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HEMATOLOGY AND SERUM CHEMISTRY COMPARISONS BETWEEN FREE-RANGING AND REHABILITATED HARBOR SEAL (*PHOCA VITULINA RICHARDSI*) PUPS

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ABSTRACT: The objectives of this study were to compare the hematology and serum chemistry values between free-ranging and stranded harbor seal (*Phoca vitulina richardsi*) pups and to ascertain how blood values of stranded pups changed during the rehabilitation process. Coincident with these comparisons, reference values were obtained for free-ranging pups. Stranded harbor seal pups ($n=28$) recovered from areas between Pebble Beach and Moss Landing, California (USA) were admitted to The Marine Mammal Center, Sausalito, from March to May 1995, 1996, and 1998. Blood samples were collected from harbor seal pups before and after rehabilitation. As a control group, wild harbor seal pups were captured at Pebble Beach and Elkhorn Slough ($n=42$) during the 1995, 1996, and 1998 pupping seasons. Mean eosinophil and calcium values of wild pups were significantly greater than those of newly admitted pups, whereas mean bands, aspartate aminotransferase, alanine aminotransferase, total bilirubin, and chloride values were significantly lower ($P\leq0.05$). Mean neutrophil, band, lymphocyte, eosinophil, basophil, calcium, phosphorus, blood urea nitrogen, potassium, total protein, and globulin values of rehabilitated pups increased significantly after 2–3 mo in captivity, whereas, mean red blood cell, hemoglobin, hematocrit, cholesterol, and total bilirubin values decreased significantly ($P\leq0.05$).

Key words: Harbor seal, hematology, *Phoca vitulina richardsi,* rehabilitation, serum chemistries.

INTRODUCTION

Pinnipeds are among the marine mammals most commonly rehabilitated after stranding (Gulland et al., 2001). Each year, many Pacific harbor seal (*Phoca vitulina richardsi*) pups come ashore on mainland beaches of California due to illness and injury (Dougherty, 1983; Seagars et al., 1986). Stranded harbor seal pups from San Luis Obispo County to Mendocino County, California (USA), are admitted for rehabilitation to The Marine Mammal Center (TMMC), Sausalito. When pups are first admitted to TMMC, blood samples are collected for hematologic and serum biochemical evaluation (Gulland, 1999), which are the most commonly used parameters for the clinical evaluation of disease and injury for pinnipeds and other marine mammals (Bossart et al., 2001).

Reference values for blood constituents, which have been established for monitoring the health of wild populations (Horning and Trillmich, 1997; Morgan et al., 1998; Rea et al., 1998; Sepúlveda et al., 1999; Trumble and Castellini, 2002) and improving the management and medical care of pinnipeds for public display, captive breeding programs, or rehabilitation centers (Roletto, 1993; St. Aubin et al., 1996; Reidarson et al., 2000), are available for a variety of free-ranging and captive species. Because reference values for free-ranging harbor seal pups are limited, however, clinically healthy rehabilitated pups at TMMC have been used to establish normal ranges for health assessment. Captivity imposes several environmental constraints upon animals, however, and demands physiologic adjustments that may be reflected in clinical laboratory values (Bossart et al., 2001). Diseases, changes in diet, handling, experimental regimes, and artificial environments such as fresh water may also stress captive animals (Engelhardt, 1979; McConnell and Vaughan, 1983). Consequently, we were interested

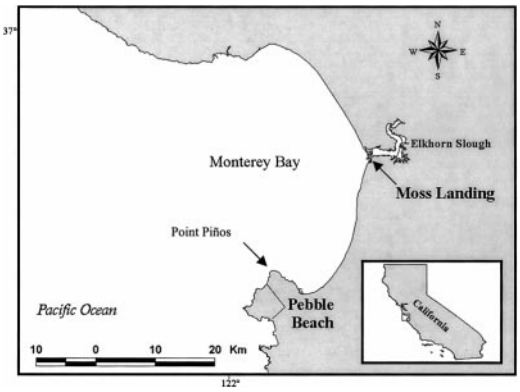

FIGURE 1. Stranded harbor seal (*Phoca vitulina*) pups from areas between Pebble Beach and Moss Landing, central California, were used for this study. Wild harbor sea pups were captured at Pebble Beach and Elkhorn Slough. Areas (including Point Piños) where three seals were recaptured are also illustrated on the map.

in investigating how ranges developed by TMMC would compare with that of wild conspecifics.

