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Energy reserve utilization in northern elephant seal (*Mirounga angustirostris*) pups during the postweaning fast: size does matter

Abstract During fasting most mammals preferentially utilize lipid reserves for energy while sparing protein reserves. This presents a potential problem for marine mammals that also depend on lipids as a major component of blubber, the primary thermoregulatory structure. Because of this dual function for lipid, rates of lipid and protein utilization should be closely regulated during the postweaning fast in northern elephant seals (*Mirounga angustirostris*). To quantify energy expenditure during the fast, we measured body mass and composition of 60 pups at 2.3 ± 0.2 days and 55.9 ± 0.3 days postweaning in 1999 and in 2000. Body condition differed significantly between years. At weaning, body mass (125.9 ± 3.8 kg) and percentage lipid content ($39.3 \pm 0.6\%$ of body mass) in 2000 were significantly greater than body mass (115.2 ± 3.1 kg) and percentage lipid content ($35.8 \pm 0.6\%$) in 1999. In general, percentage lipid content increased with body mass, and fatter pups utilized lipid at relatively higher rates during the fast. Lipid fueled 85–95% and 88–98% of energy expended by pups in 1999 and 2000, respectively. Postweaning fast duration (32–78 days) was positively correlated with body mass and hence lipid content at weaning. This suggests that body composition at weaning influences lipid utilization patterns and ultimately the duration of the postweaning fast in northern elephant seal pups.

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Introduction

The typical mammalian fasting pattern consists of three phases, categorized by the specific fuels catabolized for energy. During phase I and phase II, the body limits protein catabolism and increases fat oxidation (Cahill 1970; Young and Scrimshaw 1971; Saudek and Felig 1976; Goodman et al. 1980; Cherel et al. 1988a; Castellini and Rea 1992). After an extended period of fasting, the reduction of circulating free fatty acids triggers an increase in protein loss (Goodman et al. 1984). Terminal starvation, or phase III, occurs when 30–50% of body protein has been lost (Castellini and Rea 1992). Because entrance into phase III terminal starvation can lead to lethal levels of catabolism of critical protein sources, such as cardiac muscle, it is not considered adaptive for animals that exhibit long-term fasting.

For some species, including marine mammals, conservation of lipid reserves may also be important for survival. With the exception of sea otters, marine mammals store lipids in a layer of blubber, which serves as both the major energy store during fasting and the primary thermal barrier during immersion. An excessive reduction in the blubber layer during fasting periods on land would compromise its insulating value and result in a thermal challenge for pinnipeds when they eventually entered the ocean to forage. Thus, the balance between these two functions of the blubber layer, energy storage and thermal insulation (Nordøy and Blix 1985; Worthy and Lavigne 1987; Ryg et al. 1988; Markussen et al. 1992) may dictate survivorship of the animal.

Northern elephant seal (*Mirounga angustirostris*) pups provide excellent models for examining the relative importance of lipid reserves as an energy source and thermal barrier. These phocids have a short suckling period of 27 days and are subjected to abrupt weaning (Le Boeuf and Laws 1994). At weaning, pups are not capable of foraging independently and must rely on large lipid reserves for energy while fasting on land for up to 2.5 months as diving and swimming skills are developed (Reiter et al. 1978; Thorson and Le Boeuf 1994). Upon completion of the fast, the pups go to sea to forage with no knowledge of prey distribution or availability. If the pups are unsuccessful in capturing prey, lipid reserves from the blubber layer will be utilized further to fuel metabolism. The resulting depletion of the blubber layer will lead to increased thermoregulatory costs and an accelerated depletion of energy reserves as fasting continues at sea. Because lipid reserves serve in two conflicting roles (as an energy source to be utilized and a thermal barrier to be conserved) in these animals, lipid content at weaning and lipid allocation during fasting will undoubtedly influence pup survival. In view of this, we hypothesize that body condition at weaning influences the rate and pattern of energy reserve utilization in elephant seal pups over the postweaning fast onshore as well as during the subsequent time at sea. To determine how body energy reserves are utilized during the postweaning fast, we longitudinally measured body mass and composition of 60 northern elephant seal pups within the 1st week of weaning and again after 7–8 weeks of fasting. Particular attention was paid to individual variability to determine if pups of diverse body conditions exhibit different patterns of fasting metabolism. We also investigated inter-annual variability in pup body condition and its influence on patterns of fasting metabolism.

Materials and methods

Study area and subjects

Sixty weaned northern elephant seal pups (16 females and 14 males in 1999, 14 females and 16 males in 2000) were sampled longitudinally during the postweaning fast at Año Nuevo, CA, USA (37° 06′ 30″ N, 122° 20′ 10″ W). For each year of the study, weaned pups were selected to reflect the normal range of body mass present in the population that year. No pups that were abandoned prematurely (nursed less than 27 days) or nursed by another female after weaning were used in the study. Measurements were taken in the field within 6 days (mean 2.3 ± 0.2 days) postweaning in late January through early March and repeated on the same individuals 50–57 days (mean 53.6 ± 0.4 days) later in March and April. Due to the earlier departure of animals with low weaning mass, second measurements for one 81.5-kg and one 99.0-kg pup were taken 36 days and 46 days after the first set of measurements, respectively.

Pups were captured on the beach using a waterproof bag fitted with adjustable straps (Pernia 1984). Body mass was measured using a metal tripod and 250-kg capacity digital scale (accuracy to 0.1 kg; Measurement Systems International, Seattle, Wash., USA). After weighing, seals were initially immobilized by an intramuscular injection (1 mg kg^{-1}) of Tiletamine and Zolazepam (Telazol; Fort Dodge Animal Health, Fort Dodge, Iowa, USA). Immobilization

was maintained during morphometric measurements and isotope administration by intravenous doses of Ketamine (Ketaset; Fort Dodge Animal Health, Fort Dodge, Iowa, USA) and Diazepam (Elkins-Sinn, Cherry Hill, N.J., USA). Maintenance dosages depended on activity level of the animal and time remaining for completion of procedures.

Morphometrics

Axillary girth, standard length, and curvilinear length (American Society of Mammalogists—Society's Committee on Marine Mammals 1967) were measured. Girths at seven sites, spaced equally along the length of the body, and distances between those sites were measured. Body surface area (excluding flippers) was then calculated by the truncated cone method (Gales and Burton 1987). Pups were given a unique green identification tag (Dalton Jumbo Roto-tags, Oxon, England) in the inter-digital webbing of each hindflipper and distinctively marked with blonde hair dye (Procter & Gamble-Clairol, Samford, Conn., USA) to facilitate re-sighting.

