Aquatic Mammals 2008, *34*(2), 149-156, DOI 10.1578/AM.34.2.2008.149

Differential Hematology Profiles of Free-Ranging, Rehabilitated, and Captive Harbor Seals (*Phoca vitulina*) of the German North Sea

Ilka Hasselmeier,[1] Sonja Fonfara,[2] Jörg Driver,[3] and Ursula Siebert[1]

[1]*Research and Technology Center Westcoast of the University of Kiel, Werftstr. 6, 25761 Büsum, Germany; E-mail: ih@ftz-west.uni-kiel.de*
[2]*The GKSS Research Center, Institute for Coastal Research, Max-Planck-Str. 1, 21502 Geesthacht, Germany*
[3]*Veterinary Clinic, Boßelweg 10, 25764 Reinsbüttel, Germany*

Abstract

The hematology profile is an established tool to monitor health status and to help detect emerging diseases in animals. Knowledge of normal ranges is required, however, to evaluate blood results (e.g., WBC, neutrophils, eosinophils, monocytes, hemoglobin). Differential hematology profiles of three harbor seal (*Phoca vitulina*) groups (free-ranging animals, rehabilitated pups, and captive seals at the Seal Center Friedrichskoog), collected between 1997 and 2004, were compared. The three different groups are representative of the population of harbor seals in the German North Sea. Results indicated that these groups differed significantly from one another in their hematology profiles, thus data were re-examined with respect to hematology profile variation according to location (i.e., wild, rehabilitated, captive), age (i.e., pups, yearlings, adults), and season (i.e., spring, summer, autumn). This represents the first time that a large number of hematology profile results ($n = 793$) were collected from three groups of a single population, resulting in the establishment of baseline values (5 and 95 percentiles, median). This study is an important contribution to the understanding and assessment of the health status of harbor seals.

Key Words: captive, free-ranging, harbor seal, hematology, rehabilitation, *Phoca vitulina*

Introduction

The differential hematology profile is a common procedure used to investigate the health status of humans and animals. Hematology of marine mammals provides substantial information on the physiological condition, but it is essential to have reference values to more accurately evaluate an individual's health status. The harbor seal (*Phoca vitulina*) is one of the most common pinnipeds held in captivity, second only to California sea lions (*Zalophus californianus*) (Andrews et al., 2000). Since captive animals depend on human care, reference values are needed to provide the best possible medical attention. A shortcoming of many marine mammal hematology studies, including those of wild animals, is insufficient knowledge of their life history. Pinnipeds are known to show clinical symptoms only at a very late stage (Reynolds & Rommel, 1999; Dierauf & Gulland, 2001). Deviations of hematologic values from baseline levels might therefore be used to assess treatment or the need for more intensive testing of a suspected problem.

Relatively little has been published regarding hematologic reference values for harbor seals, and previous studies were either comprised of only one group of seals or had small sample sizes (McConnell & Vaughan, 1983; Roletto, 1993; Morgan et al., 1998; Bossart et al., 2001; Lander et al., 2003).

Since 1999, the state of Schleswig-Holstein, Germany, has funded a monitoring project to examine live harbor seals in the German North Sea. For this study, reference ranges of blood constituents for free-ranging, rehabilitated, and captive harbor seals were compared. The hematology profiles presented here will be useful for routine health examinations of harbor seals of different ages and living conditions.

The study was primarily concerned with blood values that potentially indicate diseases (Dierauf & Gulland, 2001): white blood cells (WBC) and their derivatives (e.g., lymphocytes, neutrophils, eosinophils, monocytes), red blood cells (RBC), hemoglobin (HB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and thrombocytes (PLT).

Materials and Methods

Blood was always collected from the epidural vertebral vein with a needle (1.2 × 100 mm) and syringe and immediately transferred into a tube containing ethylene diamine tetraacetic acid (EDTA). The tubes were carefully rocked and kept at room temperature until further handling. Most blood samples were processed within 1 to 12 h.

During March/April (spring), August/September (summer), and October through December (autumn) 1997-2004, 170 free-ranging harbor seals were caught with a net (3 m × 200 m), transferred into tube nets, and restrained manually (x = 45 min) for blood collection and measurements. The animals were caught on the sandbank Lorenzenplate (54° 25' N, 8° 39' E), Germany, and in Rømø, Denmark (Figure 1). Captures always occurred around noon, according to low tide at the sandbank. Three age groups were classified: (1) animals born the same year of the catch (pups), (2) seals born the year previous to the catch (yearlings), and (3) animals at least 2 y old (older). Seals are known to travel hundreds of kilometers regardless of season (Reijnders et al., 2005). As the pupping season of harbor seals in the German North Sea is at the end of May, there were no pups available in the first catching season (spring).