Acquisition of baseline data from a combination of stranded and wild animals provides a more comprehensive understanding of population health (Reddy et al., 2001). The goal of this study was to compare hematology and serum chemistry values between free-ranging and stranded harbor seal pups, controlling for age class, season, geographic location, and laboratory techniques. Specific objectives included: 1) collection of reference values for wild harbor seal pups, 2) comparison of blood profiles between wild and stranded harbor seal pups, and 3) examination of how blood profiles of stranded pups changed after rehabilitation.

MATERIALS AND METHODS

All harbor seal pups that stranded in areas between Pebble Beach (36°34′N, 121°59′W) and Moss Landing (36°48′N, 121°47′W), California, and survived treatment at TMMC, were used for this study during 1995, 1996, and 1998 (Fig. 1). Upon admission, initial assessment of pups included an examination of general condition using a pupgar scoring technique, and determination of body mass to the nearest 1.0 kg, standard length to the nearest 1.0 cm, body temperature, and hydration (Smith and Stone, 1992). Ages of pups were determined according to pelage and tooth development, umbilical regression, and mass (Gulland et al., 1997). A condition index (CI; mass × 100/length) was calculated for all pups. To examine complete blood counts (CBC) and serum chemistries of pups when first admitted ("admit"), blood samples were collected from 28 harbor seal pups within 24 hr after arriving at TMMC (n=4 males and 11 females during 1995, n=three males and five females during 1996, and n=one male and four females during 1998). Blood samples were collected from the epidural vertebral vein of pups into ethylenediaminetetraacetic acid (EDTA) vacutainers and serum separator tubes using a 20 gauge × 38 mm needle. Serum tubes were allowed to clot for 15 min before being centrifuged at 3,000 × G for 15 min. Complete blood counts of whole blood were determined using a Sysmex microcell-counter (F-800; Long Grove, Illinois, USA). Blood smears were made from whole blood samples and stained with Wright's stain. White blood cell (WBC) differentials, including segments, bands, lymphocytes, monocytes, eosinophils, and basophils, were counted manually by the laboratory technician at TMMC. Absolute values of differentials were reported. A Vet Test 8008 (Idexx Laboratories Inc., Westbrook, Maine, USA) was used to analyze serum chemistry panels. Sodium (Na), potassium (K), and chloride (Cl) values were determined using a VetLyte Electrolyte Analyser (Idexx Laboratories Inc.).

While being cared for at TMMC, harbor seal pups were housed together after they were physically stable (e.g., globulin values were within normal ranges and contagious diseases were not detected from external examination, fecal examination, hematology, or radiography) to promote social behaviors and interaction with conspecifics. Harbor seal pups were tube-fed a Multi-Milk™ formula (Pet Ag, Elgin, Illinois) and rehydrating solutions before being weaned to whole North Atlantic and Pacific herring (*Clupea* sp.) supplemented with vitamin E, pinniped multivitamins (Pinnivites®, Mazuri, Richmond, Indiana, USA), and salt tablets. Antibiotic treatment was used as needed during the rehabilitation process.

After rehabilitation, blood samples were collected again from the same pups to examine their "pre-release" values. Pups were weighed to the nearest 1.0 kg and standard length was measured to the nearest 1.0 cm. The CI also was calculated again after rehabilitation.

