 [b]	292.8
4-OH-CB107/ 4′-OH-CB108	84.85	68.97	84.2 [a]	138.5	114.3	138.8 [a]	99.5	92.6	88.6 [a]	140.4

The letters a and b indicate if there is significant difference or not between the groups. No common letter indicates significant difference (Kruskall–Wallis (pairwise comparison) 95% significance level).

Thus, *p,p′*-DDE, *trans*-nonachlor and HCB constituted 94–97% and 97–98% of the total OCPs in liver and in plasma, respectively. Among the PCBs, the concentrations of CB-153 and CB-138 were highest within all whale groups in both the liver and plasma, with levels approximately two times as high as the next most concentrated PCB congeners. The concentrations of the PCB congeners were approximately as follows: CB-153 (15%) > CB-138 (13%) ≫ CB-180 (6%) = CB-149 (6%) = CB-187 (6%) > CB-118 (5%) = CB-101/90 (5%) > CB-99 (4%) in the liver, and CB-153 (19%) > CB-138 (14%) > > CB-180 (8%) = CB-149 (8%) > CB-187 (7%) ≈ CB-118 (6%) = CB-101/90 (6%) > CB-99 (4%) in the plasma, although the relative concentrations differed slightly between the age/gender groups. The PBDE/BFR congeners found in highest concentrations in liver were BDE-47 and BDE-154. These two compounds constituted 63–67% of the total PBDEs/BFRs in the pilot whales. In plasma, only BDE-47 was found in more than 50% of the individuals (Table 2). Other BDE congeners (including BDE-154) were only found in few individuals in low concentrations (Table S4).

All OCs in the liver, except for CB-44, CB-47/-48, CB-74, CB-97, CB-196/-203, CB-199, *trans*-chlordane, Mirex and BDE-153, were significantly negatively correlated to age in females ($r_s = -0.731$ to -0.970, $p < 0.05$), whereas only CB-56/-60, BDE-49, BDE-47 and BDE-99 were significantly negatively correlated to length in females ($r_s = -0.579$ to -0.611, $p < 0.05$). In males only CB-44, CB-97, CB-110, CB-141, PeCB, HCB and BDE-49 were significantly negatively correlated to age ($r_s = -0.614$ to -0.904, $p < 0.05$) and all of them except CB-44 and including BDE-154 were also significantly negatively correlated to length ($r_s = -0.552$ to -0.859, $p < 0.05$). Some of the contaminants that were negatively correlated to age in males were those that also were found not to differ between AdF and juv >2 years when comparing the groups (Table 1).

3.1. Liver vs plasma concentrations

When pooling all individuals, the sum concentrations of the contaminant groups in the liver and plasma were highly correlated: ΣPCBs: $r_s = 0.923$, $p < 0.001$, ΣOCPs: $r_s = 0.929$, $p < 0.001$, ΣPBDEs: $r_s = 0.840$, $p < 0.001$. When analysing the age groups separately, ΣPCBs and ΣOCPs were still significantly correlated between liver and plasma in the AdF and Juv 0–2 year groups, but not in Juv >2 year group. Furthermore, ΣPBDEs were not significantly correlated between liver and plasma in any of the age groups ($p > 0.05$). Lipid adjusted levels of ΣPCBs and ΣBFRs did not differ between liver and plasma, whereas ΣOCs were significantly higher in liver than in plasma (paired t-test: $p = 0.005$). All the single compounds detected in both the liver and plasma were also highly correlated ($p < 0.001$). However, the concentrations of some individual POPs were significantly different in the plasma and liver; CB-153 ($p = 0.044$), CB-174 ($p = 0.027$) and BDE-47 ($p = 0.017$) were higher in plasma than in liver, whereas *p,p′*-DDE ($p = 0.004$) and HCB ($p < 0.001$) were higher in liver than in plasma. When analysing the age groups separately the difference between liver and plasma of the PCBs and BDE-47 was not significant in any of the age groups but HCB was still highly significantly different for all age groups and *p,p′*-DDE was still significant in the AdF and Juv 0–2 year groups (paired t-test: $p < 0.05$).

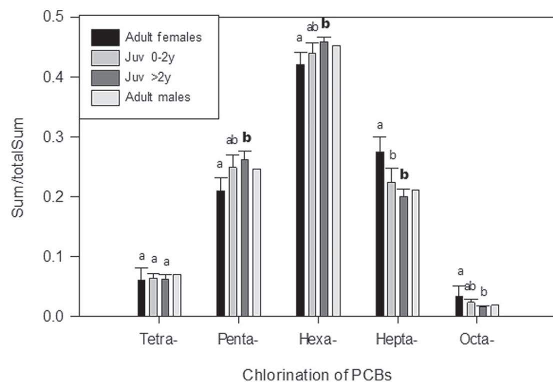

Fig. 2. Relative distribution of the tetra- to octa-chlorinated PCB homolog group concentration to the total PCB concentration ratio in liver for different age/sex groups of Faroese pilot whales. The letters a and b indicate if there is significant difference or not between the groups. No common letter indicates significant difference (Kruskall–Wallis (Mann–Whitney U-test), $p < 0.05$, bold letters $p < 0.01$).

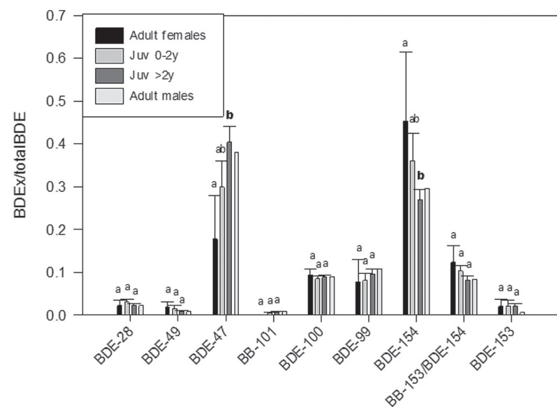

Fig. 4. Relative distribution of individual PBDE concentration to the total PBDE concentration ratios in liver for different age/sex groups of Faroese pilot whales. The letters a and b indicate if there is significant difference or not between the groups. No common letter indicates significant difference (Kruskall–Wallis (Mann–Whitney U-test), $p < 0.05$, bold letters $p < 0.01$).

3.2. Differences between age/sex groups

In liver, the concentrations in adult females were significantly lower than those in both of the juvenile groups for all the compounds ($p < 0.05$), except for CB-97, CB-110, CB-196/203, CB-199, pentachlorobenzene (PeCB), Mirex and BDE-153, which were not significantly different between the adult females and the juveniles > 2 years old (Table 1). In the two juvenile groups there were no significant differences in the concentrations of any of the measured POPs ($p < 0.05$) (Table 1). The sample size of adult males was too low to perform statistical analysis, but the levels were mostly similar to the levels in Juv >2 years.

In plasma the concentrations of all the compounds in adult females were significantly lower than in the juveniles, with only one exception, CB-183, which was not significantly different from the Juv 0–2 year group ($p = 0.107$) (Table 2). There was no statistical difference between the two juvenile groups for any of the measured POPs in the plasma.

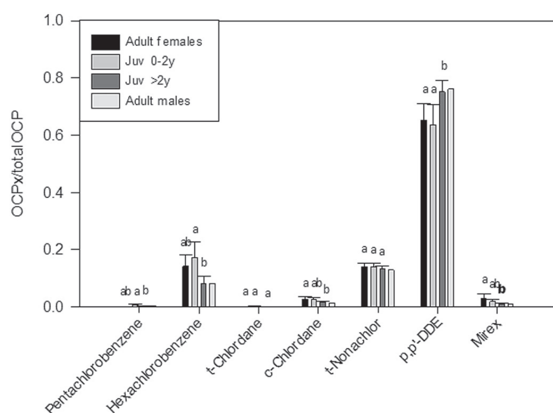

Fig. 3. Relative distribution of individual OC concentration to the total OC concentration ratios in liver for different age/sex groups of Faroese pilot whales. The letters a and b indicate if there is significant difference or not between the groups. No common letter indicates significant difference (Kruskall–Wallis (Mann–Whitney U-test), $p < 0.05$, bold letters $p < 0.01$).

3.2.1. Relative distribution of contaminants between the groups

Figs. 2–4 show the relative distributions of the measured POPs in liver of the different age/sex groups. The octa-chlorinated PCBs were found in relatively higher concentrations in the adult females than in the >2 year group ($p < 0.05$) and the hepta-chlorinated PCBs were relatively higher than both juvenile groups (AdF–Juv < 2 years: $p < 0.001$, AdF–Juv 0–2 years: $p < 0.05$) (Fig. 2). The congeners with five and six chlorine atoms (penta- and hexa-chlorinated) were relatively higher in the juveniles >2 years than in the adult female group ($p < 0.01$) (Fig. 2). For these four PCB congener homolog groups, the relative distribution in the juveniles 0–2 years was in-between the adult females and the juvenile >2 year group. PCB congeners with four chlorine atoms (tetrachlorinated) had approximately the same relative concentrations in all the age/sex groups.

The relative concentrations of *p,p′*-DDE were lower, whereas the other analysed OCPs were relatively higher, in adult females and juveniles 0–2 years than in the >2 years juveniles (Fig. 3) ($p < 0.05$). PeCB and HCB were relatively highest in the juveniles 0–2 years (Juv >2 years–Juv 0–2 years: $p < 0.05$) whereas Mirex and *cis*-chlordane were relatively highest in adult females (Mirex: AdF–Juv >2 years: $p < 0.01$, *cis*-chlordane: AdF–Juv >2 years: $p < 0.05$).

The relative distribution of the two BFR congeners found in highest concentration, BDE-47 and BDE-154, differed between the groups (Fig. 4). In adult females BDE-154 was relatively much higher than BDE-47 ($p < 0.01$). In juveniles 0–2 years the concentrations of both these congeners were approximately similar, and in juveniles >2 years the concentration of BDE-47 was relatively higher than that of BDE-154 ($p < 0.01$). BB-153/BDE-154 was also relatively lower in juveniles >2 years than in adult females ($p < 0.05$).

3.2.2. Maternal transfer

That the concentrations of contaminants were much lower in the adult female group than in the juvenile group (Tables 1, 2 and Fig. 1) can be explained by the transfer from the mother to offspring during gestation and lactation.

Comparing the concentrations of each analysed pollutant in the group of juveniles 0–2 years, with the concentrations in the adult female

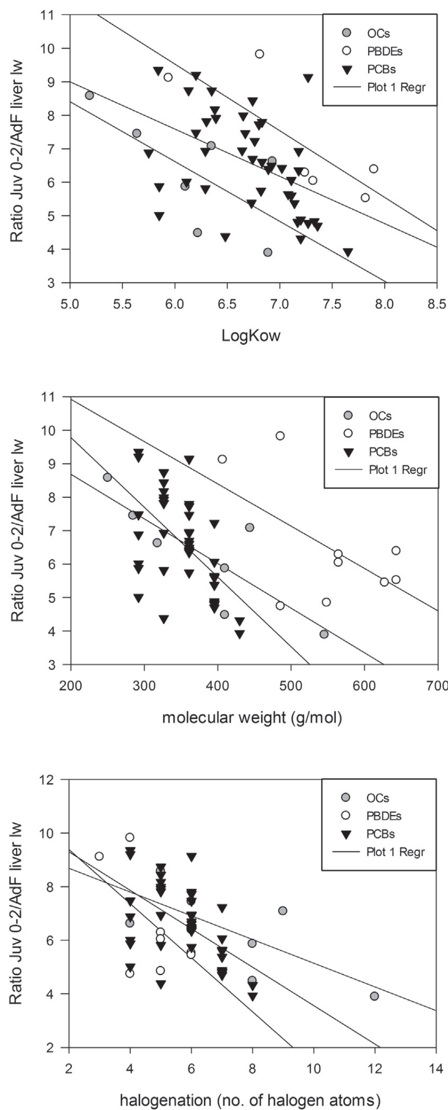

Fig. 5. In the liver of Faroese pilot whales, the ratio between mean concentrations of PCBs, OCs and PBDEs in juveniles 0–2 years old to mean concentrations in adult females versus the logK_{ow}, molecular weight and halogenation of the contaminant. LogK_{ow} values were obtained from Hawker and Connell (1988), Simpson et al. (1995), Braekevelt et al. (2003), and Shen and Wania (2005).

group showed that the levels for all the contaminants were lower in the adult females, with Juv 0–2 years/AdF ratios from 3.9 to 9.8 in liver and from 4.2 to 10.5 in plasma.

The different Juv 0–2 years/AdF ratios for the various contaminants indicated that the rate of maternal transfer differed for the individual contaminants. Fig. 5 shows the correlation in liver between relative concentrations in juveniles 0–2 years and adult females and the log K_{ow} value, molecular weight and halogenation for the different contaminant groups. The Spearman correlation factors are given in Table 3 for both the liver and plasma.

The Juv 0–2 years/AdF ratios for all the contaminants seen combined were significantly negatively correlated to all the three properties analysed for (log K_{ow}, molecular weight and halogenation degree) in liver ($p < 0.001$). However, these three properties were all positively intercorrelated ($p < 0.001$). In plasma only halogenation degree showed significant negative correlation with the Juv 0–2 years/AdF ratios ($p = 0.001$) (Table 3). When considering the different contaminant groups separately, PCBs were highly significantly correlated to all three properties in both liver and plasma ($p \leq 0.001$). However, for the OCPs only molecular weight was significant in the liver ($p < 0.05$). No significant correlation was seen for OCPs in plasma and for PBDEs in the liver. Only one PBDE compound was detected in plasma and relationship with log K_{ow}, molecular weight and halogenation degree could thus not be analysed. The number of PCB compounds was much higher than for the other two compound groups. Thus, the low number of the OCP and PBDE compounds, especially in plasma, is probably one of the reasons for not finding correlations between the physico-chemical properties and the concentrations of these compounds.

3.3. Metabolites and metabolic capacity

In liver, no OH-PCB or -PBDE metabolites were detected, and $MeSO_2$-PCB and -DDE metabolites were only detected in few samples. In plasma, OH-metabolites were detected in some of the individuals, whereas no $MeSO_2$-containing metabolites were detected in the plasma. 3-$MeSO_2$-*p,p′*-DDE was not detected in any of the samples. Table 4 gives an overview of the metabolites detected and their concentrations in liver and plasma. OH- and to a lesser extent $MeSO_2$-containing metabolites are known to be more associated with proteins rather than lipids, contrary to other POPs (PCBs, OCPs and PBDEs), which are associated with lipids. Thus, the levels of OH-PCBs and the relative levels of ΣOH-PCB to ΣPCB concentrations are reported in ww concentrations.

The measurement of metabolites 4-OH-CB107 and 4′-OH-CB108 could not be distinguished as they are both pentachloro-OH-PCBs with the same molecular weight, and their methylated derivative forms coelute in gas chromatography and are difficult to separate. This was the only OH-PCB metabolite found in more than one individual. The concentrations of 4-OH-CB107/4′-OH-CB108 were at the same level in all age/sex groups. There were large variations within all groups and no significant ($p > 0.05$) difference between them.

The mean relative concentration of the metabolite to the sum of CB-105 and CB-118, two possible precursors for the metabolite, was 4.8 (range 1.8–10.5). The levels of the possible precursors (CB-105 + CB-118) and the metabolite (4-OH-CB107/4-OH-CB108) were very weakly and not significantly correlated (Fig. 6, $r_s = 0.119$, $p = 0.56$). However, the levels of metabolites in adult females were much higher relative to the concentrations of the precursor compounds compared to the other age groups. This indicates that the metabolites are not transferred to the same extent to the juveniles as the precursor PCBs and/or that the metabolic hydroxylation is less active in the younger whales.

To estimate the overall metabolism of the contaminants the total concentration of contaminant and total concentration of metabolites can be compared. In plasma, the OH-PCB/ΣPCB concentration ratios were between 0.004 and 0.189 with a mean of 0.045, and ratios of OH-PBDE/ΣPBDE concentration ratios were between 0.21 and 37.0 and the mean ratio was 5.9. The highest ratio was found in an adult female. The OH-metabolites from PBDE were thus higher although the concentration of PBDE was much lower than of PCB. For processes that lead to OH-containing metabolites, this suggests greater metabolism of PBDEs than PCBs. The ratios of $MeSO_2$-PCBs/ΣPCBs in the liver were between 0.006 and 0.046 with a mean of 0.025 for all samples (non-detects not calculated). The $MeSO_2$-PCBs were not significantly correlated to their precursor compounds (Fig. 7).

Table 3
Spearman correlations between the ratio of the concentrations of persistent organic pollutants in juveniles 0–2 years and adult females and log K_{ow}, molecular weight and halogenation of the different compounds.

Correlations			Ratio Juv 0–2 years/AdF liver lw			Ratio Juv 0–2 years/AdF plasma lw		
POP group			LogKow	Molecular weight (g/mol)	Halogenation	$LogK_{ow}$	Molecular weight (g/mol)	Halogenation
All OCs	Spearman's rho	Correlation coefficient	−.457**	−.496**	−.512**	−.287	−.305	−.591**
		Sig. (2-tailed)	.000	.000	.000	.155	.130	.001
		N	56	59	59	26	26	26
OCPs	Spearman's rho	Correlation coefficient	−.607	−.775*	−.595	.800	0.000	−.400
		Sig. (2-tailed)	.148	.041	.159	.200	1.000	.600
		N	7	7	7	4	4	4
PBDEs	Spearman's rho	Correlation coefficient	−.600	−.152	−.260			
		Sig. (2-tailed)	.208	.696	.499			
		N	6	9	9	1	1	1
PCBs	Spearman's rho	Correlation coefficient	−.505**	−.582**	−.582**	−.550**	−.580**	−.580**
		Sig. (2-tailed)	.001	.000	.000	.010	.006	.006
		N	43	43	43	21	21	21

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

4. Discussion

4.1. Contaminant levels

The relative distribution of the different congeners and compound groups in liver and plasma of pilot whales was comparable to the distribution in blubber (Nielsen et al., 2014) and appears comparable to that previously reported in toothed whales (McKinney et al., 2006; Carballo et al., 2008; Bennett et al., 2009; Weijs et al., 2009a,b; Fair et al., 2010; Desforges et al., 2013a). The monitoring of POPs in pilot whales on the Faroe Islands has mostly consisted of analyses in blubber. POPs in liver from Faroe Islands pilot whales have previously only been reported in 15 whales from 2004 (Dam et al., 2010). The levels of POPs in whales in the present study were generally at the same levels as in the 2004 samples. *p,p′*-DDE and ΣPCB were much higher than the levels reported in livers of pilot whales from the NW Atlantic (Gulf of Maine) in 1991–96 (Weisbrod et al., 2000). Furthermore, ΣPCBs were at the same level or lower than reported in livers of stranded pilot whales from Massachusetts in 1986–90 (Tilbury et al., 1999). This geographical, inter-species comparison and time-trend comparison shows that levels in the present pilot whales were relatively high and have not declined a lot over the last decade. Levels of pollution vary between species, geographical areas and years, and comparisons between studies must reflect this. However, compared to recent analyses in cetaceans and seals, the POP levels seem to be higher than in cetaceans and seals from the Arctic (Canadian Arctic and Svalbard) and the North Sea (McKinney et al., 2006; Kelly et al., 2008b; Routti et al., 2008, 2009a; Weijs et al., 2010), but lower than in cetaceans from the more southern part of the Atlantic and the US east coast and from the highly POP contaminated St. Lawrence River (McKinney et al., 2006; Carballo et al., 2008; Montie et al., 2008; Houde et al., 2009). Also, it appears that the herein reported POP concentrations are similar to, or within the higher end of the levels found in whole blood samples from several different species of toothed whales stranded along the Japanese coast (Nomiyama et al., 2010; Ochiai et al., 2013) and the PCB concentrations reported in polar bears from Svalbard in 2008 (Bytingsvik et al., 2012).