Seal pups are sometimes stranded or left behind by their mothers for a variety of reasons (e.g., storms, disturbance by humans, short foraging trips). If pups were found along the coast of Schleswig-Holstein, they were monitored by trained seal hunters for 1 to 2 tides. If the mother did not return, they were brought to the Seal Center Friedrichskoog for rehabilitation. Hematology profiles of pups were examined when first admitted into the Seal Center Friedrichskoog (before given any medication) and shortly before release. To keep the results independent and to get more representative results, only the pre-release values were tested for reference (n = 127). After 2 to 3 mo, the pups were usually released into the wild again. Sometimes a pup would not get a health certificate due to behavioral or physical abnormalities. Those animals were kept at the center and were introduced to the other harbor seals living in captivity. Another criteria for getting a health certificate was that a pup had to be without medication for at least 10 d. Only blood values from pups with the certificate were included in this study.

The eight tested animals held at the Seal Center were also confined in tube nets for blood withdrawal. No anesthesia was used. One-hundred-six repeat samples of the eight different animals of varying ages and different sexes were collected. Blood values were tested to ascertain whether the animals living in the same environment or of different sexes and ages could be pooled. Repeated measures ANOVA analysis was used because

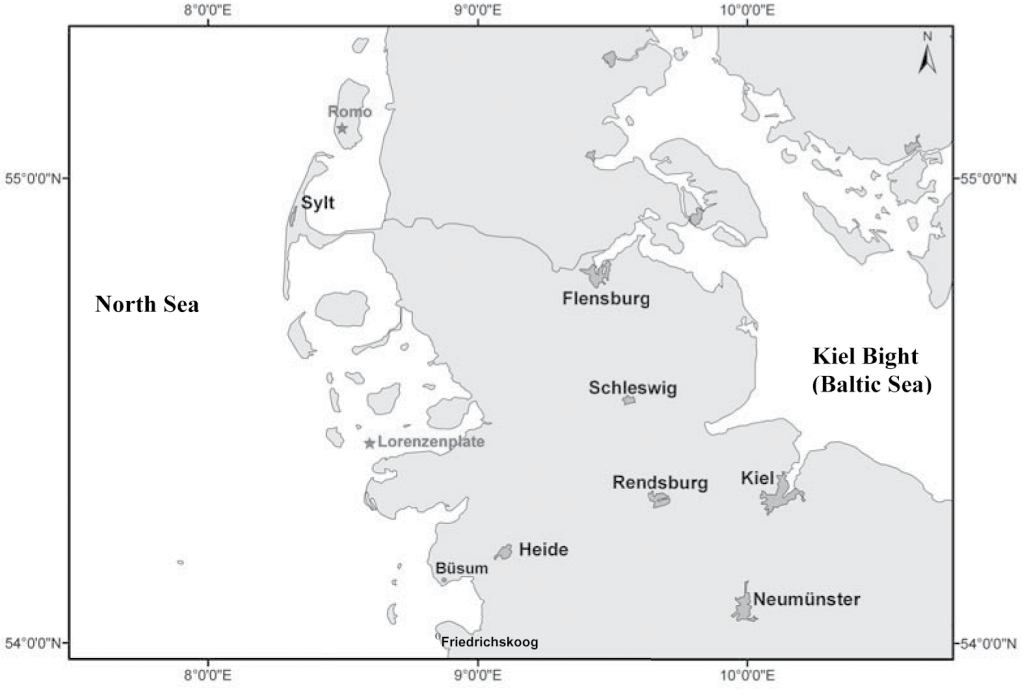

Figure 1. Study areas in the German and Danish North Sea, with the two catching locations marked with an asterisk

of the inter-dependency of the sample values. Not all of the hematology profiles were suitable for analysis and not every blood value could be measured in every animal due to clotted blood or unsuitable staining of blood smears (Tables 1 & 2).

From 1997 to 2001, the Veterinary Laboratory Ingolstadt GmbH, and later the Veterinary Laboratory in Geesthacht, used an Abbott Cell Dyn 3500 (Diamond Diagnostics, Holliston, MA, USA) to process differential hematology profiles. Beginning in 2001, a private veterinary clinic analyzed the hematology profiles using a ScilVet ABC (Scil Animal Care Company GmbH, D-68519 Viernheim, Germany). During the first phase using the ScilVet ABC, hematology profiles were simultaneously measured at the laboratory in Geesthacht to check for comparability. No differences between the two devices were found. Blood smears were dyed with Diff Quick® (Baxter Dade AG, CH-3186 Dudingen, Switzerland), and the leukocyte subgroups were counted manually (40x magnification) at all three laboratories. In some cases, no leukocyte subgroups could be counted; therefore, discrepancies in sample sizes between percentage and numerical values of leukocytes occurred.

Statistical Considerations

The median and quartile range (5 to 95%) were chosen as baseline values because the values were not normally distributed (Begemann, 1999; Mahlberg et al., 2005). In case of sample sizes $n < 10$, the min and max were depicted. For the free-ranging animals and rehabilitated pups, the sample size count reflects individuals; whereas for the captive animals, it is the number of hemograms investigated (Tables 1 & 2).

