Research Article

Postmortem Health and Pollution Investigations on Harbor Seals (Phoca vitulina) of the Islands Helgoland and Sylt

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Helgoland and Sylt are important centers of tourism in the North Sea. Harbor and grey seals are one reason for the attraction of these islands. However, little is known about these local seal groups. The present postmortem health and pollution study describes a multiparameter investigation of five ill harbor seals which were shot for animal welfare reasons. Firstly, results of pathology and blood investigations support the bad prognosis of survival made in the field. Signs of inflammation in organs, malnutrition, a high-stress level, and reduced thyroid activity were found. Secondly, metal and organic contaminants were investigated. Metal pollutants in blood, liver, muscle, and kidney tissue were not elevated. Lead and mercury concentrations showed a decreased level compared to former studies. Additionally, interesting insights were found for several organic contaminants in comparison with other studies. The Helgoland seals may be influenced by the contaminants of the Elbe plume.

1. Introduction

The ongoing and increasing use of the North Sea and its unique Wadden Sea areas for fishing, offshore wind parks, and as dumping site for dredged material containing various pollutants represents the main anthropogenic threat to this ecosystem. Besides determining contamination levels of selected environmental compartments such as sediments or the related water column, measuring body burdens of marine animals remains a widely established environmental assessment strategy [1–7]. In this context, marine mammals such as harbor seals (Phoca vitulina) are accepted indicators, in particular for medium and long-term ecosystem changes, due to their long lifespan and their role as top predators within the marine food web [8]. Correlations between bioaccumulation of environmental contaminants in the tissues of marine mammals and immunosuppressive effects enhancing the animals’ vulnerability to infectious diseases or pathogens have been described [9–11].

The tissue of seals found dead occasionally along the Wadden Sea coast line represents an important sample material which could be used for pathological investigations and for estimating contaminant body burdens in monitoring programs. However, to describe complex parameters such as the individual health status of marine mammals, an informative set of investigations has to be performed. Recently, we described a study including metals, organic contaminants, selected marker proteins as well as a number of immunological and clinical chemistry parameters to assess the health status of individual marine mammals based on blood samples [12].

Within the framework of the Trilateral Monitoring and Assessment Program (TMAP), German marine mammal monitoring activities include aerial surveys and counting of
living and dead seals providing valuable information related to
the development of the population size [13, 14]. Abt and
Engler considered the grey seal (Halichoerus grypus) pup
production at Helgoland [15]. Within the project MINOS,
Adelung and Müller investigated the diving behavior and
animal tracks of harbor seals of the Wadden Sea including
Helgoland [16].

The health monitoring focuses on pathological investiga-
tions of dead seals found along the Schleswig-Holstein
Wadden Sea coast [17] and blood sample investigations
of living seals from the sand bank Lorenzenplate [18].
Although these activities include seals from the islands Sylt
and Helgoland, the locations are not considered separately,
and little is known about the local groups of these islands. In
a previous study, we published data on the health parameter
haptoglobin measured in plasma of seals (Phoca vitulina)
from Helgoland [19].

To establish new diagnostic parameters or to conduct in vitro
experiments to understand the effects of contaminants
on a molecular level, which will provide a deeper insight into
the health status of such natural populations, the available
sample types as well as their quality and quantity are of
great importance. The seals used in the present study had to
be shot due to severe illness. Using a helicopter transport,
the time between death and necropsy was shortened, which
helped to maintain the integrity of the samples and to collect
sufficient sample amounts for new methodological develop-
ments and approaches such as proteomics or in vitro liver
cell culture assays [20–24]. The present study provides,
for the first time, a basic data set of multiple health parameters
and contaminant body burdens of animals from these areas.

