# Utilization of stored energy reserves during fasting varies by age and season in Steller sea lions

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

**Abstract:** Nine captive Steller sea lions (*Eumetopias jubatus* (Schreber, 1776), 1.75–6 years of age) were fasted for 7–14 d to test the effect of short-term fasting on changes in body mass and body condition. Trials were repeated during both the summer breeding season and the nonbreeding season in seven animals to elucidate whether there was a seasonal component to the ability of Steller sea lions to adapt to limited food resources. Mean percent mass loss per day was higher during the breeding season in juveniles (1.8% ± 0.2%·$d^{-1}$) than in subadults (1.2% ± 0.1%·$d^{-1}$), but there were no significant age-related differences during the nonbreeding season (juveniles, 1.5% ± 0.3%·$d^{-1}$; subadults, 1.7% ± 0.3%·$d^{-1}$). A decrease in the rate of mass loss occurred after the first 3 d of fasting only in subadults during the breeding season. Percent total body lipid ranged from 11% to 28% of total body mass at the initiation of fasting trials. Animals with lower initial percent total body lipid exhibited higher subsequent rates of mass loss and a lower percentage of tissue catabolism derived from lipid reserves. There was no evidence of metabolic adaptation to fasting in juveniles, which suggests that juvenile sea lions would be more negatively impacted by food limitation during the breeding season than would subadults.

**Résumé :** Neuf lions de mer de Steller (*Eumetopias jubatus* (Schreber, 1776), âgés de 1,75–6 ans) ont été soumis à un jeûne de 7 à 14 j afin de vérifier l'effet d'un jeûne de courte durée sur les changements de masse et de condition corporelles. Nous avons repris les expériences à la fois durant la saison de reproduction et durant la période non reproductive chez sept animaux afin de voir s'il y avait une composante saisonnière à l'aptitude des lions de mer de Steller de s'adapter à des ressources alimentaires réduites. La perte moyenne de masse en pourcentage par jour est plus importante durant la saison de reproduction chez les jeunes (1,8 % ± 0,2 %·$j^{-1}$) que chez les subadultes (1,2 % ± 0,1 %·$j^{-1}$), mais il n'y a pas de différence significative en fonction de l'âge durant la période non reproductive (jeunes 1,5 % ± 0,2 %·$j^{-1}$; subadultes 1,7 % ± 0,3 %·$j^{-1}$). Une diminution du taux de perte de masse se produit après les 3 premiers jours de jeûne, mais seulement chez les subadultes durant la saison de reproduction. Le pourcentage des lipides corporels totaux variait de 11 % à 28 % de la masse corporelle totale au début des expériences de jeûne. Les animaux ayant un lipide corporel total initial plus faible ont subi des taux subséquents de perte de masse plus importants et ont connu un pourcentage plus faible de catabolisme tissulaire dérivé des réserves de lipides. Il n'y a aucune indication d'une adaptation métabolique des jeunes au jeûne, ce qui indique que les jeunes lions de mer subiraient un impact négatif plus important lors d'une pénurie de nourriture durant BS que les subadultes.

[Traduit par la Rédaction]

## Introduction

Although the cause(s) of recent declines in the western Alaska population of Steller sea lions (*Eumetopias jubatus* (Schreber, 1776)) has not been irrefutably identified, a commonly proposed hypothesis contends that low food availability (at least to some age classes or during some times of the year) continues to negatively impact Steller sea lion population recovery (Loughlin and York 2000; DeMaster and Atkinson 2002; Trites and Donnelly 2003). However, assessing whether population declines are related to food limitation in Steller sea lions requires knowledge of the physiological effects of food deprivation in this species. This includes understanding how food limitation affects rates of body mass loss as well as changes in body condition (percent total body lipid content (%TBL)). It is also important to discern whether there is a seasonal or age component to how well Steller sea lions can adapt to limited food resources. There may be times of the year when low food availability will impact animals more severely owing to variation in their ability to metabolically compensate. Additionally, certain age classes may have more developed defenses to unpredictable changes in their food supply.

In the wild, Steller sea lions undergo natural periods of fasting during the summer breeding season. Adult males are thought to fast for weeks while holding breeding territories (Gentry 1970; Gisiner 1985) and reproducing females (who

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may be as young as 3 years) fast for 4–12 d directly after giving birth to a pup (Higgins et al. 1988; Milette and Trites 2003). Even while very young, Steller sea lion pups experience periodic short fasts while their mothers are foraging at sea (Rea et al. 1998; Trites and Porter 2002). Animals that undergo predictable annual fasting periods, such as those associated with breeding, would be expected to show metabolic adaptations to fasting to limit the deleterious effects of these periods of food deprivation (Castellini and Rea 1992).

Many mammalian and avian species (e.g., phocid seals, bears, penguins) decrease their overall rate of energy expenditure to conserve valuable and limited resources while fasting. Evidence of metabolic depression has been demonstrated in several phocid seal species as a decrease in the basal or resting metabolic rates measured during the postweaning fast in pups (Worthy and Lavigne 1987; Markussen et al. 1992; Rea and Costa 1992; Noren 2002). Metabolic rate decreased as fasting progressed in many species, both on a whole-body basis and when corrected for decreasing body size and changing body composition. Northern elephant seal (*Mirounga angustirostris* (Gill, 1866)) pups lost mass at a 41% slower rate during the second 4-week period of the postweaning fast than during the first month (Rea and Costa 1992). This decrease in mass loss was accompanied by a 20% decline in mass-specific metabolic rate seen in elephant seal pups over the 10-week fast (Rea and Costa 1992). Atlantic gray seals (*Halichoerus grypus* (Fabricius, 1791)) decreased the rate of mass loss by 50% after 2 weeks of fasting (from approximately 0.8 to 0.4 $\mathrm{kg \cdot d^{-1}}$), while metabolic rate decreased by 45% during the same period (Nordøy et al. 1990). Nordøy et al. (1993) showed a similar exponential decrease in body mass during 32 d of fasting in harp seal (*Pagophilus groenlandicus* (Erxleben, 1777)) pups. Subadult Steller sea lions have also demonstrated the ability to decrease their resting metabolic rate in response to captive fasting experiments (Rosen and Trites 2002), with a 16% decrease in resting metabolic rate after 1 week of fasting and a mean decrease of 31% from control levels after a second week of fasting. Arnould et al. (2001) illustrated that Antarctic fur seal (*Arctocephalus tropicalis* (Gray, 1872)) pups enter this state of depressed metabolism within 2–3 d of fasting, several days sooner than documented in most fasting phocid pups.

