Metabolic response to fasting in 6-week-old Steller sea lion pups (*Eumetopias jubatus*)

L.D. Rea, D.A.S. Rosen, and A.W. Trites

Abstract: Four Steller sea lions (*Eumetopias jubatus*) aged 6 weeks were fasted for 2.5 d to determine how young pups mobilize energy reserves during short periods of fasting similar to those experienced in the wild. At 6 weeks of age, the pups lost $5.1 \pm 0.3\%$ of their body mass during 2 d of fasting, with an average daily mass loss of 0.7 ± 0.1 kg·d^{-1}. Plasma blood urea nitrogen (BUN) concentration increased significantly from 3.0 ± 0.1 mM, after an overnight fast, to 4.8 ± 0.5 mM, after 2.5 d of fasting. It is apparent that BUN levels are quickly depressed, since after only an overnight fast, these pups showed BUN levels 2- to 4-fold lower than those measured after the same pups, when 9 months of age, had recently been fed fish. Plasma ketone body (β-HBA) concentrations of the 6-week-old pups increased significantly from 0.32 ± 0.08 to 0.42 ± 0.08 mM between 0.5 and 1.5 d of fasting. There was no significant change in mean plasma concentration beyond 1.5 d, owing to variable individual responses to extended fasting. Plasma β-HBA levels at 9 months of age ranged from 0.07 to 0.18 mM. Six-week-old Steller sea lion pups showed blood chemistry consistent with metabolic adaptation to fasting within 16 h but were unable to sustain a protein-sparing metabolism for a prolonged period. The pups appeared to revert to protein catabolism after only 2.5 d of fasting. This infers a decrease in lipid catabolism that might be due to the depletion of available lipid resources.

Résumé : Quatre jeunes Otaries de Steller (*Eumetopias jubatus*), âgées de 6 semaines, ont été soumises à un jeûne de 2,5 jours, afin de nous permettre de déterminer comment les jeunes mobilisent leurs réserves énergétiques au cours de courtes périodes de jeûne, semblables à celles qu'ils doivent subir en nature. À 6 semaines, les jeunes ont perdu $5,1 \pm 0,3$ % de leur masse totale après 2 jours de jeûne, ce qui constitue une perte quotidienne moyenne de $0,7 \pm 0,1$ kg·jour^{-1}. La concentration de l'azote uréique du plasma sanguin (BUN) a augmenté significativement, passant de $3,0 \pm 0,1$ mM après un jeûne d'une nuit, à $4,8 \pm 0,5$ mM après 2,5 jours de jeûne. Il est donc évident que les concentrations d'azote uréique du plasma sanguin déclinent rapidement puisque, après seulement une nuit de jeûne, ces concentrations étaient de deux à quatre fois moins élevées que celles qui ont été mesurées chez les mêmes individus nourris de poisson à l'âge de 9 mois. Les concentrations de corps cétoniques (β-HBA) dans le plasma des jeunes de 6 mois ont augmenté significativement de $0,32 \pm 0,08$ à $0,42 \pm 0,08$ mM entre 0,5 et 1,5 jours de jeûne. Il n'y a pas eu de changement important de la concentration moyenne dans le plasma après plus de 1,5 jours de jeûne à cause des réponses individuelles variables à un jeûne prolongé. Les concentrations plasmatiques de β-HBA à l'âge de 9 mois se situaient entre 0,07 et 0,18 mM. Chez les jeunes de 6 mois, la chimie du sang était celle d'individus dont le métabolisme s'était adapté au jeûne en moins de 16 heures, mais ces animaux se sont montrés incapables de maintenir leurs réserves de protéines pendant une période prolongée. Ils avaient déjà recours au catabolisme des protéines après seulement 2,5 jours de jeûne. Cela suppose une diminution du catabolisme des lipides qui peut être attribuable à la disparition des ressources lipidiques disponibles.

