

**SEASONAL DIFFERENCES IN PHYSIOLOGY OF
CAPTIVE STELLER SEA LIONS (*EUMETOPIAS JUBATUS*)
IN RESPONSE TO SHORT-TERM LOW ENERGY INTAKE**

by

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Abstract

Steller sea lions (*Eumetopias jubatus*) were fed restricted iso-caloric amounts of Pacific herring (*Clupea pallasii*) or walleye pollock (*Theragra chalcogramma*) for 8-9 days, four times a year. At these levels, the sea lions lost an average of 10.1% of their initial body mass while on both experimental diets for up to nine days, but at a significantly higher rate in winter and at a lower rate in summer. Decreases in body fat mass and standard metabolic rates during the trials were similar throughout the seasons and for both diets. Metabolic depression was not always observed during the trials despite the constant loss of body mass. Changes in cortisol, triiodothyronine and blood urea nitrogen (BUN) were seasonally dependent. Over the course of the trials, serum levels of cortisol and BUN increased and total triiodothyronine decreased the most in winter. Serum cortisol levels correlated negatively with both body mass and body condition suggesting that cortisol may play an important role in body fat regulation in Steller sea lions. The mean ghrelin level in Steller sea lions correlated negatively with body mass, but ghrelin did not correlate with serum leptin. My findings support the hypothesis that restricted energy intake at different times of the year differentially affects Steller sea lions, and that diet type (herring or pollock) may have seasonally-specific effects on body mass and composition. Steller sea lions may be more severely impacted by reduced energy intake in winter than at other times of the year.

Changes in iron binding capacity were significantly greater in the herring-fed group than in the pollock-fed group, and a significantly greater decrease occurred in winter and spring compared to summer and fall. Iron saturation increased in the herring-fed group and decreased in the group fed pollock. These results suggested a potential anemia from a reduced diet of pollock in Steller sea lions. Serum iron, phosphorus, hematocrit and gamma glutamyltransferase showed consistent changes during food restriction, suggesting that these may serve as indicators of nutritional stress in Steller sea lions.

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Chapter I: Steller sea lion decline in Alaska and two major hypotheses: nutritional stress and low juvenile survival

1.1 Steller sea lions

Steller sea lions (*Eumetopias jubatus*) inhabit coastal regions of the north Pacific from northern Japan, through Russia and Alaska, to northern California (Ridgeway and Harrison 1981). The population of Steller sea lions in the Gulf of Alaska and Aleutian Islands has declined by over 80%, from 282,000 animals in 1975 to 76,000 in 1992. The most recent pup counts in 2002 (5,472 pups on rookeries and 178 pups on haul-out sites; Sease and Gudmundson 2002) suggest a total population of ~29,000 sea lions in the western Alaska region (Trites and Larkin 1996). They have been listed as “Endangered” in western Alaska and “Threatened” in eastern Alaska under the US Endangered Species Act.

Steller sea lions are the largest Otariidae, with mature males reaching 1,000 kg and females averaging about 300 kg. Like other pinnipeds, Steller sea lions have an annual life cycle. Pregnant females give birth on rookeries starting in mid-May, with peak production in mid-June (Pitcher and Calkins 1981). Mating occurs shortly after pups are born, but fetuses do not begin developing until late fall. Lactation is characterized by short (~1 day) feeding trips by the mothers, alternating with short stays on land during summer (Trites and Porter 2002; Milette and Trites 2003). Pups typically wean within a year in the wild (Pitcher and Calkins 1981; Calkins and Pitcher 1982), but captive animals are capable of feeding on solid food within 3 months of birth (based on animals raised at the Vancouver Aquarium).

Moulting starts anywhere from mid-June to November, depending on age class. Juveniles are the first group to moult, followed by adult females, pups and bulls (Daniel 2003). Sea lions in southeast Alaska spend more time feeding at sea during the winter and spring than summer, presumably either due to differences in prey quality and distribution (Trites and Porter 2002) or energy requirements (Winship and Trites 2003). Energetic models suggest that all of these factors contribute to the food requirements (kg d^{-1}), which have been calculated to be highest in winter and lowest in summer (Winship and Trites 2003).

1.2 The Nutritional Stress hypothesis

The Nutritional Stress hypothesis (Alverson 1992; Merrick et al. 1997) suggests that poor nutrition, caused by changes in quality, quantity, and/or availability of prey to Steller sea lions, results in negative physiological and/or behavioral states that affect sea lion numbers (see review by Trites and Donnelly 2003). Animals affected in this manner may suffer from decreased birth and growth rates, or an increased mortality rate, which ultimately affects population numbers. This hypothesis is partly supported by studies of sea lion diets, body size, and juvenile survival rates (Trites and Donnelly 2003).

Concurrent with changes in population numbers, Steller sea lions in western Alaska appeared to have switched from a diet of fatty fish to one dominated by less fatty fish, presumably due to a shift in fish abundance or distribution (Merrick et al. 1997). In the 1970s, the summer diet of Steller sea lions around Kodiak Island was dominated by small schooling fish (60.7%), followed by gadids (32.1%), and salmon (17.9%) (Merrick et al. 1997). However, in the 1990s, gadids were the most abundant (85.2%) followed by salmon (18.5%), small schooling fish (18.5%), flat fish (13.0%) and squid or octopus (11.1%) (Merrick et al. 1997). The summer diet of Steller sea lions in western Alaska was dominated during 1990s by hexagrammidae and gadidae while the winter diet was predominantly gadidae (Sinclair and Zeppelin 2002). Health consequences related to gadid-dominated diets have been speculated upon in several studies (e.g., Geraci 1975; Thompson et al. 1997; Rosen and Trites 2000). It has been proposed that diets high in gadid species are energetically inadequate for Steller sea lions and may have contributed to the observed population decline.

Several researchers have also noted a statistical relationship between diet diversity and rate of local population decline (Merrick et al. 1997; Waite and Burkanov 2003; Winship and Trites 2003). Populations with the least diverse diet experienced the greatest rates of decline. The sea lions from areas with the greatest population declines also had the greatest calculated food intake requirements (i.e., lowest average energy density diet — Winship and Trites 2003).

Short-term changes in pinniped body size are usually associated with changes

in their hypodermal fat layer (i.e., blubber layer) because lipids represent the primary source of energy for sea lions. Dietary fat deposited into the blubber layer becomes a stored form of energy and also aids in insulation and buoyancy. Feeding on fewer fish or fish with a lower fat content may result in energy deficits and decreased fat deposits in sea lions. As a consequence, animals would have lower energy reserves to allocate to their activities. Longer-term energy deficits are associated with decreased body size when energy intake is insufficient to maintain core growth, particularly in younger animals. These demands for somatic growth may also be seasonally dependant.

Body size of Steller sea lions decreased in the 1980s compared to the 1970s (Calkins et al. 1998). For example, the size of the axillary girth of 1 year-old Steller sea lions was reduced by 10.4% and its mass by 26.9%, while those of a 14 year-old decreased by 1.7% and 3.0% respectively (Calkins et al. 1998). Although Steller sea lions in the 1980s had a lower body mass index (mass/standard length) compared to the 1970s, their blubber index (blubber thickness/standard length) did not differ (Pitcher et al. 1998). This suggests that changes in body fat reserves in Steller sea lions were insignificant, and that changes in lean mass may have had greater impacts on the population while it declined in the 1980s.

Poor nutrition during pregnancy typically results in the loss of embryos or fetuses, or restricts the rate of offspring growth. Pitcher et al. (1998) showed that nearly all sexually mature lactating females in the 1970s and 1980s were pregnant during early gestation. However, pregnancy rates had declined by nearly half by late gestation. Feeding experiments with laboratory rats demonstrated that the mean fetus weight was significantly lower in those that ate pollock (*ad lib.*) compared to those fed herring (*ad lib.*; Donnelly 2001). Similar data for Steller sea lion fetal or birth weights are not available for comparison (Trites and Donnelly 2003). However, a mathematical model has shown that low fecundity during the 1980s and 1990s may have contributed to the population decline of Steller sea lions (Holmes and York 2003).

Lower energy intake at times when food intake requirements are high — such as from March to May (Winship et al. 2002) — may also have affected juvenile

survival or growth rates. It has been suggested that low survival rates (10-20%) of the juvenile population (York 1994; Pitcher et al. 1998; Holmes and York 2003) might have been the main source of the population decline. Adequate food intake is critical for young animals because they preferentially allocate their energy to growth and the development of fundamental body structures. Nutritionally stressed juvenile sea lions would have smaller body sizes, lower fat deposition and/or less functional immune systems, and fewer chances to survive compared to older or better nourished individuals.

1.3 Adjusting to changes in energy intake

Animals possess a number of subtle means to limit the effects of energy limitation, such as changing their metabolism and/or activity levels (Øritsland 1990). In previous studies, captive Steller sea lions decreased their energy expenditure when switched to a lower energy prey diet by reducing metabolic rates (Rosen and Trites 1999; Rosen and Trites 2000). However, it is not known if Steller sea lions respond similarly to low-energy food intake at different times of the year given that sea lions likely have large natural seasonal fluctuations in their energy budgets throughout the year. Rea et al. (1999) showed that experimentally fasted juvenile sea lions lost body mass at a higher rate during the non-breeding than during the breeding season.

Animals regulate their energy budgets through a series of hormones. Among the primary hormones of interest in energy regulation are cortisol, leptin and thyroid hormones (triiodothyronine and thyroxine). All four hormones are known to play important roles in the regulation of energy metabolism, and levels are known to fluctuate seasonally (Nilssen et al. 1984; Young 1984; Ashwell-Erickson et al. 1986; Gardiner and Hall 1997; Campbell and MacArthur 1998; Fitzgerald and McManus 2000; Horton et al. 2000; Mann et al. 2000; Nieminen et al. 2000; Pulawa and Florant 2000; Ashutosh Dhanda and Kundu 2001; Huber et al. 2003; Oki and Atkinson 2004). Cortisol and thyroid hormones increase hepatic gluconeogenesis from non-carbohydrate sources (Aron et al. 2001; Greenspan 2001). Increased glucocorticoid levels are routinely associated with nutritional stress (Kitaysky et al. 1999; Kitaysky et al. 2001; Ortiz et al. 2001a, 2001b, 2003a).

Leptin is synthesized and secreted from adipose cells, and is known to increase lipid metabolism (Reidy and Webber 2000). Its function is to regulate food intake and energy balance, as demonstrated in humans and lab rodents (Horton et al. 2000; Wauters et al. 2000; Caprio et al. 2001). However, studies of its function in pinnipeds (two species of otariids and one species of phocid; Rea and Nagy 2000; Ortiz et al., 2001a, 2001b, Arnould et al. 2002) failed to show a positive relationship between body condition and leptin concentration, or a decreased level over the course of fasting. These studies suggest that leptin in pinnipeds may not play a similar role in energy regulation as shown in lab rodents and human.

Another hormone of interest is ghrelin, which is a growth-hormone peptide released from the stomach, and an antagonistic hormone to leptin (Nakazato et al. 2001). Increased levels are known to increase carbohydrate utilization and decrease fat utilization and energy expenditures (Asakawa et al. 2001; Wren et al. 2001). To date, ghrelin in pinnipeds has only been measured in northern elephant seal pups (Ortiz et al. 2003b).

Animals are assumed to defend/maintain an appropriate body mass (i.e., set-point), which varies depending on circumstances such as age or season. Body mass is thought to be maintained within this set-point through changes in food intake or energy expenditure (Adam and Mercer 2001). Changes in seasonal body condition have been speculated to occur due to observed metabolic changes triggered by hormonal changes. Endogenous, circannual metabolic cycles are directed by temperature/day-length changes that serve to control changes in mass and food consumption (Armitage and Shulenberger 1972; Ward and Armitage 1981).

Seasonal changes in body mass, body condition or metabolic rate of Steller sea lions should be related to the above mentioned hormones. Sea lions should also defend body mass set-points in response to reduced food intake. Maintaining an appropriate body mass is critical for animals to survive, particularly at a young age.