As a control group, 37 newly weaned, wild harbor seal pups (n=12 males and one female during 1995, n=three males and five females during 1996, and n=three males and 13 fe-

males during 1998) were captured using salmon nets at Pebble Beach during the months of May and June. Five additional harbor seal pups (n=four males and one female) were captured in Elkhorn Slough, Moss Landing, California on 3 May 1996, following methods of Jeffries et al. (1993) (Fig. 1). Wild harbor seal pups were weighed to the nearest 1.0 kg, and standard length was measured to the nearest 1.0 cm. As for rehabilitated pups, the CI was computed for wild pups. Blood samples were collected from wild harbor seal pups within 1 hr of capture using a vacutainer assemblage, consisting of a 8.9 cm × 18 gauge spinal needle, vacutainer holder, and an adapter. Samples of blood were stored on ice until transported to the lab, and serum separator tubes were centrifuged within 4 hr of being collected. Complete blood counts and serum chemistries were processed at TMMC within 24 hr of collection.

Blood samples were collected from three harbor seals that were recaptured during the course of this study. One free-ranging male (#170), which was captured on Pebble Beach and frequently sighted within the general vicinity thereafter, was recaptured on Pebble Beach 1 yr later. Additionally, one rehabilitated female pup (#449) was recaptured during subsequent tagging studies in Elkhorn Slough, 11 days after it had been released, and one rehabilitated male pup (#613) stranded live at Point Piños, Pacific Grove (Fig. 1), 43 days after release.

After testing for homogeneity of variances and normality (Kolmogorov-Smirnov test, Systat 8.0.1, 1999, Systat Software Inc., Richmond, California), analysis of variance (ANOVA) were used to determine whether mean CI differed among years for each of the three data sets (Zar, 1984). After pooling data across years for each data set, a paired t-test was used to compare mean CI values calculated for pups before and after rehabilitation, whereas a two-sample t-test was used to compare mean CI between pre-release pups and wild pups (Systat; Zar, 1984).

Because condition of seals did not differ among years (see below) and we were not interested in further examining annual variation of blood values, cumulative sample precision (standard error divided by the mean: SE/$\bar{x}$; Krebs, 1989) of each parameter was plotted for each seal group after pooling data across years to determine if an adequate number of blood samples were collected. Bootstrap resampling techniques (n=1,000 replicates) were used to establish reference ranges ($\bar{x}$±95% BCa percentiles) for wild pups (S-Plus, 2000 Professional, Mathsoft, Inc., Cambridge, Massachusetts, USA) (Table 1). Because homogeneity of variances and normality (Kolmogorov-Smirnov test, Systat) were not evident for some blood values, randomization tests (Resampling Statistics Inc. 4.1b4, Arlington, Virginia, USA) were used to compare mean blood values of wild pups with those of admitted rehabilitated pups. Paired t-tests were used to compare mean admit blood values of rehabilitated pups with their mean pre-release values (Systat), after mean differences of blood parameters were plotted to test for normality. It was assumed that significant differences between admit and pre-release values also occurred between wild and pre-release values when admit and wild values were statistically similar. A significance level of $P \leq 0.05$ was used for all statistical tests.

RESULTS

Mean CI values did not differ among years for admitted (F=2.57, P=0.10), pre-release (F=2.48, P=0.10), or wild (F=1.58, P=0.22) pups; thus, data were pooled across years for each group. Mean CI of admitted harbor seal pups ($\bar{x}$ CI=12.5, SE=0.3, n=27; $\bar{x}$ weight=9.1 kg, SE=0.3; $\bar{x}$ SL=72.7 cm, SE=1.5) increased significantly after rehabilitation ($\bar{x}$ CI=24.0, SE=0.8, n=27; $\bar{x}$ weight=21.1 kg, SE=0.7; $\bar{x}$ SL=87.7 cm, SE=1.l; t=0.00, P=0.00), whereas CI of pre-release rehabilitated harbor seal pups did not differ significantly from wild pups ($\bar{x}$ CI=22.0, SE=0.8, n=42; $\bar{x}$ weight=19.0 kg, SE=0.8; $\bar{x}$ SL=85.6 cm, SE=1.1; t=−1.84, P=0.07).