[Traduit par la Rédaction]

Introduction

During periods of food deprivation, animals must mobilize body reserves to maintain homeostasis and provide en-ergy to the central nervous system. Lipids will be mobilized as fuel as animals shift from initial protein catabolism to a protein-sparing metabolism. During this gradual transition, changes occur in the relative concentrations of circulating blood urea nitrogen (BUN) and ketone bodies (β-HBA) (Cahill et al. 1966). These changes in metabolic chemistry have been well documented in phocid seals (such as grey seals (Nordøy et al. 1990, 1992), harp seals (Nordøy et al. 1993), and northern elephant seals (Castellini and Costa 1990; Rea 1995)), which undergo prolonged periods of fasting as pups and show evidence of fasting-adapted metabolism (Castellini and Rea 1992). However, few data are available for fasting otariid pups.

This study was designed to simulate the brief fasting period experienced by Steller sea lion (*Eumetopias jubatus*) pups on the breeding beaches when their mothers are foraging at sea, and provides the only data available on how Steller sea lion pups mobilize energy reserves during short

L.D. Rea.[1] National Marine Mammal Laboratory, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Seattle, WA 98115, U.S.A., and Department of Biology, University of Central Florida, P.O. Box 162368, Orlando, FL 32816-2368, U.S.A.
D.A.S. Rosen and A.W. Trites. Marine Mammal Research Unit, Fisheries Center, The University of British Columbia, 2204 Main Mall, Vancouver, BC V6T 1Z4, Canada.

[1]Author to whom all correspondence should be sent at the following address: Department of Biology, University of Central Florida, P.O. Box 162368, Orlando, FL 32816-2368, U.S.A. (e-mail: lrea@pegasus.cc.ucf.edu).

periods of fasting. In Alaska, sea lions give birth to a single young in June and nurse their pup continuously for an average of 9 d before making their first foraging trip (Milette 1999). Mothers then alternate short periods on land nursing their pups (mean duration 1 d; Milette 1999) with foraging trips (mean durations of 7.1 ± 0.8 to 25.6 ± 2.2 h, depending on geographical location; Brandon and Davis 1999), during which young pups are left unattended on shore.

The purpose of our study was to investigate the effects of complete fasting on body condition and blood chemistry in Steller sea lion pups. We hypothesized that pups would exhibit patterns of changes in blood metabolite concentrations similar to those seen in species adapted to long-term fasting, such as phocid seal pups, but that metabolic shifts would occur more rapidly, owing to the short duration of the fasting intervals typically experienced by pups of this age. The sparsity of physiological data available for this species makes it particularly important to establish ranges of blood values that are typical for healthy animals over a range of physiological states (feeding and fasting). Collecting data under controlled conditions is essential for interpreting blood chemistry and hematology values of free-ranging animals and for monitoring the health of individuals in captive environments.

Methods

The four 6-week-old Steller sea lion pups (two males and two females) that took part in our study were in good general health and had been tube-feeding for approximately 4 weeks on a formulated milk diet since their introduction to the Vancouver Aquarium (Vancouver, B.C., Canada). The composition of the modified formula diet (26.7% fat, 6.3% protein, 22.8% solids; D.A.S. Rosen and A.W. Trites, unpublished data) was similar to the composition of milk collected from free-ranging Steller sea lions within 1 month of parturition (21.6% fat, 9.3% protein, 39.2% solids; Adams 1999). Animals were housed in fenced enclosures with access to fresh water for drinking.

Body mass was measured daily, using a platform scale (±0.1 kg), prior to drawing blood samples. No other morphometric measurements were collected, given the short duration of the study. We felt that the expected error in any girth measurements collected from a physically restrained pup would overshadow the small changes in axillary girth that would be demonstrated over 2 d of fasting.

All pups received a standard ration of milk formula via stomach tube in the early evening of 2 August 1998. One blood sample was collected after a 16-h overnight fast, to measure blood chemistry and hematology factors prior to prolonged fasting. Blood chemistry measured in recently weaned phocid pups does not yet show characteristic protein-sparing profiles, thus we chose to collect our first blood sample following an overnight fast, to facilitate comparison with previously published pinniped fasting studies, published veterinary blood chemistry data, and studies on fasting juvenile Steller sea lions being conducted concurrently. Subsequent blood samples were collected during the morning hours of the following 2 d. In total, the pups fasted for approximately 2.5 d, with blood samples being collected at 0.5, 1.5, and 2.5 d following their last meal.