2. Materials and Methods

2.1. Animals. Five severely ill harbor seals (Phoca vitulina)
were found by seal rangers on Helgoland (four animals) and
Sylt (one animal), both islands of the German North Sea
(Table 1, Figure 1). The animals were shot for animal welfare
reasons by an official seal ranger who is obliged to follow a
standard protocol and is permitted to kill a seal only if certain
clinical criteria are fulfilled. Immediately after death, blood
was collected into monovettes after puncture of the epidural
vertebral vein using a 20 mL syringe and a 1.2 mm × 100 mm
needle (TSK-Supra, TSK Laboratory, Japan). The tubes were
carefully agitated and kept at room temperature until further
sample processing. Blood samples were collected in serum, in
EDTA and in lithium heparin monovettes for metal analysis
(all Sarstedt AG & Co, Nümbrecht, Germany). Furthermore
length, weight, and sex were assessed. Age was estimated
based on length and weight. All animals were juveniles
(<one-year old).

After completing the first investigations and blood
drawing in the field, the animals were transferred in a
transportation box by a helicopter (helicopter type MBB
BO 105, Helicopter Service Wasserthal GmbH, Hamburg,
Germany) to the dissecting room of the Research and
Technology Centre (FTZ) in Büsum, Germany, for necropsy
examinations. Due to the rapid transportation (approximately 1 hour),
very fresh tissue sample material could be obtained for necropsy and further research studies, for
example, cultivation of liver cells [24].

Blood samples were transported immediately to the
respective laboratories or to the Helmholtz-Zentrum (HZG),
Geesthacht, Germany, for further sample processing or
storage at −80°C. Plasma samples were taken, frozen and
sub-samples sent to the respective laboratories in Spain and
Italy for the analysis of organic contaminants and thyroid
hormones.

2.2. Pathology. Necropsy was performed as soon as an animal
arrived at the FTZ Büsum, about one hour after death. Body
condition and macroscopically findings were evaluated. The
necropsy involved the investigation of intestine, stomach,
liver, spleen, lung, and thymus. Samples from liver, kidney,
and muscle tissue were collected. Sub-samples were taken
and frozen, and sent to the respective laboratory in Italy for
metal analysis.

2.3. Health Parameters in Blood Samples. For the diagnosis of
inflammatory conditions in the body, serum electrophoresis

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**Table 1: Details of harbor seals of the islands Helgoland and Sylt investigated in this study.**

| Seal code | Date of sampling | Sex  | Age (year) | Location  | Length (cm) | Weight (kg) |
|----------|------------------|------|------------|-----------|-------------|-------------|
| Pv 01    | 5.12.07          | Female | <1        | Helgoland | 101         | 17.8        |
| Pv 02    | 5.12.07          | Male   | <1        | Helgoland | 110         | 17.4        |
| Pv 03    | 29.1.08          | Female | <1        | Helgoland | 97          | 15.8        |
| Pv 04    | 29.1.08          | Male   | <1        | Helgoland | 112         | 22.6        |
| Pv 05    | 14.2.08          | Male   | <1        | Sylt      | 100         | 17.4        |

**Figure 1:** The seals were sampled on the islands Helgoland and Sylt, Germany.
and measurements of several acute phase proteins were performed. Total protein, albumin, and globulin level were investigated after standardized protocols using electrophoresis with an automated analyzer (Olympus Hite 320, Olympus Deutschland GmbH, Hamburg, Germany) at the Synlab.vet Hamburg, Germany. Further, the following acute phase proteins were measured: C-reactive protein (CRP), haptoglobin (Hp), transferrin (Tf), and carbohydrate deficient transferrin (CDT). Concentration of CRP in serum samples was analyzed at the Synlab.vet Hamburg using turbidimetry (Olympus AU 2700, Olympus Deutschland GmbH). The measurement of Hp in serum was performed at the HZG using a multispecies Hp assay from Tridelta Development Limited (Maynooth, Kildare, Ireland). The quantification of Tf and CDT was conducted in serum samples as described in former studies at the HZG [20, 22].

