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BLOOD CHEMISTRIES AND BODY CONDITION OF STELLER SEA LION PUPS AT MARMOT ISLAND, ALASKA

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This work is part of a large project focused on assessing the blood chemistry and body condition of pinnipeds in and around Alaskan waters. We have utilized a series of blood indices that reflect hydration state, blood oxygen transport, and protein, lipid and carbohydrate metabolism. In addition to total mass, animals are also examined for blubber thickness at several locations around the body. These parameters are useful for detecting significant changes in health status that might alter water balance, cause anemia, or compromise basic metabolic status. The Steller sea lion (*Eumetopias jubatus*) is of particular interest because its population has declined over the last 20 years to such an extent that the species has been designated as "threatened" under the United States Endangered Species Act (Federal Register, November 26, 1990). The cause(s) of the decline are unknown but may be linked to redistribution, disease, environmental perturbations (which may influence the quality or quantity of prey), the synergistic effects of fisheries, or other unknown causes (Braham *et al.* 1980, Merrick *et al.* 1987, Loughlin and Merrick 1989, Loughlin *et al.* 1992). Calkins and Goodwin (1988) suggested that adult female Steller sea lions in the Gulf of Alaska during 1985–1986 were anemic and smaller in body size than animals sampled ten years earlier, possibly as a result of food limitations. Also, Castellini and Calkins (1993) have shown the latter group was more lean than the animals studied in the 1970s. The best population models currently suggest that the decline in sea lion numbers results from a reduction in survival of juveniles or breeding females, or both (York, personal communication).

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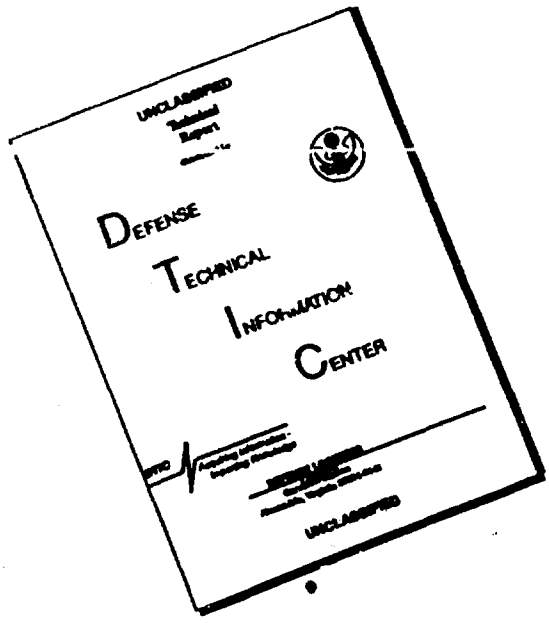

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BLOOD CHEMISTRIES AND BODY CONDITION OF STELLER SEA LION PUPS AT MARMOT ISLAND, ALASKA

This work is part of a large project focused on assessing the blood chemistry and body condition of pinnipeds in and around Alaskan waters. We have utilized a series of blood indices that reflect hydration state, blood oxygen transport, and protein, lipid and carbohydrate metabolism. In addition to total mass, animals are also examined for blubber thickness at several locations around the body.

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These parameters are useful for detecting significant changes in health status that might alter water balance, cause anemia, or compromise basic metabolic status. The Steller sea lion (*Eumetopias jubatus*) is of particular interest because its population has declined over the last 20 years to such an extent that the species has been designated as "threatened" under the United States Endangered Species Act (Federal Register, November 26, 1990). The cause(s) of the decline are unknown but may be linked to redistribution, disease, environmental perturbations (which may influence the quality or quantity of prey), the synergistic effects of fisheries, or other unknown causes (Braham *et al.* 1980, Merrick *et al.* 1987, Loughlin and Merrick 1989, Loughlin *et al.* 1992). Calkins and Goodwin (1988) suggested that adult female Steller sea lions in the Gulf of Alaska during 1985–1986 were anemic and smaller in body size than animals sampled ten years earlier, possibly as a result of food limitations. Also, Castellini and Calkins (1993) have shown the latter group was more lean than the animals studied in the 1970s. The best population models currently suggest that the decline in sea lion numbers results from a reduction in survival of juveniles or breeding females, or both (York, personal communication).

