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First health and pollution study on harbor seals (*Phoca vitulina*) living in the German Elbe estuary

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ABSTRACT

The Elbe is one of the major rivers releasing pollutants into the coastal areas of the German North Sea. Its estuary represents the habitat of a small population of harbor seals (*Phoca vitulina*). Only little is known about the health status and contamination levels of these seals. Therefore, a first-ever seal catch was organized next to the islands of Neuwerk and Scharhörn in the region of the Hamburg Wadden Sea National Park. The investigations included a broad set of health parameters and the analysis of metals and organic pollutants in blood samples. Compared to animals of other Wadden Sea areas, the seals showed higher γ-globulin levels, suggesting higher concentrations of pathogens in this near-urban area, elevated concentrations for several metals in particular for V, Sn, Pb, and Sr, and comparable ranges for chlorinated organic contaminants, except for elevated levels of hexachlorobenzene, which indicates characteristic inputs from the Elbe.

1. Introduction

Due to their role as top predators within the marine food web, marine mammals such as harbor seals (*Phoca vitulina*) can be used as indicators for ecosystem change (The Trilateral Wadden Sea Cooperation, 2010). Increasing commercial use, e.g., fisheries and offshore wind parks, as well as ongoing inputs of pollutants strongly influence the North and Baltic Sea ecosystems. The German states Schleswig–Holstein, Hamburg and Lower Saxony have declared their Wadden Sea areas as National Parks. The Hamburg Wadden Sea area includes also parts of the Elbe estuary, where harbor seals play an important role for the regional tourism.

The harbor seal population in the Elbe estuary is relatively small in comparison to other populations that can be found along the Wadden Sea coast line. In one of the latest aerial surveys conducted in 2008, on an average 427 animals were counted in the area of the Hamburg Wadden Sea (Hellwig and Krüger-Hellwig, 2008). Most animals (371) were present on the western haul out sites "Robbenplate" and "Wittsandloch". Fifty-six animals were counted on the eastern haul out site "Hundebalje". Beside regular aerial surveys since 2002, no further investigations, e.g., of the health status of these animals, have been carried out.

In 2002, the phocine distemper virus (PDV) epizootic reduced the harbor seal population to 50% in this and other areas of the Wadden Sea (Reijnders et al., 2005). Since the epidemic impact, the seal population of the Hamburg Wadden Sea area has grown continuously. However, the size of the population has not yet reached its original size before the virus outbreak (Hellwig and Krüger-Hellwig, 2008).

Whether environmental pollution-related immunosuppression might have contributed to the severity and extent of morbillivirus-caused mass mortalities among marine mammals is still under discussion (Härkönen et al., 2006; Ross, 2002). However, several studies have shown a relationship between contaminant body burdens and immunological dysfunctions (Beckmen, 1999; De Guise et al., 2006; De Swart et al., 1994; Kakuschke and Prange, 2007). Despite partly decreasing inputs of contaminants into the North Sea, the Elbe River is still the primary contributor to the contamination of its estuary and of the German Bight (Loewe et al., 2006).

Several studies concerning the health status (Hasselmeier et al., 2008; Kakuschke et al., 2010; Siebert et al., 2007) and/or

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contaminant body burdens (Ahrens et al., 2009; Griesel et al., 2008; Weijs et al., 2009) of harbor seals were conducted in the Wadden Sea. To our knowledge, we report for the first time results for seals of the Elbe estuary. Our investigation included a common set of health parameters and pollutants, applied in the studies mentioned above. In addition, a new method for the determination of transferrin (Tf) isoforms (established markers for specific disorders in humans) as a potential new biomarker for seals was applied.

2. Material and methods

2.1. Animals

The seal catch was carried out in the estuary of the river Elbe next to the islands of Neuwerk and Scharhörn in the area of the Hamburg Wadden Sea National Park (Germany) in October 2008 (Fig. 1).

The seal catch was coordinated from on board the GKSS research vessel "Ludwig Prandtl" and carried out with two Zodiac boats. Harbor seals were captured using a 120 m × 8 m net with a mesh size of 10 cm × 10 cm, adapted from a method described by Jeffries et al. (1993). Briefly, the net was spread out slowly between both Zodiac boats in a distance of around 100 m to the animals. Due to the low water depth, the net reached to the ground and the animals were not able to dive below the net. Both boats moved simultaneously towards the beach, trapping the seals within the net. After the landing of the two boats, the net was moved manually onto the shore line. The caught animals were removed from the net, transferred into tube nets, and restrained manually to assess length, weight, sex and age and to collect anal smears and blood samples. The handling for measurements and blood collection took 10–15 min for each seal. During the procedure the animals were continuously under observation of two veterinarians. After completing the investigations, the animals were released back into the wildlife. The time span between transferring all animals in tube nets and releasing back into the wildlife took 1 h.