Although metabolic depression is instrumental in decreasing overall rates of body mass loss, important changes in the catabolism of stored fuels for energy are also critical to an animal's ability to efficiently utilize lipid stores and effectively spare critical lean tissue and vital organs during fasting (see review in Castellini and Rea 1992; Rea 1995; Arnould et al. 2001; Houser and Costa 2001). The ability to conserve important protein resources by relying heavily on catabolism of lipid reserves correlates with the initial body fat of a number of species prior to fasting. In Svalbard rock ptarmigan (*Lagopus muta hyperborea* Sundevall, 1845), for example, lean subjects lost mass at a faster rate than subjects with higher initial fat contents because of an immediate reliance on lean tissue catabolism for energy (Lindgård et al. 1992). Similarly, other studies have documented that the total body lipid stores available at the onset of fasting most likely determine the limits of fasting ability (Leiter and Marliss 1982; Goodman et al. 1984; Taylor and Konarzewski 1989; Belkhou et al. 1990; Cherel et al. 1992, 1993; Rutishauser et al. 2004). In both northern elephant seals and southern elephant seals (*Mirounga leonina* (L., 1758)) (Carlini et al. 2001; Noren et al. 2003), fatter pups lost proportionately more fat during the postweaning fast than thinner conspecifics. Higher rates of protein utilization have also been documented during fasting in lactating northern elephant seals that were lean versus those that were fat (Crocker et al. 1998).

Seasonal differences in the ability to adapt metabolically to fasting may also be related to initial body fat stores. American black bears (*Ursus americanus* Pallas, 1780) adopted a highly efficient fasting metabolism (including urea recycling) during periods of winter denning but were unable to maintain their lean body mass when fasted during summer months (when they would normally have access to food) (Nelson et al. 1975). This occurred even when black bears were experimentally housed under simulated winter sleep conditions during the summer. In contrast, Atkinson et al. (1996) found that free-ranging polar bears (*Ursus maritimus* Phipps, 1744) that initiated the summer with high body fat content were able to preserve critical lean tissue (by using a higher percentage of their body energy in the form of fat) when they experienced food limitation during summer months. The latter study suggested that the limited ability of summer black bears to sustain a fasting adapted metabolism in previous studies may be more related to initial body fat stores than strictly to seasonal influences.

Our study was designed to test the hypothesis that juvenile and subadult Steller sea lions metabolically adapt to fasting by minimizing rates of mass loss and preferentially using body fat stores to spare protein as seen in other fasting-adapted species. We also investigated how the seasonal timing of food deprivation impacts changes in body mass and body fat content during controlled fasting conditions to determine whether there are particular times of the year during which Steller sea lions are more negatively impacted by food limitation.

## Methods

Nine captive Steller sea lions participated in one or more fasting trials for a maximum of 14 d to test the effect of short-term fasting on changes in body mass and condition (Table 1). Seven of these animals (four juveniles and three subadults) completed trials during both the breeding season (BS) and nonbreeding season (NBS) to allow direct comparison of physiological responses to fasting in each individual between the two seasons. Four juvenile Steller sea lions (1.75–2.5 years of age) housed at the Vancouver Aquarium (Vancouver, British Columbia) were fasted for 7 d. Five subadult sea lions (3.5–6 years of age) were fasted for 8–14 d first at the Vancouver Aquarium and later at the Alaska SeaLife Center (Seward, Alaska). The duration of fasting trials was set a priori by mass differences between juveniles and subadults and was adjusted during experiments if animals approached mass loss limits set by Animal Care protocols. To ensure the continued good health of each study animal, fasting trials were discontinued if body mass loss exceeded 3% of an individual's body mass per day for 2 con-

**Table 1.** Fasting trials on Steller sea lions (*Eumetopias jubatus*) held at the Alaska Sea Life Center (ASLC) and the Vancouver Aquarium (VA).

| Sea lion (cohort) | Animal ID | Sex | Fasting season | Location | Date of trial | Duration (d) | Age (years) |
|---|---|---|---|---|---|---|---|
| M93WO (1993) | M1 | Male | BS | ASLC | June 1999 | 14 | 6 |
| | | | NBS | VA | December 1996 | 14 | 3.5 |
| F93KI (1993) | F2 | Female | BS | ASLC | June 1999 | 13 | 6 |
| | | | NBS | VA | October 1997 | 8 | 4.5 |
| F93SU (1993) | F1 | Female | BS | VA | June 1997 | 12 | 4 |
| | | | NBS | ASLC | December 1998 | 10 | 5.5 |
| M93AD (1993) | M2 | Male | BS | VA | June 1997 | 14 | 4 |
| M93TA (1993) | M3 | Male | NBS | VA | April 1997 | 9 | 4 |
| F97SI (1997) | F3 | Female | BS | VA | July 1999 | 7 | 2 |
| | | | NBS | VA | March 1999 | 7 | 1.75 |
| M97KO (1997) | M4 | Male | BS | VA | July 1999 | 7 | 2 |
| | | | NBS | VA | March 1999 | 7 | 1.75 |
| F97HA (1997) | F4 | Female | BS | VA | July 1999 | 7 | 2 |
| | | | NBS | VA | November 1999 | 7 | 2.5 |
| M97TI (1997) | M5 | Male | BS | VA | July 1999 | 7 | 2 |
| | | | NBS | VA | November 1999 | 7 | 2.5 |

**Note:** The season during which the fast was undertaken (BS, breeding season; NBS, nonbreeding season) is noted as well as the duration of the fast and the age of the individual at the time of the study.

secutive days or if it exceeded a total mass loss of 15% of initial body mass. The first animal fasted at the Vancouver Aquarium (M1) exhibited a 20% mass loss during the 14 d fasting period. Although this level of mass loss was comparable with mass loss experienced by otariid males in the wild during the BS (Boyd and Duck 1991) and caused no deleterious medical effects to the animal, it was decided that a 15% maximum mass loss would be adopted for all subsequent studies. Five fasting trials were terminated early when the subadult animals reached this assigned maximum mass loss (Table 1).