Blood was drawn from the caudal gluteal vein directly into serum separator and heparinized Vacutainer tubes using a 1.5-in. (1 in. = 25.4 mm) 18-g hypodermic needle and Vacutainer adapter system. Hematocrit was determined immediately, using a battery-operated microhematocrit centrifuge (Compur M1100, Bayer, Germany; samples were spun at 5400 × g for 3.5 min), and hemoglobin concentration was measured spectrophotometrically, using

methanocyanide (Sigma Chemicals kit 525-A). Plasma and serum were then separated from the red blood cells by centrifugation and frozen for later analysis.

Methods for the analysis of glucose, BUN, and β-HBA concentrations and for the determination of water content and specific gravity of the plasma have been previously described by Castellini et al. (1993). Previous studies have shown that heparin is the most appropriate anticoagulant for accurately measuring blood glucose in pinnipeds, because excessive lysis of red blood cells can lead to artificially depressed glucose values when blood is collected in glycolytic inhibitor tubes (sodium fluoride – potassium oxalate) (Castellini et al. 1992). All values are presented as mean ± standard deviation (SD). Paired t tests (Number Cruncher Statistical Systems 2000) were used to determine significant changes ($p \leq 0.05$) in measured parameters during the fasting period.

Seven months following the fasting experiment, we collected blood samples from the same four individual sea lions when in a recently fed state (1.2 to 3.3 h after feeding). We did this to determine whether the changes we noted in plasma metabolite concentrations during the fasting experiment were occurring within the first 16 h after feeding. These data are included for comparison (presented at 0 d fasting). The pups were 9 months of age at this time and were feeding on whole herring (*Clupea* sp.). Blood samples were drawn from the caudal gluteal vein while the pups were manually restrained with the aid of a restraining cage.

Results

Initial body mass of the 6-week-old pups ranged from 24.3 to 36.7 kg prior to fasting. All pups showed a significant decrease in body mass during both the first and second days of fasting ($p < 0.001$; Fig. 1a). This amounted to a mean loss of 0.7 ± 0.1 kg/d and resulted in a 5.1 ± 0.3% decrease from initial mass over a 2-d period (0.5–2.5 d after feeding; Fig. 1b). At 9 months of age, the 4 pups weighed between 51.0 and 76.2 kg.
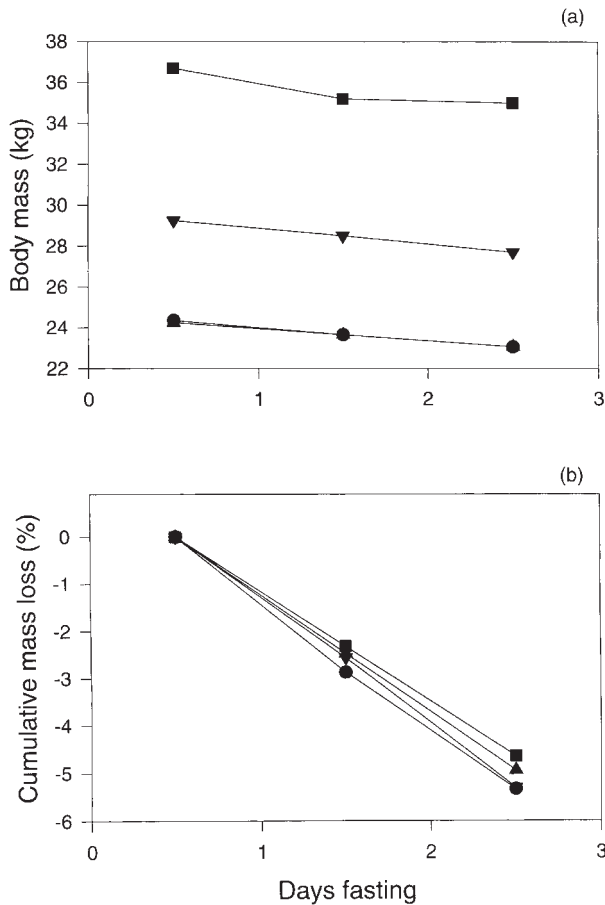
All sea lion pups had low BUN concentrations, ranging from 2.2 to 3.8 mM (3.0 ± 0.7 mM; Fig. 2a), following the overnight fast. Plasma BUN levels remained at a low mean concentration of 3.0 ± 0.1 mM after 1.5 d of fasting but increased significantly to 4.8 ± 0.5 mM after 2.5 d of fasting ($p = 0.006$). In the fed state, the mean BUN level of 9-month-old sea lions was 9.9 ± 0.9 mM, which was more than three times the mean overnight fasting value ($p = 0.0009$).