Body composition

Body composition was calculated from total body water (TBW) estimated by the hydrogen isotope dilution method (Ortiz et al. 1978; Costa 1987). An initial blood sample was taken by venapuncture of the extradural vein to determine background isotope levels. Each pup received 1.1×10^7 Bq ^3H_2O (HTO) in 3.0 ml of sterile water by injection into the extradural vein. The absolute amount of tracer injected was determined by gravimetric calibration of the syringes used for isotope administration. Labeled water was allowed to equilibrate with body water for approximately 180 min, and a second blood sample was drawn while the animal was restrained manually. Serial blood sampling demonstrates that HTO equilibration occurs within 90 min of intravenous injection in northern elephant seal pups (Houser and Costa 2001), so 180 min was considered to be an adequate equilibration period. Blood samples were kept in a cooler on ice while in the field. Samples were centrifuged for 10 min at 3,000 rpm, and the serum decanted into 5-ml screw cap vials within 3–5 h of collection. Vials were stored at −60°C until analysis.

Water from aliquots of serum (200–250 μl) was collected into scintillation vials by dry-ice distillation, following a modified method described in Ortiz et al. (1978). Scintillation cocktail (10 ml; Ecolite, ICN, Costa Mesa, Calif., USA) was added to each scintillation vial, and the tritium activity of each sample determined on a LS 6500 multi-purpose scintillation counter (Beckman Instruments, Fullerton, Calif., USA). All samples were analyzed in triplicate.

HTO dilution space was determined for each seal from the activity of the injected isotope in counts per minute (cpm) divided by the specific activity of the equilibration sample (cpm ml^{-1}). The tritium activity of pre-injection blood samples collected from each seal during the second capture was subtracted from equilibration values to account for residual tritium activity from body water measurements made during the first capture. To correct the overestimation of body water by the HTO dilution method, TBW was assumed to be 96% of HTO dilution space (Reilly and Fedak 1990; Nagy and Costa 1980). Lipid mass was calculated with the assumption that fat mass is water-free and lean mass is 73.3% water (Worthy et al. 1992; Iverson et al. 1993):

$$M_{lipid} = M_{total} - 1.37\,TBW \tag{1}$$

where M_{lipid} is lipid mass and M_{total} is total body mass. Total body mass is the sum of the respective compartments:

$$M_{total} = M_{lipid} + M_{lean} \tag{2}$$

where M_{lipid} and M_{total} are as above, and M_{lean} is lean tissue mass.

Average fasting field metabolic rate

Average fasting field metabolic rate (FMR; MJ day^{-1}) was calculated for each re-captured animal using two methods. The first method used water flux calculated using Eqs. 1–3 of Ortiz et al. (1978). Water influx was reduced by 20% to correct the overestimation of metabolic water production due to inhaled water vapor in fasting northern elephant seals (Costa 1987). Daily metabolic energy expended, E_{met}, was determined from:

$$E_{met} = r_{in} k \tag{3}$$

where r_{in} is the corrected mean daily water influx (ml day^{-1}) and k is the constant 35.3 kJ ml^{-1} (Costa et al. 1986). This method assumes that water in the environment does not enter via dermal absorption, no energy is acquired from external sources, and all energy is derived from fat oxidation. Because pups may rely on some protein oxidation for a portion of energy, this method may overestimate the actual FMR.

The second method used components of mass loss. The sum of energy provided from changes in lipid mass and protein mass between captures was used to estimate metabolic rate during this period. We assumed that pups do not acquire energy from external sources. The protein component of lean tissue mass (27%) was calculated using values for the hydration state of muscle in northern elephant seal pups (72.7 ± 0.8%, $n = 6$ pups, determined by freeze drying). Values of 18.0 kJ g^{-1} and 39.3 kJ g^{-1} for protein and fat oxidation, respectively (Schmidt-Nielsen 1997), were used to calculate total energy lost during the fasting period between captures.

For this method the total loss of protein was estimated; however, it is unknown whether the entire quantity of lost protein is utilized for energy by the pup. Because northern elephant seal pups undergo a complete molt soon after weaning (Reiter et al. 1978), mobilization of protein from body stores would be required for the production of new pelage. In fact, greater muscle protein utilization occurs during the molting fast than during the nonmolting fast of king penguins (*Aptenodytes patagonica*, Cherel et al. 1988b). Consequently, protein mobilization attributed to the molting process contributes to a measured protein loss, but does not provide energy to meet daily metabolic costs. As a result, an overestimation of energy from protein oxidation is probable when calculating metabolism using this method. To correct this error, we estimated total protein mobilized for the molt using each individual's surface area (excluding flippers) and values for the wet mass and hydration state of northern elephant seal pelage from Worthy et al. (1992). Protein mobilized for the molting process was then subtracted from total protein lost before calculating FMR and determining the percentage contribution of lipid and protein to total energy expended during the fasting period.

Fasting duration

Although we were unable to re-capture all pups for physiological measurements, we were able to estimate fasting duration for all

animals based on daily beach surveys. Seals that were not resighted were assumed to have initiated their foraging trip at sea. The last observation date was recorded as the last day of the postweaning fast. Fasting duration was determined for all pups except for two that died before the end of the fast in 2000 (one via trampling by a male elephant seal and the other by an alleged gunshot wound).

Statistical analyses

All graphical and statistical analyses were conducted using Sigma Plot and Sigma Stat Software (SPSS Science, Chicago, Ill., USA), unless otherwise noted. All percentages were arcsine transformed prior to performing statistical analyses. Student's t-tests were used to determine differences in body mass and standard length between years and between sexes within each year. Student's t-tests were also used to determine differences in the length of the postweaning fast between sexes within each year. Linear regressions were determined by least squares method and significance was determined by F-tests. Student's t-tests were used to compare slopes and y-intercepts of regressions for data from 1999 and 2000. Because lipid content varied with body mass, ANCOVA was used (Systat Version 9, Systat Software, Richmond, Calif., USA) to determine both year and sex effects on lipid content at weaning. Paired t-tests were used to compare the two methods of calculating average field metabolic rate and to compare lipid contents at weaning and near the end of the fast for re-captured pups. Forward stepwise regressions were performed to determine relative contributions of body mass and lipid content at weaning to fasting duration. All means are presented with ± 1 SEM. Results were significant at $P < 0.05$.

Results

Body condition at weaning

Body condition at weaning differed between 1999 and 2000. Although standard length at weaning did not differ between years ($P > 0.05$), body mass in 2000 was significantly greater than body mass in 1999 (Table 1). The weaning mass of males drove the difference in body mass between the 2 years. Female mass was not significantly different between 1999 and 2000 ($P > 0.05$), but male mass in 2000 was significantly greater than male mass in 1999 ($t = -3.5$, $P = 0.002$) at weaning.