Cumulative sample precision curves indicated that adequate sample sizes were collected for all blood parameters (data not reported; see Lander [1998] for an illustration of partial data). Mean eosinophil and calcium (Ca) values of wild pups were significantly greater than those of newly admitted pups, whereas bands, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (tot bili), and Cl were significantly lower ($P \leq 0.05$; Table 1). Mean WBCs, neutrophils, bands, lymphocytes, eosinophils, basophils, Ca, phosphorus, blood urea nitrogen (BUN), K, total protein, and globulins of rehabilitated pups increased significantly after 2–3 mo in captivity, whereas, mean red blood cell (RBC), hemoglobin (HGB), hemato-

crit (HCT), cholesterol, and tot bili values decreased significantly ($P \leq 0.05$; Table 1). Changes in blood values for the three animals that were recaptured are illustrated in Table 2.

DISCUSSION

Concentrations of RBC and HGB generally are greater for neonates at birth and decrease as animals grow, gain weight, and learn to dive (Castellini et al., 1996; Bossart et al., 2001). Mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) values, however, should increase because of the increased need for oxygen (Bossart and Dierauf, 1990). During this study, mean RBC and HGB values of admitted neonates at TMMC decreased significantly from time of admission to time of release, whereas MCV and MCH values remained statistically unchanged. Although rehabilitated harbor seal pups did gain weight while at TMMC, they may not have developed, nor had the need, for an increased oxygen carrying capacity because their diving activities were limited.

Many researchers have focused on the adaptive significance of erythrocyte parameters and their association with diving behavior and apnea (Castellini et al., 1986; Kopec and Harvey, 1995). McConnell and Vaughan (1983) found that RBC, HGB, and HCT values of captive common seals (*P. vitulina vitulina*) were consistently less than values of free-ranging seals. They assumed this was probably due to the decreased activity of the seals in captivity caused by the lack of space and water depth. Results from this study indicated similar trends; mean values of RBC, HGB, and HCT for pre-release pups were significantly less than mean values of wild pups. Harbor seal pups at TMMC were contained in pens that were approximately 5 m × 7 m, with pools that were only 1 m deep. Their diving activities in captivity, therefore, were restricted and not equivalent to that of wild harbor seal pups. Because blood values were not sampled during an experimental diving regime, it can only be assumed that erythrocyte values of wild harbor seal pups were greater than those of rehabilitated harbor seal pups as a result of having some diving experience. Interestingly, RBC, HGB, and HCT values increased for the two rehabilitated pups that were recaptured after they had been diving in the wild (Table 2).

A decreased RBC count also may indicate anemia due to nutritional deficiency of iron, vitamin B_{12}, or protein. It would then be expected that RBC values of newly admitted pups, rather than release values, would be significantly less than values of wild pups. However, anemia can be masked by concomitant dehydration (Bossart et al., 2001). Harbor seal pups commonly are dehydrated when admitted to TMMC, and this was a possibility, as indicated by the increased mean chloride and HCT levels (Bossart et al., 2001).

In addition to factors listed above, diet also may cause a decrease in RBC and HGB values while in captivity (McConnell and Vaughan, 1983). It is unclear whether rehabilitated pups suffered from dietary deficiencies during this study. Rehabilitated harbor seal pups were fed frozen herring (*Clupea* sp.), after being thawed, a process that destroys many vitamins (Geraci, 1975; McConnell and Vaughan, 1983). Rehabilitated pups, however, were provided with vitamins (i.e., vitamin E and pinniped multivitamins, Pinnivites®, Mazuri, Richmond, Indiana) during treatment. Furthermore, herring yields maximum weight gain and has the greatest percentage of fat among commercially available fish (Chen-Valet et al., 1990). Mean cholesterol of wild pups, however, was significantly greater than that of rehabilitated pups, possibly indicating the diet of wild pups had a greater fat concentration, depending on when seals were caught with respect to their last meal. Lower phosphorus values of admitted pups may have indicated a dietary calcium deficiency (Bossart et al., 2001), which may have corresponded to their lower calcium values. However, the increased levels of phospho-