1.4 Study goal

A basic understanding of the factors affecting energy allocation (storage, growth, etc.) is necessary to determine the potential effects of changes in prey type or

amount (energy intake) on sea lion health. My study focused primarily on the manner in which unpredicted, experimental energy restriction affects body mass and body condition of Steller sea lions at different times of the year (i.e., food restrictions other than natural fasting in a sea lion's life cycle). Given the seasonal changes in the energy budgets of Steller sea lions, the effects of energy restriction should differ with seasonal energy requirements. Additionally, given the concern over observed differences in prey composition among populations of wild Steller sea lions, I also wanted to investigate whether prey quality has an additional effect on sea lion health during periods of food restriction.

Blood chemistry usually reflects the health and nutritional status of animals and has been the principal means of monitoring the well-being of marine mammals (e.g., Geraci et al. 1979; Worthy and Lavigne 1982; Rea et al. 1998; Rea et al. 2000). Body mass and body condition (% body fat) can also be used as health status indicators, but generally reflect longer term (weekly to monthly) nutritional status. Changes in nutritional status appear at a faster rate in blood and urine chemical parameters (Milner et al. 2003). Thus, blood urea nitrogen, glucose, and iron-related parameters (serum iron, iron binding capacity, iron percent saturation) should also be measured to evaluate their response to changes in diet.

My goal was to study seasonal relationships between low energy intake, food quality, body mass, body condition (energy reserves), metabolic rate, and associated hormones. For this purpose I conducted a feeding experiment with captive female Steller sea lions at the Vancouver Aquarium Marine Science Centre, Vancouver, British Columbia. Steller sea lions were fed iso-caloric, low energy (about 20% of maintenance level) diets of either herring (high-lipid) or pollock (low-lipid) for short-term durations (9 days), four times over the course of a year. My goal was to provide information on the seasonal effects of short-term diet changes on the health and physiological status of Steller sea lions by documenting differential responses to the feeding experiment throughout the year. My hypothesis was that Steller sea lions respond differently to short-term low energy intake of different types of diet at different times of the year.

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Chapter II: Body mass and composition responses to short-term low energy intake are seasonally dependent in Steller sea lions (*Eumetopias jubatus*)

2.0 Summary

Steller sea lions (*Eumetopias jubatus*) were fed restricted iso-caloric amounts of Pacific herring (*Clupea pallasii*) or walleye pollock (*Theragra chalcogramma*) for 8-9 days, four times a year. At these levels, the sea lions lost an average of 10.1% of their initial body mass while on either experimental diet, but at a significantly higher rate in winter ($1.6 \pm 0.14 \text{ kg d}^{-1}$), and at a lower rate in summer ($1.2 \pm 0.32 \text{ kg d}^{-1}$). Decreases in body fat mass and standard metabolic rates during the trials were similar throughout the seasons and for both diet types. The majority of the body mass that was lost when eating pollock derived from decreases in lipid mass, while a greater proportion of lean tissue was lost while eating herring. However, animals fed herring in the summer preferentially used body fat reserves compared to the pollock-fed animals. Metabolic depression was not always observed during the trials despite the constant loss of body mass. This supports the hypothesis that restricted energy intake at different times of the year differentially affects Steller sea lions, and that diet type (herring or pollock) may have seasonally-specific effects on body mass and composition.

2.1 Introduction

A possible change in the abundance or type of available prey may underlie the decline of Steller sea lions (*Eumetopias jubatus*) in western Alaska (Alverson 1992; Merrick et al. 1997; Rosen and Trites 2000b; Trites and Donnelly 2003). As the sea lion population declined in the 1980s, females (pregnant and non-pregnant) exhibited decreased body size (Calkins et al. 1998), while estimated birth and juvenile and adult survival rates appeared to have been negatively impacted (Holmes and York 2003). Such changes could have been a direct result of reductions in the quality, quantity, or availability of prey — commonly known as the 'nutritional stress' hypothesis (Alverson 1992; Merrick et al. 1997; Trites and Donnelly 2003).

Health consequences related to gadid-dominated diets have been speculated upon in a number of studies (e.g., Geraci 1975; Thompson et al. 1997; Rosen and

Trites 2000b). Captive feeding experiments have revealed that Steller sea lions lose significant body mass when switched from a high-fat to a low-fat diet (squid and walleye pollock), but partially compensate by decreasing their resting metabolic rates (i.e., metabolic depression; Rosen and Trites 1999; 2000b). It has therefore been proposed that diets high in gadid and other low-energy species are nutritionally inadequate for Steller sea lions, and may contribute to the observed population decline (Trites and Donnelly 2003).

The potential effects of periods of inadequate nutrition on sea lion populations are likely further complicated by the seasonal nature of their energy budgets. In other words, the potential impact of nutritional stress may be seasonally dependent. Low juvenile survival rates (York 1994; Pitcher et al. 1998; Holmes and York 2003) might be a direct result of low energy intake at times of year when food intake requirements for immature ($< \text{age } 3$) female Steller sea lions in Southeast Alaska are high, such as from March to May (Winship et al. 2002). Adequate food intake is crucial to young animals, who allocate the bulk of their energy towards growth and development. Nutritionally-stressed sea lions may have a smaller body size, lower fat deposition and/or compromised immune systems and as a result may not survive severe (abiotic or biotic) environmental conditions. Such nutritional effects might be magnified during times of the year when required energy expenditures are greatest.

Food consumption by wild Steller sea lions likely fluctuates seasonally in response to changes in energy requirements (Winship et al. 2002) associated with such activities as breeding from late May to early July (Pitcher and Calkins 1981), periods of growth (core tissues and energy reserves) and moulting from mid-June to early November (Daniel 2003). While moulting, food intake by pinnipeds generally decreases (Lager et al. 1994), although it is unclear whether this is true for otariids. Sea lions spend proportionally more time feeding at sea during winter and spring compared to summer (Trites and Porter 2002). Foraging time for lactating females is also longer in winter than in summer, suggesting a greater effort is required to obtain food in winter due to dispersed fish distribution and/or increased energy needs (Trites and Porter 2002).

Assuming that Steller sea lions have natural seasonal fluctuations in their food intake and energy budgets (allocation of energy), they likely also display seasonal changes in their body condition and metabolic rates. However, no such information is available for Steller sea lions. Seasonal changes in energy budgets may include seasonal changes in body mass due to changes in energy expenditures (including basal metabolism), independent of changes in food/energy intake. Experiments with iso-caloric diets comprised of high- or low-fat prey demonstrated that changes in body fat of juvenile Steller sea lions was affected more by season and gender than by the amount of lipid consumed by the animals (Rosen and Trites 2001). Experimentally fasted juvenile Steller sea lions lost more body mass during the non-breeding season than during the breeding season (Rea et al. 1999). Yet it remains unclear how animals that undergo natural seasonal changes in their energy budget respond to unpredicted decreases in energy intake at different times of the year.

The role of variation in nutrition on health has important implications for Steller sea lion conservation. Measures of health that might be used to assess the status of individual animals can be confounded by seasonal differences in physiology and behaviour. For example, absolute or relative body condition may undergo natural changes that do not reflect changes in animal health *per se*. Similarly, animals may differentially defend their energy reserves from the effects of decreased energy intake at different times of the year. Thus, controlled feeding experiments are required to ensure that physiological data obtained from wild animals are properly interpreted in light of potentially critical periods of the year, when decreased energy intake might disproportionately affect sea lion health.

A primary hypothesis of my study was that captive sea lions would defend seasonally-dependent levels of body mass and condition (% total body fat). The following feeding study on young captive Steller sea lions was designed to investigate the effect of season on the effects of reduced energy intake on sea lion body mass, body condition and metabolic rate. The results of these experiments have significant bearing on understanding the importance of seasonal changes in prey availability and quality on Steller sea lion physiology.

2.2 Methods

2.2.1 Experimental design

All procedures and protocols involving animals were conducted under the authority of the University of British Columbia Animal Care Permit No. A98-0095.

Seven female Steller sea lions (SSLs) — five juveniles, 2.5-3 years old; and two sub-adults, 5.5-6 years old — participated in the study. The animals were held in an outdoor facility at the Vancouver Aquarium Marine Science Centre and had previously participated in various research projects since captured as pups. All of the study animals typically ate a daily ration of Pacific herring (*Clupea pallasii*), equivalent to approximately 5 - 6% of their body mass supplemented with vitamin tablets (1 tablet per 3 kg food; 5M26 Vita-zu tablets, Purina Test Diets, Richmond, IN). Food intake (kg d^{-1}) and body mass ($\pm 0.1 \text{ kg}$) were recorded daily. Proximate nutrient composition analyses of representative fish samples were conducted by Norwest Labs (soxhlet hexane extraction for lipid and total nitrogen estimates $\times 6.25$ for protein; Surrey, British Columbia). Gross energy content was calculated from composition analyses by appropriate conversions of lipid (39.3 kJ g^{-1}) and protein (18.0 kJ g^{-1}) content (Schmidt-Nielsen 1997).

The sea lions were divided into two experimental groups: four animals (one sub-adult and three juveniles) and three animals (one sub-adult and two juveniles). The diet of each group was alternated between Pacific herring and walleye pollock (*Theragra chalcogramma*), except for one juvenile from the first group who refused the pollock diet and was therefore fed herring exclusively. In other words, one group of animals consumed the same diet during the winter and summer season and a different group received this diet during spring and fall. When consuming herring (mean wet weight $88.9 \pm 5.4 \text{ g}$; fork length $19.5 \pm 0.3 \text{ cm}$), the sea lions were fed amounts equivalent to 1% of their initial (Day 1) body mass throughout each trial. Pollock consumption was set at 1.6-2.6% of their initial body mass (mean wet weight $29.9 \pm 6.6 \text{ g}$; fork length $15.0 \pm 1.1 \text{ cm}$). These diets were calculated to be energetically equivalent (i.e., iso-caloric; with 1% of an animal's body mass of herring equal to 1.6% pollock in the winter trials and 2.6% during the rest of the year). The sea lions were maintained on each diet for up to 9 days per experimental

trial. Previous studies (Rosen and Trites 2002) indicated the animals would lose approximately 2.3 kg d^{-1} for 9-14 days if fasting. Therefore, 9-day period was therefore chosen based on the expectation that the sea lions would lose 10-15% of their body mass under a restricted energy intake regime. Self-imposed Animal Care Protocols limited body mass loss to 15%, and consequently the trial for one animal had to end after 8 days in the summer. Two additional animals in the summer (herring-fed animals) and two in the fall (herring-fed animals) also had their trials concluded after 8 days due to logistical difficulties (staff availability, etc.), although the animals had not lost 15% of their body mass.

To examine seasonal variation in response to under-nutrition, the experimental protocol was repeated four times during the year: winter (November to December 2002), spring (February to March 2003), summer (May to June 2003), and fall (August to September 2003). Diet types were alternated for each group for each season (i.e., if an animal had been fed herring in the winter trial, she was fed pollock during the subsequent spring trial).

2.2.2 Data collection

Hydrogen isotope dilution (Reilly and Fedak 1990) was used to determine body fat content in the morning of the first and last (9th or 10th) day of each feeding trial. The dilution procedure was conducted before the first feed of the day (i.e., >16 hours after the last meal), and gas anesthesia (isoflurane) was used under veterinarian supervision to minimize stress during blood draws. A baseline serum sample (1ml blood) was obtained before an appropriate dose ($0.10 - 0.15 \text{ g kg}^{-1}$ body mass) of heavy water (D_2O) was administered intramuscularly. After a (previously validated; D.Rosen unpublished data) 2.0 to 2.5 hour equilibration period, a second serum sample was collected. Sera were separated using standard techniques (including running a centrifuge at 3500 RPM for 5 minutes) and stored at -70°C . Sera and dose samples were analyzed by Metabolic Solutions (Nashua, NH) for isotope concentrations. Total body water was converted to lean tissue mass using formulae modified from Reilly and Fedak (1990) for grey seals. Although a conversion formula specific to Otariids does not exist, use of the Reilly and Fedak

(1990) equation will yield internally consistent results that are comparable to those obtained in other studies. Lipid mass was calculated as the remainder of lean mass from total body mass. Body composition was also calculated as percentage total body fat (where: $TBF\% = \text{lipid mass} / \text{total mass} \times 100$).