For each seal group, differences among seasons (separately for different age classes) and ages (separately for different seasons) were assessed using Mann-Whitney U or Kruskal-Wallis H tests, respectively. Captive seals were tested for differences between individuals with the same tests as those used on the free-ranging animals. In general, nonparametric tests were used because most variables did not match the assumptions required for parametric tests. Analyses were conducted only for those age classes (i.e., pups, juveniles, and adult females, respectively) for which there were sufficient sample sizes. Since 16 different blood values were examined, an alpha-level adjustment was required; otherwise, 16 tests of the essential 0-hypothesis would lead to an inflated probability. For this purpose, Fisher's Omnibus test was applied, which combines a number of *p*-values into a single Chi-square distributed variable, with degrees of freedom equalling twice the number of *p*-values (i.e., blood values) (Haccou & Meelis, 1994). Exact tests were used when small samples required their use (McConnell & Vaughan, 1983; Morgan et al., 1998). All indicated *p*-values are two-tailed, and 5% was chosen as the significance level.

Results

Data of free-ranging animals of the two catch locations were pooled because of close geographical proximity between sites and no statistically significant differences in hematology profiles (Fisher's Omnibus test: $\chi^2 = 89.33$, df = 128, $p > 0.08$). Statistical tests of free-ranging harbor seals from the North Sea indicated that blood values varied among seasons and age classes. In detail, testing for differences among seasons revealed significant effects in all three age classes (Fisher's Omnibus test, pups: $\chi^2 = 81.46$, df = 32, $p < 0.001$; yearlings: $\chi^2 = 67.54$, df = 32, $p < 0.001$; older: $\chi^2 = 217.80$, df = 32, $p < 0.001$).

During the autumn, at least 50% of pups had at least 9% monocytes; whereas at least 50% of yearlings had none (Table 1). The medians of the neutrophils (%) also varied between the yearlings caught in spring (56%) and the older animals caught in summer (33%) (Table 1).

Similarly, the age-classes of free-ranging seals differed significantly in all three seasons (spring: $\chi^2 = 72.28$, df = 32, $p < 0.001$; summer: $\chi^2 = 84.32$, df = 32, $p < 0.001$; autumn: $\chi^2 = 61.42$, df = 32, $p < 0.001$). Within the group of older animals, for example, the neutrophil median ranged from 33% (summer) to 50% (spring) (Table 1).

Results of rehabilitated pups were similar to those of free-ranging animals. With a median of 69% neutrophils (pre-release value of rehabilitated pups), however, the proportion was greater than that of free-ranging animals, and it was almost twice as high as that of free-ranging animals of about the same age (pups caught in the summer). With a median of only 1%, eosinophils were lowest in the rehabilitated pups (Table 1).

Results for captive animals were more varied than expected. Within this group, only hematology profiles among the three adult females did not differ significantly ($\chi^2 = 24.10$, df = 32, $p = 0.84$). Significant differences were found between the two pups (Hinnerk and Lilli) and the juvenile (Mareike) ($\chi^2 = 51.48$, df = 32, $p = 0.02$) and also between the two adult males (Lümmel and Hein) ($\chi^2 = 58.2$, df = 32, $p = 0.003$). The WBC not only differed considerably within the captive group but also from free-ranging animals and rehabilitated pups. The percentages of neutrophils of the captive animals were greater than those of the other two groups (i.e., free-ranging seals and rehabilitated pups); whereas the percentage of eosinophils

Table 1. Differential hematology profile of white blood cells (WBC) and their derivatives from free-ranging harbor seals (w), divided into age groups (pups, yearlings, older) and seasons, including spring (spr), summer (su), and autumn (aut), and the pre-release blood withdrawal of rehabilitated seal pups (rehab_pups_last) and captive animals of the Seal Center Friedrichskoog. The first number indicates the median with the count as index, and the numbers within the parentheses depict the percentile range (5 to 95%). For the free-ranging animals (w) and the pups, the sample size count reflects individuals; whereas for the captive animals, it is the number of hemograms investigated. In cases of sample sizes < 10, min and max rather than percentiles were depicted.

