Steroid Hormones and Persistent Organic Pollutants in plasma from North-eastern Atlantic Pilot whales

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Abstract

Persistent organic pollutants (POPs) are known to have endocrine disruptive effects, interfering with endogenous steroid hormones. The present study examined nine steroid hormones and their relationships with the concentrations of selected POPs in pilot whales (*Globicephala melas*) from the Faroe Islands, NE Atlantic. The different steroids were detected in 15 to all of the 26 individuals. High concentrations of progesterone (83.3 – 211.7 pmol/g) and pregnenolone (PRE; 4.68-5.69 pmol/g) were found in three adult females indicating that they were pregnant or ovulating. High androgen concentrations in two of the males reflected that one was adult and that one (possibly) had reached puberty. In males a significant positive and strong correlation between body length and testosterone (TS) levels was identified. Furthermore, positive and significant correlations were found between 4-OH-CB107/4'-OH-CB108 and 17β-estradiol in males. In adult females significant positive correlations were identified between PRE and CB149 and t-nonachlor, between estrone and CB138, -149, -187 and p,p'-DDE, between androstenedione and CB187, and between TS and CB-99 and -153. Although relationships between the POPs and the steroid hormones reported herein are not evidence of cause-effect relationships, the positive correlations between steroids and POPs, particularly in females, suggest that POPs may have some endocrine disrupting effects on the steroid homeostasis in this species.

Key words: Pilot whales, steroid hormones, POPs, endocrine disruption.

1 Introduction

Long-finned pilot whales (*Globicephala melas*) from the Faroe Islands (NE Atlantic) are highly contaminated with persistent organic pollutants (POPs) such as PCBs, organochlorine pesticides (OCPs) and polybrominated diphenyl ethers (PBDEs), and persistent metabolites such as hydroxyl (OH)-containing PCBs (Hoydal et al., 2015). Some of these POPs and/or their metabolites have structural similarities with endogenous hormones, and can interact with hormone transport proteins or disrupt hormone metabolism. They can thus mimic or in some cases interfere with the activity of endogenous hormones (Jenssen, 2006; O'Connor and Chapin, 2003). Previously, we have shown that in pilot whales some POPs may have minor effects on circulating levels of thyroid hormones and vitamin concentrations (Hoydal et al., 2016). However, POPs can also perturb the reproductive hormone system, and thus the reproductive function of wildlife mammals (Colborn et al., 1993). Possible mechanisms for endocrine disruption are multiple and complex, and 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).

Endocrine disruptive effects of POPs related to reproductive hormones have frequently been reported in fish, amphibians and reptiles (Guillette, 2000). Contaminants can have estrogenic or anti-estrogenic effects (Yordy et al., 2010). For example p,p'-DDE and t-nonachlor have estrogenic properties whereas CB-138 and CB-180 have anti-estrogenic properties (Yordy et al., 2010). Thus the effects of exposure to a mixture of contaminants may be difficult to interpret. In wild mammals and surrogate wildlife mammalian model species, it has been suggested that POPs, and in particular PCBs and their OH-PCB metabolites) can influence circulating levels of steroid hormones (Gustavson et al., 2015; Hallanger et al., 2012; Haave et al., 2003; Ropstad et al., 2006; Sonne et al., 2014). Disturbance of steroid levels elicited by POPs has also been indicated in studies of cetaceans (Subramanian et al., 1987; Yordy et al., 2010). The developing foetus is uniquely sensitive to endocrine disruption and studies using experimental animals have indicated that exposure to PCB at environmentally relevant concentrations *in utero*, and in the suckling period, can influence reproductive functions (O'Connor and Chapin, 2003; Ropstad et al., 2006). In a recent study, high and stable PCB burdens have been associated with small populations and low fecundity rates in killer whales (*Orcinus orca*) from European waters (Jepson et al., 2016). However, it is not known if this low fecundity rate is linked to endocrine disruption caused by pollutants.

Although high body concentrations of POPs were reported to disrupt levels of testosterone in Dall's porpoise males in 1984 (Subramanian et al., 1987), only one other study appears to have followed up this work. Applying an *in vitro* approach (E-Screen) to investigate estrogenic or anti-estrogenic activity in contaminant mixtures extracted from the blubber of bottlenose dolphins, significant estrogenic activity were reported for some contaminant groups, such as the DDTs (Yordy et al., 2010). In spite of these indications that POPs may act as steroid disrupting chemicals in whales, and the implications of such endocrine disruption on fitness and population dynamics of cetaceans, to our knowledge there are no other reports of possible effects of POPs on levels of steroid hormones in neither male nor female cetaceans.

Taking into consideration the relative high concentrations of POPs reported in pilot whales from the Faroe Islands (Hoydal et al., 2015), the aim of the present study was to examine possible relationships between POP concentrations and steroid hormones in these whales. Thus, we determined the concentrations of steroid hormones constituting the circulatory steroid profile in

female and male pilot whales from the Faroe Islands taking into account age and size differences, and analysed the relationships between the hormone concentrations and the POP concentrations previously analysed in the same individuals (Hoydal et al., 2015).