Standard metabolic rate (SMR) was measured by open circuit respirometry. Measurements were obtained 1 or 2 days prior to the start of each trial, and towards the end of the trial (7th or 8th day). The animals entered and remained calm in a sealed opaque dry metabolic chamber (~1,050 L), or were placed inside a transport cage, which was then wheeled into a metabolic tent (two animals in the summer and two animals in the fall). Measurements were made under standard metabolic rate conditions for non-mature animals - postabsorptive, quiescent but awake, non-pregnant, and within their assumed thermal neutral zone (Kleiber 1975). Air was drawn at a constant rate ($150\text{--}200 \text{ L min}^{-1}$) sufficient to prevent extreme change in gas concentrations ($O_2 > 19.0\%$, $CO_2 < 1.9\%$). Air circulation was ensured by a small fan in the chamber and tent. Sea lion behaviour was monitored via a video camera.

Oxygen and carbon dioxide concentrations within a desiccated subsample of expired air were determined by a S-3A/I Solid Oxide (stabilized zirconia) Cell Analyzer (Ametek Inc., Pittsburgh, PA) and an AR-60 Infrared Gas Analyzer (Anarad Inc., Santa Barbara, CA), respectively for winter and spring trials. Expired airflow temperature was monitored by a La Crosse Technology Wireless Thermometer, and barometric pressure, humidity and environmental temperature were monitored by a Temperature Station WS-7014U (Springfield Instrument Canada, Mississauga, Ontario). These measures were used to correct airflow to STPD (Standard Temperature and Pressure, Dry). A different system was used in the summer and fall trials, consisting of a FC-1B Oxygen Analyzer, a CA-1B Carbon Dioxide Analyzer, and a 500H Flow Generator & Controller (all Sable Systems Henderson, NV). The expired air current was continuously sub-sampled, and a Sable Data Acquisition System (Sable Systems, Salt Lake City, UT) took an average concentration from 100 sub-samples every second.

The sea lions were trained to remain calm in the chamber for 1 to 2 hours

after a minimum 15-minute acclimation period. Oxygen consumption was converted to energy utilization using the equation: 1 L O₂=20.0 kJ.

For comparative purposes we chose to calculate mass-corrected standard metabolic rate as $SMR_c = SMR M^{-0.67}$ (Rosen and Trites 2002). This differs from Kleiber's (1975) inter-specific predictive equation for adult mammals where: $SMR = 70M^{0.75}$.

The dates and patterns of new hair growth during the moult were recorded for each Steller sea lion as a routine weekly observation by research staff. The mean duration of moulting was calculated from the first date of signs of new hair and the date of termination of new hair growth (Daniel 2003).

2.2.3 Data analyses

All results were calculated as mean \pm standard deviation (SD). Seasonal variations in pre-experimental (initial) values were determined using one-way repeated measure ANOVAs (SAS, version 8.2, SAS Institute, Cary, NC). Two-way repeated measures ANOVA was used to evaluate the effect of changes from the initial values in diet and season (SAS). Degrees of freedom ($df = n-k-1$) were calculated on the data using animal \times diet ($k=13$) as the primary parameters, given that an individual animal did not consume both diets within each season and one animal only consumed herring. For example, df (season \times diet) = 23 observations – 13 parameters - 1 = 14. We selected the most parsimonious model and calculated post hoc tests of specific differences (SAS). The mean percent changes from the initial values were compared by post hoc tests of specific differences (SAS). Care was taken into the model to minimize the effect of alternated diet treatment in each group. Correlations were determined using multiple linear regressions (Splus 6.1, Insightful Inc., Seattle, WA). Differences were considered significant at $P < 0.05$.

2.3 Results

2.3.1 Food intake

Steller sea lions consumed an average of 1.3 ± 0.25 kg d⁻¹ (mean \pm SD) of herring (1% of initial body mass) and 3.1 ± 0.85 kg d⁻¹ of pollock (1.6-2.6% of

initial body mass) throughout the trial. Their daily gross energy intakes (GEI) were $10.4 \pm 2.0 \text{ MJ d}^{-1}$ on the reduced herring diet and $9.4 \pm 2.5 \text{ MJ d}^{-1}$ on the reduced pollock diet, which were not statistically different ($t_{2,26}=1.18$, $P=0.25$). Protein contents were similar between the herring ($16.3 \pm 0.02 \%$ wet weight) and pollock ($14.7 \pm 0.77\%$), but lipid content was higher in the herring ($13.3 \pm 0.09 \%$) than in the pollock ($0.95 \pm 0.37 \%$) diet. Although the sea lions consumed equal calories from the herring and pollock diets, differences of composition in food intake resulted in the sea lions ingesting 2.3 times more protein from the pollock diet but only 20% of the fat compared to when they were eating herring.

2.3.2 Body mass and condition

The pre-experimental (initial) body masses of all seven Steller sea lions were fairly consistent between each trial ($F_{3,18}=1.72$, $P=0.19$). Average initial body mass increased slightly from winter ($126.3 \pm 29.9 \text{ kg}$) to spring ($131.6 \pm 20.3 \text{ kg}$), decreased for the summer trial ($128.5 \pm 22.7 \text{ kg}$) and increased again in the fall ($134.4 \pm 29.9 \text{ kg}$; Fig. 2.1).

There were significant differences in the initial percent body fat (as % total body mass) between seasons ($F_{3,18}=9.01$, $P<0.001$). The percentage of body fat increased from winter ($14.0 \pm 2.3\%$) to spring ($19.6 \pm 2.0\%$), decreased from spring to summer ($13.1 \pm 4.4\%$) and stayed constant from summer to fall ($12.6 \pm 2.6\%$; Fig. 2.1). There were significant positive relationships between body mass and total body fat for each pre- (initial $r^2=0.46$, $n=28$) and post- (final; $r^2=0.46$, $n=28$) experimental values (both when calculated as an absolute amount, in kg, Fig. 2.2; or as a percent of total body mass, % TBF, data not shown).

The sea lions lost an average of $10.1 \pm 2.5 \%$ of their initial body mass each season during the experimental trials (Fig.2.3). There were no significant differences associated with the diet type (reduced herring diet $10.7 \pm 2.9\%$ of initial body mass loss; reduced pollock diet $10.1 \pm 2.5 \%$; $F_{1,5}=0.11$, $P=0.75$). However, loss of body mass was dependent on season ($F_{3,14}=5.26$, $P=0.012$), with the greatest loss occurring during the winter trial ($13.0 \pm 2.7\%$ of initial; both diet groups pooled) followed by the fall ($9.9 \pm 2.7\%$, vs. winter, $F_{1,18}=7.15$, $P=0.016$), spring ($9.9 \pm 1.3\%$,

vs. winter, $F_{1,18}=7.43$, $P=0.014$), and summer ($9.0 \pm 2.6\%$, vs. winter, $F_{1,18}=12.57$, $P=0.002$). While daily loss of body mass (which accounts for differences in trial lengths) was also seasonally dependent ($F_{3,14}=4.26$, $P=0.025$; winter, 1.6 ± 0.14 kg d^{-1} ; spring, 1.3 ± 0.22 kg d^{-1} ; summer, 1.2 ± 0.32 kg d^{-1} ; and fall, 1.4 ± 0.33 kg d^{-1}), there was a significant interaction between season and diet ($F_{3,14}=5.07$, $P=0.014$; Table 2.1). Significant effects of diet were found in summer and fall, whereby the pollock-fed group lost body mass at a higher rate (1.4 ± 0.35 kg d^{-1}) than the herring-fed group (0.97 ± 0.18 kg d^{-1}) in the summer trial, while the rate was higher in the herring-fed group (1.6 ± 0.17 kg d^{-1}) than the pollock-fed group (1.0 ± 0.19 kg d^{-1}) in the fall trial (Table 2.1).

The sea lions lost $34.3 \pm 25.9\%$ of their initial total body fat while eating herring, and $37.3 \pm 15.0\%$ while eating pollock. There were no significant differences due to seasons ($F_{3,14}=0.47$, $P=0.71$) or diet type ($F_{1,5}=0.08$, $P=0.78$; Fig. 2.3), which is at least partially due to the high variability.

On average, 54% of the total body mass loss derived from decreases in body fat ($53.6 \pm 49.4\%$ on the reduced herring diet; and $53.7 \pm 24.7\%$ on the reduced pollock diet; $F_{1,5}=0$, $P=0.98$). Pooling the data from both diet groups showed that changes in lipid mass comprised the smallest portion of total body mass lost in winter ($39.3 \pm 16.8\%$) and the greatest in summer ($73.2 \pm 67.2\%$), although there was no significant overall seasonal effect ($F_{3,14}=1.07$, $P=0.39$; Table 2.2). However, there was a significant interaction between the effects of season and diet on the percent of total body mass loss due to changes in body fat ($F_{3,14}=4.71$, $P=0.017$). In most trials the pollock-fed group lost a greater portion of their mass from body fat compared to the herring-fed group across each season. The only exception was the herring-fed group in summer that lost body mass exclusively from body fat ($106.8 \pm 74.0\%$), while only $28.5 \pm 8.5\%$ of the body mass loss was derived from lipid in the pollock-fed group ($F_{1,14}=3.06$, $P=0.008$; Table 2.2). Within the herring-fed group, the amount of total body mass loss attributable to fat loss also differed between summer ($106.8 \pm 74.0\%$) and the other seasons: winter ($37.7 \pm 22.6\%$; $F_{1,14}=2.89$, $P=0.012$), spring ($45.7 \pm 26.4\%$; $F_{1,14}=2.58$, $P=0.022$) and fall ($24.1 \pm 14.4\%$; $F_{1,14}=3.48$, $P=0.0037$).

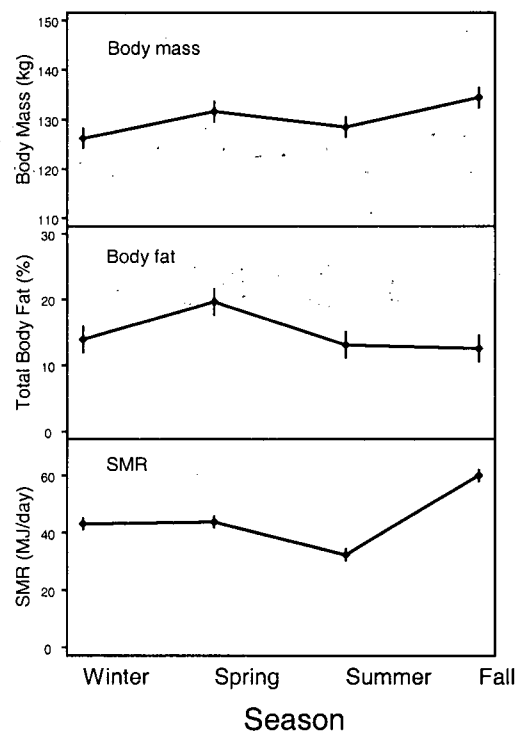


Fig. 2.1 Seasonal variation in initial body mass ($P>0.05$); total body fat (%; $P<0.001$); and standard metabolic rate (SMR; $P<0.001$). Data are shown as mean \pm SE; $n=7$ for each season.

