Searching for Stress: Hematologic Indicators of Nutritional Inadequacies in Steller Sea Lions

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Introduction

The population of Steller sea lions (*Eumetopias jubatus*) has declined more than 80% since the 1970's (Loughlin, 1998), and continues to decrease within the western portion of their range. A prevalent hypothesis to explain this decline proposes that changes in the quality and/or availability of prey have resulted in an undefined nutritional inadequacy affecting sea lion health and, ultimately, has caused changes in key life history parameters (e.g., reproduction, survival) (Trites and Donnelly, 2003).

A number of studies have attempted to identify behavioral and physiologic indicators of nutritional stress in wild sea lions, with varied results (Trites et al., 2003). In nutritional studies of most vertebrates, one of the primary diagnostic tools is the observation of changes in hematologic parameters. Ideally, blood samples can be used to not only detect nutritional stress, but also to differentiate between proximate causes. Although changes in a single blood parameter are rarely used for diagnostic purposes, a range of hematologic parameters have become standard analytic tools that, when used collectively, can provide a powerful diagnostic tool.

Unfortunately, the measurable response of an animal to a set stimulus is highly species-specific. Therefore, it is critical to undertake empirical trials to test the response of particular parameters to nutritional stress. Furthermore, the power of a parameter to be used to detect differences among wild populations also requires a set of control values to determine the extent and source of natural variation.

This experiment examined the response of a suite of hematologic parameters to experimentally induced nutritional stress in a group of captive Steller sea lions. The goal of this study was to identify a suite of parameters that could be used to diagnose comparable conditions among wild Steller sea lions.

Methods

The experiments were conducted with four captive female Steller sea lions. They were alternated

between isocaloric diets of Atka mackerel (4.9-6.7% lipid w.w.) and herring (10.3-13.4% lipid). The level of food intake (~35.6 kJ d^{-1}) was set *a priori* at a level estimated to produce a 10-15% loss of initial body mass over the 29-day trials. Body mass was measured daily (± 0.1 kg), and body composition was determined at the start and end of each trial by deuterium dilution technique.

Blood samples were also obtained at the beginning and end of each trial for clinical analyses. Plasma and serum samples were obtained under isoflurane anesthesia, processed, and sent to a commercial lab (Central Veterinary Services) for analysis. A standard suite of 39 clinical parameters was measured (Table 1). The consistency in blood parameter changes was determined by comparing the direction of change between of pre- and post-experimental samples for each of the 39 tested parameters. The direction, and not the extent of the change was the only pertinent factor in this initial analysis. The parameters that yielded significantly consistent results were then reanalyzed to determine the magnitude of the parameter change.

Results

Nine of the blood parameters measured in this study showed consistent changes over the 29-day period of induced nutritional stress. White blood cell counts, platelet counts, phosphorous levels, alkaline phosphatase levels, and serum Fe levels all showed consistent decreases, whilst red blood cell counts, hemoglobin levels, hematocrit levels, and gamma GT levels, showed consistent increases (summarized in Table 1). Only one of the blood parameters showed a significantly different response in relation to diet - blood urea nitrogen (BUN) levels showed a consistent increase on the Atka mackerel diet and a consistent decrease on the herring diet ($P=0.029$). Sea lions on the Atka mackerel diet showed a mean percentage increase in BUN level of 9.2%, and a mean percentage decrease of 4.9% on the herring diet.

Discussion

Over the last 10 yr, a great deal of scientific effort has been undertaken to discern the cause of the decline in Steller sea lion numbers. This has included ongoing studies of sea lion physiology, including diagnostic hematology. However, interpretation of results from field studies require controlled experiments with captive animals undergoing known nutritional stress.

The current study has identified consistent changes in certain blood parameters over this type of simulated nutritional stress, including differences related to prey species. Although the majority of blood parameters measured in this study showed little consistency in changes over the 29-day period of simulated nutritional stress, nine of the parameters did show consistent changes across the trials. White blood cell counts, platelet counts, phosphorous levels, alkaline phosphatase levels, and serum FE levels all showed consistent decreases, whilst red blood cell counts, hemoglobin levels, hematocrit levels, and gamma GT levels, showed consistent increases.

The response of specific hematologic parameters to nutritional stress is highly species-specific and few studies have been carried out on pinnipeds. Roletto (1993) found that diseased California sea lions (*Zalophus californianus*) had higher WBC, BUN and lower RBC, Hb, Hct, Hb concentration, and alkaline phosphatase levels. Diseased elephant seals (*Mirounga angustirostris*) had higher RBC,

Hb, Hct, WBC, and lower alkaline phosphatase. Furthermore, harbor seals (*Phoca vitulina*) showed marked changes in erythrocyte parameters with changes in the availability of clupeid prey (Thompson et al., 1997).