Additionally, several enzymes and hormones were analyzed to investigate organ functions and body condition. The enzyme activities of alkaline phosphatase (AP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (y- GT), cholinesterase, lactate dehydrogenase (LDH), alpha-amylase, lipase, creatine kinase (CK) as well as the amount of total bilirubin, cholesterol, triglyceride, creatinine, bile acid, urea, and glucose were analyzed using photometry (Olympus AU 2700, Olympus Deutschland GmbH). Cortisol was analyzed using a chemiluminescence immunoassay (CLIA, Immulite 2000, Siemens AG, Erlangen, Germany). All investigations were performed in serum samples at the Synlab.vet Hamburg with standardized methods. The thyroid hormones thyroxine (T4) and triiodothyronine (T3) were analyzed in serum samples using RIA commercial kits (Chematil, Angri, Italy) and the radioactivity counted using a Packard 1600 TR β-counter at the University of Bologna, Italy.

2.4. Element Analysis of Whole Blood and Plasma. The element analysis was performed for whole blood and plasma. For whole blood samples the sample preparation and multielement determination were done according to the procedure described in former studies [3]. For plasma analysis, the whole blood was centrifuged (3000 g, Centrifuge 5804G, Eppendorf AG), diluted (1 + 9) by MilliQ water, and filtered (<0.2 μm, Nylon ProFillTMPlus, MedChrom GmbH, Flörheim-Dalsheim, Germany). Twenty elements were analyzed in whole blood and plasma. Al, Be, Cd, Co, Cr, Mg, Mn, Mo, Ni, Pb, and V were analyzed using an ICP-MS with a collision cell (Agilent 7500c ICP-MS, Agilent Technologies). 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).

2.5. Metal Hypersensitivities. Metal-specific lymphocyte proliferation was investigated using a lymphocyte transformation test (LTT). The special modification, MELISA (Memory Lymphocyte Immunostimulation Assay), was performed as previously described in the Laboratory Center Bremen, Germany [25–27]. The mitogen- and nonstimulated lymphocyte proliferation was tested as well as the metal-specific proliferation after stimulation with the following metals/metal species: Al, Au, Be, Cd, Cr, Cu, ethylmercury (EtHg), mercurychloride (HgCl), methylmercury (MeHg), phenylmercury (PhHg), Mo, Ni, Pb, Sn, and Ti. The stimulation index (SI) was calculated as follows: SI = metal-stimulated proliferation (cpm)/non-stimulated proliferation (cpm).

SI > 3 was regarded as a positive hypersensitivity response.
SI 2-3 is interpreted as a possible sensibilisation.
SI < 0.1 is interpreted as a suppressive effect.

In this study, the test was performed for three of five animals (Pv 01, 02, 05).

2.6. Metal Analysis of Liver, Kidney, and Muscle Tissue. Selected toxic elements were analyzed in liver, kidney, and muscle tissues at the University of Bologna, Italy. Subsamples (0.7 g) of each tissue were digested with a Milestone MLS 1200 mega microwave oven using 4 mL nitric acid and 1 mL hydrogen peroxide. Measurements of As, Hg, Pb, and Cd were performed by inductively coupled plasma optical emission spectrometry (ICP-OES) using a Perkin Elmer Optima 2100 DV instrument, coupled with a CETAC U5000AT+ ultrasound nebulizer for Hg. Two blanks were run during each set of analysis to check for the purity of the chemicals. The accuracy of the method was verified with a reference material (CRM 278: lyophilized mussel, Community Bureau of Reference, BCR, Brussels). All values of the reference material were within certified limits.

2.7. Chlorinated Pesticides and PCBs in Plasma. Aliquots of plasma were subjected to solid-phase extraction (SPE) and analyzed by gas chromatography-mass spectrometry (GC-MS) following the procedure described in our previous study [12]. The measurements were performed at the University of Las Palmas de Gran Canaria, Spain. 17 chlorinated pesticides and metabolites as well as 27 polychlorinated biphenyl congeners (PCBs) were included in this study. Limits of quantification (LOQs) were determined from 10-fold standard deviations of blanks. LOQs were 50 ng L−1 for methoxychlor and DDT including metabolites, and 10 ng L−1 for the rest of the analytes.