Animals were housed in separate research tanks filled with seawater and maintained on a normal training schedule using ice cubes instead of fish to reward them for completing tasks such as walking onto a platform scale and entering a restraint cage. Training practices to accomplish the goals of this study were developed by the training staff at the Vancouver Aquarium and are outlined in Christen et al. (1999).

At the onset of the study, blood samples and body mass measurements were collected from each sea lion following an overnight fast. These data provide a control sample for each individual prior to the period of prolonged fasting. Body mass was then measured daily on a platform scale (±0.1 kg). Body composition (%TBL) was estimated using an isotope dilution technique (Costa 1987) at the initiation and completion of each experimental fasting study. This technique provided a direct measure of the change in body condition resulting from food limitation during the BS and NBS. However, as animals were supplied with a fresh water intake during fasting (ice cubes during training sessions and access to drinking water), this technique could not be used as a measure of fasting metabolism. On the first day of study (after an overnight fast), a 10 mL blood sample was drawn into serum separator blood collection tubes to measure background levels of deuterium oxide in each animal. Each animal was then injected intramuscularly with a precisely measured mass of sterile deuterium oxide (99.9% $^2H_2O$). A postinjection blood sample was collected to measure the resulting specific activity of deuterium in serum after 2 h of equilibration. Beginning in 1999, a second postinjection sample was collected at approximately 2.25 h to verify equilibration. Blood samples were centrifuged after 1 h and serum frozen for later isotope analysis. Deuterium analysis was completed in Dr. Kenneth Nagy's laboratory at the University of California at Los Angeles for all studies conducted from 1996 to 1997 and blood samples were analyzed at a commercial laboratory (Metabolic Solutions, Inc., Nashua, New Hampshire) beginning in 1998. No deuterium dilution was conducted on animal F4 at the end of the NBS fast. Total body water was calculated from hydrogen isotope dilution space using the calculation of Bowen and Iverson (1998), which incorporates the results of an otariid-specific validation experiment conducted by Arnould et al. (1996*b*) into a general pinniped model using a larger sample size. Using the Bowen and Iverson (1998) model, interspecific comparisons of energy derived from change in body composition may be more consistent with other pinniped studies. Percent body fat and lean tissue were calculated according to Reilly and Fedak (1990). Animals were held in a restraint cage during blood collection, deuterium injection, and the equilibration period and were held under isofluorane anesthesia (Heath et al. 1996) for all, or part, of this restraint period at the discretion of the attending veterinarian. Calculations of the percentage of energy lost during fasting that was derived from either lean or lipid mass loss were based on the assumptions that the energy density of lipid was 39.9 $MJ \cdot kg^{-1}$, that lean body mass was composed of 22.3% protein, and that the energy density of that protein was 23.6 $MJ \cdot kg^{-1}$ (Atkinson et al. 1996). Morphometric measurements (standard length, axillary and hip girths) were taken opportunistically at the onset and completion of each trial. All measurements were collected while the animals were awake and positioned on target in a ventrally recumbent position. A body condition index (CI, no units) was calculated using the formula CI = (AG / SL) × 100, where AG is axillary girth (cm) and SL is standard length (cm) (Pitcher 1986; Ryg et al. 1990). A body density index (DI, kg·cm$^{-3}$) was also calculated using the formula DI = $M$ / (SL × (AG × AG)) × $10^6$, where $M$ is mass (kg), SL is standard length (cm), and AG is axillary girth (cm) following Castellini and Calkins (1993) as modified by Fadely (1997). Larger values for CI reflect a greater relative girth, while larger values for DI infer a greater mass per volume, consistent with a higher density and, therefore, leaner body condition. To normalize these ratios, CI and DI data were arcsine transformed prior to statistical analysis. Blubber depth was also measured in several animals at the beginning and end of each study using a portable ultrasound (Scanoprobe, Ithaca, New York). Blubber depth was measured at three points: at both a dorsal and a lateral point along the axillary girth ring and at a lateral hip location.

Two-way repeated-measures design ANOVA and Tukey multiple range tests were used to determine significant differences in body morphology, initial CI and DI, initial percent body fat content, and percent contribution of fat to energy expenditure between age classes and in relation to the season of study. Paired $t$ tests were used to test for significant differences in rates of body fat loss between the fasts conducted during the BS and those undertaken in the NBS. Paired $t$ tests were also used to determine significant changes in mass, total body fat mass, %TBL, daily rates of mass loss, CI, and DI and to determine if decreases in blubber depth at the axillary girth ring lateral site were greater during the NBS fasting trials than during the BS fasting trials. To accommodate nonmatched trials, two-sample $t$ tests were also used to determine if changes in CI and DI were greater in NBS trials than in BS trials for all data. Linear regression analysis was used to determine whether there were significant relationships between daily rates of absolute or percent mass loss or the percentage of energy derived from fat and the initial %TBL content of the subjects. In multiple linear regression analyses, one study (either BS or NBS if both were available) was selected randomly for each individual and included for statistical consideration. Statistics were considered significant at $p < 0.05$.

## Results

### Mass loss

Controlled fasting trials on juvenile and subadult Steller sea lions resulted in rates of mass loss ranging from 1.4 to 3.6 kg·d$^{-1}$ in animals with initial body mass ranging from 75.0 to 329.0 kg. Total mass loss experienced during these 7–14 d studies ranged from 10.0 to 49.5 kg, or a total of 8.4%–21.2% of initial body mass. The relative rates of mass loss (to correct for differences in body size and trial length) ranged from 1.0% to 2.0% of initial body mass per day.

There were no significant seasonal differences documented in subadult animals in the absolute rate of mass loss (BS = 2.3 ± 0.9 kg·d$^{-1}$, NBS = 2.6 ± 0.2 kg·d$^{-1}$; $p = 0.68$, $n = 4$) or when daily mass loss was expressed as a proportion of initial mass (BS = 1.2% ± 0.1%·d$^{-1}$, NBS = 1.7% ± 0.3%·d$^{-1}$; $p = 0.09$) (Fig. 1). Contrary to expectation, both the absolute and relative rates of mass loss experienced by the juvenile sea lions in the BS (2.2 ± 0.5 kg·d$^{-1}$ and 1.8 ± 0.2%·d$^{-1}$, respectively) were significantly higher than experienced during the NBS (1.7 ± 0.2 kg·d$^{-1}$ and 1.5 ± 0.3%·d$^{-1}$, respectively) ($p < 0.05$) (Fig. 1). This differential seasonal response to fasting for subadult and juvenile sea lions resulted in no significant age-related differences in relative rates of mass loss between subadults and juveniles in the NBS ($p = 0.27$), while during the BS, relative rates of body mass loss in juveniles (1.8%·d$^{-1}$) were significantly higher than in subadult sea lions (1.2%·d$^{-1}$) ($p < 0.001$).