Mean β-HBA concentration was 0.32 ± 0.08 mM after the overnight fast (Fig. 2b). The β-HBA levels of the 4 pups increased significantly to 0.42 ± 0.08 mM after 1.5 d of fasting ($p = 0.02$). At 2.5 d, β-HBA concentrations had dropped in two of the pups and risen in the other two, resulting in no significant change in mean concentration. When the pups were older and recently fed, β-HBA concentrations were significantly lower, ranging from 0.07 to 0.18 mM ($p = 0.007$).

Plasma-glucose concentrations did not change significantly during the fast, although fasting values (6.1 ± 1.3 mM) were significantly lower than values found in the recently fed pups (8.1 ± 0.6 mM; $p = 0.007$).

Hematocrit increased significantly from 34.8 ± 2.0% at 0.5 d after feeding to 36.3 ± 2.2% at 2.5 d after feeding ($p = 0.02$); however, hemoglobin concentrations, which ranged from 12.0 to 14.3 mg/L, did not change significantly as the fast progressed. Also, no significant change in mean corpuscular hemoglobin concentration was detected during fasting (range 36.8–38.6 mg/L). Owing to the difficulty of collecting

Fig. 1. Change in mass of four 6-week-old Steller sea lion pups (Hazy, ●; Kodiak, ■; Sitka, ▲; and Timber, ▼) during 2.5 d of complete fasting, represented as change in body mass (kg) (*a*) and cumulative percent body mass loss (*b*).
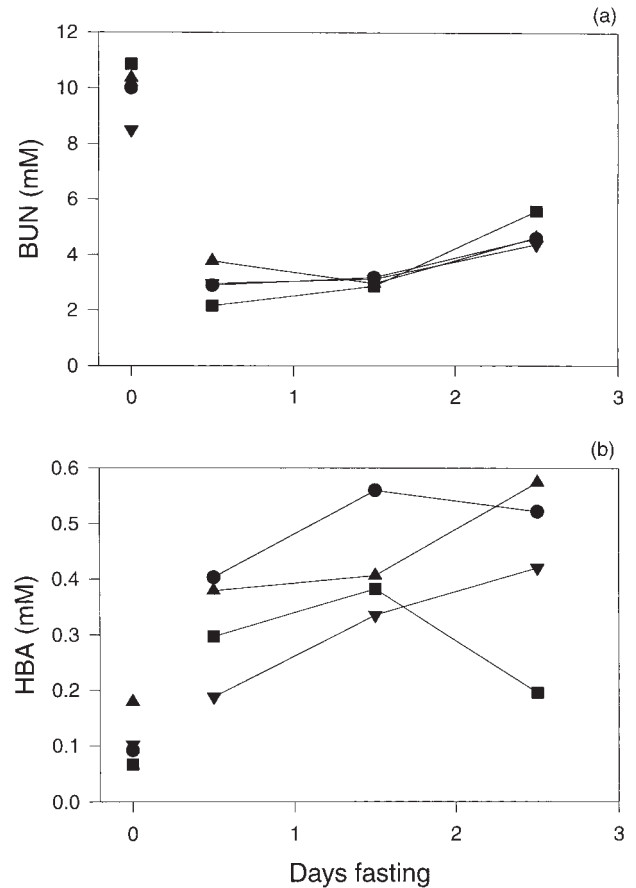
Fig. 2. Changes in plasma concentrations (mM) of blood urea nitrogen (BUN) (*a*) and ketone bodies (β-HBA) (*b*) in four 6-week-old Steller sea lion pups (Hazy, ●; Kodiak, ■; Sitka, ▲; and Timber, ▼) during 2.5 d of complete fasting. Concentrations shown for 0 d of fasting were measured in the same pups at 9 months of age shortly after feeding.

blood samples from the older animals, no data were available on hematocrit or hemoglobin for recently fed pups.

