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Hydroxylated and methoxylated polybrominated diphenyl ethers in long-tailed ducks (*Clangula hyemalis*) and their main food, Baltic blue mussels (*Mytilus trossulus* × *Mytilus edulis*)

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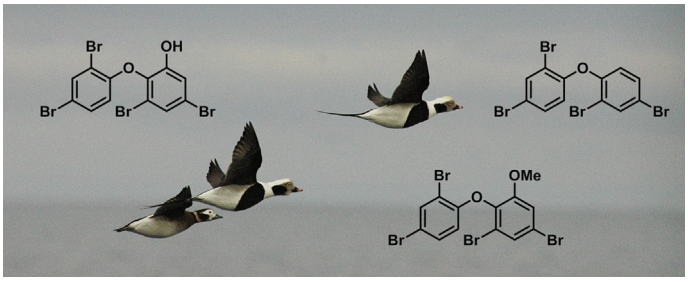
HIGHLIGHTS

- OH-PBDEs, MeO-PBDEs and PBDEs in blue mussel and long-tailed duck were measured.
- OH- and MeO-PBDE levels were higher in birds sampled the Baltic than in the Arctic.
- OH- and MeO-PBDE levels were higher in mussels in the Baltic Sea than in the Arctic.
- Levels of OH-PBDEs in Baltic blue mussels were significantly higher in summer.
- The PBDE profile in benthic feeding sea ducks differs from other marine birds.

GRAPHICAL ABSTRACT



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ABSTRACT

Long-tailed ducks (*Clangula hyemalis*) that breed in northern Europe and western Siberia and commonly winter in the Baltic Sea, are threatened by a significant population decrease. The ducks are, by primarily feeding on Baltic blue mussels (*Mytilus trossulus* × *Mytilus edulis*) while wintering in the Baltic Sea, potentially subjected to high levels of toxic hydroxylated polybrominated diphenyl ethers (OH-PBDEs). To assess long-tailed ducks exposure to polybrominated phenols (PBPs), polybrominated anisoles (PBAs), hydroxylated polybrominated diphenyl ethers (OH-PBDEs), their methylated counterparts (MeO-PBDEs) and polybrominated diphenyl ethers (PBDEs), livers of ten long-tailed ducks wintering in the Baltic Sea were analysed. Pattern and levels of analytes in long-tailed ducks (liver) and blue mussels sampled in March and May at nine sites in the Baltic Sea were compared. The geometric mean concentration (ng/g l.w.) in livers of long-tailed ducks and Baltic blue mussels were: Σ_2PBPs: 0.57 and 48; Σ_2PBAs: 0.83 and 11; Σ_7OH-PBDEs: 6.1 and 45; Σ_7MeO-PBDEs: 3.8 and 69; Σ_7PBDEs: 8.0 and 7.2, respectively. Based on an estimated daily intake of 450 g fresh blue mussel meat, long-tailed ducks daily dietary intake of brominated substances while foraging in the Baltic Sea in March–May was estimated to; 390 ng Σ_2PBPs, 90 ng

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Σ_2PBAs, 370 ng Σ_7OH-PBDEs, 590 ng Σ_7MeO-PBDEs and 59 ng Σ_7PBDEs. The low levels of PBPs, PBAs, OH-PBDEs and MeO-PBDEs in the long-tailed duck livers compared to blue mussel, despite a continuous daily intake, suggest that these compounds are poorly retained in long-tailed ducks.

1. Introduction

Both breeding and wintering sea duck populations, i.e. long-tailed duck (*Clangula hyemalis*), common eider (*Somateria mollissima*), velvet scoter (*Melanitta fusca*) and common scoter (*Melanitta nigra*), have decreased dramatically in the Baltic Sea over the past 20 years (Skov et al., 2011; Kilpi et al., 2015). The number of long-tailed ducks wintering in the Baltic Sea has decreased from approximately 4.3 million birds in 1992–93 to about 1.5 million birds in 2007–09. This represents a population decline of approx. 65% and today the long-tailed duck is classified as vulnerable species on the IUCN global red list of threatened species (BirdLife International, 2014). There may be several reasons for the population declines of sea ducks in the Baltic Sea, such as changed predation pressures and changes in the quantity and quality of food (Skov et al., 2011; Ottvall, 2012). Furthermore, thiamine deficiency has been observed in common eiders breeding in the Baltic Sea (Balk et al., 2009).

The Baltic blue mussel (*Mytilus trossulus* × *Mytilus edulis*) is the dominant benthic invertebrate on hard bottoms and a very important food source for common eiders and long-tailed ducks (Stempniewicz, 1995; Kube and Skov, 1996; Žydelis and Ruskyte, 2005; Skov et al., 2011). Blue mussels in the Baltic Proper have previously been reported to contain a large number of anthropogenic and naturally produced brominated substances e.g. polybrominated phenols (PBPs), polybrominated anisoles (PBAs), hydroxylated polybrominated diphenyl ethers (OH-PBDEs), methoxylated polybrominated diphenyl ethers (MeO-PBDEs) and polybrominated diphenyl ethers (PBDEs) (Malmvarn et al., 2005; Loefstrand et al., 2010; Loefstrand, 2011). Several other halogenated natural organic compounds in blue mussels have also been reported from the southern Baltic Sea (Hauler et al., 2014). Hence, the sea ducks may, by primarily feeding on Baltic blue mussels while wintering in the Baltic, potentially be subjected to high levels of brominated organic compounds.