Among other mammals, bobcats showed decreased phosphorus levels, and increased Hb levels and RBC counts during periods when their primary species of lagomorph prey was scarce (Knick and Seal, 1993). These results indicate hemato-concentration and have been found previously in wolves (DelGuidice, et al., 1991) and badgers (Harlow and Seal, 1981). In contrast, herring gulls showed decreased Hct and Hb levels with experimentally induced fasting (Totzke et al., 1999).

Surprisingly, the blood metabolites that commonly reflect short-term nutritional status - glucose, BUN, or protein levels - did not show consistent changes over the experimental trials in this current study. However, blood urea nitrogen (BUN) did show significantly different patterns over the two different diets (it was the only parameter to do so). It showed a consistent increase on the Atka mackerel diet, and a decrease on the herring diet. BUN is one of the most widely used indices of nutritional status. It is a good indicator of protein nutrition, being directly related to protein ingested during periods when energy intake is above maintenance levels. However, when energy levels drop below maintenance levels, as in this current study, blood urea nitrogen is likely to rise as a result of tissue catabolism. During both diet trials, the animals lost substantial protein stores (around 8 kg). Therefore, it is surprising that BUN levels decreased during the herring diet trials.

BUN has been tested as an index of nutritional status in cottontails (*Sylvilagus* sp.); it was higher in animals that were on restricted diets and were losing weight, indicating protein catabolism. Similarly, white-tailed deer (*Odocileus virginianus*) and moose (*Alces alces*) on starvation diets showed increases in BUN levels. Chinstrap penguins (*Pygoscelis antarctica*) showed increased levels of plasma nitrogen wastes (urea) that result from protein catabolism throughout the fasting period (Alonso-Alvarez et al., 2003). Blood samples from Steller sea lion pups caught in Alaska, revealed that BUN levels were significantly higher in the Gulf of Alaska than Southeast Alaska (Rea et al., 1998). It was suggested in this study that the higher levels measured in the Gulf of Alaska were indicative of animals that had undergone a short fasting period. Similarly, herring gulls (*Larus argentatus*) showed significantly higher levels of BUN during periods of fasting (Totzke et al., 1999). High urea concentrations were found in Eurasian badgers (*Meles meles*) that were in poor nutritional condition (Domingo-Roura et al., 2001).

Previous studies, many with ruminant mammals, have shown that there are significant changes in blood characteristics with nutritional status. However, it is equally clear that there is no overwhelming choice of blood parameter to indicate nutritional stress across different species. Therefore, species-specific empirical tests such as the one carried out in the current study are essential to place results from wild studies in a biologically meaningful context.

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Literature Cited

Alonso-Alvarez, C., M. Ferrer, J. Vinuuela, and J.A. Amat. 2003. Plasma chemistry of the chinstrap penguin *Pygoscelis antarctica* during fasting periods: a case of poor adaptation to food deprivation? Polar Biol. 26: 14-19.

DelGuidice, G.D., L.D. Mech, and U.S. Seal. 1991. Gray wolf density and its association with weights and hematology of pups from 1970 to 1988. J. Wildl. Dis. 27: 630-636.

Domingo-Roura, X., C. Newman, F. Calafell, and D.W. Macdonald. 2001. Blood biochemistry reflects seasonal nutritional and reproductive constraints in the Eurasian badger (*Meles meles*). Physiol. Biochem. Zool. 74: 450-460.

Harlow, H.J. and U.S. Seal. 1981. Changes in hematology and metabolites in the serum and urine of the badger, *Taxidea taxus*, during food deprivation. Can. J. Zool. 59: 2123-2128.

Knick, S.T., et al. 1993. Hematologic, biochemical, and endocrine characteristics of bobcats during a prey decline in southeastern Idaho. Can. J. Zool. 71: 1448-1453.

Loughlin, T.R. 1998. The Steller sea lion: A declining species. Bios. Cons. 1: 91-98.

Rea, L.D., M.A. Castellini, B.S. Fadely, and T.R. Loughlin. 1998. Health status of young Alaska Steller sea lion pups (*Eumetopias jubatus*) as indicated by blood chemistry and hematology. Comp. Biochem. Physiol. a-Mol. Int. Physiol. 120: 617-623.

Thompson, P.M., D.J. Tollit, H.M. Corpe, R.J. Reid, and H.M. Ross. 1997. Changes in haematological parameters in relation to prey switching in a wild population of harbour seals. Funct. Ecol. 11: 743-750.