The work reported here considered the possibility that significant metabolic disorders could be affecting Steller sea lion pups as newborns and decreasing their survival during their first month of life. In accordance with our routine sampling protocols, four separate levels of health status which might be indicative of such major disorders were considered: hydration state, blood metabolic chemistry, blood oxygen transport, and blubber depth. There are a variety of diseases or nutritional deficiencies in marine mammals that are indicated by gross changes in these factors. For example, in northern fur seal pups, malnutrition is marked by low values for subcutaneous blubber, dehydration in all tissues, and viscous blood (Keyes 1965). In general, hydration state in the plasma and the whole blood responds readily to alterations in water balance in pinnipeds (Castellini *et al.* 1990). Plasma chemistry can demonstrate excessive protein metabolism caused by starvation (increases in blood urea nitrogen [BUN]), breakdowns in lipid metabolic regulation (free fatty acids [FFA] and ketone bodies [β-HBA]), and carbohydrate regulation (glucose), all of which are altered in pinnipeds that are fasting for extended periods or have begun to starve (Castellini and Rea 1992). For instance, Hubbard (1968) reported that the BUN level in an anorexic Steller juvenile was off-scale at over 160 mg%, compared to a normal range of about 9–30 mg%. In seals, extreme fasting can increase blood ketone levels to over 1.5 mM, although not to values as high as seen in other fasting mammals (Castellini and Costa 1990). Blood hematocrit and hemoglobin were determined to test for possible anemia, dehydration, and/or iron deficiency. Blubber and skinfold thickness were determined to investigate potential gross problems with nutrition or thermoregulation. Using these clinical precedents, gross changes in the measured blood parameters could indicate a variety of pathological or nutritional problems (for review of marine mammal clinical blood pathology, see Bossart and Dierauf 1990). For many of these values, comparative data were available that were collected from Steller pups over 20 yr ago in California (Hubbard 1968), before their population in Alaska started to decline.

Table 1. Blood chemistries from Steller pups at Marmot Island, AK and reference values.

Sample date	Subject ID no.	Mass (kg)	SG WB (gm/ml)	SG PL (gm/ml)	Plasma %water	Blood %water	RBC %water	HCT (%)	Hb (gm%)	MCHC	Ketones (mM)	Glucose (mM)	FFA (mM)	BUN (mg%)
6/26/90	801	17	1.049	1.021	94.3	78.3	66.9	60.0	19.4	32.3	0.32		1.75	14.1
6/27/90	802	28	1.061	1.024	94.0	83.7	70.8	46.5	15.3	32.9	0.44	8.4	0.69	10.2
6/27/90	803	27	1.049	1.018	93.3	80.5	68.3	53.0	18.1	34.2	0.15	9.1	0.22	16.2
6/27/90	804	27	1.042	1.021	93.6	81.0	67.4	50.0	16.7	33.4	0.27	8.1	0.42	14.0
6/27/90	805	21	1.055	1.018	93.1	83.4	70.2	44.7	14.8	33.1	0.21	12.4	0.59	11.0
6/27/90	806	21	1.044	1.022	94.2	82.9	67.7	44.7	14.8	33.1	0.43	7.7	0.57	16.0
6/27/90	807	21		1.018	93.8			55.0	17.5	31.8	0.45	5.9	0.54	10.9
6/27/90	808	33		1.019	93.6			51.5	17.0	33.0	0.31	7.2	0.79	4.8
6/27/90	809	32		1.014	94.1			48.5	16.2	33.4	0.24	8.2	0.37	8.3
6/30–7/4/91	301	25		1.016	92.9						0.25	11.4	1.34	14.0
6/30–7/4/91	303	20		1.017	93.1						0.2	8.6	0.73	14.3
6/30/91	305	22		1.012	93.2						0.16	9.9	1.42	12.9
6/30–7/4/91	307	25		1.016	93.3						0.23	9.6	1.82	19.0
6/30–7/4/91	309	28		1.013	92.7						0.19	9.6	1.57	11.5
7/4/91	311	24		1.013	93.1						0.13	7.7	2.11	11.8
6/30–7/4/91	313	21		1.014	93.0						0.18	8.7	1.54	12.6
6/30/91	315	19		1.018	93.3						0.11	7.8	2.05	19.9
7/4/91	317	14		1.018	93.5						0.35	6.2	3.3	15.4
Mean			1.050	1.017	93.4	81.6	68.5	50.4	16.6	33.0	0.26	8.6	1.2	13.2
SD			0.006	0.003	0.4	1.9	1.5	4.8	1.5	0.6	0.10	1.6	0.8	3.5
(a) Steller pups (1968)			na	na	na	na	na	48.6	16.2	33.3	na	5.9	na	21.3
(b) Northern fur seal			na	na	91.1	77.1	70.9	46.3	16.3	35.3	na	8.3	na	40.8
(c) California sea lion			1.063	1.025	90.3	77.2	69.8	48	12–20	32–38	na	8.6	na	17.5
(d) Suckling elephant seal pup			1.071	1.022	90–93	72.9	67–68	48.4	18–22	33–39	0.1	7–9	1–3	25–35