A slight but significant increase ($p = 0.026$) in plasma water concentration was evident between 1.5 and 2.5 d of fasting. A much greater difference was seen between values in fasting pups and those measured in older feeding animals ($p < 0.001$; Fig. 3*a*). Similarly, the specific gravity of the plasma was significantly higher in feeding animals than in fasting pups ($p = 0.001$), while there was no significant change during fasting (Fig. 3*b*).

Discussion

The mean daily rate of mass loss of approximately 2.5% of initial body mass/d measured in this study is similar to rates of mass loss reported for Galapagos fur seals (*Arctocephalus galapagoensis*; 2.9%/d based on a mean mass loss of 181 g/d (Trillmich 1986) and a mean pup mass of 6.1 kg at 1 month of age (Horning and Trillmich 1997)). In comparison, the absolute rate of mass loss in Steller sea lion pups was similar to that measured in fasting northern elephant seal pups during the first half of their postweaning fast (0.7 ± 0.2 kg/d; Rea 1995), but represents a much higher percentage of the Steller sea lion pups' initial body mass. Northern elephant seals lost only 0.7 ± 0.2% of their initial
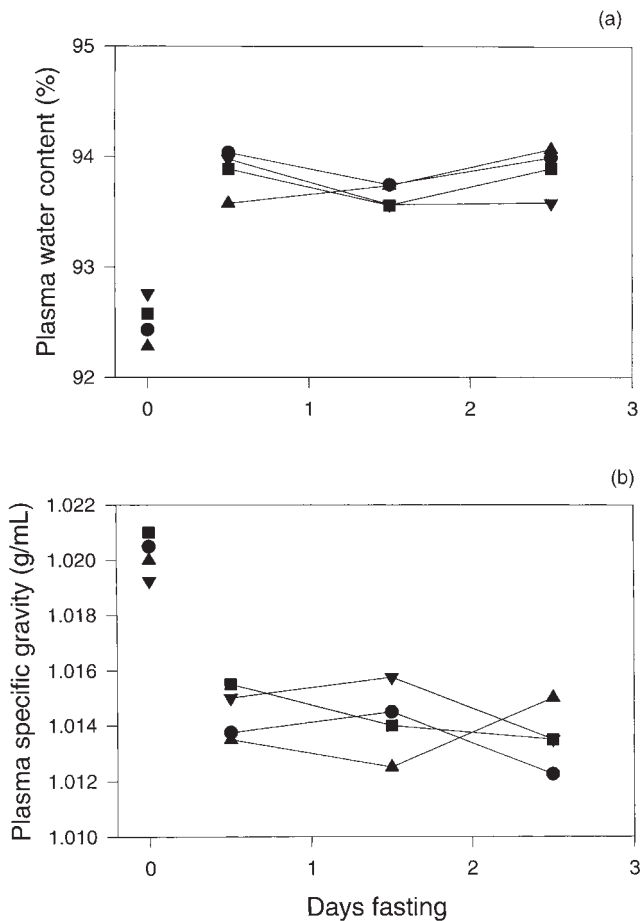
body mass/d ($n = 9$, range 0.4–1.0%/d) compared with a mean daily rate of mass loss of 2.5% measured in this study. This higher rate of mass loss and the smaller initial body size of the otariid pups means that they could not survive the long periods of fasting (up to 3 months) undertaken by their phocid counterparts. One-month-old Steller sea lion and Galapagos fur seal pups experience female absences of only 24 h (range of 5–53 h; Milette 1999) and 30 h (Trillmich 1986), respectively. Such high rates of mass loss should be considered when calculating growth rates and energy transfer efficiencies for pups in the wild. For example, given a relatively long 2-d foraging trip during each nursing–foraging cycle, females must provide their pups with adequate resources during the short period they spend on land, to offset a mass deficit of 5% before any net growth can be realized.

Previous studies on fasting phocid pups reported a gradual decrease in plasma BUN concentrations, indicating a gradual decline in the animals' dependance on protein catabolism. In this study, in Steller sea lion pups that had fasted overnight, circulating levels of BUN had already reached low levels (compared with levels after feeding when the pups were 9 months old) by the time the first blood sample was drawn. This suggests that Steller sea lion pups shifted rapidly from utilizing protein stores for energy to utilizing lipid stores.