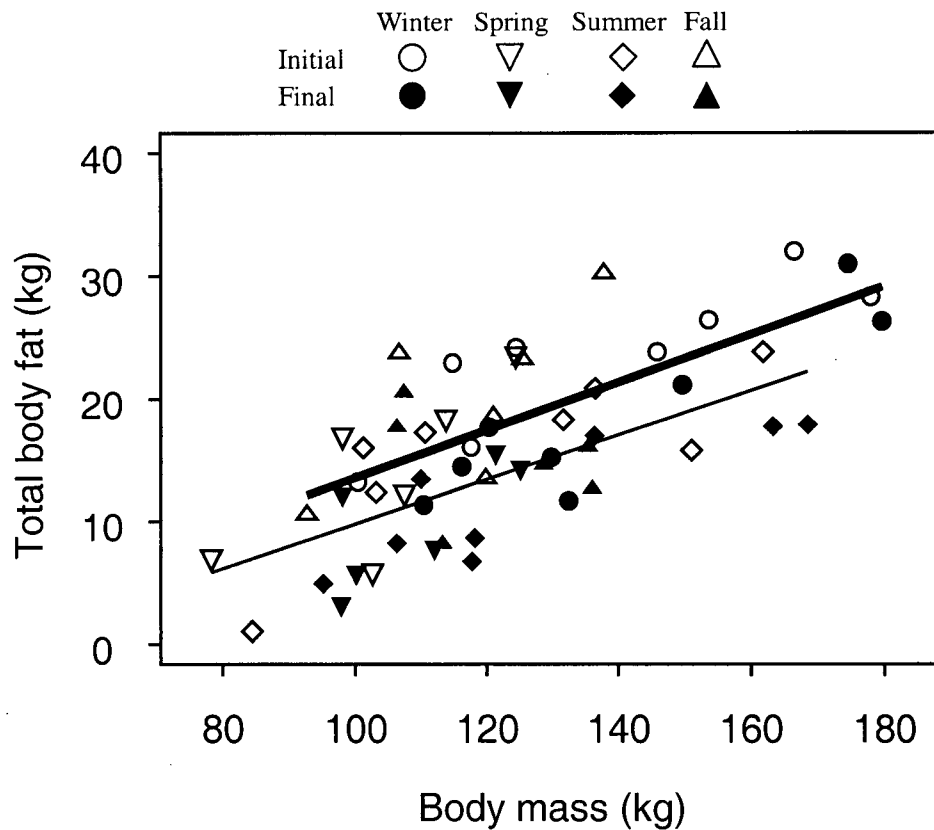


Fig. 2.2 The relationship between body mass (kg) and total body fat mass (kg). Open symbols indicate pre-experimental (initial) values and closed symbols indicate post-experimental (final) values. There were significant linear relationships within data for the initial values ($r^2=0.46$; thick line, $n=28$), and final values ($r^2=0.46$, thin line, $n=28$).

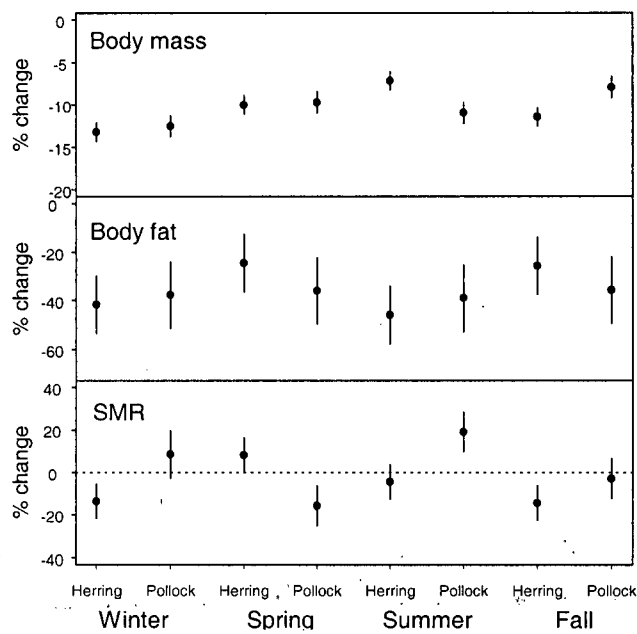


Fig. 2.3 Mean percent changes (\pm SE) in body mass, total body fat mass, and standard metabolic rate (SMR) for each diet type within each season. Sample sizes were $n=4$ for the herring-fed groups and $n=3$ for the pollock-fed groups for each season, except for the SMR for the pollock-fed group in winter ($n=2$). There was a significant effect of season on mean percent changes of body mass ($P<0.05$). There were no significant interactions between season and diet for any of the variables.

Table 2.1 Mean daily rate of body mass (BM) loss, total body fat (TBF) loss and lean mass (LM) loss.

		Winter		Spring		Summer		Fall	
		Herring	Pollock	Herring	Pollock	Herring	Pollock	Herring	Pollock
BM	Mean	1.6 ^p	1.6 ^p	1.3 ^{pq}	1.3 ^{pq}	0.97 ^q	1.4 ^{pr}	1.6 ^p	1.0 ^{qr}
kg day ⁻¹	SD	0.13	0.22	0.11	0.37	0.18	0.35	0.17	0.19
TBF	Mean	0.60 ^x	0.66 ^x	0.61 ^x	1.0 ^x	0.97 ^x	0.42 ^x	0.38 ^x	0.69 ^x
kg day ⁻¹	SD	0.41	0.11	0.10	0.54	0.52	0.20	0.48	0.14
LM	Mean	0.95 ^{ac}	0.95 ^{ac}	0.72 ^{ac}	0.26 ^{bc}	0.01 ^b	0.99 ^{ad}	1.2 ^a	0.35 ^{bcde}
kg day ⁻¹	SD	0.32	0.24	0.11	0.40	0.62	0.15	0.49	0.14

Different letters within row indicate values are significantly ($P < 0.05$) different.

Table 2.2 Body fat mass loss measured as percentage of body mass loss.

		Season			
Diet		Winter	Spring	Summer	Fall
All (%)	Mean	39.3	59.8	73.2	42.3
	SD	16.8	24.1	67.2	31.1
Herring (%)	Mean	37.7 ^a	45.7 ^a	106.8 ^b	24.1 ^a
	SD	22.6	26.4	74.0	14.4
Pollock (%)	Mean	41.4 ^a	78.5 ^{ab}	28.5 ^a	66.6 ^{ab}
	SD	8.6	6.2	8.5	32.5

Different letters within herring and pollock indicate values are significantly ($P < 0.05$) different.

On average, Steller sea lions lost $0.69 \pm 0.52 \text{ kg d}^{-1}$ of lean mass and $0.66 \pm 0.38 \text{ kg d}^{-1}$ of total body fat mass. Daily rates of lean mass loss were greater than rates of total body fat mass loss in all trial groups, except for the spring and fall pollock-fed groups and the summer herring-fed group (Table 2.1).

There was no effect of either season or diet on both daily rates of lean mass loss (season, $F_{3,14}=2.49$, $P=0.10$; diet, $F_{1,5}=0.31$, $P=0.60$; Table 2.1) and total body fat mass loss (season, $F_{3,14}=0.62$, $P=0.61$; diet, $F_{1,5}=0.14$, $P=0.73$; Table 2.1). However, there was a significant interaction of season and diet on daily rate of lean mass loss ($F_{3,4}=8.32$, $P=0.002$, Table 2.1). In the spring and fall trials, mean daily rates of lean mass loss were higher in the herring-fed group than in the pollock fed group, however, the trend was reversed in the summer trial (Table 2.1).

2.3.3 Standard metabolic rate

The pre-experimental standard metabolic rates varied throughout the year ($F_{3,17}=27.0$, $P<0.001$). Although no changes were noted from winter ($43.1 \pm 4.1 \text{ MJ d}^{-1}$) to spring ($43.8 \pm 6.8 \text{ MJ d}^{-1}$), metabolism decreased from spring to summer ($32.4 \pm 8.0 \text{ MJ d}^{-1}$), and increased from summer to fall ($60.1 \pm 10.6 \text{ MJ d}^{-1}$; Fig. 2.1). Average standard metabolic rate was 4.1 times higher than that predicted by Kleiber's calculation for adult, terrestrial mammals. Neither pre- nor post-experimental standard metabolic rate correlated with body mass ($P>0.05$).

We obtained both initial and final standard metabolic rates from only two animals fed pollock during the winter trial, because one animal did not remain calm in the chamber for the entire period of oxygen consumption measurement. Absolute changes between standard metabolism at the start and end of the feeding trials differed significantly between the herring-fed groups and the pollock-fed groups ($F_{1,5}=7.84$, $P=0.038$; interaction season \times diet, $F_{3,13}=6.44$, $P=0.006$). However, statistical significance was lost when the data were transformed into percentages (Fig. 2.3). Metabolism was decreased ($-5.7 \pm 19.1\%$) when eating herring, and increased slightly ($0.7 \pm 14.6\%$) when eating pollock.

Scaling the standard metabolic rate according to $M^{0.67}$ showed that neither season nor diet affected changes in mass corrected standard metabolic rate (season,

$F_{3,13}=1.46$, $P=0.27$; diet, $F_{1,5}=2.41$, $P=0.18$).

2.3.4 Molting observations

Six of the seven animals showed signs of new hair growth in late July, and one animal showed evidence in late August. The end date of molting ranged from September 30th to November 4th (5 out of the 7 animals completed molting in October). Mean duration of molting was 80.6 ± 14.6 days.

2.4 Discussion

2.4.1 Seasonal fluctuations of initial values

As expected, the sea lions showed seasonal fluctuations in their pre-experimental body condition (Fig. 2.1), as commonly seen in other mammals that have pronounced seasonal life histories (Young 1984; Korhonen 1988; Ryg et al. 1990; Lager 1994; Campbell and MacArthur 1998; Pulawa and Florant 2000). However, there were no significant seasonal changes in body mass, nor did the apparent trends follow the changes in body condition (Fig. 2.1).

The sea lions in my study had initial body lipid stores ranging from 7.2 % to 22.2%, which were comparable with the total body fat stores of shot animals from the wild population (5-17%; Pitcher et al. 2000). These were also similar to the level reported for lactating Antarctic fur seals (*Arctocephalus gazella*; <16%; Arnould et al. 2002), but leaner than Antarctic and Galapagos fur seals (*Arctocephalus galapagoensis*) at parturition (22% and 26%, respectively; Costa and Trillmich 1988), or phocid species (up to 50% of body mass—however, most data are from lactating mothers; Ryg et al. 1990; Oftedal et al. 1993; Lydersen et al. 1995; Oftedal et al. 1996; Ortiz et al. 2001). Experiments with lemmings have shown that laboratory animals have greater capabilities for storing fat than those in the wild, where they must allocate additional energy to reproduction and thermoregulation (Batzli and Esseks 1992). Since my animals were not reproducing and were housed in a temperate outdoor facility, it is possible that they allocated more energy to lipid reserves than did their wild counterparts.

The correlation between body mass and total body fat might suggest that

body mass is a good indicator of total fat mass, at least among healthy young Steller sea lions of a similar age. However, the finding that separate significant linear relationships (Fig. 2.2) were found in both pre- (good condition) and post-experiment (poor condition) animals suggests that we cannot rely on body mass to estimate Steller sea lions' body fat mass. While my study has greater strength given its longitudinal design, it also had a small sample size; therefore further study is required on the relationship between body mass and body fat mass.

Seasonal increases in the blubber layer were not merely a product of increasing energy intake, given that an increase in blubber during the winter-spring interval was not accompanied by an increase in energy intake. Animal health records for the study animals (unpublished data) showed that food intake varied between individuals prior to the trials, which means that we cannot exclude the possible effects of previous research on the animals when interpreting my results. Still, the lack of correlation between pre-experimental energy intake and lipid stores indicates seasonal changes in energy expenditures. In all likelihood, these expenditures in my captive animals were associated with the moulting period, and/or reflected by changes in standard metabolic rate.

The higher level of total body fat I observed in spring (prior to the new hair growth; Fig. 2.1) was similar to the pattern seen in phocid seals (although usually with an increase in food intake; Lager et al. 1994; Boily 1996). It is unclear whether increases in fat stores occur in preparation for the expenses associated with the breeding or moulting period, as poor condition has been shown to delay both processes (Stewart and MacDonald 1997). The primary need for increased energy reserves during moulting in pinnipeds is often assumed to be related to associated periods of fasting or decreased food intake (e.g., Worthy et al. 1992; Lager et al. 1994; Boily 1996). Steller sea lions in my study did not fast during their moult (unpublished data), nor are they known to fast in the wild (Merrick et al. 1997; Sinclair and Zeppelin 2002). Data from other captive Steller sea lions suggests that voluntary food intake is generally lower during warmer months and higher during colder months (Kastelein et al. 1990; Nitto et al. 1998).

The standard metabolic rates of the sea lions in my study showed seasonal

variations (Fig. 2.1), as do other mammalian species (Baily 1965; Armitage and Shulenberger 1972; Ward and Armitage 1981; Campbell and MacArthur 1998). In my study, standard metabolic rate was lowest in the summer and highest in the fall. Rosen and Renouf (1998) suggested that seasonal changes in resting metabolism were more related to the total amount of energy used (food plus tissue catabolism), rather than just the energy ingested. Similarly, changes in standard metabolism in my sea lions were clearly not the sole source of changes in energy expenditures. For example, the percentage of body fat increased between winter and spring, despite no significant change in metabolism (and an apparent decrease in food intake). Lower metabolic rates were recorded in winter, as was observed in other species such as the woodchuck (*Marmota monax*; Bailey 1965), reindeer (*Rangifer tarandus*; Nilssen et al. 1984), and harbour seal (*Phoca vitulina*; Hedd et al. 1997; Rosen and Renouf 1998). Lowering metabolism during winter possibly serves to decrease the catabolism of fat reserves and preserves a minimum blubber layer for thermoregulation. However, resting metabolic rates of grey seals (*Halicoerus grypus*) were highest in winter (Boily and Lavigne 1997), as were those of other phocid species including harbour seals and spotted seals (*Phoca largha*) (Ashwell-Erickson et al. 1986).