High exposure to POPs is known to be related to effects on different physiological systems (Letcher et al., 2010). The concentrations in the pilot whale liver and plasma in the present study are higher than the levels shown to affect hormone and vitamin levels in other marine mammals, including belugas (*Delphinapterus leucas*) (Villanger et al., 2011). The lw concentrations of PBDEs in the pilot whale liver and plasma were several times higher than the concentrations in blubber of beluga showing negative relationship to thyroid hormones (Villanger et al., 2011). The PCB levels in plasma were several times higher than found in grey seal pups showing negative effects on retinol levels (Jenssen et al., 2003). The concentrations were also higher than or at the same levels as in serum from Californian sea lions showing impacts on Vitamin A (retinol) concentrations (Debier et al., 2005) and in the liver and plasma of ringed seals from the Baltic showing effects on the thyroid hormone and vitamin A levels (Routti et al., 2008, 2009a, 2010).

Table 4
Concentration of hydroxyl-(OH−) and methylsufonyl-($MeSO_2$-) containing PCB and/or PBDE metabolites detected in the liver and plasma samples of Faroese pilot whales in ng/g wet weight.

Matrix		n	Mean ± std. dev. ng/g ww	Min–max ng/g ww
Liver	3′-$MeSO_2$-CB49	10	2.65 ± *0.64*	(1.69–4.03)
	4′-$MeSO_2$-CB49	7	0.61 ± *0.19*	(0.42–1.00)
	4-$MeSO_2$-CB64	10	1.43 ± *0.44*	(1.03–2.40)
	3-$MeSO_2$-CB70	2	1.63 ± *0.14*	(1.53–1.73)
	3′-$MeSO_2$-CB101	12	1.88 ± *1.10*	(0.78–4.63)
	4-$MeSO_2$-CB70	8	1.39 ± *0.46*	(1.02–2.23)
	4′-$MeSO_2$-CB101	7	0.72 ± *0.33*	(0.52–1.39)
	4′-MeSO2-CB87	1	0.66	
Plasma	3-OH-CB118	1	0.46	
	4-OH-CB107/4′-OH-CB108	14	0.54 ± *0.11*	(0.39–0.71)
	4′-OH-BDE17	10	1.77 ± *1.40*	(1.04–5.68)
	6′-OH-BDE49	1	0.8	

n = number of individuals in which the metabolite was detected.

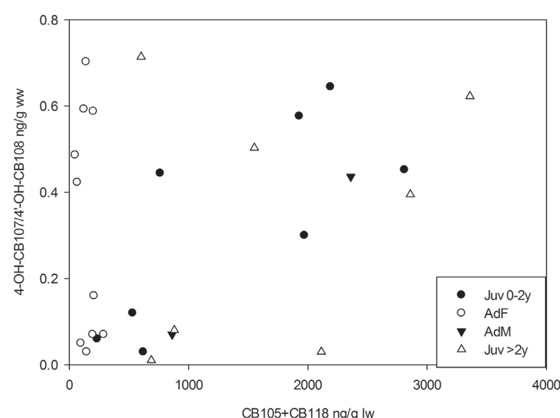

Fig. 6. Correlation between the 4-OH-CB107/4′-OH-CB108 metabolite concentrations and their precursor PCBs (CB-105 and CB-118) in Faroese pilot whale plasma.

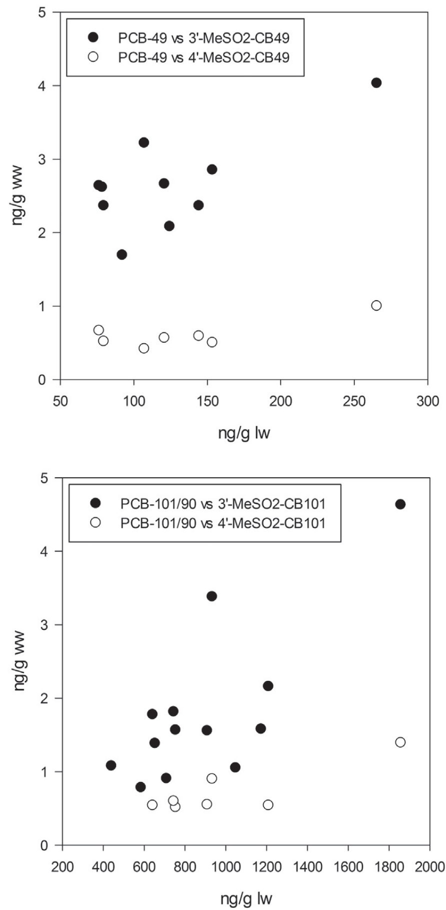

Fig. 7. Correlations between detected $MeSO_2$-PCB metabolites and their precursor PCBs in Faroese pilot whale liver.

Furthermore, the concentrations were highly exceeding the threshold concentrations for effects on circulatory retinol (1530 ng/g lw) and hepatic vitamin A (1660 ng/g lw), and the integrated toxicity reference values of 1630 ng/g lw, suggested by Desforges et al. (2013b) from analyses in beluga. The PCB concentrations in juvenile pilot whales also exceeded the threshold levels for reproductive effects of 10,000 ng/g lw reported for bottlenose dolphins (Hall et al., 2006).

4.2. Partitioning between liver and plasma

The lw concentrations of the detected contaminants in this study were mostly similar in the liver and plasma and correlated significantly in these two tissues. The OCPs *p,p′*-DDE and HCB were however significantly higher in liver, whereas CB-153, CB-174 and BDE-47 were significantly higher in plasma. Kelly et al. (2008b) also reported ΣDDTs and Σchlorobenzenes in beluga to be higher in the liver than in the blood, whereas ΣPCBs were higher in the blood than in the liver. Different partitioning of contaminants between tissues is dependent on the properties of the compounds such as polarity/lipophilicity and the molecular size and mass leading to different toxicokinetics. Lipophilic pollutants that enter the blood are quickly distributed to tissues with higher lipid content such as the liver and adipose tissue (Boon et al., 1994; AMAP, 2004). POPs measured in blood will thus be reflective of the recent exposure, but also of the release of compounds from tissue stores since mobilization of the fat deposits which are associated with starvation, disease, or growth, and for the females also with pregnancy and lactation, can lead to rapid release of the stored contaminants into the blood. The concentrations of lipids and lipophilic contaminants in the blood may thus change as a result of several factors such as feeding status, metabolism and the overall health condition of the animal, in addition to the reproduction status.

In bottlenose dolphins it has been shown that compounds seem to be selectively mobilized from blubber to blood according to their chemical properties (Yordy et al., 2010). DDTs and chlordanes have been found to be more strongly accumulated in the blubber compared to PCBs, PBDEs, Mirex and HCB, and penta-, hexa- and hepta-PCBs and tetra-PBDE homolog groups were most prone to mobilization (Yordy et al., 2010). Thus, the higher levels of some PCBs in plasma samples of the current pilot whales could indicate non-equilibrium conditions after mobilization of contaminants from blubber.

Blubber was not analysed in the present study, but nine of the individuals in the present study (all juveniles) have been previously analysed for 14 of the PCBs (CB-28, -52, -99, -101, -105, -118, -128, -138, -153, -156, -170, -180, -183 and -187) and five of the OCPs (HCB, *trans*-chlordane, *cis*-chlordane, *p,p′*-DDE and Mirex) in blubber in another study (Nielsen et al., 2014). Comparing these data with the liver and plasma concentrations of those compounds, shows that all the OCPs and PCBs except CB-28, -52, -101, -153, and -156 were significantly lower in blubber than in the liver when lipid adjusted concentrations were compared. All these PCBs and OCPs correlated between blubber and plasma ($p < 0.02$) and lw concentrations were significantly lower in the blubber than in the plasma ($p < 0.05$) except for *cis*-chlordane which was borderline significant ($p = 0.058$).

The mobilization of blubber in the analysed individuals is likely since body fat content and the lipid content in the blubber of pilot whales have shown to be highest during the winter and lowest in the summer (Lockyer, 1993). The samples from the present study were from July and September when the pilot whales are in their lowest body condition, since they have been using their energy stores that have been built up during the winter. For mature females and males the energy use is especially connected to mating and reproductive activities, respectively (Lockyer, 1993, 2007).

Xenobiotics are principally biotransformed in the liver to facilitate excretion, and liver is the tissue containing the highest concentrations of metabolising enzymes and especially cytochrome P450 monooxygenases. The higher concentrations of *p,p′*-DDE and HCB in the liver compared to plasma can be related to the binding to these liver proteins. Cetaceans are known to have low activity of CYP2B (Tanabe et al., 1988; Boon et al., 1989, 1992) which metabolize DDE, and *p,p′*-DDE will thus likely be metabolized very slowly, leading to accumulation instead of removal in the liver cells. HCB can be metabolized via CYP3A mediation (Den Besten et al., 1993) and subsequently via glutathione conjugation (To-Figueras et al., 1997), which is also expressed mostly in the liver. Thus, the high concentrations of *p,p′*-DDE and HCB in liver may be linked to very low activities of these two CYP enzymes in pilot whales.

Our results showed that in liver both BDE-47 and BDE-154 were found at the highest concentrations whereas BDE-47 was the only congener found in plasma (in most samples). Analyses in rodents have shown that BDE-47 is quickly distributed from the blood to the adipose tissue (Örn and Klasson Wehler, 1998). Analyses of PBDEs (BDE-28, -47, -99, -100, -153, -154) in blubber of four of the individual pilot whales from the present study showed much higher levels of BDE-47 than the other congeners in blubber, with BDE-47 and BDE-154 constituting 25–50% and 5–13% of the ΣPBDE concentration, respectively (Nielsen et al., 2014). PBDEs have been shown to exhibit a much lower degree of trophic magnification compared to PCBs in an Arctic food web (Kelly et al., 2008a,b). In that study BDE-47 was the only congener

which showed trophic magnification although low, partly because BDE-47 is formed via debromination of higher brominated congeners (Kelly et al., 2008a). Generally, however, lower brominated PBDEs, particularly BDE-47 and BDE-153, seem to biomagnify to a higher degree compared to higher brominated PBDEs (de Wit et al., 2010).

In young individuals of harbour porpoise higher chlorinated PCBs, higher brominated PBDEs and HCB were found to be preferentially stored in the liver, whereas other compounds mainly were found in blubber (Weijs et al., 2010). Furthermore, Weijs et al. (2009a) found that PBDE patterns in the blood differed from patterns in blubber and that this probably was due to a selective retention of some congeners in other tissues than blood. This is in accordance with the higher levels of HCB and PBDEs in the liver in the present pilot whales.

4.3. Differences between age groups

The age/gender groups analysed in the present study were mostly adult females and juveniles (mostly males) and the differences thus mainly reflect the differences between the exposure in suckling (Juv 0–2 years) and non-suckling (Juv >2 years) calves compared to the adult females. Several mechanisms can be interacting leading to differences between these age groups such as differences in prey preferences and differences in metabolic ability, but the significantly lower POP concentrations in adult females than in the juveniles can largely be explained by the transfer from the mother to offspring during gestation and to a larger extent during lactation (Borrell et al., 1995; Wells et al., 2005). This is in general accordance with that previously found in marine mammals (e.g. Hansen et al., 2004; Weijs et al., 2009b; Fair et al., 2010; Yordy et al., 2010). However, the transfer rates differ between different compounds as shown by the different Juv 0–2 years/AdF ratios and different relative levels of compounds in the different age groups. Congeners that are transferred to a larger extent through milk and/or gestation than through solid food would be expected to be found in relatively higher concentrations in juveniles 0–2 years than in the juveniles >2 years and to be relatively lower in the adult females. This was the case for PeCB and HCB. Other compounds, such as BDE-47 and penta- and hexa-chlorinated PCBs, were found in relatively low concentrations in adult females, and in relatively higher concentrations in juveniles 0–2 years and in even higher concentrations in juveniles >2 years and the adult males. This could indicate that these compounds are transferred to a relatively large extent through milk and/or gestation, but are also accumulated from the solid food and that they are not metabolized and thus concentrate in the tissue. PCBs with no vicinal H atoms in *ortho- and meta-* or *meta- and para*-positions and congeners with no vicinal H atoms at *meta- and para*-position and ≥2 ortho-Cl atoms (metabolic groups I and II) are considered to be persistent since they are not metabolized by CYP enzymes (Boon et al., 1997; Desforges et al., 2013a). Several of these PCB congeners (CB-99, CB-128, CB-138, CB-146, CB-153) are penta- or hexa-chlorinated PCBs and the relative higher levels in the subadult whales could be due to the recalcitrant properties of these PCBs. Analyses in humans have shown that PCBs have half-lives of 7–9 years for the most recalcitrant and 3–4 years of the metabolizable congeners in children (Grandjean et al., 2008). Relative concentrations of *p,p′*-DDE were significantly higher in juveniles >2 years than in juveniles 0–2 years. This would indicate relatively high exposure from the solid food and/or low metabolic transformation of this compound. Higher chlorinated PCBs and higher brominated PBDEs, which along with Mirex are those with the highest log K_{ow} values, were relatively high in adult females. This indicates that they are transferred to a lesser extent through lactation and gestation (Figs. 2–4). Larger relative concentrations of higher chlorinated PCBs in adult females compared to juveniles are in accordance with previous findings in pilot whale blubber from the Faroe Islands (Dam and Bloch, 2000).

The higher relative levels of heptachlorinated PCBs and *p,p′*-DDE and lower relative concentrations of pentachlorinated PCBs in adult females compared to juveniles 0–2 years have also been found in seal species from different geographic locations and is reflective of selective retention of compounds with higher log K_{ow} values in female blubber relative to milk and selective transfer of contaminants with lower log K_{ow} values (Wolkers et al., 2002, 2004, 2006).

The lack of significant difference in the concentrations of most POP compounds between the two juvenile groups was probably due to the large variations within the age/gender groups for most of the compounds. The separation of the two juvenile groups was made to distinguish between juveniles that are still exposed to contaminants through suckling and those that are not. It was however not possible to separate these two groups of juveniles thoroughly, since the stomach content was not analysed and the splitting of the juvenile group at the age of two was based on former studies of the age of weaning in pilot whales from the Faroe Islands (Desportes and Mouritsen, 1993).

Suckling in Faroe Island pilot whales has generally been shown to proceed until the calves are between 1.5–3.5 years old (Martin and Rothery, 1993). There can be large variations in the age at which pilot whale calves are weaned. Calves only a few months old have been found with traces of solid food in the stomach and calves of three years of age have been found that did not seem to have started to consume solid food yet (Desportes and Mouritsen, 1993). Thus, the period when the calf is receiving only milk and both milk and solid food can be short or long. The variations of POP levels in the two juvenile groups can thus in part be due to differences in feeding pattern at the same age. Calves from primiparous females also receive higher concentrations of POPs than the subsequent calves (Aguilar and Borrell, 1994; Wells et al., 2005) and levels in the new-born calves can thus be higher if they are from the first pregnancy than from the subsequent pregnancies. Thus, the initial exposure of the calves from transfer during gestation and lactation can differ due to the body load of the mother and between her first pregnancy and her subsequent pregnancies. Unfortunately, we do not have information of the pregnancy history of the females in this study, but examining the ovaries could give us that information and sampling of the ovaries should be attempted in future studies.

The contaminant pattern will also be influenced by the biotransformation capacity of the compounds in the species and by the relative levels in the prey. It is difficult to analyse the influence of solid food on the relative contributions of POPs since to our knowledge, there is a dearth of published reports on POPs in the prey of pilot whales, which is preferentially squid (*Todarodes sagittatus* and *Gonatus* sp.) but also fish such as greater Argentine (*Argentina silus*) and blue whiting (*Micromesistius poutassou*) (Desportes and Mouritsen, 1993) and because the exposure of the prey is dependent on local source in the area (Weisbrod et al., 2001). Concentrations of PCBs have been reported in squid (*Todarodes* and *Gonatus*) from the Faroese shelf (Dam et al., 2001) showing very low levels. However, only the mantle of the *Todarodes* was analysed, and since PCBs are mostly located in the liver in squid, the concentrations were not representative of the dietary intake exposure of the whales. Thus the dietary influence of individual PCBs was not possible to assess based on this particular study. Studies of trophic magnification in an Arctic food web show that recalcitrant PCBs are biomagnified to a high extent whereas PBDEs are not (Kelly et al., 2008b). Analyses of arctic beluga have shown that lighter congeners of PCBs and PBDEs were less important in the beluga compared to their prey due to metabolic transformation, resulting in trophic magnification of recalcitrant congeners and concurrent trophic elimination of metabolizable congeners (Desforges et al., 2013a).

Young animals can have less ability to metabolize xenobiotics due to a less developed enzyme system (Wolkers et al., 2002; Weijs et al., 2010; Bytingsvik et al., 2012). The contaminants that were found to decrease significantly with age in the present male pilot whales, CB-44, -97, -110 and CB-144, PeCB, HCB, and BDE-49 could indicate that they

were those that were most easily transferred from the mother to offspring. However, these compounds were not those with the highest Juv 0–2 years/AdF ratios. The PCB congeners that decreased with age were congeners with vicinal H atoms in the *meta*- and *para*-positions and with only two *ortho*-Cl atoms. These have been found to be metabolized in harbour porpoises in contrary to PCB congeners with vicinal H atoms in *meta*- and *para*-positions but with three or four *ortho*-Cl (Boon et al., 1994). The decreasing levels could thus be due to increasing ability of biotransformation with age.