Blood was collected into monovettes after puncture of the epidural vertebral vein using a 20 mL syringe and a 12 mm × 100 mm needle (TSK-Supra, TSK Laboratory, Japan). The tubes were carefully agitated and kept at room temperature until further sample processing. Most blood samples were processed within 1–12 h. Swabs taken from the anus were used for microbiological investigations.

During this catch five animals were caught and coded sequentially (Table 1). The age was estimated based on length and weight and the animals were grouped into seals <1 year, between 1 and 2 years, and >2 years.

2.2. Hematology

For hematology, EDTA monovettes (Sarstedt AG & Co., Nümbrecht, Germany) were used. A basic hematology profile (white blood cells [WBC], red blood cells [RBC], hemoglobin [HGB], hematocrit [HCT], mean cellular volume [MCV], mean cellular hemoglobin [MCH], mean cellular hemoglobin concentration [MCHC], thrombocytes, and reticulocytes), was analyzed at Synlab.vet Hamburg in Geesthacht, Germany, using a Sysmex XT – 2000 analyser (Sysmex Deutschland GmbH, Norderstedt, Deutschland). The leukocyte subgroups (neutrophiles, eosinophiles, lymphocytes, and monocytes) were counted manually.

2.3. Lymphocyte proliferation assay

The MELISA® (Memory Lymphocyte Immunostimulation Assay), a modification of the lymphocyte transformation test (LTT), was performed as previously described in the Laboratory Center

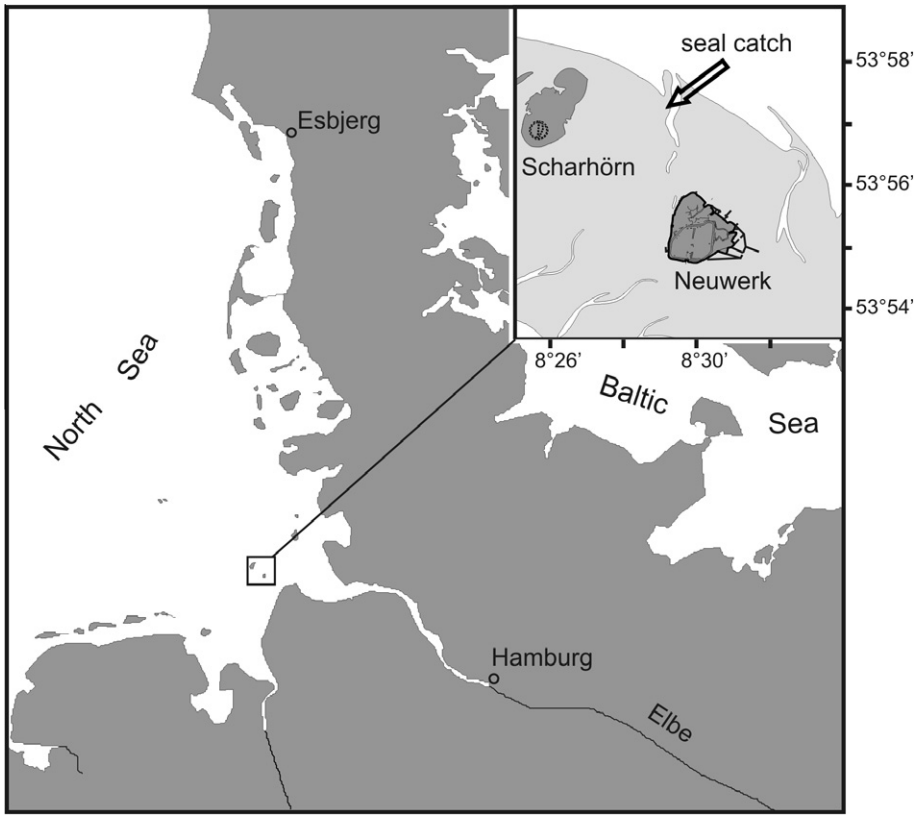

Fig. 1. Sampling location in the estuary of the river Elbe.

Table 1
Details of the harbor seals of this study caught in the Elbe estuary in 2008.