Totzke, U., M. Fenske, O. Huppop, H. Raabe, and N. Schach. 1999. The influence of fasting on blood and plasma composition of herring gulls (*Larus argentatus*). Physiol. Biochem. Zool. 72: 426-437.

Trites, A. and C.P. Donnelly. 2003. The decline of Steller sea lions *Eumatopias jubatus* in Alaska: a review of the nutritional stress hypothesis. Mammal Rev. 33: 3-28.

Table 1. Summary of changes in the 39 tested blood parameters over a 29-day experimentally induced nutritional stress in a group of captive Steller sea lions.

Parameter	P (consistency)	Direction of Change	Mean Pre Trial Levels	Mean Post Trial Levels	Mean Change (%)
White cell count	0.013	Decrease	6.1 (1.36)	5.4 (1.05)	-9.66
Red cell count	0.0001	Increase	4.4 (0.23)	4.6 (0.24)	5.72
Hemoglobin	0.0001	Increase	161.2 (6.81)	169.8 (7.99)	5.122
Hematocrit	0.0001	Increase	0.5 (0.03)	0.5 (0.02)	5.04
Mean corp. volume	0.424	-	106.6 (3.42)	105.3 (2.34)	-0.83
Mean corp. Hb	0.18	-	37.0 (1.22)	36.8 (1.04)	-0.49
Mean corp. Hb conc.	0.424	-	347.6 (9.11)	349.4 (4.79)	0.19
RDW	0.99	-	17.3 (2.6)	16.9 (2.19)	-1.13
Platelet count	0.013	Decrease	310.2 (77.37)	288.1 (74.09)	-7.159
Mean platelet volume	0.791	-	9.7 (2.15)	10.2 (1.99)	5.07
Glucose	0.424	-	6.7 (0.47)	6.4 (0.53)	-5.45
Blood urea nitrogen	0.99	-	7.7 (0.79)	7.4 (0.9)	-0.26
Creatinine	0.18	-	101.7 (11.79)	96.1 (7.64)	-2.82
Sodium	0.424	-	149.3 (2.16)	149.8 (1.53)	0.49
Potassium	0.99	-	3.7 (0.16)	3.7 (0.17)	0.068
Calcium	0.791	-	2.4 (0.1)	2.4 (0.09)	0.19
Phosphorus	0.013	Decrease	2.2 (0.33)	1.9 (0.22)	-10.66
Total protein	0.99	-	72.1 (2.9)	73.2 (2.12)	1.5
Albumin	0.99	-	39.8 (1.6)	40.8 (1.34)	2.43
Globulin	0.791	-	32.3 (1.78)	32.3 (1.67)	0.45
Albumin/Globulin ratio	0.791	-	1.3 (0.07)	1.3 (0.09)	1.36
Bilirubin total	0.424	-	3.3 (0.79)	3.8 (1.11)	26.8
Alkaline Phosph.	0.013	Decrease	87.9 (20.72)	78.2 (14.74)	-9.59
SGPT (alt)	0.057	-	75.9 (31.7)	83.2 (22.6)	12.46
SGOT (ast)	0.99	-	15.3 (8.4)	16.5 (9.18)	29.18
Chloride	0.99	-	111.4 (2.1)	111.2 (1.75)	-0.29
Carbon dioxide	0.791	-	24.8 (2.37)	24.2 (2.37)	-1.84
Calcium osmolality	0.18	-	299.1 (4.26)	299.4 (3.32)	0.28
Anion gap	0.99	-	17.0 (2.52)	18.3 (2.1)	1.22
Creatine phosphate	0.791	-	93.8 (14.42)	112.6 (12.5)	21.7
Gamma GT	0.0018	Increase	93.8 (14.42)	112.6 (12.51)	21.71
Serum Fe	0.0001	Decrease	22.3 (3.65)	16.1 (3.4)	-28.8
IBCT	0.18	-	59.3 (10.63)	50.6 (6.37)	-15.16
Iron saturation (%)	0.791	-	27.7 (4.48)	24.1 (2,78)	-10.48
CD-Neutrophils	0.791	-	4.1 (1.02)	3.8 (0.93)	-31.08
CD-Lymphocytes	0.424	-	1.7 (0.32)	1.4 (0.39)	-26.1
CD-Monocytes	0.99	-	0.4 (0.14)	0.3 (0.11)	-12.6
CD-Eosinophils	0.18	-	0.2 (0.26)	0 (0.06)	-23.01
CD-Basophils	0.791	-	0 (0.02)	0 (0.02)	373.7