Establishing a reference interval for acute phase proteins, cytokines, antioxidants and commonly measured biochemical and hematologic parameters in the northern fur seal (*Callorhinus ursinus)*

Valerie Johnson[1], A Russell Moore[2], Rachel Conway[2], Tonya Zeppelin[3], Tom Gelatt[3], Colleen Duncan[2]

[1.] Department of Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, Michigan 48824

[2.] Department of Microbiology, Immunology and Pathology, College of Veterinary Medicine, Colorado State University, Fort Collins, Colorado 80524

[3.] National Marine Fisheries Service, Alaska Fisheries Science Center, Marine Mammal Lab, Seattle, Washington, 98115

This manuscript is a result of research funded by the NOAA fisheries AK region under award NA16NMF4390028 to Colorado State University

Abstract

Over the past several decades there has been a precipitous decline of northern fur seals (*Callorhinus ursinus;* NFS) at their breeding grounds on the Pribilof Islands in the Bering Sea. The cause of this decline is likely multifactorial and could include changes in environmental parameters, prey abundance and distribution as well as exposure to pathogens and pollutants. Evaluation of inflammatory markers and antioxidant levels of the current population of fur seals in addition to hematologic and biochemical profiles could provide important information regarding health and subclinical or clinical disease in this population. Serum and plasma samples were obtained from clinically healthy adult female NFS and references intervals were determined for multiple parameters that can be altered in response to the presence of disease and environmental stressors. We established a reference interval for cytokines involved in acute inflammation and infection (TNFa, IL1, IL6, IL8, KC, IL10, C-reactive Protein) by utilizing commercially available canine cross-reactive antibodies. Reference intervals were also established for reactive oxygen species (hydrogen peroxide and malondialdehyde), as well as antioxidant levels (vitamin E and selenium) and acute phase proteins evaluated by serum electrophoresis. To improve the ability to compare and interpret indicators of health and disease in this species, we developed reference intervals for commonly utilized hematologic and biochemical tests in addition to the aforementioned markers of oxidative stress and inflammatory biomarkers. There were several animals identified as outliers indicating that they may have had subclinical illness or inflammation. Further investigation utilizing these tests in clinically ill animals and comparison to animals that exhibit normal behavior and no overt signs of illness could increase our understanding of the utility of measuring these parameters in this species.

Introduction

The northern fur seal, once abundant on the shores of the Pribilof islands during the breeding season has undergone a precipitous decline over the past couple decades (Spraker and Lander, 2010). Northern fur seals are highly migratory and only come ashore during the summer to give birth and breed, making it difficult to definitively determine the cause of the observed decrease in numbers. Various contributory factors have been postulated to impact marine mammal populations such as infectious disease environmental pollutants and decreased availability of food from fishing and/or climate change (Beckmen et al., 2003; Chatterton et al., 2020; Dufresne et al., 2010; Duncan et al., 2014a; Foster et al., 2018). While it is likely that the NFS decline is related to a combination of factors, determining the elements involved are critical to understanding potential threats to population recovery.

It is known that environmental pollutants, nutritional stress, injury, and infectious disease cause physiologic stress that leads to an acute phase response that may be undetectable in a routine complete blood count (CBC) and biochemistry profile (Dupont et al., 2013; Petersen et al., 2004). Reactive oxygen species have been identified as a marker of physiologic stress and when increased due to disease or other environmental stressors they can overwhelm endogenous antioxidant defense systems (Auten and Davis, 2009). Oxidative stress has been identified in elephant seals during breeding fasts and has also been proposed as a biomarker to quantify physiologic stress caused by disease or environmental factors in pinnipeds (Sharick et al., 2015). Biomarkers can be utilized to detect subclinical illness and predict susceptibility to infectious disease and have been extensively evaluated in other species (Andaluz-Ojeda et al., 2012; Bozza et al., 2007; Johnson et al., 2016). Recent technology has made it possible to evaluate multiple biomarkers simultaneously with the use of species-specific cytokine assays that are able to assess 10-30 different cytokines utilizing a small amount of serum. A commercially available canine multiplex cytokine panel has been validated for 3 types of pinnipeds (Levin et al., 2014). This technology is frequently utilized in humans as well as domestic animal species including cats, dogs and horses and provides multiple biomarkers that can be utilized to detect inflammation and have been correlated with prognosis for several disease processes (Andaluz-Ojeda et al., 2012; Bozza et al., 2007; Hall et al., 2015; Johnson et al., 2016; Kjelgaard-Hansen and Jacobsen, 2011; Wagner and Freer, 2009). Acute phase proteins are produced during inflammation and other disease processes and are increasingly being utilized to monitor inflammation, severity of disease and response to treatment in a variety of species (Eckersall and Schmidt, 2014; Gebhardt et al., 2009;

Petersen et al., 2004). C-reactive protein is one such acute phase protein that is commonly utilized in many species to identify inflammation and assess response to treatment in inflammatory and infectious disease (Gebhardt et al., 2009; Petersen et al., 2004). CRP levels have been demonstrated to be elevated in NFS infected with lungworm which cannot be detected antemortem and have been identified as an important marker of inflammation in other pinniped species (Kakuschke et al., 2013; Sheldon et al., 2017). C-Reactive Protein (CRP) in harbor seals has also been demonstrated to cross-react with a commercially available human ELISA and our study revealed a similar cross-reactivity in NFS utilizing a canine CRP ELISA (Funke et al., 1997; Gelain and Bonsembiante, 2019; Kakuschke et al., 2013).

While infectious and environmental factors have been evaluated in NFS, there is currently limited information regarding acute phase proteins, cytokines, and antioxidant levels in this species. Although available assays may be employed, meaningful interpretation of results requires a basic knowledge of normal values. The objective of this research was to determine reference values for testing that can be utilized to assess overall health of the individual animals tested for multiple parameters including biochemical, hematologic, oxidant status, and acute phase proteins. This information may engender an improved understanding of the cause of the observed decline in population and a possible opportunity to intervene to maintain the overall health of a critical ecosystem (Seguel et al., 2019).

