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Mineral elements and essential trace elements in blood of seals of the North Sea measured by total-reflection X-ray fluorescence analysis ☆

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Abstract

Mineral and essential trace elements are involved in numerous physiological processes in mammals. Often, diseases are associated with an imbalance of the electrolyte homeostasis. In this study, the concentrations of mineral elements (P, S, K, Ca) and essential trace elements (Fe, Cu, Zn, Se, Rb, Sr) in whole blood of harbor seals (*Phoca vitulina*) were determined using total-reflection X-ray fluorescence spectrometry (TXRF). Samples from 81 free-ranging harbor seals from the North Sea and two captive seals were collected during 2003–2005.

Reference ranges and element correlations for health status determination were derived for P, S, K, Ca, Fe, Cu, and Zn level in whole blood. Grouping the seals by age, gender and sample location the concentration levels of the elements were compared.

The blood from two captive seals with signs of diseases and four free-ranging seals showed reduced element levels of P, S, and Ca and differences in element correlation of electrolytes were ascertained.

Thus, simultaneous measurements of several elements in only 500 μL volumes of whole blood provide the possibility to obtain information on both, the electrolyte balance and the hydration status of the seals. The method could therefore serve as an additional biomonitoring tool for the health assessment.
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Keywords: Total-reflection X-ray fluorescence; Blood; Marine mammals; North Sea; Health; Biomonitoring tool

1. Introduction

According to the Trilateral Dutch–German–Danish Monitoring and Assessment Program (TMAP), seals are used as biological parameters for monitoring the state of the coastal ecosystem of the North Sea [1,2]. A Seal Management Plan (SMP) [3,4] has been developed in order to describe the population in the Wadden Sea Coast of the North Sea.

The usual way to estimate the health status of seals in the North Sea is by veterinary investigation of wild living animals and carcasses found at the coasts [5]. One part to assess the condition of these animals is the determination of element distribution in tissues of seals during dissections. Numerous reports have documented the concentrations of various elements in liver, kidney, muscle or the blubber following necropsies of marine mammals. However, less work has been published on harbor seals [6–9]. In order to evaluate the present status of seals in the North Sea, it is necessary to investigate free-ranging animals. Studies of living animals are limited by the availability of sample types and quantity. In fact, sampling is restricted to blood, saliva, faeces, body secretion or hair. Some studies described the influences of environmental parameters on blood chemistry as well as hematology in harbor seals [10,11]. However, such data on seals of the North Sea are still fragmentary. Current, normal ranges of mineral and essential elements in the whole blood of free-ranging seals in the Wadden Sea are lacking. Furthermore, health assessment requires the definition of blood chemistry reference ranges for each specific population [12].