TABLE 1. Mean ± standard deviation (SD), range, and sample size (*n*) of hematology and serum chemistry values of wild harbor seal pups and rehabilitated harbor seal pups when first admitted to TMMC (Admit) and just before release (Pre-release). Reference ranges (mean ± 95% confidence intervals (CI) generated using bootstrap resampling are also presented for wild pups. Asterisks (*) indicate a significant difference ($P \leq 0.05$) between the two groups they are centered. Abbreviations as in text.

	Wild pups				
	Mean ± SD	Mean ± 95% CI	Range	*n*	*P*
CBC					
WBC $10^3/\mu l$	8.7 ± 3.2	8.7 ± 0.9	4.6–20.8	42	0.49
RBC $10^6/\mu l$	5.4 ± 0.5	5.4 ± 0.1	3.8–6.4	42	1.01
HGB g/dl	21.1 ± 1.7	21.1 ± 0.4	16.2–24.8	42	0.77
HCT %	61.0 ± 6.7	61.0 ± 1.6	42.4–78.8	42	0.69
MCV (U3)	113.2 ± 6.1	113.2 ± 1.6	100.9–122.7	42	0.12
MCH (UUG)	39.3 ± 2.9	39.4 ± 0.6	31.8–46.8	42	0.66
MCHC g/dl	34.8 ± 2.6	34.8 ± 0.6	28.6–38.9	42	0.49
Differential					
Neutrophils	5,159.8 ± 2,054.8	5,164.0 ± 736.0	2,401.0–13,520.0	40	0.16
Bands	97.2 ± 159.7	79.7 ± 47.0	0.0–656.0	40	*0.00
Lymphocytes	2,536.2 ± 1,411.8	2,538.0 ± 411.0	972.0–6,448.0	40	0.39
Monocytes	463.0 ± 389.6	460.6 ± 107.4	0.0–1480.0	40	0.83
Eosinophils	272.0 ± 394.8	268.5 ± 149.6	0.0–2132.0	40	*0.05
Basophils	187.3 ± 272.8	188.0 ± 90.7	0.0–1148.0	40	0.13
Chemistry					
AST IU	42.3 ± 19.6	42.5 ± 4.7	9.0–105.0	40	*0.02
Alk. Phos IU	472.6 ± 383.3	470.7 ± 127.9	87.0–1,778.0	41	0.08
ALT IU	21.6 ± 7.7	21.6 ± 2.2	12.0–43.0	34	*0.04
Chol mg/dl	265.9 ± 63.7	266.7 ± 14.7	119.3–399.4	41	0.64
Creat mg/dl	0.6 ± 0.1	0.6 ± 0.0	0.3–1.0	41	0.19
Glucose mg/dl	175.4 ± 30.4	175.3 ± 7.3	96.2–239.6	41	0.51
Ca mg/dl	10.3 ± 0.7	10.3 ± 0.2	8.6–11.5	42	*0.01
Phos mg/dl	7.1 ± 1.5	7.1 ± 0.4	4.9–10.5	41	0.80
Tot Bili mg/dl	0.5 ± 0.3	0.5 ± 0.1	0.0–2.0	41	*0.05
BUN mg/dl	43.4 ± 9.5	43.5 ± 2.4	24.2–63.5	42	0.47
Na mmol/l	155.3 ± 3.2	155.4 ± 0.7	149.2–162.6	39	0.09
K mmol/l	4.7 ± 0.5	4.7 ± 0.1	3.7–6.2	39	0.19
Cl mmol/l	109.5 ± 2.0	109.5 ± 0.5	106.4–114.6	39	*0.04
Albumin g/dl	3.1 ± 0.2	3.1 ± 0.1	2.5–3.6	42	0.20
Tot protein g/dl	6.5 ± 0.5	6.5 ± 0.1	5.4–7.6	42	0.14
Globulin g/dl	3.4 ± 0.4	3.4 ± 0.1	2.5–4.3	39	0.33

rus found for pre-release and wild pups may have just reflected skeletal growth (Bossart and Dierauf, 1990; Converse et al., 1994), and increased calcium levels in pre-release pups may have just been a result of increased total protein concentrations (Bossart et al., 2001).