PBDEs have been reported to exert both endocrine and neurotoxic effect (Talsness, 2008; Dingemans et al., 2011; European Food Safety Authority, 2011). However, there is growing concern regarding OH-PBDEs as these compounds seem to be more biological active than PBDEs (Legler, 2008; Dingemans et al., 2011). For example, OH-PBDEs have strong affinity to the thyroid hormone receptor (Marsh et al., 1998) and the thyroid hormone transporting proteins; transthyretin (TTR) and thyroid binding globulin (TBG) (Meerts et al., 2000; Marchesini et al., 2008). Several OH-PBDEs identified in Baltic marine wildlife were also recently found to be potent disrupters of oxidative phosphorylation (OXPHOS), acting via protonophoric uncoupling and/or inhibition of the electron transport chain *in vitro* (Legradi et al., 2014). As OXPHOS is the main metabolic pathway used by eukaryotic cells to produce energy i.e. adenosine triphosphate (ATP) under aerobic conditions, low dose exposure over an extended period of time to compounds that disturb OXPHOS can result in altered metabolism and weight loss (Wallace and Starkov, 2000; Harper et al., 2001). In blue mussels and fresh water clam *(Pisidium amnicum)*, altered metabolic activity has been shown after exposure to pentachlorophenol (PCP), a well-known uncoupler of OXPHOS (Wang and Widdows, 1993; Heinonen et al., 2003) Hence, OH-PBDEs may have potential to affect the nutritional value of blue mussels as food for sea ducks via disturbed OXPHOS.

In the Baltic Sea, the dominating cyanobacteria species (*Aphanizomenon flos-aquae* and *Nodularia spumigena)* and filamentous red, brown and green macroalgae species e.g. *Ceramium tenuicorne, Pilayella littoralis* and *Cladophora glomerata* are producers of OH-PBDEs (Malmvarn et al., 2005, 2008; Loefstrand, 2011). In marine bacteria and algae, OH-PBDEs have been shown to be formed via dimerization of naturally biosynthesized PBPs (Agarwal et al., 2014; Lin et al., 2014). PBPs (e.g. 2,4-diBP and 2,4,6-triBP) are also industrially produced and used as reactive intermediates in production of brominated resins and polymers and may enter the marine environment via waste products (WHO, 2005). Further, OH-PBDEs present in marine biota may originate from metabolic transformation of bioaccumulated PBDEs (Hakk and Letcher, 2003), which have become widespread environmental contaminants due to their past extensive use as additive flame retardants (de Wit et al., 2010; Law et al., 2014).

Seasonal variations in the concentration of OH-PBDEs and MeO-PBDEs in macroalgae and blue mussels indicate that biogenic production is an important source in the Baltic Proper. Studies on blue mussels in the Baltic Sea have shown that the concentrations of OH-PBDEs and MeO-PBDEs increase during spring and can reach high concentrations in summer, (Loefstrand et al., 2011). Similar seasonal variation of OH-PBDEs and MeO-PBDEs has also been observed in macroalgae from the Baltic Sea (Loefstrand, 2011). In mid-summer, concentrations as high as 3500 ng/g l.w. and 420 ng/g l.w. of ΣOH-PBDEs and ΣMeO-PBDEs, respectively, have been reported in blue mussels collected at a coastal area in the northern Baltic Proper (Loefstrand et al., 2011).

Given the toxicity of OH-PBDEs and of other brominated substances it is of concern that high levels have been found in Baltic blue mussels, an important food source for several sea duck species in the Baltic Sea. The aim of this study was to analyse the concentrations, profiles and retention of anthropogenic and naturally produced brominated substances (i.e. PBPs, PBAs, OH-PBDEs, MeO-PBDEs and PBDEs) in livers of long-tailed ducks wintering in the Baltic Sea. To assess the birds' dietary intake of brominated substances before breeding, blue mussels collected from sites used by long-tailed ducks for foraging in spring (March–May) were analysed.

2. Materials and methods

2.1. Study species

The long-tailed duck is a small sea duck found in the northern hemisphere. The west Siberia/north Europe population of long-tailed duck breeds predominantly in fresh water habitats in western Siberia and northern Europe. During the non-breeding season long-tailed ducks favours brackish and marine areas and the vast majority of the west Siberian/north European population of long-tailed duck stay in the Baltic Sea for the winter (Skov et al., 2011). The birds migrate to the wintering sites in the Baltic Sea in October. In winter and spring, long-tailed ducks forage on mussel banks

located offshore and in more shallow coastal areas (Skov et al., 2011) where their diet consist mainly of bivalves, supplemented with other benthic organisms, crustaceans, small fish and fish eggs (Stempniewicz, 1995; Kube and Skov, 1996; Žydelis and Ruskyte, 2005; Skov et al., 2011). Sea ducks swallow mussels whole, but since it is only the soft body that is of nutritional value they must consume large quantities of mussels each day to maintain their energy balance. The daily intake of blue mussel meat can be estimated from measurements of the ducks basic metabolic rates and the energy content in the diet as described in the Supporting information to this study. In April, large flocks of long-tailed ducks gather in the northern Baltic Proper, Gulf of Finland and Gulf of Riga before they in mid-May commence their northward migration to their Arctic breeding grounds (Skov et al., 2011).

The Baltic blue mussel (*Mytilus trossulus* × *Mytilus edulis*) is a suspension feeding bivalve that successfully has adapted to the brackish water environment in the Baltic Sea. The Baltic blue mussel differs from blue mussels (*Mytilus edulis*) in the North Sea, i.e. genetically (Johannesson et al., 1990), physiologically (Tedengren et al., 1990) and morphologically (Kautsky et al., 1990). The small size, i.e. usually smaller than 35 mm in length, and slow growth of Baltic blue mussels are thought to be related to the low salinity in the Baltic Sea (Kautsky et al., 1990). The salinity gradient also restricts the distribution of blue mussels in the Baltic Sea, and their abundance decreases rapidly north of the Åland archipelago. However, in the Baltic Proper blue mussels are commonly found on hard bottoms along the coast and at offshore banks, where they have been estimated to compose approximately 90% of the animal biomass at depths down to 25 m (Kautsky, 1981).

2.2. Samples

Ten long-tailed ducks were collected at two sites in the Baltic Sea (Fig. 1). Eight individuals were collected in February 2000 (site no. 3) and two individuals in May 2009 (site no. 8). Blue mussels from the Baltic Proper were collected in March 2012 (site no. 1–7) and May 2011 (site no. 5–6, 8–9) (Fig. 1). All sampling sites are known to be used by long-tailed ducks for foraging and include both coastal areas as well as mussel banks in open waters. Blue mussels were also collected in June 2011 (site no. 10). The blue mussels and livers of long-tailed ducks were stored frozen (−20 °C) until analysis. More information regarding samples and sampling is presented in Supporting Information.