	WBC (/µl)	Lym (#)	Lym (%)	Mono (#)	Mono (%)	Neutro (#)	Neutro (%)	Eos (%)
w_pups_su	9.5_{15} (6.5-13.5)	2.3_{5} (1.3-3.0)	38.0_{17} (21.0-50.0)	0.2_{5} (0.1-0.2)	2.0_{17} (0.0-7.0)	5.6_{5} (3.3-6.8)	36.0_{5} (24.0-54.0)	18.0_{17} (10.0-31.0)
w_pups_aut	9.1_{12} (6.8-12.5)	1.0_{10} (0.5-2.5)	20.0_{12} (6.5-25.0)	0.3_{10} (0.1-0.5)	9.0_{12} (0.0-19.0)	7.0_{10} (4.0-11.3)	51.0_{10} (34.0-60.0)	16.0_{12} (13.0-32.0)
w_yearlings_spr	10.7_{28} (8.0-15.0)	2.6_{28} (0.9-4.1)	28.0_{27} (6.5-43.0)	0.2_{28} (0.1-0.5)	0.0_{27} (0.0-4.0)	7.9_{27} (5.4-12.7)	56.0_{27} (35.0-68.0)	17.0_{27} (8.0-29.0)
w_yearlings_su	9.0_{8} (5.6-11.9)	2.8_{2} (2.6-3.0)	35.5_{8} (23.0-45.0)	0.1_{2} (0.1-0.1)	3.0_{8} (2.0-12.0)	5.7_{2} (5.4-5.9)	44.0_{2} (40.0-48.0)	16.0_{8} (9.0-20.0)
w_yearlings_aut	9.3_{13} (6.3-13.1)	1.8_{10} (1.0-2.6)	24.0_{13} (11.0-44.0)	0.3_{10} (0.1-0.7)	1.0_{13} (0.0-12.0)	6.5_{10} (3.6-20.4)	50.0_{10} (32.0-80.0)	21.0_{13} (3.0-34.0)
w_older_spr	9.9_{25} (8.5-15.0)	2.3_{25} (0.9-4.0)	30.0_{23} (18.0-48.0)	0.2_{25} (0.1-0.7)	1.0_{23} (0.0-6.0)	7.3_{25} (5.8-13.1)	50.0_{23} (40.0-66.0)	14.0_{23} (6.0-26.0)
w_older_su	9.2_{35} (7.0-14.1)	2.7_{21} (1.6-4.1)	37.0_{37} (14.0-60.0)	0.2_{21} (0.1-0.3)	6.0_{37} (0.0-36.0)	5.9_{21} (3.8-7.8)	33.0_{21} (20.0-60.0)	15.0_{37} (7.0-25.0)
w_older_aut	10.0_{28} (7.6-13.8)	1.7_{24} (1.0-2.5)	19.0_{27} (10.0-46.0)	0.4_{24} (0.1-0.9)	1.0_{28} (0.0-21.0)	7.8_{24} (5.2-10.8)	46.0_{23} (33.0-69.0)	18.0_{27} (9.0-32.0)
rehab_pups_last	9.0_{127} (6.1-12.3)	2.5_{95} (0.7-4.2)	25.0_{71} (8.0-43.0)	0.2_{95} (0.1-0.5)	3.0_{71} (0.0-11.0)	6.6_{95} (4.7-8.9)	69.0_{71} (53.0-86.0)	1.0_{71} (0.0-6.0)
Lümmel (male, adult)	11.3_{22} (7.2-17.9)	1.6_{5} (0.6-4.5)	19.0_{20} (12.0-26.0)	0.3_{5} (0.2-0.6)	2.0_{20} (0.0-9.0)	8.0_{15} (5.3-10.8)	75.0_{20} (63.0-82.0)	3.0_{17} (1.0-8.0)
Nell/Deern/Lütte (females, adults)	7.7_{34} (4.9-16.5)	0.6_{7} (0.3-2.2)	15.0_{36} (10.0-31.0)	0.2_{7} (0.1-0.2)	2.0_{36} (1.0-10.0)	6.3_{6} (2.8-7.6)	77.0_{36} (63.0-85.0)	4.0_{32} (1.0-10.0)
Hein (male, pup→adult)	12.1_{20} (8.4-15.7)	2.2_{9} (1.0-3.7)	22.0_{18} (12.0-38.0)	0.3_{9} (0.1-0.4)	3.0_{18} (1.0-11.0)	8.8_{9} (6.5-13.3)	66.0_{18} (52.0-82.0)	4.0_{15} (0.0-9.0) Hein
Mareike (female, juvenile)	8.9_{7} (7.8-12.2)	2.5_{7} (0.8-4.0)	22.0_{5} (11.0-36.0)	0.3_{7} (0.1-0.3)	8.0_{5} (2.0-16.0)	7.0_{7} (5.2-8.5)	62.0_{7} (46.0-85.0)	8.0_{5} (4.0-12.0)
Lilli (female, pup)	8.3_{5} (4.9-9.6)	2.3_{5} (1.2-3.5)	16.0_{5} (10.0-18.0)	0.2_{5} (0.1-0.3)	12.0_{5} (6.0-18.0)	5.7_{5} (3.6-6.9)	70.0_{5} (68.0-70.0)	2.0_{5} (2.0-2.0)
Hinnerk (male, pup)	10.4_{4} (9.0-11.4)	2.3_{4} (1.6-3.3)	28.0_{4} (24.0-30.0)	0.3_{4} (0.2-0.5)	6.0_{4} (4.0-12.0)	7.1_{4} (6.8-9.2)	64.0_{4} (62.0-64.0)	2.0_{4} (0.0-4.0)

Table 2. Differential hematology profile of red blood cells (RBC) and their derivatives from free-ranging harbor seals (w), divided into age groups (pups, yearlings, older) and seasons, including spring (spr), summer (su), and autumn (aut), and the pre-release blood withdrawal of rehabilitated seal pups (rehab_pups_last) and captive animals of the Seal Center Friedrichskoog. The first number indicates the median with the count as index, and the numbers within the parentheses depicted the percentile range (5 to 95%). For the free-ranging animals (w) and the pups, the sample size count reflects individuals; whereas for the captive animals, it is the number of hemograms investigated. In case of sample sizes < 10, min and max rather than percentiles were depicted.