Fig. 3. Plasma water content (%) (*a*) and specific gravity (*b*) in four 6-week-old Steller sea lion pups (Hazy, ●; Kodiak, ■; Sitka, ▲; and Timber, ▼) during 2.5 d of complete fasting. Values shown for 0 d of fasting were measured in the same pups at 9 months of age shortly after feeding.

larly northern elephant seal pups) is partially due to their larger body size and higher body fat content (Rea 1995).

Consistent with the rapid decrease in protein catabolism, sea lion pups also show an immediate increase in circulating levels of β-HBA. This suggests an increased reliance on lipid catabolism within the first 16 h of fasting. Comparable concentrations in elephant seal pups do not occur until 3 weeks into the postweaning fast (Rea 1995).

The variability in individual response seen in β-HBA levels during the second day of fasting may mean that at least one of the pups had switched back to catabolizing higher amounts of protein to provide his energy needs during fasting. The same pup showed the largest increase in circulating BUN levels during the second day of fasting, as well, which also suggests an increased reliance on burning proteins. All metabolite concentrations (BUN, β-HBA, and glucose) measured during this study fell within the range of values documented in free-ranging Steller sea lion pups studied in Alaska; thus, this study validates using plasma β-HBA levels of above 0.3 mM to indicate fasting in free-ranging sea lion pups (Rea et al. 1998).

The slight increase in hematocrit seen after 2.5 d of fasting was not due to dehydration, since plasma water content increased slightly during this time. Phocid pups also show no indication of dehydration during prolonged fasting (Castellini et al. 1990). The main differences seen between feeding and fasting animals in this study may be due to age-related changes in water balance. Adult female Steller sea lions show a mean plasma water content of $90.7 \pm 13.7\%$ ($n = 57$) and a mean plasma specific gravity of 1.026 ± 0.006 g/mL ($n = 57$), which is consistent with the trends of decreasing plasma water concentration and increasing plasma specific gravity with age (Castellini and Rea 1996).

In summary, 6-week-old Steller sea lion pups showed evidence of rapid metabolic adaptation to fasting but were unable to sustain a protein-sparing metabolism for a prolonged period at this age. These data suggest that pups were reverting to protein catabolism after only 2.5 d of fasting. This infers a decrease in lipid catabolism that may be due to the depletion of available lipid resources.

Acknowledgements

In species adapted to prolonged fasting, circulating BUN levels are held at low levels for several weeks. Subsequent increases in BUN concentration are typically shown in fasting animals that are making a transition from stage II fasting (the protein-sparing phase), in which lipids are the primary source of energy, to a stage in which protein is catabolized to provide the needed gluconeogenic precursors for glucose production. This is thought to happen when available lipid reserves are depleted.

The moderate increase in plasma BUN concentration seen after only 2.5 d of fasting suggests that Steller sea lions have a limited ability to spare protein stores. This might be due to sea lions having a smaller body size (and thus a higher metabolic rate per unit body mass) and lower body fat content than phocid seals. At the onset of fasting, Steller sea lion pups were approximately 11–15% body fat ($n = 3$; unpublished data derived by water space measurement using deuterium oxide dilutions; L. Rea), which is similar to body fat content measured in free-ranging pups (2.3–10%; Brandon and Davis 1999; Trites and Jonker 1999). In contrast, prior to fasting, northern elephant seal pups are approximately 48% body fat (Rea and Costa 1992). There is evidence that the extreme protein-sparing ability of phocid pups (particu-

References

Adams, T.C. 1999. Appendix B.7: proximate composition of Alaskan Steller sea lion milk during first four weeks post-partum. *In* Final Report on the Steller Sea Lion Physiology Workshop, held

at Seattle, Wash., 8–10 February 1999. *Edited by* A. Didier. Pacific States Marine Fisheries Commission, 45 S.E. 82nd Drive, Suite 100, Gladstone, OR 97027–2522, U.S.A. pp. 24–26.