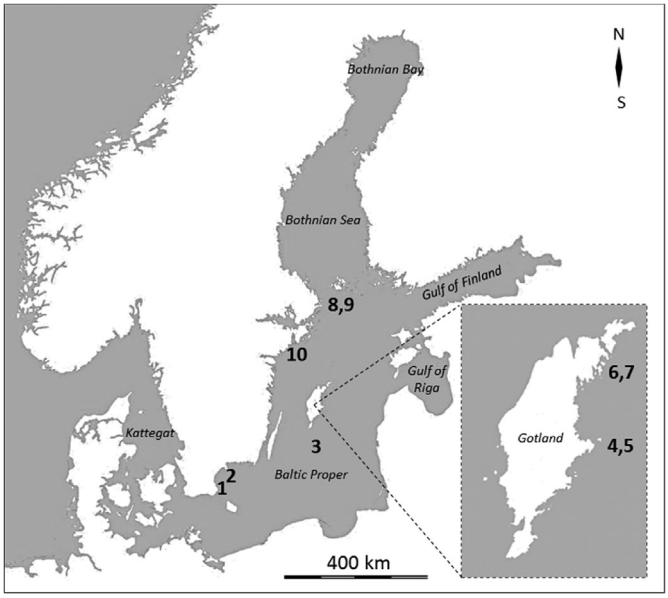

Fig. 1. The Baltic Sea, with the island Gotland magnified. The sampling sites of Baltic blue mussels are indicated by numbers 1–10. Coordinates and sampling depths are presented in Table S1, Supporting Information.

2.3. Chemicals

Details regarding chemicals and standards used are given in Supporting Information.

2.4. Extraction, derivatization and clean up

Blue mussels (1–2 cm in length) from each sampling site were thawed and the soft body was removed from the shell. Blue mussels collected from the same site and date were pooled and the soft body tissue was homogenised and divided into aliquots of 10 g. Livers from collected long-tailed ducks were homogenised individually and divided into aliquots of 5 g.

The extraction of brominated substances was performed according to the liquid–liquid extraction method developed by Jensen et al. (2009), with the minor modification that *iso*-hexane (*iso*-hx) was used instead of *normal*-hexane (*n*-hx) (Jensen et al., 2009). Prior to extraction, surrogate standards (4′-OH-BDE121 and 4′-MeO-BDE121) were added to each sample. The extraction procedure and the amount of surrogate standards used are described in detail in Supporting Information.

After extraction, the lipid content was determined gravimetrically. Due to high lipid content, the liver samples from long-tailed duck were cleaned up by gel permeation chromatography (GPC) prior to partitioning with potassium hydroxide as described in Supporting Information.

Neutral and phenolic compounds were separated into two fractions by partitioning each sample with potassium hydroxide (0.5 M in 50% ethanol) (Jensen et al., 2009). The procedure is described in detail in Supporting Information. The isolated phenolic compounds were methylated with diazomethane in excess, for 3 h at room temperature and in darkness as described by Hovander and coworkers (Hovander et al., 2000).

Residues of lipids in the mussel and liver samples were removed by concentrated sulfuric acid (98%) treatment, as described in the Supporting Information. The neutral and phenolic fractions of blue mussels were further cleaned up on silica:sulfuric acid gel columns as described in supporting information. Volumetric standard (BDE-139 and BDE-138) was added to each blue mussel and liver sample, respectively, prior to instrumental analysis in the same amount as the added surrogate standard.

2.5. Instrumental analysis

The samples were analysed by gas chromatography/mass spectrometry (GC/MS) using electron capture negative ionization (ECNI) and selected ion monitoring (SIM), scanning bromine ions (m/z 79 and 81). Identification and quantification were performed using authentic reference standards. Detailed description of the instrumental settings is given in Supporting Information.

2.6. Quality assurance/quality control

The blue mussel samples mean recoveries and relative standard deviation (RSD) of the surrogate standards; 4′-OH-BDE121 and 4′-MeO-BDE121, were 85% (RSD 20%) and 72% (RSD 15%), respectively. The long-tailed duck liver sample mean recoveries and RSD of the surrogate standards; 4′-OH-BDE121 and 4′-MeO-BDE121, were 72% (RSD 13%) and 69% (RSD 8%), respectively. The limit of detection was set to three times the background noise (signal to noise ratio = 3). A procedural solvent blank was prepared in parallel with

Table 1
Concentrations (ng/g l.w.) of PBPs, PBAs, OH-PBDEs, MeO-PBDEs and PBDEs in livers of long-tailed ducks and blue mussels in the Baltic Sea. For long-tailed ducks (n = 10), the concentrations are presented as geometric mean and range (min–max), the number of samples above limit of quantification is given within brackets when applicable.

