Energy Reallocation during and after Periods of Nutritional Stress in Steller Sea Lions: Low-Quality Diet Reduces Capacity for Physiological Adjustments

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ABSTRACT

Two groups of female Steller sea lions (groups H and P) were subjected to periods of energy restriction and subsequent refeeding during winter and summer to determine changes in energy partitioning among principal physiological functions and the potential consequences to their fitness. Both sea lion groups consumed high-quality fish (herring) before and after the energy restrictions. During restrictions, group H was fed a lower quantity of herring and group P a caloric equivalent of low-quality fish (pollock). Quantitative estimates of maintenance and production energies and qualitative estimates of thermoregulation, activity, and basal metabolic rate were measured. During summer, all animals compensated for the imposed energy deficit by releasing stored energy (production energy). Group H also optimized the energy allocation to seasonal conditions by increasing activity during summer, when fish are naturally abundant (foraging effort), and by decreasing thermoregulation capacity when waters are warmer. During winter, both groups decreased the energy allocated to overall maintenance functions (basal metabolic rate, thermoregulation, and activity together) in addition to releasing stored energy, but they preserved thermoregulatory capacity. Group H also decreased activity levels in winter, when foraging in the wild is less efficient, unlike group P. Overall, sea lions fed pollock did not change energy allocation to suit environmental conditions as readily as those fed herring. This implies that a low energy-density diet may further reduce fitness of animals in the wild during periods of nutritional stress.

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Introduction

The first law of thermodynamics (which defines the conservation of mass and energy; Lucas 1992) states that all of the energy acquired through food (energy intake) is exclusively partitioned between waste, production, and metabolic work (energy outputs). Therefore, animals that do not consume sufficient energy to optimally satisfy their energy output (demands) have to reduce and differentially reallocate energy among these output variables. The way that animals prioritize the allocation of energy between their fundamental physiological functions when energy intake is reduced ultimately has consequences for reproduction and survival.

Marine mammals, such as Steller sea lions (*Eumetopias jubatus*), are known to experience predictable periods of decreased energy intake as a normal part of their life cycles (such as fasting during the breeding season). The balance between energy intake and the cost of existence is further affected by the environment in which the animals live (Prestrud 1991; Cuyler and Øritsland 1993; Patterson et al. 1998). For example, while animal energy requirements vary by season (Winship et al. 2002), so do the distribution patterns and nutritional quality of their prey (Anthony et al. 2000; Kitts et al. 2004). Animals are physiologically equipped for such predictable events but may be less prepared to contend with unpredictable or extended changes in environmental conditions (Trillmich and Ono 1991; Trites and Donnelly 2003; Soto et al. 2006).

Animals must adjust their behavior and physiology in proportion to the energy deficit they incur (Boyd 2002). Pinnipeds can optimize net energy retention (i.e., reduce potential debt) by increasing digestive efficiency and reducing the energy they lose as waste (Lawson et al. 1997; Trumble et al. 2003). However, if this adjustment is insufficient, animals must determine how the net energy they ingest is partitioned between the principal physiological functions of growth, stored energy, reproduction, thermoregulation, voluntary activity, and basal maintenance (Lavigne et al. 1982). Potential specific strategies might entail reducing energy expenditures through suppressing reproductive functions (Pitcher et al. 1998) or reducing production work (i.e., decreasing body growth or sacrificing body energy stores; Stini 1969; Calkins et al. 1998). Reducing activity levels and time spent in thermally challenging environments is another strategy (Limberger et al. 1986; Nash 1998), as is decreasing basal metabolic rate or minimizing thermoregulatory costs through social aggregation (Ohata and Miller 1977; Rosen and Trites 1999). The set of strategies actually employed can affect the individual's capacity to survive and reproduce in the wild

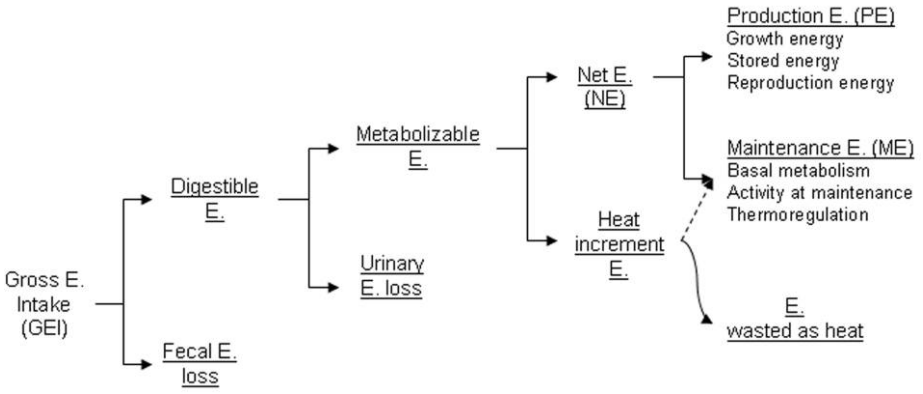

Figure 1. Schematic and simplified partitioning of the gross energy intake in an animal (based on Kleiber 1975; Lavigne et al. 1982). E = energy.

depending on how optimal these decisions are relative to environmental conditions.

While it is relatively easy to speculate on a myriad of theoretical strategies that marine mammals can invoke to balance their energy budgets when faced with suboptimal nutrition, no studies have teased apart how marine mammals actually partition energy. With this in mind, we manipulated the diets of captive Steller sea lions to reveal the energetic priorities that they make under optimal and suboptimal nutritional conditions. We considered the influence of prey type as well as season on energetic priorities and strategies with the ultimate goal of elucidating the physiological and life-history consequences associated with the different decisions they make.

Material and Methods

Experimental Design

All research was conducted under permit A04-0169 issued by the University of British Columbia Animal Care Committee and followed the experimental design detailed in Jeanniard du Dot et al. (2008). In brief, experiments were conducted at the Vancouver Aquarium (British Columbia, Canada) in the summer of 2005 (June–August) and winter of 2006 (January–March) on eight trained, captive, female Steller sea lions (3–5 yr old). The animals were randomly divided into group H and group P.

Each seasonal experiment consisted of three phases of 28 d each. In the 28-d baseline phase, all of the animals were fed their usual daily ration of Pacific herring (*Clupea pallasi*), at levels set by husbandry staff to ensure animal health and training. The baseline phase was followed by a 28-d restriction treatment during which the two groups of sea lions received a reduced level of gross energy intake (approximately 260 kJ kg^{-1} d^{-1} for 3-yr-old and 230 kJ kg^{-1} d^{-1} for 5-yr-old animals) designed to elicit a total mass loss not exceeding 15% of the initial body mass over the food restriction period, as per animal care protocols (the difference in restricted energy intake between age groups was aimed at buffering age/size differences during energy restriction; Schmidt-Nielsen 1990; Rea et al. 2007). Animals in group H were fed exclusively Pacific herring during the restriction while group P was fed solely walleye pollock (*Theragra chalcogramma*) but at isocaloric levels that ensured both groups obtained the same net energy intake. The restriction period was followed by a 28-d controlled refeeding period during which each sea lion received the same initial intake of Pacific herring that they had received during the baseline phase. A subsample of each batch of herring and pollock were analyzed for their proximate chemical composition (soxhlet hexane extraction for lipids and total nitrogen extraction × 6.25 for protein; Norwest Labs, Surrey, British Columbia). Gross energy content of the fish was calculated using the energy conversion factors provided by Schmidt-Nielsen (1997)—39.3 kJ g^{-1} for lipid and 18 kJ g^{-1} for protein.

Bioenergetic Partition

General Partition of the Energy Budget. The energy budget of sea lions is, by definition, balanced between the energy ingested and the energy spent (Fig. 1). To determine which physiological functions Steller sea lions altered during an energy restriction treatment and in what proportion, changes in net energy intake (NE), production energy (PE), and maintenance energy (ME) were estimated in relation to baseline levels. Similar calculations were performed for changes occurring during the controlled refeeding when sea lions increased their energy intake compared with their intake in the previous restriction phase. In both cases, changes in energy intake and allocation to physiological functions were calculated for the first 2 wk of each phase as well as for the entire 28-d periods (cumulative changes encompassing those measured during the first 2 wk).

Estimation of the NE. Net energy is the biologically useful energy that can be utilized for all biological functions through either ME or PE (see below). NE was calculated as the gross energy intake (GEI) minus the energy lost through feces (calculated

through digestive efficiency; DE), urine (UrE), and heat (HiE):

$$NE = PE + ME = GEI \times DE - (UrE + HiE). \quad (1)$$

Food intake (± 0.025 kg d^{-1}) of each animal was recorded daily (Table 1), and these data were combined with prey proximate composition values to calculate average GEI of Steller sea lions over relevant periods (Table 2). DE, UrE, and HiE were not directly measured and were obtained from published estimates for Steller sea lions and other pinnipeds. DE was considered to be 95.4% for herring and 93.9% for pollock (Rosen and Trites 2000*a*). UrE was estimated at 7% of the digestible energy for all animals during the baseline and the controlled refeeding phase, based on several phocid seal values (Parsons 1977; Lavigne et al. 1982). During the restriction, group P was assumed to lose more energy through UrE (higher protein intake from food and higher protein loss from body stores), and UrE was estimated to be 10% of digestible energy. In these calculations, HIF was used as an approximation of HiE. Based on data from Rosen and Renouf (1997) and Rosen and Trites (2000*b*), HIF was adjusted with quantity and quality of food ingested. Energy lost through heat was considered to be 12.5% of GEI during the baseline and the controlled refeeding phases. During the restriction, HIF was assumed to be 10% of GEI for group H and 15.5% for group P. HIF in Steller sea lions is not recycled for thermoregulation and is totally lost as dissipated heat (Rosen and Trites 2003).