Brandon, E.A.A., and Davis, R.W. 1999. Appendix B.5: neonatal growth and condition and female attendance patterns. *In* Final Report on the Steller Sea Lion Physiology Workshop, held at Seattle, Wash., 8–10 February 1999. *Edited by* A. Didier. Pacific States Marine Fisheries Commission, 45 S.E. 82nd Drive, Suite 100, Gladstone, OR 97027–2522, U.S.A. pp. 20–22.

Cahill, G.F., Herrera, M.G., Morgan, A.P., Soeldner, J.S., Steinke, J., Levy, P.L., Reichard, G.A., and Kipnis, D.M. 1966. Hormone-fuel interrelationships during fasting. J. Clin. Invest. **45**: 1751–1769.

Castellini, J.M., Castellini, M.A., and Kretzmann, M.B. 1990. Circulatory water concentration in suckling and fasting northern elephant seal pups. J. Comp. Physiol. B, **160**: 537–542.

Castellini, M.A., and Costa, D.P. 1990. Relationships between plasma ketones and fasting duration in neonatal elephant seals. Am. J. Physiol. **259**: R1086–R1089.

Castellini, M.A., and Rea, L.D. 1992. The biochemistry of natural fasting at its limits. Experientia (Basel), **48**: 575–582.

Castellini, M.A., and Rea, L.D. 1996. Blood chemistry patterns associated with development in Steller sea lions. *In* Abstracts of the International Symposium and Workshop on Otariid Reproductive Strategies and Conservation, National Zoological Park, Smithsonian Institution, Washington, D.C. p. 11. (Abstr.)

Castellini, M.A., Castellini, J.M., and Kirby, V.L. 1992. Effects of standard anticoagulants and storage procedures on plasma glucose values in seals. J. Am. Vet. Med. Assoc. **201**: 145–148.

Castellini, M.A., Davis, R.W., Loughlin, T.R., and Williams, T.M. 1993. Blood chemistries and body condition of Steller sea lion pups at Marmot Island, Alaska. Mar. Mamm. Sci. **9**: 202–208.

Horning, M., and Trillmich, F. 1997. Development of hemoglobin, hematocrit, and erythrocyte values in Galápagos fur seals. Mar. Mamm. Sci. **13**: 100–113.

Milette, L.L. 1999. Behaviour of lactating Steller sea lions (*Eumetopias jubatus*) during the breeding season: a comparison between a declining and stable population in Alaska. M.Sc. thesis, The University of British Columbia, Vancouver.

Nordøy, E.S., Ingebretsen, O.C., and Blix, A.S. 1990. Depressed metabolism and low protein catabolism in fasting grey seal pups. Acta Physiol. Scand. **139**: 361–369.

Nordøy, E.S., Stijfhoorn, D.E., Raheim, A., and Blix, A.S. 1992. Water flux and early signs of entrance into phase III of fasting in grey seal pups. Acta. Physiol. Scand. **144**: 477–482.

Nordøy, E.S., Aakvaag, A., and Larsen, T.S. 1993. Metabolic adaptations to fasting in harp seal pups. Physiol. Zool. **66**: 926–945.

Rea, L.D. 1995. Prolonged fasting in pinnipeds. Ph.D. thesis, University of Alaska, Fairbanks.

Rea, L.D., and Costa, D.P. 1992. Changes in standard metabolism during long-term fasting in northern elephant seal pups (*Mirounga angustirostris*). Physiol. Zool. **65**: 97–111.

Rea, L.D., Castellini, M.A., Fadely, B.S., 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, **120**: 617–623.

Trillmich, F. 1986. Attendance behavior of Galapagos sea lions. *In* Fur seals: maternal strategies on land and at sea. *Edited by* R.L. Gentry and G.L. Kooyman. Princeton University Press, Guildford, N.J. pp. 168–185.

Trites, A.W., and Jonker, R.A.H. 1999. Appendix B.9: morphometric measurements and body conditions of healthy and starveling Steller sea lion pups (*Eumetopias jubatus*). *In* Final Report on the Steller Sea Lion Physiology Workshop, held at Seattle, Wash., 8–10 February 1999). *Edited by* A. Didier. Pacific States Marine Fisheries Commission, 45 S.E. 82nd Drive, Suite 100, Gladstone, OR 97027–2522, U.S.A. p. 29.