Species	Long-tailed duck	Blue mussel	Blue mussel	Blue mussel	Blue mussel	Blue mussel	Blue mussel	Blue mussel	Blue mussel	Blue mussel	Blue mussel	Blue mussel	Blue mussel
Sampling date		2012-03-06	2012-03-06	2012-03-07	2012-03-16	2012-03-16	2012-03-01	2012-03-01	2011-05-16	2011-05-16	2011-05-08	2011-05-08	2011-06-28
n	10	1 pool	1 pool	1 pool	1 pool	1 pool	1 pool	1 pool	1 pool	1 pool	1 pool	1 pool	1 pool
Sampling site	8 and 3	1	2	3	4	5	6	7	5	6	8	9	10
Lipid content (%)	5.8	1.2	1.1	1.5	1.6	1.4	1.4	1.2	3.2	3.9	2.6	3.5	3.4
Range	*3.8–7.2*												
2,4-diBP[a]	0.0054	10	14	2.7	1.0	2.2	2.9	4.9	1.0	0.56	1.3	4.0	44
Range	*n.d – 0.48 (5)*												
2,4,6-triBP[b]	<LOQ	140	240	48	24	35	58	53	17	22	30	31	52
Range	*<LOQ – 2.6 (6)*												
Σ PBPs	**0.57**	**150**	**250**	**51**	**25**	**37**	**61**	**58**	**18**	**23**	**31**	**35**	**96**
Range	*0.22–3.1*												
2′-OH-BDE68[c]	<LOQ	5.4	7.3	3.4	3.8	4.3	5.2	4.6	1.2	1.0	2.0	2.6	110
Range	*<LOQ – 0.27 (1)*												
6-OH-BDE47	4.2	37	48	25	26	27	31	30	9.6	8.9	13	18	340
Range	*2.3–6.5 (10)*												
6-OH-BDE90[d]	<LOQ	2.9	3.9	2.7	3.3	3.5	3.8	3.4	0.88	1.0	2.9	2.9	130
Range	*<LOQ – 0.74 (2)*												
6-OH-BDE99	0.68	5.5	7.2	4.7	6.4	7.2	8.4	7.0	1.4	1.6	5.8	6.0	320
Range	*0.10–2.3 (10)*												
2-OH-BDE123[e]	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	27
Range													
6-OH-BDE85[f]	<LOQ	13	28	7.2	13	15	20	16	3.4	4.8	3.5	3.8	440
Range													
6-OH-BDE137[g]	<LOQ	2.1	3.4	1.1	3.5	3.9	6.0	3.8	0.53	0.77	1.6	1.6	180
Range	*n.d – 0.015 (1)*												
Σ OH-PBDEs	**6.1**	**65**	**98**	**44**	**55**	**61**	**75**	**64**	**17**	**18**	**29**	**35**	**1500**
Range	*3.4–8.0*												

(*continued on next page*)

Table 1 (*continued*)

Species	Long-tailed duck	Blue mussel	Blue mussel	Blue mussel	Blue mussel	Blue mussel	Blue mussel	Blue mussel	Blue mussel	Blue mussel	Blue mussel	Blue mussel	Blue mussel
Sampling date		2012-03-06	2012-03-06	2012-03-07	2012-03-16	2012-03-16	2012-03-01	2012-03-01	2011-05-16	2011-05-16	2011-05-08	2011-05-08	2011-06-28
n	10	1 pool	1 pool	1 pool	1 pool	1 pool	1 pool	1 pool	1 pool	1 pool	1 pool	1 pool	1 pool
Sampling site	8 and 3	1	2	3	4	5	6	7	5	6	8	9	10
Lipid content (%)	5.8	1.2	1.1	1.5	1.6	1.4	1.4	1.2	3.2	3.9	2.6	3.5	3.4
Range	*3.8–7.2*												
2,4-diBA[h]	<LOQ	2.6	1.7	0.40	0.40	0.53	1.0	0.36	0.69	0.33	0.40	1.9	5.3
Range	*n.d – 0.15 (5)*												
2,4,6-triBA[i]	<LOQ	17	21	5.4	5.3	8.6	8.0	5.2	15	13	8.0	23	66
Range													
Σ PBAs	**0.83**	**19**	**23**	**5.8**	**5.7**	**9.1**	**9.0**	**5.6**	**16**	**13**	**8.4**	**25**	**71**
Range	*0.63–1.2*												
2′-MeO-BDE68[j]	0.19	10	15	14	15	13	16	12	4.6	4.4	17	16	26
Range	*<LOQ – 0.34 (9)*												
6-MeO-BDE47	1.6	31	43	45	44	41	50	38	17	16	75	69	62
Range	*0.66–2.3 (10)*												
6-MeO-BDE90	0.29	2.4	5.1	2.8	6.1	5.2	6.8	4.7	1.2	1.4	6.3	6.6	10
Range	*0.18–0.64 (10)*												
6-MeO-BDE99	0.96	3.9	9.5	2.8	11	9.4	13	8.6	2.3	2.6	13	14	21
Range	*0.63–2.8 (10)*												
2-MeO-BDE123[k]	<LOQ	0.35	1.0	0.17	0.48	0.41	0.47	0.4	0.087	0.10	0.45	0.47	1.6
Range	*<LOQ – 0.041 (1)*												
6-MeO-BDE85[l]	<LOQ	3.1	8.1	1.9	8.3	6.5	8.3	5.3	1.1	1.2	5.1	5.0	17
Range													
6-MeO-BDE137	0.30	1.7	4.9	1.2	5.5	5.1	6.5	4.3	0.66	0.7	3.1	3.0	82
Range	*0.079 – 0.75 (10)*												
Σ MeO-PBDEs	**3.8**	**52**	**86**	**67**	**90**	**81**	**100**	**74**	**27**	**27**	**120**	**110**	**220**
Range	*2.3–6.9*												
BDE-28[m]	<LOQ	<LOQ	0.35	<LOQ	<LOQ	0.37	0.1	<LOQ	<LOQ	0.073	0.16	<LOQ	n.d.
Range	*<LOQ – 0.18 (1)*												
BDE-47[n]	1.6	3.8	5.9	2.9	3.0	3.1	3.7	3.9	1.3	1.2	1.7	1.4	1.7
Range	*<LOQ – 2.9 (7)*												
BDE-100[o]	1.6	1.2	2.0	<LOQ	0.97	1.0	1.3	1.3	0.34	0.36	n.d.	n.d.	1.0
Range	*0.44–3.6 (10)*												
BDE-99[p]	2.0	4.3	8.1	2.8	3.6	3.9	5.4	5.2	0.94	0.98	2.4	2.2	11
Range	*<LOQ – 1.9 (8)*												
BDE-154	1.2	0.46	0.69	0.53	0.43	0.50	0.52	0.59	0.19	0.18	0.18	0.14	0.24
Range	*0.48–1.9 (10)*												
BDE-153	1.0	0.38	0.74	0.30	0.31	0.33	0.42	0.51	0.12	0.11	0.16	0.12	n.d.
Range	*0.30–2.9 (10)*												
BDE-183	0.36	0.23	0.41	0.32	0.28	0.36	0.24	0.34	0.15	0.13	0.12	0.087	n.d.
Range	*0.070–0.67 (10)*												
Σ PBDEs	**8.0**	**10**	**18**	**6.9**	**8.7**	**10**	**12**	**12**	**3.1**	**3.0**	**4.8**	**3.9**	**14**
Range	*3.1–15*												