	RBC (t/l)	HB (g/dl)	HCT (%)	MCH (pg)	MCHC (g/dl)	MCV (fl/µm³)	RDW (%)	PLT (g/l)
w_pups_su	4.8_{15} (4.0-5.6)	20.0_{15} (17.5-22.0)	58.0_{15} (45.0-62.0)	41.5_{15} (37.4-45.2)	34.0_{15} (32.0-38.7)	112.0_{7} (110.0-119.0)	16.0_{5} (15.0-17.0)	298.0_{15} (175.0-478.0)
w_pups_aut	5.0_{12} (4.6-5.6)	20.5_{12} (18.0-22.5)	55.0_{12} (51.0-64.0)	40.4_{12} (37.0-44.4)	36.8_{12} (33.0-39.4)	109.0_{12} (106.0-115.0)	16.0_{8} (15.0-19.0)	380.0_{12} (276.0-433.0)
w_yearlings_spr	4.8_{28} (3.8-5.4)	19.0_{28} (14.5-21.5)	54.0_{28} (39.0-61.0)	39.9_{28} (34.4-43.5)	35.6_{28} (33.5-41.1)	110.0_{28} (101.0-116.0)	15.0_{18} (15.0-17.0)	380.0_{28} (265.0-495.0)
w_ yearlings_su	4.6_{8} (3.8-6.3)	19.0_{8} (17.0-20.0)	53.0_{8} (45.0-73.0)	41.2_{8} (40.4-45.0)	35.8_{8} (34.2-37.9)	116.0_{7} (112.0-119.0)	16.0_{2} (15.0-16.0)	297.0_{8} (121.0-373.0)
w_yearlings_aut	4.9_{13} (3.9-5.3)	20.5_{13} (16.0-22.0)	54.0_{13} (43.0-64.0)	41.0_{13} (39.0-44.7)	36.8_{13} (32.6-38.6)	112.0_{13} (107.0-119.0)	16.0_{4} (15.0-16.0)	326.0_{13} (195.0-535.0)
w_older_spr	4.7_{25} (4.3-5.3)	20.5_{25} (18.0-22.0)	53.0_{25} (48.0-59.0)	44.1_{25} (36.3-46.7)	38.1_{25} (32.4-41.4)	114.0_{25} (109.0-119.0)	15.0_{16} (14.0-16.0)	292.0_{25} (228.0-407.0)
w_older_su	4.3_{35} (3.7-4.7)	18.5_{35} (16.5-19.5)	50.0_{35} (43.0-55.0)	43.6_{35} (36.6-47.4)	38.0_{35} (33.8-39.2)	116.0_{29} (112.0-124.0)	16.0_{21} (14.0-18.0)	341.0_{35} (248.0-407.0)
w_older_aut	4.7_{29} (4.3-5.4)	21.0_{29} (18.0-23.0)	56.0_{29} (47.0-65.0)	42.8_{29} (41.0-46.5)	37.3_{29} (32.4-38.9)	114.0_{29} (112.0-121.0)	15.0_{17} (15.0-16.0)	314.0_{29} (230.0-373.0)
rehab_pups_last	4.8_{127} (3.8-5.7)	16.5_{127} (13.5-21.0)	46.0_{127} (36.0-59.0)	36.0_{127} (28.4-39.8)	36.4_{126} (28.0-39.0)	99.0_{116} (82.0-112.0)	16.0_{94} (15.0-19.0)	583.0_{124} (316.0-743.0)
Lümmel (male, adult)	4.8_{21} (3.8-5.7)	20.0_{21} (17.0-22.0)	56.0_{22} (46.0-69.0)	40.5_{22} (38.0-48.6)	35.0_{19} (31.9-40.7)	118.0_{18} (107.0-123.0)	14.0_{8} (13.0-15.0)	329.0_{22} (155.0-499.0)
Nell/Deern/Lütte (females, adults)	5.1_{34} (3.7-5.9)	21.0_{34} (15.5.24.5)	59.0_{34} (45.0-68.0)	41.0_{34} (38.7-45.8)	35.0_{23} (32.0-39.7)	117.0_{34} (106.0-125.0)	14.0_{7} (13.0-15.0)	246.0_{31} (115.0-458.0)
Hein (male, pup→adult)	4.8_{20} (4.2-6.0)	19.0_{20} (16.0-21.0)	51.0_{20} (46.0-61.0)	39.0_{20} (30.0-48.6)	35.7_{20} (32.8-41.9)	108.0_{20} (93.0-117.0)	14.0_{9} (13.0-17.0)	370.0_{20} (148.0-582.0)
Mareike (female, juvenile)	5.2_{7} (4.0-5.7)	21.4_{7} (15.0-24.0)	59.0_{7} (42.0-60.0)	45.8_{7} (4.0-53.5)	39.1_{7} (35.0-45.5)	117.0_{7} (92.0-120.0)	15.0_{7} (14.0-22.0)	294.0_{7} (263.0-683.0)
Lilli (female, pup)	5.0_{5} (4.3-5.5)	21.0_{4} (19.5-24.0)	53.0_{5} (47.0-63.0)	46.6_{5} (43.9-49.7)	40.8_{5} (37.8-46.5)	114.0_{5} (100.0-117.0)	16.0_{5} (13.0-17.0)	387.0_{5} (136.0-494.0)
Hinnerk (male, pup)	4.7_{4} (4.0-5.2)	19.0_{4} (17.5-21.0)	48.0_{4} (41.0-53.0)	40.2_{4} (39.8-44.7)	39.8_{4} (39.4-42.9)	102.0_{4} (100.0-104.0)	15.0_{4} (14.0-22.0)	392.0_{4} (360.0-477.0)