2 Materials and methods

Blood plasma from 26 pilot whales (12 females and 14 males) was sampled in connection with traditional hunt in the Faroe Islands on two different occasions; on 23/7-2010 and 02/09-2011 (Table S1). Immediately post-mortem, blood samples were collected into clean heat treated (at 450 ^{o}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 ^{o}C until analysis. The length of the animals was measured and the sex was registered. Age was determined by counting growth layer groups formed annually in dentine and cement of teeth as described in Lockyer (1993). The individuals were grouped into sex and age groups according to their length and/or age, based on the mean length and age of sexual maturity (Desportes et al., 1993; Martin and Rothery, 1993). Thus males smaller than 494 cm and younger than 14 years and females smaller than 375 cm and younger than eight years were categorized as juveniles. The sampling in 2010 consisted of 5 adult females, 1 juvenile female, 2 adult males and 3 juvenile males, and the sampling in 2011 consisted of 5 adult females, 1 juvenile female and 9 juvenile males (Table S1). For more information on the sampling procedures, see Hoydal et al. (2015).

2.1 Steroid hormone analysis

Plasma samples were analysed for steroid hormones using solid phase extraction (SPE) followed by GC-MS/MS according to the fully validated method, including quality criteria, described by Hansen et al. (2011). The steroids analysed were the progestagens pregnenolone (PRE) and progesterone (PRO), the androgens androstenedione (AN), dehydroepiandrosterone (DHEA), dihydrotestosterone (DHT) and testosterone (TS), and the estrogens estrone (E1), 17α-estradiol (αE2) and 17β-estradiol (βE2).

Briefly, SPE columns (Agilent Technologies, Bond Elut – C18, 500 mg, 3 ml) were conditioned with 2 x 2.5 ml heptane, 2 ml acetone, 2 x 2.5 ml methanol and 2 x 3 ml of pH 3 adjusted water. Approximately 2-2.5 ml of sample was added to 6 ml of pH 3 adjusted water and 50 µl internal standard (mixture of six deuterated steroid analogues 0.4 ng/µl) and the pH in the solution was adjusted to approximately 3 with 1 M H_2SO_4 and the sample solutions were applied on the columns. The samples were eluted with 2 x 2.5 ml acetone and the eluate was evaporated to dryness under a gentle stream of N_2 and at 60 ^{o}C. The evaporated samples were reconstituted in 200 µl $CHCl_3$ and applied on new SPE columns (aminopropyl cartridges (Waters Sep-Pak, Vac 6cc (500 mg) NH_2 cartridges)), which had been prepared by adding 2 x 2 ml heptane. The samples were eluted by adding 5 ml of $CHCl_3$:isopropanol (2:1), and the fatty acids and phospholipids were retained in the column. The samples were evaporated to dryness under a gentle N_2 flow and at 60 ^{o}C. The evaporated samples were reconstituted in 50 µl $CHCl_3$ + 450 µl heptane and applied to the silica column (1 g of silica dissolved in heptane in glass columns (Merck LiChrolut, 3 ml) with filters (Macherey-Nagel Chromabond filters) on the bottom). Then, 5 ml of heptane were added into the column to remove sterol esters, followed by addition of 10 ml of heptane:acetone (90:10) to remove

sterols and stanols. Then the steroids were eluted from the columns using 5 ml of heptane: acetone (65:35).

Derivatisation of the samples was performed by evaporating the samples to appr. 1 ml (N_2, 60 ^oC). A volume of 100 µl of the derivatisation standard (0.2 ng/µl AE2 (MeOH)) was added and the samples were evaporated to dryness. A derivatisation reagent was made by 1 ampule of N-methyl-N-(trimethyl-silyl)-trifluoro acetamide, 2 µl of N-trimethylimidazole and 50 µl of 20 mg 1,4 dithioerythritol in 500 µl pyridine (DTE) and 50 µl of the reagent was added to the evaporated samples. The vials were placed in the oven at 60 ^oC for one hour for derivatisation and the samples were evaporated to dryness under N_2 and at 60 ^oC. After that they were dissolved in 200 µl heptane with 0.1 ng /µl MeE1 (injection standard) and transferred to GC vials and analysed by GC-MS/MS (Bruker Scion TQ). Limits of quantification for individual steroids and procedures for quality control are described in details in Nossen et al. (2016). Recoveries are reported in Hansen et al. (2011).

2.2 Analysis of POPs in plasma

The plasma samples were previously analysed for 175 different POPs (i.e. PCBs, PBDEs and OCPs) and their relevant metabolites (i.e. OH-metabolites) at the Organic Contaminants Research Laboratory/Letcher Labs at the National Wildlife Research Centre, Carleton University in Ottawa, Canada. The samples were cleaned up in multiple steps and three fractions were extracted; phenolic, neutral and sulfonic fractions. The extractions were based on methods described elsewhere (Gabrielsen et al., 2015; Gebbink et al., 2008a, 2008b; McKinney et al., 2006) with modifications and analysed using gas chromatography (GC) and quadrupole mass spectrometry (MS). The results are fully reported elsewhere (Hoydal et al., 2015).