4.3.1. The influence of physico-chemical properties of POPs on maternal transfer

Correlations between POP concentrations and log K_{ow}, MW and halogenation degree show that these physico-chemical properties are important factors for the transfer of contaminants from mothers to offspring. As these three properties are all positively intercorrelated it is difficult to conclude on their individual importance. The transfer between mother and their calves is dependent on the partitioning of the compounds between maternal blubber and her blood and between maternal blood and milk. The lipophilicity of compounds has been found to be closely related to the partitioning between blubber and milk in adult female bottlenose dolphins (Yordy, 2009). Analyses in seals have shown selective transfer of POPs from maternal inner blubber to serum and that this was strongly dependent on log K_{ow} values with less lipophilic compounds more efficiently transferred (Vanden Berghe et al., 2012). Yordy (2009) found similar results of congener specific POP mobilization and off-loading to milk in adult bottlenose dolphin females, showing that ΣPBDEs, HCB, tri- to hexa-chlorinated PCBs and tetra- to penta-brominated PBDEs had similar concentrations in blubber and milk whereas ΣPCBs, ΣDDTs, ΣCHLs, Mirex, hepta- to deca-chlorinated PCBs and hexabrominated PBDEs were higher in blubber. This is in accordance with the result of the present study showing relatively higher concentrations in adult females of hepta- and octa-chlorinated PCBs, hexabrominated PBDEs, *p,p′*-DDE and Mirex which all have log K_{ow} values of around 6.9 or higher.

Our result showed however that for the maternal transfer of OCPs, the molecule weight (MW) was the important factor. The OCPs are a more diverse contaminant group than PCBs and PBDEs and other properties probably explain the difference in maternal transfer. Compounds with large MW can have less ability to cross membranes due to their size, although they have high Log K_{ow} (Kannan et al., 1998; Weijs et al., 2010). Although none of the three factors analysed was significant for the maternal transfer of the PBDEs they show very different relative distributions for the different age groups. In females BDE-154 is much higher than BDE-47 whereas the opposite is the case for the Juv >2 year group. This indicates selective retention of this large molecule compared to the smaller BDE-47.

The exposure of pilot whales to POPs commences early during the gestation period, and the physiochemical properties of the compounds also seem to be important in the transfer during gestation. Borrell et al. (1995) found differences in the ratios of *p,p′*-DDE/ΣDDT and ΣDDT/ΣPCB between pilot whale mothers and foetuses indicating that less lipid soluble compounds were transferred more easily through the placenta than more lipophilic compounds. PCBs and OCPs were analysed in blubber of three pilot whale mother/foetus pairs in 2000 (Hoydal and Dam, 2003). Further analysing the data from these mother–foetus pairs suggests that during the pregnancy lower chlorinated PCBs and most OCs were transferred to the foetus to concentrations in foetus blubber reaching the same levels or higher than in the mother, whereas the higher chlorinated PCBs and Mirex are retained in the mother leading to higher levels in the mother than in the foetus. When comparing the mother/foetus ratios with their log K_{ow} values the relationships were clearly positively correlated. The mother/foetus ratios for PCB congeners with log K_{ow} values higher than 6.5 were most highly correlated to the log K_{ow} values (r^2: 0.82, 0.88 and 0.92) in the three mother foetus pairs respectively) and thus showing the same influence of log K_{ow} on the partitioning between mother and foetus as previously reported between blubber and milk in bottlenose dolphins from Sarasota Bay, Florida (Yordy, 2009) for which blubber/milk ratios increased dramatically for compounds with log $K_{ow} > 6.5$ whereas the partitioning of compounds with log K_{ow} values <6.5 appeared non-selective.

Distribution of POPs in mothers and their foetuses has been examined in pilot whale mother/foetus pairs from Australia (Weijs et al., 2013). Although the authors concluded that the higher chlorinated PCBs were preferentially transferred through gestation since foetus/mother relationships of higher chlorinated PCBs increased with the duration of pregnancy, the lowest foetus/mother ratios were identified for PBDEs and octa- and nona-chlorinated PCBs (Weijs et al., 2013) which are all large molecules with high log K_{ow} values.

4.4. Metabolites

To our knowledge, the present study is the first to report POPs and their metabolites in plasma of pilot whales. In the present study, only the co-eluting metabolites 4-OH-CB107/4-OH-CB108, were detected and the mean concentration (0.54 ng/g ww (non-detects not included, otherwise 0.32)) and the mean ΣOH-PCB to ΣPCB concentration ratios (0.045) were low and comparable to levels in other toothed whales (Montie et al., 2008; Nomiyama et al., 2010; Ochiai et al., 2013). This indicates low metabolic capacity in pilot whales as reported for other cetacean species. The concentrations of OH-PCB metabolites in cetaceans have been found only in low concentration or not detectable which is in striking contrast to the often high PCB concentrations. Concentrations of OH-PCBs between 0.019 and 0.25 ng/g ww were reported in toothed whales from the Japanese coast (Nomiyama et al., 2010), and bottlenose dolphins have been shown to have concentrations of approximately 4 and 58 ng/g ww depending on the location (Montie et al., 2008). The ΣOH-PCB to ΣPCB concentration ratios were 0.02 and 0.15 in bottlenose dolphins (Montie et al., 2008) dependent on the sampling location, and ratios in other toothed whale species ranged between 0.001–0.056 (Ochiai et al., 2013). For ceteceans, this indicates low PCB metabolic capacity and/or rapid clearance of OH-PCB metabolites formed. The metabolite profiles in cetacean blood have also been shown to be entirely different from profiles found in pinnipeds, polar bear, terrestrial mammals and humans (Fängström et al., 2002; Gebbink et al., 2008a,b; Routti et al., 2008, 2009a; Letcher et al., 2009; Weijs et al., 2009a; Nomiyama et al., 2010; Ochiai et al., 2013; Gabrielsen et al., 2015). The OH-metabolite composition has also been reported to differ between toothed whales and baleen whales, with the lower chlorinated OH-PCBs (tri- to penta-chlorinated) being the dominant OH-PCBs in toothed whales (60–80% of the total OH-PCBs), whereas octachlorinated OH-PCB were dominating in baleen whales (Nomiyama et al., 2010). Toothed whales thus seem to preferentially metabolize lower chlorinated PCBs and accumulate their OH-metabolites in the blood (Tanabe et al., 1988; Nomiyama et al., 2010).

The reason for only detecting 4-OH-CB107/4-OH-CB108 in the present study is probably due to higher detection limits in our analyses, which were between 0.01 and 0.5 ng/g ww, as compared to the detection limit in the study of Nomiyama et al. (2010), which was 0.6 pg/g ww. In a study of harbour porpoises from the North Sea, also only 4-OH-CB107 and one more metabolite (4-OH-CB130) was found in the serum in only a limited number of samples and in low concentrations (Weijs et al., 2009a). In various seals species has 4-OH-CB107 been found to be the dominating metabolite (Routti et al., 2008; Weijs et al., 2009a; Gabrielsen et al., 2011) although several other metabolites were detected in the seals. Analyses in hooded seals (*Cystophora cristata*) show that the levels of 4-OH-CB107 was higher in plasma of hooded seal mothers than in their pups, and the levels in pups were suggested to stem from maternal transfer rather than biotransformation processes (Gabrielsen et al., 2011). In polar bears, 4-OH-CB107 is not a dominating

metabolite (Sandau, 2000; Gebbink et al., 2008b; Letcher et al., 2009; Bytingsvik et al., 2012; Gabrielsen et al., 2015).

In a study of PBDEs and metabolites in bottlenose dolphins (Houde et al., 2009), 15 and 16 different OH-PBDE metabolites were detected in concentrations of 0.62 and 1.15 ng/g ww and the ΣOH-PBDE to ΣPBDE concentration ratios were 0.12 and 0.04 (Houde et al., 2009). It is difficult to compare the results from that particular study to the results of the present study since we only detected one metabolite, 4-OH-BDE17, in a few samples, but then at mean concentration 1.77 ng/g ww (range 1.04–5.68 ng/g ww). However, it should be noted that 4-OH-BDE17 was not the metabolite found in highest concentrations in the bottlenose dolphins, and that the ΣOH-PBDE to ΣPBDE concentration ratio was higher in our study than reported in the bottlenose dolphins. In harbour porpoises from the North Sea low levels of PBDEs were detected and no OH-PBDE metabolites (Weijs et al., 2009a). OH-PBDEs were not detected in livers of beluga from two Canadian populations with highly contrasting e.g. PCB and PBDE exposure (McKinney et al., 2006).

PCBs and $MeSO_2$-PCB metabolites have previously been analysed in blubber of cetaceans from the Irish Sea and Aegean Sea (Mediterranean), including one pilot whale from the Irish Sea (Troisi et al., 1998). The $MeSO_2$-PCB to PCB concentration ratios differed from 0.1 to 0.01 in different species with the pilot whale in the middle with a ratio of 0.05, along with the white-sided dolphin (*Lagenorhyncus acutus*). This previously reported ratio is in accordance with the mean ratio in liver in the present study. $MeSO_2$-PCB has also been analysed in beluga and in ringed seal and the metabolites in the liver from the present study were, as for the PCBs, higher than in the livers of beluga whale from Canadian Arctic but much lower than in beluga from St. Lawrence River (McKinney et al., 2006). The $MeSO_2$-PCB levels in ringed seals from Svalbard were similar to the levels in the pilot whales from the present study, whereas the seals from the Baltic had higher levels (Routti et al., 2008).

The metabolites measured in pilot whales can be produced by the transformation of precursor compounds in the individual or it can be transferred with the diet or from the mother with milk or in the uterus. Correlation between the precursor compound and the metabolite is sometimes used as an indication of that the metabolites are produced in the individual by biotransformation (Nomiyama et al., 2010). It is however difficult to determine OH-metabolites to parent PCB relationships since OH-metabolites can be formed through direct insertion of oxygen molecule or through 1,2-shift mechanisms involving an intramolecular migration or shift of the chlorine thus altering the original chlorine substitution pattern of the PCB (Letcher et al., 2000; Sandau, 2000). The precursor of 4-OH-CB107 can be CB-107, CB-105 and CB-118 (Jaspers et al., 2008; Nomiyama et al., 2010). Of these, only CB-105 and CB-118 were analysed in this study and the relationship between the concentration of the co-eluting 4-OH-CB107/4-OH-CB108 to the concentration of the sum of these two congeners were not found to be significantly correlated. Several of the adult females had high metabolite concentrations relative to the precursor concentration (Fig. 6) compared to the other age groups. This could indicate that the metabolites are not transferred as easily through lactation as the precursor compounds. Analyses in humans have shown that OH-PCB are poorly transferred to mothers milk in Faroese mothers and although the exposure of parent PCBs is high, the exposure to nursed children to OH-metabolites via mothers milk is low (Fängström et al., 2005).

OH-PBDEs can originate from metabolism of PBDEs but also from production in marine organisms such as algae and sponges along with their MeO-PBDE analogues (Wiseman et al., 2011). The only OH-PBDEs detected in this study were 4′-OH-BDE17 in 10 individuals and 6′-OH-BDE68 in one individual. OH-PBDEs from natural production are characterized by the hydroxyl group being in an *ortho* position and the bromines in the non-hydroxylated ring being in 2-, 4-, or 2,4-positions, whereas OH-PBDEs having the hydroxyl group in a *meta*- or *para*-position and bromine substituted in 2-, 4-, or 2,4-positions are likely biotranformation products of PBDEs (Malmberg et al., 2005). According to this the 6′-OH-BDE-68 compound is probably of natural origin whereas 4′-OH-BDE17 is more likely a product from metabolism of PBDE. 4′-OH-BDE17 is a possible metabolite of BDE-47 as it has been detected in faeces of BDE-47 treated rats (Marsh et al., 2006) but was not detected in microsomes from hepatic rat cells exposed to BDE-47 (Moffatt et al., 2011). Co-occurrence of OH-PBDEs and MeO-PBDEs suggests metabolic relationship between these, and possibly inter-conversion of OH- and MeO-PBDEs can take place in exposed organisms (Wiseman et al., 2011). Rotander et al. (2012b) detected 2′-MeO-BDE68 and 6-MeO-BDE47 in blubber of pilot whales from the Faroe Islands. The metabolites were thought to be of natural origin (Rotander et al., 2012b). How or if such MeO-metabolites can be linked to the OH-PBDEs found in plasma is not known.

5. Conclusion

The present study of chlorinated and brominated POPs and their metabolites in liver and plasma of pilot whales shows that although the use of most of these compounds have been regulated for several decades, the concentrations are still high in pilot whales from the North-east Atlantic and are found at levels leading to concern for the health of the whales. The levels are generally four to ten times higher in juveniles than in adult females largely due to maternal transfer through lactation and gestation.

The relative distribution of POPs differed among the age groups, and was influenced by the maternal transfer. POPs with high lipid solubility (e.g. higher chlorinated PCBs, higher brominated PBDEs) were transferred from the adult females to their offspring to a lesser extent than compounds that are less lipid soluble. However, OH- and/or $MeSO_2$-PCB and PBDE metabolites of the pollutants were found in very low concentrations indicating a very low metabolic capacity in pilot whales as reported in other cetaceans.

The pilot whales are thus exposed to high concentrations POPs at a young age when they are in critical periods of their development which leads to special concern. The juvenile pilot whales are however not exposed to high levels of OH- and/or $MeSO_2$-PCB and PBDE metabolites, which are linked to several of the effects of POPs in marine mammals.

Although the levels in liver and plasma were correlated, and the concentrations in these two matrices did not differ when the concentrations were normalized to lipids, some single compounds were differently accumulated in the liver and plasma and a smaller number of compounds was detected in plasma due to the low lipid content. This has to be taken into account when only analysing one of the matrices.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2015.03.056.

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Relationships between concentrations of selected organohalogen contaminants and thyroid hormones and vitamins A, E and D in Faroese pilot whales

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ABSTRACT

Pilot whales (*Globicephala melas*) from the Faroe Islands, North-East Atlantic, have high body concentrations of organohalogenated compounds (OHCs), such as polychlorinated biphenyls (PCBs), organochlorinated pesticides (OCPs) and brominated flame retardants (BFRs). The aim of the present study was to examine if and to what extent blood plasma and liver concentrations of several groups of these OHCs are related to concentrations of relevant nutritional and hormonal biomarkers in pilot whales. Thyroid hormones (THs: total and free thyroxine and total and free triiodothyronine) and vitamin A (retinol), D (25-hydroxyvitamin D_3) and E (α-tocopherol) were analysed in plasma ($n=27$) and vitamin A (total vitamin A, retinol and retinyl palmitate) and E (α- and γ-tocopherol) were analysed in liver ($n=37$) of Faroe Island pilot whales. Correlative relationships between the biomarkers and OHC concentrations previously analysed in the same tissues in these individuals were studied. The TH concentrations in plasma were significantly higher in juveniles than in adults. Vitamin D concentrations in plasma and α- and γ-tocopherol in liver were higher in adults than in juveniles. Multivariate statistical modelling showed that the age and sex influenced the relationship between biomarkers and OHCs. Some significant positive relationships were found between OHCs and thyroid hormone concentrations in the youngest juveniles ($p<0.05$). In plasma of juvenile whales α–tocopherol was also positively correlated with all the OHCs ($p<0.05$). Only few significant correlations were found between single OHCs and retinol and vitamin D in plasma within the age groups. There were significant negative relationships between hepatic PBDE concentrations and retinol (BDE-47) and γ–tocopherol (BDE-49, -47, -100, -99, -153) in liver. The relationships between OHCs and THs or vitamins suggest that in pilot whales OHCs seem to have minor effects on TH and vitamin concentrations.

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1. Introduction

Pilot whales (*Globicephala melas*) from the Faroe Islands, North-East Atlantic, belonging to the toothed whales (*Odontoceti*), have high body concentrations of organohalogenated compounds (OHCs), such as polychlorinated biphenyls (PCBs), organochlorinated pesticides (OCPs) and brominated flame retardants (BFRs), such as polybrominated diphenyl ethers (PBDEs) (Borrell and Aguilar, 1993; Dam and Bloch, 2000; Hoydal et al., 2015; van Bavel et al., 2001). Several OHCs are known to have disrupting effects on biological processes such as reproduction, immunefunction and neurodevelopment (Weisglas-Kuperus, 1998). Although the mechanisms behind these effects are not fully understood, some of these anthropogenic compounds are known to disrupt endocrine systems, and influence levels of enzymes, hormones and vitamins (Colborn et al., 1993; Letcher et al., 2010). These endocrine disrupting effects may in turn result in health effects that reduce reproductive success and survival of the

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afflicted populations.

It has been shown that OHCs, such as PCBs, interfere with the regulation and homeostasis of thyroid hormones (THs), vitamin A (retinoids) and vitamin E (tocopherols) in various marine mammals including toothed whale species, such as beluga (*Delphinapterus leucas*) (Desforges et al., 2013; Villanger et al., 2011b), seals and polar bears (*Ursus maritimus*) (Gabrielsen et al., 2015; Jenssen et al., 2003; Simms et al., 2000; Sørmo, 2005; Villanger et al., 2013). Furthermore, vitamin D has been shown to be influenced by body concentrations of OHCs in grey seals (*Halichoerus grypus*) (Routti et al., 2008). Due to their physiological importance, these variables have been proposed to be relevant biomarkers of effects of OHC exposure (Borrell et al., 2002; Debier et al., 2005; Rolland, 2000; Simms and Ross, 2000). A biomarker is defined as a biological response that can be related to either exposure to, and/or toxic effects from environmental chemical or chemicals (Peakall, 1994).

In earlier studies of pilot whales OHC concentrations in blubber, liver and plasma were reported to be several times higher in juveniles than in adult females (Dam and Bloch, 2000; Hoydal et al., 2015), in line with earlier findings of mammalian OHC transfer from mother to offspring through gestation and lactation (Borrell et al., 1995). Thus there is an enhanced concern for the health of the juveniles since THs and vitamins are important for growth and development in young individuals, including that of the brain (Brouwer et al., 1998; Howdeshell, 2002; Zoeller et al., 2002).