Seal code	Date of blood sampling	Sex	Age (year)	Total length (cm)	Reduced length (cm)	Weight (kg)
W 01/08 Pv	10.10.2008	Male	<1	96	51	25
W 02/08 Pv	10.10.2008	Male	>2	130	85	48
W 03/08 Pv	10.10.2008	Male	>2	147	89	49
W 04/08 Pv	10.10.2008	Male	<1	112	56	24
W 05/08 Pv	10.10.2008	Male	1–2	119	78	39

Bremen, Germany (Kakuschke et al., 2005, 2006, 2008a,b) and briefly described in the Supporting Information S1. The mitogen- and non-stimulated lymphocyte proliferation was tested as well as the metal-specific proliferation of following metals/metal species: Al, Be, Cd, ethylmercury (EtHg), mercurychloride (HgCl), methylmercury (MeHg), phenylmercury (PhHg), Mo, Ni, Pb, Sn, and Ti. The metals were tested at two concentration levels. Level II is the 1:1 dilution of level I. The concentrations of level I are given in μg/well: Al (40), Be (50), Cd (6), EtHg (0.5), HgCl (0.5), MeHg (0.5), PhHg (0.5), Mo (25), Ni (5), Pb (25), Sn (25) and Ti (50). The stimulation index (SI) was calculated as followed: SI = metal-stimulated proliferation (cpm)/non-stimulated proliferation (cpm). SI $\geqslant$ 3 was regarded as a positive hypersensitivity response.

2.4. Serum protein electrophoresis, investigations on acute phase proteins, and serology

Serum protein electrophoresis was done at the Synlab.vet Hamburg with an automated analyzer (Olympus Hite 320, Olympus Deutschland GmbH, Hamburg, Germany).

C-reactive protein (CRP) was measured at the Synlab.vet Hamburg using turbidometry (Olympus AU 2700, Olympus Deutschland GmbH).

For the measurement of haptoglobin (Hp), a multispecies Hp assay from Tridelta Development Limited (Maynooth, Kildare, Ireland) was used. The Hp concentrations were quantified in EDTA plasma samples collected by using EDTA monovettes according to the manufacturer's instructions. Colorimetric measurements were performed using a photometer (Multilabel Counter WALLAC 1420, Perkin Elmer). All samples were analyzed in duplicate at the GKSS, Geesthacht.

The serology included the analysis of *Brucella* spp. and distemper virus antibodies and was performed at Synlab.vet using an immunofluorescence antibody test (IFAT).

2.5. Determination of transferrin isoforms

Tf isoforms were analyzed in serum at the GKSS as described recently (Grebe et al., 2010). Briefly, the procedure utilizes a strong anion-exchange (SAX) chromatography hyphenated with inductive-coupled plasma mass spectrometry (ICP-MS). The setup consisted of a high performance liquid chromatograph (Agilent 1100 series, Agilent Technologies, Waldbronn, Germany) and an ICP-MS (Agilent 7500cs, Agilent Technologies, Tokyo, Japan).

Seal blood was sampled in Serum Gel S monovettes (Sarstedt AG & Co.). Tf in blood samples was saturated with iron by incubation with $FeCl_3$ solution. After the precipitation of lipoproteins the samples were centrifuged and the resulting supernatant was diluted with starting buffer (20 mM Bis–Tris, pH 6.5). After the separation with a linear gradient of ammonium acetate on a SAX column (Poros HQ 2.1 × 100 mm, 10 μm particles, Applied Biosystems, Foster City, USA), Tf isoforms were measured using element-specific detection of ^{56}Fe. Interferences were reduced by using the collision cell with 5 mL min^{-1} H_2.

The evidence for being Tf isoforms with differing degrees of sialination was provided by specific enzymatic digestions and partial mass spectrometric determination of the amino acid sequence of seal Tf found in the SAX fractions (Grebe et al., 2010).

2.6. Clinical chemistry and bacteriology

Clinical chemistry and bacteriology were performed at the Synlab.vet Hamburg. The enzyme activities of alkaline phosphatase (AP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (γ-GT), cholinesterase, glutamate dehydrogenase (GLDH), lactate dehydrogenase (LDH), alpha-amylase, lipase, creatine kinase (CK) as well as the amount of total bilirubin, cholesterol, creatinine, bile acid, urea, uric acid, triglyceride, glucose, and inorganic phosphate were analyzed using photometry (Olympus AU 2700). Chloride was quantified by potentiometry. Cortisol and thyroxin were analyzed using a chemiluminescence immunoassay (CLIA, Immulite 2000, Siemens AG, Erlangen, Germany), and folic acid and vitamin B12 using an electrochemiluminescence immunoassay (ECLIA, Immulite 2000). Swabs (Heinz Herenz Medizinal bedarf GmbH, Hamburg) from the anus were investigated microbiologically by Synlab.vet Hamburg.

2.7. Element analysis of whole blood

For the element analysis blood samples were collected in special Lithium Heparin (LH) monovettes for metal analysis (Sarstedt AG & Co.) and stored at −80 °C. Twenty-five elements were analyzed in whole blood samples following the procedure described in our previous study at the GKSS (Griesel et al., 2008).