References: (a) Hubbard 1968; (b) Hubbard 1968, Castellini and Castellini 1989; (c) Hubbard 1968, Castellini and Castellini 1989, Bossart and Dierauf 1990; (d) Costa and Ortiz 1982, Castellini and Castellini 1989, Castellini and Costa 1990, Castellini *et al.* 1990, Castellini and Rea 1992.

Key: SG = specific gravity; WB = whole blood; PL = plasma; RBC = red blood cells; HCT = hematocrit; Hb = hemoglobin; MCHC = mean corpuscular hemoglobin concentration; FFA = free fatty acids; BUN = blood urea nitrogen

Eighteen newborn (2–3 wk postpartum) Steller sea lion pups were studied on Marmot Island, northeast of Kodiak Island, in the Gulf of Alaska in late June and early July, 1990 and 1991. Nine pups were studied each year. Marmot Island is an historical breeding area for Steller sea lions but population levels have dropped over 67% in the last 10 years (Loughlin *et al.* 1992). We studied pups near their nursing areas by herding their mothers into the surf zone and then working with the pups that remained on the beach. The pups were placed in a small hoop net, weighed to the nearest 0.5 kg on a hanging scale and then moved back onto the sand where they were manually restrained while body measurements and blood samples were taken. Blood samples (about 10 ml) were drawn from the pelvic venous plexus with 18 gauge × 2 inch needles into heparinized syringes and then transferred into heparinized blood collection tubes. The blood was immediately sampled for hematocrit and hemoglobin and then chilled on ice for return to the field camp (6 h, maximum time). In 1991, blood samples from five of the pups were collected at two times, one week apart, and the average values are reported. In 1990, blubber thickness was measured using a portable ultrasound transducer (Ultrasonics, Inc.; specified accuracy ±1 mm) at six key locations around the body (dorsal and lateral at the chest, axillary, and mid-trunk). In 1991, skinfold calipers were used to measure blubber and skin thickness at the same six sites and the data were plotted against body weight to test whether blubber was being laid down with increasing size. The entire procedure for each pup was completed within 10–15 min. At the field camp hematocrit values were measured in duplicate using standard clinical micro-hematocrit centrifugal techniques, and the samples were then prepared for return to the University of Alaska at Fairbanks.

Hemoglobin levels were determined using cyanmethemoglobin spectrophotometric assay techniques (SIGMA kit #525). Mean corpuscular hemoglobin concentration was calculated by dividing the hemoglobin concentration by the HCT. Plasma glucose was assayed using a modified reaction of the hexokinase-glucose-6-phosphate dehydrogenase assay (Castellini and Castellini 1989). FFA in plasma were assayed spectrophotometrically using a linked enzymatic assay (NEFA-C; WAKO Pure Industrial Chemicals). BUN was measured using an enzymatic endpoint determination (SIGMA kit #66-20). β-HBA was assayed using a modified form of the β-hydroxybutyrate dehydrogenase reaction (Castellini and Costa 1990). Water concentrations in the plasma and whole blood were determined using dry weight/wet weight techniques, and red blood cell (RBC) water was determined using relationships between plasma and whole blood water concentration, HCT levels, and the specific gravity of plasma and whole blood (Castellini and Castellini 1989, Castellini *et al.* 1990). Specific gravity of the plasma and whole blood was determined by measuring the mass of 200 μl of sample on a 4-place electronic scale (Harris *et al.* 1987, Castellini *et al.* 1990).