When each year was analyzed separately, there was no difference in standard length at weaning between

Table 1 Body mass (M_b) and standard length (SL) of northern elephant seal pups at weaning (measurements taken 2.3 ± 0.2 days postweaning) and after fasting approximately 2 months (measurements taken 55.9 ± 0.3 days postweaning) in 1999 and 2000

	Pups	Range	Mean ± 1 SEM	Statistics
Weaning M_b (kg)				
1999	30	77.0–141.2	115.2 ± 3.1	2000 > 1999, $t = -2.2$,
2000	30	70.0–159.5	125.9 ± 3.8	$P = 0.034$
Weaning SL (cm)				
1999	30	126–155	144 ± 1	No SD, $P > 0.05$
2000	30	131–164	147 ± 1	
Fasted M_b (kg)				
1999	23	51.8–104.4	85.3 ± 2.5	2000 > 1999, $t = -2.5$,
2000	20	57.0–114.5	94.8 ± 2.8	$P = 0.015$
Fasted SL (cm)				
1999	23	125–161	149 ± 2	No SD, P > 0.05
2000	20	133–163	150 ± 2	

males and females ($P > 0.05$). Similarly, male weaning mass (mean: 113.0 ± 5.0 kg, $P = 14$) did not differ from female weaning mass (mean: 117.1 ± 4.0 kg, $P = 16$) in 1999 ($P > 0.05$). In contrast, male weaning mass (mean: 134.2 ± 3.6 kg, $P = 16$) was significantly greater than female weaning mass (mean: 116.4 ± 6.2 kg, $P = 14$) in 2000 ($t = -2.6$, $P = 0.016$).

Overall, lipid content at weaning ranged from 28.5 to 43.9% of body mass, and increased with body mass (Fig. 1) according to the following equation in 1999:

$$\text{Lipid content}_{\text{weaning}}(\%) = 22.7 + 0.1 \, M_{\text{bweaning}}$$
$$\left(F = 21.0, \ r^2 = 0.45, \ P < 0.001, \ n = 29 \text{ pups} \right) \quad (4)$$

and in 2000:

$$\text{Lipid content}_{\text{weaning}}(\%) = 24.0 + 0.1 \, M_{\text{bweaning}}$$
$$\left(F = 32.4, \ r^2 = 0.54, \ P < 0.001, \ n = 30 \text{ pups} \right) \quad (5)$$

where lipid content is in percentage of total body mass and M_{b} is in kilograms. Although slopes of Eqs. 4 and 5 were not significantly different ($P > 0.05$), pups born in 2000 had significantly greater lipid content at weaning than pups born in 1999 ($F = 10.9$, $P = 0.002$; Fig. 1).

When the sexes were analyzed separately, the trends were identical. Lipid content increased significantly with body mass for males and for females in 1999 (males: $F = 13.5$, $P = 0.004$; females: $F = 6.4$, $P = 0.024$) and in 2000 (males: $F = 7.1$, $P = 0.018$; females: $F = 25.1$, $P < 0.001$). Furthermore, both male and female pups born in 2000 had significantly greater lipid content at weaning than pups of identical sex born in 1999 (males: $F = 9.1$, $P = 0.006$; females: $F = 5.2$, $P = 0.03$).

When each year was analyzed separately, there was no difference in lipid content at weaning between males and females in 1999 ($P > 0.05$) or in 2000 ($P > 0.05$). These results demonstrate that males and females had similar body mass and lipid content at weaning in 1999 and that despite the sex difference in body mass, males and females also had similar lipid content at weaning in 2000. Because of these findings, body energy reserve utilization patterns during the fast are presented for males and females combined.

Body condition after fasting 2 months

Similar to results at weaning, standard length near the end of the fast did not differ between years ($P > 0.05$), but body mass in 2000 was significantly greater than body mass in 1999 (Table 1). Again, the mass of males drove the difference in body mass between the two years. Female mass was not significantly different between 1999 and 2000 ($P > 0.05$), but male mass in 2000 was significantly greater than male mass in 1999 ($t = -2.9$, $P = 0.009$) near the end of the fast.

When each year was analyzed separately, there was no difference in standard length near the end of the fast between males and females ($P > 0.05$). Similarly, male mass (mean: 86.2 ± 2.6 kg, $n = 10$) did not differ from female mass (mean: 84.7 ± 3.9 kg, $n = 13$) near the end of the fast in 1999 ($P > 0.05$). Although male mass (mean: 98.3 ± 3.1 kg, $n = 12$) was still greater than female mass (mean: 89.6 ± 4.9 kg, $n = 8$) near the end of the fast in 2000, this difference was no longer significant ($P > 0.05$).

To quantify changes in body composition during the fast, only longitudinal data from re-captured pups were used. Slopes and y-intercepts for relationships between lipid content and body mass at weaning for re-captured pups in 1999 and 2000 were not significantly different from those of all pups in their cohort at weaning, Eqs. 4 and 5, respectively ($P > 0.05$). Analogous to data from all pups at weaning, re-captured seals born in 2000 had significantly greater lipid content at weaning than pups born in 1999 ($F = 10.2$, $P = 0.003$). As a result, we consider re-captured pups as a good representation of all pups sampled for each year.

For re-captured pups, lipid content ranged from 32.2 to 46.0% of total body mass near the end of the fast. Similar to data at weaning, lipid content near the end of the fast increased with body mass in 1999:

$$\text{Lipid content}_{\text{fasted}}(\%) = 30.0 + 0.1 \, M_{\text{bfasted}}$$
$$\left(F = 4.9, \ r^2 = 0.21, \ P = 0.039, \ n = 21 \text{ pups} \right) \quad (6)$$

and in 2000:

$$\text{Lipid content}_{\text{fasted}}(\%) = 27.9 + 0.1 \, M_{\text{bfasted}}$$
$$\left(F = 9.3, \ r^2 = 0.35, \ P = 0.007, \ n = 19 \text{ pups} \right) \quad (7)$$

where lipid content is in percentage of total body mass and M_{b} is in kilograms. In contrast to data at weaning, the significant difference in lipid content between years did not persist near the end of the fast ($P > 0.05$).
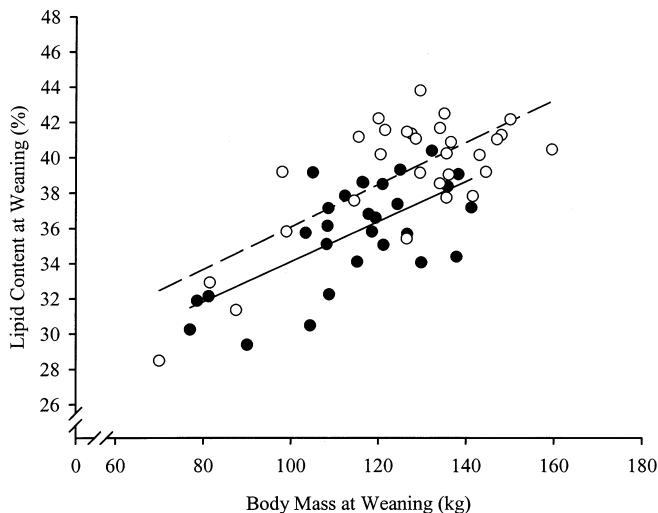