Estimation of PE. PE is the portion of the NE allocated to productive functions such as structural growth (GrE), storage of body energy reserves (StE), or reproductive functions (ReE, pregnancy, lactation, etc.):

$$PE = GrE + StE + ReE. \quad (2)$$

Changes in body mass and composition were used to calculate changes in PE (as ReE = 0 in our experiment). Body mass of each animal (± 0.1 kg) was recorded daily and body composition was measured at the end of the baseline and every 2 wk until the end of the controlled refeeding during the summer. Briefly, this was done using the deuterium dilution method (Reilly and Fedak 1990), conducted before the sea lions' first meal of the day (~18-h overnight fast) and using equations from Arnould et al. (1996) to calculate percent lipid and lean tissue mass from body water data (procedure performed while animals were under anesthesia; for more details, see Jeanniard du Dot et al. 2008). These values were then combined with body mass measures to calculate absolute mass of lipid and lean tissue compartments.

Energy content of proteins and lipids released during food restriction (negative StE and GrE values) were calculated ac-

Table 1: Gross energy intake (GEI), body mass, composition, and energy content measured during the different phases of the summer 2005 and winter 2006 experiments

Season, Diet Group	Phase	GEI (kJ d^{-1})	Body Mass (kg)	Lipid Mass (kg)	Lean Mass (kg)	Body Energy (MJ)
Summer:						
H	B4	35.43 (5.75)	108.88 (21.18)	22.48 (5.79)	86.39 (15.55)	1,266.6 (294.7)
	R2	27.07 (3.92)	104.23 (20.03)	17.11 (4.99)	93.53 (18.25)	1,089.2 (277.9)
	R4	27.07 (3.92)	98.65 (18.97)	12.03 (2.48)	86.62 (16.84)	859.6 (166.6)
	CR2	34.65 (6.07)	96.28 (17.63)	16.47 (2.64)	84.90 (18.04)	1,025.0 (185.1)
	CR4	34.65 (6.07)	96.10 (17.12)	17.35 (5.36)	78.75 (11.77)	1,031.6 (262.6)
P	B4	40.73 (7.13)	131.35 (23.59)	22.39 (4.60)	108.96 (19.46)	1,365.6 (260.5)
	R2	31.64 (4.64)	128.45 (23.60)	19.83 (4.10)	108.62 (19.66)	1,264.2 (246.7)
	R4	31.64 (4.64)	120.40 (21.81)	17.82 (4.50)	102.58 (17.45)	1,158.2 (252.9)
	CR2	39.78 (6.74)	118.10 (21.66)	19.98 (2.97)	98.12 (19.06)	1,222.2 (195.3)
	CR4	39.78 (6.74)	118.33 (21.76)	16.68 (2.79)	101.65 (19.00)	1,109.3 (194.5)
Winter:						
H	B4	47.59 (5.21)	121.70 (19.73)	24.94 (5.00)	96.76 (14.79)	1,409.9 (261.5)
	R2	30.34 (3.78)	115.45 (18.81)	20.78 (4.18)	94.67 (14.80)	1,238.0 (228.2)
	R4	30.34 (3.78)	110.50 (18.88)	16.22 (3.08)	94.28 (16.15)	1,058.0 (187.4)
	CR2	45.33 (5.71)	114.85 (18.10)	25.19 (3.91)	89.66 (14.85)	1,387.2 (210.1)
	CR4	45.33 (5.71)	119.50 (17.74)	26.89 (4.58)	92.61 (13.22)	1,467.0 (238.1)
P	B4	47.53 (4.58)	143.60 (21.94)	27.49 (4.32)	116.11 (18.39)	1,597.3 (240.0)
	R2	34.35 (3.94)	139.85 (21.76)	27.65 (4.94)	112.20 (17.49)	1,585.6 (262.6)
	R4	34.35 (3.94)	134.30 (21.19)	26.31 (4.63)	107.99 (17.23)	1,514.3 (249.0)
	CR2	46.74 (4.66)	136.25 (20.65)	28.97 (4.20)	107.28 (17.79)	1,614.8 (223.1)
	CR4	46.74 (4.66)	139.75 (20.14)	30.27 (4.73)	109.48 (16.20)	1,675.8 (246.9)

Note. B4, R4, CR4 = measurements made during the fourth week of baseline, restriction, and controlled refeeding; R2, CR2 = measurements during the second week of same periods. Numbers in parentheses are the standard errors of the mean.

Table 2: Relative contribution of production energy (PE) and maintenance energy (ME) in percent to the compensation of the energy deficit compared to the baseline during the restriction and relative allocation of the energy gain compared to the restriction during the controlled refeeding

Diet Group, Sampling Time (wk)	Restriction				Controlled Refeeding			
	Summer		Winter		Summer		Winter	
	PE (%)	ME (%)	PE (%)	ME (%)	PE (%)	ME (%)	PE (%)	ME (%)
Group H:								
2	198^{A} (64)	-48^{AB} (9)	83^{A} (13)	10^{B} (9)	220^{A} (78)	-31^{B} (27)	182^{A} (50)	-36^{B} (15)
4	166^{A} (41)	16^{B} (51)	86^{A} (24)	32^{AB} (7)	67 (54)	-75^{AB} (35)	131^{A} (17)	-19^{B} (14)
Group P:								
2	75 (44)	9^{B} (24)	5^{B} (14)	51^{AB} (9)	132 (115)	-50^{AB}(17)	83^{A} (35)	-8^{B} (23)
4	95^{A} (23)	20^{B} (23)	26^{AB}(1)	46^{B} (16)	-4^{B} (31)	-41^{B} (35)	79^{A} (28)	-8^{B} (22)

Note. Calculations were performed during either the first 2 wk of the treatments or the entire 4 wk. Values in parentheses are the standard errors of the means; A = values different from 0%; B = values significantly different from 100%. During the restriction: 100% = energy used by PE or ME was equal to the entire energy intake deficit; 0% = none of the energy deficit was compensated for by a change in the PE or ME functions. A negative percentage means that the energy allocated to PE or ME increased despite the energy restriction. A percentage over 100% means that the energy expenditure for PE or ME decreased to a greater extent than the calculated energy deficit. During the controlled refeeding: 100% = the entire newly increased energy intake (compared with the restriction) is allocated to PE or ME; 0% = no additional energy is allocated to PE or ME, compared with the restriction allocation. A negative percentage means that the energy allocation to PE or ME decreased even though the energy intake increase. A percentage over 100% means that more than the energy intake's increase is allocated to PE or ME.

cording to standard biochemical estimates (18 and 39.3 kJ g^{-1}; Kleiber 1975). When increases in lipids or lean tissues occurred (positive StE and GrE values), additional costs of deposition were calculated as 2.38 kJ necessary per kJ of proteins deposited and 1.17 kJ per kJ of lipids following experimentally derived values in Roberts and Young (1988). These values are close to theoretical values (2.37 kJ kJ^{-1} for proteins and 1.08 kJ kJ^{-1} for lipids) and within range of the values found experimentally in a variety of mammals (Pullar and Webster 1977).

Estimation of ME. ME comprises the energy allocated to essential physiological functions such as for the basal maintenance of tissues and biochemical processes (BME), for thermoregulation (ThE), or voluntary activity (AcE). ME can also be calculated in reference to body composition changes and daily metabolic rate (DMR) data:

$$\text{ME} = \text{BME} + \text{AcE} + \text{ThE} = \text{DMR} - \text{HIF} - \text{DepE}. \quad (3)$$

DMR is a measure of the total energy expenditure of animals over 24 h. It comprises BME, AcE, and ThE, as well as energy lost as heat (HIF) and the energy utilized to deposit protein or lipid body mass (DepE).

DMR was measured every 2 wk concurrent with body composition determinations by enclosing the sea lions in a large metabolic chamber containing a small pool and with enough room for the animals to perform their daily routine for 22–24 h. Both DMR and resting metabolic parameters (see below) were measured by open circuit respirometry. Fans were used to provide good air circulation in the chamber and the behavior of the animals was recorded using a video camera. A thermometer measured the indoor temperature, and a water system was installed to spray mist (when inside temperature reached 30°C or a 5°C difference with outside temperature) in the chamber to prevent the temperature from increasing too much compared with the outside. After an adequate equilibration time (~2 h), air was drawn from the metabolic chamber at a constant rate (using a FlowKit model 500H flow generator and controller; Sable Systems, Salt Lake City, UT) sufficient to keep levels of O_2 above 18.5% in the chamber (250–350 L min^{-1}). Desiccated outflow subsamples of O_2 and CO_2 concentrations were analyzed by Sable System FC-1B and CA-1B analyzers. Outside air baseline measurements were taken before and after the metabolic rate recording to correct for any drift in the system. Data was recorded and analyzed using Sable data acquisition system.

During DMR measurements, the animals were fed twice a day through a feeding tube that was otherwise sealed. When the recording period did not reach 24h, the data were linearly extrapolated between the first and the last data point for the remaining hours. Mean metabolic rate was also compared between day hours (6 a.m.–8 p.m.) and night hours (8 p.m.–6 a.m.) to assess diurnal differences. The mass-corrected metabolic rate was calculated using the scaling factor of 0.714 calculated by Hunter (2005). To correct for initial daily metabolic rate differences between sea lions, rates of change compared with respective baseline measurement were calculated.

DMR data were converted into energy consumption (conversion: 20.2 kJ L^{-1} O_2) to be incorporated into equation (3). To estimate changes in ME allocation over time, individual DMR measurements (calculated on a 24-h basis) were multiplied by 14 for first 2–wk period and by 28 for the full 4-wk period. The energy used to deposit tissues (DepE) was estimated as 0.17 kJ kJ^{-1} of lipids deposited and 1.38 kJ kJ^{-1} of deposited proteins, respectively (Roberts and Young 1988). These values represent only the costs associated with tissue deposition and are independent of the actual energy deposited. HIF values were the same as those previously described to estimate NE.