n.d. = not detected, <LOQ = below limit of quantification. [a]LOD (long-tailed duck) = 0.00047 ng, [b]LOQ (long-tailed duck) = 0.16 ng, [c]LOQ (long-tailed duck) = 0.24 ng, [d]LOQ (long-tailed duck) = 0.15 ng, [e]LOD (long-tailed duck) = 0.11 ng, LOD (blue mussel) = 0.0012 ng, [f]LOQ (long-tailed duck) = 0.21 ng. [g]LOD (long-tailed duck) = 0.00067 ng, gLOQ (long-tailed duck) = 0.0034 ng, [h]LOD (long-tailed duck) = 0.0025 ng, LOQ (long-tailed duck) = 0.0075 ng, [i]LOQ (long-tailed duck) = 0.45 ng, [j]LOQ (long-tailed duck) = 0.036 ng, [k]LOQ (long-tailed duck) = 0.011 ng, [l]LOQ (long-tailed duck) = 0.18 ng, [m]LOQ (long-tailed duck) = 0.050 ng, LOQ (blue mussel) = 0.024 ng, [n]LOQ (long-tailed duck) = 0.51 ng, [o]LOD (blue mussel) = 0.0057 ng, LOQ (blue mussel) = 0.028 ng, [p]LOQ (long-tailed duck) = 0.25 ng.

each batch samples. The limit of quantification (LOQ) was set to three times the limit of detection (LOD) or five times the mean concentration in the procedural solvent blanks. The procedural solvent blanks (n = 2) treated together with the liver samples were found to contain small amounts of OH-PBDEs, MeO-PBDEs and PBDEs. Background levels in solvent blanks were subtracted from the samples when present. LOD and LOQ values are presented as footnotes in Table 1.

2.7. Statistical analysis

When calculating means and sums, concentrations below LOQ and LOD were assigned a value equal to half the LOQ or LOD, respectively. Measurements were log-transformed prior to statistical testing, which included un-paired student t-test and pearson product moment correlation. The significance level α of 0.05 was Bonferroni adjusted and set to 0.005 to reduce the risk of making type I error when doing repeated statistical t-tests.

3. Results

3.1. Blue mussels

The concentrations (ng/g l.w.) of PBPs, PBAs, OH-PBDEs, MeO-PBDEs and PBDEs in blue mussels are presented in Table 1. The lipid content (arithmetic mean) in blue mussels collected in March (1.3%, n = 7 sites) was lower than in mussels collected in May (3.3%, n = 4 sites) and June (3.4%, n = 1 site). Geometric mean (GM) concentrations of Σ_2PBPs, Σ_2PBAs, Σ_7OH-PBDEs, Σ_7MeO-PBDEs and Σ_7PBDEs in the pools of Baltic blue mussels collected in March and May (n = 11), are given in Table 2 and Fig. S1. Concentrations of Σ_2PBPs, Σ_2PBAs, Σ_7OH-PBDEs, Σ_7MeO-PBDEs and Σ_7PBDEs measured in one blue mussel pool collected in June (site no.10, Fig. 1) are also presented in Table 2.

3.2. Long-tailed duck

The concentrations (ng/g l.w.) of PBPs, PBAs, OH-PBDEs, MeO-PBDEs and PBDEs in ten long-tailed duck livers are presented in Table 1. As the livers of long-tailed ducks collected in year 2000 (n = 8) and 2009 (n = 2) showed similar concentrations, these data are presented together. The lipid content in duck livers ranged from 3.8 to 7.2%. Geometric mean concentrations of Σ_2PBPs, Σ_2PBAs, Σ_7OH-PBDEs, Σ_7MeO-PBDEs and Σ_7PBDEs in the long-tailed duck livers (n = 10) are given in Table 2 and Fig. S1.

4. Discussion

This study shows that long-tailed ducks, while foraging on blue mussels in the Baltic Proper are exposed to several compound classes of brominated organic compounds via their mussel diet (Table 1).

Data regarding other mussel feeding sea ducks exposure to brominated substances are in fact scarce. Two studies from the northern hemisphere have previously reported levels of PBDEs and MeO-PBDEs in livers from mussel feeding sea ducks i.e. in common eider and white-winged scoter (*Melanitta deglandi*) (Kelly et al., 2008a, 2008b). Compared to these studies, the mean concentration of Σ_7MeO-PBDEs (3.8 ng/g l.w.) in livers of long-tailed duck sampled in the Baltic Sea was approximately 2–3 times higher than in livers from white-winged scoter (Σ_{10}MeO-PBDEs: 2.1 ng/g l.w.) and common eider sampled in the Canadian arctic (Σ_{10}MeO-PBDEs: 1.3 ng/g l.w) (Kelly et al., 2008a). By contrast, the mean concentration of Σ_7PBDEs (8.0 ng/g l.w.) in livers of long-tailed ducks was lower than in livers of white-winged scoters (Σ_{15}PBDEs: 71 ng/g l.w.) and eiders (Σ_{15}PBDEs: 20 ng/g l.w.) (Kelly et al., 2008b). A wide range of anthropogenic contaminants have also been reported in common eider eggs from two coastal areas in Norway (Huber et al., 2015). The mean concentrations of Σ_{17}PBDEs in eggs at each site were 0.84 and 1.1 ng/g w.w, respectively. In long-tailed duck livers, OH-PBDEs (Σ_7OH-PBDEs: 6.1 ng/g l.w) were found at similar concentrations as PBDEs, whereas OH-PBDEs were not detected in sea ducks sampled in the Canadian arctic (Kelly et al., 2008a). OH-PBDEs were not analysed in the study of eider eggs but both simple bromophenols (e.g. 2,4-diBP and 2,4,6-triBP) and 2,4,6-triBA were found (Huber et al., 2015).