Thyroid hormones consist of thyroxine (T4) and triiodothyronine (T3) and control metabolism, cell differentiation and growth and are essential for normal reproduction and important in thermoregulation (Gregory and Cyr, 2003; Rolland, 2000). The synthesis of THs is controlled through negative feedback by the HPT axis (hypothalamus-pituitary-thyroid axis) (Kirby, 1990) where thyrotropin releasing hormone (TRH) secreted from the hypothalamus triggers the release of thyroid stimulating hormone (TSH, thyrotropin) from the pituitary gland (St Aubin, 1987). TSH stimulates the production of the THs (T4 and T3) in the thyroid gland, which subsequently when released into the blood stream can inhibit the production of TSH from the pituitary (Kirby, 1990). Retinoids (Vitamin A and its metabolites) are essential for various physiological functions including growth and development, reproduction, vision, epithelial maintenance and immune function (Novak et al., 2008; Simms and Ross, 2000). In marine mammals retinoids are derived from the diet mostly as retinyl esters (RE) and are often referred to as “dietary hormones” (Simms and Ross, 2000). The vitamins E and D also control important organism functions. Vitamin E refers to a group of tocopherols that function as chain breaking antioxidants preventing the propagation of free radical reactions (Brigelius-Flohe and Traber, 1999) with α-tocopherol as the most active form in mammals. Vitamin D_3 has several important roles in the organism, including mineral homeostasis, and is involved in calcium metabolism and bone mineralization together with other endocrine hormones. The predominant form of vitamin D is 25-hydroxyvitamin D_3 (25(OH)D3). Vitamin D can be produced by cutaneous exposure to ultraviolet-b light in terrestrial mammals, but fish eating marine mammals like pilot whales are able to satisfy their vitamin D_3 requirements from the diet (Kenny et al., 2004). Similarly vitamin E is also derived from the diet (Debier and Larondelle, 2005).

Several mechanisms can be involved in the OHC-mediated disruption of THs and vitamins (Brouwer et al., 1998; Liu et al., 2014). Thyroxine (T4), produced by the thyroid gland, is transported in plasma to target tissues by binding to the thyroxine plasma transport protein (TTR), albumin or thyroxine binding globulin (TBG). When delivered at the target cell T4 is deiodinated by T4-monodeiodinase to triiodothyronine (T3), which is the active hormone. The T4-TTR complex is transported in the blood plasma in a complex with retinol bound to the retinol binding protein (RBP). Hydroxy metabolites of some OHCs like PCBs or PBDEs can inhibit thyroxine (T4) binding to thyroxine plasma transport protein (TTR) by competing with T4 for TTR binding sites with the result of loss in T4 and retinol-RBP from the body (Brouwer et al., 1989a; Bytingsvik et al., 2013; Murk et al., 1998). TBG is the principal transport protein in marine mammals (St Aubin, 2001) and has been measured in cetaceans, including Short-finned pilot whale (*Globicephala macrorhynchus (scammoni))* (Ridgway and Patton, 1971) whereas efforts on analysing TTR in beluga have not been successful (St Aubin, 2001). OHCs can also interact with thyroid gland function and morphology, leading to effects on the synthesis and secretion of T4 (Brouwer et al., 1998), including effects on deiodination enzymes (Gabrielsen et al., 2015). Also thyroid metabolism can be affected by OHC exposure by induction of enzymes leading to increased glucuronidation of hepatic T4 and increased biliary secretion and elimination of T4 (Brouwer et al., 1998).

OHCs and their metabolites can reduce uptake of dietary vitamin A, decrease liver vitamin A stores, disrupt circulatory transport to tissues and/or increase glomerular filtration and excretion of vitamin A metabolites (Simms and Ross, 2000). In marine mammals, retinoids are stored mainly in the liver, but also in tissues like blubber, mainly as retinyl esters (Borrell et al., 2002). Although there is great variation in the vitamin supply from the diet and in the liver or extrahepatic tissue stores, vitamin A levels remain constant in plasma apparently due to homeostatic regulation. Thus, body depletion of retinoids can better be evaluated through concentrations in depot tissues such as liver and blubber (Borrell et al., 2002) and negative correlations have been found between OHC concentrations and retinoid levels in blubber (Nyman et al., 2003; Tornero et al., 2004b; Tornero et al., 2005a). However, relationships between contaminants and retinol levels in plasma have also been reported in marine mammals (Braathen et al., 2004; Brouwer et al., 1989b; Jenssen et al., 2003) and Greenland sharks (*Somniosus microcephalus)* (Molde et al., 2013). Analyses in beluga from Arctic Canada found that PCB correlated negatively with vitamin A in liver, but positively in blubber and plasma (Desforges et al., 2013). The negative relationship between vitamin A and PCB in liver was thought to be related to up-regulation of hepatic enzymes involved in vitamin A metabolism (Desforges et al., 2013).

In consideration of the high levels of OHCs that have been reported in Faroe Island pilot whales, the aim of the present study was to examine how the concentrations of several groups of OHCs are related to levels of THs, vitamin A, E and D status. Plasma and liver from pilot whales sampled in 2009–2011 on the Faroe Islands were analysed in the present study. Plasma samples were analysed for concentrations of thyroid hormones (total and free T4 and T3) and vitamin A (retinol), E (α-tocopherol) and D (25(OH)-D3). Liver samples were analysed for vitamin A (total vitamin A, retinol and retinyl palmitate) and E (α- and γ-tocopherol). Correlative relationships among these biomarkers and plasma and liver concentrations of the PCBs, PBDEs, OCPs and their metabolites were studied.

2. Materials and methods

Plasma and/or liver were sampled from 37 pilot whales 2009, 2010 and 2011 on the Faroe Islands. The sampling of liver and plasma from 27 individuals in 2010 and 2011 was previously described in Hoydal et al. (2015). In addition to these 27 individuals; 10 livers from pilot whales were sampled in 2009 and 2010. An overview of the individuals analysed and their biological data is given in Table S1.

The individuals were divided into adult females, adult males, sub-adults >2 years and calves 0–2 years according to their length and/or age as described in Hoydal et al. (2015). The grouping into adults and juveniles was done using the animal lengths based on the studies of age at sexual maturity by Martin and Rothery (1993) and Desportes et al. (1993). The further division of the juveniles into the two age groups (0–2y and >2y) was based on their different feeding and exposure pattern, since the calves 0–2 years old are expected to be suckling and exposed to contaminants via milk, whereas the sub-adults >2 years old are expected to be weaned and exposed via their solid food based on analyses of stomach content by Desportes and Mouritsen (1993).

2.1. OHC concentrations

The 27 individuals, from which both liver and plasma were collected, were previously analysed for OHCs and metabolites and the results were reported in Hoydal et al. (2015). In the present study these results were calculated in molar concentrations (Table S2) and were used in the statistical analyses of relationships between contaminants and hormone and vitamin levels in the pilot whales.

2.2. Analysis of thyroid hormones in plasma

Measurements of plasma concentration of total thyroxine (TT4), total tri-iodothyronine (TT3), free thyroxine (FT4) and free tri-iodothyronine (FT3) were conducted at the Department of Biology, NTNU, using solid-phase ^{125}I radioimmunoassay (Coat-A-Count, Diagnostic Products, Los Angeles, CA, USA) and the analyses were carried out according to the protocols provided with the kits. The detection and quantification were performed on a gamma counter (Cobra Auto-Gamma, Packard Instruments Company, Dowers Grove, IL, USA). The samples were analysed in triplicates (TT4, FT4) or duplicates (TT3, FT3). The quality control material Lyphocheck Immunoassay Plus Control from Bio-Rad in three concentrations (level 1, 2 and 3) was analysed together with the samples. One of the controls (concentration level 2) and one of the 26 samples were analysed two times (in duplicate or triplicate) to measure the intra-assay stability of the analysis. If the differences between replicates were too large (CV >15%) the analyses were repeated for these samples. The inter-assay CVs for repeated analyses were 6.8–8.1% for TT4, 1.21–3.5% for TT3, 1.3–12.1% for FT4, and 5.3–9.0% for FT3.

2.3. Analysis of vitamin A, E and D in plasma

The plasma samples were analysed for retinol (vitamin A), α-tocopherol (vitamin E) and 25(OH)D3 (vitamin D) at Department of Analytical Chemistry, Gdańsk University of Technology, Poland. 500 μL of plasma was mixed with 0.5 mL of ethanolic internal standard mixture and vortexed for 30 s Precipitated proteins were centrifuged at 6000 rpm for 1 min and 1 mL of hexane was added. After vortexing for 45 s and centrifugation the hexane layer was collected. Hexane extraction was repeated two more times. Pooled hexane extract was evaporated to dryness in a gentle stream of nitrogen. Dry residue was dissolved in 0.5 mL of ethanolic 2,6-di-tert-butyl-4-methylphenol (BHA) solution (30 μg/mL) and analysed by HPLC-MS-MS.

The samples were analysed on an Agilent 1200 HPLC system coupled with an AB Sciex Instruments 4000 Q TRAP Mass spectrometer. The column used was an Agilent XDB C18 (1.8 μm, 4.6 × 50 mm). A gradient mobile phase was used (Component A: 10 mmol ammonium acetate in methanol-water mixture (90% methanol), Component B: 10 mmol ammonium acetate in methanol-methyl tert-butyl ether (80% methanol) and the flow rate of 1.0 mL/min. The injection volume was 5 μL.

2.3.1. Standards and calibration

Standards of retinol acetate, retinol palmitate, DL-α-tocopherol, δ-tocopherol, γ-tocopherol and DL-α-tocopherol acetate were supplied by Supelco, (Bellefonte, PA). Standard of retinol and BHT were purchased from Sigma-Aldrich, (St. Louis, MO, USA). HPLC-isocratic grade methanol and absolute ethanol were obtained from VWR Prolabo, (France). N-hexane used in extraction procedure was purchased from Merck, Lichrosolv, (Germany).

The primary solutions of standards were prepared by dissolution of pure substances in ethanol. The UV absorbances were measured and concentrations calculated using specific molar absorptivities (25(OH)D3 and 25(OH)D3-d6: $\varepsilon=18300$ at $\lambda=265$ nm, retinol: $\varepsilon=52480$ at $\lambda=325$ nm, retinyl-palmitate: $\varepsilon=49260$ at $\lambda=325$ nm, α -tocopherol and α -tocopherol -d6: $\varepsilon=3265$ at $\lambda=292$ nm).

A mixture of standards (25(OH)D3, retinol, retinyl-palmitate and α -tocopherol) was prepared by mixing of appropriate volumes of primary solutions and 0.5 mL of ethanolic BHA solution (3 mg/mL) and diluting with ethanol to a final volume of 50 mL. Separate mixture of internal standards (25(OH)D3-d6 and α -tocopherol -d6) was prepared in the same way.

Seven calibration solutions were prepared from the mixture of standards. Varying aliquots of the mixture together with 0.200 mL of internal standards mixture were added to ethanolic BHA solution (30 μg/mL). The concentration ranges for particular compounds were as follows: 1.7–170 ng/mL for 25(OH)D3, 10–950 ng/mL for retinol, 130–2500 ng/mL for retinyl-palmitate and 0.34–33 μg/mL for α -tocopherol.

External calibration curves were constructed by injecting calibration solutions in triplicate and calculating ratio of analyte's peak area to internal standard peak area as a function of analyte's concentration ($A/A_{IS}=f(c)$, where A – analyte's peak area, A_{IS} – internal standard peak area, c – analyte's concentration). 25(OH)D3-d6 was used as an internal standard for 25(OH)D3 and retinol, while α -tocopherol -d6 was used in case of α -tocopherol and retinyl-palmitate. Calibration curves were linear within the studied concentration ranges. Coefficients of determination were higher than 0.999 for all analytes except retinyl-palmitate ($R^2=0.995$). The performance of the method was examined analysing standard reference material SRM968e. The recoveries are given in the supplementary material (Table S3).

Limits of detection (LOD) were estimated as concentrations giving signal to noise ratio of 3. For 25(OH)D3, retinol, retinyl-palmitate and α -tocopherol the detection limits were 0.002, 0.005, 0.1 and 0.07 μg/mL, respectively.

2.4. Analysis of vitamin A and E in liver

2.4.1. Total vitamin A

Livers were analysed for total vitamin A (total retinol) at the Analytical Services of the University of Barcelona (Centres Científics i Tecnològics de la Universitat de Barcelona), by the method described in Tornero et al. (2004a). Pieces of liver were crushed with a mortar or scissors. Approximately 100 mg were taken from each homogenate and saponified overnight in 5 mL ethanolic KOH solution (1 g KOH, 2 mL distilled water, 2 mL ethanol and 20 mg ascorbic acid) in a mechanical shaker under a nitrogen atmosphere. The retinoids were extracted by adding 8 mL of diisopropyl ether and shaking again for 30 min. The aqueous phase was removed and the organic extract was washed three times by adding 5 mL of aqueous phosphate buffer and shaking the solution by hand and removing the aqueous phase. After the first wash 1 mL of internal standard (retinyl acetate) was added to the extract. After this the organic extract was evaporated on a heating block under nitrogen flow and reconstituted by adding 1 mL of methanol with 0.05% butylated hydroxyl toluene (BHT). Then the samples were

filtrated into eppendorf vials using a syringe with a filter (0.22 μm) on the tip.

The samples were transferred to HPLC vials and analysed on a Waters Acquity Ultra Performance LC analyser with the software Empower PRO from Waters. The column used was a Phenomenex Kinetex 2.6 μm C18 100A (silica), 150 × 4.60 mm. Mobile phase was methanol and water and the flow rate 0.5 mL/min. The injection volume was 10 μl and the quantifying wavelength was 325 nm. A seven point standard curve was made from synthetic retinol diluted in the internal standard solution. The samples with the highest concentrations were diluted 2, 3 or 5 times to get a concentration inside the standard curve. The CVs between replicates ranged from 0.74 to 38.2% with a mean of 8.7%. CVs for repeated analyses of standards were between 0.1% and 9.9%.

2.4.2. *Vitamin A and E*

The livers were analysed for different forms of vitamin A and E (retinol, retinyl palmitate, α-tocopherol and γ-tocopherol) by reverse-phase HPLC with fluorescence detection (PerkinElmer200 series, USA) at the Department of Biology, NTNU. Vitamins were extracted from liver samples with a modified liquid–liquid extraction technique described by Murvoll et al. (2005).

Briefly, following thawing, approximately 1 g of liver tissue was homogenised (Glas-Col homogenizer system, model: 099C K54, Terre Haute, IN, USA) with addition of deionized water (MilliQ) in an ice-bath. Subsequently, to 350–400 mg of liver:water homogenate 100 μL of internal standard solution (IS, retinol acetate 25 μg/mL) and 1000 μL of hexane with 0.04% of BHT (2,6-di-tert-butyl-4-methylphenol, Sigma Aldrich, St. Louis, MO, USA) were added. The samples were vortex mixed for 10 s and further homogenised with a 5 mm steel beads in a Qiagen TissueLyser (QIAGEN, CA, USA), run at 30 Hz for 1.5 min. Subsequently, the samples were sonicated (high-intensity ultrasonic processor, four microtips; Sonics and Materials, Newtown, CT). The ultrasound processor was set to give pulses of 2 s, followed by 0.5 s without any pulse. Total sonication time was 2 min. The amplitude was 21% of maximum for this instrument. After the sonication 400 μL of ethanol (with 0.04% of BHT) was added and the samples were again vortex mixed for 10 s After a centrifugation for 3 min at 13,100 rpm (Eppendorf centrifuge 5415D, Eppendorf AG, Hamburg, Germany), 900 μL of hexane layer was collected. Then, the entire extraction process was repeated with reduced (300 μL instead of 400 μL) volume of ethanol with 0.04% of BHT. The two extracts were pooled and evaporated to dryness at 40 °C in an automated evaporation system (TurboVap® LV) under gentle stream of nitrogen (ca. 10 min). After evaporation, to each vial 1000 μL of 100% methanol was added. Samples were mechanically shaken (Vibramax 110, Heidolph,Germany) for 1 min and 150 μL of aliquot was transferred to HPLC amber vial. The whole extraction process was conducted under dim light conditions.

The chromatography was carried out using a HPLC instrument equipped with a fluorescence detector, an autosampler with a Peltier sample tray , a pump, vacuum degasser, and a column (Chrompack Intersil, ODS-3, 150 × 4.6 mm, 5 μm) from Varian, Inc. (Lake Forest,CA) connected to a guard column (ChromGuard SS 10 × 3 mm) also from Varian. Data were collected with Turbochrom Work station version 6.1.2 software. The chromatography was carried out using a step gradient elution mode in which eluent A was methanol (Sigma-Aldrich) and eluent B methanol–water (98:2, v/v). The following gradient was used during the run: 100% A at a flow rate 1 mL/min (0–4 min), 1.2 mL/min (4–10 min) and 1.5 mL/min (10–15 min), 15–35 min 100% B at a flow rate 1.5 mL/min and 35–55 min 100 A at the flow rate 2.0 mL/min. The column was at 21 ºC. Retinol and retinol acetate were detected using an excitation wavelength of λ=325 nm and an emission wavelength of λ=470 nm at medium sensitivity. These settings were maintained from injection to 8 min. At 8 min, the excitation wavelength was changed to λ=295 nm and emission wavelength to λ=330 nm in order to optimize the detection of tocopherols. These conditions were maintained until 20 min. At 20 min the settings were changed to the initial fluorescence detector conditions in order to detect retinyl palmitate and they were maintained until the end of the chromatographic run. All vitamins were quantified on the base of peak area ratios over internal standards as obtained from calibration curves.

Standards consisted of retinol acetate (IS) retinyl palmitate, α-tocopherol and γ-tocopherol (Supelco, Bellefonte, PA,USA) and retinol (Sigma Aldrich). The standards were dissolved individually in 100 mL of absolute ethanol, while retinyl acetate was dissolved in 100 mL of ethanol with an addition of 0.04% BHT. Standard solutions were stored at −70 °C. The internal standard (IS) working solutions (WS) in methanol (retinol acetate 25 μg/mL) were prepared daily by appropriate dilution of the stock solution. Calibration curve consisted of a six-point linear calibration line ($R^2 > 0.99$, each measured in duplicate) derived from a mixture of analytes prepared by diluting standard solutions in methanol: retinol 0.02–15 μg/mL, retinyl palmitate 0.1–30 μg/mL, α- and γ-tocopherol 0.1–20 μg/mL. The final results were calculated as the mean of the concentrations determined for two replicate samples.

CVs for the replicate analyses of vitamin A and E in liver were within the range 0.2–9.0 (mean 3.4) for retinol, 0.3–7.7 (mean: 2.6) for α-tocopherol, 0.2–7.0 (mean: 2.34) for γ-tocopherol and 0.1–12.7 (mean: 2.18) for retinyl palmitate. The CVs were below 12.7 for all samples, except for sample 8 (11–31.3), 9 (19.0–24.4), US49 (15.9–25.3) and for retinyl palmitate also sample 4 (21.2) and US21 (22.4).

2.5. *Statistics*

The statistical programme SPSS (IBM, version 21) was used for univariate data analyses. Since most of the biomarkers and the contaminant concentrations (Hoydal et al., 2015) were not normally distributed, non-parametric analyses were used. Differences in hormone and vitamin concentrations among the age and sex groups were analysed with Kruskal-Wallis 1-way ANOVA with pairwise comparison. Correlations among the individual biomarkers and between biomarkers and OHCs were examined using Spearman correlations. Significant levels were set to $p \leq 0.05$.