The elements were determined with two different analytical methods. Al, Be, Bi, Cd, Co, Cr, Cs, Li, Mg, Mn, Mo, Na, Ni, Pb, Sn, and V were analyzed using an ICP-MS equipped with a collision cell (Agilent 7500c ICP-MS, Agilent Technologies). The standard mode was used for Al, Be, Cs, Li, Na, Pb, Sn, and V. For the other elements, better results were obtained using He as collision gas (flow rate 3.0 mL min^{-1}). Measurements of As, Ca, Cu, Fe, K, Rb, Se, Sr, and Zn were performed by total-X-ray-fluorescence spectrometry (TXRF) (Atomika TXRF 8030 C, FEI Company, Oberschleissheim, Germany).

For internal quality control, the reliability of the analytical procedures was checked with the human reference material Seronorm™ Trace Elements Whole Blood L-2 (SERO AS, Billingstad, Norway) and/or Clin Check® Whole Blood Control Level II (Recipe, Chemicals + Instruments, Munich, Germany). In addition the laboratory successfully completed the NIST/NOAA 2005 and 2007 Interlaboratory Comparison Exercise for Trace Elements in Marine Mammals (Christopher et al., 2007).

2.8. Chlorinated pesticides and PCBs in plasma

Aliquots of LH plasma were subjected to solid-phase extraction (SPE) and analyzed by gas chromatography–mass spectrometry (GC–MS). Twenty chlorinated pesticides and metabolites as well

as 19 polychlorinated biphenyl congeners (PCBs) were included in this study (Table 4). Standards were purchased from Dr. Ehrenstorfer (Augsburg, Germany), Riedel-de Haën (Seelze, Germany), and Sigma–Aldrich Laborchemikalien GmbH (Steinheim, Germany). The measurements were performed at the University of Las Palmas de Gran Canaria, Spain.

Two-ml aliquots of plasma were applied to 60 mg (3 mL) Oasis® HLB cartridges (Waters Corporation, Milford, USA) mounted in a vacuum manifold (Waters Corporation). Before the application of the plasma samples, the HLB cartridges were cleaned and conditioned as indicated by the manufacturer. Samples were then passed through the cartridge by gravity flow. The adsorbed pesticides and PCBs were eluted with 1 mL of methylene chloride. After a gentle nitrogen blow down and immediate resolubilization in 200 µl *n*-hexane, the resulting final extracts were subsequently analyzed by GC–MS.

GC–MS was performed with a TRACE DSQ (Thermo-Finnigan) instrument. The GC column was a fused silica capillary column BPX5 (crosslinked 5% phenyl methylpolysiloxane, SGE Inc., Austin, USA) with a length of 30 m, 0.25 mm i.d. and a film thickness of 0.25 µm. Helium at a flow rate of 2.1 ml min^{-1} was used as carrier gas. Temperatures were programmed as follows: initial oven temperature of 80 °C held for 1 min, ramped at 10 °C/min to 300 °C and held for 9 min. Injector and transfer line were set at 200 and 310 °C, respectively. Standards and samples were injected (2 µl) in the splitless mode.

Two chromatographic runs were performed for each sample to obtain mass spectra in two different ionization modes. DDT and metabolites, methoxychlor, and PCB congeners 28, 52, 101, and 118 were ionized in electron impact mode at 70 eV with an ion source temperature of 200 °C. For the rest of analytes included in this study, negative chemical ionization was applied using methane as reactant gas at a flow rate of 2.5 mL min^{-1}. The MS was operated in selected ion monitoring mode.

For the quantification of target analytes, six-level calibrations were generated from standard solutions. PCB 202 was used as internal and tetrachloro-m-xylene as surrogate standard.

Limits of quantification (LOQs) were determined as 10-fold standard deviations of blanks. LOQs for DDT and metabolites, methoxychlor, and PCB congeners 28, 52, 101, 118 and 138 were 10 pg mL^{-1} and for PCB congeners 153 and 180 5 pg mL^{-1}. LOQs for the rest of analytes were 1 pg mL^{-1}. The recovery rates were higher than 85% for all the chlorinated pesticides and between 58% and 67% for the PCB congeners.

3. Results and discussion

The aim of the present study was to investigate the health status in combination with measuring body burdens of harbor seals living in the German Elbe estuary, an area where seals have not been previously investigated. However, this area is strongly influenced by anthropogenic activities such as shipping or dredging and shows a high pollution level compared to offshore regions of the North Sea.

3.1. Hematology profile

The hematology profile of the animal W 01/08 Pv showed an elevated number of WBC in general, and neutrophiles and monocytes in particular, compared to the other animals of this study (Table 2) and other investigations on harbor seals (De Swart et al., 1995; Engelhardt, 1979; Hasselmeier et al., 2008). Interestingly, this animal revealed also increased levels for cortisol (Table S2),

Table 2
Immunological investigations of seals of the Elbe estuary.

