Polybrominated Diphenyl Ethers (PBDEs) and Hexabromocyclododecane (HBCD) in marine and freshwater biota samples from the German Environmental Specimen Bank

Lohmann N[1], Rolle S[1], Ruedel H[2], Teubner D[3], Koschorreck J[4]

[1] Eurofins GfA Lab Service GmbH, Hamburg, Germany; [2] Fraunhofer Institut fuer Molekularbiologie und Angewandte Oekologie (Fraunhofer IME), Schmallenberg, Germany; [3] Trier University, Biogeography, Trier, Germany; [4] German Federal Environment Agency, Berlin, Germany

Introduction

The German environmental specimen bank (ESB) is one of the largest cryoarchives for environmental samples and covers different ecosystems across Germany [1]. It allows for retrospective analysis of spatial and temporal trends in contaminant monitoring. The examples of polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD) are presented: Spatial and trend data from soft body of blue mussels, herring gull eggs, filets from eelpout and bream from more than 20 sampling sites in Germany are compared and - where possible – compared against the respective European environmental quality standard (EQS) of the EU Water Framework Directive [2] for fish.

Materials and methods

Samples. Samples of blue mussels (*Mytilus edulis*), herring gull eggs (*Larus argentatus*), eelpout (*Zoarces vivparus*) and bream (*Abramis brama*) were collected annually between 1996 and 2015 by the ESB Project Team, Trier University, Germany. The soft body of the mussels, egg content of gull eggs and muscle tissue of fish samples were processed, cryomilled and archived in sub-samples at temperatures below -150°C by the Fraunhofer Institute for Molecular Biology and Applied Ecology (Fraunhofer IME), Department Environmental Specimen Bank, Schmallenberg, Germany. Sampling and processing was performed under well-defined and reproducible conditions according to standard operating procedures [3-7]. Sampling areas (fig. 1) were in the North and Baltic Sea, the rivers Rhine, Saar, Danube and Elbe with the tributaries Mulde and Saale and Lake Stechlin as well Lake Belau as low anthropogenically influenced area [8].

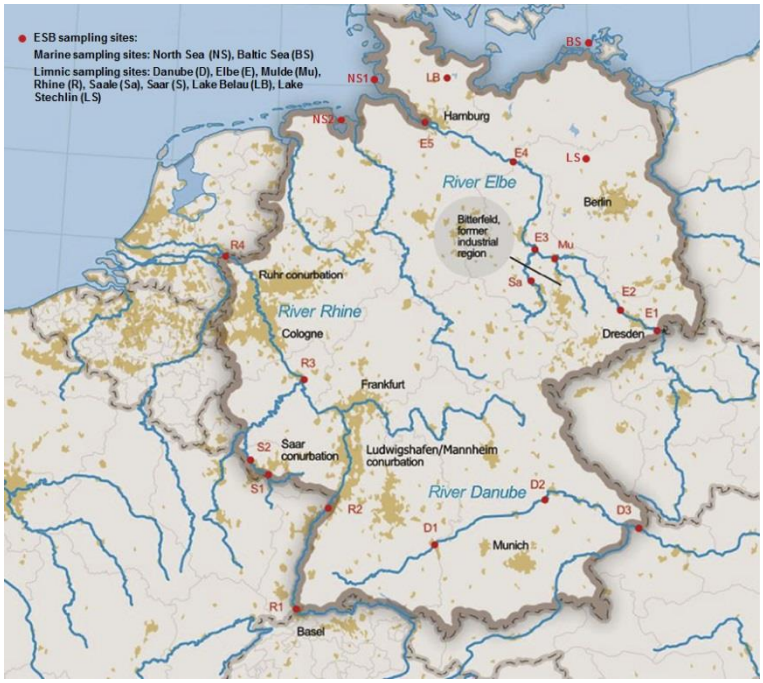

Figure 1: Sampling sites

Analysis of PBDEs. Fish and mussel samples were analysed for PBDEs using means of gas chromatography and mass spectrometry (GC/MS) analogue to an accredited method described before [9]. After addition of a mixture of ^{13}C-labeled internal standards, the extraction was performed by means of Soxhlet, followed by a clean-up procedure using concentrated sulphuric acid combined with column chromatography involving alumina. Further ^{13}C-labeled internal standards were added for determination of the recovery of the internal standards added before. PBDEs were analysed by means of GC/MS. For each substance 2 isotope masses were measured. The identification of PBDEs was based on retention time and isotope ratio. The quantification was carried out by means of isotope dilution method with the use of internal and external standards including a multi-point calibration. Recoveries measured for the internal standards used range between 50 and 120 %. Results for BDE 28, 47, 99, 100, 153 and 154 are presented in this publication as the EQS is related to these congeners, in addition also BDE 66, 183 and 209.