Assumptions and Approximations. A number of assumptions and gross approximations were invoked because the experimental design did not allow for all parameters to be calculated. First, calculations of StE and GrE differentiated only lipid and lean tissue changes and could not account for skeletal growth.

The values used for HIF, DE, and UrE were based on estimates from various studies. DE, HIF, and UrE were adjusted to fish quality and biomass intake between diet groups and phases (Lawson et al. 1997; Trumble et al. 2003) as explained earlier but were not specifically measured for this experiment. Measurements of DMR and body composition taken in the middle and at the end of each treatment were assumed to reflect constant changes between measurements over the 2-wk periods, and there was also an assumption that there were no significant changes during the baseline phase when only a single measurement was taken. For the DMR data, there was also the inherent assumption that the animals behaved similarly outside of the metabolic chamber. DMR measurements were also taken a few days (1–3 d) after the body composition measurements such that maintenance and production energies considered in the same budget equation were estimated a few days apart.

Finally, the energetic cost of protein turnover was ignored in calculating production energy, although it is included in DMR, and it was assumed to be similar during all the phases (an attempt to directly measure this variable was unsuccessful). The model also did not consider the potential use of amino acids for the production of lipids.

Qualitative Energetic Estimates

ThE, AcE, and BME were not directly measured and could not be estimated quantitatively to be incorporated into the energy budget equations (eq. [3]), but respirometry measurements were performed to estimate qualitative changes in the energy allocated to these functions.

Thermoregulatory Capacity. Thermoregulation capacity was tested in lieu of direct ThE measurements by determining the metabolic rate of animals caged in a swim mill at different water temperatures for 50 min. Water temperatures were regulated within 0.4°C of the 8° and 2°C target temperatures, which respectively represent the lower range of summer and winter sea surface temperature encountered by sea lions in the North Pacific (DFO 2007). Metabolic rates in waters of 8° or 2°C (MR_8 and MR_2) were measured on each animal every 2 wk in the same week that the body composition and the DMR data were obtained. MR_8 and MR_2 were measured using similar respirometry methods as the DMR data (see above), with the exception that animals breathed into a small hermetically sealed dome above the water level, and the air flow rate was 200 L min^{-1}. Animals were postabsorptive, and their activity levels were reduced and relatively consistent between and within animals. The first 5 min of the recordings were ignored to avoid potential change in metabolic rate due to the primary excitement of the animals entering the swim mill. The following 10 min were considered the "baseline" for the metabolic rate (before thermal challenge). Differences in oxygen consumption between the 10-min baseline and last 10 min of the trials were used as an index of thermoregulatory capacity (capacity of the animals to keep body temperature constant without having to increase metabolic rate).

Standard Metabolic Rate. Measurements of standard metabolic rate in air (SMR_A) were measured only during the winter experiment, every 2 wk in the same week as the other measurements. The animals were enclosed for 45 min (including a 5-min equilibration time) in a small sealed, dry metabolic chamber containing a fan for air circulation and a video camera. Respirometry methods used were similar to those described above ("Thermoregulatory Capacity"). Measurements were performed on postabsorptive animals (>16 h after last meal). Levels of activity in the chamber, monitored by video, varied between animals but were consistent throughout the experiment for the same animal. SMR_A was calculated from the 20 min in the middle of the recording, to eliminate potential initial excitement and final impatience behaviors. Rates of change were also calculated to buffer individual variability.

Exercise Metabolism. Metabolism after exercise was measured to assess changes in fitness of the animals throughout the experimental conditions, and was performed only during the summer trials. The sea lions were trained to run between two trainers as quickly as possible for 20 laps (total distance of 290 ± 10 m). To keep the animals interested, trainers rewarded them with small pieces of fish between every few laps. Prey ingestion was kept minimal to limit the potential effect of HIF. The metabolic rates at the end of the running time were measured with animals in the swim mill (water temperature 8°C) because the small volume of the respirometry dome allowed an immediate measurement of postexercise peak oxygen consumption. Oxygen debt after the exercise was determined by integrating the increase in oxygen consumption above the average postrecovery baseline values. Time to reach baseline was assessed as the period until two sequential 1-min averages of $\dot{V}O_2$ averages were within a 5% margin of one another. Changes in oxygen debt compared with the baseline value were calculated to correct for initial individual variability. Trials when animals did not enter the dome right away at the end of the running time were discarded.

Statistical Analyses

Our study employed a repeated-measures design. Consequently, effects of diet, season, phase (and age, even though the experiments were designed to buffer effects of age/size) or interaction between these terms on dependent variables (DMR, SMR_A, MR_8, MR_2, etc.) were estimated using mixed effect models. All of the models' assumptions were verified as per Pinheiro and Bates (2000), and changes during the restrictions and the controlled refeedings were compared with baseline measurements. The first set of analyses investigated the effect of diet type and experimental phase on the different dependent variables strat-

ified by seasons. Fixed effects were diet, phase, and the interaction between these parameters, and random effects were individual animals, diet, and phase, depending on the best model fit estimated by ANOVA and AIC. The second set of analyses investigated the within-group differences along the experimental time line stratified by diet and season.

The relative contribution of changes in PE and ME to compensate for the energy intake deficit during the restriction was statistically estimated using one-sample *t*-tests. To estimate the relative contribution of PE or ME to energy deficit (or subsequently energy intake increase), the hypotheses H_0 ($\mu = 0\%$; no contribution) and H_0' ($\mu = 100\%$; total contribution) were tested with one-sample *t*-tests. All data values presented are means ± SE and statistical significance of each parameters estimates was set at $\alpha = 0.05$.

Results

Bioenergetic Partition

Energy Intake, Body Mass, and Body Composition. Experimental diet, body mass, and body composition data are summarized in Table 1 (further details available in Jeanniard du Dot et al. 2008). In brief, body mass changes were similar between the two diet groups ($P = 0.07$; or age groups $P > 0.13$), and body mass loss reached approximately 10%–15% of the baseline body mass during the summer and winter restrictions. Both groups started the restrictions with similar average body fat ($P = 0.34$ in summer and $P = 0.88$ in winter). Initial body condition of the animals (or age) did not impact subsequent rates of lipid or protein catabolism (all $P > 0.08$), although diet type did. During the summer restriction, animals in group H lost exclusively body fat when losing mass. Group P initially lost mostly body fat but overall lost significantly less body fat and more lean mass than group H after 2 and 4 wk of restriction (both $P < 0.001$). The higher contribution of lipids to compensate for the deficit in energy intake by group H led to a decrease in total body energy (a change from 1,200 to 800 MJ in summer and from 1,400 to 1,000 MJ in winter) and was significantly greater than group P ($P < 0.05$). During winter, group H again lost significantly more body fat than group P (both $P < 0.01$) due to the tendency for group P to exclusively catabolize lean mass during the restriction. This translated into a more or less stable total body energy content between 1,500 and 1,600 MJ throughout the restriction for group P in this season, whereas group H dropped from 1,400 to 1,050 MJ.

During the subsequent controlled refeeding in summer (when the sea lions returned to the baseline diet), the animals remained stable at the same mass and body energy content they attained at the end of the restriction ($P = 0.24$), but the difference in body composition between the two groups became less significant ($P > 0.06$). During winter, animals displayed compensatory growth and restored their body energy status during the controlled refeeding phase (mass gain rates increased by 7.29% ± 1.43% compared with baseline rates, mostly achieved by gaining body fat). They finished the experiment with slightly more lipids than before the restriction even though the difference was not statistically significant ($P = 0.20$). Changes in body energy were then incorporated into the bioenergetic calculations (eqq. [1]–[3]).

Daily Metabolic Rate. During summer, the sea lions in both diet groups started the experiment with similar mass-corrected daily metabolic rates ($P = 0.68$), which averaged 1,094.01 ± 79.35 kJ $kg^{-0.714}$ d^{-1} (range 872–1,577 kJ $kg^{-0.714}$ d^{-1}). DMR of group P stayed constant throughout the experiment, when considered either on a 24-h basis or split into day or night hours (all $P > 0.30$; Fig. 2*a*, 2*b*). DMR of group H, however, increased (and was significantly higher than for group P; all $P = 0.03$) by the second week of the restriction (+18% ± 3%, $P = 0.003$) and remained elevated until the middle of the controlled refeeding (+19% ± 6%, $P = 0.01$). The increase in total DMR was entirely due to an increase of metabolic rate during day hours (all $P < 0.001$ except for the last measurement at the end of the controlled refeeding $P = 0.90$; Fig. 2*b*), up to a 37% ± 3% increase over baseline levels during the restriction. In contrast, metabolic rates at night were constant throughout the study (all $P > 0.05$), with an average overall value of 784 ± 21 kJ $kg^{-0.714}$ d^{-1}. DMR returned to the pre-restriction values at the end of the controlled refeeding ($P = 0.22$).

During winter, both diet groups started the experiment with similar DMR (1,235 ± 64 kJ $kg^{-0.714}$ d^{-1}; $P = 0.34$). In both groups, the baseline averages were higher in winter than in summer (average difference = 141.15 ± 70.03 $kg^{-0.714}$ d^{-1}, $P = 0.002$). DMR of group P during winter stayed stable most of the time (similarly to summer trends, $P > 0.09$; Fig. 2*d*), except at week 2 of the deprivation, when a significant decrease occurred (−15% ± 5% at 1,073 ± 122 kJ $kg^{-0.714}$ d^{-1}, $P = 0.02$; Fig. 2*c*).