The rate of mass loss also varied within a fasting trial for some animals. Subadult animals that fasted during the BS showed a significant decrease in the rate of percent mass loss per day between the initial 3 d of fasting (1.5% ± 0.3%·d$^{-1}$) and the period of fasting from day 6 to day 9 (1.1% ± 0.3%·d$^{-1}$) ($p = 0.004$). No additional significant decrease in rate of percent mass loss was seen as the fasts progressed during the BS (day 10 to day 12: 1.2% ± 0.1%, $n = 4$; day 13 to day 14: 0.87% ± 0.3%, $n = 2$; $p > 0.05$). This reduction in the rate of mass loss with the duration of fasting was not seen in subadults during the NBS ($p = 0.45$). In the juvenile cohort, no significant change in rate of mass loss was seen during fasting in the BS ($p = 0.16$); however, during the NBS fasts, these smaller animals actually increased their rate of mass loss from 1.4% ± 0.5%·d$^{-1}$ during the first 3 d of fasting to 1.8% ± 0.5%·d$^{-1}$ on the last 2 d (days 6 and 7) of the fast ($p = 0.038$).

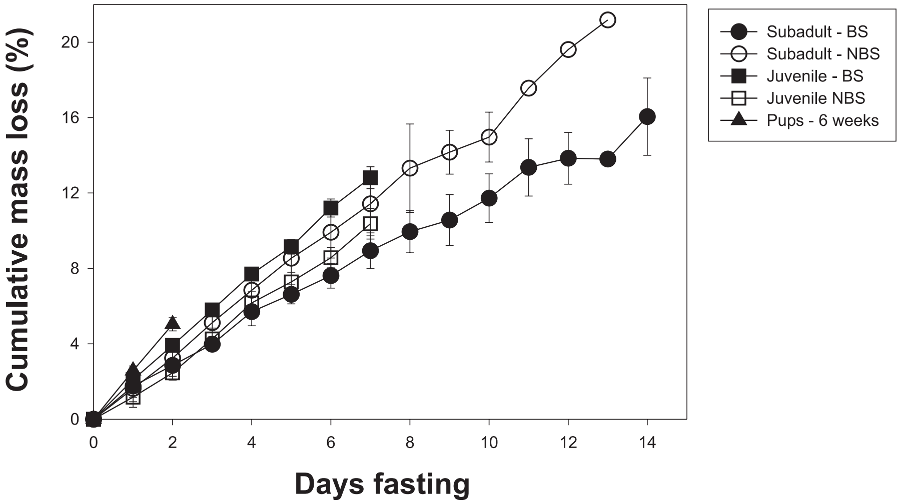
### Morphometrics

Standard length remained relatively constant in all animals during the short study periods, with minor changes attributed to minor inconsistencies in positioning of the animal (Table 2). Axillary girth decreased 4–11 cm (7.4 ± 2.6 cm) (Table 2) during the 8–14 d fasting periods of the subadults and 6–13 cm (8.4 ± 2.9 cm) during the 7 d of fasting for the juvenile sea lions. During these same periods, blubber thickness decreased by 4–10 mm (5.8 ± 2.3 mm) at the lateral point on the axillary girth ring in the subadult animals and 5–10 mm (7.2 ± 1.9 mm) in the juveniles (Table 2). There was no significant difference in the magnitude of these changes in blubber depth between the BS and NBS fasts ($p = 0.29$). Significantly greater decreases in blubber depth were seen at the axillary lateral location (5.8 ± 2.3 mm) than at the hip lateral point (2.6 ± 2.1 mm) ($p = 0.007$) in subadults. Similarly, juveniles showed a greater decrease in blubber thickness at the axillary point (7.2 ± 1.9 mm) than at the hips (3.4 ± 1.5 mm) ($p < 0.001$).

The calculated CI ((AG / SL) × 100) was significantly higher in juveniles prior to fasting (70.1 ± 2.8) than in subadult sea lions (61.5 ± 3.1) ($p < 0.001$). CI decreased in all cases over the duration of the fast ($p < 0.001$). However, there were no significant differences in the magnitude of these changes between seasons (BS = 3.8 ± 2.2, NBS = 3.0 ± 1.2; $p = 0.42$) or between age classes ($p = 0.32$).

The calculated DI ($M$ / (SL × (AG × AG)) × $10^6$) was significantly lower in juvenile sea lions than in subadults at the onset of fasting during the BS (43.5 ± 3.5 and 49.6 ± 3.6 kg·cm$^{-3}$, respectively; $p = 0.008$) and the NBS (44.0 ± 1.7 and 51.1 ± 2.6 kg·cm$^{-3}$ respectively; $p = 0.009$). In contrast with changes seen in CI during fasting, there was no significant change in DI during either season over the fasting period ($p = 0.30$).

**Fig. 1.** Cumulative percent body mass loss during the breeding season (BS) and nonbreeding season (NBS) fasts in subadult and juvenile Steller sea lions (*Eumetopias jubatus*). Comparative data are provided for 6-week-old Steller sea lion pups (Rea et al. 2000).