Multivariate analyses were performed using the statistical programme SIMCA-P+(Umetrics, version 12.0, 2008). PCA analysis was used to study the relationship between individual contaminants and the biomarkers (THs and vitamins) and the biological factors (age, length). OPLS (orthogonal partial least squares regression) modelling was used to analyse the influence of the contaminants and the biological factors (X-variables) on the biomarkers (Y-variables). 27 OHCs in plasma, lipid%, length and age were included in the OPLS model to investigate their combined effect on the biomarkers in blood. Separate OPLS model for liver included 59 OHC variables, lipid%, length and age. For each OPLS model, R^2 and Q^2 values were calculated, where R^2 shows the goodness of fit (R^2X: variation of X explained by the model, R^2Y: variation of Y explained by the model) and Q^2 shows the goodness of prediction (cross-validation of the model) (Eriksson et al., 2013). R^2 value >0.7 and a Q^2 value >0.4 denote a highly significant model when analysing biological data (Lundstedt et al., 1998). In addition the significance of the models was analysed by CV-ANOVA. If the initial model was not significant (CV-ANOVA, $p < 0.05$) the least significant variables were removed one by one until a significant model was obtained. If significance was not obtained, the model was defined as not-significant. All variables were centred and scaled before the analysis. The contaminant variables were log transformed to approximate normal distribution.

3. Results

3.1. Thyroid hormones in plasma

The mean concentrations ± standard deviation of TT4, FT4, TT3 and FT3 were 62.78 ± 29.2 nmol/L, 7.56 ± 3.4 pmol/L, 1.21 ± 0.6 nmol/L and 1.63 ± 1.0 pmol/L, respectively. There were no significant differences in the concentrations of THs among the four age/gender groups (adult females, adult males, juv > 2y, juv 0–2y: Table 1).

When comparing THs between juveniles (n = 15) and adults (n = 12, pooled adult males and females), the concentrations of all four THs were significantly higher in juveniles than in adults ($p < 0.01$ for TT3 and FT3, $p < 0.05$ for TT4 and FT4). Also the free active form of the hormone (FT3) in comparison to total T3 and free T4 was higher in juveniles than in adults ($p < 0.05$).

Of the THs analysed; TT4, FT4, TT3, and FT3, were all highly inter-correlated ($p < 0.001$). No significant correlations were found between TH concentrations and length or age of the animals (Fig. 1).

3.2. Vitamin A, E and D in plasma

Concentrations of vitamins in plasma in relation to length of the individuals are shown in Fig. 2. The concentrations of 25(OH) D3 were significantly higher in adult females than in juveniles 0–2 years ($p = 0.02$), whereas the concentrations of retinol and α-tocopherol in plasma did not differ among the different age/gender groups (Table 1). Retinol and 25(OH)D3 in plasma were positively correlated with the age of the animals ($r_s = 0.522$, $p = 0.018$ and $r_s = 0.529$, $p = 0.024$, respectively). There were no differences in plasma vitamin concentrations when comparing all the juvenile animals (n = 15) and the adults (n = 12), except for 25(OH)D3 which was significantly higher in adults than in juveniles ($p = 0.03$). In plasma, retinol was positively correlated to α-tocopherol ($r_s = 0.614$, $p = 0.001$) and to 25(OH)D3 ($r_s = 0.437$, $p = 0.033$). Furthermore, α-tocopherol and 25(OH)D3 were also significantly correlated ($r_s = 0.544$, $p = 0.006$). The vitamin concentrations in plasma were not correlated to the concentrations of THs in plasma.

3.3. Vitamin A and E in liver

The α-tocopherol concentrations in liver differed significantly between the age/sex groups (Kruskal-Wallis, $p = 0.021$), but not in the pairwise comparison (Table 2). The liver concentrations of retinol, retinyl palmitate and γ-tocopherol in the pilot whales did not differ significantly between the four age/sex groups. However, α- and γ-tocopherol and retinol in liver were significantly higher in adults (n = 12, pooled adult males and females) than in all the juveniles (n = 15) ($p < 0.05$). Furthermore, α- and γ-tocopherol in liver were significantly correlated to the length of the animals ($r_s = 0.390$, $p = 0.021$ and $r_s = 0.346$, $p = 0.042$, for α- and γ-tocopherol respectively). Correlations between age and vitamins in liver were not significant. Fig. 3 shows the relationship between vitamin concentrations in liver and the length of the individuals.

The hepatic concentrations of vitamins, except for α-tocopherol and retinyl palmitate, were inter-correlated in all animals ($p < 0.01$). On a molar basis the retinyl palmitate constituted around 27% (25–75 percentiles: 22–31%) and retinol around 4% (25–75 percentiles: 2–5%) of the total vitamin A. Thus on a molar basis, the median retinyl palmitate concentration was 6 times

Table 1
Mean, standard deviation (SD), median, ranges and 25–75 percentiles of plasma concentrations of thyroid hormones, vitamin A, E and D in pilot whales from the Faroe Islands.

		Adult females n = 10	**Juv 0–2y n = 8**	**Juv > 2y n = 7**	**Adult males n = 2**
TT4, nmol/l	**mean** ± SD	**50.6** ± *16.35*	**82.57** ± *35.02*	**71.1** ± *19.75*	**51.11** ± *4.41*
	median (min–max)	**51.56** (22.23–79.02)	**95.51** (30.38–118.24)	**64.22** (48.6–101.01)	**51.11** (47.99–54.23)
	25–75 percentiles	40.5 – 61.8	43.3 – 112.6	56.8 – 92.0	–
FT4, pmol/l	**mean** ± SD	**6.11** ± *1.97*	**9.71** ± *3.86*	**8.68** ± *2.65*	**6.68** ± *0.73*
	median (min–max)	**5.60** (3.43–10.18)	**10.78** (5.05–15.03)	**8.29** (5.05–12.79)	**6.68** (6.16–7.2)
	25–75 percentiles	4.86 – 7.07	5.37 – 12.9	6.73 – 11.0	–
TT3, nmol/l	**mean** ± SD	**0.89** ± *0.26*	**1.57** ± *0.68*	**1.51** ± *0.53*	**1.07** ± *0.20*
	median (min–max)	**0.80** (0.54–1.43)	**1.72** (0.57–2.65)	**1.37** (0.83–2.35)	**1.07** (0.93–1.21)
	25–75 percentiles	0.71 – 1.06	0.89 – 1.96	1.15 – 1.99	–
FT3, pmol/l	**mean** ± SD	**1.04** ± *0.48*	**2.23** ± *1.07*	**2.14** ± *1.07*	**1.49** ± *0.69*
	median (min–max)	**0.90** (0.58–2.17)	**2.3** (0.85–3.64)	**1.98** (0.67–3.37)	**1.49** (1.0–1.98)
	25–75 percentiles	0.69 – 1.32	1.11 – 3.35	1.28 – 3.37	–
TT4:FT4	**mean** ± SD	**8.28** ± *1.31*	**8.5** ± *2.11*	**8.3** ± *0.74*	**7.73** ± *1.51*
	median (min–max)	**8.13** (6.48–10.69)	**7.87** (5.82–11.79)	**8.08** (7.51–9.63)	**7.73** (6.67–8.80)
TT3:FT3	**mean** ± SD	**0.91** ± *0.19*	**0.72** ± *0.10*	**0.79** ± *0.24*	**0.77** ± *0.23*
	median (min–max)	**0.90** (0.59–1.24)	**0.71** (0.55–0.85)	**0.72** (0.56–1.25)	**0.77** (0.61–0.93)
TT4:TT3	**mean** ± SD	**57.8** ± *14.27*	**55.86** ± *22.65*	**49.78** ± *12.76*	**48.29** ± *4.89*
	median (min–max)	**57.2** (31.1–77.2)	**52.8** (31.76–104.73)	**50.71** (27.44–64.22)	**48.29** (44.83–51.75)
FT4:FT3	**mean** ± SD	**6.19** ± *1.06*	**4.68** ± *1.18*	**4.72** ± *1.77*	**5.17** ± *2.91*
	median (min–max)	**6.12** (4.7–7.86)	**4.94** (2.91–5.95)	**4.48** (2.39–7.56)	**5.17** (3.11–7.22)
Retinol [nmol/mL]	**mean** ± SD	**0.24** ± *0.05*	**0.18** ± *0.08*	**0.26** ± *0.07*	**0.22** ± *0.09*
	median (min–max)	**0.24** (0.17–0.35)	**0.20** (0.09–0.28)	**0.24** (0.17–0.35)	**0.22** (0.16–0.29)
	25–75 percentiles	0.19 – 0.27	0.10 – 0.24	0.21 – 0.34	–
α –tocopherol [nmol/mL]	**mean** ± SD	**27.86** ± *7.43*	**19.83** ± *10.77*	**31.01** ± *12.38*	**17.88** ± *3.64*
	median (min–max)	**29.00** (14.97–38.15)	**17.68** (9.27–42.84)	**36.2** (10.99–41.98)	**17.88** (15.3–20.45)
	25–75 percentiles	23.7 – 32.8	11.5 – 24.8	17.0 – 40.8	–
25(OH)-D3 [nmol/mL]	**mean** ± SD	**0.26** ± *0.07*	**0.12** ± *0.07*	**0.23** ± *0.11*	**0.20** ± *0.03*
	median (min–max)	**0.24*** (0.18–0.40)	**0.15*** (0.02–0.19)	**0.23** (0.09–0.36)	**0.20** (0.18–0.22)
	25–75 percentiles	0.21–0.28	0.03–0.17	0.11–0.36	–

* Significant difference between groups (Kruskall-Wallis 1-way ANOVA, $p < 0.05$).

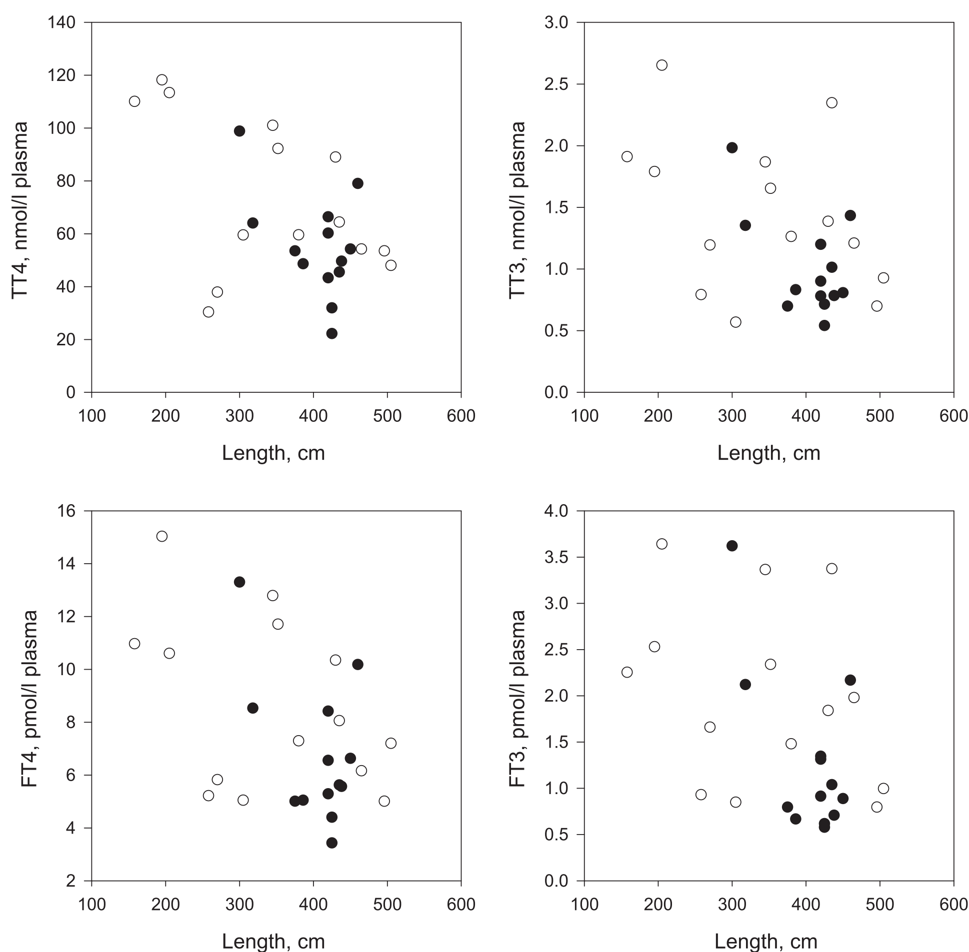

Fig. 1. Plasma concentrations of total and free thyroxin (TT3 and FT4) and triiodothyronine (TT3 and FT3) in male and female pilot whales (*Glopicephala melas*) in relation to the length of the individuals. Closed and open circles represent females and males, respectively.

higher than retinol concentration in liver (25–75 percentiles: 4–12 times). In the liver; concentration of α-tocopherol was on average 15 times higher than γ-tocopherol on a molar basis (25–75 percentiles: 12–17 times higher).

3.4. Relationship between vitamins in liver and plasma

The retinol concentrations in liver and plasma were significantly positively correlated ($r_s = 0.418$, $p = 0.038$). The retinol concentration in liver was on average 250 times higher than in plasma (25–75 percentiles: 80–400 times, assuming that the plasma density is 1.025 g/mL). There were no other significant correlations between vitamins in liver and vitamins in plasma.

α-Tocopherol in plasma was not correlated to the concentrations in liver of either α-tocopherol or γ-tocopherol. The level of α-tocopherol in liver was on average 2 times higher than in plasma (range: 0.9–4.5 times).

3.5. Relationship between hormones, vitamins and OHCs

The PC1 explained 79% and 68% of the variation in liver and plasma respectively, while PC2 explained 7% and 10% of the variation in liver and plasma, respectively. The plot of scores representing liver samples (Fig. 4A) showed a clear separation of the adult females group from the other age groups along the PC1 axis. The corresponding loading plot showed that the differences in the hepatic OHC concentrations, size and age and vitamin E concentrations between adult females and the rest of whales were responsible for the observed clustering of the samples along PC1 (Fig. 4B). Differences in size and age as well as variation between different hepatic OHCs accumulation (e.g p,p′-DDE, CB-110, BDE-49) were responsible for separation of Juv > 2y from 0 to 2y along the PC2 axis.

The plots of scores representing plasma samples, similarly as for the liver samples, showed a clustering and separation of the adult females from the other whales along the PC1 axis (Fig. 5A). The loading plot (Fig. 5B) showed that the clustering of the adult

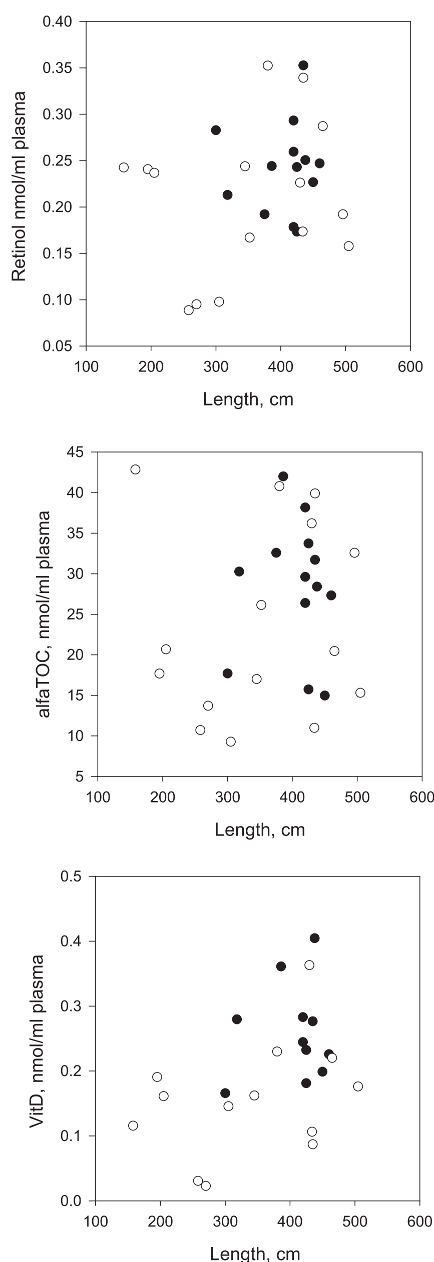

Fig. 2. Plasma concentrations of vitamin A (retinol), α–tocopherol (alfaTOC) and vitamin D (VitD) in male and female pilot whales (*Glopicephala melas*) in relation to the length of the individuals. Closed and open circles represent females and males, respectively.

females was due to the differences in OHC concentration and THs and in the age and size between the adult females and the other whales. Also the relative levels of THs, TT3:FT3 and FT4:FT3, explained the clustering. Differences in TH concentrations and vitamin levels seemed to be responsible for the observed separation of the juvenile groups, Juv 0–2 and Juv >2, along the PC2 axis.

3.5.1. Relationships between OHCs and THs

Significant OPLS models (CV-ANOVA, $p<0.05$) were found for the THs (TT4: $R^2X=0.742$, $Q^2=0.292$; FT4: $R^2X=0.742$, $Q^2=0.259$; TT3: $R^2X=0.768$, $Q^2=0.387$; FT3: $R^2X=0.742$, $Q^2=0.372$) (Fig. 6). The models showed that all the THs were negatively associated with the biological variables age and length. In contrast, the THs were positively associated with the plasma concentrations of the OHCs included in the OPLS. Spearman correlation analysis confirmed that all PCBs, OCPs and PBDEs (BDE47) in plasma were significantly positively correlated with TT4, FT4, TT3 and FT3 ($p<0.05$).

Although no significant OPLS models were identified for the FT4:FT3 and the TT3:FT3 ratios, Spearman correlation analysis showed that the FT4:FT3 ratio was significantly negatively associated with all analysed contaminants ($p<0.05$), and that the TT3: FT3 ratio was significantly negatively associated with several PCB congeners (CB-74, -95, -99, -101/90, -118, -146, -149, -151, -153, -170/190, -174, -179, -180, -187), HCB, cis-chlordane and p,p'-DDE ($p<0.05$). The significant correlations between OHCs and THs are given in Table 3.

Since age and length were important variables in the OPLS models, further OPLS analyses were performed for the age groups separately (AdF: n=10 and Juveniles: n=15). However, no significant models were identified. When dividing the individuals into the four age/sex groups (and excluding the adult males since there were only two individuals), Spearman correlation analyses did identify correlations between some of the contaminants and THs mostly in the juveniles 0–2 years age group (Table 3). In adult females (n=10) only HCB was significantly positively correlated to TT3 and this was also found for all the juveniles (n=15).