Changes in DMR of group H were different in winter versus summer (all $P < 0.001$). In winter, DMR averages were lower than the baseline levels for all subsequent measurements (all $P < 0.02$). This was true for the metabolic rate on a 24-h basis and also when data was split into day and night hours (Fig. 2*d*). It decreased by 23.60% ± 2.90% at the end of the restriction compared with the baseline (917 ± 40 kJ $kg^{-0.714}$ d^{-1}) by 15.15% ± 3.65% (1,017 ± 38 kJ $kg^{-0.714}$ d^{-1}) at the end of the controlled refeeding and by 21.82% ± 1.61% during the ad lib. phase. Decreases in DMR were also greater for group H than for group P at the end of the restriction ($P = 0.01$), at the end of the controlled refeeding ($P = 0.01$), and during the ad lib. phase ($P < 0.01$). Age did not impact relative changes in DMR ($P > 0.58$)

Energy Redistribution. Bioenergetic calculations were performed to determine the reallocation of energy expenditures relative to the net energy deficit during the restriction phase (compared with baseline energy intake) and the increase in net energy during the controlled refeeding phase (relative to restriction levels) by combining data on DMR, energy intake, and body composition (see above). Age was never found to impact PE or ME in either season (all $P > 0.12$). During the summer experiment, the calculated total net energy deficit during the re-

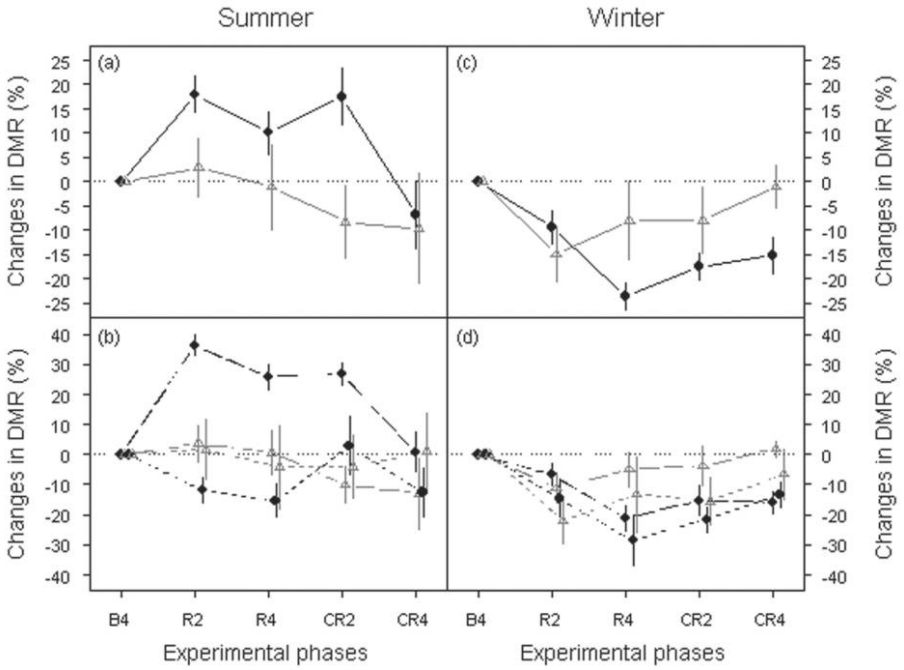

Figure 2. Mean ± SE changes in daily metabolic rates (DMR) compared to the baseline measurement (B4) at weeks 2 and 4 of the restriction (R) and the controlled refeeding (CR) of Steller sea lions in group H (*circles*) or in group P (*triangles*) in the summer (*a*, *b*) and in the winter (*c*, *d*) experiments. *a*, *c*, DMR measured on a 24-h basis; *b*, *d* metabolic rates separated between daytime (*dashed lines*; 6 a.m.–8 p.m.) and nighttime hours (*dotted lines*; 8 p.m.–6 a.m.).

striction phase (compared with the energy the sea lions were getting during the baseline phase) averaged 121.5 ± 17.5 MJ during the first 14 d and 216.8 ± 46.3 MJ over the entire 28-d phase for group H and 139.8 ± 31.0 MJ during the first 2 wk and 277.0 ± 70.1 MJ for the whole phase for group P.

In summer, the decrease in energy intake during the restriction phase did not significantly affect the proportion of energy that animals in group P allocated to ME (i.e., energy for thermoregulation, voluntary activity, and basal metabolism) either after 2 or 4 wk of energy restriction (9% ± 24% and 20% ± 23%, contribution to net energy deficit not significantly different from 0%, $P = 0.87$ and 0.81, respectively; Table 2). Surprisingly, sea lions in group H increased the energy they allocated to ME after 2 wk of restriction, which effectively increased the calculated potential energy deficit by approximately 48% ($P = 0.03$) relative to changes in food intake alone (Table 2). After 4 wk of deprivation, calculations for one out of four animals were dramatically opposite to the others (F03RO). Consequently, with a low statistical power, the variability was too large to see any significant difference from either a 100% or 0% contribution of ME to the deficit (all $P > 0.2$). The bioenergetic calculations showed that the entire energy intake deficit was covered by releasing energy stored in the body (tissue catabolism) after both 2 and 4 wk of restriction (166% ± 41% and 95% ± 23% coverage for groups H and P, not significantly different from 100% coverage or from one another, $P < 0.5$).

During the winter restriction, the total net energy deficit averaged 182.3 ± 19.7 MJ over the first 2 wk and 364.5 ± 39.4 MJ over the whole phase for group H, and 181.8 ± 26.6 MJ over the first 2 wk and 350.5 ± 57.1 MJ over the whole phase for group P. Group P significantly decreased its energy allocated to ME during restriction by a level equivalent to 50% ± 17% of the energy intake deficit for the first 2 wk of restriction (different from 0%, $P = 0.03$; different from 100%, $P = 0.02$) and 46% ± 16% after 4 wk (different from 100%, $P = 0.002$). This group also offset 26% ± 1% of the energy deficit by releasing stored energy after 4 wk of restriction (contribution of PE significantly lower than 100%, $P < 0.0001$). In comparison, group H significantly decreased the overall energy allocated to ME over the restriction phase at a level equivalent to 32% ± 7% of the energy deficit, but the change was significant only when calculated over the entire 4-wk period ($P = 0.01$), not over the initial 2-wk period ($P = 0.36$). This group compensated for the majority of the energy intake deficit via tissue catabolism (PE). The calculated contributions for the first 2 wk of restriction (83% ± 13%) or the whole month (86% ± 24%) are significantly different from 0 but not from 100% (both $P > 0.3$). Overall during the winter trials, the offset to the energy intake deficit from the decrease in energy allocated to ME was significantly greater for group P than for group H after 2 wk of restriction ($P = 0.05$) but not when calculated for the entire 4 wk of restriction ($P = 0.77$). The energy released from body stores was always greater for group H than for group P (both $P < 0.05$).

In summer, the total net increase in energy intake during

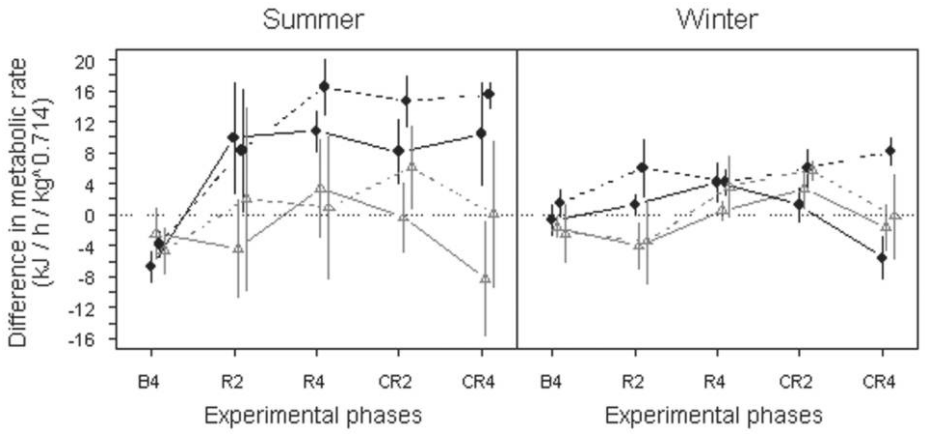

Figure 3. Mean ± SE metabolic rate differences between the beginning and the end of the measurement time after 45 min in water at 8°C (*dotted lines*) and 2°C (*solid lines*) for Steller sea lions in group H (*circles*) and group P (*triangles*) during the restriction (R2 and R4) and the controlled refeeding (CR2 and CR4) in the summer 2005 and winter 2006 experiments.

the controlled refeeding (energy "surplus" compared with the restriction phase) averaged 189.1 ± 63.8 MJ (across animals) for group H and 257.4 ± 76.7 MJ for group P over the whole phase. In winter, the energy intake "surplus" reached 334.3 ± 43.5 MJ for group H and 306.2 ± 69.8 MJ for group P over the 4-wk controlled refeeding. In summer, the change in energy allocated to either ME or PE could not be determined due to high individual variability and low statistical power (different from neither 0% nor 100% contribution). This was due to the dissimilar behavior of one animal in each diet group compared to the others (F03RO and F00YA). Group H, however, seemed to allocate newly available energy mostly to PE, at least during the first 2 wk, and not to ME, which mostly had negative calculated proportions of energy allocation (see Table 2).

In winter, however, both diet groups allocated the majority of their net energy intake surplus to PE (i.e., to body energy stores and/or somatic growth). Group H allocated 131% ± 17% and group P 79% ± 28% of the net energy difference to production energy (not different from 100%, $P > 0.15$ for both groups; Table 2). Energy allocation to maintenance energy did not increase in the winter, either after 2 or 4 wk of recovery for any of the groups (19% ± 14% for group H and 8% ± 22% for group P, not different from 0%; all $P > 0.2$).

Qualitative Energetic Estimates

Thermoregulation Capacity. During summer, average metabolic rates during the baseline phase were similar between the two diet groups in water at 8°C (average all animals pooled: 50.0 ± 1.2 kJ $kg^{-0.714}$ h^{-1}) and at 2°C (48.9 ± 1.3 kJ $kg^{-0.714}$ h^{-1}; $P = 0.68$ and 0.34, respectively). The baseline values for the average metabolic rates were also not higher for the 2°C temperature than for the 8°C ($P = 0.44$).