Quality control (QC) samples consisted of bovine (*Bos taurus*) serum spiked with known concentrations of many of the targeted neutral, phenolic and sulfonate analytes. Based on the spiked internal standards, the mean percent recoveries of all analytes from plasma were generally > 80 %. Method limits of quantification (MLOQs) were based on 10 times the signal to noise ratio, and method limits of detection were based on 3 times the signal to noise ratio. MLODs for plasma of PCBs, OCs and FRs generally ranged from 0.02 to 0.5 ng/g wet weight (ww) for halogenated phenolic contaminants. MLOQs for plasma of PCBs, OCs and FRs generally ranged from 0.1 to 3 ng/g ww, and 0.1 to 1.7 ng/g ww for halogenated phenolic contaminants.

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. The absorbance was measured at 540 nm, the maximum absorbance. Of the 175 different POPs analysed for, 27 were detected as contaminants in plasma samples from more than 50 % of the individuals and the concentrations of these POPs were used in the present study. A detailed description of the analysis and the POP results are reported in Hoydal et al. (2015). Because the interactions of chemicals with biological systems occur at the molecular levels, both hormones and POP concentrations are shown as molar concentrations on a ww basis in the tables and figures.

2.3 Data analysis

A principle component analysis (PCA) was performed using the statistical program SIMCA-P+ (Umetrics, version 12.0, 2008) to examine relationships among the variables (i.e. the biological variables length and age, the steroid concentrations and the concentrations of the 27 different POPs

detected in plasma). The PCA also provided information on differences among the sexes and age-groups with respect to steroid hormone and POP concentrations. The variables were centred and scaled before the analysis and the eigenvalues and eigenvectors were computed based on the correlation matrix. The statistical program SPSS (IBM, version 21) was used for further statistical analyses of the data. Most of the steroids were not normally distributed, thus non-parametric methods for statistical analyses were used. Based on the interpretation of the PCA, possible correlations between steroids were tested using Spearman rank analysis (r_S). Bonferroni correction was not applied when comparing associations between multiple variables because of the increased probability of producing false negatives (Moran, 2003). Significant levels were set to $p \leq 0.05$. Samples, in which the steroid hormones were not detected, were not included in the analysis.

3 Results

Except for αE2, all the analysed steroids (i.e., PRO, PRE, AN, DHEA, DHT, TS, E1 and βE2) were detected in more than 50 % of the individuals. Due to the low detection rate αE2 was excluded from the further statistical analyses. Concentrations of the eight remaining steroid hormones were above the detection limit in 15 to all 26 animals (Table 1). Thus, not all hormones were detected in all individuals. DHEA was detected in 12 males but only in 7 females (see Table 1).

The plasma concentrations of the progestagens PRE and PRO were between 0.16-5.69 and 0.14-211.7 pmol/g, respectively, (Figure 1**Fejl! Henvisningskilde ikke fundet.**A and B). It should be noted that both PRE and PRO concentrations were particularly high in three of the 10 adult females (US 41, US 20 and US 18) (4.68-5.69 and 83.3 -211.7 pmol/g in PRE and PRO respectively). In the other adult females, the concentrations of PRE and PRO were below 2.23 and 1.98 pmol/g, respectively.

The concentrations of the androgens TS, AN, DHEA and DHT were between 0.29-21.9, 0.09-3.66, 0.01-1.42, and 0.09-2.48 pmol/g, respectively (Figure 1C-F). However, three of the male pilot whales (US6, US19 and US47) had somewhat higher AN concentrations than the rest (3.66, 2.87, 2.50 pmol/g), and two of these (US19, US6) also had somewhat higher concentrations of DHEA (1.42 and 0.66 pmol/g), TS (21.9 and 15.9 pmol/g) and DHT (2.48 and 0.92 pmol/g). The highest androgen levels were found in one male characterized as a juvenile (US19) and one male characterized as an adult (US6) (Figure 1). These males were sampled in July 2010.

For the estrogens, the concentrations of βE2 and E1 ranged between 0.04-0.45 and 0.05-0.19 pmol/g, respectively (Figure 1G and H). For these hormones, some individuals appeared to have somewhat elevated concentrations compared to the others (Figure 1). The highest concentrations of E1 and βE2 were found in two juvenile males (US 26 and US 25).

Although, the steroid hormones (as well as the POPs) are lipophilic compounds, the concentrations in plasma were not correlated to the lipid content. Only the correlation between E1 and lipid content in females was close to significance (r_s=0.62, p=0.054), but not when including all individuals (r_s=0.011, p=0.958).

3.1 Inter-correlative relationships between steroids

Inter-correlations between the steroid hormone levels were investigated for females and males separately (see Table S2). Correlation analyses showed that in females, the progestagens were significantly positively correlated, as were the estrogens, whereas the androgens were not

significantly inter-correlated. PRO was also positively and significantly correlated with E1 (r_s=0.636, p=0.048) and PRE was negatively and significantly correlated with DHT (r_s=-0.648, p=0.043). βE2 correlated positively with DHEA (r_s=0.786, p=0.036) in females.