### %TBL

%TBL ranged from 11% to 28% of total body mass at the initiation of fasting trials in both subadults and juveniles (Table 3). There was no apparent seasonal or age-dependent pattern in the body condition of the animals prior to fasting studies ($p = 0.78$ and $p = 0.89$, respectively), and it is likely that any differences in body fat content were strongly influenced by the diet and feeding regime prior to these studies. A significant decrease in %TBL was seen in subadults ($p < 0.001$) and juveniles ($p < 0.001$) (Table 3). The latter does not include data from F4 or M5 during the NBS fast, as a final body composition could not be obtained. In both subadults and juveniles, there was no significant difference between seasons in the relative rate of fat loss (expressed as a percentage of initial fat mass per day) ($p = 0.82$ and $p = 0.97$, respectively). Similarly, absolute body fat mass decreased significantly during fasting ($p< 0.001$) regardless of the season of study. The daily rate of absolute total body lipid loss did not differ significantly between the BS (2.1 ± 0.4 kg·d$^{-1}$) and NBS (1.7 ± 0.8 kg·d$^{-1}$) ($p = 0.34$) in subadults. Although the daily rate of fat loss did not differ significantly between seasons in juveniles ($p = 0.09$), there was a tendency for higher rates of mass loss (2.7 ± 0.1 kg·d$^{-1}$) during the NBS than during the BS (1.8 ± 0.6 kg·d$^{-1}$). This was an opposite trend to that seen in fasting subadults; however, there was a very small sample size for juveniles in the NBS ($n = 2$).

Lipids contributed 68.8%–97.9% of energy during fasting in subadults and 82.5%–100% of energy mobilized in juveniles during fasting (Table 3). Owing to the wide variation seen between individuals, there was no significant difference in the percentage of total energy expended that was derived from fat catabolism between seasons (BS = 90.8% ± 5.6%, NBS = 88.0% ± 12.5%; $p = 0.80$) or between age classes (subadults = 87.9% ± 10.0%, juveniles = 91.9% ± 6.5%; $p = 0.26$). However, there was a significant positive relationship between the percentage of energy derived from fat reserves and the %TBL prior to fasting ($r^2 = 0.42$, $p = 0.017$) (Fig. 2*a*). There was a significant negative correlation between the rate of absolute body mass loss (kg·d$^{-1}$) and the %TBL of the sea lion at the initiation of fasting ($r^2 = 0.34$, $p = 0.018$) (Fig. 2*b*). A slightly weaker correlation was seen when mass loss was represented on a percent loss per day basis ($r^2 = 0.27$, $p = 0.04$) (Fig. 2*c*). Correlations were not significant for either absolute or percent mass loss per day ($p > 0.05$) when sexes or age classes were separated for analysis.

## Discussion

As expected, all animals experienced a decrease in body mass during fasting. The range of mass loss (1.0%–2.0%·d$^{-1}$) in both juveniles and subadults was higher than that previously reported for young phocid seals that undergo prolonged fasting (Reilly 1991; Nordøy et al. 1993; Rea 1995; Noren et al. 2003) but similar to rates of mass loss seen in fasting northern fur seal pups (Donohue et al. 2000), juvenile male northern fur seals (Baker et al. 1994), and otariid males fasting during the BS (Boyd and Duck 1991). However, the observed rates of mass loss were lower than those reported in Antarctic fur seal pups (Arnould et al. 1996*a*; Guinet et al. 2000; Beauplet et al. 2003; Rutishauser et al. 2004) and 6-week-old Steller sea lion pups (Rea et al. 2000). This would be expected for larger, older animals that presumably have lower mass-specific metabolic rates (Kleiber 1975). Although the subadult sea lions exhibited slightly greater rates of absolute daily mass loss than juveniles (subadults, 2.5 kg·d$^{-1}$; juveniles, 2.0 kg·d$^{-1}$), they exhibited lower relative loss rates when calculated as a percentage of initial body mass (subadults, 1.4%; juveniles, 1.7%). The latter measurement is probably more reflective of the impact of the fasting period on the overall health (and survivability) of a fasting otariid. However, these age-related trends were complicated by an underlying seasonal effect.

Overall, the impact of the fasting episode among juvenile animals appeared to be greater during the BS, when relative rates of mass loss were significantly greater than in the NBS (1.87%·d$^{-1}$ versus 1.46%·d$^{-1}$). Given the small sample size and different seasonal trends in each individual, there was no statistically significant difference in juvenile body fat content between the BS and NBS. However, in two juveniles (M4 and F3), the trend was for initial %TBL to be

**Table 2.** Changes in body morphometrics in subadult and juvenile Steller sea lions during the breeding season (BS) and nonbreeding season (NBS) fasting trials.

| Animal ID | Fasting season | Days fasting | Mass (kg) | Standard length (cm) | Axillary girth (cm) | Hip girth (cm) | Condition index | Density index | Axillary lateral blubber depth (cm) | Axillary dorsal blubber depth (cm) | Hip blubber depth (cm) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| M1 | BS | 0 | 329.0 | 244 | 165 | 109 | 67.8 | 49.8 | 15 | 15 | 11 |
| | | 14 | 280.0 | 243 | 162 | 106 | 66.7 | 43.9 | na | na | na |
| | NBS | 0 | 165.7 | 204 | 126 | 59 | 61.9 | 51.3 | 14 | 14 | 10 |
| | | 14 | 130.6 | 203 | 115 | na | 56.7 | 48.6 | 10 | 10 | 10 |
| M2 | BS | 0 | 150.7 | 200 | 125 | 55 | 62.5 | 48.2 | 27 | na | 18 |
| | | 14 | 128.7 | 196 | 115 | 55 | 58.7 | 49.7 | 22 | 12 | 13 |
| M3 | NBS | 0 | 156.9 | 203 | 120 | na | 58.9 | 54.1 | 15 | 13 | 13 |
| | | 9 | 132.7 | 199 | 113 | 56 | 56.8 | 52.2 | 8 | 10 | 10 |
| F1 | BS | 0 | 137.7 | 195 | 118 | na | 60.3 | 51.1 | 28 | 15 | 18 |
| | | 12 | 117.3 | 193 | 108 | na | 55.7 | 52.6 | 18 | 13 | 13 |
| | NBS | 0 | 167.5 | 207 | 133 | na | 64.3 | 47.8 | 20 | 15 | 11 |
| | | 9 | 144.0 | 204 | 124 | 85 | 60.8 | 45.9 | 12 | 10 | 11 |
| F2 | BS | 0 | 164.0 | 207 | 126 | 85 | 60.9 | 49.9 | 16 | 15 | 8 |
| | | 13 | 140.0 | 210 | 119 | 84 | 56.7 | 47.1 | 12 | 10 | 7 |
| | NBS | 0 | 140.2 | 203 | 116 | na | 57.1 | 51.3 | na | na | na |
| | | 8 | 117.4 | 202 | 111 | na | 55.0 | 47.2 | 13 | 8 | 10 |
| M4 | BS | 0 | 145.4 | 185 | 140 | 76 | 75.7 | 40.1 | 20 | 18 | 13 |
| | | 7 | 126.0 | 183 | 127 | 76 | 69.4 | 42.7 | 8 | 10 | 8 |
| | NBS | 0 | 125.6 | 182 | 127 | 77 | 69.9 | 42.9 | na | na | na |
| | | 7 | 112.8 | 180 | 121 | 71 | 67.2 | 42.8 | 25 | 18 | 10 |
| M5 | BS | 0 | 150.0 | 191 | 137 | 80 | 71.7 | 41.6 | 30 | 20 | 15 |
| | | 7 | 133.4 | 191 | 130 | 79 | 67.8 | 41.6 | 15 | 10 | 10 |
| | NBS | 0 | 163.6 | 203 | 138 | 90 | 68.0 | 42.3 | 25 | 13 | 13 |
| | | 7 | 149.8 | na | na | na | na | na | na | na | na |
| F3 | BS | 0 | 111.0 | 174 | 120 | 66 | 69.0 | 44.3 | 20 | 15 | 13 |
| | | 7 | 96.2 | 175 | 108 | 69 | 61.7 | 47.1 | 13 | 8 | 10 |
| | NBS | 0 | 104.2 | 172 | 119 | 66 | 69.0 | 43.2 | na | na | na |
| | | 7 | 94.2 | 167 | 113 | 62 | 67.4 | 44.6 | 20 | 13 | 8 |
| F4 | BS | 0 | 88.4 | 159 | 109 | 72 | 67.6 | 48.1 | 20 | 13 | 8 |
| | | 7 | 75.6 | 155 | 101 | 66 | 65.2 | 47.8 | 10 | 8 | 10 |
| | NBS | 0 | 93.0 | 164 | 111 | 76 | 67.7 | 46.0 | 20 | 10 | 10 |
| | | 7 | 81.2 | 161 | 103 | 75.5 | 64 | 47.5 | 14 | 10 | 8 |