The greater BUN values of pre-release pups may have been a result of a high protein diet (Bossart et al., 2001). Mean total protein of this group was greater than that of wild pups, which may have been a result of greater values of globulins in pre-release pups. Globulins consist of many proteins involved in a variety of physiologic responses and increased levels may have been due to stimulation of globulin production during recovery from disease. Lower values of globulin and decreased numbers of lymphocytes found for admitted pups may have been a result of decreased immune stimulation (Christopher et al., 1999; Bossart et al., 2001).

Mean total bilirubin of admitted harbor seal pups was significantly greater than that of wild harbor seal pups, but it is com-

TABLE 1. Extended.

Admit pups				Pre-release pups		
Mean ± SD	Range	*n*	*P*	Mean ± SD	Range	*n*
9.7 ± 3.9	4.6–19.5	27	*0.00	15.0 ± 5.3	7.6–30.4	27
5.4 ± 0.4	4.7–6.4	27	*0.00	4.5 ± 1.1	3.2–9.5	27
20.9 ± 2.0	14.7–23.9	27	*0.00	17.5 ± 1.7	13.2–20.4	27
59.8 ± 6.7	47.9–77.7	25	*0.00	47.5 ± 6.4	35.4–59.4	25
108.8 ± 5.9	98.3–122.6	26	0.62	109.8 ± 7.1	101.8–131.3	26
38.8 ± 3.0	33.6–46.7	26	0.12	40.3 ± 5.0	21.3–48.7	26
35.5 ± 2.7	30.1–42.2	26	0.18	37.0 ± 5.0	19.7–43.9	26
6,702.7 ± 3,101.4	3,220.0–15,210.0	24	*0.0	10,155 ± 4,357.3	5,332.0–21,280.0	24
211.1 ± 233.9	0.0–630.0	24	*0.02	496.3 ± 551.4	0.0–1,221.0	24
2,077.3 ± 1,046.6	708.0–5,500.0	24	*0.00	3,152.0 ± 1,132.9	735.0–5,587.0	24
409.2 ± 644.6	0.0–2,172.0	24	0.22	568.9 ± 661.9	0.0–2,128.0	24
53.2 ± 101.2	0.0–2,196.0	24	*0.03	476.0 ± 852.8	0.0–3,344.0	24
61.9 ± 121.8	0.0–390.0	24	*0.01	251.70 ± 306.4	0.0–1,057.0	24
94.8 ± 122.0	31.0–626.0	22	0.51	77.6 ± 25.6	42.0–138.0	22
244.0 ± 185.7	21.0–936.0	26	0.08	294.4 ± 194.3	52.0–878.0	26
54.2 ± 52.0	12.0–206.0	26	0.31	45.4 ± 20.3	15.0–94.0	26
248.3 ± 71.1	37.1–353.8	23	*0.00	195.1 ± 36.6	142.9–288.0	23
0.4 ± 0.2	0.2–1.0	26	0.43	0.5 ± 0.2	0.3–1.1	26
164.8 ± 58.3	77.2–353.4	26	0.06	140.8 ± 26.5	75.7–190.7	26
9.3 ± 0.8	7.9–11.1	26	*0.00	10.7 ± 1.0	8.9–14.0	26
6.9 ± 1.3	4.5–9.3	24	*0.01	8.4 ± 1.3	5.8–10.8	24
3.1 ± 5.0	0.0–22.1	26	*0.03	0.7 ± 1.2	0.01–6.3	26
39.6 ± 15.6	16.0–74.2	26	0.09	48.5 ± 8.4	26.6–64.3	26
158.8 ± 6.2	150.0–175.5	25	0.60	158.1 ± 4.7	148.0–166.2	25
4.3 ± 0.6	3.4–5.4	25	*0.00	4.9 ± 0.5	3.8–5.7	25
113.1 ± 6.1	102.0–129.5	25	*0.00	108.8 ± 2.9	102.0–112.0	25
2.9 ± 0.5	2.1–3.8	26	*0.01	3.3 ± 0.5	2.6–5.4	26
6.1 ± 0.9	4.9–9.7	26	*0.00	7.9 ± 1.0	6.1–9.8	26
3.3 ± 0.8	2.4–6.6	25	*0.00	4.7 ± 1.0	2.7–6.3	25