The physiological relationship between metabolic rate and body mass is complicated (Darveau et al. 2003; Banavar et al. 2003; West et al. 2003). My sea lions did not show any significant relationship between body mass and standard metabolic rate. Metabolic rate in otariids has correlated positively with body mass in some studies (Donohue et al. 2000), but not in others (Beauplet et al. 2003). Donohue et al. (2000) concluded that the major contributing factor in the metabolic rate relationship in northern fur seal pups was the proportion of lean mass. The lack of positive relationship between metabolism and body mass in my study is probably due to the longitudinal study design, as suggested by Boily (1996), because body mass change is usually affected by changes in total body fat.

Moulting is often described as an energetically "expensive" activity due to an increased metabolism associated with increased growth or thermoregulatory costs (Worthy et al. 1992; Lager et al. 1994; Boily 1996). However, quantitative

evidence is equivocal (Ashwell-Erickson et al. 1986; Boily 1996; Boily and Lavigne 1997; Rosen and Renouf 1998). Metabolic studies on otariids have shown that pups have elevated metabolic rates during moult (Donohue et al. 2000; Beauplet et al. 2003), but no such studies have been conducted on moulting juveniles or adults. Comparing my animals prior to the new hair growth (summer) and at the end of new hair growth (fall) showed elevated body mass and standard metabolic rate, but no changes in total body fat. The lack of change in observed fat metabolism and the contribution of increased core tissues to increases in total body mass suggest an offsetting shift from thermoregulation to growth expenditures.

2.4.2 Seasonal effects of short-term low energy intake

The natural seasonal fluctuations I observed in body condition and metabolic rates might lead to the prediction that sea lions would suffer disproportionately from unexpected food restriction during periods of lower body fat reserves, such as winter, summer or fall. Alternately, it might be argued that decreased food intake would have a greater impact on Steller sea lions in the (presumably) high-energy requirement seasons such as summer (reproduction), or in the thermally demanding winter period.

The most obvious impacts of food restriction are decreases in body mass. However, the source of that mass loss is also important. Lipid from the hypodermal blubber layer is the primary internal energy source for pinnipeds since it yields more energy than protein on a per unit mass. Conversely, lean body mass is generally conserved (except for the demands of gluconeogenesis) as prolonged protein catabolism eventually leads to death (Øritsland 1990). However, a minimal blubber layer is required for thermoregulation, and otariids possess a much thinner blubber layer than phocid seals. Therefore, a sea lion in a negative energy state must balance its catabolism of internal tissues by taking into account their energy density, the physiological consequences of depletion, and the extent of the energy deficit. These considerations likely result in disparate priorities for tissue use at different times of the year.

The results of my study illustrate that Steller sea lions respond differently to

short-term, low energy intake at different times of the year. Differences in body mass loss attributable to diet type (herring or pollock) observed in some seasons also suggest that diet composition has a greater impact on Steller sea lion health at certain times of the year.

Both absolute body mass loss and rate of mass loss were highest in winter and lowest in summer. Interestingly, both relative body fat loss (-43%) and increases in metabolism (5.6%) were greatest (although not statistically significant) in the summer (Fig. 2.3). There are several reasons why Steller sea lions may be more susceptible to food restriction in the winter than in the summer.

Differences in rates of body mass loss may relate to higher thermoregulatory demands during the winter. Standard metabolism (which would include any inadvertent costs due to thermoregulation) was higher in the winter than the summer, although it was highest in the fall. There were no significant seasonal differences in rates of lipid catabolism that might be expected if the blubber layer were being conserved for thermoregulatory purposes. The higher relative rates of lipid loss of the herring-fed group during the summer and lower rates during other months may have been the product of lower absolute changes in body mass and/or greater relative use of lean tissue rather than conservation of lipid stores *per se*.

Alternatively, Steller sea lions may be most susceptible to food deprivation during periods when decreased food intake is less likely to occur naturally, and less susceptible when decreased food intake is part of their normal life history. During the summer breeding period, female sea lions fast for 1-2.5 days while nursing their pup on shore (which presents a concurrent energetic challenge) (Milette and Trites 2003). Winter months are characterized by high core growth and acquisition of lipid reserves, probably supported by high rates of food intake (Winship et al. 2001). Voluntary food intake of Steller sea lions in captivity is generally lower in warmer months and higher in colder months (Kastelein et al. 1990; Nitto et al. 1998). Therefore, it is possible that natural differences in energy state (and the related physiological states) predispose the sea lions to be more susceptible to unpredicted food restriction during the winter months and less during the summer.

It is interesting to note that the period when Steller sea lions appear to have

the greatest ability to endure lower energy intake coincides with the period when the average energy density of their diet in the wild is the highest. This is partly due to changes in intra-specific composition. For example, the lipid and energy content of adult pollock was greatest in summer (July/August; 5.3% lipid, dry matter basis) and fall (5.4%, September –November) and lowest in winter and spring (5.1% and 5.2%, respectively) (Kitts et al. *in press*). Additionally, the species composition of the diet of sea lions in southeast Alaska changes, with an increase in high-energy density prey during the summer months. As a result of these seasonal changes in energy requirements and prey energy density, the predicted food requirements (kg d^{-1}) may be lowest in summer and highest in winter and spring particularly for the populations in southeast Alaska based on dietary information (Winship and Trites 2003). Therefore, my results might also suggest that Steller sea lions appear to be more susceptible to restricted energy intake in cold periods when energy and food requirements are greatest, than in warm periods when energy and food requirements are lower. However, the significant differences in daily rates of body mass loss and lean tissue mass loss between the herring and pollock-fed groups in the summer trial may suggest that sea lions would be better able to conserve lean tissue in response to a reduced amount of food when consuming herring than pollock during this period, while this relationship was reversed in the fall.

The anatomical sources of the changes in body mass are noteworthy because decreases in body mass were not necessarily primarily derived from body fat. First, higher body mass loss did not always mean higher total body fat mass loss, which raises concerns over using percent lipid mass as a measure of sea lion health (often referred to as a 'condition index') among wild populations. This is supported by my finding that there were distinct linear relationships between body and lipid mass for sea lions in good (pre-experimental) and poor (food restricted) condition. Second, the animals were, contrary to my expectations, catabolizing a large proportion of core tissues when losing body mass. This was contrary to the general model for fasting pinnipeds (e.g., Cherel 1992; Noren and Mangel 2004) that predicts Steller sea lions would preferentially lose body fat except for the protein needed for gluconeogenesis.

Additionally, the variation in the rate of lipid loss was not directly related to either prey lipid density or intake levels. The greatest loss in body fat occurred amongst the summer herring-fed group (over 100% of body mass loss came from body fat mass). The high proportion (>100%) of mass loss from body fat means that animals actually increased lean mass tissues while they lost body fat. For example, one animal in summer lost 11.4 kg of body mass and 13.2 kg of total body fat mass (while gaining 1.8 kg of lean mass) over a 9 day study period, which means body fat mass loss accounted for 116% of her total body mass loss. The reason for the high rate of lipid loss is not clear — the pollock-fed group lost only 28% of its body mass loss from total body fat during the same period. It is also unclear why the herring-fed group used such a high proportion of lipid while the pollock-fed group used such a high proportion of lean mass — or what caused the diet-related difference. The differences between these values are greater than inherent inaccuracies of the deuterium dilution technique.

Although Steller sea lions ate the same energy from the herring and pollock diet, the energy source differed between them. When the sea lions ate pollock, they consumed 2.3 times more protein, but only 20% of the lipid that the herring group received. Energy available for metabolism was also different from gross energy intake. Since digestive efficiency and dry matter digestibility was higher in herring (95.4 % and 90.1 %, respectively) than pollock (93.9% and 86.5%, respectively; Rosen and Trites 2000a) during full-sized meals, the sea lions may have received less energy from the pollock diet than from the herring diet in the end. It is interesting that significant differences in daily rates of body mass and lean tissue loss due to diet types were observed only in the warmer months (Table 2.1; summer and fall). This indicates that the effects of diet composition were more prominent in summer and fall, and diminished in winter and spring.

A reduction in metabolism (metabolic depression) is an option for animals to limit their energy expenditures and therefore body mass loss in response to reduced energy intake (Øritsland 1990). Short-term restricted feeding experiments with Steller sea lions have demonstrated that animals either increase or decrease their metabolic rate depending on the circumstance. Metabolic depression has been shown

to occur in fasting Steller sea lions (Rosen and Trites 1999) and harbour seals (Markussen et al. 1992). Alternatively, some Steller sea lions exhibited elevated metabolic rates in response to an energetically restricted diet (~50-70% of their normal intake for ~30 days; Rosen and Trites 2002). In my study, the pollock-fed group increased their metabolism and the herring-fed group decreased it as a whole (although these were not statistically different). In addition, the absolute changes in standard metabolic rate that occurred during the feeding trials showed significant changes associated with the diet types. Rosen and Trites (2002) suggested that the differential response exhibited between fasting (decreased metabolism) and food restricted (increased metabolism) sea lions was partly due to daily food intake rather than mass loss. It is feasible that the greater food intake (by mass) during the pollock trials was sufficient to trigger a 'foraging' response, while the lower food intake during the herring trials were low enough to trigger a 'fasting' response.

However, changes in standard metabolic rate were not constant between seasons within the same diet. For example, the herring-fed group increased and the pollock-fed group decreased their metabolic rate in spring, while the trend was reversed in winter and summer. It is worth noting that whenever one of the diet groups lost a greater amount of total body fat than the other group, they also depressed their metabolic rate (regardless of the season). Changes in metabolism were likely independent of gross energy intake, as suggested by Rosen and Renouf (1998).

It is important to note that the pollock-fed group consumed the equivalent of 1.6% of their body mass during the winter trials. Although gross energy intake was not statistically different from the herring-fed group, caution needs to be exerted in interpreting the differences found in the pollock-fed group during winter. During this period, neither body condition nor metabolic rate showed significant differences. However, standard metabolic rate increased in the pollock-fed group, while decreasing in the herring-fed group. Similarly, although body mass change was different between the diet groups in summer and fall, neither total body fat mass nor standard metabolic rates explained these differences. It might be explained by circulating hormones which may play an important role in energy metabolism, such

as leptin (Reidy and Webber 2000).

Finally, my study only reports the gross effects of seasonal food restriction on body mass, composition, and metabolism. Understanding the proximate mechanisms that regulate the differences observed between seasons is equally important. Specifically, it is important to investigate whether the observed physical differences are related to an interaction between circulating hormones, body condition and metabolic rate, particularly given the roles hormones serve to control a sea lion's energy budget. This likely means that the relationship between circulating hormones, body condition and metabolic status also changes seasonally. Understanding this relationship will not only provide a clearer understanding of sea lion physiology, but may result in concentrations of hormones in the blood circulation being useful indicators of the relative energetic status of Steller sea lions in the wild.

2.5 Conclusion

Steller sea lions underwent natural seasonal fluctuations in body condition and metabolic rate. Sea lions lost a greater proportion of body mass during the colder seasons and a smaller proportion during the warmer seasons, which coincides with the predicted food/energy requirements for the wild Steller sea lions. This suggests that Steller sea lions may be more susceptible to nutritional stress during the winter. Neither body fat mass loss nor changes in metabolic rate explained the observed loss of body mass, suggesting that other mechanisms influence changes in body mass. Overall, type of diet (under conditions of severely restricted intake) did not significantly affect changes in body mass, body condition, or metabolism. However, there were seasonal effects of diet types on daily rates of body mass loss.