Fig. 1 Lipid content in relation to total body mass at weaning for northern elephant seal pups. Data are presented for pups in 1999 (*filled circles*) and in 2000 (*empty circles*). Each point represents a single pup. Least-squares regressions for 1999 (*solid line*) and 2000 (*dashed line*) are described in the text
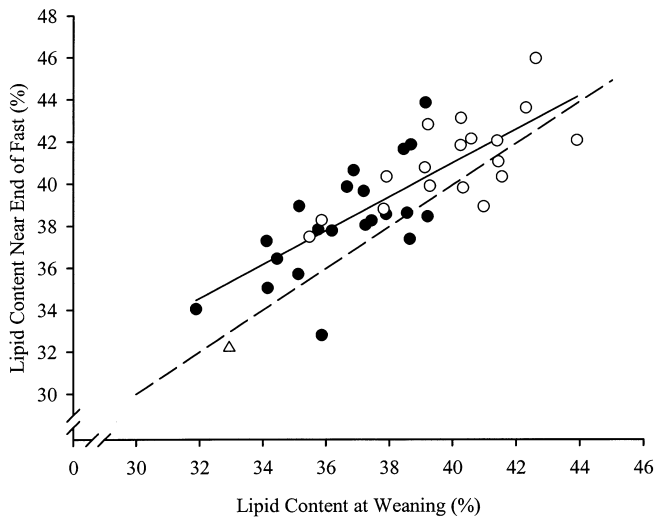
Fig. 2 Lipid content near the end of the fast in relation to lipid content at weaning for re-captured northern elephant seal pups. Data are presented for pups in 1999 (*filled circles*) and in 2000 (*empty circles*). Each point represents a single pup. The least-squares regression for both years combined (*solid line*) and a line of equality (*dashed line*) are shown. The equation for the least-squares regression is presented in the text. The one pup (*open triangle*) that was re-captured comparatively early (36 days after the first capture) due to its small body mass is not included in the regression but is presented for comparison. After the second capture, this animal remained on the beach for only 6 days before initiating its foraging trip

Lipid content near the end of the fast was positively related to lipid content at weaning (Fig. 2):

$$\text{Lipid content}_{\text{fasted}}(\%) = 8.6 + 0.8 \text{ lipid content}_{\text{weaning}}(\%)$$
$$(F = 67.4, \ r^2 = 0.65, \ P < 0.001, \ n = 39 \text{ pups}) \tag{8}$$

where lipid content is in percentage of total body mass. For most pups, percentage lipid content near the end of the fast was greater than percentage lipid content at weaning ($t = -4.7$, $P < 0.001$, $n = 40$ pups). In general, lean pups tended to have relatively greater increases in percentage lipid content than fat pups during the fasting period (Fig. 2). However, some pups weaned with high lipid content ($> 38\%$) actually reduced their percentage lipid content during the postweaning fast (Fig. 2).

Utilization of body reserves during the fast

The observation that most pups had relatively greater lipid content near the end of the fast than at weaning seems paradoxical. However, the results are a consequence of the relatively greater loss of lean tissue, which is comprised mostly of water. Thus, proportionately, lipid content increased.

For all seals, lean tissue was the largest component of mass lost (Table 2); however, the relative contribution of lipid and lean tissue to mass lost over the fasting period varied between individuals. When the hydration state of lean tissue is considered, protein was a smaller component of mass lost than lipid (Table 2). To quantify these losses in terms relative to initial body compartment masses, pups lost approximately 15–37% (mean: $25.7 \pm 0.7\%$) of initial lipid stores and 22–36% (mean: $29.8 \pm 0.5\%$) of initial protein stores. Protein mobilized for the molt was approximately 48–85% (mean: $60.3 \pm 1.3\%$) of lost protein mass, representing 16–20% (mean: $17.8 \pm 0.2\%$) of initial protein reserves.

Pups lost approximately 24–34% (mean: $28.4 \pm 0.4\%$) of their weaning mass. Heavier pups at weaning showed lower mass loss per unit of body mass during the fasting period than lighter animals (Fig. 3). There were no significant differences between slopes and y-intercepts of regressions for each year ($P > 0.05$); therefore, data from both years were combined. The relationship between mass specific rate of mass loss and weaning mass is:

$$\text{Rate of mass loss} = 7.6 - 0.02 \ M_{\text{bweaning}}$$
$$(F = 23.6, \ r^2 = 0.38, \ P < 0.001, \ n = 41 \text{ pups}) \tag{9}$$

where rate of mass loss is in g day^{-1} kg^{-1} and M_{b} is in kilograms (Fig. 3).

Fasting metabolism

Energy was derived primarily from lipid reserves for all pups during the fasting period. After correcting for protein mass utilized for the molt, which was approximately 9–16% (mean: $11.5 \pm 0.3\%$) of total energy lost, daily metabolism fueled by lipid ranged from 85 to 95% (mean: $89.7 \pm 0.7\%$) and 88–98% (mean: $92.5 \pm 0.7\%$) in

Table 2 Mass lost during 53.6 ± 0.4 days of fasting in northern elephant seal pups

	Pups (n)	Weaning mass (kg)	Mass lost (kg)	Proportion of mass lost (%)
Body mass	42	78.6–159.5 (124.8 ± 2.5)	24.5–45.0 (35.2 ± 0.7)	100
Lean tissue mass	40	53.5–94.8 (76.8 ± 1.4)	16.0–28.7 (22.9 ± 0.5)	51.0–76.0 (65.5 ± 0.9)
Water mass	40	39.1–69.2 (56.1 ± 1.0)	11.7–20.9 (16.7 ± 0.4)	37.0–55.0 (47.7 ± 0.7)
Protein mass	40	14.5–25.6 (20.7 ± 0.4)	4.3–7.8 (6.2 ± 0.1)	14.0–20.0 (17.7 ± 0.2)
Lipid mass	40	25.1–64.7 (47.6 ± 1.3)	7.4–16.6 (12.1 ± 0.4)	24.0–49.0 (34.5 ± 0.9)

For each mass the range is given with mean $\pm$ 1 SEM (in parentheses). Data for 1999 and 2000 are combined.