mon for neonatal harbor seals to have high bilirubin values (Dierauf et al., 1984). Increased total bilirubin values may indicate RBC breakdown, liver dysfunction, bile flow impairment in the common bile duct, and other health problems (Kopec and Harvey, 1995; Bossart et al., 2001). Gerber et al. (1993), however, found that admitted harbor seal pups with bilirubin levels between 3.0 and 15.0 mg/dl, had no ill effects. This was probably due to sudden breakdown of fetal erythrocytes and saturation of bilirubin pathways. After treatment, bilirubin values of rehabilitated pups during this study decreased and were similar to values of wild pups.

The stressful effect of restraint and handling must be considered when measuring blood parameters and interpreting these results (Chapple et al., 1991). Stress resulting from fear, excitement, apprehension, or handling can cause splenic contraction in phocids, resulting in an increase of erythrocytes into circulation (Geraci and Smith, 1975; Castellini et al., 1986; Ponganis, 1992). Mean RBC, HGB, and

TABLE 2. Hematology and serum chemistry values at time of capture and recapture for one wild pup (#170) and at time of release and recapture for two rehabilitated pups (#449 and #613). Abbreviations as in text.

	Pup #170		Pup #449[a]		Pup #613	
	Initial capture	Recapture	Pre-release	Recapture	Pre-release	Recapture
CBC						
WBC $10^3/\mu l$	7.5	11.2	9.2	7.7	18.1	16.5
RBC $10^6/\mu l$	5.1	4.5	4.8	5.4	3.8	5.3
HGB g/dl	21.4	17.3	19.4	21.3	15.2	20.7
HCT %	58.3	55.8	47.7	56.3	38.6	52.5
MCV (U3)	114.8	123.7	99.4	104.6	102.1	99.6
MCH (UUG)	42.1	38.4	40.4	39.6	40.2	39.3
MCHC g/dl	36.7	31.0	40.7	37.8	39.4	39.4
Differential						
Neutrophils	4,725.0	5,376.0	5,336.0	4,312.0	14,118.0	11,550.0
Bands	225.0	0.0	0.0	77.0	0.0	0.0
Lymphocytes	2,400.0	4,704.0	2,208.0	2,387.0	2,715.0	1,320.0
Monocytes	75.0	0.0	644.0	693.0	1,086.0	825.0
Eosinophils	0.0	896.0	368.0	231.0	0.0	1,155.0
Basophils	75.0	224.0	644.0	0.0	181.0	1,650.0
Chemistry						
AST IU	105.0	64.0	281.0	—	73.0	108.0
Alk. Phos IU	296.0	38.0	239.0	181.0	114.0	58.0
ALT IU	32.0	29.0	95.0	—	32.0	31.0
Chol mg/dl	235.7	182.9	285.0	278.1	182.0	214.0
Creat mg/dl	0.5	0.4	0.6	0.7	1.1	0.9
Glucose mg/dl	185.6	223.4	164.0	176.1	146.0	103.0
Ca mg/dl	10.5	9.5	10.1	9.7	8.9	10.0
Phos mg/dl	8.4	3.8	9.5	7.2	8.9	5.9
Tot Bili mg/dl	0.5	0.8	0.9	1.4	1.0	0.3
BUN mg/dl	43.2	33.3	65.0	—	46.0	29.0
Na mmol/l	151.6	162.1	154.0	155.3	148.0	149.0
K mmol/l	5.3	4.5	5.7	4.8	5.3	4.3
Cl mmol/l	111.4	115.0	109.0	110.3	102.0	99.0
Albumin g/dl	3.1	2.8	4.2	3.1	2.8	2.6
Tot protein g/dl	6.4	11.0	8.1	6.8	9.0	7.8
Globulin g/dl	3.3	8.2	3.9	3.7	6.2	6.1