**Note:** Owing to animal behavior, some data were not attainable and are indicated by na.

**Table 3.** Contribution of lean body mass and total body lipid mass to energy loss during fasting in subadult and juvenile Steller sea lions during the breeding season (BS) and nonbreeding season (NBS).

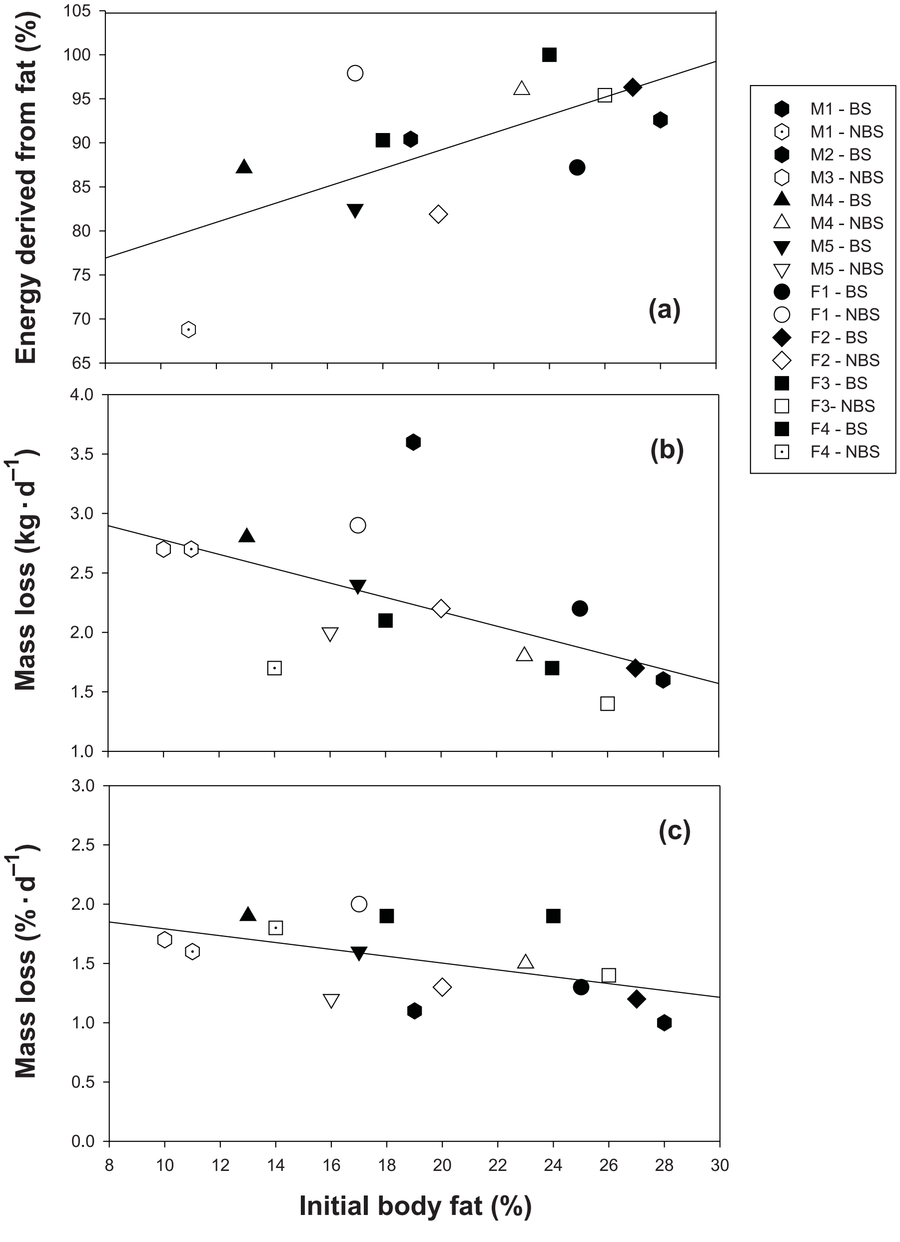
| Animal ID | Fasting season | Duration of fast (d) | Initial total body lipids (%) | Final total body lipids (%) | Lean mass loss (kg) | Lipid mass loss (kg) | Total energy loss (MJ) | Energy derived from lean loss (%) | Energy derived from lipid loss (%) |
|---|---|---|---|---|---|---|---|---|---|
| M1 | BS | 14 | 19 | 13 | 21.7 | 27.3 | 1187 | 9.6 | 90.4 |
| | NBS | 14 | 11 | 8 | 27.1 | 8.0 | 457 | 31.2 | 68.8 |
| M2 | BS | 14 | 28 | 22 | 8.2 | 13.8 | 586 | 7.4 | 92.6 |
| F1 | BS | 12 | 27 | 23 | 10.7 | 9.7 | 439 | 12.8 | 87.2 |
| | NBS | 9 | 25 | 15 | 3.2 | 20.3 | 813 | 2.1 | 97.9 |
| F2 | BS | 13 | 25 | 16 | 5.3 | 18.7 | 763 | 3.7 | 96.3 |
| | NBS | 8 | 17 | 13 | 14.2 | 8.6 | 413 | 18.1 | 81.9 |
| M4 | BS | 7 | 13 | 8 | 10.2 | 9.2 | 415 | 12.9 | 87.1 |
| | NBS | 7 | 23 | 17 | 3.1 | 9.7 | 399 | 4.0 | 96.0 |
| M5 | BS | 7 | 17 | 14 | 10.2 | 6.4 | 306 | 17.5 | 82.5 |
| F3 | BS | 7 | 18 | 12 | 6.6 | 8.2 | 357 | 9.7 | 90.3 |
| | NBS | 7 | 26 | 21 | 2.6 | 7.4 | 303 | 4.6 | 95.4 |
| F4 | BS | 7 | 24 | 12 | 0 | 12.1 | 473 | 0 | 100 |