In males, all the androgens were positively and significantly inter-correlated, except for AN and DHT. In the males, the estrogens were also positively and significantly correlated, whereas the progestagens were not significantly correlated. However, PRO was positively and significantly correlated with E1 and βE2 (r_s=0.818, p=0.002; r_s=0.827, p=0.002, respectively) and PRE was positively and significantly correlated with βE2 (r_s=0.644, p=0.013) and DHEA (r_s=0.622, p=0.031).

The only identified significant correlations between the biological variables age and length and the plasma steroid concentrations, was a positive correlation between TS and length in males (r_s=0.578, p=0.030).

3.2 Relationships between circulating POP and steroid concentrations

In the PCA, the PC1 explained 59.7% of the overall variation, whereas the PC2 explained 7.9% but was not significant (Figure 2). The score plot (Figure 2A) showed a separation of adult and juvenile females along the PC1 axis. In the loading plot (Figure 2B), all the POPs, except for 4-OH-CB107, were clustered in the opposite direction of age and length along PC1. This indicates negative relationships between POP concentrations and age. Indeed, when comparing the loading and score plots, the individuals with the highest POP concentrations generally were juvenile individuals, whereas POP concentrations were lowest in the adult females. The 4-OH-CB107/4'-OH-CB108 metabolite was placed close to origin indicating that the plasma concentration of this metabolite was not influenced by age and length. The loading plot also showed that the androgens were separated from the estrogens along the PC1. When comparing the loading and score plot, the two adult males were characterized by having high concentrations of the androgens (AN, DHEA, DHT and TS), and low concentrations of the estrogens.

Although the PCA indicated inverse relationships between many of the POPs and some of the steroid hormones, such as PRO, BE2 and PRE, along PC1, the sex and age of the animals had an obvious impact on the results. Testing the relationship by the correlation between the t(1) (PC1) scores and length and age, did however, not show a significant relationship, but close to significant (p=0.06 and p=0.07 for length and age, respectively). The POP concentrations were, however, significantly lower in adult females, compared to the juveniles and adult males (Hoydal et al., 2015). Thus, because both steroid hormone concentrations and POP levels were influenced by sex and age, possible relationships among the POPs and the hormones were examined within each of the sexes. The statistical analyses showed that there were positive correlations between 4-OH-CB107/4'-OH-CB108 and βE2 in males (r_s=0.724, p= 0.003, n=14; Figure 3). Visually the correlation seems to be driven by two outliers, but when removing the outliers the correlation was even more significant (r_s=0.806, p=0.002). No other significant relationships between other POPs and hormones were identified in either of the sexes. However, when investigating only the adult females, significant positive correlations were identified between PRE and CB149 and t-nonachlor, between E1 and CB138, -149, -187 and p,p'-DDE, and between AN and CB187, TS and CB99 and -153 (Figure 3, Table 2). The significant correlations between POPs and PRE were, however, highly influenced by the very high levels in three individuals, and when removing these individuals the correlations still had high r_s values (0.696 and 0.600 for CB149 and t-nonachlor respectively), but they were not significant when

the data from these three individuals were removed. Similarly the TS correlations seemed to be driven by one high value and when removing this value the correlations were not significant (although the correlations were close to significant, p=0.058 and 0.067 for CB99 and -153 respectively). When investigating only juvenile males, βE2 was positively correlated with 4-OH-CB107/4'-OH-CB108 (r_s=0.680, p= 0.015, n=12) as was found for all males. For juvenile females and for adult males the sample sizes were too low to investigate relationships between hormone concentrations and POPs within these groups.

Although no significant correlation was found between lipid content in plasma and the concentrations of steroid hormones or POPs, the correlations between lipid adjusted hormones and POPs were tested. These analyses showed both significant negative and positive correlations between POPs and steroids in females, and positive correlations between βE2, PRE and PRO and POPs (mostly 4-OH-CB107/4'-OH-CB108) in males (see Table S3). PRE and t-nonachlor and E1 and CB-149 were still positively correlated in adult females when adjusting for lipid content, as were βE2 and 4-OH-CB107/4'-OH-CB108 in males.

4 Discussion

In the Faroe Island pilot whales, plasma steroid hormone concentrations and POP concentrations were influenced by the sex and age of the animals. Thus, relationships between the plasma POP concentrations and the steroid concentrations were investigated within each of the sexes, and when a sufficient sample size was available, also within the age classes of the sexes (i.e., adult females and juvenile males).