Materials and Methods

Sampled Populations: Samples were collected from 161 lactating adult female NFS from 2 rookeries on St Paul Island Alaska; Polovina Cliffs and Zapadni Reef, between August and October in 2015-2017. All work described in this manuscript was conducted in accordance with and under the authority of the United States Marine Mammal Protection Act (National Marine Fisheries Service, NMFS Permit 14327-01) and the NMFS Alaska/Northwest Fisheries Science Centers Institutional Animal Care and Use Committee (IACUC #s A/NW 2013-3 and A/NW 2013-3/2016-2). Seals were individually captured, weighed and physically restrained during sample collection. All animals were weighed to the nearest 0.2 kg (Dyna-Link, Measurement Systems International, Seattle, WA, USA) and lactation was confirmed during handling. Lactating adult female NFS with normal body weights were presumed to be healthy based on those parameters and were included in this study. NFS that were included in this study were tagged with flipper tags for identification in subsequent years as part of the vital rates monitoring program conducted by the National Oceanic and Atmospheric Association (NOAA). Tag numbers were recorded, and no individuals were captured more than once during

the two years of the study and thus all individuals were sampled at one time only. Blood was collected from the dorsal pedal vein using a 21 gauge butterfly catheter and collected directly into vacutainer tubes containing ethylenediaminetetraacetic acid (EDTA) and no additives tubes. Samples were placed in a cooler with ice packs for up to 4 hours. Blood samples were centrifuged for 10 minutes, serum or plasma aliquots were extracted, and samples were frozen at -80C for up to 2 years until sample processing. Multiple aliquots were obtained for each animal and aliquots utilized for all testing underwent no more than two freeze/thaw cycles prior to testing. CBCs were performed on blood collected into EDTA tubes and the tubes were subsequently centrifuged. Plasma was removed and samples processed as described below. Samples with excess hemolysis or lipemia were excluded from the study. All individuals in all years had a CBC performed on the day of blood collection and samples were kept on ice until processing. Samples were collected in 2016 and 2017 from 92 animals at the Zapadni Reef rookery and these samples additionally had routine biochemistry, serum protein electrophoresis, cytokine analysis and evaluation of markers of oxidative stress and antioxidant status. To our knowledge, these methods have not been fully validated in this species.

Routine CBC: Routine CBC was performed on whole blood from EDTA tubes using a VetScan® HM5 Hematology Analyzer (Abaxis, Union City, CA, USA) using canine settings. The analyzer was shown to be operating within established laboratory quality assurance protocols prior to sample analysis.

Routine Biochemistry: One serum aliquot was thawed and used for routine biochemistry profile, serum protein electrophoresis, cytokine analysis, evidence of oxidative stress and antioxidant levels. Serum biochemistry was performed using a Cobas c501 (Roche Diagnostics, Indianapolis, IN, USA) analyzer and commercially available kits. Evaluated measurands and the analytical method are included in Table 1. The analyzer was shown to be operating within established laboratory quality assurance protocols. Samples were analyzed at Colorado State University Clinical Pathology Department which routinely conducts testing on a large variety of domestic and exotic species.

Serum Protein electrophoresis: Serum TP concentration was determined using the biuret method. Agarose gel electrophoresis was performed according to manufacturer's instructions using Sebia Hydragel Protein (E) gels on a Sebia Hydrasys system (Sebia, France). Resulting gels were stained with Amido black and scanned using a flat-bed scanner (Epson Perfection V700 Photo, Epson America, Inc, Long Beach, CA, USA) and Phoresis software (version 8.6.3, Sebia, France). A concurrently run pooled normal canine serum sample was used as quality assurance and to aid in location of

fraction demarcations. The electrophoretic gel and resulting electrophoretograms were reviewed by a single reviewer to screen for visibly atypical patterns. Serum and EDTA plasma samples of 5 individuals were evaluated to identify the location of fibrinogen.

Cytokine analysis: Serum samples were analyzed utilizing commercially available canine multiplex bead technology for 13 cytokines including granulocyte-monocyte colony stimulating factor (GM-CSF), interferon gamma (IFNγ), monocyte chemoattractant protein 1 (MCP1), tumor necrosis factor alpha (TNFα), interferon inducible protein 10 (IP10), keratinocyte chemoattractant like protein (KC) and interleukins (IL) 2, IL6, IL7, IL8, IL10, IL15, IL18 (EMD Millipore, Billerica MA, USA). Because these assays had not been fully validated for use in this species, an initial set of 20 samples were tested. Absence of detectible results in all 20 samples was interpreted as evidence of a lack of cross-reactivity or other interference and these cytokines were not evaluated in the remaining samples, specifically IL-2, IL-7, IL-15 and IP-10. All samples were run in duplicate with the appropriate quality controls and standards included in the multiplex kit. Samples were diluted and tests were conducted as described in the manufacturer's instructions.

C-reactive protein was measured using a commercially available canine ELISA according to manufacturer's instructions (BD biosciences, Franklin Lake NJ, USA) and colorimetric readings obtained on a Syntergy HT spectrophotometer (BioTrek Instruments, Winooski VT, USA). The lower limit of the standard curve was adopted as the lower reportable limit. If needed, samples were diluted to bring the test results within the range of the provided standards. Results were expressed in the units provided by the kit.

Biomarkers of oxidative stress: Oxidative stress was assessed using commonly available non-species specific commercially available colorimetric kits. Malondialdehyde, a byproduct of lipid peroxidation and marker of oxidative stress was measured on stored serum samples according to manufacturer's instructions (Abcam, Cambridge, MA, USA). Hydrogen peroxide was measured as an additional marker of oxidative stress from stored samples using a commercially available kit according to manufacturer's instructions (Oxiselect, Cell Bioloabs, San Diego, CA, USA). The lower limit of the standard curve was adopted as the lower reportable limit. If needed, samples were diluted to bring the test results within the range of the provided standards. Results were expressed in the units provided by the kit.

Laboratory analysis for antioxidant levels: Levels of commonly assessed antioxidants vitamin E and selenium were performed by the Colorado State University Veterinary Diagnostic laboratory. Serum samples were stored at -80 until

time of submission and then kept at 4°C until analysis within 72 hours of submission. Vitamin E levels were determined utilizing high performance liquid chromatography (Catignani, 1986). Selenium levels were measured using atomic absorption with hydride generation (Poole et al., 1977).

Reference interval generation: All values were entered into Microsoft Excel and evaluated using the Reference Value Advisor V 2.1 Macroset, according to published guidelines (Geffre et al., 2011). Stringent outlier analysis was performed using multiple iterations of Horn's algorithm with Tukey's interquartile fences, as needed. Data distribution and symmetry was determined using visual inspection of the histograms and an Anderson-Darling test with p value < 0.05 as evidence of non-Gaussian distribution in samples of > 60 individuals and <0.20 as evidence of non-Gaussian distribution in samples of < 60 individuals (Le Boedec, 2016). Summary statistics, 95% reference limits, and 90% confidence interval of the reference limits were calculated using methods dependent on sample distribution (Friedrichs et al., 2012). CBC measurands were evaluated for partitioning by rookery based on the percentage of partitioned results outside of combined reference intervals as suggested by Lahti and further evaluated using a t-test (Lahti et al., 2004). When the lower reference limit was below the lower limit of detection, the 90% confidence interval of the lower limit could not be calculated.