The concentration of naturally produced brominated compounds (i.e. OH-PBDEs and MeO-PBDEs) were higher in Baltic blue mussels than reported in Arctic blue mussels (*M. edulis*). On average, the mean concentration of Σ_7OH-PBDEs and Σ_7MeO-PBDEs in Baltic blue mussels collected during March and May were 45 and 69 ng/g l.w., respectively, which is approximately 5 times higher levels for MeO-PBDEs than in Arctic blue mussels, in which OH-PBDEs were not detected (Kelly et al., 2008a). Interestingly, the levels of anthropogenic PBDEs in Arctic blue mussel (Σ_{15}PBDEs: 5.4 ng/g l.w.) (Kelly et al., 2008b) and Baltic blue mussels (Σ_7PBDEs: 7.2 ng/g l.w.) were found to be more similar. The concentrations of Σ_7OH-PBDEs and Σ_7MeO-PBDEs in the Baltic mussels during March and May were found to be significantly correlated (n = 11, r = 0.61, p = 0.048) which was expected given their natural origin. The concentrations of Σ_7MeO-PBDEs and Σ_7PBDEs were not significantly correlated. This can be expected as PBDEs have an anthropogenic origin. Surprisingly, the levels of Σ_7OH-PBDEs and Σ_7PBDEs (n = 11, r = 0.97, p < 0.0001) and Σ_2PBPs and Σ_7PBDEs (n = 11, r = 0.77, p = 0.005) were found to be strongly correlated in this study, for which we have no explanation. If the OH-PBDEs were formed from metabolism of PBDEs the much higher levels of OH-PBDEs compared to PBDEs found in

Table 2
Geometric mean (GM) concentrations and range (min–max) of ΣPBPs, ΣPBAs, ΣOH-PBDEs, ΣMeO-PBDEs and ΣPBDEs in livers of long-tailed ducks (n = 10) and Baltic blue mussels collected during March and May (site no. 1–9, n = 11), presented on both lipid weight and fresh weight basis. Concentrations in one pool of blue mussel collected in June (site no.10) are also presented.

	Long-tailed duck (ng/g l.w.)		Blue mussels (March–May) (ng/g l.w.)		Blue mussels (June) (ng/g l.w.)	Long-tailed duck (ng/g f.w.)		Blue mussels (March–May) (ng/g f.w.)		Blue mussels (June) (ng/g f.w.)
n samples	10		9 (pools)		1 (pool)	10		9 (pools)		1 (pool)
Compound class	GM	Range	GM	Range		GM	Range	GM	Range	
Σ_2PBPs	0.57	0.22–3.1	48	18–252	96	0.032	0.016–0.18	0.87	0.39–2.7	3.3
Σ_2PBAs	0.83	0.63–1.2	11	5.6–25	71	0.047	0.045–0.054	0.20	0.067–0.086	2.4
Σ_7OH-PBDEs	6.1	3.4–8.0	45	17–98	1500	0.34	0.19–0.47	0.82	0.54–1.2	53
Σ_7MeO-PBDEs	3.8	2.3–6.9	69	27–120	220	0.21	0.086–0.38	1.3	0.63–4.0	7.5
Σ_7PBDEs	8.0	3.1–15	7.2	3.0–18	14	0.45	0.13–0.74	0.13	0.10–0.20	0.49

Σ_2PBP/As: 2,4-diBP/A, 2,4,6-triBP/A.
Σ_7OH/MeO-PBDEs: 2′-OH/MeO-BDE68, 6-OH/MeO-BDE47, 6-OH/MeO-BDE90, 6-OH/MeO-BDE99, 2-OH/MeO-BDE123, 6-OH/MeO-BDE85, 6-OH/MeO-BDE137.
Σ_7PBDEs: BDE-28, BDE-47, BDE-100, BDE-99, BDE-154, BDE-153, BDE-183.

Baltic blue mussels would suggest a strong metabolism of PBDEs in blue mussels which is unlikely.

The mean lipid weight normalized concentrations of Σ_2PBPs, Σ_2PBAs, Σ_7OH-PBDEs and Σ_7MeO-PBDEs (Fig. S1) were found to be significantly lower in livers of long-tailed duck than in blue mussels collected in March–May (t-test, $P < 0.001$) whereas the difference in Σ_7PBDEs was not significant (t-test, $P = 0.7$). The results were similar when comparisons were made on fresh weight basis, with the exception that the concentration of Σ_7PBDEs were significantly higher in long-tailed duck than in blue mussels (t-test, $P < 0.001$). Given that dietary intake of blue mussels is the main exposure route for brominated substances in long-tailed duck while foraging in the Baltic Proper, the relatively high estimated daily intake of Σ_2PBPs (390 ng), Σ_2PBAs (90 ng), Σ_7OH-PBDEs (370 ng) and Σ_7MeO-PBDEs (590 ng) (for details regarding calculations see supporting information) compared to the measured concentrations in livers of long-tailed ducks suggest that these compounds have low retention in the long-tailed duck liver. However, for PBDEs the lipid weigh normalized concentration in long-tailed duck liver and blue mussel did not differ significantly which indicate that the more lipophilic PBDEs have higher retention than MeO-PBDEs in long-tailed duck liver tissue.

In long-tailed ducks, no correlation between the sum concentrations of the different substance classes (i.e. Σ_2PBPs, Σ_2PBAs, Σ_7OH-PBDEs, Σ_7MeO-PBDEs and Σ_7PBDEs) were found. Furthermore, the concentrations of 6-MeO-BDE47 and BDE-47 were not correlated in the long-tailed ducks. However, others have reported significant correlations between these two compounds in glaucous gull (*Larus hyperboreus*) (Verreault et al., 2005) and white-tailed sea eagle (*Haliaeetus albicilla*) (Jaspers et al., 2013). Jaspers et al. suggested that metabolic formation of MeO-PBDEs in fish might explain the trophic transfer of MeO-PBDEs along with PBDEs up the food chain (Jaspers et al., 2013). Hence the lacks of correlation observed in long-tailed ducks may be due to the fact that long-tailed ducks, by primarily feeding on bivalves and benthic organisms represent a different food web and a lower trophic level than glaucous gull and white-tailed sea eagle.