lay between the free-ranging and rehabilitated animals.

Red blood cells and related blood values appeared more homogeneous within groups (Table 2). Noteworthy differences were only found within the group of rehabilitated pups. The HB of rehabilitated pups was less than in the other groups. Accordingly, MCH, MCHC, and MCV were also lower. The RBC distribution width, however, was the same as in the free-ranging and captive animals. Another conspicuous value was that of the platelets. With at least 50% of the rehabilitated pups having ≥ 583 (g/l) platelets, they had considerably larger values than the free-ranging animals and most of the captive seals.

Discussion

Overall, differences among hematology profiles for the three harbor seal groups highlighted the need to distinguish among different groups, ages, and seasons in order to develop proper baseline hematology profile values for harbor seals. This observation was comparable to other studies of free-ranging and captive groups (McConnell & Vaughan, 1983; Bossart et al., 2001; Lander et al., 2003; Trumble et al., 2006).

Usually, hematology profile values were depicted as means with respective standard deviations (SD). To establish reference ranges, however, it seemed more sensible to provide the median and interpercentile ranges as is regularly done for ranges of human blood chemistry (Begemann, 1999; Mahlberg et al., 2005).

The erythrocyte hematology profile was more homogeneous than the leukocyte profile. The latter is influenced more by physiological changes in the animals (e.g., stress, bacterial or viral diseases). In the free-ranging individuals, this is especially true since their life histories were not known. Even though animals were immediately released if they appeared sick or too stressed, seals with minor injuries or infections could have been part of the sample. The large number of animals, however, should have handled potentially skewed data for this reason.

Young free-ranging animals (about 3 mo of age) had the highest proportion of lymphocytes. Maternal antibody flow during nursing time lasts 4 to 6 wks (Reynolds & Rommel, 1999). After 3 to 4 mo, the immune system should be developed for the most part (Tizard, 2000). The abundance of pathogens to which the pups were exposed stimulated the immune reaction. An increase in lymphocytes observed in dogs (2 mo of age) probably results from an immune response due to numerous new antigens after weaning (Mischke, 2003). Interestingly, lymphocytes in all three age groups of seals peaked during the summer. These results corroborated a study by Fonfara (Sonne et al., 2007), who found that cytokines of the specific immune response in the same study group of seals were also elevated during the summer. As the study was done in 2004 and 2005, this might still be a consequence of the seal die-off in 2002.

Monocytes did not seem to vary much within or among groups. However, differences in the interpercentile ranges were variable, especially for free-ranging adult seals caught during summer (0 to 36%). Considerable variation of monocytes is common in manually prepared blood smears (Roletto, 1993; Bishop & Morado, 1995).

Free-ranging seals of about 1½ y had the largest proportion of eosinophils. These cells are mostly responsible for parasitic defense (Begemann, 1999; Mahlberg et al., 2005). The animals of this age group had the highest infestation of parasites. This was confirmed by pathological findings during dissections performed at the Research and Technology Center Westcoast, Germany (Lehnert et al., 2007).

Unlike in McConnell & Vaughan (1983), the WBC counts of rehabilitated pups (9.0/µl) were lower than those of young free-ranging animals (9.5/µl). This discrepancy may have resulted from McConnell & Vaughan averaging all blood samples collected during rehabilitation. In this study, only the pre-release values were taken into consideration for comparison. When pups were first admitted, however, their immune systems were impaired as indicated by little or no IgG (Hasselmeier et al., in prep.) and a lower WBC count (7.6/µl). The results of this study possibly support the conclusion of McConnell & Vaughan (1983) that rehabilitated pups are more susceptible to infections than free-ranging pups. *In vitro* stimulated immune cells of blood samples from admitted pups showed, however, that they are capable of initiating a defense mechanism (Kakuschke et al., 2005).