Results/Discussion

Most measurands displayed Gaussian distribution and contained > 60 reference individuals after removal of outliers; nonparametric methods were preferred for generation of 95% reference interval and bootstrap methods were used for generation of 90% confidence intervals of the reference limits, as needed. Reference intervals are provided in Tables 2-4. Reference ranges were similar to those derived for other species. Evaluation of the subgroup percentage of values outside common reference intervals suggested the need for partitioning of red blood cell concentration (RBC), mean corpuscular hemoglobin (MCH), platelets (PLT), red cell distribution width (RDWcv), and platelet distribution width (PDWsd). A t-test indicated a statistical difference between rookeries for these measurands. Reference intervals partitioned by rookery are presented in Table 5. The significant differences in complete blood counts from animals that resided at different rookeries was an unexpected finding. Both red blood cell and platelet concentrations were significantly lower in animals at Zapadni rookery when compared to Polvina Cliffs rookery. This could be due to diet, pathogens or other factors but suggests that hematology reference intervals may need to be established for different

166 geographical locations utilized by NFS and may not be able to be generalized across their range. Interestingly, a study
167 evaluating hematologic parameters in 24 NFS in Russia revealed RBC concentration similar to that of the Zapadni
168 rookery with a mean MCH lower than both Polvina and Zapadni rookeries. PLT, RDWcv and PDWsd were not measured
169 in this study (Norberg et al., 2011). This further supports the development of hematology reference intervals for
170 separate geographical locations. There were no differences between rookeries for any of the other biochemical,
171 oxidant, cytokines or other acute phase proteins examined.

172 Serum protein electrophoretic profiles were similar to results obtained in other seals (Ross et al., 1993). Most samples
173 had a single α-1. Most of the samples (65/86) had a variably distinct α-2a and α-2b peak as shown in Figure 1. These
174 two peaks could not be consistently measured and were combined as a single α-2 for the purposes of reference interval
175 generation. Comparison of paired serum and EDTA plasma samples localized fibrinogen in the β-γ region as shown in
176 Figure 2.

177 The canine cytokine multiplex analysis has been evaluated for use in pinnipeds and was determined to have better
178 cross-reactivity with most pinniped cytokines than the human test (Levin et al., 2014). Similar to results in the other
179 pinniped species, several of the cytokines had no measurable results in initial samples that were evaluated (IL2, IL7, IL15,
180 and IP10). Levin et al described validation of the canine cytokine multiplex for the harbor seal, gray seal and harp seal.
181 None of these species displayed detectable IL7 or IP10 secretion from lymphocytes even when stimulated in contrast to
182 canine lymphocytes which is consistent with our findings in the NFS (Levin et al., 2014). IL2 was not detectable in the
183 harp seal and was only detected in harbor seals and gray seals after stimulation with conconavalin A which activates
184 lymphocytes. No species had detectable IL2 when stimulated with lipopolysaccharide (LPS). IL15 was detected in harp
185 seals but only detectable in gray seals and harbor seals after stimulation with conconavalin A but not LPS. It would
186 therefore not be surprising that there was a lack of cross-reactivity in these antibodies in NFS. We presumed that lack of
187 cross-reactivity was present as it would be unlikely that the results would be zero for all the individuals tested but it is
188 also possible that levels were too low to be detected. In healthy adults, low levels of inflammatory cytokines would be
189 expected. Because traditional methods for outlier detection could not be evaluated, outliers were classified as an
190 individual displaying more than 3 cytokines that were significantly elevated when compared with the rest of the
191 evaluated animals (Table 6).

Serum cytokines are a more sensitive way to identify and monitor an inflammatory or anti-inflammatory response to immune activation, inflammation and infection (Bozza et al., 2007). In clinical veterinary studies these correlations can be difficult to assess due to low numbers of patients and the lack of an established reference interval (Gebhardt et al., 2009). Healthy dogs were utilized to establish reference values for commonly measured cytokines in canine patients (Johnson et al., 2016). Cytokine multiplex technology allows evaluation of many variables with a small amount of sample and cross-reaction of the canine multiplex with multiple pinniped species has been demonstrated (Levin et al., 2014). This technology has been utilized in multiple species for a variety of immune mediated, infectious, inflammatory and neoplastic diseases but has not been widely utilized in marine mammals to this date (Kjelgaard-Hansen and Jacobsen, 2011). The few studies that have evaluated cytokine expression and acute phase proteins in marine mammal species demonstrate that similar to other species they have potential to serve as biomarkers for subclinical or early disease processes as well as prognostic indicators and further investigation in this area is warranted (Gelain and Bonsembiante, 2019).

Interestingly, there were four animals that had elevations in more than 3 inflammatory cytokines which suggests subclinical inflammation. All four animals had elevations in granulocyte-monocyte chemoattractant protein (GM-CSF) which is an inflammatory cytokine involved in recruitment and activation of multiple inflammatory cells including macrophages and neutrophils (Hotchkiss et al., 2013). These animals also had an increase in interleukin 6 (IL-6) a pro-inflammatory acute phase cytokine. Although this cytokine can have some anti-inflammatory properties as well as pro-inflammatory it is stimulated by presence of pathogen associated molecular patterns (PAMPs) and is an important mediator of the acute phase response through stimulation of production of acute phase proteins and increasing production of neutrophils (Cohen, 2002). Two of these animals had elevations in keratinocyte chemoattractant (KC) which is a pro-inflammatory cytokine that is generally produced in inflammatory states and functions to recruit neutrophils to the site of inflammation or infection (Son et al., 2007). Another two animals had an increase in tumor necrosis factor alpha (TNFα) which is a pro-inflammatory cytokine that is associated with acute and chronic inflammation and infection and functions to increase production of other inflammatory cytokines and acts on the central nervous system to induce fever and other symptoms of inflammation or infection (Cohen, 2002). Additionally two NFS had an increase in monocyte chemoattractant protein (MCP1) (Bozza et al., 2007; Duffy et al., 2010; Yadav et al., 2010). This cytokine is a pro-inflammatory cytokine that acts to attract monocytes and macrophages to the site of inflammation

or infection and is present in acute infections as well as chronic inflammatory conditions. Three of the animals had an increase in interleukin 18 (IL-18) another pro-inflammatory cytokine. This cytokine is produced by macrophages and stimulates an inflammatory cell mediated immune response through activation of T cells. It can be stimulated by infection or chronic inflammation (Krishnan et al., 2014). In humans and dogs with sepsis or other infectious or inflammatory conditions it has been suggested that utilizing panels of cytokines is more predictive of severity of inflammation or infection (Andaluz-Ojeda et al., 2012; Johnson et al., 2016). Therefore, the fact that all these animals had an increase in multiple pro-inflammatory mediators strongly suggests the presence of an infectious or inflammatory condition. Of the four animals with elevated cytokines some had mild abnormalities on routine biochemical tests that would likely have been considered insignificant. NFS 320p had a mildly increased neutrophil total count of 9.81 (range 3.71-9.34) with a normal neutrophil percentage and a slightly decreased hemoglobin of 141 (range 158-198) with a normal hematocrit. NFS 015x did not have a chemistry or protein electrophoresis performed due to limited sample obtained but had a normal complete blood count. NFS 010x had an elevated creatinine kinase (CK) of 602 (range 96-337). In captured wild animals CK is often attributed to the stress of capture and this animal had a normal CBC and chemistry profile (Hotchkiss et al., 2013). NFS 012x also had an increased CK of 1398, a slightly elevated calcium of 11.0 (range 8.1-10.6) with a normal CBC and no other biochemical abnormalities. This animal also had a mild increase in the alpha-1% on protein electrophoresis of 6.1 (3.4-5.5) with a corresponding increase in the alpha-1 concentration of .43 (range .22-.37). Increase of the alpha-1 fraction suggests acute inflammation but the alteration was mild and the CBC normal (Sheldon et al., 2017). All four animals had normal vitamin E and Selenium levels and no evidence of oxidative stress. The increased sensitivity to detect inflammation or immunosuppression in clinically normal animals is of great interest and a larger study examining cytokine profiles in addition to testing for common bacterial and viral diseases to investigate possible causes for the observed information would be informative.