During the baseline phase, thermoregulatory costs—measured as the difference in the rate of oxygen consumption between the beginning and the end of the trial—had a negative value at both 8°C (−4.60 ± 1.91 kJ $kg^{-0.714}$ h^{-1}) and 2°C (−4.27 ± 1.52 kJ $kg^{-0.714}$ h^{-1}), reflecting that metabolic expenditure was lower at the end of the thermal trial than at the start (Fig. 3). Group H exhibited significant increases in thermoregulatory costs during the restriction and the controlled refeeding treatments at both 8°C (all $P < 0.04$) and 2°C (all $P < 0.002$). At the end of the restriction phase, thermoregulatory costs for group H were 10.80 ± 2.59 kJ $kg^{-0.714}$ h^{-1} in the 8°C water and 16.46 ± 3.50 kJ $kg^{-0.714}$ h^{-1} in water at 2°C after 1 h in the water. In contrast, group P did not increase its thermoregulatory costs at either 8° or 2°C (all $P > 0.2$) compared with the initial values at the beginning of the trial. When comparing diet groups, group H increased its thermoregulatory costs significantly more than group P in 2°C water, starting at week 4 of the restriction until the end of the controlled refeeding (all $P < 0.01$). At 8°C, thermoregulatory costs for group H were significantly greater than for group P only at the end of the controlled refeeding ($P < 0.01$).

During the winter baseline phase, both experimental groups had similar metabolic rates, compared with one another and at both temperatures ($P = 0.34$ at 8°C and $P = 0.2$ at 2°C, total average of 1,017 ± 50 kJ $kg^{-0.714}$ h^{-1} at 8°C and 986 ± 47 kJ $kg^{-0.714}$ d^{-1} at 2°C), which were lower than the summer baseline values ($P = 0.01$ and 0.007, respectively). There were no significant changes in thermoregulation costs (change in metabolism over a single measurement) during any of the phases relative to baseline values, nor any differences attributable to diet group (all $P > 0.07$; Fig. 3). Thermoregulatory costs were 56 ± 35 and 93 ± 45 kJ $kg^{-0.714}$ d^{-1} in waters of 8° and 2°C, respectively, after 1 h in the water at the end of the restriction and −87 ± 47 and 94 ± 73 kJ $kg^{-0.714}$ d^{-1} at the end of the ad lib. period. Age never impacted relative changes in thermoregulation at any temperature tested (all $P > 0.08$)

Standard Metabolic Rate. SMR_A baseline values (during the winter trial only) averaged 73 ± 4 kJ $kg^{-0.714}$ h^{-1} with no differences between diet groups (Fig. 4*a*). There were no significant changes

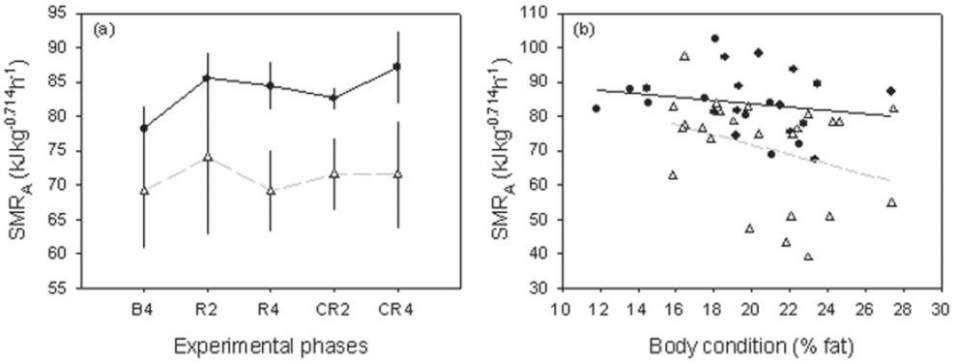

Figure 4. Standard metabolic rates in air (SMR_A) of Steller sea lions in group H (*circles*) and group P (*triangles*) during the different experimental phases (*a*) and related to the body condition (total body fat %) of the animals (*b*). SMR was measured throughout all the phases only during the winter experiment. *a*, Bars represent the standard error of the means; *b*, lines represent the function from the linear mixed effects models run for group H (*solid line*) and group P (*dashed line*). Relationships fitted by linear mixed effect models were not significant (group H: $P = 0.21$; group P: $P = 0.88$) and were not significantly different from one another ($P = 0.52$).

in SMR_A during subsequent experimental phases or attributable to diet group (or age group), whether expressed as absolute values or as rates of changes compared with the baseline measurement (all $P > 0.08$). SMR_A values were not significantly different from baseline values during the restriction (78.04 ± 4.55 kJ $kg^{-0.714}$ h^{-1}), the controlled refeeding (79.34 ± 5.32 kJ $kg^{-0.714}$ h^{-1}), or the ad lib. treatments (74.58 ± 4.27 kJ $kg^{-0.714}$ h^{-1}; all $P > 0.06$). SMR_A was not significantly correlated with body mass or body condition of the animals (all $P > 0.1$; Fig. 4*b*).

Exercise Metabolic Rate. There were no differences attributable to diet (or age) in the baseline oxygen debt after a period of standard exercise ($P = 0.68$); the debt averaged 4.10 ± 0.84 L (range from 2.41 to 8.23 L). Neither the diet, age, nor the experimental phase affected the O_2 debt of the animals when expressed as absolute values or as rates of change compared with the baseline values (all $P > 0.1$). The time to reach the stable O_2 consumption rate after exercise ranged from 5 to 10 min, and the experimental treatments did not affect the time to recover after exercising (all $P > 0.09$).

Discussion

Animals can employ a limited set of options to balance their energy budgets when facing a decrease in gross energy intake. The simplest strategy is to adjust their digestive efficiencies to maintain a constant net energy intake during periods of nutritional stress. If this is insufficient, energy must be released from body stores and/or spared by reducing the energy allocated to physiological functions including activity, thermoregulation, and basal metabolism. Time of year and quality of diet may interplay with these processes and affect an animal's energetic decisions. Our study provides both qualitative and quantitative information on how Steller sea lions partition energy when faced with energetic stress, and it shows that energy restriction induces different patterns of energy allocation dependent on season and quality of diet.

Energetic Priorities during Summer

When faced with energy restrictions, animals can utilize a range of bioenergetic adjustments including increased digestive efficiencies, behavioral adjustments to reduce activity or thermal costs, decreases in basal metabolism, and changes in growth and in foraging effort (Mrosovsky and Sherry 1980; Keiver et al. 1984; Worthy and Lavigne 1987; McCarter and McGee 1989; Nordøy et al. 1990; Oritsland 1990; Markussen et al. 1992; Rosen and Trites 1999, 2002; Ali et al. 2003). During the summer restriction, all animals in our study (independent of diet type) relied exclusively on internal energy reserves to balance their budgets when faced with energy deficits caused by reduced energy intake.

Depletion of internal energy reserves to counteract the net energy intake reduction often occurs in animals, but it is not usually the sole adjustment observed. A reduction in ME is a common response of nutritionally stressed animals to save energy and thus reduce the amount of body components required to mobilize during nutritional stress (Worthy and Lavigne 1987; McCarter and McGee 1989; Nordøy et al. 1990; Markussen et al. 1992; Rosen and Trites 1999, 2002). During our summer experiment, ME did not decrease significantly for any of the diet groups, meaning there was no overall evidence of energy expenditure sparing during the restriction from thermoregulation, activity, and basal metabolism. Counterintuitively, allocation to ME increased after 2 wk of restriction for group H, as has been observed in other species during nutritional stress (Mrosovsky and Sherry 1980; Keiver et al. 1984; Ali et al. 2003). This increase in energy allocated to ME may have been why animals in group H seemed to release more energy from their body than the actual gross energy deficit (198%, even though it was not significantly greater than 100% of the energy deficit; see Table 2).

Changes in ME, particularly increases during nutritional stress, are generally thought to be associated with increases in activity (Mrosovsky and Sherry 1980; Keiver et al. 1984; Ali et al. 2003), a major component in the energy budget of pinnipeds

(Costa and Williams 1999). For example, immature Steller sea lions spend 40%–75% of their time swimming, and mature animals spend 70%–80% of this time in the water in winter (Merrick and Loughlin 1997; Swain and Calkins 1997; Trites and Porter 2002), which represents up to 65% of their daily energy expenditure (Trites and Porter 2002; Winship et al. 2002). In our study, voluntary activity was not directly measured, but DMR provided qualitative data about changes in the costs of activity. DMR increased by about 35% during daylight hours (20% on average on a 24-h basis) for the animals fed herring during the energy restriction. These changes were probably due to an increase in activity since the animals were not thermally challenged in the chamber and the levels of changes were too high to be attributable only to changes in basal metabolism.

Increases in voluntary activity during an energy restriction are typical of a "hunger response." The reaction of increased activity from animals in group H that received a reduced amount of fish may mirror an innate reaction that wild animals may also display to increase their foraging effort in an attempt to find more food and counteract the state of energy restriction (Rosen and Trites 2002). Changes in activity in animals in group H also appeared to reflect a flexible strategy to optimize their increased energy expenses against the probability of catching food in their known captive setting, since the increases in activity were seen only during the daytime, when they usually received food from the trainers, but it was decreased during the night hours, when the probability for getting food was null. The overall significant increase in activity may explain why group H increased maintenance energy. In contrast, the sea lions fed pollock did not display any significant change in activity levels, at least as assessed from the DMR data. This means that they did not initiate a hunger response in order to "optimize" the chances to get more food as per natural setting, nor did they decrease their activity levels to spare energy.