higher in the NBS (Table 3). This would be consistent with higher dependency on lipid metabolism occurring when animals had higher %TBL, resulting in lower rates of relative mass loss during fasting as has been previously documented in polar bears (Atkinson et al. 1996) and ptarmigan (Lindgård et al. 1992). When initial %TBL values were similar during both seasons (M5 began fasting with 17% in the BS and 16% in NBS), higher rates of relative mass loss were still seen during the BS than during the NBS ($1.58\%\cdot d^{-1}$ versus $1.21\%\cdot d^{-1}$) for this individual. When the fast was initiated with higher %TBL during the BS than during the NBS (F4 began fasting with 24% in the BS and 14% in the NBS), the difference in relative rates of mass loss was much smaller ($1.93\%\cdot d^{-1}$ versus $1.81\%\cdot d^{-1}$ for the BS and NBS, respectively). Although each juvenile consistently showed lower relative rates of mass loss in the NBS, the magnitude of the seasonal difference was accentuated if animals entered the NBS fast with high body fat content and minimized if the fast was initiated in less robust body condition. Similarly, the higher relative rates of mass loss seen in juveniles during the BS relative to subadults may be at least partly related to lower %TBL content in juveniles than in subadults during this season (18.0% ± 4.5% versus 24.8% ± 4.0%, respectively), although potential age related differences in basal metabolic rates should not be overlooked as a contributor to higher rates of mass loss in younger animals.

The lack of seasonal difference in mass loss demonstrated by the subadults may have been due to other physiological adjustments apparent during the BS that served to decrease the rate of mass loss. Although differences were not significant, subadults tended to have higher %TBL content during the BS than during the NBS, which may have enabled these animals to more effectively rely upon lipid metabolism to spare protein reserves, minimizing overall mass loss. This is also supported by evidence that subadult sea lions are able to maintain low levels of blood urea nitrogen in the plasma during 14 d of fasting during the BS, whereas blood urea nitrogen is seen to increase after the first week of fasting during the NBS (L.D. Rea, D.A.S. Rosen, and A.W. Trites, unpublished data). During the BS fasts, subadult sea lions also showed the capability to decrease their rate of mass loss after the first 3 d of fasting. This decrease in rate of mass loss may be due to an increased reliance on lipid metabolism during early fasting as seen in fasting-adapted species (Cherel et al. 1988; see review in Castellini and Rea 1992) or be a consequence of metabolic depression or both. Previous analyses have shown evidence of metabolic depression limiting mass loss in response to fasting in captive Steller sea lions (Rosen and Trites 2002).This decrease in rate of mass loss was not apparent during the NBS studies (when subadult body fat stores tended to be lower), and, although not statistically significant, the trend was for the rate of mass loss to increase during the latter parts of the NBS fast (day 6 to day 9). A similar increase in rates of mass loss have been documented in other species when they have reached the limit of their protein sparing abilities, coincident to depletion of the lipid reserves and increased reliance on protein oxidation (Robin et al. 1988; Nordøy et al. 1992). In all four fasting trials conducted during the NBS, subadults reached the maximum 15% mass loss prior to completing the prescribed 14 d of fasting. This result suggests that subadult Steller sea lions are not as able to adapt their metabolism to a limited food environment during the NBS, resulting in higher rates of catabolism of their energy reserves. If these fasting experiments were not terminated early to ensure the continued good health of the captive sea lions, one would expect that the rate of mass loss would have increased further as animals exceeded the limit of their ability to spare protein and would have likely reverted to catabolizing predominantly protein tissue to meet their energetic needs, potentially damaging vital organs.

Unlike subadults, the rate of mass loss did not decrease significantly after the first 3 d of fasting during BS trials in juvenile sea lions (and increased near the end of the NBS fast). Since the fasting duration typically experienced by juvenile Steller sea lions in the wild is short, it is possible that the ability to physiologically adapt to fasting is limited in this species until later in development when it is needed (e.g., during active breeding). This result would also suggest

**Fig. 2.** Percentage of energy that is derived from body lipid stores and rates of body mass loss experienced by subadult and juvenile Steller sea lions during the breeding season (BS) and non-breeding season (NBS) fasts were correlated with initial percent total body lipid content. (*a*) The relationship between percentage of energy that is derived from body lipid stores and the initial percent body fat is described by the equation $Y = 1.338X + 61.998$ ($r^2 = 0.429$, $p = 0.04$). (*b*) The relationship between rate of mass loss, expressed as absolute body mass loss per day, and initial percent body fat content is described by the equation $Y = -0.068X + 3.509$ ($r^2 = 0.850$, $p < 0.001$). (*c*) The relationship between rate of mass loss, expressed as percent body mass loss per day relative to initial body mass, and initial percent body fat content is described by the equation $Y = -0.034X + 2.153$ ($r^2 = 0.488$, $p = 0.036$).



that metabolic responses shown by the subadult sea lions may also be less developed than those of fully mature sea lions that undergo various durations of fasting during the BS. Juvenile sea lions were even more poorly adapted to conserve resources during the NBS, showing significant increases in the rate of mass loss by the end of the shorter 7 d trials. It is possible that the added metabolic demands for growth and development result in a higher mass-specific metabolic rate that precludes young sea lions from depressing their metabolism sufficiently during fasting to conserve body reserves. This is also evident in the higher rates of percent mass loss seen in 6-week-old Steller sea lion pups during captive fasting (5.1% ± 0.3% over 2 d of fast; Rea et al. 2000).