Reactive oxygen species and antioxidants are parameters that reflects an individual's health status. Oxidative stress occurs in marine mammals in response to illness or injury (Sharick et al., 2015). Increased exposure to environmental contaminants has been demonstrated to increase oxidative stress in marine mammals (Li et al., 2005). Therefore, an important part of a complete health assessment should include evaluation of both markers of oxidation and antioxidant levels. We evaluated markers of oxidative stress malondialdehyde and H2O2 as well as antioxidants vitamin E and selenium to establish baseline reference intervals in NFS. A commonly utilized biomarker of acute inflammation, C-

Reactive Protein (CRP) was analyzed utilizing a canine commercially available ELISA. CRP has been identified as a biomarker in elephant seals with lungworm and therefore is likely to be a potential biomarker of inflammation in other pinniped species (Eckersall and Schmidt, 2014; Funke et al., 1997; Sheldon et al., 2017). Elevated CRP was not detected in the animals tested in this study.

Current best practices were used for collection of sample, analysis, and construction of reference intervals. Given the challenges in assuring the health in wildlife, a more aggressive approach was taken for outlier identification and removal. While this should help minimize the effects of subclinical disease, the clinical utility of these reference intervals should be assessed. It was noteworthy that 4 animals were identified as outliers as previously defined based on their cytokine profiles, yet all 4 animals had no abnormalities detected with commonly utilized biochemical tests and blood cell counts. It is possible that these animals had an underlying subclinical inflammatory stimulus which could not be detected by conventional methods. It is also possible that this could be a normal variation for this species and analysis of greater numbers of animals as well as clinically ill animals would assist in determination of the variability in this species as well as utility in serving as a marker of immune health.

In conclusion we have developed a reference interval for a comprehensive evaluation of multiple parameters of health in the NFS that includes evaluation of hematology, biochemistry, oxidative stress and antioxidant function, acute phase proteins and inflammatory markers. Development of reference intervals for these parameters in this species is important to develop a baseline for the monitoring of the global health of the Northern Fur Seal. The decline of the population on the Pribilof Islands may be indicative of a decline in health of the marine ecosystem due to environmental factors contributing to increased predation, introduction of a novel pathogen or an effect of lack of resources due to human interactions such as fishing (McHuron et al., 2019; Spraker and Lander, 2010). Infectious disease has been identified in the NFS population in Alaska including polyoma virus, *Coxiella* and *Brucella* species but the contribution of these infections to population decline is not known (Duncan et al., 2014a; Duncan et al., 2013; Duncan et al., 2014b; Minor et al., 2013; Van Bressem et al., 2009). Marine pollutants have also been demonstrated to affect immune function in NFS and other pinnipeds and could contribute to increased susceptibility to infectious disease (Beckmen et al., 2003; Desforges et al., 2016; Dufresne et al., 2010; Dupont et al., 2013). This population of animals is monitored yearly and identification tags are placed. Therefore samples from animals who return and are sampled in subsequent

years as well as identification of animals who do not return provide a unique opportunity to evaluate the utility of these biomarkers in this population. Animals with increased markers of inflammation such as the animals identified in this study could subsequently be assessed for presence of known infectious disease. Further evaluation of this population utilizing these techniques could allow important insight into the factors leading to the precipitous decline of this species and a better understanding with respect to the benefit of possible interventions.

Table 1 Methods

Measurand	**Method**
Sodium	Indirect Ion Specific Electrode (ISE)
Potassium	Indirect ISE
Chloride	Indirect ISE
Bicarb	Phosphoenolpyruvate
Anion Gap	Calculated
Osmolality (calculated)	Calculated
Calcium	BAPTA (2,2′,2″,2‴-[Ethane-1,2-diylbis(oxy-2,1-phenylenenitrilo)]tetraacetic acid
Phosphorus	Phosphomolybdate
Magnesium	Xylidyl blue
Urea nitrogen	Urease Ultraviolet (UV)
Creatinine	Alkaline picrate, International Federation of Clinical Chemistry and Laboratory Medicine (IFCC)-isotope dilution mass spectrometry (IDMS) standardized
Total billirubin	Diazonium ion, blanked
Aspartate aminotransferase (AST)	UV without P5P (pyridoxal-5-phosphate)

Creatine kinase (CK)	N-acetyl-cystein (NAC) activated
Gamma-glutamyl transferase (GGT)	Gamma-glutamyl-carboxy-nitroanilide
Alkaline phosphatase (ALP)	p-nitrophenyl phosphate (pNPP), 2-amino-2-methylpropanol (AMP) buffer
alanine aminotransferase (ALT)	UV without P5P (pyridoxal-5-phosphate)
Glucose	Hexokinase
Amylase	Ethylidene-4-nitrophenyl-α-D-maltoheptaoside (G7 PNP), blocked
Cholesterol	Cholesterol oxidase, esterase, peroxidase
Total protein	Biuret
Albumin	Bromcresol green
Globulin	Calculated
Albumin: Globulin ratio (A/G)	Calculated
Iron	Ferrozine-no proteinization

277

278

Table 2: Hematology reference intervals from adult female Northern fur seals at St Paul Island, Alaska.