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Chapter III: Seasonal differences of changes in hormone levels in response to short-term restricted diet in Steller sea lions (*Eumetopias jubatus*)

3.0 Summary

Steller sea lions (*Eumetopias jubatus*) were fed an iso-caloric, restricted diet of Pacific herring (*Clupea pallasii*) or walleye pollock (*Theragra chalcogramma*) for up to 9 days, four times per year, resulting in a loss of approximately 10% of their initial body mass. Changes in cortisol, triiodothyronine and blood urea nitrogen (BUN) were seasonally dependent ($P < 0.05$). Over the course of the trials, serum levels of cortisol and BUN increased ($217.6 \pm 231.1\%$, $11.4 \pm 24.4\%$, respectively) and total triiodothyronine decreased ($-35.6 \pm 31.9\%$) the most in winter (Nov/Dec). Total thyroxine and glucose levels decreased as a whole, but changes were neither diet related nor season dependent. Leptin levels increased in the herring-fed group ($12.2 \pm 21.4\%$) and decreased in the pollock-fed group ($-2.8 \pm 23.5\%$), although these differences were not statistically significant. Contrary to expectations, there was no relationship between leptin and body conditions. Ghrelin showed a negative relationship with body mass. Serum cortisol levels correlated negatively with both body mass and body condition suggesting that cortisol may play an important role in body fat regulation in Steller sea lions. The observed changes in hormone levels support the morphological data suggesting that Steller sea lions may be more severely impacted by reduced energy intake during winter than during other times of the year.

3.1 Introduction

Distinct changes have been noted in the metabolic rates, body mass and body condition of many mammals with seasonal life cycles (e.g., Ashwell-Erickson et al. 1986; Rosen and Renouf 1998; Fitzgerald and McManus 2000). In Chapter II, I reported the effects on body mass, body condition and metabolism of captive Steller sea lions fed a restricted iso-caloric diet of either herring or pollock for up to 9 days in different seasons. I found that sea lions lost greater amounts of body mass in winter (November/December) and smaller amounts in summer (May/June), but that the changes in body mass did not correspond to changes in total body fat mass or

metabolic rate.

Hormones serve as a mechanism for mediating seasonal changes in physiology, including altering and defending seasonal set-point in body mass, composition and metabolism (Armitage and Shulenberg 1972; Ward and Armitage 1981; Adam and Mercer 2001). Hormonal changes may also explain why the decreases in Steller sea lion body mass varied by season when food intake levels were held constant.

Key hormones in regulating mammalian energy expenditure include cortisol, thyroid hormones, leptin and ghrelin. Cortisol is one of the major glucocorticoids that increase glucose production from non-carbohydrate sources, such as proteins (gluconeogenesis; Aron et al. 2001). Increased cortisol levels can also indicate high levels of stress (Aron et al. 2001; St. Aubin 2002; Hunt et al. 2004; Mashburn and Atkinson 2004) including nutritional stress (Kitaysky et al. 1999; Kitaysky et al. 2001). The principal thyroid hormones, triiodothyronine (T3) and thyroxine (T4), affect the metabolism of mammals (such as through thermogenesis to maintain body temperature), as well as alter growth rates (McNabb 1992). Leptin is a product of the obese (OB) gene and is primarily synthesized and secreted into circulation by adipose cells (Doyon et al. 2001). In humans and lab rodents, leptin regulates body fat by altering energy expenditure and rates of food intake (see Reidy and Webber 2000 for review). The role of each of these hormones in regulating metabolism and body condition of sea lions is not known.

Ghrelin is a recently discovered growth hormone-releasing peptide that is produced in and released from the stomach (Kojima et al. 1999). Ghrelin likely works in an antagonistic fashion to leptin (Nakazato et al. 2001), given that ghrelin levels correlate negatively with leptin levels (Tshöpp et al. 2001; Beck et al. 2002).

Understanding how hormone concentrations of healthy sea lions vary seasonally and how they respond to changes in prey intake and composition is essential to evaluating the nutritional status of free-ranging animals and understanding the impact of potential changes in prey availability. Hormones that change in concentration in response to food intake might provide a simple means to assess the nutritional status of wild sea lions.

The >80% decline of Steller sea lions in western Alaska (Trites and Larkin 1996) may be related to inadequate diets, due to either an overall shortage of food or a physical inability to consume sufficient amounts of low-energy density prey (such as walleye pollock, *Theragra chalcogramma*) to meet daily energy requirements (Alverson 1992; Merrick et al. 1997; Rosen and Trites 2000; Trites and Donnelly 2003).

Previous experiments with captive pinnipeds have shown a variety of responses (both within and between studies) in metabolism, body mass, and metabolism to changes in prey quantity and/or composition (Kirsch et al. 2000; Rosen and Trites 2000; Trumble et al. 2003; Chapter II). Many of these discrepancies may be explained by the interaction between quantity and quality of prey, and endogenous seasonal changes in hormones and other physiological responses.

The following study used a 12-month captive feeding regime with seven young (2-6 years old) female Steller sea lions to determine whether three particular hormones (cortisol, thyroid hormones, ghrelin and leptin) play a significant role in regulating seasonal set points or changes in body mass or condition in Steller sea lions. Glucose and blood urea nitrogen were also measured to document their role in energy utilization. I sought to identify whether there were particular times of the year when restriction in quantity or quality of diet combined with natural seasonal changes in hormone concentrations to produce a more severe effect on sea lions than at other times of the year. My results provide insights into the importance of seasonal cycles and contribute significantly to understanding the physiology and ecology of Steller sea lions. They may also provide valuable insights into understanding the role of nutrition in the decline of Steller sea lions in Alaska.

3.2 Methods

3.2.1 Animals

All procedures and protocols involving animals were conducted under the authority of a University of British Columbia Animal Care Permit No.A98-0095.

Study animals were held in an outdoor facility at the Vancouver Aquarium Marine Science Centre, British Columbia, Canada, and had previously participated in various research projects since being brought in as pups from northern Vancouver Island, British Columbia. The animals were usually fed Pacific herring (*Clupea pallasii*; daily equivalent amounts of about 5 - 6% of their body mass) with vitamin supplements (1 tablet per 3 kg food; 5M26 Vita-zu tablets, Purina Test Diets, Richmond, IN). Food intake (kg d^{-1}) and body mass ($\pm 0.1 \text{ kg}$) were recorded daily.

Seven female Steller sea lions (five juveniles, 2.5-3 years old; and two sub-adults, 5.5-6 years old) were divided into two experimental diet groups: four animals (one sub-adult and three juveniles) began the experiments on a diet of Pacific herring (*Clupea pallasii*) and three animals (one sub-adult and two juveniles) were fed walleye pollock (*Theragra chalcogramma*). The diet of each group was alternated with each subsequent trial between herring and pollock except for one juvenile in the first group that fed exclusively on herring because she refused the pollock diet. Three animals were therefore fed herring in the winter switched to a pollock diet in the spring, back to herring in the summer, followed by pollock in the fall, and vice versa for the 3 animals fed pollock in winter. Proximate nutrient composition analyses of representative fish samples were conducted by Norwest Labs (soxhlet hexane extraction for lipid and total nitrogen estimates $\times 6.25$ for protein; Surrey, British Columbia). The herring averaged $8.1 \pm 0.05 \text{ kJ g}^{-1}$ and $13.3 \pm 0.1\%$ lipid (wet weight), and the pollock $3.0 \pm 0.2 \text{ kJ g}^{-1}$ and $1.0 \pm 0.4\%$ lipid. The amount of herring fed was set at the equivalent of 1% of the sea lions' initial (Day 1) body mass. Pollock consumption was set at 1.6% (winter) and 2.6% (rest of the study) of their initial body mass, which provided an energy intake equivalent to the herring diet (i.e., iso-caloric). The sea lions were maintained on each diet for up to 9 days per experimental trial. Under these diets, sea lions lost approximately 10% of their initial body mass, but the magnitude of loss was seasonally dependent (Table

Table 3.1 Mean \pm standard deviations of absolute and percent body mass loss during feeding of herring or pollock (n=7 for each season; Chapter II).

Body mass loss	Season			
	Winter	Spring	Summer	Fall
kg	15.8 \pm 1.6	13.0 \pm 2.3	11.3 \pm 2.9	13.0 \pm 2.8
%	13.0 \pm 2.7	9.9 \pm 1.3	9.0 \pm 2.6	9.9 \pm 2.7

Mass loss was significantly greater in the winter trials ($F_{3,4}=5.26$, $P=0.012$).

3.1). I terminated one trial of the study (summer and fall) on 4 animals prematurely after only 8 days of feeding due to logistical constraints, and one because she reached the maximum mass loss (15%) dictated by the Animal Care Permit.

The experimental protocol was repeated four times over the course of the year during winter (November to December 2002), spring (February to March 2003), summer (May to June 2003), and fall (August to September 2003).

3.2.2 Data collection

Blood samples were obtained in the morning of the first and last (9th or 10th) day of each feeding trial, and were drawn before the first feed of the day (i.e., >16 hours after the last meal). All of the hormones measured in this experiment were known to have diurnal fluctuations (Gardiner and Hall 1997; Horton et al. 2000; Ashutosh Dhandha and Kundu 2001; Oki and Atkinson 2004; Mashburn and Atkinson 2004). In my study, blood samples were mostly collected in the morning, with a few collected around noon. Blood was drawn from the caudal gluteal vein of anesthetized animals (isoflurane) into serum separator collection tubes and EDTA under veterinarian supervision. Sera were separated using standard techniques (including running a centrifuge at 3500 RPM for 5 minutes) and stored at -70°C .

3.2.3 Laboratory and statistical analyses

Blood urea nitrogen (BUN) and glucose levels were measured as routine clinical analyses at a commercial veterinary lab (Central Veterinary Laboratory,

Langley, B.C.). Duplicate undiluted sera were tested for hormone concentrations by using a commercially available radioimmunoassay for cortisol, total T3, total T4 (Coat A Count, DPC, Los Angeles, CA), and leptin (Multi-species Leptin RIA kit, Linco Research Inc., St. Charles, MO). Leptin concentration was expressed as human equivalent (HE) because the antibody was raised against human leptin. Intra-assay coefficients of variation were calculated for each hormone by running quality controls in duplicates (for cortisol 24.5%, n=6; for TT3 6.1%, n=8; for leptin two different concentrations; 3.1% n=7 and 19.3% n=5) except in the case of TT4. Inter-assay coefficients of variation for TT4 duplicate quality control samples were <5.0%. Before performing leptin analyses with a Multi-species leptin RIA kit, several extra serum samples were tested for leptin with canine leptin sandwich ELISA (Iwase et al. 2000; Dr. Masayuki Saito, University of Hokkaido, Japan). The leptin concentrations were below the detection limit of this assay kit (1 ng ml⁻¹) and, therefore a multi-species leptin RIA kit was used instead.

Plasma ghrelin was measured using a Ghrelin (Canine) RIA kit (Phoenix Pharmaceuticals, Inc; Belmont, CA). The procedures (centrifuge tubes containing aprotinin) to prevent proteinase activity were not conducted. The intra-assay coefficient of variation for ghrelin was not obtained from multiple samples (>2), and acceptable levels (CV<10%) of ghrelin were recovered from only 18 out of 56 samples. Only values from these 18 samples are presented.

Results are reported as mean \pm standard deviation (SD). Seasonal variations in parameters were determined using one-way repeated measure ANOVAs (SAS, version 8.2, SAS Institute, Cary, NC). Two-way repeated measures ANOVAs evaluated the effect of changes from the initial values in diet and season. We selected the most parsimonious model and calculated post hoc tests of specific differences. The mean percent changes from the initial values were compared by post hoc tests of specific differences. Care was taken into the model to minimize the effect of alternated diet treatment in each group. Since all hormones are assumed to play important role in body mass/body fat regulation, correlations were determined using multiple linear regressions (Splus 6.1, Insightful Inc., Seattle, WA). Differences were considered significant at $P < 0.05$.

3.3 Results

3.3.1 Seasonal fluctuations in initial serum levels

Mean pre-experimental (initial) cortisol levels were slightly higher in summer ($79.7 \pm 38.4 \text{ ng ml}^{-1}$, mean \pm standard deviation) and fall ($68.6 \pm 20.6 \text{ ng ml}^{-1}$), and lower in winter ($58.2 \pm 25.6 \text{ ng ml}^{-1}$) and spring ($57.1 \pm 23.5 \text{ ng ml}^{-1}$; Fig. 3.1), although these differences were not statistically significant ($F_{3,18}=1.58$, $P=0.2$). Negative correlations were noted between cortisol level and body mass ($r^2=0.08$; Fig. 3.2A), absolute total body fat ($r^2=0.29$; Fig. 3.2B) and percent total body fat ($r^2=0.31$; data not shown).