	W 01/08 Pv	W 02/08 Pv	W 03/08 Pv	W 04/08 Pv	W 05/08 Pv
Hematology profile					
White blood cells (WBC, $\times10^9$ L^{-1})	19.9	10.4	7.0	11.5	9.4
Red blood cells (RBC, $\times10^{12}$ L^{-1})	5.01	5.08	6.40	5.68	5.07
Hemoglobin (HGB, g L^{-1})	193	221	261	217	195
Hematocrit (HCT, L L^{-1})	0.56	0.60	0.71	0.62	0.56
Mean cellular volume (MCV, μm^3)	112.2	118.7	110.2	109.2	109.5
Mean cellular hemoglobin (MCH, pg)	38.5	43.5	40.8	38.2	38.5
Mean cellular hemoglobin concentration (MCHC, g dL^{-1})	34.3	36.7	37.0	35.0	35.1
Thrombocytes ($\times10^9$ L^{-1})	333	301	178	115	110
Reticulocytes (μL^{-1})	35,070	81,280	32,000	39,760	86,190
Neutrophiles (μL^{-1})	14,726	3432	4060	7360	5076
Lymphocytes (μL^{-1})	2587	5928	2030	3220	2914
Monocytes (μL^{-1})	796	312	210	460	nd
Eosinophiles (μL^{-1})	1791	728	700	460	1410
Lymphocyte proliferation					
Non-stimulated proliferation (cpm)	–	2936	1801	801	1022
PWM-stimulated proliferation (cpm)	–	139,121	120,072	198,268	229,449
Stimulation index	–	41	43	283	225
Serum protein electrophoresis					
Albumin absolute (g L^{-1})	27.8	32.3	32.8	30.2	31.6
α-Globulin absolute (g L^{-1})	13.7	8.9	9.0	14.1	7.6
β-Globulin absolute (g L^{-1})	6.0	12.5	12.0	5.1	12.1
γ-Globulin absolute (g L^{-1})	26.5	21.3	22.1	29.6	26.7
Ratio albumin/globulin	0.60	0.75	0.76	0.62	0.68
Total protein (g L^{-1})	74	75	76	79	78
Acute phase proteins					
C-reactive protein (mg L^{-1})	100	35	30	62	57
Haptoglobin (g L^{-1})	0.93	0.71	0.67	0.56	0.13
Serology					
Antibodies against *Brucella* spp.	<1:50	<1:50	<1:50	<1:50	<1:50
Antibodies against distemper virus	<1:50	<1:50	<1:50	<1:50	<1:50

nd = not detected.

CRP and Hp (Table 2). As other measured parameters did not differ markedly to results of the other seals and no obvious impairment was present on physical examination (data not shown), this result is most likely consistent with a stress-leukogram (Jackson, 2010).

3.2. Lymphocyte proliferation

The lymphocyte proliferation was similar to the range measured previously in other seals of the North Sea (Kakuschke et al., 2005). However, W 04/08 Pv and W 05/08 Pv showed higher stimulation indices compared to the older seals W 02/08 Pv and W 03/08 Pv (Table 2). Further parameters indicate no differences between both age groups.

Additionally, seals of the Elbe estuary were investigated for metal-specific hypersensitivity reactions as described for Wadden Sea seals and different groups of animals living in the Seal Station Friedrichskoog (Schleswig Holstein, Germany) (Kakuschke et al., 2005, 2006, 2008a,b). For one seal (W 03/08 Pv) Sn- and Ti-specific hypersensitivity reactions were found (Fig. S1). As shown below, the Sn concentrations in blood of the Elbe seals were elevated and might induce hypersensitivities. However, this result was not present in other seals of this study, and investigations of a larger number of animals from this geographical area are necessary to confirm this relationship and to evaluate the influence of metal pollutants on the immune system.

3.3. Serum proteins

The total protein, albumin, and albumin/globulin ratio were comparable to other investigations on harbor seals (Table 2) (Engelhardt, 1979; Hasselmeier, 2006). Interestingly, α-, β-, and γ-globulins showed differences compared to other studies on harbor seals: α- and β-globulins were lower, and, in particular, γ-globulins were higher in our study on harbor seals of the Elbe estuary compared to animals of other regions of the Wadden Sea (Engelhardt, 1979; Hasselmeier, 2006). Gamma-globulins are the group of immunoglobulins consisting of different antibodies and are elevated in various inflammatory, infectious, and neoplastic conditions. This result suggests that seals sampled at near-urban sites might have an activated humoral immune system caused by higher exposure to pathogens. The role of the biological pollution on the immune system was also shown in a study on harbor seals captured from remote and near-urban sites in British Columbia, Canada, and Washington State, USA (Mos et al., 2006).