Measurand	Conventional Units	n	Mean	SD	Median	Min	Max	p-value	Distribution	Method	LRL of RI	URL of RI	CI 90% of LRL	CI 90% of URL
PCV	%	159	47.57	3.76	47.27	38.09	56.88	0.21	G	NP	41.3	55.1	38.09-41.75	53.92-56.88
RBC conc.	10^6/µL	160	5.07	0.449	5.05	4.19	6.16	0.08	G	NP	4.29	5.96	4.19-4.37	5.85-6.16
Hemoglobin	g/dL	152	179	9.7	180	154	203	0.64	G	NP	158	198	154-163	194-203
MCV	fL	161	94	4	94	82	103	0.06	G	NP	85	101	82-89	101-103
MCHC	g/dL	160	376	23.3	380	332	436	0	NG	NP	334	433	332-339	417-436
MCH	pg	160	35.4	2.68	35.3	30.1	42.4	0.1	G	NP	30.7	41.4	31.1-31.2	39.8-42.4
WBC conc.	10^3/µL	150	8.98	1.88	9.03	4.43	13.37	0.14	G	NP	5.3	13.22	4.43-6.11	12.77-13.37
Neutrophil	%	161	74.1	5.82	74.6	60.4	86	0.06	G	NP	61.9	85.5	60.4-63.5	82.7-85.0
Neutrophil	10^3/µL	153	6.59	1.42	6.64	3.22	10.54	0.74	G	NP	3.71	9.34	3.22-4.29	9.03-10.54
Lymphocyte	%	160	20.1	5.53	19.2	8.6	33.9	0.003	NG	NP	11.3	32.6	8.6-12.9	30.2-33.9
Lymphocyte	10^3/µL	159	1.84	0.634	1.75	0.65	3.43	0.014	NG	NP	0.76	3.27	0.65-0.90	2.96-3.43
Monocyte	%	161	5.4	3.28	5.6	0.6	10.0	0	NG	NP	0.6	9.8	0.6-0.6	9.6-10.0
Monocyte	10^3/µL	161	0.5	0.327	0.51	0.03	1.25	0	NG	NP	0.06	1.13	0.03-0.04	1.00-1.25
Eosinophil	%	152	0.23	0.13	0.2	0	0.6	0	NG	NP	0.1	0.5	0-0.1	0.5-0.6
Eosinophil	10^3/µL	153	0.02	0.014	0.02	0	0.06	0	NG	NP	0	0.06	0-0.01	0.05-0.06
Basophil	%	150	0.1	0.06	0.1	0	0.2	0	NG	NP	0	0.2	0-0	0.2-0.2
Basophil	10^3/µL	159	0.009	0.007	0.01	0	0.02	0	NG	NP	0	0.02	0-0	0.02-0.02
Platelet conc.	10^3/µL	161	366	66.2	360	193	555	0.06	G	NP	244	497	183-268	474-555
MPV	fL	158	8.91	0.92	8.9	7.2	11.3	0.18	G	NP	7.3	10.7	7.2-7.5	10.4-11.3
PCT	%	160	0.33	0.062	0.33	0.18	0.47	0.01	NG	NP	0.22	0.44	0.18-0.24	0.42-0.47
RDWsd1	fL	161	54.7	2.81	54.7	47.7	61.7	0.05	NG	NP	49.2	60.9	47.7-50.0	60.2-61.7
RDWcv1	%	159	15.2	0.8	15.2	13.6	17.1	0.08	G	NP	13.7	16.8	13.3-14.0	16.7-17.1
PDWsd	fL	159	11.4	1.91	11.3	8.0	17.1	0.03	NG	NP	8.5	15.5	8.0-8.5	14.8-17.1
PDWcv	%	161	36.1	1.9	36.4	32.3	40.7	0.002	NG	NP	32.7	39.6	32.3-33.1	39.0-40.7

G, Gaussian; NG, non-Gaussian; NP, nonparametric; RI, reference interval; LRL, lower reference limit; URL, upper reference limit; PCV, packed cell volume; MCHC, mean cell hemoglobin concentration; WBC, white blood cell; conc., concentration; TS, total solids; TP, total protein; PCT, plateletcrit; RDWsd1, Red cell Distribution Width standard deviation; RDWcv1, Red cell Distribution Width coefficient of variance, PDWsd, Platelet Distribution Width standard deviation, PDW CV, Platelet Distribution Width coefficient of variance.

Table 3: Biochemistry reference intervals from adult female Northern fur seals at St Paul Island, Alaska.

Measurand	Conventional Units	n	Mean	SD	Median	Min	Max	p-value	Distribution	Method	LRL of RI	URL of RI	LRL 90% CI	URL 90% CI
Sodium	mEq/L	75	146	4	146	136	153	0.007	NG	NP	137	153	136-138	151-153
Potassium	mEq/L	88	4.08	0.423	4.09	2.974	5.06	0.55	G	NP	3.01	4.91	2.97-3.33	4.67-5.06
Chloride	mEq/L	89	99.2	5.18	100.3	89.7	109.1	0.003	G	NP	88.1	107.9	86.7-90.4	106.7-109.1
Bicarb	mEq/L	89	19.3	2.7	19.1	13.4	24.9	0.19	G	NP	13.5	24.5	13.4-15.3	23.5-24.9
Anion Gap	mEq/L	84	29	3.8	29	22	39	0.009	NG	NP	23	39	22-24	36-39
Osmolality (calc)	mOsm/Kg	82	296	12.5	299	264	312	<0.001	NG	NP	267	312	264-271	309-312
Calcium	mg/dL	83	9.4	0.61	9.4	8.1	10.7	0.66	G	NP	8.1	10.6	8.1-8.4	10.4-10.7
Phosphorus	mg/dL	88	4.7	1.18	4.6	1.8	7.9	0.97	G	NP	2.4	7.1	1.8-3.0	6.6-7.9
Magnesium	mg/dL	82	2.4	0.21	2.3	1.9	2.8	0.01	NG	NP	2.0	2.8	1.9-2.0	2.8-2.8
Urea nitrogen	mg/dL	85	32	4.8	32	21	45	0.1	G	NP	23	44	21-25	40-45
Creatinine	mg/dL	90	0.8	0.13	0.8	0.5	1.1	<0.001	NG	NP	0.5	1.1	0.5-0.6	1.0-1.1
Total billirubin	mg/dL	89	0.1	0.04	0.1	0	0.2	<0.001	NG	NP	0.1	0.2	0-0.1	0.2-0.2
AST	U/L	82	75	20	73	36	129	0.08	G	NP	45	125	36-47	108-129
CK	U/L	68	186	62.9	178	92	345	0.02	NG	NP	96	337	32-108	311-345
GGT	U/L	87	202	53	205	81	334	0.97	G	NP	91	312	81-115	286-334
ALP	U/L	78	70	14.2	70	36	100	0.24	G	NP	40	99	36-44	93-100
ALT	U/L	89	30	21.3	34	3	96	<0.001	NG	NP	4	91	3-6	60-96
Glucose	mg/dL	89	151	21.2	150	96	203	0.78	G	NP	103	200	96-115	182-203
Amylase	U/L	38	194	51.9	192	92	325	0.89	G	R	84	297	62-108	269-324
Cholesterol	mg/dL	90	237	37.8	228	148	334	0.01	NG	NP	179	331	148-185	296-334
Total protein	g/dL	89	6.7	0.54	6.7	5.5	8.2	0.38	G	NP	5.6	7.9	5.5-5.8	7.4-8.2
Albumin	g/dL	90	3.7	0.27	3.8	3.2	4.5	0.07	G	NP	3.2	4.4	3.2-3.3	4.1-4.5
Globulin	g/dL	88	2.9	0.4	2.9	2.2	4.0	0.17	G	NP	2.2	3.9	2.2-2.3	3.7-4.0
A/G		87	1.3	0.159	1.3	0.9	1.64	0.31	G	NP	0.93	1.6	0.90-1.07	1.50-1.64
Iron	ug/dL	36	123	36.3	121	58	194	0.88	G	R	48	198	34-66	179-215

G, Gaussian; NG, non-Gaussian; NP, nonparametric; R, robust

Table 4: Agarose gel serum protein electrophoresis, cytokine, and antioxidant reference intervals from adult female Northern fur seals at St Paul Island, Alaska.