The congener profile of OH-PBDEs, MeO-PBDEs and PBDEs in long-tailed ducks and their main food (i.e. blue mussels collected in March and May) are presented in Fig. 2. Although the mussels were found to contain several OH-PBDEs and MeO-PBDEs, two specific congeners, i.e. 6-OH-BDE47 and 6-MeO-BDE47 dominated in long-tailed duck. The difference in congener profile between blue mussel and long-tailed duck might be due to metabolic processes, e.g. *in vivo* debromination and/or selective elimination in the ducks.

In Baltic blue mussels the PBDE congener profile was dominated by BDE-99 and BDE-47, two of the major PBDE congeners in commercial PentaBDE mixtures (La Guardia et al., 2006) whereas in livers of long-tailed ducks the congeners BDE-47, BDE-100, BDE-99, BDE-154 and BDE-153 were found at approximately equal concentrations (Fig. 2). Interestingly, the PBDE profile found in long-tailed duck was similar to the profiles earlier found in common eider and white-winged scoter (Kelly et al., 2008b). Previously, BDE-47 has been found to dominate the congener profile in birds feeding on aquatic organisms (Chen and Hale, 2010) and in fish and fish feeding mammals (de Wit et al., 2010). However, the similar and broader PBDE profiles observed in eiders, white-winged scoters and long-tailed ducks indicate that benthic feeding sea ducks might have a different PBDEs exposure and/or metabolism compared to other marine birds. This finding should be recognised in future risk assessments.

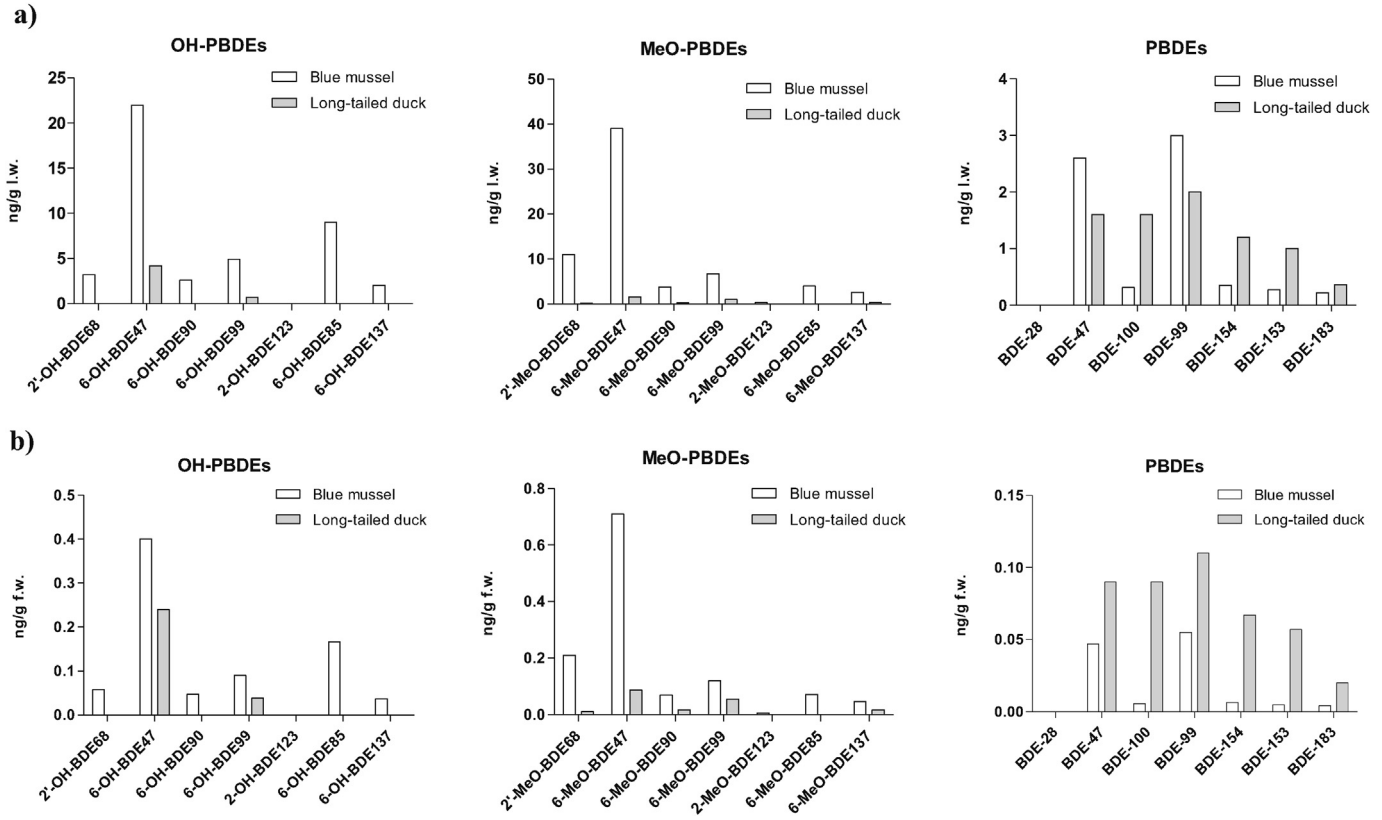

Fig. 2. Geometric mean concentrations of congeners of OH-PBDEs, MeO-PBDEs and PBDEs in Baltic blue mussels collected during March and May (site no. 1–9, n = 11) and in livers of long-tailed ducks (n = 10) presented on **a)** lipid weight basis and **b)** fresh weight basis. Only compounds which were quantified in ≥50% of the samples are presented. Please note the different scales on the y-axes.