3.4. Transferrin isoforms

Isoforms of Tf, an iron-transport glycoprotein in mammals, has been investigated by us, to our knowledge, for the first time in seals. It is well known that human Tf can be separated into several isoforms based on differences in their carbohydrate moities and particularly their number of negatively-charged terminal sialic acid residues (Del Castillo Busto et al., 2009; Helander et al., 2001). Altered distributions of Tf isoforms, in particular Carbohydrate Deficient Transferrin (CDT, defined as the sum of α-, mono- and disialotransferrin) and their elevated concentrations in serum are used in human medicine as biomarkers, e.g., for damage to the liver and liver diseases (Arndt, 2001; Helander et al., 2001; Murawaki et al., 1997).

The patterns of eight isoforms found in the seal serum samples are depicted in Fig. 2.

With an increasing degree of sialination, the isoforms elute at higher retention times from the anion-exchange column (Grebe et al., 2010). Supporting information on retention times and relative peak areas is given in Table S1 and all five chromatograms in Fig. S2.

Despite the small set of samples, two distinctly different sets of Tf isoform patterns were observed (Fig. 2). For two animals (W 02/08 Pv and W 04/08 Pv), the relative amounts of lower sialinated isoforms 1 and 2 (CDT) added up to more than 30% and 23%, respectively, while for the other three animals CDT was below 1%. The two animals with high CDT levels also exhibited higher levels of creatine kinase whereas the other diagnostic clinical parameters showed no notable differences.

Due to the small set of samples, our case study does not allow an interpretation of the different Tf isoform patterns. However, in

Table 3
Element profile in whole blood samples (concentrations are given in $\mu g\,L^{-1}$) of seals caught in the Elbe estuary compared to our previous study on seals of the German Bight (Griesel et al., 2008).

	W 01/08 Pv	W 02/08 Pv	W 03/08 Pv	W 04/08 Pv	W 05/08 Pv	Seals German Bight
Al	17.2	29.9	16.2	14.5	13.1	<0.17–499
As	564	283	190	459	190	42.0–592
Be	1.28	1.39	1.20	1.18	1.04	<0.08–1.80
Bi	2.20	2.50	1.86	1.86	1.65	
Ca	59.1×10^3	66.5×10^3	45.3×10^3	74.3×10^3	48.2×10^3	$29.8–55.0 \times 10^3$
Cd	0.90	1.05	0.85	0.84	0.87	<0.12–3.10
Co	0.58	0.89	0.72	0.80	0.65	<0.02–7.56
Cr	6.36	7.56	5.96	4.24	5.02	1.52–84.9
Cs	0.74	1.98	1.23	0.68	1.15	
Cu	1.09×10^3	1.54×10^3	1.06×10^3	0.70×10^3	1.13×10^3	$0.53–1.40 \times 10^3$
Fe	670×10^3	993×10^3	810×10^3	244×10^3	797×10^3	$520–1137 \times 10^3$
K	249×10^3	323×10^3	236×10^3	194×10^3	244×10^3	$131–197 \times 10^3$
Li	4.52×10^3	3.90×10^3	4.78×10^3	8.20×10^3	3.93×10^3	
Mg	57.2×10^3	72.1×10^3	52.8×10^3	31.6×10^3	61.1×10^3	
Mn	88.6	127	146	23.2	144	67–151
Mo	7.82	8.88	6.26	6.30	8.58	1.27–22.8
Na	3.31×10^6	3.86×10^6	3.09×10^6	3.31×10^6	3.13×10^6	
Ni	3.78	5.92	4.61	3.34	3.60	<0.38–25.7
Pb	11.4	8.88	3.63	3.80	7.81	<0.02–4.52
Rb	77.0	115	80.7	65.6	83.4	52–149
Se	0.97×10^3	1.85×10^3	1.57×10^3	0.58×10^3	1.05×10^3	$0.52–2.26 \times 10^3$
Sn	1.81	1.66	1.01	0.81	0.93	<0.06–0.47
Sr	77.9	73.2	43.4	125.4	70.4	25–70
V	4.94	7.38	4.70	4.44	4.98	<0.05–1.30
Zn	3.46×10^3	4.98×10^3	4.21×10^3	1.36×10^3	3.97×10^3	$2.73–4.57 \times 10^3$

Table 4
Chlorinated pesticides and PCBs in plasma in ng L^{-1}.