In the adult female pilot whales, some positive correlations between steroids and POPs were observed (Figure 3, Table 2). This indicates that POPs may have some endocrine disturbing effects on the steroid homeostasis. It should be noted that in an *in vitro* study examining estrogenic and anti-estrogenic effects of POPs in bottlenose dolphins, p,p'-DDE and t-nonachlor were identified as estrogenic compounds, whereas PCB-138 was identified as an anti-estrogenic compound (Yordy et al., 2010). Furthermore, that study showed that there were positive correlations between some groups of POPs in blubber extracts of bottlenose dolphins and E-screen estrogenic activity. The results from the present study appear to be in accordance with the results reported in a study of sledge dog (*Canis familiaris*) bitches, in which concentrations of progestagens, androgens and estrogens in adult female dogs were found to be higher in the POP exposed group than in the control group (Sonne et al., 2014). It was suggested that a reduction in the gonadal (and potentially also adrenal) steroidogenesis, induced by POP exposure may be compensated, or perhaps even slightly overcompensated, by the brain via HPG (and hypothalamic-pituitary-adrenal (HPA) axis) feed-back communicated by gonadotropins. This hypothesis of "compensatory hypogonadism" predicts that elevated blood gonadotropin levels, in particular LH levels, would be expected in exposed individuals, in cases where no significant change, or even minor increases, in steroid levels are observed (Sonne et al., 2014).

In contrast to the results reported in the adult female pilot whales, a study on adult female polar bears (*Ursus maritimus*) showed no relationships between plasma concentrations of PCB-congeners, pesticides (including p,p'-DDE) or PBDEs and neither PRE, E1, AN, TS, nor any of the other steroid

hormones (Gustavson et al., 2015). However, in that particular study significant negative relationships were identified between circulating concentrations of OH-PCBs and plasma concentrations of PRE and AN in the adult female polar bears. In female pilot whales, plasma concentrations of OH-PCBs are very low (Hoydal et al., 2015), presumably due to their low biotransformation capacities combined with relatively low concentrations of the substrates (i.e. PCBs) compared to in juveniles and males (Hoydal et al., submitted). Thus, based on the results from the present study, it is suggested that the plasma concentrations of OH-PCBs in pilot whales are below a hypothesized threshold level for effects on steroids such as PRE and AN.

In contrast to in the adult female pilot whales, in males significant relationships between 4-OH-CB107/4'-OH-CB108 and βE2 were identified. OH-PCBs have been shown to inhibit sulfotransferases, involved in the metabolism of estrogens in humans cells (Kester et al., 2000). The positive correlation between 4-OH-CB107/4'-OH-CB108 and βE2 in males could thus indicate inhibition of the estrogen metabolism in these individuals.

Previously, negative relationships between POPs (DDE, PCB) and TS concentrations have been reported in adult male Dall's porpoises (*Phocoenoides dalli*) (Subramanian et al., 1987). In the present study, only two of the 14 males were adults. Thus, we could not statistically investigate relationships between the POPs and TS in adult males. Nevertheless, no relationships were identified when investigating both adult and juvenile males. Since the results herein indicated, in accordance with previous reports in male pilot whales, that plasma concentrations of androgens, including TS, is higher in adult males than in juvenile males prior to puberty (Desportes et al., 1994, 1993) the present study cannot conclude if plasma androgen concentrations in male pilot whales are influenced by plasma POP concentrations. Nevertheless, it should be noted that in the 12 juvenile males, there were no relationships between neither POPs and TS, nor any of the other androgens.

With respect to the plasma concentrations of the steroid hormones in the present pilot whales, only concentrations of TS in males have previously been analysed in Faroese pilot whales (Desportes et al., 1994, 1993). In those studies TS concentrations were less than 1 ng/ml (approximately 3.5 pmol/g) in immature males younger than 8 years, and increasing sharply during puberty from 2 to 29 ng/ml (approximately 6.9 – 100 pmol/g) which was maintained in mature males, although with great variability (Desportes et al., 1994). This is in accordance with the present study in which TS levels were less than 1.2 ng/g (4.3 pmol/g) in all males except for three individuals which had concentrations between 2.2 and 6.3 ng/g plasma (7.5 – 21.9 pmol/g). Two of these individuals were characterized as mature from the length or age (Desportes et al., 1993) whereas the third one, which had the highest TS concentration, was characterized as immature from the length. This may indicate that this individual was in puberty, although it was smaller than pilot whales characterized as maturing in the previous studies (Desportes et al., 1994, 1993).

The two individuals with elevated βE2 concentrations were juvenile males. Such high concentrations of βE2 were not observed in adult males, likely due to a decrease in aromatase activity in the males by the onset of puberty. This change in the estrogen/androgen ratio has been demonstrated in male polar bears, shifting the ratio by a factor of approximately 60 in favour of androgens when aromatase activity decreases at the unset of puberty (Ciesielski et al., 2017).

Three adult females had somewhat higher PRO concentration compared to the other individuals. Because high levels of PRO indicate pregnancy these three whales could have been pregnant or ovulating (Trego et al., 2013). There were no correlations between the POPs and PRO in the adult females, and it is possible that the reproductive status of the females may have inflicted on the identified relationships between the POPs and the hormone concentrations in the adult females. PRE was also elevated in the same individuals that had high PRO concentrations, and the correlations between PRE and POPs were driven by these three PRE concentrations. Similarly one of these individuals also had high TS concentration. This high TS concentrations also had high influence on the correlations between TS and POPs. Thus, care should be taken when interpreting the relationships between some of the POP compounds and PRE, E1, AN and TS in the adult female pilot whales.