In juveniles 0–2 years of age (n=8), TT4 correlated positively with CB-52, -74, -92, -95, -101/90, -105, -128, -149, -151, p,p'-DDE and BDE47 ($p<0.05$). TT3 correlated positively with CB-87, -92, -99 and trans-nonachlor ($p<0.05$), FT3 correlated positively with CB-87, -92, -99, p,p′-DDE and trans-nonachlor, FT3 ($p<0.05$) and TT4:FT4 ratio correlated positively with CB-74, -118 and -183. No correlations were found between THs and contaminants in plasma in juveniles >2 years (n=7).

A negative correlation was found between 4-OH-CB107/4′-OH-CB108 and the TT4:TT3 ratio in adult females ($p<0.05$), but not in the juvenile groups.

3.5.2. Relationships between vitamins and OHCs

The OPLS models were not significant for any of the vitamins in neither plasma nor liver. Spearman correlation analyses did, however, identify significant ($p<0.05$) correlations between the concentrations of some of the contaminants and vitamins. In the plasma, α-tocopherol was significantly positively correlated to concentrations of CB-87, -146 and -183 ($p<0.05$). There were no significant correlations between plasma retinol or 25(OH)D3 and any of the contaminants (Table 4). In liver, retinol was negatively correlated to BDE47, and γ-tocopherol was negatively correlated to BDE-47, -49, -100, -99 and -153 ($p<0.05$), whereas no significant correlations were found between α-tocopherol or retinyl palmitate and contaminants ($p>0.05$).

Within the age groups, there were also some statistically significant ($p<0.05$) relationships between vitamins and OHCs (Table 4). In plasma of adult females, retinol was positively correlated to HCB and negatively to CB-146. α-Tocopherol was negatively correlated to CB-74 and positively correlated to CB-87. In liver of adult females retinol was positively correlated to CB-74 and CB-196/203, retinyl palmitate was positively correlated to CB-196/203 but negatively correlated to BDE-28, -47, -49, and -153, and α-tocopherol was positively correlated to CB-171 and Mirex.

Table 2
Mean, standard deviation (SD), median, range and 25–75 percentiles of liver concentrations of vitamin A, and E pilot whales from the Faroe Islands.

	*	Adult females n=13	Juv 0–2y n=7	Juv >2y n=8	Adult males n=7
Retinol [nmol/g]	**mean** ± SD	**99.42** ± *56.84*	**68.65** ± *56.50*	**53.97** ± *70.98*	**122.2** ± *114.73*
	median (min–max)	120.3 (13.2–179.5)	45.69 (8.05–176.0)	18.06 (4.57–173.5)	87.32 (13.1–370.1)
	25–75 percentiles	45.4–148.4	32.3–92.4	11.9–129.9	77.9–131.3
α –tocopherol [nmol/g]	**mean** ± SD	**51.16** ± *15.54*	**38.80** ± *12.53*	**38.88** ± *11.26*	**56.49** ± *8.88*
	median (min–max)	48.09 (27.5–76.4)	34.74 (24.3–58.1)	35.58 (27.82–58.32)	60.92 (45.98–67.54)
	25–75 percentiles	41.7–63.4	28.0–52.6	30.3–49.9	46.9–63.9
γ -tocopherol [nmol/g]	**mean** ± SD	**3.41** ± *1.02*	**2.69** ± *0.60*	**2.72** ± *0.77*	**3.55** ± *0.63*
	median (min–max)	3.74 (1.76–4.76)	2.59 (1.66–3.51)	2.56 (1.92–4.42)	3.51 (2.81–4.45)
	25–75 percentiles	2.41–4.28	2.47–3.18	2.19–2.94	3.00–4.34
Retinol palmitate [nmol/g]	**mean** ± SD	**554.1** ± *362.80*	**360.4** ± *175.47*	**660.8** ± *504.86*	**845.2** ± *511.12*
	median (min–max)	483.2 (89.8–1430)	367.4 (145.9–571.5)	737.0 (9.01–1581)	748.9 (354.8–1881)
	25–75 percentiles	243.7–750.3	195.6–532.4	165.8–899.0	419.2–990.5
Total vitamin A [nmol/g]	**mean** ± SD	**2067** ± *1392*	**1604** ± *742*	**2665** ± *2009*	**3786** ± *3367*
	median (min–max)	2133 (274.3–4485)	1641 (365–2746)	3049 (42.9–5639)	2569 (1338–10370)
	25–75 percentiles	707.5–2889	1065–2171	819.7 – 4413	1499–3884

* For total retinol: AdF: n=12, Juv =0-2y: n=8, Juv >2y: n=9, AdM: n=7.

In plasma of the juveniles, in the 0-2y group α-tocopherol was positively correlated to all detected contaminants except for CB-118 and BDE-47 (Table 4). When pooling all the juveniles (0–2y and >2y groups) (n=15) α-tocopherol was positively correlated with all the detected contaminants ($p < 0.05$). 4-OH-CB107/4′-OH-CB108 was positively correlated with 25(OH)D3 in the 0-2y group (Table 4).

In liver of the juveniles in the 0-2y group there were no significant correlations between vitamins and contaminants. However, in the >2y group γ-tocopherol was negatively correlated to all measured contaminants, except for CB-44, -47/48, -56/60, -66, -97, -110, -141, PeCB, HCB, c-Chlordane, and BD-E49. When pooling all the juveniles (n=15) γ-tocopherol was negatively correlated to CB-172, BDE-47 and -100 ($p < 0.05$).

4. Discussion

4.1. THs

The mean plasma thyroid hormone concentrations in Faroe Island pilot whales determined in this study were somewhat higher but mostly within the same ranges as those reported previously in pilot whale from the same area in 2003-04, except for FT3 which was higher than previously reported (Dam et al., 2010). However, compared to other odontoceti species such as beluga and bottlenose dolphin (*Tursiops truncatus*) plasma TH concentrations in the present pilot whales were mostly lower (Fair et al., 2011; Flower et al., 2015; Villanger et al., 2011b). Numerous variables can influence the TH concentrations, such as season, water temperature, geographical area, and biological variables such as age, sex, stress and pregnancy (Fair et al., 2011; Flower et al., 2015; St. Aubin and Geraci, 1989, 1988). It is therefore difficult to directly compare concentrations between different studies.

As shown in Fig. 1 and the 25−75 percentiles in Table 1, there were large variations in the TH concentrations, also within the age/sex groups and in particular for the juveniles 0–2 years group. While the 25−75 percentile ranged by a magnitude of approximately 1.5 in adult females the difference was 2.5–3 magnitudes in the juveniles 0–2 years. The variation in juveniles >2 years was similar to that in adult females except for FT3 which had higher variation. FT3 was the TH with highest variation within the age groups.

The TH levels were found to be significantly higher in juveniles than in adults in the present study. Higher levels of THs in juveniles compared to adults or decreasing TH concentrations with age, have also been found in wild and captive bottlenose dolphins (Fair et al., 2011; West et al., 2014) beluga (Flower et al., 2015) and other mammals (Gabrielsen et al., 2011; Hall et al., 1998), as well as humans (Kapelari et al., 2008). This most likely reflects the importance of THs for processes like growth and development (Rolland, 2000) as well as heat production, since young individuals have a smaller size and thus smaller volume relative to surface. In the study of Fair et al. (2011) age was found to be a more important factor influencing circulating TH concentrations in bottlenose dolphins than sex, reproductive status, geographic location and ocean temperature. In the present study, the age and length were important variables in the OPLS models for the THs, in particular for TT4 and FT4, showing negative relationship, demonstrating the important influence of age on the TH levels.

The influence from sex on TH was not analysed in the present study since the males were mostly juveniles and the adults were mostly females. Differences in THs between sexes have been found in cetaceans showing higher TH concentrations in males (Flower et al., 2015), whereas other studies have not found differences between the sexes or the results have been inconclusive (Fair et al., 2011; West et al., 2014).

4.1.1. Relationship between OHCs and THs

The OPLS models showed positive correlations between THs and plasma OHC concentrations but negative relationship between age and length and THs. The adult group of the pilot whales in the present study consisted mostly of adult females, which generally had four to ten times lower contaminant levels than the juveniles (Table 2S; Hoydal et al. 2015), whereas the TH levels were significantly higher in the juveniles. The positive relationship between contaminants and thyroid hormones found in the present study could thus be explained by the difference between these two age groups and not by an effect of the contaminants on the hormones. The previous analyses of pilot whales (Dam et al., 2010) also showed positive correlations between liver PCB TEQ values (calculated for mono- ortho-PCBs) and plasma TT3 and FT4. These analyses had not been corrected for age differences (Dam et al., 2010). However, when correcting for age in the present study, by treating the age groups separately, positive correlations between THs and several OHCs in plasma were still seen in the juvenile 0-2y group and between HCB and TT3 in both the adult females group and in the juveniles.

These positive associations are in contrast to the negative relationships between THs and OHCs previously reported in other odontocetes (Schwacke et al., 2012; Villanger et al., 2011b) and other mammals (Braathen et al., 2004; Debier et al., 2005; Tabuchi et al., 2006), and the lowered TH levels documented in wildlife

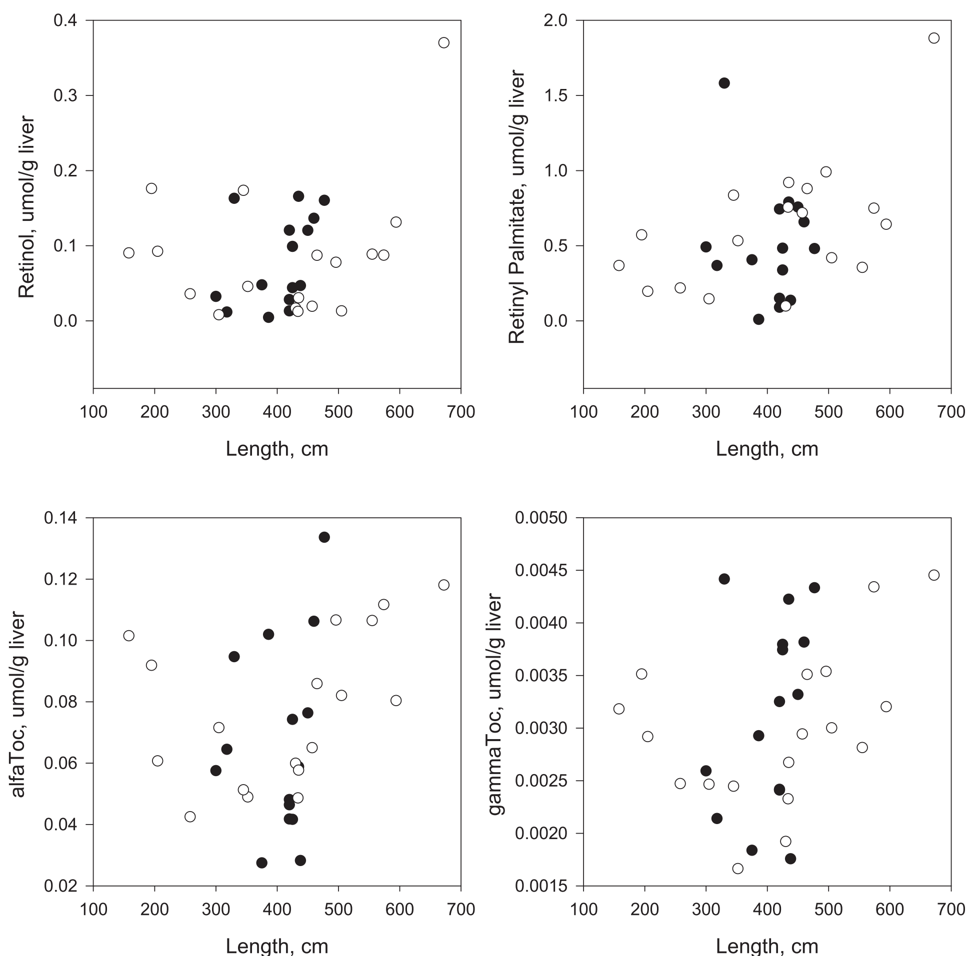

Fig. 3. Liver concentrations of vitamin A (retinol, and retinyl palmitate), α–tocopherol (alfaToc) and γ-tocopherol (gammaToc) in male and female pilot whales (*Glopicephala melas*) in relation to the length of the individuals. Closed and open circles represent females and males, respectively.

mammals experimentally exposed to OHCs as compared to their controls (Brouwer et al., 1989b; Kirkegaard et al., 2011). In bottlenose dolphins from Georgia, US, with higher PCB contamination than the present pilot whales, negative correlations between ∑PCBs and TT4, FT4 and TT3 were found (Schwacke et al., 2012). Negative relationships between several PBDE congeners and CB-105 and TT4, FT4 and TT3 were also reported in a study of beluga whales from Svalbard (Villanger et al., 2011b), where lower OHC concentrations than in the present study were reported.

It should, however, be noted that significant positive correlations between OHCs and THs, as reported herein, also have been found in other marine mammals. In seals positive correlations were found between hydroxylated OHC metabolites and FT3 (Routti et al., 2010a) and between PBDEs and TT4 and TT3 (Hall et al., 2003). The concentrations of PBDEs in pilot whales in the present study were similar to those found in seals (Hall et al., 2003) and the concentrations of OH-metabolites were low, although the levels of PCBs and pesticides were relatively high (Hoydal et al., 2015). Furthermore, in polar bears from East Greenland with lower concentrations of PCBs than in the present pilot whales, positive correlations between ortho-chlorinated PCBs and TT4 were found, although negative correlations between OHCs and THs were also found (Villanger et al., 2011a).

The positive relationship between OHCs and THs could indicate stimulation of TSH secretion from the pituitary and/or disturbance of the feedback (T4 and T3 effect on the TSH release) by OHCs. Villanger et al. (2011b) reported negative correlation between TSH and FT4 in beluga whales from Svalbard reflecting the inhibition of TSH release from the pituitary by FT4.

The negative associations between TT3:FT3 and FT4:FT3 and the OHCs (Fig. 5 and Table 3) reflect a relative increase in free T3 with higher OHC concentrations. The relative concentrations of FT3 to TT3 and FT4 were also found to be significantly higher (i.e. the TT3:FT3 and FT4:FT3 ratios were lower) in juveniles featuring higher OHC concentrations than adults. Thus, age related differences in TH concentration and OHC exposure could explain the observed relationships.

Higher concentration of free THs relative to total THs could result from the binding of contaminants or their metabolites to transport proteins. This in consequence may inhibit the binding of THs to proteins and the binding to the retinol:RBP complex which has been suggested as a mechanism responsible for TH disruption (Brouwer

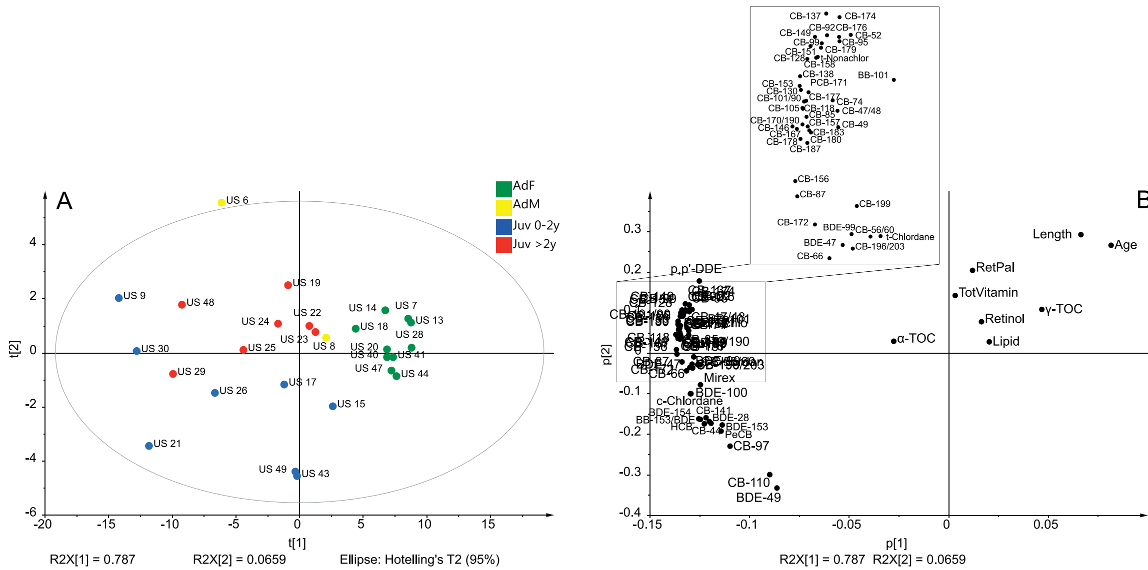

Fig. 4. Score plot (A) and loading plot (B) of vitamins and the contaminants (Hoydal et al., 2015) analysed in pilot whale (*Glopicephala melas*) liver.

et al., 1998). However, this effect is mostly thought to be associated with PCBs and PBDEs OH-metabolites, due to their structural similarity to THs (Brouwer et al., 1998; Liu et al., 2014). In pups of hooded seals the TT3:FT3 ratio was found to be negatively correlated to OH-PCBs, particularly 3′-OH-CB138 (Gabrielsen et al., 2011; Villanger et al., 2013). On the contrary TT4:FT4 ratio was positively correlated to BDE-99 and 4-OH-CB107 in the same study. This mechanism does however seem unlikely for explaining the relatively higher free T3 levels in the present pilot whales since some of the PCBs, found to be negatively correlated to the TT3:FT3 ratio and important variables in the OPLS model for FT3, were recalcitrant compounds such as CB-146, -153, -180, and -187 (Structural group I) and DDE. These compounds are found not to be metabolised to any significant degree in cetaceans (Boon et al., 1997; Tanabe et al., 1988) and OH-metabolites would thus not be produced from these compounds. 4-OH-CB107/4′-OH-CB108 (co-eluting) were the only OH-metabolites detected in the pilot whales and it has been found to be the only or most prevalent OH-metabolite in other cetaceans as well (Houde et al., 2006; Kunisue et al., 2007; McKinney et al., 2006; Montie et al., 2009; Murata et al., 2007). The concentrations in pilot whales (mean: 0.32 ng/g ww =0.9 pmol/g ww) were comparable or slightly lower than in the hooded seals, although the PCB concentrations were much higher in the pilot whales (Gabrielsen et al., 2011; Hoydal et al., 2015; Villanger et al., 2013). Compared to the TT4 concentrations of 22.2–118.2 pmol/g the binding affinity of the OH-metabolites would have to be 20–100 times higher than the T4 binding affinities to the transport proteins in order to

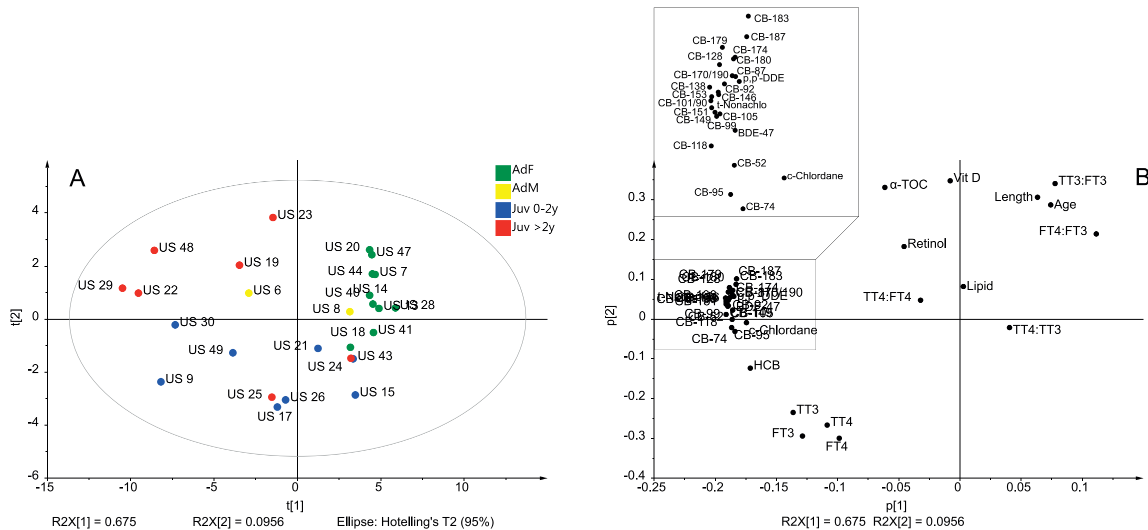

Fig. 5. Score plot (A) and loading plot (B) of thyroid hormones and vitamins and the contaminants (Hoydal et al., 2015) analysed in pilot whale (*Glopicephala melas*) plasma.