It is important to keep in mind that our experimental diets differed not only in terms of fish quality but also in terms of biomass intake. The animals in group P consumed approximately 60% more fish than group H for the same energy intake during the restriction and slightly more fish (5%) than during their own baseline herring intake. This difference in satiation levels (intrinsic consequence of a low-energy fish for isocaloric diets compared with high-energy fish, even in the wild) could explain why group P did not display an increase activity associated with a hunger response.

If the increase in activity observed in the laboratory for group H translated into increased time in the water (to increase foraging effort in the wild), it would have several secondary energetic implications (Rosen et al. 2007), such as increasing the thermoregulation costs associated with spending more time in a thermally conductive environment looking for food. It is thus important to assess the changes in thermoregulatory capacity occurring during the restriction concomitantly with the changes in activity levels. Thermoregulation costs in nondeprived sea lions (thus considered with "optimal" body condition, around 20% body fat in summer) were insignificant in water as cold as 2°C. However, thermoregulation capacity decreased (metabolic costs for thermoregulation increased) during the restriction while animals were losing mass, especially at the lower water temperatures for group H. Unlike animals in group P, the animals fed herring had to increase their metabolic production of heat to stay warm after 1 h in the water (no change in behavior in the cage). Their insulation did not seem to be sufficient to adequately isolate the body core after 15 or 28 d of losing mass.

The animals fed herring almost exclusively mobilized lipids (probably mostly derived from the hypodermal blubber layer) as fuel to compensate for the energy intake deficit during the summer (Table 1). As the subcutaneous lipid layer provides sea lions with insulative capacity (Heath et al. 1977) in addition to being an energy reserve (Beck et al. 2000; Hamilton et al. 2004), it is not surprising that the thermoregulatory capacity of this group decreased. However, given summer water temperatures in the North Pacific (11°–17°C at the surface and 9°–11°C at 40-m depth; DFO 2007), it is questionable whether this decrease in thermoregulatory capacity would have significant consequences in the wild. Animals consuming pollock, on the other hand, lost a significantly lower amount of lipids and greater amount of lean mass to compensate for the energy intake deficit (Table 1). The lower reliance on lipids as an energy reserve allowed them to preserve a stable insulative capacity in cold water. However, this means that group P lost more lean tissues than group H, and it is known that catabolism of proteins from muscle mass can affect organ integrity and activity and foraging efficiencies (Vaz 2003). Consequently, the reliance on catabolism of protein reserves could ultimately have a more drastic and permanent effect on the fitness of sea lions. We therefore expected that these animals would have an increase in postexercise oxygen debt. However, the sea lions did not show any noticeable change during the restriction in the capacity of sea lions to recover after a forced exercise, measured either as an increase in their O_2 debt or time needed to recover. It is feasible that the few minutes of forced activity may not have been challenging enough to detect changes in fitness for animals that regularly spend hours (sometimes days) foraging at sea.

During the controlled refeeding phase, the individual variability was too large to estimate the allocation of energy toward either PE or ME. A closer inspection of the data showed that one animal in each diet group yielded results that were opposite to the others. This trend combined with the low statistical power associated with a small sample size prevented our drawing conclusions about the reallocation of energy. Nevertheless, calculations suggested that energy was more likely allocated to PE (at least for the first 2 wk into the controlled refeeding; Table 2) even though the animals did not regain weight during this period in summer (see Table 1) and thus probably did not allocate a significant amount of energy to growth in mass (StE /GrE, included in PE). Instead, newly available energy could have been allocated to growth in length (GrE) without clear impacts on our measured mass data (Jeanniard du Dot et al. 2008). Alternatively, the molting period in sea lions occurs

in late summer (August/September) and might have affected the energy budget of our animals during the summer controlled refeeding. Kumagai et al. (2006) hypothesized that an increase in resting metabolic rate (RMR) in August/September among seven Steller sea lions was attributable to the energy requirement for molting, and Williams et al. (2007) found a 1.3-fold increase in RMR during the molting periods in California sea lions (*Zalophus californianus*). Although increase in energy requirements during molting is not consensual in pinnipeds (Ashwell-Erickson et al. 1986; Rosen and Renouf 1998) and the lack of clear statistic evidence makes this interpretation only speculative, energy allocation to PE on refeeding without observed mass gain from the animals could be linked to molting and the energy necessary for growing new hair and epidermis.

Allocation to maintenance functions (at least activity and thermoregulation) did not change in the controlled refeeding phase compared with that in the restriction period despite the increased energy intake. Activity levels assessed from the DMR showed that group H still displayed a "hunger response" 2 wk into the controlled refeeding even though their energy intake was higher. This indicates that the increased intake still did not lead to satiation. Thermoregulation capacity did not return to the initial baseline levels during the refeeding period, suggesting that restoration of this function was not a priority in summer. Activity reached baseline levels only at the end of the month-long recovery period. The animals in group P however displayed no change in thermoregulation capacity compared with the restriction or the baseline periods, once again showing a lack of adjustment to new energetic conditions.

Energetic Priorities during Winter

Repercussions of the energy deficit on the sea lions' physiological functions appeared different during winter than during summer. The degree to which the animals compensated for the overall energy deficit by releasing energy stored in their body (tissue catabolism) was lower during winter than summer. Unlike in summer, a significant part of the energy deficit was also compensated for by a decrease in ME. Both experimental groups showed this energy-sparing decrease in maintenance energy, which seemed to start earlier during the restriction for group P than for group H (although was not greater over the entire restriction phase).

Converting from the body composition data, it is apparent that the animals in group P released a significantly lower amount of stored energy during winter than did animals in group H (Table 2), even though both groups were losing body mass at the same rate. This difference resulted from the tendency of group P to rely almost exclusively on catabolism of lean tissues and minimally on their lipid reserves to compensate for the energy deficit (Table 1), while animals in group H lost significantly more lipid mass. As protein is less energy dense than fat, identical mass losses translated into a lower quantity of energy released from the bodies of sea lions in group P than group H. The "traditional" physiological response of most animals to nutritional deprivation or fasting is to rely predominantly on lipid stores and only as a last resort on the lean tissue reserves (Castellini and Rea 1992), which was the response of group H but not of group P.

Group P did not enter the fast-adapted metabolism that conserves protein and catabolize lipid reserves when fed the pollock diet, despite evident mass loss, as was also seen in previous studies (Rosen and Trites 2005). In this study, however, sea lions were not fasted but were moderately restricted and still fed daily, with animals in group P receiving a full fish intake (in terms of biomass), unlike group H. This difference could explain why group H entered the fast-adapted metabolic response but group P did not (Jeanniard du Dot et al. 2008). It is interesting to note that the same sea lions exposed to a more acute energy restriction in an earlier study by Kumagai et al. (2006) showed opposite tendencies, with animals fed pollock losing more lipids than animals fed herring. Levels of daily energy intake and of satiation probably interact to explain the disparity with Kumagai's results. The fact that group P responded to energy restriction by losing as much mass as group H for a lower energy output may be maladaptive from a long-term bioenergetic perspective, especially when integrity of protein core tissues is a health priority.

Thermoregulation capacity in water (capacity to keep body temperature constant without having to increase metabolic rate) was a priority during the winter throughout the experiment for all the animals. Pinnipeds have anatomical and adaptive mechanisms that minimize the amount of energy required for seasonally dependent thermoregulation needs (Irving 1969; Blix and Steen 1979; Whittow 1987). During the colder winter season, the insulative layer of blubber was thicker for all sea lions (measured by ultrasound; data not shown), and both diet groups retained a greater proportion of lipid stores during mass loss compared with summer (Table 1). The anatomical and physiological adjustments of insulation to external conditions was also reflected by lower baseline metabolic rates in 2° and 8°C waters in winter, when these two water temperatures were more relevant to natural conditions, compared with summer (DFO 2007).

Since the energy allocated to thermoregulation was stable during the winter energy restriction, the observed decrease in ME for the two diet groups must have derived from a decrease in voluntary activity and/or BME. Basal metabolism was not assessed per se in our study, but SMR_A was. Since activity levels (monitored in the metabolic chamber) were consistent within animals throughout all phases of the experiment, the stable SMR_A suggests that changes in basal metabolism were not significant enough to affect the standard metabolism. Consequently, the decreases in maintenance energy allocation were probably mostly due to a decrease in activity levels rather than changes in BME. During winter, both experimental groups showed lower activity levels—as estimated from the DMR data—during the restriction phase. This decrease in voluntary activity during nutritional stress has been observed in other animals (van Dijk et al. 2002; Karakas et al. 2006). In our study, the depression in activity lasted longer for sea lions in group H than for those in group P, which indicates that the restricted

pollock diet did not trigger extended energy saving as did the herring diet, nor was it sustained for as long as needed (only for 2 wk of the 4-wk restriction). This may lead to deleterious effects occurring earlier for group P than for group H over the long term.

It is interesting that in our study, similar levels of decreased energy intake evoked three different strategies for sea lions eating different diets in different seasons. Group H reacted to the energy restriction in opposite ways in winter and summer. These sea lions—deprived in terms of both energy and biomass intake—displayed a foraging response in the summer and a metabolic depression in the winter. In contrast, the group P animals eating a high biomass of fish with a low-energy intake did not alter their activity levels in summer and altered them only slightly during winter. This suggests that diet quality, biomass intake, and season are determining factors underlying the metabolic response of sea lions during nutritional stress, and this must be taken into account when predicting the physiological effects of such events.

During the winter controlled refeeding, bioenergetic calculations indicated that the majority of the increased energy intake was allocated to PE, while the allocation to ME did not change from the lower levels displayed during the restriction. Unlike in summer, all of the animals regained mass when switched back to their baseline diet by greatly increasing their growth rate (relative to baseline), almost exclusively in the form of increased lipid mass (Table 1). Such a differential response in the gain of lipid versus lean mass has been observed in other animals (Xie et al. 2001). In our case, it probably represents the combined effect of maximizing energy stores in expectation of another period of restriction (Metcalfe and Monaghan 2001), a strategy to restore thermoregulation capacity as quickly as possible (ultimately allowing more energy to be spent on production functions; Guinet et al. 1998; Pitcher et al. 1998) and the natural physiological limit in rate of protein accretion.