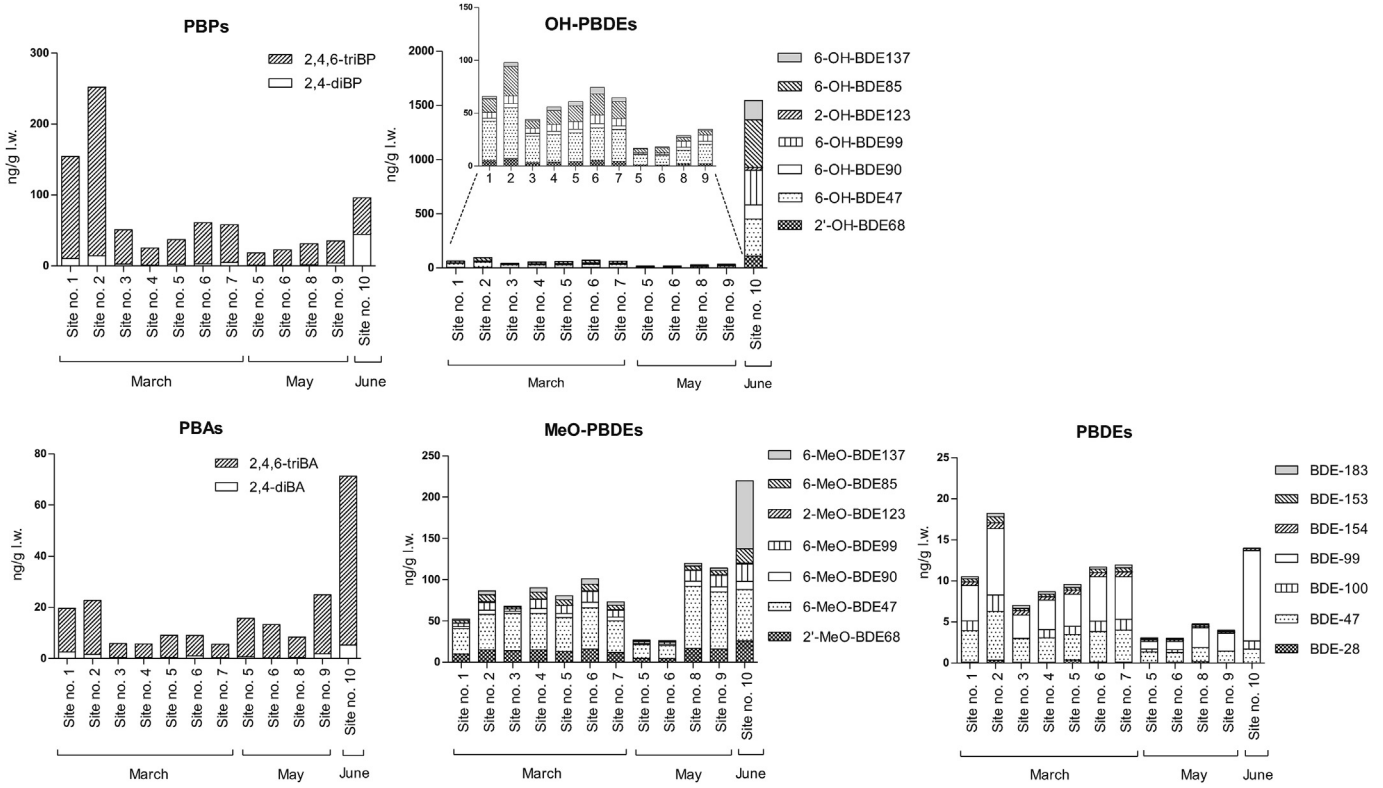

Fig. 3. Concentrations (ng/g l.w.) of PBPs, PBAs, OH-PBDEs, MeO-PBDEs and PBDEs in blue mussels from different sampling sites in the Baltic Sea collected in March 2012, May 2011 and June 2011, presented on lipid weight basis. The site numbers refers to the sampling sites shown in Fig. 1. The concentrations are presented on fresh weight basis in Fig. S1.

The highest concentration of OH-PBDEs and MeO-PBDEs was found in blue mussels sampled in June (site no. 10) as shown in Fig. 3 and in Fig. S2. With biogenic production of OH-PBDEs and MeO-PBDEs by e.g. cyanobacteria and algae, and with previous studies showing that the concentration of OH-PBDEs and MeO-PBDEs varies seasonally in Baltic blue mussel (Loefstrand et al., 2011) and algae (Loefstrand, 2011) this result was expected. The high concentrations of Σ_7OH-PBDEs (1500 ng/g l.w. or 53 ng/g f.w.) and Σ_7MeO-PBDEs (220 ng/g l.w. or 7.5 ng/g f.w.) in June 2011 are similar to the concentrations of Σ_7OH-PBDEs (50 ng/g f.w) and Σ_7MeO-PBDEs (6.0 ng/g f.w.) in blue mussels collected from the same location in June 2008 (Loefstrand et al., 2011). This indicates that blue mussel's high exposure to OH-PBDEs at this location during summer is an annually recurring phenomenon.

Although relatively low concentrations of brominated substances was found in the livers of long-tailed ducks, their continuous dietary exposure to these compounds while foraging in the Baltic Proper may still be of some concern as PBDEs and OH-PBDEs have been associated with both neurotoxic and endocrine disrupting effects (Dingemans et al., 2011; European Food Safety Authority, 2011). Several OH-PBDEs present in the Baltic marine environment have also been found to disrupt OXPHOS *in vitro* and to show strong synergistic effects when combined as mixtures (Legradi et al., 2014). Even if the chronic low dose exposure to compounds disturbing OXPHOS may not affect the long-tailed ducks directly there might be indirect effects via their food. Further studies are needed to assess if blue mussels' exposure to OH-PBDEs affect their nutritional value as food for sea ducks and if other, potentially more exposed species of sea ducks, e.g. the Baltic breeding common eider, are affected.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.chemosphere.2015.10.012.

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