	W 01/08 Pv	W 02/08 Pv	W 03/08 Pv	W 04/08 Pv	W 05/08 Pv
α-HCH	nd	9	2	2	3
HCB	271	2730	1860	1160	1400
β-HCH	5	12	4	5	6
γ-HCH	nd	9	2	3	3
δ-HCH	nd	6	4	nd	4
Heptachlor	nd	8	2	3	4
Aldrin	nd	9	2	3	4
Heptachlor epoxide	nd	4	1	2	1
trans-Chlordane	nd	2	<1	<1	nd
cis-Chlordane	nd	2	nd	nd	nd
Dieldrin	nd	9	3	2	3
Endrin	nd	2	nd	nd	nd
Endosulfan	nd	3	1	1	1
2,4′-DDE	nd	<10	<10	nd	nd
4,4′-DDE	1770	12,000	6000	2030	3800
2,4′-DDT	nd	nd	nd	nd	nd
4,4′-DDT	nd	430	121	nd	nd
2,4′-DDD	nd	nd	nd	nd	nd
4,4′-DDD	nd	nd	nd	nd	nd
Methoxychlor	nd	nd	nd	nd	nd
PCB 28	nd	92	73	21	66
PCB 52	11	39	37	30	73
PCB 77	nd	nd	10	nd	nd
PCB 81	nd	nd	nd	nd	nd
PCB 101	19	320	433	89	125
PCB 105	nd	59	21	5	15
PCB 114	nd	nd	nd	nd	nd
PCB 118	6	115	83	31	39
PCB 123	nd	nd	nd	nd	nd
PCB 126	nd	nd	nd	nd	nd
PCB 138	299	1000	1720	464	1040
PCB 153	216	3280	4950	1010	2890
PCB 156	nd	18	24	8	21
PCB 157	nd	6	7	nd	6
PCB 167	nd	nd	5	nd	5
PCB 169	nd	nd	nd	nd	nd
PCB 170	13	153	271	38	148
PCB 180	<50	738	635	105	387
PCB 189	nd	nd	nd	nd	nd

nd = not detected.

analogy to their application as biomarkers in human medicine, Tf isoforms could be a potential biomarker as well for seals.

3.5. Clinical chemistry and bacteriology

Most of the results of the clinical chemistry measured in this study were within the ranges described in other studies on harbor seals (Table S2) (Bossart et al., 2001; Trumble and Castellini, 2002). For several enzyme activities the animal W 05/08 Pv showed elevated values compared to the other four animals of this study. However, most diagnostic parameters showed no remarkable differences.

3.6. Element profile in whole blood samples

Essential and non-essential/toxic elements were analyzed in whole blood samples.

Firstly, interesting results for the essential trace elements were found (Table 3). For the seals W 01/08 Pv, W 02/08 Pv, W 03/08 Pv, and W 05/08 Pv, the values for Fe and Zn in whole blood were comparable to results of our previous studies on Wadden Sea seals living on the sandbank Lorenzenplate (Schleswig–Holstein, Germany) and on Römö (Denmark), whilst the concentrations of K and Cu were higher (Griesel et al., 2008). Contrarily, animal W 04/08 Pv showed normal K and Cu concentrations, lower values for Fe and Zn compared to published values and lower concentrations of essential trace elements such as Mg, Mn, and Se in comparison to the other seals of this study. Furthermore, several Ca concentrations measured in this study were higher compared to our previous studies on free ranging seals (Griesel et al., 2009). However, the concentrations were comparable to those measured in harbor seal pups (Kakuschke et al., 2009).

Secondly, among the toxic metals, interesting differences in comparison to other Wadden Sea areas of the North Sea and further inshore areas in the world were found.

The concentrations of V and Sn in blood samples were significantly higher in the Elbe seals compared to our previous study on animals of other Wadden Sea areas: The levels of V were more than two times higher than those from seals living on the sandbank Lorenzenplate and on Römö (Griesel et al., 2008). Compared to marine mammals of other inshore areas, e.g., to manatees (*Tricheus manatus latirostris*) of the upper Crystal River, Florida, the V blood concentrations for the Elbe animals were also elevated (Stavros et al., 2008). However, blood samples of northern fur seals (*Callorhinus ursinus*) from northeast Japan revealed higher V concentrations than our results (Saeki et al., 1999). Furthermore, elevated Sn concentrations were measured in blood of Elbe seals compared to seals caught at the Lorenzenplate and Römö (Griesel et al., 2008). However, in blood samples of Florida manatees of the upper Crystal River up to 3 μg Sn kg^{-1} ww blood were measured (Stavros et al., 2008). Similar higher Sn concentrations were found in the liver of cetaceans from Japanese coastal water compared to animals from offshore northwest North Pacific (Takahashi et al., 2000). Furthermore, elevated Sn concentrations were found in liver samples of harbor porpoises (*Phocoena phocoena*) from the river Elbe in comparison to samples taken from North Sea porpoises (Fahrenholtz et al., 2009). These results suggest that Sn levels may be correlated to the high shipping traffic in estuaries or