1999 and 2000, respectively, and increased with lipid content at weaning (Fig. 4). There were no significant differences between slopes and *y*-intercepts of the
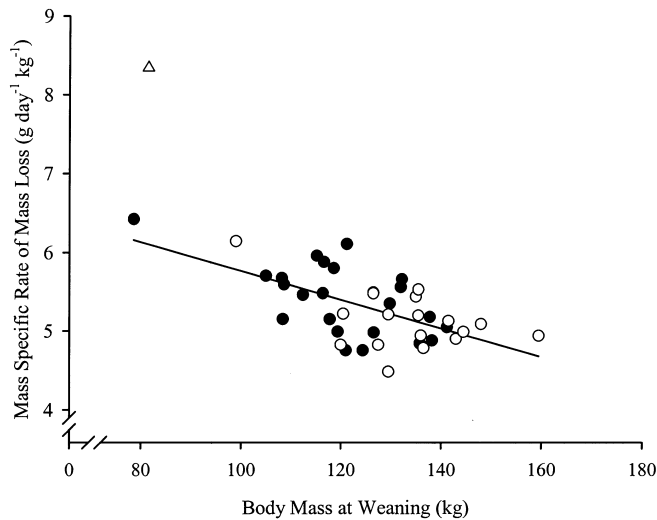

Fig. 3 Mass specific rate of mass loss over the fasting period between captures in relation to weaning mass for northern elephant seal pups. Data for pups in 1999 (*filled circles*) and in 2000 (*empty circles*) are presented. Each point represents a single pup. The least squares regression for both years combined (*solid line*) is described in the text. An outlying animal with the highest mass specific rate of mass loss (*open triangle*) is not included in the regression but is presented for comparison (see legend to Fig. 2)

regressions for each year ($P > 0.05$); therefore, data from both years were combined. The relationship between the contribution of lipid to total energy expended and lipid content at weaning is (Fig. 4):

$$\text{Contribution of lipid}(\%) = 59.3 +$$
$$0.8 \text{ lipid content}_{\text{weaning}}(\%) \tag{10}$$
$$\left(\text{F} = 32.2, \text{ r}^2 = 0.47, \text{ P} < 0.001, \text{ n} = 39 \text{ pups}\right)$$

where the contribution of lipid represents the percentage of total energy expended over the fasting period that was supplied by lipids after correcting for protein mass lost for the molt. Lipid content is in percentage of total body mass at weaning.

For both methods of FMR calculation, FMR increased significantly with average body mass (where average body mass is calculated by methods of Ortiz et al. 1978; Fig. 5). FMR calculated by the water flux method (Ortiz et al. 1978) ranged from 8.3 to 25.0 MJ day^{-1} (mean: 17.4 ± 0.5 MJ day^{-1}) and was approximately two times higher than FMR calculated by the components of mass loss method ($t = 16.9$, $P < 0.001$; Fig. 5). The latter ranged from 6.4 to 12.7 MJ day^{-1} (mean: 9.8 ± 0.3 MJ day^{-1}). FMR calculated from water flux increased with average body mass according to:

Fig. 4 Energetic contribution of lipid to total energy used during the fasting period in relation to lipid content at weaning for northern elephant seal pups. Data for pups in 1999 (*filled circles*) and in 2000 (*empty circles*) are shown. Each point represents a single pup. The least-squares regression for both years combined is denoted by the *solid line* and described in the text. An outlying animal (*open triangle*) is not included in the regression but is presented for comparison, as explained in the legend to Fig. 2. The *horizontal dotted line* indicates the minimum level of lipid contribution to fasting metabolism in northern elephant seal pups reported by previous studies that measured urine output (Adams and Costa 1993) and urea turnover (Pernia et al. 1980; Houser and Costa 2001)
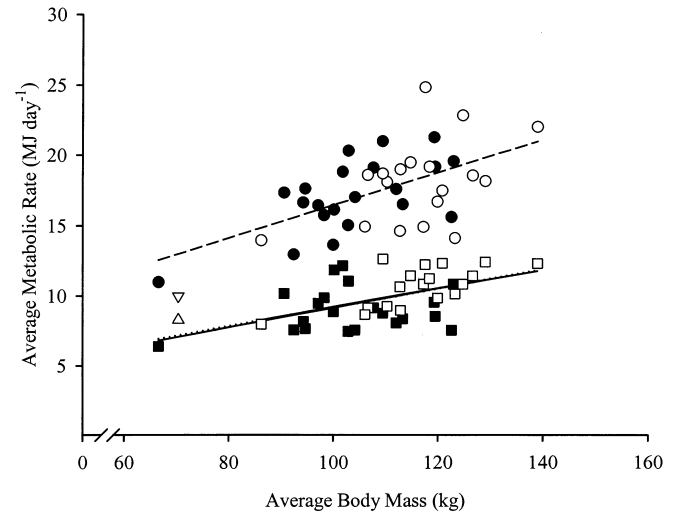

Fig. 5 Average field metabolic rate (FMR) in relation to average body mass over the fasting period for northern elephant seal pups. FMR calculated by water flux (following methods of Ortiz et al. 1978) for pups in 1999 (*filled circles*) and in 2000 (*empty circles*) and calculated from mass loss for individual pups in 1999 (*filled squares*) and in 2000 (*open squares*) are presented. Each point represents a single pup. Least-squares regressions for metabolic rates calculated from water flux (*dashed line*) and from mass loss (*dotted line*) are shown and described in the text. The outliers for FMR calculated from water flux (*upright open triangle*) and calculated from mass loss (*inverted open triangle*) are not included in the regressions but are presented for comparison, as explained in the legend to Fig. 2. Predicted basal metabolic rates (BMRs) for adult terrestrial animals of the same body mass from Kleiber (1975) are presented by the *solid line* for comparison

$$FMR = 4.7 + 0.1 \text{ average } M_b$$

$$\left(F = 17.8, \ r^2 = 0.32, \ P<0.001, \ n = 39 \text{ pups}\right) \quad (11)$$

while FMR calculated from lost lipid and protein mass increased with average body mass according to:

$$FMR = 2.3 + 0.07 \text{ average } M_b$$

$$\left(F = 16.2, \ r^2 = 0.31, \ P<0.001, \ n = 39 \text{ pups}\right) \quad (12)$$

where FMR is in MJ day^{-1} and M_b is in kilograms for both equations. FMRs calculated from Eq. 12 are nearly identical to basal metabolic rates (BMR) predicted by Kleiber (1975) for adult terrestrial animals of similar body mass (Fig. 5).