Among species adapted to prolonged fasting, protein is generally spared during periods of mass loss by increasing reliance upon lipid reserves for energy (see review in Castellini and Rea 1992). In our study, there was no significant difference in the rate of loss of fat mass between the BS and NBS fasts, although for subadults, the trend was to have higher reliance upon lipid reserves during the BS (consistent with the trend for subadults to have higher initial %TBL content during the BS). Contrary to this pattern, juveniles tended to have a higher rate of fat loss in the NBS, although not statistically significant. Other studies with captive Steller sea lions have demonstrated seasonal priorities in tissue catabolism during more extended periods of food restriction (Rosen and Trites 2005; Kumagai et al. 2006), with greater reliance during certain seasons on lipid mass loss to sustain (or even increase) core tissue mass.

The decreases in body mass during fasting resulted in both a detectable decrease in the thickness of the blubber layer, particularly at the axillary lateral location, and a decrease in axillary girth. This suggests that morphometric measurements taken at the axilla would be more sensitive to changes in body condition in Steller sea lions than those taken at the hip. In harbor seals (*Phoca vitulina* L., 1758), the greatest seasonal variability in blubber depth was found in the neck region of males and in the shoulder region of females (Fadely 1997); however, these correlated poorly with girth measurements (also see Rosen and Renouf 1997). In our study, the calculated CI was responsive to decreases in overall body resources during fasting, although this index did not seem sensitive enough to reflect subtle differences in daily rates of mass loss between age classes during the BS. Similarly, the calculated DI failed to reflect small decreases that occurred during fasting in the %TBL as measured by deuterium dilution. Previous studies also showed the inability of CI and DI to distinguish subtle differences between young Steller sea lion pups and animals that were visibly emaciated (starveling pups), particularly when the general body size was similar (Rea 1995).

Regression analysis suggested that 85% of the variability seen in the rate of absolute body mass loss during fasting could be explained by the initial body condition (%TBL) of the animal. This relationship suggests that individuals with greater relative lipid reserves prior to fasting lost mass at a lower rate than those with lower initial lipid levels. In Steller sea lions, body condition (%TBL) prior to fasting also strongly correlated with an animal's ability to utilize lipid reserves and spare protein. In this study, over 43% of the variability in the percentage of energy derived from lipids during fasting was attributed to the initial body fat content of sea lions. Among phocid seals, the %TBL content of pups prior to prolonged fasting influences the proportion of lipids and lean tissue used for energy during fasting (Rea and Costa 1992; Rea 1995; Noren et al. 2003). This relationship has also been demonstrated in fasting polar bears (Atkinson et al. 1996), fasting penguins (Robin et al. 1988; Cherel et al. 1992, 1993), and other fasting mammals and birds (Goodman et al. 1984; Lowell and Goodman 1987; Belkhou et al. 1990; Lindgård et al. 1992; Arnould et al. 2001). Ptarmigan with high fat content used protein to meet 9.4% of their energy needs, whereas lean birds obtained almost 30% of their energy from lean tissue catabolism. Fasting pinnipeds typically derive 85%–98% of their energy during fasting from lipid reserves (Boyd and Duck 1991; Noren et al. 2003; Field et al. 2005). The one example of exceptionally high lean tissue catabolism in this study was exhibited by a subadult who initiated fasting in the NBS with 11% TBL. This suggests that Steller sea lions may have a threshold body condition below which they are less capable of metabolically adapting to fasting. Although this low body condition animal lost 18.5% of its initial lean tissue stores, it did not reach a point of overfasting, defined by Cahill et al. (1979) and Cherel et al. (1988) as using >30% of original lean body mass.

Although the range of TBL reserves measured in this group of captive animals is wide (11%–28%), it is within the range of TBL stores seen in free-ranging Steller sea lions (Rea 2002). It is likely that the body composition of these captive animals was less related to any seasonal influence that could be affecting body composition of animals in the wild and more influenced by the captive diet or experimental manipulations conducted prior to the fasting experiments. It has been well documented that many species that undergo prolonged fasting experience hyperphagia prior to fasting that allows the accumulation of energy reserves, predominantly in the form of lipid stores. If the body condition of these captive animals did not match the optimum body reserves to ensure metabolic adaptation to fasting, this may minimize the contrast seen between the results of captive fasting in the BS and NBS. Contrary to being a detriment to our study, this wide range of body fat contents allowed us to investigate the impact of lower body condition on the ability to attain and maintain a fasting-adapted state. This mimics the situation that young animals may face in the wild in a food-limited environment.

In conclusion, our study has shown that subadult captive Steller sea lions are capable of adapting to fasting during the BS through a decrease in the relative rate of mass loss owing, in part, to metabolic depression (Rosen and Trites 2002). This capability is only evident during the BS when this species would naturally undergo periods of fasting in the wild. There was no evidence of metabolic adaptation to fasting in juvenile sea lions during the BS as indicated by a decrease in relative rates of mass loss and shown by the elevated relative rates of mass loss in juveniles during the BS compared with subadults. These results suggest that juvenile sea lions would be more negatively impacted by food limitation in the environment during the BS than would subadult conspecifics and that both cohorts would be equally affected during the NBS. It therefore seems that body condition prior to fasting likely plays a significant role in determining an animal's ability to metabolically adapt to fasting as demonstrated by lean animals using more protein reserves for energy during fasting.

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