Activity levels that were depressed during the restriction seemed to return to prerestriction levels during the controlled refeeding and the ad lib. phase for the animals fed pollock. They remained below baseline levels for those fed herring until the end of the trial. Synergistically, the delay in restoring energy devoted to activity and maintenance expenditures after a depression is known to help increase growth rates above their normal values after nutritional stress (Condit and Ortiz 1987; Farbridge et al. 1992; Yambayamba et al. 1996; Ali et al. 2003). More energy is available to be allocated to production since expenditures for maintenance energy are kept minimal. Consequently, the strategy of maintaining low activity levels during the controlled refeeding phase adopted by group H seemed to be more energetically efficient than the strategy invoked by group P, although this strategy may be difficult to invoke in the wild due to other concurrent demands.

The seasonal difference in capacity to display compensatory growth on refeeding reflects a difference in energetic priorities. In winter, most energy available during the controlled refeeding was allocated to StE, with a tendency to spare energy allocated to thermoregulation and activity. In summer however, compensatory growth in terms of mass (StE) was not a priority (mass did not recover on refeeding), while energy allocation to thermoregulation and activity remained high. There were clues in our study suggesting that sea lions may allocate energy preferentially to GrE in summer rather than storage (StE), from elevated levels of IGF-1 in this season (Richmond et al. 2006) and from greater increases in length measurements (Jeanniard du Dot et al. 2008). In summer, the sea lions needed a period of ad lib. food and hyperphagia to display compensatory growth. This implies that energy restriction might be more difficult to overcome in summer than winter (discussed more thoroughly in Jeanniard du Dot et al. 2008) and that sea lions may have to increase their foraging effort.

Relevance of Energetic Strategies

Energetic priorities chosen by the sea lions were dependent on the type of fish consumed in the diet during the restrictions and on the season during which the nutritional stress occurred. During summer, the animals fed exclusively herring compromised on thermoregulation capacity and allocated more energy to activity, even though it meant spending more energy (thereby increasing their energy deficit). In contrast, during winter this group spared energy by decreasing overall activity while maintaining thermoregulation capacity as much as possible given the unavoidable mass loss. Increasing foraging effort while nutritionally stressed (as seen in the summer) is a feasible strategy only if the probability of finding fish is high—otherwise it represents a waste of already scarce energy and ultimately results in a shorter survival time. Increasing foraging effort is probably a profitable behavioral adaptation in summer in a natural environment, based on natural prey cycles. Prey populations tend to be more abundant, accessible, and spatially predictable in summer (Sigler et al. 2004; Womble et al. 2005), and they often have higher energy content (Anthony et al. 2000; Kitts et al. 2004) than in winter. Prey being scarcer and less predictable in winter, the lower probability of finding fish would render it more profitable to reduce the energy expenditure spent on foraging in this season.

Group H's strategy to compromise thermoregulation capacity and to allocate energy to other functions probably reflected the fact that thermoregulatory needs are the lowest at this time of the year. Water temperature on the coast of British Columbia, the Gulf of Alaska, and the Aleutian Islands ranges from 11° to 17°C at the surface and from 9° to 11°C at 40-m depth in summer (DFO 2007). Temperatures tested during the thermoregulation trials were lower compared with those in natural conditions in the summer, which means that even if group H compromised their thermoregulation capacity at 8° and 2°C, they may not have to compromise at warmer (more relevant) water temperatures. In addition, the animals were physically unable to move much during the metabolic measurements and may not have been able to offset the thermoregulation needs by the heat released from activity as observed in the wild (Hind and Gurney 1997; Rosen et al. 2007). An increase in foraging resulting in an increase in activity levels as observed for group

H could compensate for decreases in thermoregulation capacity. In winter, however, sea temperatures in the North Pacific range from 4° to 10°C at the surface and from 3° to 8°C at 40-m depth (DFO 2007). Capacity to stay warm in water must be a priority at these temperatures. Poor heat management may force sea lions to increase the production of heat through metabolism and thus burn additional fuel. If lipids from the insulative blubber layer are mobilized, the animal may find itself in a deleterious cycle of increasing energy demands (Rosen et al. 2007).

Energetic strategies of sea lions fed herring during both seasons appeared to adjust to prevailing conditions in their natural environment. In contrast, the animals fed pollock did not alter their energetic allocation as much. Thermoregulation and activity levels stayed more or less stable regardless of the need to optimize these functions to a given season. Unlike the sea lions fed herring, animals fed pollock did not make clear adjustments to adequately respond to the energy deficits they encountered. This suggests that a decrease in biomass intake concomitant to a decrease in energy intake is needed to trigger relevant and adaptive responses to energy deficits. Contradictory signals related to a decrease in energy intake associated with a constant biomass intake (such as for group P during the restriction) seemed to offset the capacity of sea lions to adjust to nutritional stress, independent of the season. A large (but energetically inadequate) ration of low-energy fish such as pollock for sea lions in the wild may prevent their body from triggering physiological and behavioral mechanisms needed to adjust to low energy intake and ultimately decrease the fitness of animals in their natural environment. The "nonadjustments" observed for sea lions consuming inadequate amounts of pollock could thus result in detrimental outcomes if the nutritional stress was carried over longer periods of time than the 28-d time frame of our study.

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Literature Cited

Ali M., A. Nicieza, and R.J. Wootton. 2003. Compensatory growth in fishes: a response to growth depression. Fish Fish 4:147–190.

Anthony J.A., D.D. Roby, and K.R. Turco. 2000. Lipid content and energy density of forage fishes from the northern Gulf of Alaska. J Exp Mar Biol Ecol 248:53–78.

Arnould J.P.Y., I.L. Boyd, and J.R. Speakman. 1996. Measuring the body composition of antarctic fur seals (*Arctocephalus gazella*): validation of hydrogen isotope dilution. Physiol Zool 69:93–116.

Ashwell-Erickson S., F.H. Fay, R. Elsner, and D. Wartzok. 1986. Metabolic and hormonal correlates of molting and regeneration of pelage in Alaskan harbor and spotted seals (*Phoca vitulina* and *Phoca largha*). Can J Zool 64:1086–1094.

Beck C.A., D. Bowen, and S.I. Iverson. 2000. Seasonal changes in buoyancy and diving behaviour of adult grey seals. J Exp Biol 203:2323–2330.

Blix A.S. and J.B. Steen. 1979. Temperature regulation in newborn polar homeotherms. Physiol Rev 59:285–304.

Boyd I.L. 2002. Energetics: consequences for fitness. Pp. 247–277 in A.R. Hoelzel, ed. Marine Mammal Biology: An Evolutionary Approach. Blackwell Sciences, Oxford.

Calkins, D.G., E.F. Becker, and K.W. Pitcher. 1998. Reduced body size of female Steller sea lions from a declining population in the Gulf of Alaska. Mar Mamm Sci 14:232–244.

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

Condit R. and C.L. Ortiz. 1987. The physiological transition from fasting to feeding in weaned elephant seal pups. Mar Mamm Sci 3:207–219.

Costa D.P. and T.M. Williams. 1999. Marine mammals energetics. Pp. 176–217 in J.E. Reynolds and S.A. Rommel, eds. Biology of Marine Mammals. Smithsonian Institution, Washington, DC.

Cuyler L.C. and N.A. Øritsland. 1993. Metabolic strategies for winter survival by Svalbard reindeer. Can J Zool 71:1787–1792.

DFO (Department of Fisheries and Oceans, Ottawa). 2007. Contours of Average Temperature and Salinity in the Gulf of Alaska. http://www.pac.dfo-mpo.gc.ca/sci/osap/data/alaska/default_e.htm.

Farbridge K.J., P.A. Flett, and J.F. Leatherland. 1992. Temporal effects of restricted diet and compensatory increased dietary intake on thyroid function, plasma growth hormone levels and tissue lipid reserves of rainbow trout *Oncorhynchus mykiss.* Aquaculture 104:157–174.

Guinet C., J.P. Roux, M. Bonnet, and V. Mison. 1998. Effect of body size, body mass, and body condition on reproduction of female South African fur seals (*Arctocephalus pusillus*) in Namibia. Can J Zool 76:1418–1424.

Hamilton J.L., R.M. Dillaman, W.A. McLellan, and D.A. Pabst. 2004. Structural fiber reinforcement of keel blubber in harbor porpoise (*Phocoena phocoena*). J Morphol 261:105–117.

Heath M.E., S.M. McGinnis, and D. Alcorn. 1977. Comparative thermoregulation of suckling and weaned pups of the northern elephant seal, *Mirounga angustirostris.* Comp Biochem Physiol 57:203–206.

Hind A.T. and W.S.C. Gurney. 1997. The metabolic cost of swimming in marine homeotherms. J Exp Biol 200:531–542.

Hunter A.M.J. 2005. A Multiple Regression Model for Predicting the Energy Requirements of Marine Mammals. MS thesis. University of British Columbia, Vancouver.

Irving L. 1969. Temperature regulation in marine mammals. Pp. 147–174 in H.T. Andersen, ed. The Biology of Marine Mammals. Academic Press, New York.

Jeanniard du Dot T., D.A.S. Rosen, and A.W. Trites. 2008. Steller sea lions show diet-dependent changes in body composition during nutritional stress and recover more easily from mass loss in winter. J Exp Mar Biol Ecol 367:1–10.

Karakas A., E. Serin, and B. Gunduz. 2006. Food restriction affects locomotor activity in Mongolian gerbils (*Meriones unguiculatus*). Turk J Biol 30:23–28.

Keiver K.M., K. Ronald, and F.W.H. Beamish. 1984. Metabolizable energy requirements for maintenance and fecal and urinary losses of juvenile harp seals (*Phoca groenlandica*). Can J Zool 62:769–776.