3. Results and Discussion

3.1. Pathology. All five harbor seals showed comparable pathologic findings; for example, they showed signs of acute catarrhal enteritis including swollen mesenteric lymph nodes. In three animals (Pv 03–05), single Acanthocephala spp. were found in the small intestine. In four animals (Pv 01, 03–05), nematodes were found in the stomach. The liver of all five seals showed multifocal small white partly confluent foci of 1 to 4 mm in diameter. In three animals (Pv 02–04), the spleen was pulpous hyperplastic. Additionally, all five
Table 2: Element profile in whole blood (WB) and plasma (P) [μg L⁻¹] samples of seals investigated in this study compared to our previous study on seals of the German Bight [3].

| Element | Pb | Pb | Pb | Pb | Pb_
|---------|----|----|----|----|-----|
|         |     |     |     |     |      |
| Al      | 1.17 | 1.72 | 1.32 | 0.80 | —  |
| P       | 8.43 | —   | —   | —   | 2.81 |
| As      | 287  | 449 | 290 | 732 | —  |
| P       | 130  | 1010 | 478 | 1940 | 285 |
| Be      | <0.08 | <0.08 | <0.08 | <0.08 | —  |
| P       | <0.08 | <0.08 | <0.08 | <0.08 | <0.08 |
| Ca      | 45.8 \(\times\) 10³ | 59.2 \(\times\) 10³ | 70.2 \(\times\) 10³ | 53.7 \(\times\) 10³ | —  |
| P       | 37.6 \(\times\) 10³ | 82.5 \(\times\) 10³ | 79.3 \(\times\) 10³ | 89.2 \(\times\) 10³ | 71.1 \(\times\) 10³ |
| Cd      | 0.70  | 0.70 | 0.71 | 0.70 | —   |
| P       | 0.66  | 0.75 | 0.84 | 0.64 | 0.65 |
| Co      | 0.86  | 0.85 | 0.83 | 0.84 | —   |
| P       | 0.53  | 1.05 | 0.86 | 1.00 | 0.69 |
| Cr      | —     | 0.89 | —   | 0.41 | —   |
| P       | 5.02  | 5.96 | 4.62 | 6.10 | 5.67 |
| Cu      | 717   | 668 | 669 | 779 | —   |
| P       | 471   | 645 | 652 | 851 | 552 |
| Fe      | 457 \(\times\) 10³ | 369 \(\times\) 10³ | 147 \(\times\) 10³ | 380 \(\times\) 10³ | —   |
| P       | 131 \(\times\) 10³ | 356 \(\times\) 10³ | 2.09 \(\times\) 10³ | 1.63 \(\times\) 10³ | 1.41 \(\times\) 10³ |
| K       | 171 \(\times\) 10³ | 216 \(\times\) 10³ | 139 \(\times\) 10³ | 216 \(\times\) 10³ | —  |
| P       | 142 \(\times\) 10³ | 305 \(\times\) 10³ | 157 \(\times\) 10³ | 290 \(\times\) 10³ | 157 \(\times\) 10³ |
| Mg      | 3580 \(\times\) 10³ | 3210 \(\times\) 10³ | 2490 \(\times\) 10³ | 3530 \(\times\) 10³ | —  |
| P       | 12.3 \(\times\) 10³ | 22.5 \(\times\) 10³ | 18.7 \(\times\) 10³ | 22.6 \(\times\) 10³ | 15.9 \(\times\) 10³ |
| Mn      | 8.12  | 3.21 | 2.68 | 5.37 | —   |
| P       | 3.45  | 4.98 | 4.65 | 4.33 | 3.70 |
| Mo      | 1.54  | 1.19 | 1.09 | 1.25 | —   |
| P       | 15.2  | 10.6 | 7.98 | 9.17 | 8.07 |
| Ni      | 0.72  | 0.85 | 0.80 | 0.84 | —   |
| P       | 2.54  | 16.94 | 8.20 | 5.34 | 4.10 |
| Pb      | 1.35  | 2.47 | 1.24 | 1.48 | —   |
| P       | 0.04  | 0.14 | <0.02 | <0.02 | <0.02 |
| Rb      | 63.0  | 126 | 55.0 | 127 | —   |
| P       | 37.1  | 102 | 37.3 | 94.7 | 39.8 |
| Se      | 476   | 330 | 530 | 420 | —   |
| P       | 513   | 701 | 607 | 793 | 840 |
| Sr      | 61    | 67 | 126 | 85 | —   |
| P       | 59.8  | 108 | 152 | 140 | 145 |
| V       | 0.88  | 0.85 | 0.89 | 0.88 | —   |
| P       | 1.66  | 3.62 | 5.77 | 4.58 | 4.61 |
| Zn      | 2500  | 1840 | 1360 | 2340 | —  |