Analysis of HBCD. Fish samples were analysed for HBCD. After addition of the internal standards, ^{13}C-labeled α- and γ-HBCD, the extraction was performed by means of Soxhlet using a mixture of appropriate polar and non-polar solvents for ultratrace-analyses. The extract was further purified with sulphuric acid, followed by alumina clean-up. The final extract was reduced nearly to dryness under a gentle stream of nitrogen after addition of ^{13}C-labeled β-HBCD and dissolved in methanol. The measurements were performed using liquid chromatography and tandem mass spectrometry (incl. electro spray ionization) (LC/ESI-MS/MS). Identification and quantification was performed similar as described for PBDEs above.

Determination of extractable lipids. The determination of the extractable lipids was performed gravimetrically within an analytical procedure for determination of PBDEs (blue mussels only) or determination of hexachlorobenzene (HCB) described before [10, 11] (results of HCB not being reported here [12]).

Results and discussion

Levels of PBDEs in bream from German rivers and lakes were above the EQS (0,0085 ng/g) at all sampling sites. In contrast, levels of HBCD in bream of the same freshwater sampling sites were all well below the EQS (167 ng/g). Normalisation of analytical results from bream to a fish with a lipid content of 5% and trophic level of 4.0 as suggested by the European Guidance for biota monitoring under the WFD did not have an effect on EQS compliance.

Levels and patterns of PBDEs and HBCD in blue mussel, herring gull eggs, eelpout and bream show differences between species (fig. 2 and 3). After normalisation to lipid basis the levels for both contaminants are lowest for blue mussel, followed by eelpout, and by herring gull eggs and bream at much higher concentrations.

The PBDE-pattern of bream (freshwater species), which is dominated by BDE-47, can be discriminated against the marine samples where BDE-209 and BDE-99 are present in significantly higher amounts. With regard to the HBCD-pattern, α-HBCD is more present in the bream samples, β- and γ-HBCD are more distinctive for the marine samples.

Current trends for HBCDD and PBDE appear to decrease in herring gull eggs from the North and Baltic Sea but not in eelpout samples, and vary in bream from the freshwater sampling sites.

References

1. German Federal Environment Agency (Umweltbundesamt): German Environmental Specimen Bank – Concept, Berlin, October 2008. (http://www.umweltprobenbank.de/de/documents/11426 accessed 15 May 2017)
2. Directive 2013/39/EU amending Directives 2000/60/EC and 2008/105/EC (*OJ* L 226, 24.08.2013, p. 1)
3. Klein et al: Guideline for Sampling and Sample Treatment – Bream (Abramis brama), Trier, 2012. (http://www.umweltprobenbank.de/de/documents/publications/11544 accessed 15 May 2017)
4. Wagner et al: Richtlinie zur Probenahme und Probenbearbeitung Miesmuschel (Mytilus edulis), 2011. (https://www.umweltprobenbank.de/en/documents/publications/20492 accessed 15 May 2017)
5. Klein et al: Guideline for Sampling and Sample Treatment Eelpout (Zoarces viviparus), 2010. (https://www.umweltprobenbank.de/en/documents/publications/14526 accessed 15 May 2017)
6. Paulus et al: Guideline for sampling and sample treatment Herring gull (Larus argentatus), 2010. (https://www.umweltprobenbank.de/en/documents/publications/11893 accessed 15 May 2017)
7. Rüdel et al: Guidelines for Sampling and Sample Processing: Pulverisation and Homogenisation of Environmental Samples by Cryomilling, 2009. (https://www.umweltprobenbank.de/en/documents/publications/11939 accessed 15 May 2017)
8. Wenzel et al. (2004) *Environmental Science & Technology* **38**, 1654-1661.
9. Päpke et al. (2011) *Organohalogen Compounds* **73**, 416-419
10. Schröter-Kermani et al. (2004) *Organohalogen Compounds* **66**, 1756-1759
11. Neugebauer et al. (2011) *Organohalogen Compounds* **73**, 1340-1343
12. Lohmann et al. (2014). *Organohalogen Compounds* **76**, 1545-1548