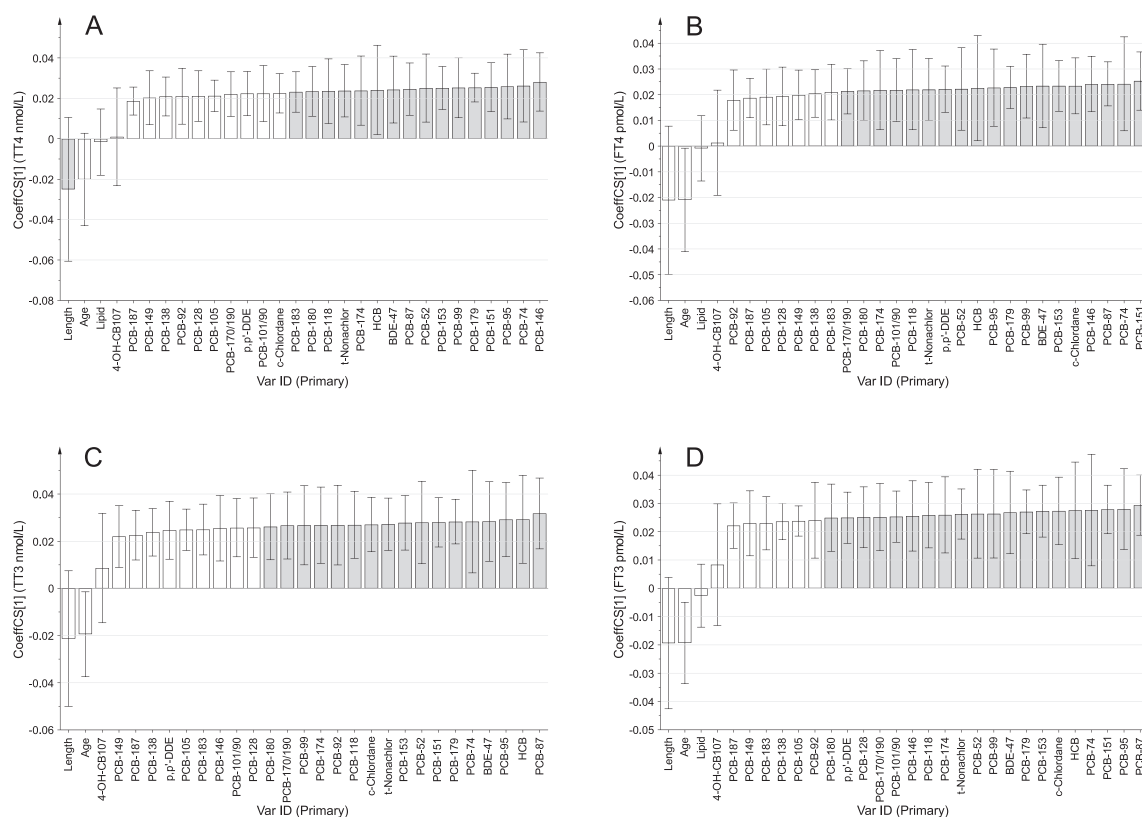

Fig. 6. Regression coefficient plots of the OPLS model showing regression coefficient (CoeffCS) values of each variable indicating the direction and strength of the relationships between individual X-variables and the Y-variable A: TT4, B: FT4, C: TT3 and D: FT3. The dark grey bars present CoeffCS values of variables with VIP values > 1, which indicate high importance.

cause disruption of the system. Analyses have shown binding affinities of hydroxylated PCBs and T4 to TTR to be of similar magnitude in humans, whereas only few OH-PCBs bind to TBG and then with 100 times lower binding affinity (Cheek et al., 1999).

Overall, the TH levels in the pilot whales in the present study do not seem to be negatively affected by the relative high OHC exposure. THs disruption by OHCs has been described as binding of OH-metabolites to thyroid hormone transport proteins or thyroid hormone receptors due to structural similarity (Brouwer et al., 1998; Liu et al., 2014). Hence, the very low concentrations OH-metabolites found in pilot whales (Hoydal et al., 2015) are probably below threshold concentration that could affect THs. Low concentrations of OH-metabolites in cetaceans results from their generally low ability to metabolise OHCs. Thus the negative effects of OHCs on THs that have been found in other cetacean species (Schwacke et al., 2012; Villanger et al., 2011b) indicate that other mechanisms than binding to transport proteins are involved and that other parameters such as TSH hormone concentrations and deiodinase activities are relevant and need to be considered when analysing the effects of OHCs on THs.

4.2. Vitamins

The total vitamin A levels in liver of pilot whales were somewhat higher than those reported in common dolphin (*Delphinus delphis*) from North-West Spain and harbour porpoises (*Phocoena*

Table 3
Significant correlations ($p > 0.05$) between POPs and THs analysed by Spearman correlation (AdF: Adult females, Juv 0–2: Juveniles 0–2 years old, Juv > 2: Juveniles more than 2 years old). ↑ Positive correlation,↓ Negative correlation.

		All individuals	AdF	Juv 0–2	Juv > 2
TT4	↑	All OHC compounds		CB-52, -74, -92, -101, -105, -128, -151, ppDDE, BDE-47	
TT3	↑	All OHC compounds	HCB	CB-87, -92, -99, *t*-nonaCHL	
FT4	↑	All OHC compounds			
FT3	↑	All OHC compounds		CB-87, -92, -99, *t*-nonaCHL, ppDDE	
TT4:FT4	↑			CB-74, -118, -183	
TT3:FT3	↓	All OHC compounds			
TT4:TT3	↓		4-OH-CB107/4′-OH-CB108		
FT4:FT3	↓	All OHC compounds			

Table 4
Significant correlations ($p > 0.05$) between POPs and vitamins analysed by Spearman correlation (AdF: Adult females, Juv 0–2: Juveniles 0–2 years old, Juv > 2: Juveniles more than 2 years old). ↑ Positive correlation,↓ Negative correlation.

Plasma:		All	AdF	Juv 0–2	Juv > 2
Retinol	↑		HCB		
	↓		CB-146		
a-TOC	↑	CB-146, -87, -183	CB-87	All OHC compounds (excl. CB-118, BDE-47)	
	↓		CB-74		
25OHD3	↑			4-OH-CB107/4′-OH-CB108	
Liver:					
Retinol	↑		CB-74, -196/203		
	↓	BDE-47			
Retinyl palmitate	↑		CB-196/203		
	↓		BDE-28, -49, -47, -153		
a-TOC	↑		CB-171, Mirex		
g-TOC	↓	BDE49, -47, -100, -99, -153			All OHC compounds (excl. CB-56/60, -66, -97, -110, -141, PeCB, HCB, cCHL, BDE-49)

phocoena) from Canada (Tornero et al., 2005b, 2004a) and similar to those found in beluga from the Northwest Territories in Canada (Desforges et al., 2013). The retinol and retinyl palmitate concentrations in liver of the pilot whales were similar, or somewhat higher, than those found in the Canadian beluga and the retinol levels in plasma were similar to levels in beluga (Desforges et al., 2013) and in captive and free-ranging bottlenose dolphins from Sarasota, Florida in 1991–1996 (Crissey and Wells, 1999).

In liver, the concentrations of α–tocopherol and γ-tocopherol (vitamin E) in pilot whale were higher than those in Canadian beluga (Desforges et al., 2013), while in plasma α–tocopherol concentrations were similar to those in bottlenose dolphins from Florida (Crissey and Wells, 1999).

The vitamin D concentration in plasma (mean: 79.6 ng/mL, range: 9.1–162.0 ng/mL) was higher than previously reported in two pilot whales (40.4 ng/mL) (Keiver et al., 1988), although the previously reported values were within the low range of the present study concentrations. Higher or similar levels of vitamin D3 in comparison to pilot whales in the present study have been reported in beluga (157.7 ng/mL) and bottlenose dolphin (293.1 ng/mL) (Keiver et al., 1988). Due to differences in analysing methods between these older analyses referred to and the present study the concentration comparisons have to be used with caution. To our knowledge no newer analyses of vitamin D3 in cetaceans have been reported.

4.2.1. *Relationship between OHCs and vitamin levels*

Since both contaminants and vitamins are derived from the diet, this has to be borne in mind when assessing correlative relationships between OHCs and vitamins. The inter-correlation between the vitamins in plasma and most of the vitamins in liver could be a reflection of a similar source from the diet. During gestation, placental transfer of vitamin A and E is limited and thus vitamin levels are low at birth (Debier and Larondelle, 2005). This is however followed by a rapid increase of vitamin A and E with milk ingestions as the concentrations in milk and especially colostrum are high (Debier and Larondelle, 2005; Debier et al., 2002). The apparent higher concentrations of retinol and α-tocopherol in liver of the three smallest individuals compared to the other small individuals (Fig. 3) could be a reflection of this.

4.2.1.1. Vitamin A. In liver, BDE-47 was found to be negatively correlated to retinol, and when separating the age groups BDE-28, -49, -47 and 153 were negatively correlated to retinyl palmitate in adult females. CB-196/203 was, on the other hand, positively correlated to retinyl palmitate and CB-74 and -196/203 to retinol in liver in adult females. In plasma; HCB was positively and CB-146 negatively correlated to retinol in adult females.

PCB and vitamin A relationships in marine mammals have been investigated in cetaceans (Desforges et al., 2013; Tornero et al., 2006; Tornero et al., 2005a), seals (Debier et al., 2012; Jenssen et al., 2003; Nyman et al., 2003; Routti et al., 2010b, 2005; Vanden Berghe et al., 2010), and polar bears (Bechshøft et al., 2015, 2011; Braathen et al., 2004). These studies have shown both positive and negative relationships depending on tissue, exposure and biological factors. Desforges et al., (2013) found negative correlations between PCBs and vitamin A (retinyl esters) in liver, but positive correlations in plasma. Nyman et al. (2003) also found that PCBs in liver were negatively correlated to retinol and retinyl palmitate in grey seals. Moreover, PCBs in grey seal pups were negatively correlated to retinol or total vitamin A in plasma or serum (Jenssen et al., 2003; Vanden Berghe et al., 2010). However, Nyman et al. (2003) showed positive correlations between PCBs and retinol in grey seal plasma. Negative correlations between PCBs and vitamin A in liver were not found in the present study although the PCB concentrations in the pilot whales were much higher than the integrated toxicity reference value on 1.6 mg/kg proposed by Desforges et al. (2013), based on 5% effect concentrations for both vitamin A and E.

Relationships between PBDE and vitamin A have been less investigated and to our knowledge, among marine mammals only seals have been studied (Vanden Berghe et al., 2013). Negative relationships between PBDEs and retinol have been found in laboratory rats and mice and in birds (Fernie et al., 2005). Negative correlation between BDE47 and hepatic retinol was found in captive PBDE exposed American kestrels and BDE99 and -100 were negatively correlated to retinol in plasma (Fernie et al., 2005). In the blood of snapping turtles (collected in 2001–2004) from 12 wetland sites on the Canadian side of the Laurentian Great Lakes of North America, some OHC concentrations were correlated with THs and/or vitamin A (Letcher et al., 2015). For example, significant negative (e.g. cis-chlordane) or positive (e.g. BDE-99) correlations were reported with TT4. Dehydroretinol concentrations were positively correlated with PCP, cis-chlordane, trans-nonachlor, CB-28, -44, -49, -52, -74 and -151. In contrast, 4′-OH-BDE49 and 4-OH-CB107/4′-MeO-CB108 concentrations were negatively correlated with dehydroretinol concentrations, and retinol was positively associated with Σ46PCB, Σ28OH-PCB, PCP, HCB, cis-chlordane, trans-nonachlor, 4-OH-CB187 and 6′-OH-BDE49 concentrations. In adult lactating female seals, vitamin A was found to

be positively correlated to ∑PCBs and ∑PBDEs in inner blubber and serum (Vanden Berghe et al., 2013). BDE-47 and 4-OH-CB-107 were also positively correlated to vitamin A in serum in the same study.

The decreasing vitamin A levels in pilot whales associated with increasing PBDE levels could indicate a higher need for retinol (at least in adult females) due to exposure to this contaminant. However no changes in the plasma retinol levels were found. Furthermore, retinol in liver was positively correlated to retinol in plasma and retinyl palmitate in liver. This indicates that the vitamin A stores in the liver were not depleted by an increased requirement for retinol.

4.2.1.2. Vitamin D. Positive correlation between 4-OH-CB107/4′-OH-CB108 and CB-172 and 25(OH)D3 in 0-2y juveniles was the only association found between vitamin D and OHCs. Vitamin D_3, absorbed in the intestine, is metabolised in the liver to 25(OH)-vitamin D_3 (25(OH)D3) and then in the kidney to 1,25-dihydroxyvitamin D_3 (1,25$(OH)_2$D). The latter is the most active metabolite of vitamin D, involved in the regulation of serum calcium homeostasis (DeLuca, 2004). Levels of 1,25$(OH)_2$D have been shown to be negatively correlated with PCBs and DDT in Baltic grey seals (Routti et al., 2008). This could be due to either inhibition of the 1,25$(OH)_2$D metabolite formation or its enhanced renal clearance induced by the contaminants (Routti et al., 2008). In the present study the observed relation between 25(OH)D3 and OHCs could be explained by inhibition of the 1,25$(OH)_2$D metabolite formation (thus increasing 25(OH)D3 levels) by 4-OH-CB107/4′-OH-CB108 and CB-172. However, since very low concentrations of OH-CB107/4′-OH-CB108 were observed and correlations were only found in the 0-2y age group consisting of very few individuals it is likely that the correlative relationship found herein cannot be regarded as an effect of OHCs on vitamin D homeostasis.

4.2.1.3. Vitamin E. The most pronounced relationship between vitamins and OHCs was the positive correlation between α–tocopherol and all the analysed contaminants in plasma of juvenile pilot whales. α–Tocopherol has antioxidant properties and since several OHCs, including PCBs, OCPs and PBDEs have abilities to induce oxidative stress (Abdollahi et al., 2004; Fernie et al., 2005; Valavanidis et al., 2006), the increase in plasma α–tocopherol with increasing OHC concentrations could be a result of organism defence against oxidative stress induction by OHCs. Previously α–tocopherol has been found to be positively correlated to PCB concentrations in plasma and blubber of ringed- and grey seals (Nyman et al., 2003) and PCBs in plasma of Greenland shark (Molde et al., 2013). However, the plasma α–tocopherol concentrations in juveniles were not significantly different from the concentrations in adult females, although the OHC levels in adult females were lower and not correlated with α–tocopherol concentrations. The higher plasma vitamin E levels would require a higher supply of vitamins from food or from vitamin E stores in the body. The α–tocopherol levels in the liver were however not correlated to the OHC concentrations or to the plasma α–tocopherol concentrations, indicating that the vitamin E amounts in the blood were sufficient to cope with this higher requirement.

The negative correlation between γ-tocopherol in liver and BDE-47, -49, -99, -100 and -153 and between γ–tocopherol and most of the contaminants analysed in juveniles >2 years could indicate depletion of γ-tocopherol stores in the liver due to contaminant exposure. In comparison with α–tocopherol, γ-tocopherol is found in much lower concentrations in the body and has distinct chemical reactivity and metabolism and biological activities (Jiang et al., 2001). γ-Tocopherol has higher anti-inflammatory properties compared to α–tocopherol (Jiang et al., 2001) and is much more easily metabolised in the liver and excreted with urine compared to α–tocopherol, which by binding to a transfer protein (α–TTP) is transported to peripheral tissues (Jiang et al. 2001). The catabolism of γ-tocopherol is cytochrome P450 mediated, most likely by CYP3A (Parker et al., 2000). Strong CYP3A protein expression has been reported in pilot whale liver (Celander et al., 2000). The decrease in liver γ-tocopherol with increasing contaminants levels could thus be a result of CYP enzyme induction. Some OHCs induce and are metabolised by CYP3A (Maurel, 1996) and the induction by OHCs could thus lead to increased CYP3A enzyme concentration and a higher metabolism of γ-tocopherol.

5. Conclusion

The present study reports concentrations of THs and vitamins A, E and D in pilot whales from the North-East Atlantic and the associations between OHC concentrations and these biomarkers. The results indicate that although pilot whales are highly exposed to OHCs the thyroid hormone system and the examined vitamins do not seem to be significantly disrupted. The multivariate analyses showed that both hormones and OHCs and to some extent also vitamins were influenced by the length or age of the animals and that the age/sex groups had to be treated separately when analysing for relationships with OHCs. Due to the resulting small number of individuals in each age/sex group the results have to be interpreted with caution. In juveniles there were significant positive relationships between plasma concentrations of α–tocopherol and all the OHCs analysed, indicating a response against oxidative stress. Vitamin A did not seem to be related to OHC concentrations in neither in liver nor plasma, except for negative correlations with PBDEs in adult females, indicating that some PBDEs (and/or other compounds) may affect hepatic function. In conclusions, it appears that the high body burdens of OHCs in Faroese pilot whales may have minor effects on levels of circulating THs and vitamin A, possibly due to the low capacity of pilot whales to form OH-PCBs.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.envres.2016.04.012.