The initial levels of total T3 (TT3) did not differ significantly between seasons ($F_{3,17}=0.97$, $P=0.43$), although they gradually increased from winter ($0.70 \pm 0.12 \text{ ng ml}^{-1}$) to fall ($0.79 \pm 0.17 \text{ ng ml}^{-1}$; Fig. 3.1). TT3 levels correlated positively with body mass ($r^2=0.15$, $P<0.01$; Fig. 3.3), but did not correlate with either absolute or percent total body fat ($P>0.05$). The majority of the total T4 (TT4) measurements were lower than the detection limit of the assay kit (10 ng ml^{-1}). Values above this level did not reveal significant fluctuations in the initial TT4 levels (range $8.0 \pm 3.0 - 8.9 \pm 2.5 \text{ ng ml}^{-1}$; $F_{3,18}=0.50$, $P=0.69$; Fig. 3.1), nor correlations with body mass or total body fat ($P>0.05$). The TT3/TT4 ratio, a measurement of deiodination, ranged from 0.094 in the summer to 0.116 in the fall, but did not vary across seasons ($F_{3,17}=0.56$, $P=0.65$).

There were no seasonal differences in pre-experimental leptin levels (range: $2.52 - 2.92 \text{ ng ml}^{-1}$ HE; $F_{3,18}=0.37$, $P=0.77$, Fig. 3.1) nor did they correlate with body mass or total body fat ($P>0.05$).

Pre-experimental glucose levels fluctuated seasonally ($F_{3,18}=5.41$, $P=0.008$). The highest level was observed in spring ($7.01 \pm 0.8 \text{ mmol l}^{-1}$), followed by summer ($6.9 \pm 0.7 \text{ mmol l}^{-1}$), winter ($6.9 \pm 0.6 \text{ mmol l}^{-1}$), and fall ($6.1 \pm 0.49 \text{ mmol l}^{-1}$).

Pre-experimental levels of BUN were statistically similar across the season ($F_{3,18}=2.11$, $P=0.13$), ranging from $7.2 \pm 0.61 \text{ mmol l}^{-1}$ in the fall to $8.5 \pm 1.3 \text{ mmol l}^{-1}$ in summer.

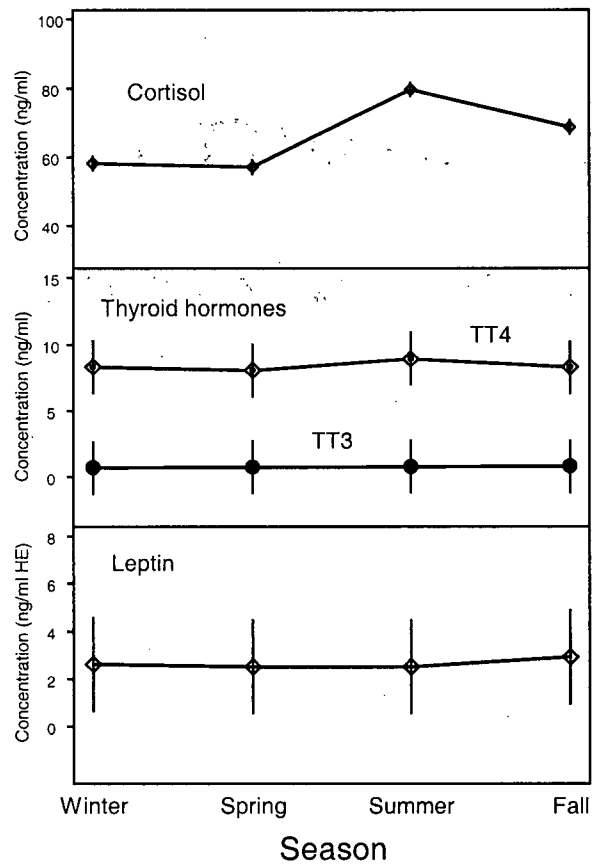


Fig. 3.1 Seasonal variations in the initial levels of cortisol, thyroid hormones, and leptin concentrations. Data are shown as mean \pm SE ($n=7$ for each season).

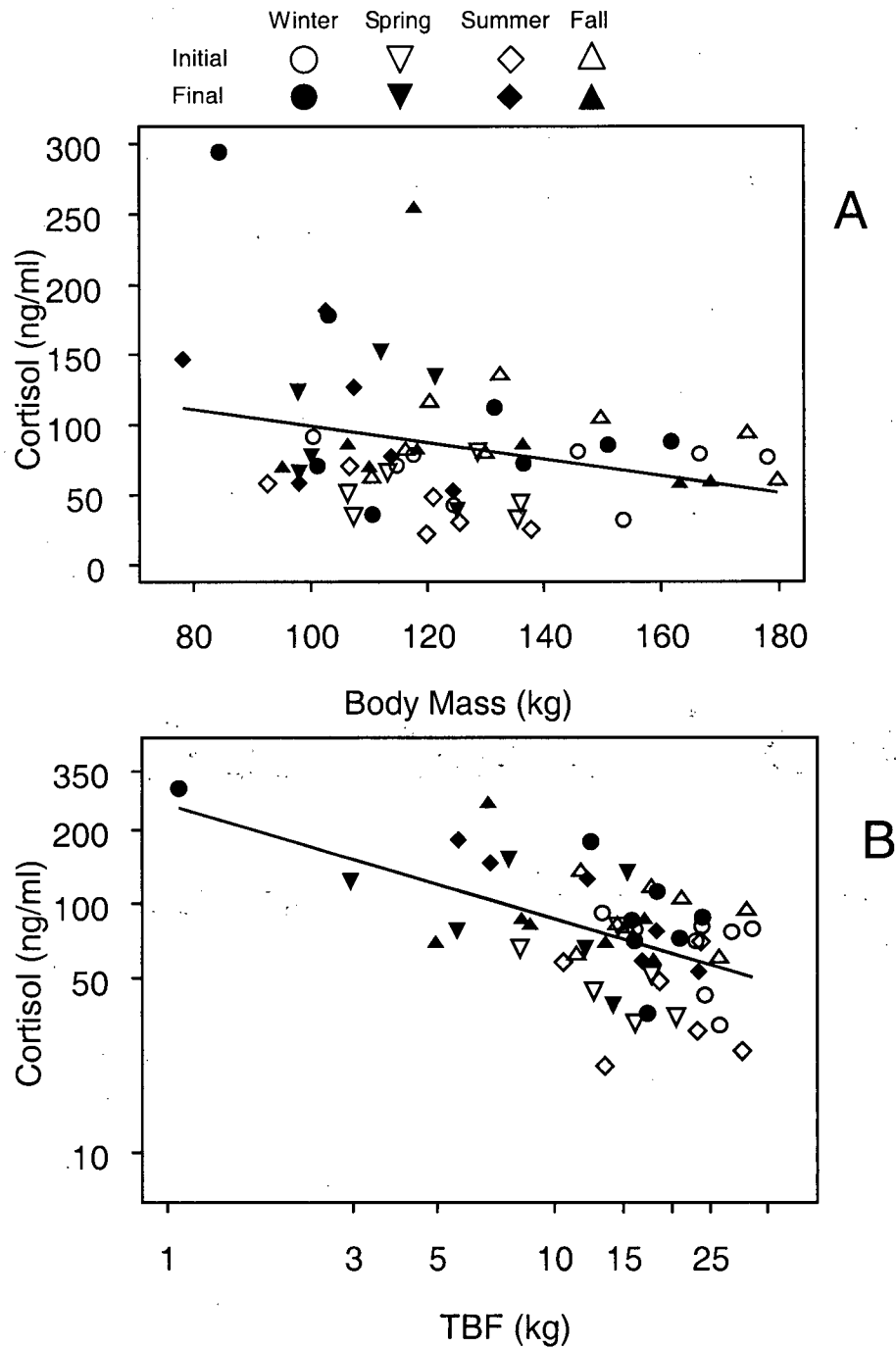


Fig. 3.2 The relationships of cortisol concentration with body mass and body condition. Open symbols indicate pre-experimental (initial) values and closed symbols indicate post-experimental (final) values. (A) The relationships between body mass (kg) and cortisol concentration (ng ml^{-1}) ($n=28$). There was a significant relationship ($r^2=0.08$) when all values (initial and final) were combined. (B) The relationships between total body fat (\log_e ; TBF; kg) and cortisol concentrations (\log_e ; ng ml^{-1}) ($n=28$). There was a significant relationship ($r^2=0.29$) when all values were combined.

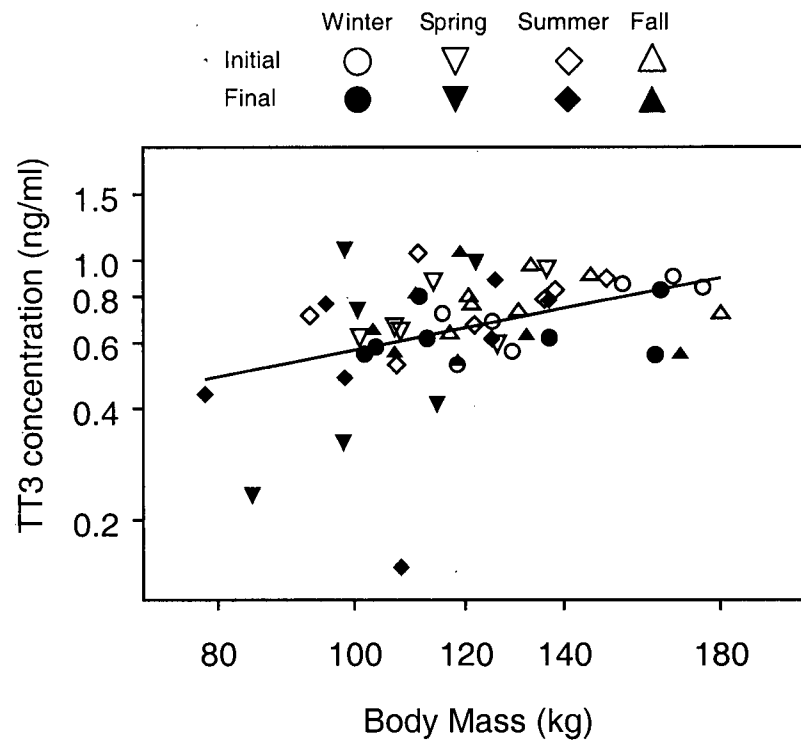


Fig. 3.3 The relationship between body mass (kg) and TT3 concentrations (n=26). There were significant relationships within data for all values (initial and final) combined ($r^2=0.15$).