animals showed alterations in the lung with hyperplastic lung lymph nodes and alveolar oedema and emphysema. In the bronchia of the animals Pv 01, 03, and 05, both, inflamed areas and nematodes, were present. One animal (Pv 02) showed a multifocal purulent bronchopneumonia without parasitic burden. The lungs of seal Pv 04 were not collabated and contained a stiff edem fluid. In two animals (Pv 01, 02), only little thymic tissue was found.
3.2. Health Parameters in Blood Samples. Serum electrophoresis showed in all five animals high amounts of the total protein concentrations compared to results in free-living seals (See supplementary materials available at doi:10.5402/2012/106259, Table S1) [28, 29]. Compared to free-living harbor seals of the Elbe estuary, the albumin-to-globulin ratios of all five animals were lower [12], indicating higher amount of globulins in relation to albumin in the sick animals than in clinically healthy seals. The concentrations of the acute phase protein CRP were high or in the upper range compared to reference date described for harbor seals [30]. The Hp concentrations were distinctly elevated for four of five animals (Table S1). However, the T3 and T4 values are lower concentrations, possibly due to diurnal variations were found (Table S1) [28, 29]. Compared to reference values for harbor seals [31]. Both enzymes in seals are mainly ALT in the serum of all five animals compared to reference variations were found (Table S1).

3.3. Elements in Whole Blood and Plasma. Addition to whole blood data concentrations in plasma samples was published for the first time in this study. Either plasma data to compare the results are not available for seals of this area. In the whole-blood samples, the concentrations of Cr, Fe, Mn, and Zn were significantly lower than the range measured in free-living seals of the German and Danish Wadden Sea (Table 2) [3]. As these elements are essential for mammals, the results reflect the malnutrition of the seals. Toxic metals were not significantly elevated (Table 2). Most of the elements showed no significant differences compared to our previous study on seals of the Wadden Sea [3]. Compared to animals of the Elbe estuary, many essential as well as toxic elements showed lower concentrations, possibly due to differences in location and feeding habitats as well as malnutrition and possible liver lesions [12].

3.4. Metal Hypersensitivities. The non- and PWM-stimulated lymphocyte proliferations were within the range measured for seals in the Wadden Sea (Table S2) [25]. All metal-specific SI values were in the normal range >0.1 and <3 (Table S2). Nevertheless, both Helgoland seals showed a possible metal-specific hypersensitivity with SI ≥ 2, seal Pv 01 to Cr, Mo, and Pb (multiple reactivity) and seal Pv 02 to Ni (single reactivity). Interestingly, comparing the animals of this study, Pv 01 had the highest Mo and Pv 02 the highest Ni concentration in plasma (Table 2).

3.5. Metal Concentrations in Liver, Kidney, and Muscle Tissue. All concentrations were below toxic thresholds (Table 3) [34]. In particular, the Cd concentrations in muscle as well as the Pb levels in all three sample types were very low or below the detection limit. An acute intoxication of the seals investigated from heavy metals can be excluded. Unfortunately, metal concentrations in tissues of harbor seals of the German North Sea have not been reported in the literature in the last years. However, compared to studies of the seventies, a clear trend is remarkable. While the concentrations of Cd remained in the same low range, the Pb and Hg values decreased significantly [35, 36].