High eosinophil values were not expected for rehabilitated pups because they were fed inspected and frozen fish and therefore should not have been exposed to parasites. Occasional necropsies of pups that died of various infections during rehabilitation indicated no infection with parasites. Additionally, water from Friedrichskoog Harbor was cleaned mechanically (different sizes of pebbles) and chemically (ozone) before it was released into the pools.

The MCV was another conspicuously lower blood value of the rehabilitated pups. The MCV of harbor seals is greater than terrestrial animals or humans but lower than that of other phocids such as elephant seals (*Mirounga angustirostris*, *M. leonina*) (Wickham et al., 1989; Knickel et al.,

2002; Braun & Dormann, 2003). The larger size of RBCs in free-ranging and captive seals is most likely an adaptation to diving and more activity during their development (Wickham et al., 1989; Dierauf & Gulland, 2001; Lander et al., 2003).

Unexpectedly, the variability within the captive group of harbor seals was very high. Only the blood values of the three adult females could be pooled. Like the rehabilitated pups, all captive animals had a very low proportion of eosinophils. As they were fed the same inspected and frozen fish and the water in the pool was prepared the same way as it was for the rehabilitation pools, they also should not have been exposed to parasites. The relatively high percentage of eosinophils in Mareike cannot be explained; she came into the Seal Center as a weaned pup of a few months of age and therefore might have had prior contact with parasites. As she was treated with anthelmintics and fed the same inspected fish as the other animals, the presence of eosinophile cannot be explained by parasites. The lifespan of an eosinophilic granulocyte is approximately 10 d, but the retention period in the peripheral blood is only 10 h (McConnell & Vaughan, 1983). MELISA tests have shown a significant allergization to molybdenum, however (A. Kakuschke, pers. comm.). As the remaining white hematology profile did not show any sign of infections, this might be the first indication of an allergic reaction in harbor seals. More samples should be collected to further investigate this phenomenon.

The red hematology profile was more homogeneous than the leukocyte ranges. This applied to the blood values within as well as among the three different groups. McConnell & Vaughan (1983) and Lander et al. (2003) found the greatest differences between free-ranging and captive seals in the RBC and HB values. These results could not be entirely corroborated with this study. Only the HB of rehabilitated pups was considerably lower than in the other groups. When admitted, the median levels of RBC (6.1 t/l) and HB (21.1 g/dl) were considerably higher than prior to their release. This phenomenon was also described in other studies (McConnell & Vaughan, 1983; Bossart et al., 2001). The pups' lower levels of HB prior to release, compared to free-ranging pups of approximately the same age were most likely due to lack of activity during their rehabilitation. As diet can also have an influence on RBC and HB (Geraci, 1975; Messow & Hermanns, 1990), the diet seemed adequate at the Seal Center. There were only minor or no differences in RBC and HB values.

Conclusions

These data support other studies dealing with questions of health status. This study showed that it is vitally important to separate animals into different (sub)groups and use proper statistical tests when examining blood values. Analysis techniques must be consistent to facilitate comparisons between different studies. It also seemed important to verify whether captive animals could be pooled as has been done in many previous analyses (McConnell & Vaughan, 1983; Dierauf & Gulland, 2001). The life history of an animal could be complex or not available. In addition, the number of samples plays an essential role in establishing baseline data. This study is an important contribution to the understanding and assessment of the health status of harbor seals. The three different groups are representative of the harbor seal population in the German North Sea and of captive harbor seals, but the data also can be consulted with respect to other populations. Several potential subgroups are still underrepresented, so further investigations and comparisons should be undertaken. Continuous, ongoing efforts are also necessary to maintain a stable monitoring program.

Acknowledgments

The authors thank the Seal Center Friedrichskoog for collecting the necessary blood samples from rehabilitated pups and their resident animals. We also thank the veterinarian laboratories in Geesthacht and Ingolstadt for their support. Roger Mundry is acknowledged for his untiring statistical support. The wild captures were supported by the National Park Service and the Schleswig-Holstein Ministry of Agriculture, Environment and Rural Areas. The authors are also grateful to all the helpers who participated in the seal catches.

Literature Cited

Andrews, B. F., Duffield, D. A., & McBain, J. F. (1997). Marine mammal management: Aiming at year 2000. *IBI Report*, *7*, 125-130.

Begemann, M. (1999). *Praktische hämatologie—Klinik, therapie, methodik.* Stuttgart, Germany: Thieme-Verlag.

Bishop, D. H., & Morado, J. F. (1995). Results on blood cell morphology and differential blood cell counts from seventeen Steller sea lion *Eumetopias jubatus* pups. *Journal of Diseases of Aquatic Organisms*, *23*, 1-6.