Kitts D.D., M.D. Huynh, C. Hu, and A.W. Trites. 2004. Seasonal variation in nutrient composition of Alaskan walleye pollock. Can J Zool 82:1408–1415.

Kleiber M. 1975. The Fire of Life: An Introduction to Animal Energetics. Krieger, Huntington, NY.

Kumagai S., D.A.S. Rosen, and A.W. Trites. 2006. Body mass and composition responses to short-term low energy intake are seasonally dependent in Steller sea lions (*Eumetopias jubatus*). J Comp Physiol B 176:589–598.

Lavigne D.M., W. Barchard, S. Innes, and N.A. Øritsland. 1982. Pinniped bioenergetics. Pp. 191–235 in Mammals in the Seas: Small Cetaceans, Seals, Sirenians, and Sea Otters. Series 5. FAO Fisheries, Rome.

Lawson J.W., E.H. Miller, and E. Noseworthy. 1997. Variation in assimilation efficiency and digestive efficiency of captive harp seals (*Phoca groenlandica*) on different diets. Can J Zool 75:1285–1291.

Limberger D., F. Trillmich, H. Biebach, and R.D. Stevenson. 1986. Temperature regulation and microhabitat choice by free ranging Galapagos fur seal pups (*Arctocephalus galapagoensis*). Oecologia 69:53–59.

Lucas A. 1992. Bioénergétique des Animaux Aquatiques. Masson, Paris.

Markussen N.H., M. Ryg, and N.A. Oritsland. 1992. Metabolic rate and body composition of harbour seals, *Phoca vitulina*, during starvation and refeeding. Can J Zool 70:220–224.

McCarter R.J. and J.R. McGee. 1989. Transient reduction of metabolic rate by food restriction. Am J Phys 257:E175–E179.

Merrick R.L. and T.R. Loughlin. 1997. Foraging behavior of adult female and young-of-year Steller sea lions in Alaskan waters. Can J Zool 75:776–786.

Metcalfe N.B. and P. Monaghan. 2001. Compensation for a bad start: grow now, pay later? Trends Ecol Evol 16:254–260.

Mrosovsky N. and D.F. Sherry. 1980. Animal anorexias. Science 207:837–842.

Nash L.T. 1998. Vertical clingers and sleepers: seasonal influences on the activities and substrate use of *Lepilemur leucopus* at Beza Mahafaly Special Reserve, Madagascar. Folia Primatol 69:204–217.

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

Ohata C.A. and L.K. Miller. 1977. Some temperature responses of northern fur seal (*Callorhinus ursinus*) pups. J Mammal 58:438–440.

Øritsland N.A. 1990. Starvation survival and body composition in mammals with particular reference to *Homo sapiens*. Bull Math Biol 52:643–655.

Parsons J.L. 1977. Metabolic studies on ringed seals (*Phoca hispida*). MS thesis. University of Guelph.

Patterson B.R., L.K. Benjamin, and F. Messier. 1998. Prey switching and feeding habits of eastern coyotes in relation to snowshoe hare and white-tailed deer densities. Can J Zool 76:1885–1897.

Pinheiro J.C. and D.M. Bates. 2000. Mixed-Effects Models in S and S-PLUS. Springer, New York.

Pitcher K.W., D.G. Calkins, and G.W. Pendleton. 1998. Reproductive performance of female Steller sea lions: an energetics-based reproductive strategy? Can J Zool 76:2075–2083.

Prestrud P. 1991. Adaptations by the arctic fox (*Alopex lagopus*) to the polar winter. Arctic 44:132–138.

Pullar J.D. and A.J.F. Webster. 1977. Energy cost of fat and protein deposition in rat. Br J Nutr 37:355–363.

Rea L.D., D.A.S. Rosen, and A.W. Trites. 2007. Utilization of stored energy reserves during fasting varies by age and season in Steller sea lions. Can J Zool 85:190–200.

Reilly J.J. and M.A. Fedak. 1990. Measurement of the body composition of living gray seals by hydrogen isotope dilution. J Appl Physiol 69:885–891.

Richmond J.P., T. Jeanniard du Dot, D.A. Rosen, and S.A. Zinn. 2006. Effect of prey composition on the endocrine response to nutritional restriction and re-alimentation in Steller sea lions (*Eumetopias jubatus*). Pp. 136–141 in Proceedings of the 6th Biennial Symposium of the Comparative Nutrition Society, Keystone, Colorado.

Roberts S.B. and V.R. Young. 1988. Energy costs of fat and protein deposition in the human infant. Am J Clin Nutr 48: 951–955.

Rosen D.A.S. and D. Renouf. 1997. Seasonal changes in blubber distribution in Atlantic harbor seals: indications of thermodynamic considerations. Mar Mamm Sci 13:229–240.

———. 1998. Correlates of seasonal changes in metabolism in Atlantic harbour seals (*Phoca vitulina concolor*). Can J Zool 76:1520–1528.

Rosen D.A.S. and A.W. Trites. 1999. Metabolic effects of low-energy diet on Steller sea lions, *Eumetopias jubatus*. Physiol Biochem Zool 72:723–731.

———. 2000*a*. Digestive efficiency and dry-matter digestibility in Steller sea lions fed herring, pollock, squid, and salmon. Can J Zool 78:234–239.

———. 2000*b*. Pollock and the decline of Steller sea lions: testing the junk-food hypothesis. Can J Zool 78:1243–1250.

———. 2002. Changes in metabolism in response to fasting and food restriction in the Steller sea lion (*Eumetopias jubatus*). Comp Biochem Physiol B 132:389–399.

———. 2003. No evidence for bioenergetic interaction between digestion and thermoregulation in Steller sea lions *Eumetopias jubatus*. Physiol Biochem Zool 76:899–906.

———. 2005. Examining the potential for nutritional stress in young Steller sea lions: physiological effects of prey composition. J Comp Physiol B 175:265.

Rosen D.A.S., A.J. Winship, and L.A. Hoopes. 2007. Thermal and digestive constraints to foraging behavior in marine mammals. Philos Trans R Soc B 362:2158–2168.

Schmidt-Nielsen K. 1990. Animal physiology: adaptation and environment. 4th ed. Cambridge University Press, Cambridge.

———. 1997. Animal physiology: adaptation and environment. 5th ed. Cambridge University Press, Cambridge.

Sigler M.F., J.N. Womble, and J.J. Vollenweider. 2004. Availability to Steller sea lions (*Eumetopias jubatus*) of a seasonal prey resource: a prespawning aggregation of eulachon (*Thaleichthys pacificus*). Can J Fish Aquat Sci 61:1475–1484.

Soto K.H., A.W. Trites, and M. Arias-Schreiber. 2006. Changes in diet and maternal attendance of South American sea lions indicate changes in the marine environment and prey abundance. Mar Ecol Prog Ser 312:277–290.

Stini W.A. 1969. Nutritional stress and growth: sex difference in adaptive response. Am J Phys Anthropol 31:417–426.

Swain U. and D.G. Calkins. 1997. Foraging behaviour of juvenile Steller sea lions in the northeastern Gulf of Alaska: diving and foraging trip duration. Pp. 92–106 in Steller sea lion recovery investigations in Alaska, 1995–1996. Division of Wildlife Conservation, Alaska Department of Fish and Game, Anchorage.

Trillmich F. and K. Ono, eds. 1991. Pinnipeds and El Niño: Responses to Environmental Stress. Springer, New York.

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

Trites A.W. and B.T. Porter. 2002. Attendance patterns of Steller sea lions (*Eumetopias jubatus*) and their young during winter. J Zool Lond 256:547–556.

Trumble S.J., P.S. Barboza, and M.A. Castellini. 2003. Digestive constraints on an aquatic carnivore: effects of feeding frequency and prey composition on harbor seals. J Comp Physiol B 173:501–509.

van Dijk P.L.M., G. Staaks, and I. Hardewig. 2002. The effect of fasting and refeeding on temperature preference, activity and growth of roach, *Rutilus rutilus*. Oecologia 130:496–504.

Vaz M. 2003. Undernutrition. Pp. 341–348 in M.J. Gibney, I. Macdonald, and H.M. Roche, eds. Nutrition and Metabolism. Blackwell, Oxford.

Whittow G.C. 1987. Thermoregulatory adaptations in marine mammals: interacting effects of exercise and body mass. A review. Mar Mamm Sci 3:220–241.

Williams T.M., M. Rutishauser, B. Long, T. Fink, J. Gafney, H. Mostman-Liwanag, and D. Casper. 2007. Seasonal variability in otariid energetics: implications for the effects of predators on localized prey resources. Physiol Biochem Zool 80:433–443.

Winship A.J., A.W. Trites, and D.A.S. Rosen. 2002. A bioenergetic model for estimating the food requirements of Steller sea lions *Eumetopias jubatus* in Alaska, USA. Mar Ecol Prog Ser 229:291–312.

Womble J.N., M.F. Willson, M.F. Sigler, B.P. Kelly, and G.R. VanBlaricom. 2005. Distribution of Steller sea lions *Eumetopias jubatus* in relation to spring-spawning fish in SE Alaska. Mar Ecol Prog Ser 294:271–282.

Worthy G.A.J. and D.M. Lavigne. 1987. Mass loss, metabolic rate, and energy utilization by harp and gray seal pups during the postweaning fast. Physiol Zool 60:352–364.

Xie S., X. Zhu, Y. Cui, R.J. Wootton, W. Lei, and Y. Yang. 2001. Compensatory growth in the gibel carp following feed deprivation: temporal patterns in growth, nutrient deposition, feed intake and body composition. J Fish Biol 58:999–1009.

Yambayamba E.S., M.A. Price, and G.R. Foxcroft. 1996. Hormonal status, metabolic changes, and resting metabolic rate in beef heifers undergoing compensatory growth. J Anim Sci 74:57–69.