**Table 3: Heavy metal concentrations in liver, kidney, and muscle samples [mg kg⁻¹ w.w.] samples.**

| Metal | Species  | Liver | Kidney | Muscle | Liver | Kidney | Muscle |
|-------|----------|-------|--------|--------|-------|--------|--------|
| As    | Pv 01    | 0.065 | 0.091  | 0.068  | 0.044 | 0.004  | 0.111  |
|       | Pv 02    | 0.064 | 0.085  | 0.062  | 0.005 | 0.005  | 0.087  |
|       | Pv 03    | 0.044 | 0.058  | 0.005  | 0.005 | 0.005  | 0.053  |
|       | Pv 04    | 0.021 | 0.004  | <0.002 | 0.005 | 0.005  | 0.010  |
|       | Pv 05    | 0.114 | 0.037  | 0.043  | 0.038 | 0.002  | 0.054  |
| Cd    | Pv 01    | <0.002| <0.002 | <0.002 | <0.002| <0.002 | <0.002 |
|       | Pv 02    | <0.002| <0.002 | <0.002 | <0.002| <0.002 | <0.002 |
| Hg    | Pv 01    | 0.040 | 0.025  | 0.003  | 0.006 | 0.024  | 0.041  |
|       | Pv 02    | 0.066 | 0.038  | 0.006  | 0.013 | 0.017  | 0.023  |
|       | Liver    | <0.0001| <0.0001| 0.018  | <0.0001| <0.0001| <0.0001|
| Pb    | Pv 01    | <0.0001| <0.0001| <0.0001| <0.0001| <0.0001| <0.0001|
|       | Muscle   | 0.004 | <0.0001| 0.006  | <0.0001| <0.0001| <0.0001|
3.6. Chlorinated Pesticides and PCBs in Plasma. Plasma concentrations of chlorinated pesticides including metabolites and PCBs for the investigated diseased seals from Helgoland (Pv 01–Pv 04) and Sylt (Pv 05) are given in Table 4. The mean currents in the German Bight transport water masses northwards along the coastline [37], carrying with them inputs from major estuaries, for example, the Rhine, Weser, or Elbe estuaries. Thus, waters around Helgoland can partly be influenced by these riverine inputs as well as the region of Sylt to a lesser extent.

In the comparison with other studies (Figure 2), hexachlorobenzene (HCB) concentrations span a range of three magnitudes. Plasma concentrations of diseased seals of Helgoland and Sylt were lower than in animals living in the Elbe estuary [12] but were higher than serum concentrations in seals of the North Sea [38]. In particular, the Helgoland seals of this study may be influenced by the Elbe plume.

DDT and metabolite concentrations (4,4′-DDE shown in Figure 2) were similar in all three studies. PCBs (PCB 138 shown in Figure 2) for diseased seals were in the same range as in the North Sea study, but slightly elevated compared to seals from the Elbe estuary. Interestingly, dieldrin and other cyclodiene insecticides and metabolites (endosulfan, chlordane, and heptachlor epoxide) showed higher plasma concentrations in the diseased seals of this study compared to animals of the Elbe estuary.

4. Conclusion

Although harbor and gray seals are tourist attractions of the islands Helgoland and Sylt, little is known about these local seal groups. This multiparameter investigation provides a first combined data set on health parameters and
contaminant burdens of five severely ill, juvenile harbor seals from Helgoland and Sylt, which were shot for animal welfare reasons. As expected, the results of pathology and blood investigations support the bad prognosis of survival made in the field. Although the levels of organic contaminants seem to be influenced by the Elbe plume, the concentrations of metal pollutants indicate similar or lower body burdens compared to former studies. However, further investigations are necessary to evaluate the results, and routinely investigations of these seal groups should be performed.

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