A "-" indicates that the value could not be calculated due to presence of multiple measurands below the lower limit of detection.

Measurand	Conventional Units	n	Mean	SD	Median	Min	Max	p-value	Distribution	Method	LRL of RI	URL of RI	LRL 90% CI	URL 90% CI
A/G (SPE)		83	0.81	0.098	0.81	0.59	1.04	0.737	G	NP	0.6	1.03	0.59-0.67	0.98-1.04
Albumin (SPE)	%	84	44.8	3.11	44.8	37.1	53.2	0.974	G	NP	37.5	51	37.1-40.6	49.5-53.2
Albumin (SPE)	mg/dL	80	2.96	0.245	2.94	2.47	3.53	0.312	G	NP	2.53	3.53	2.47-2.55	3.37-3.53
Alpha 1	%	83	4.6	0.45	4.5	3.4	5.8	0.087	G	NP	3.4	5.5	3.4-3.7	5.4-5.8
Alpha 1	mg/dL	83	0.3	0.037	0.3	0.22	0.39	0.321	G	NP	0.22	0.37	0.22-0.24	0.37-0.39
Alpha 2	%	84	19.6	1.31	19.6	16.7	22.6	0.276	G	NP	17	22.5	16.7-17.8	21.5-22.6
Alpha 2	mg/dL	84	1.28	0.152	1.27	0.9	1.64	0.54	G	NP	1.01	1.61	0.90-1.06	1.51-1.64
Beta	%	85	12	1.07	12.1	0.92	13.9	0.259	G	NP	9.8	13.8	9.2-1.3	13.6-13.9
Beta	mg/dL	84	0.79	0.101	0.79	0.52	1.00	0.72	G	NP	0.55	0.99	0.52-0.64	0.93-1.00
Gamma	%	85	19	2.17	19.2	11.8	27.1	0.72	G	NP	13.25	25.5	11.8-14.1	23.7-27.1
Gamma	g/dL	86	1.25	0.258	1.25	0.77	2.01	0.09	NG	NP	0.83	1.81	0.77-0.89	1.62-2.01
Selenium	ppm	48	0.31	0.11	0.27	0.16	0.58	< 0.001	NG	NP	0.17	0.57	0.16-0.19	0.54-0.58
Vit E	ug/ml	66	12.8	4.1	12.5	5.1	24	0.117	G	NP	6.2	22	5.1-7.0	19.0-24.0
H2O2	uM	90	0.024	0.074	0	0	0.436	<0.001	NG	NP	0	0.349	0-0	0.094-0.436
MDA	nmol/ml	78	92	35	90	23	188	0.307	G	NP	32	182	23-45	143-188
CRP	ng/ml	79	-	-	<0.0031	<0.00312	0.023	<0.001	NG	NP	<0.00312	0.018	-	0.003-0.023
KC	pg/ml	70	1431	906	1288.9	59	3788	0.005	NG	NP	116	3671	59-307	3129-3788
GM-CSF1	pg/ml	81	-	-	<9.2	<9.2	165.3	<0.001	NG	NP	<9.2	124.3	-	57.9-165.3
IFN-γ	pg/ml	81	-	-	<10.5	<10.5	713.51	<0.001	NG	NP	<10.5	564.3	-	24.0-713.5
IL-6	pg/ml	81	-	-	<3.7	<3.7	36.17	<0.001	NG	NP	<3.7	32.65	-	12.51-36.17
IL-8	pg/ml	80	-	-	<21.7	<21.7	2065.5	<0.001	NG	NP	<21.7	435.9	-	262.2-2065.5
IL-10	pg/ml	80	-	-	<8.5	<8.5	314.84	<0.001	NG	NP	<8.5	117.63	-	51.57-314.84
IL-15	pg/ml	80	-	-	<9	<9	768.29	<0.001	NG	NP	<9	555.48	-	<9-768.29
IL-18	pg/ml	80	-	-	<5.8	<5.8	55.95	<0.001	NG	NP	<5.8	43.38	-	37.08-55.95
MCP1	pg/ml	80	-	-	<21	<21	79.19	<0.001	NG	NP	<21	67.03	-	34.58-79.19
TNF-α	pg/ml	80	-	-	<6.1	<6.1	39.75	<0.001	NG	NP	<6.1	<6.1	-	<6.1-39.75

G, Gaussian; NG, non-Gaussian; NP, nonparametric

Table 5: Partitioned data of hematologic values that differed by geographical location (different rookeries).

Measurand	Conventional Units	Rookery	n	Mean	SD	Median	Min	Max	p-value[b]	Distribution[b]	Method[c]	LRL of RI[a]	URL of RI[a]	CI 90% of LRL	CI 90% of URL
RBC conc.	$10^6/\mu L$	Polvina	103	5.15	0.455	5.16	4.19	6.16	0.6	G	NP	4.25	6.01	4.19-4.36	5.86-6.16
		Zapadni	57	4.93	0.404	4.84	4.26	5.88	0.043	NG	NP	4.3	5.87	4.26-4.41	5.65-5.88
MCH	pg	Polvina	102	35	2.26	35.2	30.2	39.8	0.59	G	NP	30.6	39.47	30.2-31.2	390-39.8
		Zapadni	57	36.2	35.7	35.7	30.1	42.4	0.129	NG	NP	30.4	42.1	30.1-31.6	41.4-42.4
Platelet conc.	$10^3/\mu L$	Polvina	103	380	63	374	261	555	0.51	G	NP	266	501	261-281	481-555
		Zapadni	57	343	61.5	328	215	467	0.011	NG	NP	226	466	215-258	449-467
RDWcv1		Polvina	103	15.3	0.78	15.3	13.9	17.1	0.04	NG	NP	14	16.88	13.9-14.16	16.7-17.1
		Zapadni	55	15	0.76	15	13.6	17.1	0.717	G	NP	13.6	16.9	13.6-13.8	16.3-17.1
PDWsd		Polvina	102	117	1.94	11.7	8.5	17.1	0.24	G	NP	8.5	16	8.5-8.8	15.0-17.1
		Zapadni	57	10.8	1.7	10.8	8.0	14.3	0.1	NG	NP	8.0	14.1	8.0-8.5	13.7-14.3

G, Gaussian; NG, non-Gaussian; NP, nonparametric

Table 6: Cytokine panels of outliers. Asterisks denote values identified as outliers when compared to the remaining population by visual inspection of the data and Q-Q plots. Animals were determined to be outliers if 3 or more cytokine levels were above the established reference ranges.