[a] Stranding and morphometric details for pup 449 admitted to TMMC in 1997 were not included in analyses in Table 1 and can be found in Harvey and Lander (1999).

HCT values of wild and admit pups that were captured may have been greater than mean values of pre-release pups as a result of splenic contraction and acute stress, which is caused by release of adrenaline. Once seals have been in captivity and are habituated to people and handling, this stress response may not occur. Rehabilitated pups, however, were subjected to the long-term stress of captivity. Because it was unlikely that rehabilitated pups were suffering from an increased endo- or ecto-parasitic load, it is possible the observed increase of neutrophils found for pups after rehabilitation may have resulted from chronic stress, which causes cortisol release from the adrenal cortex (Schalm, 1965). Abnormal WBC counts may indicate inflammation, infection, certain drug toxicities, stress, or strenuous exercise. Although mean WBC count of rehabilitated pups before release was significantly greater than that of wild pups, rehabilitated pups were believed to be free of clinical

diseases; hence, this difference may have been due to subclinical infection or stress factors.

Differences between the seal groups may have also resulted from individual variation, sex (proportions) or age (weeks) related biases, or differences in handling of blood samples (e.g., blood samples collected from pups at TMMC were centrifuged shortly after allowing to clot for 15 min, whereas blood samples collected from wild harbor seal pups were centrifuged as much as 4 hr after collection). However, the latter may not have been problematic because Fadely (1997) found that elapsed time between sample collection and processing (up to 8 hr) did not account for any of the variation found for harbor seal blood values when other factors such as age, sex, and season were considered. It should also be noted that three small, nursing wild pups were included in analyses, and it was possible that a few other pups were not weaned. Additionally, when sampling the presumably healthy, wild population, it was possible that values indicative of illness or disease were included in the range of values of individuals, skewing the reference range towards that of diseased individuals (Kopec and Harvey, 1995). Unlike rehabilitated harbor seal pups, individual history of wild pups was unknown and it is uncertain whether sick pups were sampled.

Blood data collected during this study were difficult to interpret. As predicted, some blood values of rehabilitated pups at admittance differed from wild pups, but some blood values also differed significantly between wild pups and rehabilitated pups that were defined as clinically healthy. It is possible that small differences between the two groups may have resulted in statistically significant findings as a result of having narrow ranges (Tocidlowski et al., 2000). The importance of statistically different values, therefore, may be limited and further studies should be conducted to investigate the biologic significance of these data. Rehabilitated harbor seal pups undergo a variety of clinical and physiologic changes while in captivity and these factors may be more important when deciphering health data. For example, some rehabilitated pups recovered from illness while at TMMC. Furthermore, growth most likely differed between the groups, despite the nonsignificant difference found for condition. It was obvious that rehabilitated pups did not attain weaning weight in captivity as quickly as wild pups do during lactation, which typically occurs in 4–6 wk.

Baseline data sets established for wild and rehabilitated pups during this study can be used to improve care and treatment of animals in captivity. These data also may serve as a reference point for future evaluation of juvenile health, should an oil spill or unusual mortality event occur. To be of value in population health assessment, however, future studies should be conducted to quantify reference values for a greater sample of seals. Additionally, sex, age, seasonal, and geographic related differences need to be investigated further for this species.

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