Bossart, G. D., Reidarson, T. H., Dierauf, L. A., & Duffield, D. A. (2001). Clinical pathology. In L. A. Dierauf & F. M. D. Gulland (Eds.), *CRC handbook of marine mammal medicine* (pp. 383-436). Boca Raton, FL: CRC Press. 1,063 pp.

Braun, J., & Dormann, A. (Hrsg.) (2003). *Klinikleitfaden innere medizin* (9. Auflage). München, Germany: Urban und Fischer Verlag.

Dierauf, L. A., & Gulland, F. M. D. (Eds.). (2001). *CRC handbook of marine mammal medicine* (2nd ed.). Boca Raton, FL: CRC Press.

Geraci, J. R. (1975). Pinniped nutrition, *Rapport Proces-verbaux des Reunions Conseil International pour l'Exploration de la Mer*, *169*, 312-323.

Haccou, P., & Meelis, E. (1994). *Statistical analysis of behavioural data*. Oxford, UK: Oxford University Press.

Kakuschke, A., Valentine-Thon, E., Griesel, S., Fonfara, S., Siebert, U., & Prange, A. (2005). Immunological impact of metals in harbor seals (*Phoca vitulina*) of the North Sea. *Environmental Science and Technology*, *39*, 7568-7575.

Knickel, U. R., Wilczek, C., & Jöst, K. (2002). Laborwerte. In *MemoVet Praxis-Leitfaden Tiermedizin* (4th ed.) (pp. 245-247). Stuttgart: Schattauer.

Lander, M. E., Harvey, J. T., & Gulland, F. M. D. (2003). Hematology and serum chemistry comparisons between free-ranging and rehabilitated harbor seal (*Phoca vitulina richardii*) pups. *Journal of Wildlife Diseases*, *39*, 600-609.

Lehnert, K., Raga, J. A., & Siebert, U. (2007). Parasites in harbour seals (*Phoca vitulina*) from the German Wadden Sea between two Phocine Distemper Virus epidemics. *Helgoland Marine Research*, *61*, 239-245.

Mahlberg, R., Gilles, A., & Läsch, A. (2005). *Hämatologie* (2. Auflage). Weinheim, Germany: Wiley-VCH Verlag GmbH & Co., KGaA.

McConnell, L. C., & Vaughan, R. W. (1983). Some blood values in captive and free-living common seals (*Phoca vitulina*). *Aquatic Mammals*, *10*, 9-13.

Messow, C., & Hermanns, W. (1990). Entzündung. In L-C. Schulz (Ed.), *Lehrbuch der allgemeinen pathologie für tierärzte und studierende der tiermedizin* (pp. 275-311). Stuttgart, Germany: Ferdinand Enke Verlag Stuttgart. 462 pp.

Mischke, R. (2003). *Praktische hämatologie bei hund und katze.* Dorfles-Esbach, Germany: Schlütersche Verlag. 216 pp.

Morgan, L., Kumaresan, S., Thomas, C., & MacWilliams, P. (1998). Hematology and chemistry reference values for free-ranging harbor seals (*Phoca vitulina*) and the effects of hemolysis on chemistry values of captive harbor seals. *Journal of Zoo and Wildlife Medicine*, *29*, 394-400.

Reijnders, P. J. H., Reineking, B., Abt, K. F., Brasseur, S. M. J. M., Camphuysen, K., Scheidat, M., et al. (2005). Marine mammals. In K. Essink, C. Dettmann, H. Farke, K. Laursen, G. Lüerβen, H. Marencic et al. (Eds.), *Wadden Sea quality status report 2004* (pp. 317-330). Wilhelmshaven, Germany: Ecosystem No. 19, Common Wadden Sea Secr.

Reynolds, J. E., III, & Rommel, S. A. (Eds.). (1999). *Biology of marine mammals.* Washington, DC: Smithsonian Institution Press.

Roletto, J. (1993). Hematology and serum chemistry values for clinically healthy and sick pinnipeds. *Journal of Zoo and Wildlife Medicine*, *24*, 145-157.

Sonne, C., Fonfara, S., Dietz, R., Kirkegaard, M., Letcher, R. J., Shahmiri, S., et al. (2007). Multiple cytokine and acute-phase protein gene transcription in West Greenland sledge dogs (*Canis familiaris*) dietary exposed to organic environmental pollutants. *Archives of Environmental Contamination and Toxicology*, *53*, 110-118.

Tizard, I. R. (2000). *Veterinary immunology: An introduction* (6th ed.). Oxford, UK: W. B. Saunders Company.

Trumble, S. J., Castellini, M. A., Mau, T. L., & Castellini, J. M. (2006). Dietry and seasonal influences on blood chemistry and hematology in captive harbor seals. *Marine Mammal Science*, *22*(1), 104-123.

Wickham, L. L., Elsner, R., White, F. C., & Cornell, L. H. (1989). Blood viscosity in phocid seals: Possible adaptations to diving. *Journal of Comparative Physiology, Part B*, 159, 153-158.