Animal ID	GM-CSF1	IFN-γ	KC	IL-6	IL-8	IL-10	IL-15	IL-18	MCP1	TNF-α
320P	185.09*	<10.5	6798.1*	54.97*	<21.7	<8.50	388.49	98.36*	<21	<6.10
012X	548.12*	<10.5	729.1	248.39*	<21.70	104.69	1135.6	321.38*	716.96*	58.91*
015X	320.61*	<10.5	725.1	125.91*	<21.70	<8.50	<9.0	<5.80	169.88*	139.34*
010X	490.16*	<10.5	4719.1*	339.77*	<21.70	<8.50	665.15	135.57*	<21	59.96*

Acknowledgements

We would like to acknowledge R. Ream, M. Williams, M. Lander, and the science field team from NOAA Fisheries and biologists from the Ecosystem Conservation office, St. Paul AK for their assistance with capture, restraint and acquisition of samples. In addition, we would like to acknowledge research assistants Lauren Prince and Jacqueline Harrison for laboratory sample analysis at Colorado State University.

References

Andaluz-Ojeda, D., Bobillo, F., Iglesias, V., Almansa, R., Rico, L., Gandia, F., Resino, S., Tamayo, E., de Lejarazu, R.O., Bermejo-Martin, J.F., 2012. A combined score of pro- and anti-inflammatory interleukins improves mortality prediction in severe sepsis. Cytokine 57, 332-336.

Auten, R.L., Davis, J.M., 2009. Oxygen toxicity and reactive oxygen species: the devil is in the details. Pediatr Res 66, 121-127.

Beckmen, K.B., Blake, J.E., Ylitalo, G.M., Stott, J.L., O'Hara, T.M., 2003. Organochlorine contaminant exposure and associations with hematological and humoral immune functional assays with dam age as a factor in free-ranging northern fur seal pups (Callorhinus ursinus). Mar Pollut Bull 46, 594-606.

Bozza, F.A., Salluh, J.I., Japiassu, A.M., Soares, M., Assis, E.F., Gomes, R.N., Bozza, M.T., Castro-Faria-Neto, H.C., Bozza, P.T., 2007. Cytokine profiles as markers of disease severity in sepsis: a multiplex analysis. Crit Care 11, R49.

Catignani, G.L., 1986. An HPLC method for the simultaneous determination of retinol and alpha-tocopherol in plasma or serum. Methods Enzymol 123, 215-219.

Chatterton, J., Med, C.Z., Pas, A., Alexander, S., Leech, M., Uddstrom, L., Harvey, C., Masters, N., Dennison, S., Roe, W.D., 2020. Mycobacterial Disease and Subsequent Diagnostic Investigations in a Group of Captive Pinnipeds in New Zealand. J Zoo Wildl Med 51, 177-187.

Cohen, J., 2002. The immunopathogenesis of sepsis. Nature 420, 885-891.

Desforges, J.P., Sonne, C., Levin, M., Siebert, U., De Guise, S., Dietz, R., 2016. Immunotoxic effects of environmental pollutants in marine mammals. Environ Int 86, 126-139.

Duffy, A.L., Olea-Popelka, F.J., Eucher, J., Rice, D.M., Dow, S.W., 2010. Serum concentrations of monocyte chemoattractant protein-1 in healthy and critically ill dogs. Vet Clin Pathol 39, 302-305.

Dufresne, M.M., Frouin, H., Pillet, S., Lesage, V., De Guise, S., Fournier, M., 2010. Comparative sensitivity of harbour and grey seals to several environmental contaminants using in vitro exposure. Mar Pollut Bull 60, 344-349.

Duncan, C., Dickerson, B., Pabilonia, K., Miller, A., Gelatt, T., 2014a. Prevalence of Coxiella burnetii and Brucella spp. in tissues from subsistence harvested northern fur seals (Callorhinus ursinus) of St. Paul Island, Alaska. Acta Veterinaria Scandinavica 56.

Duncan, C., Goldstein, T., Hearne, C., Gelatt, T., Spraker, T., 2013. Novel polyomaviral infection in the placenta of a northern fur seal (Callorhinus ursinus) on the Pribilof Islands, Alaska, USA. J Wildl Dis 49, 163-167.

Duncan, C.G., Tiller, R., Mathis, D., Stoddard, R., Kersh, G.J., Dickerson, B., Gelatt, T., 2014b. Brucella placentitis and seroprevalence in northern fur seals (Callorhinus ursinus) of the Pribilof Islands, Alaska. J Vet Diagn Invest 26, 507-512.

Dupont, A., Siebert, U., Covaci, A., Weijs, L., Eppe, G., Debier, C., De Pauw-Gillet, M.C., Das, K., 2013. Relationships between in vitro lymphoproliferative responses and levels of contaminants in blood of free-ranging adult harbour seals (Phoca vitulina) from the North Sea. Aquat Toxicol 142-143, 210-220.

Eckersall, P.D., Schmidt, E.M., 2014. The final hurdles for acute phase protein analysis in small animal practice. J Small Anim Pract 55, 1-3.

Foster, G., Nymo, I.H., Kovacs, K.M., Beckmen, K.B., Brownlow, A.C., Baily, J.L., Dagleish, M.P., Muchowski, J., Perrett, L.L., Tryland, M., Lydersen, C., Godfroid, J., McGovern, B., Whatmore, A.M., 2018. First isolation of Brucella pinnipedialis and detection of Brucella antibodies from bearded seals Erignathus barbatus. Dis Aquat Organ 128, 13-20.

Friedrichs, K.R., Harr, K.E., Freeman, K.P., Szladovits, B., Walton, R.M., Barnhart, K.F., Blanco-Chavez, J., American Society for Veterinary Clinical, P., 2012. ASVCP reference interval guidelines: determination of de novo reference intervals in veterinary species and other related topics. Vet Clin Pathol 41, 441-453.

Funke, C., King, D.P., Brotheridge, R.M., Adelung, D., Stott, J.L., 1997. Harbor seal (Phoca vitulina) C-reactive protein (C-RP): purification, characterization of specific monoclonal antibodies and development of an immuno-assay to measure serum C-RP concentrations. Vet Immunol Immunopathol 59, 151-162.

Gebhardt, C., Hirschberger, J., Rau, S., Arndt, G., Krainer, K., Schweigert, F.J., Brunnberg, L., Kaspers, B., Kohn, B., 2009. Use of C-reactive protein to predict outcome in dogs with systemic inflammatory response syndrome or sepsis. J Vet Emerg Crit Care (San Antonio) 19, 450-458.

Geffre, A., Concordet, D., Braun, J.P., Trumel, C., 2011. Reference Value Advisor: a new freeware set of macroinstructions to calculate reference intervals with Microsoft Excel. Vet Clin Pathol 40, 107-112.

Gelain, M.E., Bonsembiante, F., 2019. Acute Phase Proteins in Marine Mammals: State of Art, Perspectives and Challenges. Front Immunol 10, 1220.

Hall, S.A., Stucke, D., Morrone, B., Lebelt, D., Zanella, A.J., 2015. Simultaneous detection and quantification of six equine cytokines in plasma using a fluorescent microsphere immunoassay (FMIA). MethodsX 2, 241-248.

Hotchkiss, R.S., Monneret, G., Payen, D., 2013. Immunosuppression in sepsis: a novel understanding of the disorder and a new therapeutic approach. Lancet Infect Dis 13, 260-268.