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Paper V

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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 Zoology	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	Reflectometric 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 Zoology	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 Botany	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 Botany	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 Botany	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 Botany	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 Botany	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
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 Botany	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:
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 Zoology	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 Zoology	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	PhD 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	PhD Biology	Interactions between marine osmo- and phagotrophs in different physicochemical environments
2005	Åslaug Viken	PhD Biology	Implications of mate choice for the management of small populations

2005	Ariaya Hymete Sahle Dingle	PhD Biology	Investigation of the biological activities and chemical constituents of selected *Echinops* spp. growing in Ethiopia
2005	Anders Gravbrøt Finstad	PhD Biology	Salmonid fishes in a changing climate: The winter challenge
2005	Shimane Washington Makabu	PhD 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	PhD 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	PhD 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	PhD Biology	Acesta Oophaga and Acesta Excavata – a study of hidden biodiversity
2006	Bjørn Henrik Hansen	PhD Biology	Metal-mediated oxidative stress responses in brown trout (*Salmo trutta*) from mining contaminated rivers in Central Norway
2006	Vidar Grøtan	PhD Biology	Temporal and spatial effects of climate fluctuations on population dynamics of vertebrates
2006	Jafari R Kideghesho	PhD Biology	Wildlife conservation and local land use conflicts in western Serengeti, Corridor Tanzania
2006	Anna Maria Billing	PhD Biology	Reproductive decisions in the sex role reversed pipefish *Syngnathus typhle*: when and how to invest in reproduction
2006	Henrik Pärn	PhD Biology	Female ornaments and reproductive biology in the bluethroat
2006	Anders J. Fjellheim	PhD Biology	Selection and administration of probiotic bacteria to marine fish larvae
2006	P. Andreas Svensson	PhD Biology	Female coloration, egg carotenoids and reproductive success: gobies as a model system
2007	Sindre A. Pedersen	PhD 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	PhD Biology	Photosynthetic responses as a function of light and temperature: Field and laboratory studies on marine microalgae
2007	Tomas Holmern	PhD Biology	Bushmeat hunting in the western Serengeti: Implications for community-based conservation
2007	Kari Jørgensen	PhD Biology	Functional tracing of gustatory receptor neurons in the CNS and chemosensory learning in the moth *Heliothis virescens*
2007	Stig Ulland	PhD 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	PhD Biology	Spatial and temporal variation in herbivore resources at northern latitudes
2007	Roelof Frans May	PhD Biology	Spatial Ecology of Wolverines in Scandinavia
2007	Vedasto Gabriel Ndibalema	PhD Biology	Demographic variation, distribution and habitat use between wildebeest sub-populations in the Serengeti National Park, Tanzania
2007	Julius William Nyahongo	PhD Biology	Depredation of Livestock by wild Carnivores and Illegal Utilization of Natural Resources by Humans in the Western Serengeti, Tanzania
2007	Shombe Ntaraluka Hassan	PhD Biology	Effects of fire on large herbivores and their forage resources in Serengeti, Tanzania
2007	Per-Arvid Wold	PhD 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	PhD 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	PhD Biology	The Svalbard reindeer (*Rangifer tarandus platyrhynchus*) and its food base: plant-herbivore interactions in a high-arctic ecosystem
2008	Jiska van Dijk	PhD Biology	Wolverine foraging strategies in a multiple-use landscape
2008	Flora John Magige	PhD Biology	The ecology and behaviour of the Masai Ostrich (Struthio camelus massaicus) in the Serengeti Ecosystem, Tanzania
2008	Bernt Rønning	PhD Biology	Sources of inter- and intra-individual variation in basal metabolic rate in the zebra finch, (*Taeniopygia guttata*)
2008	Sølvi Wehn	PhD Biology	Biodiversity dynamics in semi-natural mountain landscapes - A study of consequences of changed agricultural practices in Eastern Jotunheimen
2008	Trond Moxness Kortner	PhD 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	PhD Biology	Statistical Modelling of Gene Expression Data
2008	Anna Kusnierczyk	PhD Biology	*Arabidopsis thaliana* Responses to Aphid Infestation
2008	Jussi Evertsen	PhD Biology	Herbivore sacoglossans with photosynthetic chloroplasts
2008	John Eilif Hermansen	PhD 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	PhD Biology	Somatic embryogenesis in *Cyclamen persicum.* Biological investigations and educational aspects of cloning
2008	Line Elisabeth Sundt-Hansen	PhD Biology	Cost of rapid growth in salmonid fishes

2008	Line Johansen	PhD Biology	Exploring factors underlying fluctuations in white clover populations – clonal growth, population structure and spatial distribution
2009	Astrid Jullumstrø Feuerherm	PhD Biology	Elucidation of molecular mechanisms for pro-inflammatory phospholipase A2 in chronic disease
2009	Pål Kvello	PhD 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	PhD Biology	Extreme Frost Tolerance in Boreal Conifers
2009	Johan Reinert Vikan	PhD Biology	Coevolutionary interactions between common cuckoos *Cuculus canorus* and *Fringilla* finches
2009	Zsolt Volent	PhD Biology	Remote sensing of marine environment: Applied surveillance with focus on optical properties of phytoplankton, coloured organic matter and suspended matter
2009	Lester Rocha	PhD Biology	Functional responses of perennial grasses to simulated grazing and resource availability
2009	Dennis Ikanda	PhD Biology	Dimensions of a Human-lion conflict: Ecology of human predation and persecution of African lions (*Panthera leo*) in Tanzania
2010	Huy Quang Nguyen	PhD 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	PhD Biology	Intraspecific competition in stream salmonids: the impact of environment and phenotype
2010	Sverre Lundemo	PhD Biology	Molecular studies of genetic structuring and demography in *Arabidopsis* from Northern Europe
2010	Iddi Mihijai Mfunda	PhD Biology	Wildlife Conservation and People's livelihoods: Lessons Learnt and Considerations for Improvements. Tha Case of Serengeti Ecosystem, Tanzania
2010	Anton Tinchov Antonov	PhD Biology	Why do cuckoos lay strong-shelled eggs? Tests of the puncture resistance hypothesis
2010	Anders Lyngstad	PhD Biology	Population Ecology of *Eriophorum latifolium*, a Clonal Species in Rich Fen Vegetation
2010	Hilde Færevik	PhD Biology	Impact of protective clothing on thermal and cognitive responses
2010	Ingerid Brænne Arbo	PhD Medical technology	Nutritional lifestyle changes – effects of dietary carbohydrate restriction in healthy obese and overweight humans
2010	Yngvild Vindenes	PhD Biology	Stochastic modeling of finite populations with individual heterogeneity in vital parameters
2010	Hans-Richard Brattbakk	PhD Medical technology	The effect of macronutrient composition, insulin stimulation, and genetic variation on leukocyte gene expression and possible health benefits
2011	Geir Hysing Bolstad	PhD Biology	Evolution of Signals: Genetic Architecture, Natural Selection and Adaptive Accuracy
2011	Karen de Jong	PhD Biology	Operational sex ratio and reproductive behaviour in the two-spotted goby (*Gobiusculus flavescens*)
2011	Ann-Iren Kittang	PhD 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	PhD Biology	Stochastic modeling of mating systems and their effect on population dynamics and genetics
2011	Christopher Gravningen Sørmo	PhD Biology	Rho GTPases in Plants: Structural analysis of ROP GTPases; genetic and functional studies of MIRO GTPases in Arabidopsis thaliana
2011	Grethe Robertsen	PhD Biology	Relative performance of salmonid phenotypes across environments and competitive intensities
2011	Line-Kristin Larsen	PhD 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	PhD Biology	Regulation in Atlantic salmon (*Salmo salar*): The interaction between habitat and density
2011	Torunn Beate Hancke	PhD Biology	Use of Pulse Amplitude Modulated (PAM) Fluorescence and Bio-optics for Assessing Microalgal Photosynthesis and Physiology
2011	Sajeda Begum	PhD Biology	Brood Parasitism in Asian Cuckoos: Different Aspects of Interactions between Cuckoos and their Hosts in Bangladesh
2011	Kari J. K. Attramadal	PhD Biology	Water treatment as an approach to increase microbial control in the culture of cold water marine larvae
2011	Camilla Kalvatn Egset	PhD Biology	The Evolvability of Static Allometry: A Case Study
2011	AHM Raihan Sarker	PhD Biology	Conflict over the conservation of the Asian elephant (*Elephas maximus*) in Bangladesh
2011	Gro Dehli Villanger	PhD Biology	Effects of complex organohalogen contaminant mixtures on thyroid hormone homeostasis in selected arctic marine mammals
2011	Kari Bjørneraas	PhD Biology	Spatiotemporal variation in resource utilisation by a large herbivore, the moose
2011	John Odden	PhD Biology	The ecology of a conflict: Eurasian lynx depredation on domestic sheep
2011	Simen Pedersen	PhD Biology	Effects of native and introduced cervids on small mammals and birds
2011	Mohsen Falahati-Anbaran	PhD Biology	Evolutionary consequences of seed banks and seed dispersal in *Arabidopsis*
2012	Jakob Hønborg Hansen	PhD Biology	Shift work in the offshore vessel fleet: circadian rhythms and cognitive performance
2012	Elin Noreen	PhD Biology	Consequences of diet quality and age on life-history traits in a small passerine bird
2012	Irja Ida Ratikainen	PhD Biology	Foraging in a variable world:adaptions to stochasticity
2012	Aleksander Handå	PhD Biology	Cultivation of mussels (*Mytilus edulis):*Feed requirements, storage and integration with salmon (*Salmo salar*) farming
2012	Morten Kraabøl	PhD Biology	Reproductive and migratory challenges inflicted on migrant brown trour (*Salmo trutta* L) in a heavily modified river
2012	Jisca Huisman	PhD Biology	Gene flow and natural selection in Atlantic salmon
	Maria Bergvik	PhD Biology	Lipid and astaxanthin contents and biochemical post-harvest stability in *Calanus finmarchicus*
2012	Bjarte Bye Løfaldli	PhD Biology	Functional and morphological characterization of central olfactory neurons in the model insect *Heliothis virescens*.

2012	Karen Marie Hammer	PhD Biology	Acid-base regulation and metabolite responses in shallow- and deep-living marine invertebrates during environmental hypercapnia
2012	Øystein Nordrum Wiggen	PhD Biology	Optimal performance in the cold
2012	Robert Dominikus Fyumagwa	Dr. Philos Biology	Anthropogenic and natural influence on disease prevalence at the human –livestock-wildlife interface in the Serengeti ecosystem, Tanzania
2012	Jenny Bytingsvik	PhD Biology	Organohalogenated contaminants (OHCs) in polar bear mother-cub pairs from Svalbard, Norway. Maternal transfer, exposure assessment and thyroid hormone disruptive effects in polar bear cubs
2012	Christer Moe Rolandsen	PhD Biology	The ecological significance of space use and movement patterns of moose in a variable environment
2012	Erlend Kjeldsberg Hovland	PhD Biology	Bio-optics and Ecology in *Emiliania huxleyi* Blooms: Field and Remote Sensing Studies in Norwegian Waters
2012	Lise Cats Myhre	PhD Biology	Effects of the social and physical environment on mating behaviour in a marine fish
2012	Tonje Aronsen	PhD Biology	Demographic, environmental and evolutionary aspects of sexual selection
	Bin Liu	PhD Biology	Molecular genetic investigation of cell separation and cell death regulation in *Arabidopsis thaliana*
2013	Jørgen Rosvold	PhD Biology	Ungulates in a dynamic and increasingly human dominated landscape – A millennia-scale perspective
2013	Pankaj Barah	PhD Biology	Integrated Systems Approaches to Study Plant Stress Responses
2013	Marit Linnerud	PhD Biology	Patterns in spatial and temporal variation in population abundances of vertebrates
2013	Xinxin Wang	PhD Biology	Integrated multi-trophic aquaculture driven by nutrient wastes released from Atlantic salmon (*Salmo salar*) farming
2013	Ingrid Ertshus Mathisen	PhD Biology	Structure, dynamics, and regeneration capacity at the sub-arctic forest-tundra ecotone of northern Norway and Kola Peninsula, NW Russia
2013	Anders Foldvik	PhD Biology	Spatial distributions and productivity in salmonid populations
2013	Anna Marie Holand	PhD Biology	Statistical methods for estimating intra- and inter-population variation in genetic diversity
2013	Anna Solvang Båtnes	PhD Biology	Light in the dark – the role of irradiance in the high Arctic marine ecosystem during polar night
2013	Sebastian Wacker	PhD Biology	The dynamics of sexual selection: effects of OSR, density and resource competition in a fish
2013	Cecilie Miljeteig	PhD Biology	Phototaxis in *Calanus finmarchicus* – light sensitivity and the influence of energy reserves and oil exposure
2013	Ane Kjersti Vie	PhD Biology	Molecular and functional characterisation of the IDA family of signalling peptides in *Arabidopsis thaliana*
2013	Marianne Nymark	PhD Biology	Light responses in the marine diatom *Phaeodactylum tricornutum*
2014	Jannik Schultner	PhD Biology	Resource Allocation under Stress - Mechanisms and Strategies in a Long-Lived Bird
2014	Craig Ryan Jackson	PhD Biology	Factors influencing African wild dog *(Lycaon pictus*) habitat selection and ranging behaviour: conservation and management implications

2014	Aravind Venkatesan	PhD Biology	Application of Semantic Web Technology to establish knowledge management and discovery in the Life Sciences
2014	Kristin Collier Valle	PhD Biology	Photoacclimation mechanisms and light responses in marine micro- and macroalgae
2014	Michael Puffer	PhD Biology	Effects of rapidly fluctuating water levels on juvenile Atlantic salmon (*Salmo salar* L.)
2014	Gundula S. Bartzke	PhD Biology	Effects of power lines on moose *(Alces alces)* habitat selection, movements and feeding activity
2014	Eirin Marie Bjørkvoll	PhD Biology	Life-history variation and stochastic population dynamics in vertebrates
2014	Håkon Holand	PhD Biology	The parasite *Syngamus trachea* in a metapopulation of house sparrows
2014	Randi Magnus Sommerfelt	PhD Biology	Molecular mechanisms of inflammation – a central role for cytosolic phospholiphase A2
2014	Espen Lie Dahl	PhD Biology	Population demographics in white-tailed eagle at an on-shore wind farm area in coastal Norway
2014	Anders Øverby	PhD Biology	Functional analysis of the action of plant isothiocyanates: cellular mechanisms and in vivo role in plants, and anticancer activity
2014	Kamal Prasad Acharya	PhD Biology	Invasive species: Genetics, characteristics and trait variation along a latitudinal gradient.
2014	Ida Beathe Øverjordet	PhD Biology	Element accumulation and oxidative stress variables in Arctic pelagic food chains: Calanus, little auks (alle alle) and black-legged kittiwakes (Rissa tridactyla)
2014	Kristin Møller Gabrielsen	PhD Biology	Target tissue toxicity of the thyroid hormone system in two species of arctic mammals carrying high loads of organohalogen contaminants
2015	Gine Roll Skjervø	Dr. philos Biology	Testing behavioral ecology models with historical individual-based human demographic data from Norway
2015	Nils Erik Gustaf Forsberg	PhD Biology	Spatial and Temporal Genetic Structure in Landrace Cereals
2015	Leila Alipanah	PhD Biology	Integrated analyses of nitrogen and phosphorus deprivation in the diatoms *Phaeodactylum tricornutum* and *Seminavis robusta*
2015	Javad Najafi	PhD Biology	Molecular investigation of signaling components in sugar sensing and defense in *Arabidopsis thaliana*
2015	Bjørnar Sporsheim	PhD Biology	Quantitative confocal laser scanning microscopy: optimization of in vivo and in vitro analysis of intracellular transport
2015	Magni Olsen Kyrkjeeide	PhD Biology	Genetic variation and structure in peatmosses (*Sphagnum*)
2015	Keshuai Li	PhD Biology	Phospholipids in Atlantic cod (*Gadus morhua* L.) larvae rearing: Incorporation of DHA in live feed and larval phospholipids and the metabolic capabilities of larvae for the de novo synthesis
2015	Ingvild Fladvad Størdal	PhD Biology	The role of the copepod *Calanus finmarchicus* in affecting the fate of marine oil spills
2016	Thomas Kvalnes	PhD Biology	Evolution by natural selection in age-structured populations in fluctuating environments
2016	Øystein Leiknes	PhD Biology	The effect of nutrition on important life-history traits in the marine copepod *Calanus finmarchicus*

2016	Johan Henrik Hårdensson Berntsen	PhD Biology	Individual variation in survival: The effect of incubation temperature on the rate of physiological ageing in a small passerine bird
2016	Marianne Opsahl Olufsen	PhD Biology	Multiple environmental stressors: Biological interactions between parameters of climate change and perfluorinated alkyl substances in fish
2016	Rebekka Varne	PhD Biology	Tracing the fate of escaped cod (*Gadus morhua* L.) in a Norwegian fjord system
2016	Anette Antonsen Fenstad	PhD Biology	Pollutant Levels, Antioxidants and Potential Genotoxic Effects in Incubating Female Common Eiders (*Somateria mollissima*)
2016	Wilfred Njama Marealle	PhD Biology	Ecology, Behaviour and Conservation Status of Masai Giraffe (***Giraffa camelopardalis tippelskirchi***) in Tanzania
2016	Ingunn Nilssen	PhD Biology	Integrated Enviromental Mapping and Monitoring: A Methodological approach for endusers.
2017	Konika Chawla	PhD Biology	Discovering, analysing and taking care of knowledge.
2017	Øystein Hjorthol Opedal	PhD Biology	The Evolution of Herkogamy: Pollinator Reliability, Natural Selection, and Trait Evolvability.
2017	Ane Marlene Myhre	PhD Biology	Effective size of density dependent populations in fluctuating environments
2017	Emmanuel Hosiana Masenga	PhD Biology	Behavioural Ecology of Free-ranging and Reintroduced African Wild Dog (Lycaon pictus) Packs in the Serengeti Ecosystem, Tanzania
2017	Xiaolong Lin	PhD Biology	Systematics and evolutionary history of Tanytarsus van der Wulp, 1874 (Diptera: Chironomidae)
2017	Emmanuel Clamsen Mmassy	PhD Biology	Ecology and Conservation Challenges of the Kori bustard in the Serengeti National Park
2017	Richard Daniel Lyamuya	PhD Biology	Depredation of Livestock by Wild Carnivores in the Eastern Serengeti Ecosystem, Tanzania

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