Because interactions between endocrine disrupting chemicals and hormones occur at the molecular level, for instance through binding to hormone receptors, in toxicology it is regarded relevant to relate effects to exposure concentrations using wet weight concentrations of both contaminants and biological response variables, and on a molar basis. Although both steroid hormones and most POPs are lipophilic, it would not be toxicological relevant to present the concentrations on a molar lipid weigh basis. Furthermore, in the present study neither the POPs nor the hormone concentrations correlated with the blood lipid content. Thus, the present study indicates that the blood concentrations of the analysed POPs affects the blood concentrations of some steroid hormones in pilot whales. However, the relationships between the POPs and the steroid hormones reported herein are not evidence of cause-effect relationships. Nevertheless, the findings can be regarded as indicative of the possibilities that POPs may have disruptive effects on steroid hormones in pilot whales, allowing for future studies to test this hypothesis.

5 Conclusion

The analyses of circulating steroid hormones in pilot whales in this study are important since very few analyses of steroid hormones in cetaceans have been reported. Although correlations are not the same as causation, the positive correlations that were found between POPs and steroids could indicate that POPs may have some endocrine disturbing effects on the steroid homeostasis in pilot whales, or in other toothed whales that have high body burdens of POPs, such as killer whales. However, further analyses are needed to determine the extent and implications of such possible endocrine effects of POPs, and the hypothesis of compensatory hypogonadism should be tested. Also, our study shows the significant influence of sex and size/age, and possibly maturation and reproductive status, on steroid profiles. Thus, basic studies on the reproductive endocrinology of pilot whales are needed.

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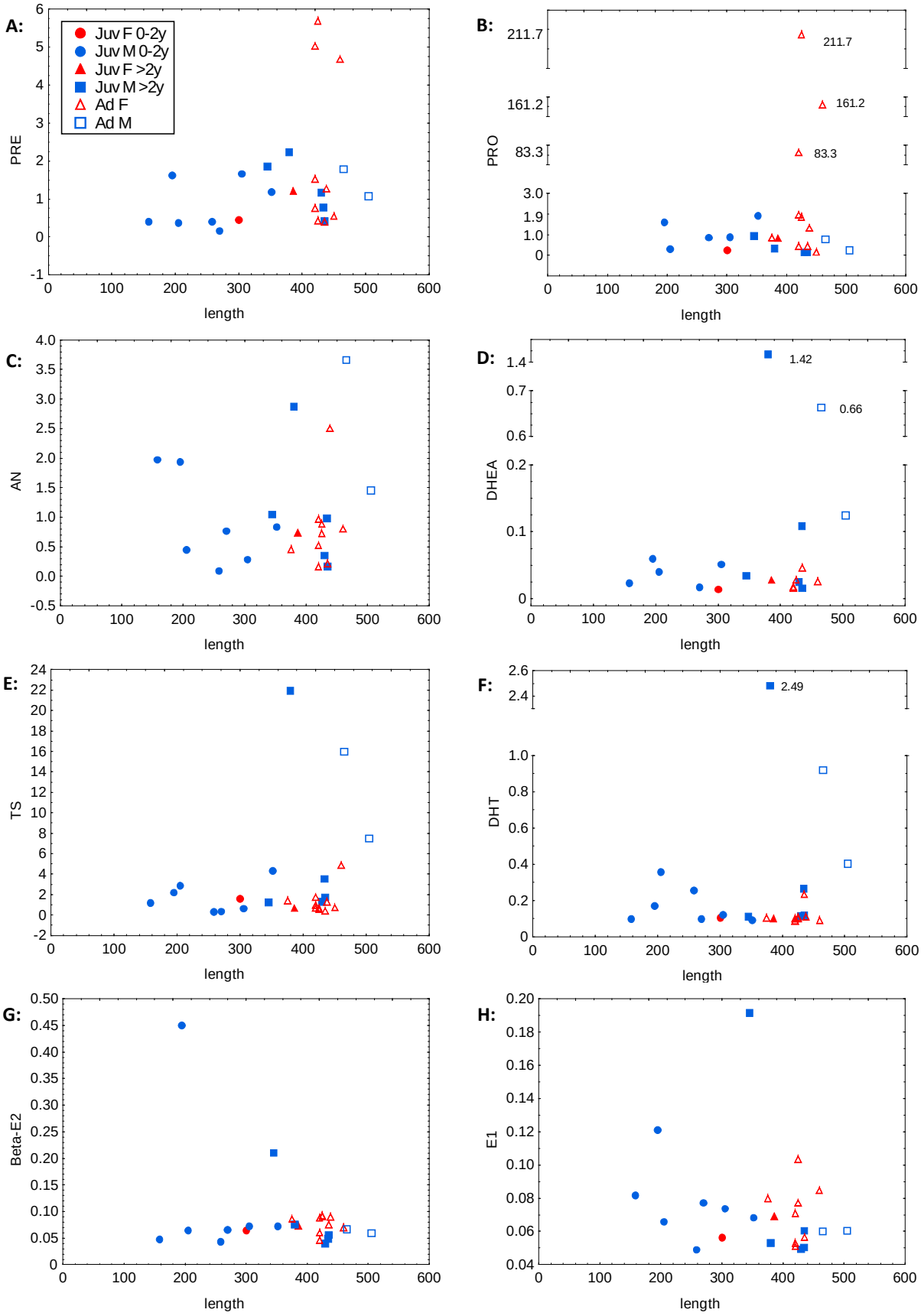
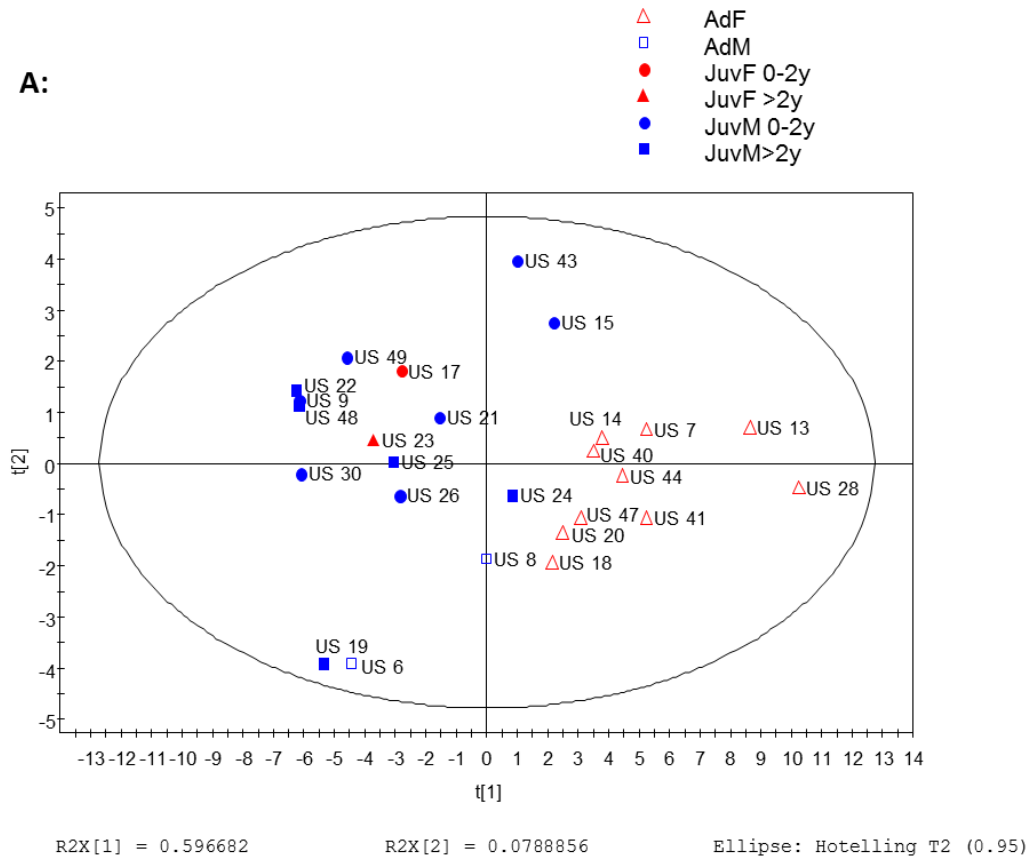