Johnson, V., Burgess, B., Morley, P., Bragg, R., Avery, A., Dow, S., 2016. Comparison of cytokine responses between dogs with sepsis and dogs with immune-mediated hemolytic anemia. Vet Immunol Immunopathol 180, 15-20.

Kakuschke, A., Profrock, D., Prange, A., 2013. C-reactive protein in blood plasma and serum samples of harbor seals (Phoca vitulina). Mar Mammal Sci 29, E183-E192.

Kjelgaard-Hansen, M., Jacobsen, S., 2011. Assay validation and diagnostic applications of major acute-phase protein testing in companion animals. Clin Lab Med 31, 51-70.

Krishnan, S.M., Sobey, C.G., Latz, E., Mansell, A., Drummond, G.R., 2014. IL-1beta and IL-18: inflammatory markers or mediators of hypertension? Br J Pharmacol 171, 5589-5602.

Lahti, A., Petersen, P.H., Boyd, J.C., Rustad, P., Laake, P., Solberg, H.E., 2004. Partitioning of nongaussian-distributed biochemical reference data into subgroups. Clin Chem 50, 891-900.

Le Boedec, K., 2016. Sensitivity and specificity of normality tests and consequences on reference interval accuracy at small sample size: a computer-simulation study. Vet Clin Pathol 45, 648-656.

Levin, M., Romano, T., Matassa, K., De Guise, S., 2014. Validation of a commercial canine assay kit to measure pinniped cytokines. Vet Immunol Immunopathol 160, 90-96.

Li, C.S., Wu, K.Y., Chang-Chien, G.P., Chou, C.C., 2005. Analysis of oxidative DNA damage 8-hydroxy-2'-deoxyguanosine as a biomarker of exposures to persistent pollutants for marine mammals. Environ Sci Technol 39, 2455-2460.

McHuron, E.A., Sterling, J.T., Costa, D.P., Goebel, M.E., 2019. Factors affecting energy expenditure in a declining fur seal population. Conserv Physiol 7, coz103.

Minor, C., Kersh, G.J., Gelatt, T., Kondas, A.V., Pabilonia, K.L., Weller, C.B., Dickerson, B.R., Duncan, C.G., 2013. Coxiella burnetii in northern fur seals and Steller sea lions of Alaska. J Wildl Dis 49, 441-446.

Norberg, S.E., Burkanov, V.N., Tuomi, P., Andrews, R.D., 2011. Hematology of Free-Ranging, Lactating Northern Fur Seals, Callorhinus ursinus. J Wildlife Dis 47, 217-221.

Petersen, H.H., Nielsen, J.P., Heegaard, P.M., 2004. Application of acute phase protein measurements in veterinary clinical chemistry. Vet Res 35, 163-187.

Poole, C.F., Evans, N.J., Wibberley, D.G., 1977. Determination of selenium in biological samples by gas-liquid chromatography with electron-capture detection. J Chromatogr 136, 73-83.
Ross, P.S., Pohajdak, B., Bowen, W.D., Addison, R.F., 1993. Immune function in free-ranging harbor seal (Phoca vitulina) mothers and their pups during lactation. J Wildl Dis 29, 21-29.
Seguel, M., Perez-Venegas, D., Gutierrez, J., Crocker, D.E., DeRango, E.J., 2019. Parasitism Elicits a Stress Response That Allocates Resources for Immune Function in South American Fur Seals (Arctocephalus australis). Physiol Biochem Zool 92, 326-338.
Sharick, J.T., Vazquez-Medina, J.P., Ortiz, R.M., Crocker, D.E., 2015. Oxidative stress is a potential cost of breeding in male and female northern elephant seals. Funct Ecol 29, 367-376.
Sheldon, J.D., Johnson, S.P., Hernandez, J.A., Cray, C., Stacy, N.I., 2017. Acute-Phase Responses in Healthy, Malnourished, and Otostrongylus-Infected Juvenile Northern Elephant Seals (Mirounga Angustirostris). J Zoo Wildl Med 48, 767-775.
Son, D.S., Parl, A.K., Rice, V.M., Khabele, D., 2007. Keratinocyte chemoattractant (KC)/human growth-regulated oncogene (GRO) chemokines and pro-inflammatory chemokine networks in mouse and human ovarian epithelial cancer cells. Cancer Biol Ther 6, 1302-1312.
Spraker, T.R., Lander, M.E., 2010. Causes of mortality in northern fur seals (Callorhinus ursinus), St. Paul Island, Pribilof Islands, Alaska, 1986-2006. J Wildl Dis 46, 450-473.
Van Bressem, M.F., Raga, J.A., Di Guardo, G., Jepson, P.D., Duignan, P.J., Siebert, U., Barrett, T., Santos, M.C., Moreno, I.B., Siciliano, S., Aguilar, A., Van Waerebeek, K., 2009. Emerging infectious diseases in cetaceans worldwide and the possible role of environmental stressors. Dis Aquat Organ 86, 143-157.
Wagner, B., Freer, H., 2009. Development of a bead-based multiplex assay for simultaneous quantification of cytokines in horses. Vet Immunol Immunopathol 127, 242-248.
Yadav, A., Saini, V., Arora, S., 2010. MCP-1: chemoattractant with a role beyond immunity: a review. Clin Chim Acta 411, 1570-1579.

Figure 1 – Representative serum protein electrophoretograms adult female Northern fur seals.

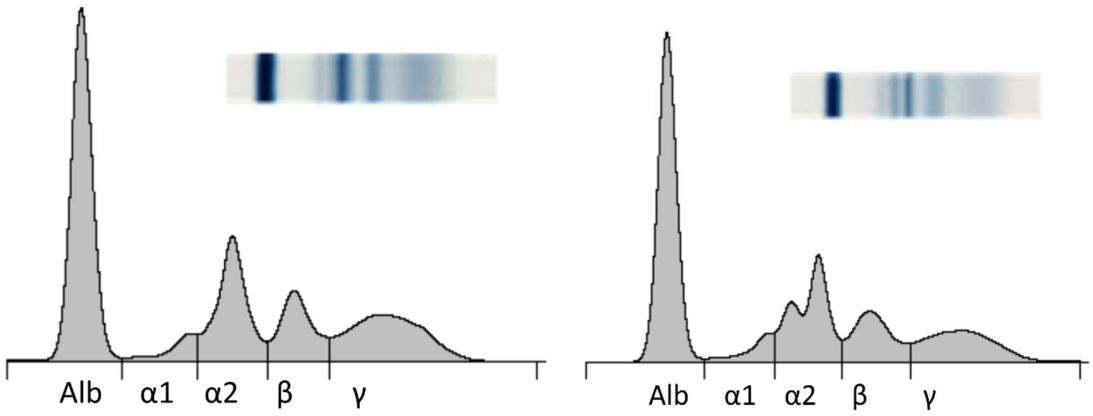

Figure 2: Comparison of paired serum and EDTA plasma from a single patient. Note the presence of a fibrinogen peak in the β-γ region of the plasma sample (pink tracing) which is not present in the serum sample (grey tracing).