Postweaning fast duration

Duration of the postweaning fast ranged from 45 to 78 days (mean: 62 ± 2 days) and from 32 to 77 days (mean: 67 ± 2 days) in 1999 and 2000, respectively. Within each year, the postweaning fast duration did not differ between males and females ($P > 0.05$).

There were no significant yearly differences in slopes and y-intercepts ($P > 0.05$) for the relationships of body mass and percentage lipid content at weaning versus fasting duration. Therefore, data from both years were combined. Total fasting duration increased with body mass and percentage lipid content at weaning (Fig. 6 A, B) according to:

$$\text{Fasting duration} = 17.5 + 0.4 \ M_{bweaning}$$

$$\left(F = 64.9, \ r^2 = 0.54, \ P<0.001, \ n = 58 \text{ pups}\right) \quad (13)$$

where fasting duration is in days and M_b is in kg at weaning, and:

$$\text{Fasting duration} = 2.1 \text{ lipid content}_{weaning}(\%) - 12.8$$

$$\left(F = 57.9, \ r^2 = 0.51, \ P<0.001, \ n = 57 \text{ pups}\right) \quad (14)$$

where fasting duration is in days and lipid content is in percentage of total body mass at weaning. Because fasting duration correlates directly with both body mass and percentage lipid content at weaning, it can be predicted by a linear combination of body mass ($R = 0.73$, $P < 0.001$) and percentage lipid content ($\Delta R = 0.27$, $P = 0.003$) at weaning according to the following equation:

$$\text{Fasting duration} = \left(0.2 \ M_{bweaning} \right.$$

$$\left. + 1.1 \text{ lipid content}_{weaning}\right) - 6.2$$

$$\left(F = 42.0, \ r^2 = 0.61, \ P<0.001, \ n = 57 \text{ pups}\right) \quad (15)$$

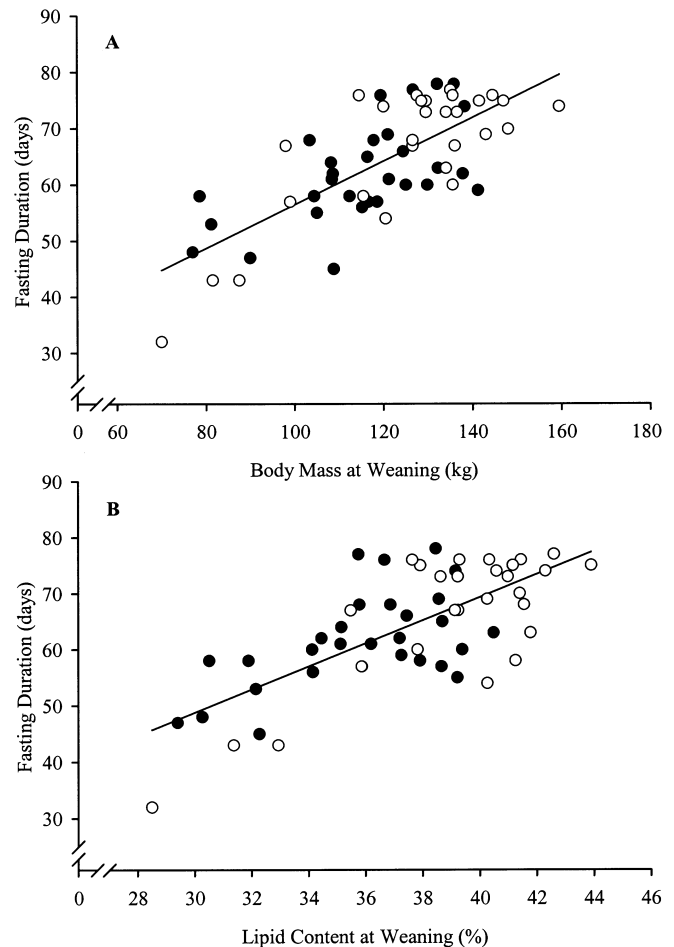where fasting duration, M_b, and lipid content are as above.

Fig. 6 Total fasting duration in relation to body mass (**A**) and lipid content (**B**) at weaning for northern elephant seal pups. Data for pups in 1999 (*filled circles*) and in 2000 (*empty circles*) are shown. Each point represents a single pup. Least-squares regressions (*solid lines*) are described in the text

Discussion

Energy utilization during the postweaning fast

Body mass and percentage lipid content are both important factors that influence metabolism of northern elephant seals during the postweaning fast. In contrast to results for southern elephant seals (*Mirounga leonina*, Carlini et al. 2001) and a previous study on northern elephant seals (Kretzmann et al. 1993), the present study shows that heavier pups have a greater proportion of body lipid at weaning. This discrepancy may be attributed to the fact that we measured approximately twice the number of seals as the previous investigators (Carlini et al. 2001; Kretzmann et al. 1993), and we focused on a broader range of body mass than Kretzmann et al. (1993). However, results similar to ours have also been demonstrated for a smaller number of gray seal (*Halichoerus grypus*) pups (Reilly 1991). We find that these differences in body condition result in variable patterns

of fat and protein utilization for northern elephant seals during the postweaning fast.

The significant relationship between lipid content at weaning and the energetic contribution of lipid to total metabolism during the fast (Fig. 4) suggests that the relative catabolism of fat and protein during the postweaning fast depends on body composition (i.e., the proportion of lipid and lean tissue) at weaning. Consistent with previous work (Ortiz et al. 1978; Costa and Ortiz 1982; Castellini et al. 1987; Houser and Costa 2001), fat oxidation satisfied the majority of energy requirements during the postweaning fast for northern elephant seal pups in the present study. In fact, for many pups weaned with $>38\%$ lipid, protein contributed $\leq 6\%$ to total metabolism (Fig. 4), which is similar to the $<4\%$ value reported previously (Pernia et al. 1980; Adams and Costa 1993; Houser and Costa 2001). Yet, unlike previous studies that measured protein catabolism from urine output (Adams and Costa 1993) and urea turnover (Pernia et al. 1980; Houser and Costa 2001), protein catabolism measured by changes in body composition was more than a negligible fraction of total metabolism for many pups. Indeed, the contribution of protein to total metabolism increased to 12–15% for pups weaned with $<35\%$ lipid. These findings indicate that fatter pups utilize proportionally more fat and spare proportionally more protein than leaner pups during the postweaning fast. As a result, pups that are weaned with low body mass and low percentage lipid content not only demonstrate relatively higher protein catabolism than heavier, fatter pups but also demonstrate proportionately greater body mass loss (Fig. 3). This greater rate of mass loss is a consequence of the relatively higher catabolism of protein-yielding tissue (e.g., muscle), which has approximately one eighth the energetic density of adipose tissue.