Figure 1 Plasma concentrations (pmol/g ww) of progestagens (A-B), androgens (C-F) and estrogens (G-H) in female and male pilot whales (*Globicephala melas*) in relation to the length (cm) of the animals. See Table 1 for hormone abbreviations.



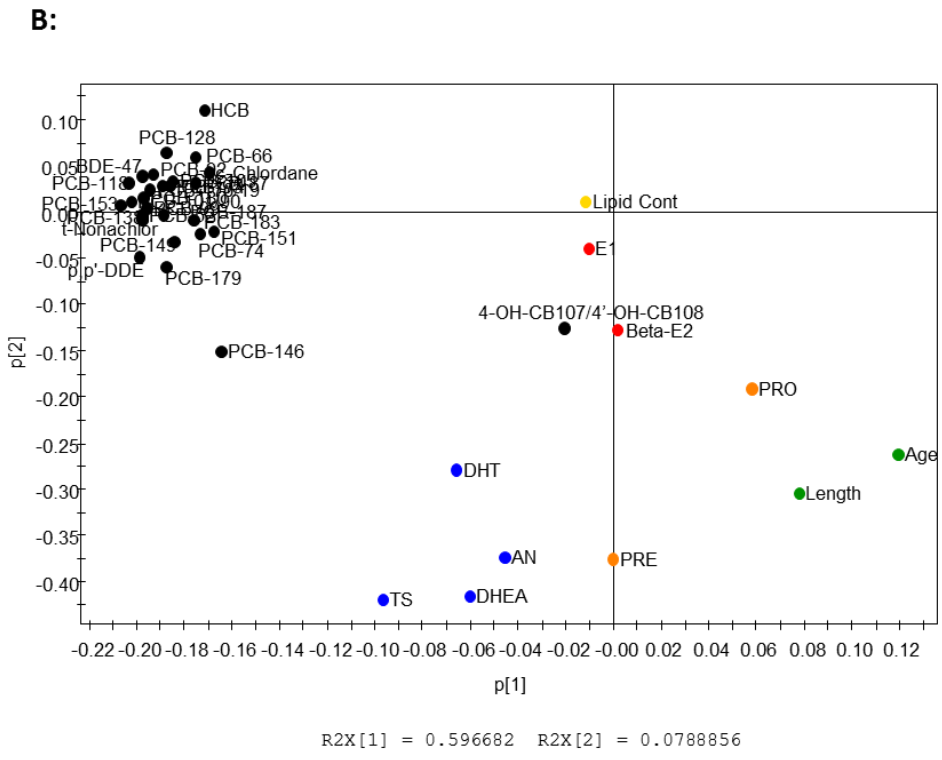


Figure 2 Principal Component Analysis showing the score plot (A) and loading plot (B) of steroid hormones and POP concentrations (log transformed) in plasma of pilot whale (*Globicephala melas*). See Table 1 for hormone abbreviations.

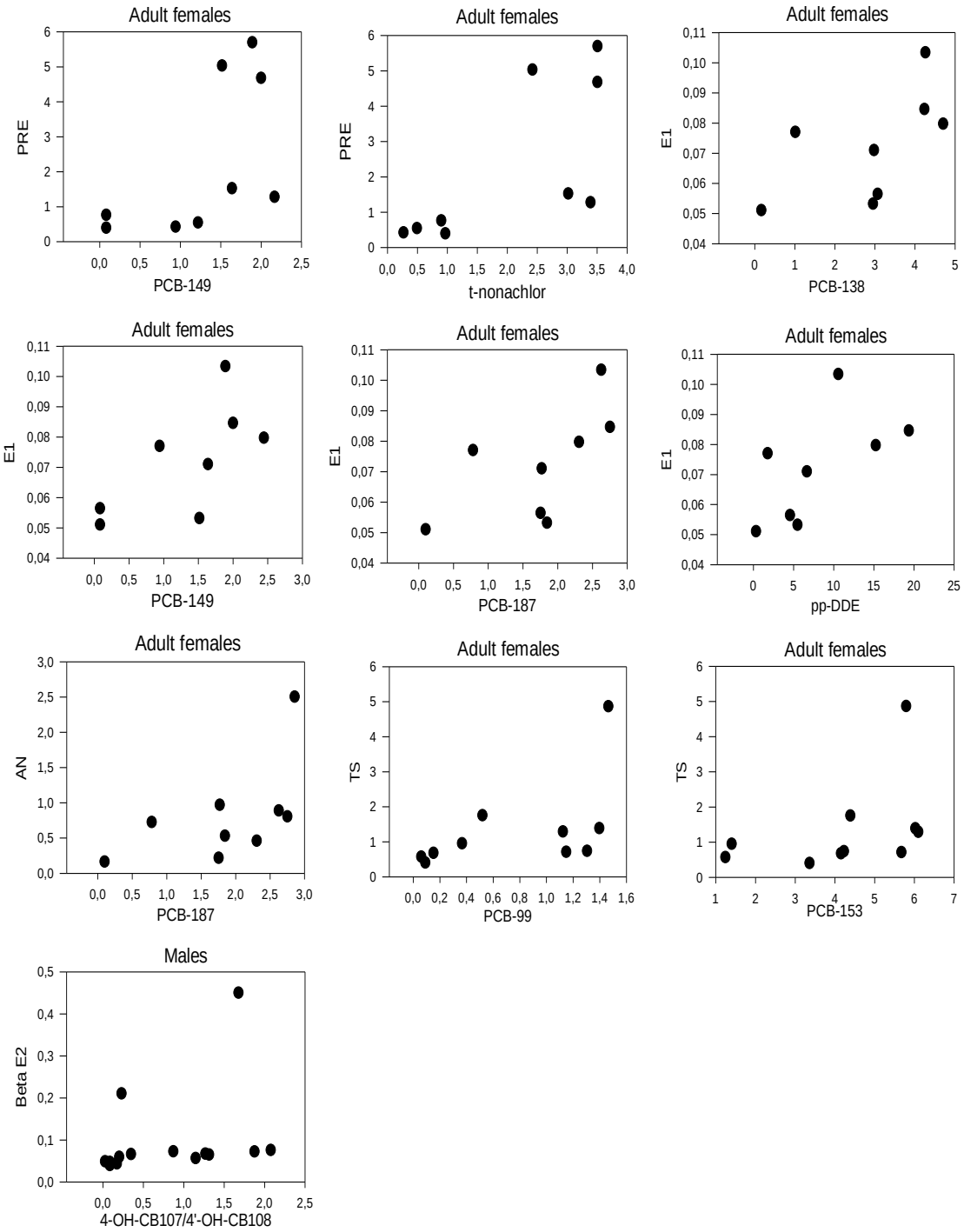


Figure 3 Significant correlations between steroid hormones and single POPs (molar concentrations) in pilot whale (*Globicephala melas*) plasma. See Table 1 for hormone abbreviations.