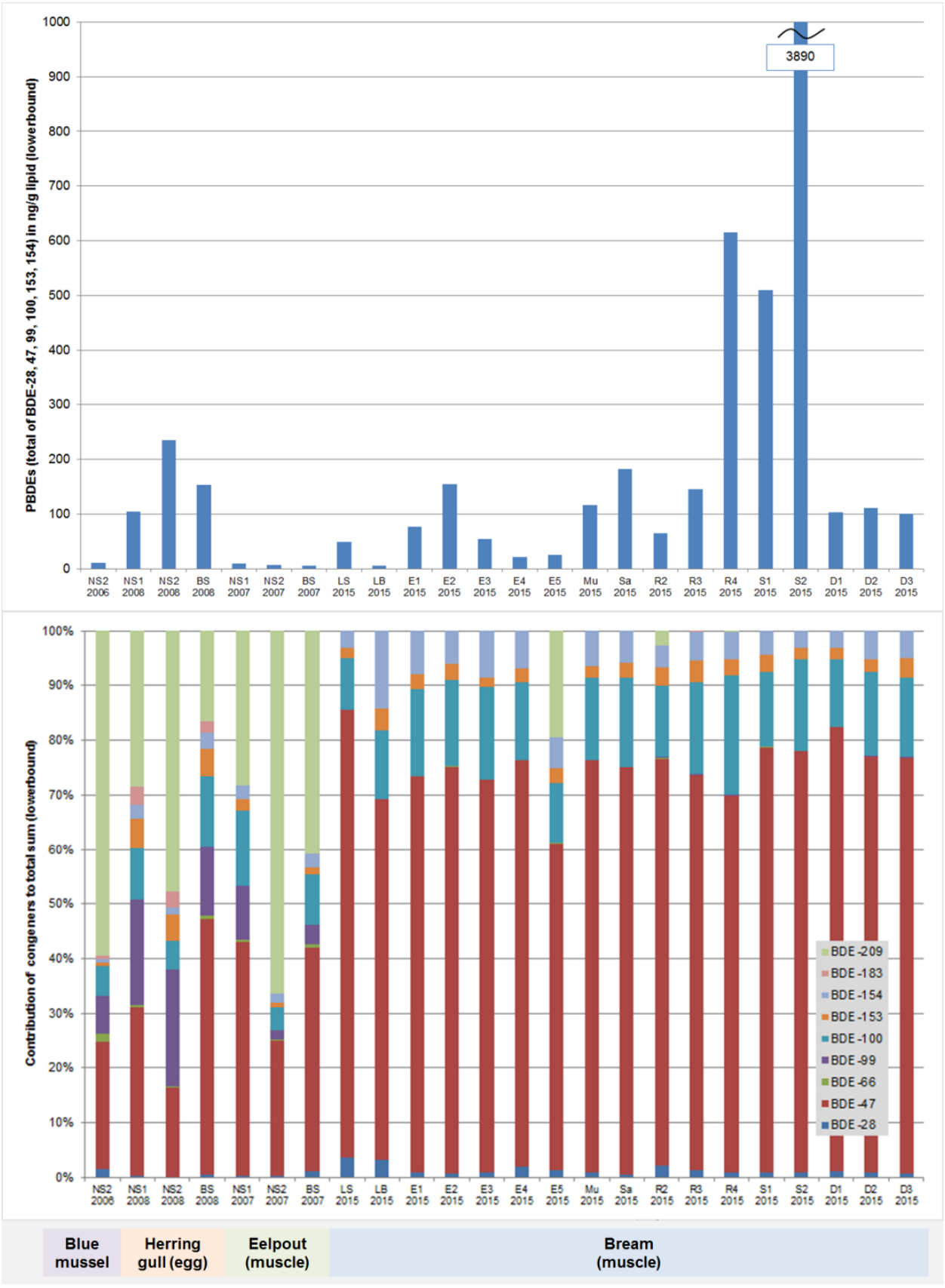

Figure 2: PBDE-Concentration and pattern in marine and freshwater ESB-samples