Similar results have been found for other seals. Relatively fatter lactating female northern elephant seals (Crocker et al. 2001) and weaned southern elephant seal pups (Carlini et al. 2001) lose proportionally more fat than thinner conspecifics during fasting periods. The reverse is also true. Lactating adult female northern elephant seals with lower percentages of adipose tissue (Crocker et al. 1998) and lean gray seal pups (Øritsland et al. 1985) maintain higher rates of protein utilization during a fast.

Intraspecific differences in fasting metabolism also exist for other mammalian species and birds. Specifically, individuals that are relatively fatter at the beginning of a fast are able to achieve and maintain a lower rate of protein catabolism than leaner individuals (Goodman et al. 1980, 1984; Robin et al. 1988; Cherel et al. 1992; Atkinson et al. 1996; Hilderbrand et al. 2000). This enhanced ability of fatter individuals to conserve protein is attributed to higher levels of circulating lipid fuels and free fatty acids (Goodman et al. 1980, 1984).

For phocid pups, the pattern of energy utilization during the postweaning fast generally reflects species-specific adaptations to different breeding habitats and thermal demands (Worthy and Lavigne 1987). For example, harp seals (*Phoca groenlandica*) that spend much of the fast in very cold water (Worthy and Lavigne 1987) exhibit significant catabolism of lean body mass, while large blubber depots are maintained (Stewart and Lavigne 1980; Worthy and Lavigne 1987). As a result, harp seal pups are able to maintain thermal balance in cold water without increasing their metabolic rate above that observed in air (Worthy and Lavigne 1987). Hooded seals (*Cystophora cristata*), also born on ice, lose approximately equal masses from the core and the blubber layer (Bowen et al. 1987). Similarly, harbor seals (*Phoca vitulina*) enter cold water soon after birth and demonstrate both fat and lean tissue catabolism during the first 2 weeks of the fast (Muelbert and Bowen 1993). In contrast, weaned gray seals (Nordøy and Blix 1985; Øritsland et al. 1985; Worthy and Lavigne 1987) and southern elephant seals (Bryden 1969) fast on land and lose mass primarily from the blubber layer.

Within phocid species, individual differences in tissue catabolism may also serve an adaptive functional role for thermoregulation. For phocids, poor body condition results in increased metabolic costs in water (Irving and Hart 1957). Conserving lipid reserves by catabolizing some lean tissue safeguards the insulating value of the blubber layer, thereby reducing overall metabolic costs. This may be especially important for northern elephant seal pups that are weaned with relatively low lipid content. By maintaining higher levels of protein catabolism, lean pups become proportionately fatter during the postweaning fast. An increase in percentage lipid content may ensure that these pups have sufficient lipid stores for thermoregulation during the first foraging trip at sea. As a result, northern elephant seal pups with lipid contents ranging from 34 to 44% at the end of the postweaning fast are not thermally challenged in 3.8°C water (Noren 2002).

Field metabolic rate during the postweaning fast

An interesting finding in this study was the similarity between FMR calculated from mass loss for northern elephant seals pups and BMR predictions from Kleiber (1975). Although most immature and mature marine mammals demonstrate metabolic rates that are considerably higher than predicted levels (Whittow 1987; Donohue et al. 2000), previous studies that measured oxygen consumption of resting northern elephant seal pups found similar results (Kohin et al. 1999; Noren 2002). The similarity between calculated FMR, measured resting metabolic rate (RMR), and BMR predicted for adult terrestrial animals of similar body mass is quite surprising. FMR includes costs of basal metabolism, locomotion, and other expenses; and as a consequence, FMR is usually higher than BMR (Nagy 1987) and RMR. However, the apparent similarity of FMR, RMR, and predicted BMR may be explained in

part by adaptations specific to the stage of development for elephant seals. Previous studies have shown that a reduction in metabolism occurs during the postweaning fast, which is an adaptation for energy conservation during long-term abstinence from food (Heath et al. 1977; Rea and Costa 1992). Further, these pups spend much of their time on the beach asleep and in periods of apnea, which may also reduce energy expenditure during the postweaning fast (Blackwell and Le Boeuf 1993). Northern elephant seals are also adapted for long, continuous deep diving (Le Boeuf et al. 1988, 1993), for which a lower mass-specific metabolic rate would be an advantage (Kooyman 1989). All of these mechanisms could contribute to reducing metabolism in northern elephant seal pups during the postweaning fast.

Although we corrected for most errors inherent in the measurement of body composition and water influx with isotopically labeled water (see Nagy and Costa 1980), other sources of error could be significant to the calculations of FMR in the present study. Unlike fasting gray seal pups, for which daily energy expenditure obtained from doubly labeled water is approximately 3% lower than that calculated from changes in body composition (Reilly 1991), FMR determined by water flux using tritium is about 2 and 1.6 times greater than that calculated from changes in body composition for northern elephant seal pups (present study) and molting female southern elephant seals (Boyd et al. 1993), respectively. Similarly, FMR determined by water flux using D_2O is 2.1 times greater than that calculated from changes in body composition for southern elephant seal pups (Carlini et al. 2001). In the studies on elephant seals, FMR calculated by water flux following methods of Ortiz et al. (1978) may overestimate actual metabolic rate. This is likely due to the underlying assumption that energy is derived solely from lipid oxidation, which provides approximately twice the energy of protein oxidation. Because a portion of energy may be derived from protein catabolism, FMR calculations from water flux, with the assumption that all water influx is from fat oxidation, can lead to elevated estimates of FMR, particularly for leaner seals. For example, if lipid fuels 95% and protein fuels 5% of total metabolism, then FMR calculated from water influx from lipid and protein oxidation is 13% less than FMR calculated with the assumption that all water influx is from fat oxidation. Similarly, FMR is reduced by 25% when lipid fuels 85% and protein fuels 15% of total metabolism. FMR calculated from protein and lipid mass loss offers a solution to the above error. However, the determination of body composition from total body water by the HTO dilution method is not always precise, and this may affect calculations of FMR. For example, if the body composition for seals in this study was underestimated by 2% lipid content at weaning, then FMR calculated from changes in body composition is underestimated by 11–29%. An underestimate of 5% body lipid content at weaning results in a 24–50% underestimate of FMR calculated from changes in body composition. Further-

more, the calculation to correct for protein utilized for the molt is only an approximation and is therefore another source of error. Both of the above factors may reduce the accuracy of FMR calculated from changes in body composition. Thus, FMRs calculated by the two methods in this study may represent the maximum and minimum values for these seals, with the actual FMRs falling within these limits.