Table 1 contains the measured values and mean (±SD) values for all the blood assay data. Not all values could be collected for every sea lion. For comparative purposes the table also has values from 11 Steller pups sampled over 22 yr ago and reference values for other pinnipeds. Whole blood, plasma

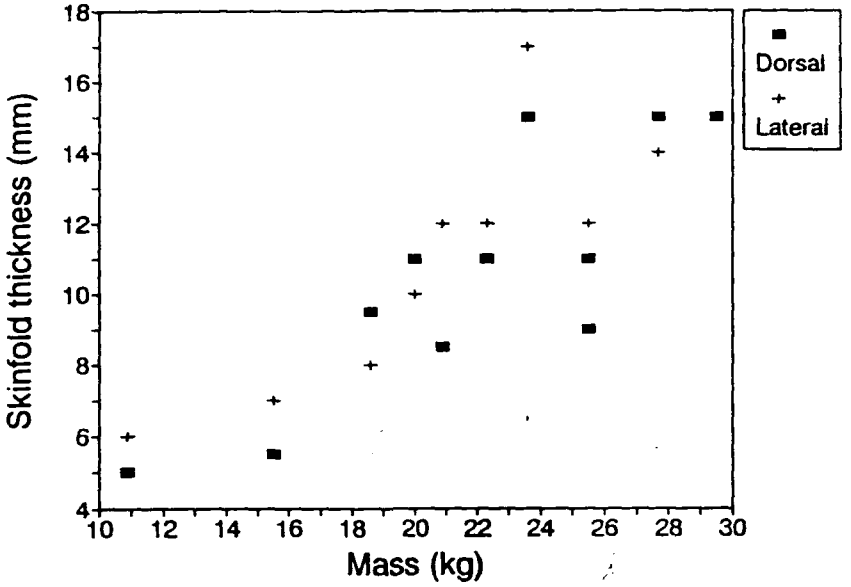

Figure 1. Plot of axillary skinfold thickness against body mass for Steller sea lion pups in 1991.

and red cell values of water content and specific gravities for the Steller sea lion pups were normal compared to other pinnipeds and, if anything, appeared to be higher in hydration state. No obvious differences were noted in hematocrit or hemoglobin levels or in the amount of hemoglobin per RBC. Plasma ketone levels in fasting or starving pinnipeds have only recently been investigated (Castellini and Costa 1990, Nordoy and Blix 1991), but the low levels in Steller pups did not indicate ketosis brought about by starvation. Plasma glucose and FFA levels were more variable and might have reflected the time since the pups last nursed. However, because the results were in the range of reference values, there were no gross indicators of carbohydrate or lipid irregularities. Similarly, the plasma urea nitrogen levels appeared lower than reference values and did not demonstrate the increased protein catabolism that is brought on by starvation in pinnipeds (Nordoy *et al.* 1992).

The average dorsal blubber thickness at mid-trunk *via* ultrasound (skin subtracted) for the nine pups in 1990 was 3 ± 1 mm. In 1991, axillary skinfold thickness was plotted against body weight (Fig. 1) and indicated that skin and blubber were being laid down with increasing pup size. Blubber thickness values for healthy newborn fur seal pups (Scheffer 1961) were recorded to be about 3 mm, the same depth as obtained in this study. Unfortunately, this is the only information available on blubber thickness in newborn otariids.

These results suggest several possibilities about the health status of Steller sea lions. First, the pups appear to be healthy during their early period of suckling,

and there is no indication that they are severely compromised; they were strong, agile and active. Second, there does not seem to be any indication of a major disease in neonatal sea lions in this region of Alaska that impacts blood metabolite chemistry, blood chemistry associated with iron metabolism and oxygen carrying capacity, or hydration state.

The limitations of this study are that not all possible metabolic disorders were screened. It is important to note that these samples were not analysed for viral or bacterial disease or immune indications of infection, although it seems unlikely that a problem serious enough to reduce the survival of the pups in their first month of life would have escaped detection. There was no indication that these 18 pups were the survivors from earlier newborn mortality as there has not been any increase in the numbers of carcasses on the beaches that would indicate a spreading disease (Loughlin, personal observation). It is important to realize that these data reflect the health status of pups during their first few weeks of life. It is conceivable that a metabolic problem could occur at the juvenile stage and we are currently addressing this issue.

We conclude that the blood chemistries and body condition of newborn Steller sea lion pups at Marmot Island were within normal ranges for pinnipeds, and that they have not changed over the last 22 yr compared to control animals from California. There were no indications of severe metabolic disorders that impacted these newborns. Whether such problems exist at the juvenile and adult stages, or in other regions of the sea lion range, is yet to be determined. To that end, we are currently expanding our sampling range into wider areas of the Gulf of Alaska, southeastern Alaska, and into the Aleutians. Work has begun on examining juveniles and adults, and samples have also been submitted for expanded blood chemistry and immune analysis using routine veterinary screening procedures.

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