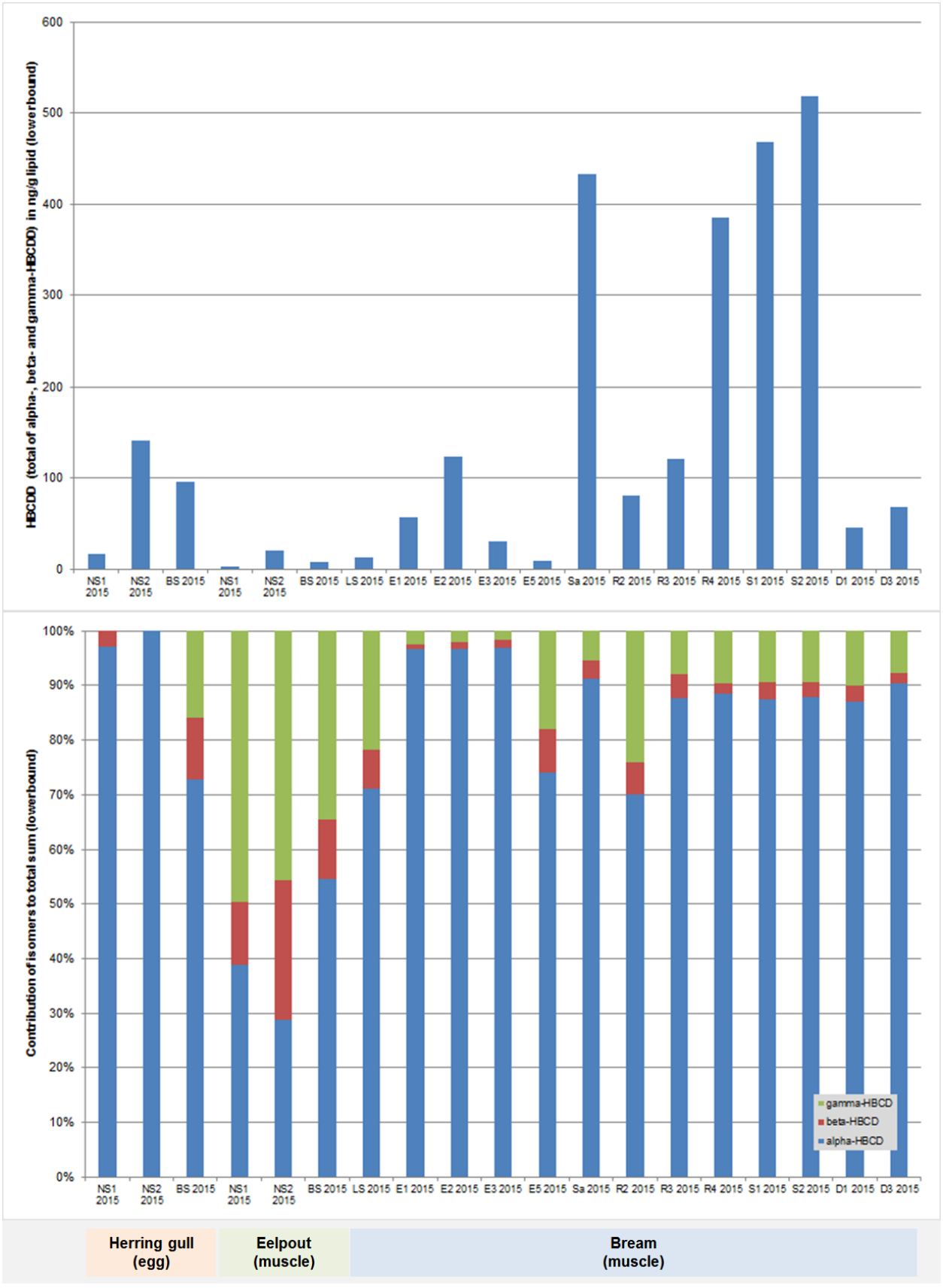

Figure 3: HBCD-Concentration and pattern in marine and freshwater ESB-samples