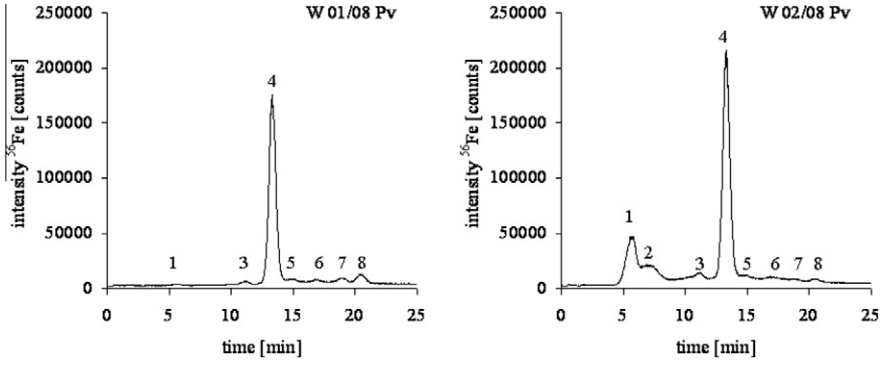

Fig. 2. Anion-exchange chromatograms of the separated Tf isoforms (1–8) from seals of the Elbe estuary measured by ICP-MS (^{56}Fe), one typical chromatogram for each group: group I (W 01/08 Pv, W 03/08 Pv, W 05/08 Pv); group II (W 02/08 Pv, W 04/08 Pv).

inshore areas. Despite its ban in 2003, most ships are still covered with antifouling paint containing tributyltin (TBT). Parts of these biocides are incorporated into marine organisms. Terlizzi et al. describe the impact of antifouling technologies on the marine environment (Terlizzi et al., 2001).

Furthermore, the concentrations of Pb and Sr also showed differences between samples of Elbe seals and animals of other Wadden Sea areas. Pb concentrations in blood of Elbe seals were similar to concentrations measured in seal pups found along the coasts of Schleswig–Holstein and seals from the island Römö, whereas seals caught on the Lorenzenplate revealed lower Pb concentrations (Griesel et al., 2008; Kakuschke et al., 2009). Stavros et al. (2008) suggested that Pb concentrations in blood of Florida manatees may be caused by increased Pb concentrations transported via rivers.

Al concentrations were likewise higher in Elbe and Römö animals compared to animals of the Lorenzenplate (Griesel et al., 2008). For most animals caught on the Lorenzenplate and Römö, the Be concentrations were below the detection limit, whereas all five seals of this study revealed concentrations >1 $\mu g\,L^{-1}$.

Additionally, while the As concentrations in blood of the Elbe seals were within the range measured in seals from Lorenzenplate and Römö, the values were higher than the median levels calculated for these seals (Griesel et al., 2008).

Despite the small number of seals investigated, the results of this study suggest that animals living in estuaries and inshore habitats with industrial emissions and sewage, shipping traffic and dredging tasks are exposed to higher levels of contaminants compared to animals living offshore.

3.7. Chlorinated pesticides and PCBs in plasma

Plasma concentrations of the investigated chlorinated pesticides (including some common metabolites) and PCBs are given in Table 4. As the sampling area of this study lies in the Elbe estuary and is supposed to be influenced by riverine inputs, a comparison with results for seals in bordering coastal areas is of special interest. Weijs et al. (2009) reported serum concentrations of hexachlorobenzene (HCB), 4,4′-DDT and metabolites, and PCBs: medians and ranges (minimum–maximum) in $ng\,L^{-1}$ for 47 harbor seals from Helgoland, Lorenzenplate, and Römö were <20 for HCB, 2750 (722–8440) for 4,4′-DDE, and 7670 (1700–34,200) for PCB 138.

In comparison, the corresponding plasma concentrations for the investigated seals in the Elbe estuary are slightly lower for PCBs, in the same range for 4,4′-DDT and metabolites, and 10 to 100-fold higher for HCB.

The increased HCB levels of the investigated seals are possibly caused by inputs of the Elbe River. In suspended particulate matter of the lower Elbe River, HCB is dominating over PCBs and DDT metabolites. In addition, in sediments of the German North Sea, variable concentration patterns are observed. Further away from the Elbe estuary, HCB concentrations decrease relative to concentrations of PCBs and DDT metabolites (Loewe et al., 2006).

4. Conclusion

The Elbe River is one of the major rivers releasing organic and inorganic pollutants into the coastal areas of the German North Sea, and distinctive toxic effects on biota in bordering coastal areas may be expected. This investigation represents the first health and pollution study of seals living in the Elbe estuary. It indicates significant differences in comparison to the results obtained during the investigations of animals from other Wadden Sea areas. The seals in the Elbe estuary show higher γ-globulin levels suggesting higher concentrations of pathogens in this near-urban area, elevated blood concentrations for several metals in particular for V, Sn, Pb, and Sr, and elevated levels of HCB, which indicates characteristic inputs from the River Elbe.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.marpolbul.2010.07.011.

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