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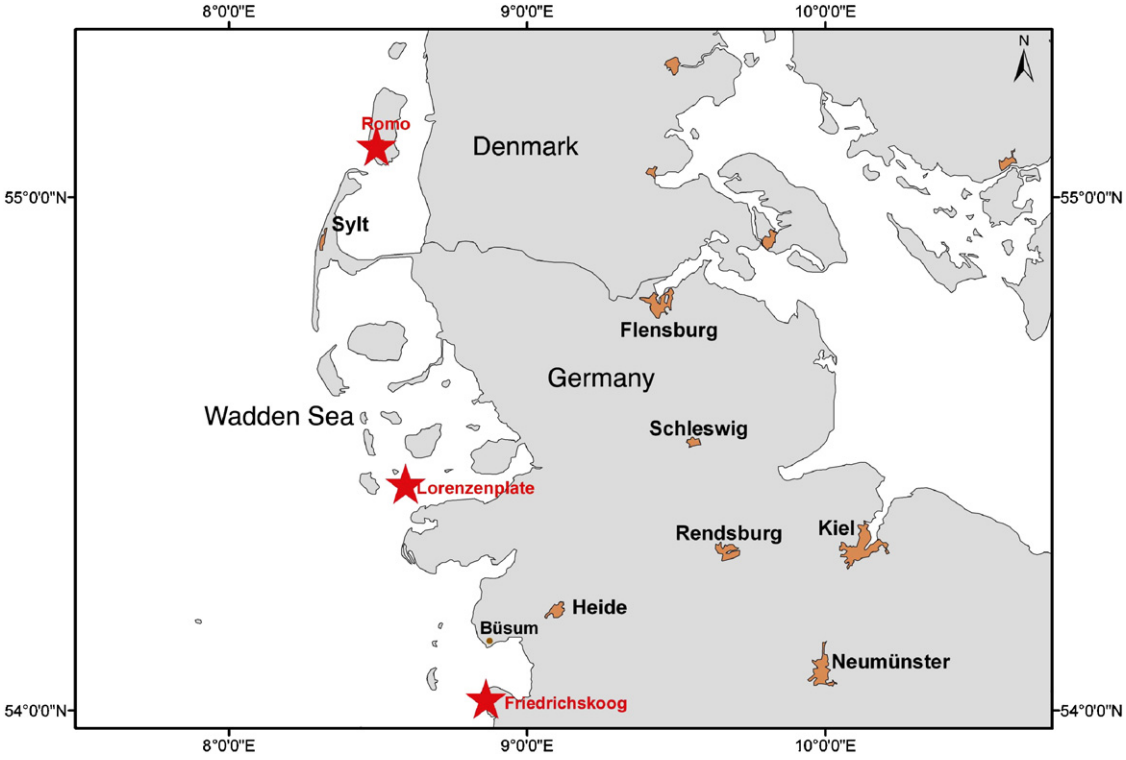

Fig. 1. Sampling locations.

Sampling in field on a sand bank, without laboratory equipment and difficulties getting useable serum or plasma, requires an analytical method for whole blood.

Due to its versatility and multi-element capacity, total-reflection X-ray fluorescence (TXRF) is used for the qualitative and quantitative analysis of elements in biomedical samples. Since only a few elements have been detected in the whole blood [13,14], serum [15,16] and plasma [17,18] of human beings, developing a multi-element method for the whole blood of marine mammals is a new task. By analyzing the mineral elements P, S, K, and Ca and the essential trace elements Fe, Cu, Zn, Se, Rb, and Sr in the whole blood of living seals more information about their electrolyte balance and nutritional status needs to be obtained. Furthermore, reference ranges for the seals investigated were developed, as well as correlations between elements were calculated.

Table 1
Number of animals investigated, per haul-out site/location, gender and age classes

Location	Gender	Age		Overall number
		Adult	Juvenile	
Lorenzenplate	M	20	12	32
	F	5	17	22
Lorenzenplate total		25	29	54
Rømø	M	21	1	22
	F	3	2	5
Rømø total		24	3	27
Overall number		49	32	81

M–male; F–female.

The aim of this study was to investigate the suitability of measuring mineral and essential trace elements as a monitoring tool and investigating the applicability of this results for a supplementary tool for the diagnosis of the health status of harbor seals of the Wadden Sea. These results should indicate physiological ranges of the elements, which can be used to detect electrolyte imbalance, the hydration status and, along with medical checks, the condition of the animals.

2. Materials and methods

2.1. Sampling and preparation

Fresh whole blood samples were collected from 81 harbor seals (*Phoca vitulina*). The animals were captured by net during

Table 2
Results of the reference material Clin Check whole blood level II ($n=10$)

Element	Certified		Measured		LOD (µg/L)
	Mean value (µg/L)	Control range (µg/L)	Mean value (µg/L)	±S.D. (µg/L)	
Cr	7.1	6.0–8.2	<LOD		200
Mn	27	22–32	<LOD		250
Co	4.7	3.7–5.7	<LOD		270
Ni	8.3	6.4–10.2	<LOD		85
Zn	2449	1954–2939	2201	247	71
Se	171	137–205	154	17.0	45
Cd	4.3	3.5–5.1	<LOD		
Pb	304	258–350	281.0	23.0	150
Hg	15	12–18	–	–	–

S.D.=standard deviation; LOD=limit of detection

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