In the present study, metabolic rates and the ratio of protein to lipid utilization for northern elephant seals pups were averaged over the entire fasting period. Consequently, the values presented here are means and may not be appropriate assessments for specific intervals of the fast, as FMR and energy utilization patterns appear to change during fasting. For example, fasting female northern elephant seals have higher levels of protein catabolism later in lactation (Crocker et al. 1998). Likewise, the relative contribution of lipids and proteins to metabolism as well as the location of these fuels (i.e., carcass, viscera, or blubber) change during the postweaning fast in harp seal pups (Worthy and Lavigne 1983).

Implications for postweaning fast duration

As shown previously for southern (Arnbom et al. 1993; Carlini et al. 2001) and northern (Kretzmann et al. 1993) elephant seal pups, we found that postweaning fast duration increased with body mass. Northern elephant seal pups weaned with low body mass and low lipid content fasted for at least 1 month while some of the heaviest and fattest pups fasted for over 2 months. Variation in fasting metabolism appeared to serve as a critical adaptation that enabled pups of all body conditions to remain on land for a prolonged period of time. Two physiological processes tie pups to land immediately after weaning. First, pups shed their black lanugo soon after being weaned (Reiter et al. 1978). Northern elephant seals haul-out on land for the molt process, and the postweaning molt requires one to three weeks for completion (Reiter et al. 1978). Second, pups undergo critical physiological changes, such as increasing mass specific blood volume, hematocrit, hemoglobin concentration, and muscle myoglobin concentration, during the postweaning fast (Thorson and Le Boeuf 1994). These changes result in an increase in oxygen stores (Thorson and Le Boeuf 1994) that are important for increasing dive duration (Kooyman 1989). Although pups weaned with low body mass and lipid content fasted for only 5–6 weeks, compared to the 10- to 11-week fast for others, it is likely that without conserving lipid stores these smaller pups would have been forced to leave the beach even earlier due to depleted energy reserves. By remaining on land for at least 4 weeks after weaning, these pups were able to molt completely and acquire 93% of the total oxygen storage capacity of pups at 8 weeks postweaning (Thorson and Le Boeuf 1994). Larger pups that remain on the beach and fast for

11 weeks after weaning may have an advantage in terms of capacity for diving in comparison to smaller pups that must terminate the fast earlier and begin foraging with lower oxygen stores.

Inter-annual variability

The inter-year differences in pup body mass and percentage lipid content at weaning observed in our study may be due to differences in oceanic and atmospheric conditions. The 1st year of this study (1999) was a post-El Niño year; the 2nd year (2000) was considered a "normal" year. Adult female northern elephant seals had longer trips, and mass gain decreased for the 1998 post-lactation foraging trip compared to previous years (Crocker et al. 1999). Females also arrived at Año Nuevo later for the 1999 breeding season than in previous seasons (D. Crocker, unpublished observation). This implies that the foraging trip prior to the 1999 breeding season may have been challenging for pregnant females. Milk fatty acid signatures from female northern elephant seals in 1999 also suggest that a shift in diet from previous years may have occurred (Pettinger 2000). Any one of these factors could have affected female body condition prior to giving birth in 1999. Because pup mass is directly related to maternal body mass and composition in elephant seals (Arnbom et al. 1993; Deutsch et al. 1994; Crocker et al. 2001), it is not surprising that pups born in 1999 not only weighed less but also had proportionately less lipid than pups born in 2000.

An interesting finding in this study was that male body mass was significantly greater than female body mass at weaning in 2000 (normal year), but there was no sex difference in weaning mass in 1999 (post-El Niño year). Similarly, previous studies have demonstrated a sex difference in the weaning mass of northern elephant seals (Le Bouef et al. 1989) while others have reported no difference (Kretzmann et al. 1993). Likewise, for southern elephant seals, differences in male and female weaning mass were observed for some years but not others (Arnbom et al. 1993; Carlini et al. 2001). In contrast, there was no sex difference in percentage lipid content at weaning in 1999 or in 2000 for northern elephant seals in the present study. Lack of a sex difference in body composition at weaning has also been reported previously for northern (Kretzmann et al. 1993) and southern (Carlini et al. 2001) elephant seals. Although mothers may be able to increase the body mass of their male offspring, they are perhaps limited in their ability to increase the percentage body lipid content of male pups. These results suggest that differential maternal investment may occur in years when the foraging trip prior to giving birth is fairly successful but may not occur when this foraging trip is relatively difficult. However, this interpretation should be taken with heed, as results from previous studies on northern (Kretzmann et al. 1993; Deutsch et al. 1994) and southern (Fedak et al. 1996) elephant seals indicate that there is no significant variation in reproductive effort with offspring sex. Instead, maternal body mass and composition are important factors in determining reproductive effort and pup size at weaning (Arnbom et al. 1993; Deutsch et al. 1994; Crocker et al. 2001). It is possible that the difference in male and female weaning mass observed in 2000 may be related to significant differences in maternal body condition that we were unable to measure or correct for in this study.

The lack of an inter-year difference in percentage lipid content for pups near the end of the fast is attributed to differences in body reserve utilization patterns. Thin seals born in 1999 were more likely to conserve lipid reserves than fat seals born in 2000. Regardless of lipid levels at weaning, pups appear to leave the beach with similar body composition. It is possible that a critical body composition level cues pups to initiate foraging. This has been suggested for female northern elephant seals, who initiate the post-lactation foraging trip when their body composition reaches approximately 23% adipose tissue (Crocker et al. 2001).

In conclusion, this study demonstrates that both body mass and body composition influence the utilization of fat and protein reserves as well as the duration of the postweaning fast in northern elephant seal pups. Plasticity in energy reserve utilization may be a critical adaptation for this species since it enables all pups to remain on land after weaning to develop the physiological and behavioral capabilities required for the first foraging trip. Variable patterns of energy reserve utilization and unequal postweaning fast durations may also ensure that all pups initiate the first foraging trip with adequate insulation for thermal balance in the ocean.

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