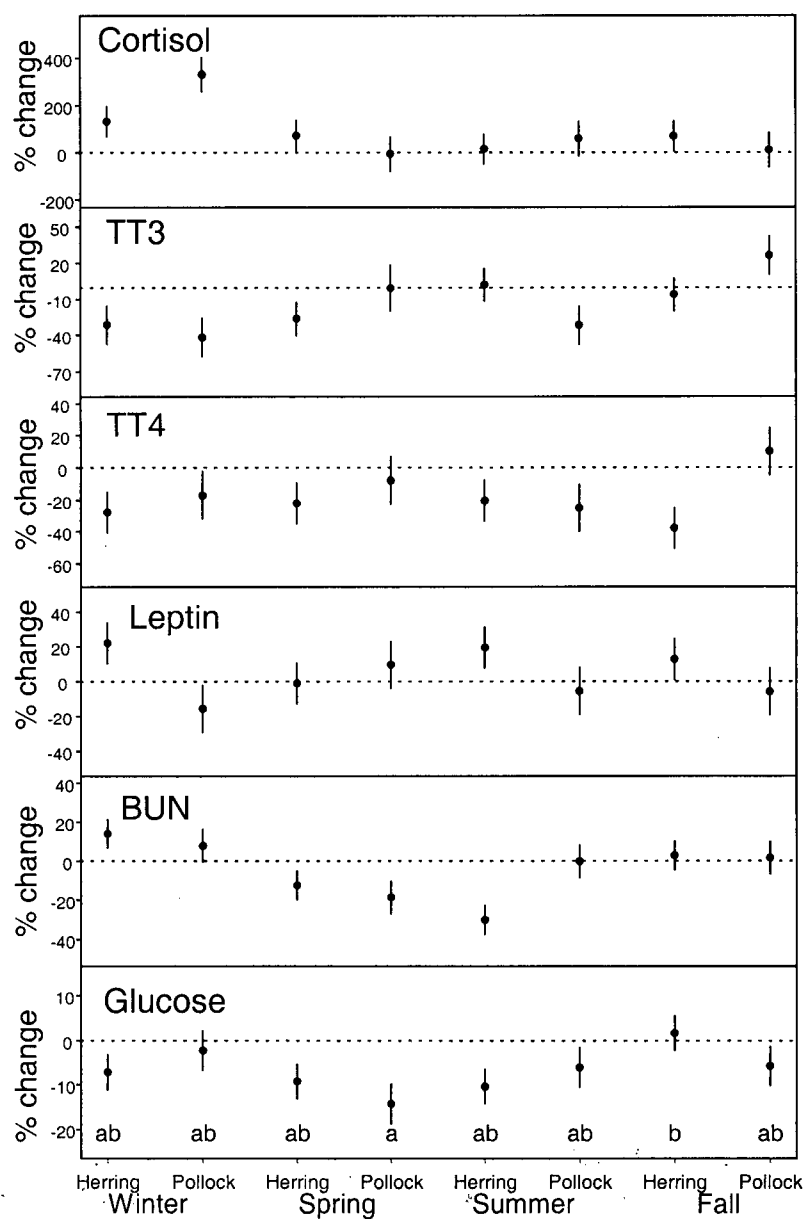


Fig. 3.4 Mean percent changes (\pm SE) in cortisol, TT3, TT4, leptin, BUN and glucose after feeding each diet type within each season. $n=4$ for the herring-fed group, except for winter ($n=3$), and $n=3$ for the pollock-fed group except for spring ($n=2$). Different letters in glucose indicate significant differences.

3.3.2 Changes in serum profile after the feeding trials

All groups, except for the spring pollock-fed group, exhibited increased cortisol levels following short-term restricted consumption of experimental diets (Fig. 3.4). Grouping by diet showed that serum cortisol levels increased by $71.7 \pm 85.8\%$ for the herring-fed group and by $100.3 \pm 204.3\%$ for the pollock-fed group, although they were statistically similar ($F_{1,5}=0.33$, $P=0.59$). Pooling the data by season (combining data from both diet groups) showed that seasons affected the degree of change in cortisol levels ($F_{3,14}=4.65$, $P=0.019$). The greatest increase in cortisol was in winter ($217.6 \pm 231.1\%$), followed by fall ($44.6 \pm 77.8\%$), spring ($39.2 \pm 66.0\%$), and summer ($34.5 \pm 65.3\%$).

The TT3 levels of the sea lions generally decreased after the feeding trials (Fig. 3.4). Exceptions were those fed pollock in the spring and fall trials, and those fed herring in the summer trial. Pooling both diet groups showed a general decrease in TT3 level ($-11.5 \pm 42.9\%$ for the pollock-fed group and $-14.6 \pm 21.9\%$ for the herring-fed group; $F_{1,5}=0.12$, $P=0.74$). However, percent changes in TT3 were dependent on season ($F_{3,12}=3.73$, $P=0.042$). Changes in TT3 were greatest in winter ($-35.6 \pm 31.9\%$) followed by spring ($-16.8 \pm 18.6\%$) and summer ($-12.4 \pm 30.0\%$). The sea lions increased their TT3 levels by $7.92 \pm 33.5\%$ in the fall.

TT4 levels decreased over most of the feeding trials, except for the fall pollock-fed group (Fig. 3.4). Changes in TT4 levels were similar across season (winter $-23.3 \pm 19.9\%$, spring $-16.1 \pm 14.1\%$, summer $-22.5 \pm 25.5\%$, and fall $-17.4 \pm 42.7\%$; $F_{3,14}=0.29$, $P=0.82$). The herring-fed group lost a greater percentage of TT4 ($-28.1 \pm 17.8\%$) than the pollock-fed group ($-8.8 \pm 32.3\%$), but this difference was not significant ($F_{1,5}=3.62$, $P=0.12$). Overall, changes in the TT3/TT4 ratio were not affected by season or diet type (0.01 ± 0.04 when all values were combined; $P>0.05$).

Leptin levels increased in the herring-fed group by $12.2 \pm 21.4\%$ and decreased in the pollock-fed group by $-2.8 \pm 23.5\%$ during the trials, although these differences were not significant ($F_{1,5}=3.95$, $P=0.10$; Fig. 3.4). Pooling both diet groups showed that leptin levels increased in all seasons with the greatest increase occurring in summer ($8.8 \pm 20.7\%$) and the smallest increase in spring ($3.5 \pm 29.4\%$)

although these differences were not significant ($F_{3,14}=0.03$, $P=0.99$).

The mean ghrelin level in Steller sea lions was 1.0 ± 0.36 ng ml⁻¹ (all 18 acceptable samples, Table 3.2). Unfortunately, I was unable to determine the effects of diet treatment on ghrelin levels because both initial and end values were successfully recovered from only four sets, including one animal in the summer, one in the spring and two in the fall trials (Table 3.2). Of these four, three animals decreased ghrelin levels following the restricted diet intake, and one animal in the fall trial showed increased ghrelin levels. Ghrelin levels (both pre- and post-experimental) correlated negatively with body mass ($r^2=0.27$, $P=0.026$; Fig. 3.5), but did not show a significant correlation with leptin levels ($P>0.05$).

There were significant seasonal effects on changes in BUN levels ($F_{3,14}=5.09$, $P=0.014$, Fig. 3.4). While BUN levels increased in winter ($11.4 \pm 24.4\%$) and fall ($2.34 \pm 4.73\%$) trials, they decreased over the course of the spring (-15.1 ± 6.6) and summer trials ($-17.2 \pm 17.4\%$). Although decreases in BUN were greater overall in the herring-fed group (with all seasons pooled; $-7.0 \pm 21.5\%$) than in the pollock-fed group ($-1.4 \pm 15.4\%$), diet type was not a significant factor ($F_{1,5}=0.53$, $P=0.50$).

Steller sea lions experienced decreased glucose levels during all trials, except for the herring-fed group in the fall (Fig. 3.4). Pooling both diet groups revealed that, while the average change was greatest in the spring trial ($-11.4 \pm 8.5\%$) and the least in the fall ($-1.5 \pm 7.6\%$), there was no overall effect of season ($F_{3,4}=1.61$, $P=0.23$). Both diet groups had similar decreases in percentage glucose under the restricted diet (6~7% decrease; $F_{1,5}=0.09$, $P=0.78$). However, there was a significant interaction of diet and season ($F_{3,14}=3.50$, $P=0.044$).

Table 3.2 Ghrelin concentrations (ng ml⁻¹) in Steller sea lions.

Animal	Season	Initial	End	Diet
F97HA	Winter	0.67	-	Pollock
F97SI	Winter	0.69	-	Herring
F97SI	Spring	1.12	1.05	Pollock
F00BO	Spring	-	1.14	Pollock
F00TS	Spring	-	1.53	Herring
F00ED	Summer	-	0.99	Pollock
F97SI	Summer	0.73	-	Herring
F00BO	Summer	1.23	1.19	Herring
F00BO	Fall	-	1.54	Pollock
F00NU	Fall	0.48	-	Pollock
F00TS	Fall	0.81	-	Pollock
F97SI	Fall	0.72	0.50	Pollock
F00ED	Fall	1.42	1.60	Herring
F00YA	Fall	-	1.08	Herring

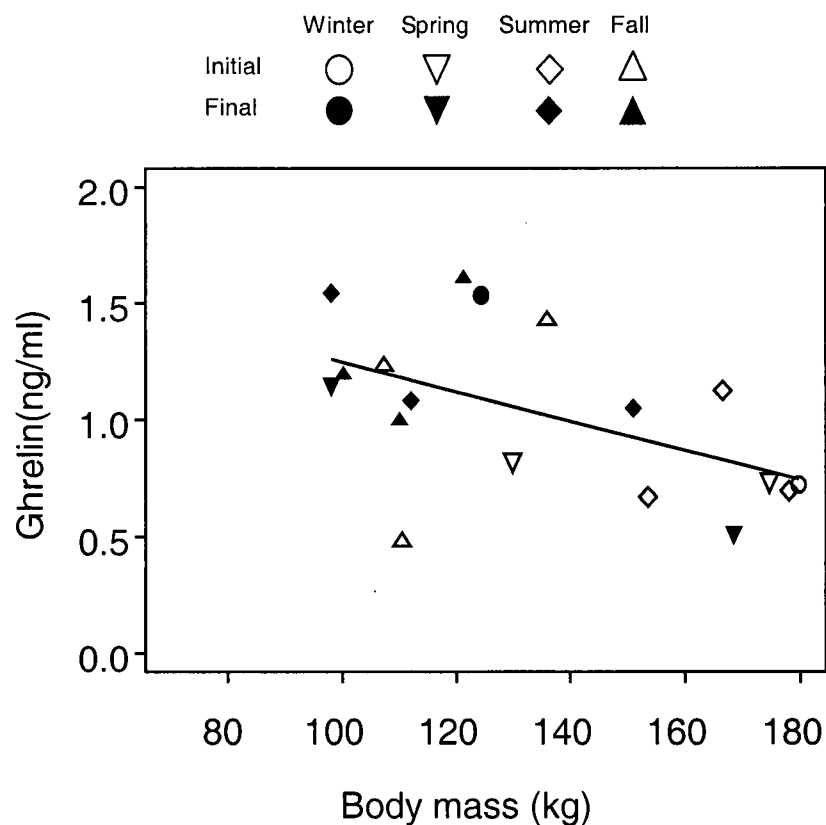


Fig. 3.5 The relationship between body mass and plasma ghrelin level ($r^2=0.27$, $P=0.026$).

3.4 Discussion

3.4.1 Seasonal fluctuations in initial serum levels

The initial level of glucose was the only serum parameter that fluctuated significantly across season. The highest levels of glucose observed in the spring and lowest observed in the fall corresponded with the highest and lowest levels in total body fat mass, respectively (Chapter II). Since neither cortisol nor thyroid hormones — which are meant to increase gluconeogenesis (cortisol) or glucose turnover and absorption (thyroid hormones) — were related to the seasonal fluctuations of glucose level in this study, it is unknown why this significant fluctuation of glucose was observed. Insulin and glucagons are other important hormones which regulate glucose levels in the blood (Campbell 1996). Future studies should consider these hormones.

Cortisol is the principal glucocorticoid in marine mammals, and it is usually elevated in response to stress (St. Aubin 2002). The sea lions in my study showed slight but insignificant seasonal fluctuations in the pre-experimental serum cortisol levels (Fig. 3.1). However, initial and final cortisol values were negatively related to body mass and absolute and relative measures of body fat (Fig. 3.2). When body mass decreased between seasons, cortisol levels increased (and vice versa). The lowest cortisol levels were observed in the spring, and corresponded with the highest level of total body fat. These data suggest that, while the seasonal variation in body mass and composition was 'natural' (in that it was self-regulating), the lower set-point was associated with higher levels of this hormone. The higher concentrations may be indicative of seasonal changes in physiological control (e.g., a greater utilization of protein) rather than physiological stress *per se*.

It is interesting to note that when cortisol levels decreased from summer to fall, standard metabolic rate rose to its highest level (Chapter II). Similarly, the high summer cortisol levels were associated with the lowest observed standard metabolic rates (Chapter II). It is possible that cortisol acts as a metabolic suppressor in Steller sea lions or, conversely, that high metabolic rates occur during periods of minimal stress, although this is not clear in other mammals.

The ranges of initial cortisol concentrations in my Steller sea lions

(22.1-134.3 ng ml⁻¹) were similar to those reported for other captive Steller sea lions (73.0 – 94.1 ng ml⁻¹ Mashburn and Atkinson 2004) and for wild Antarctic fur seals (65 ng ml⁻¹ Liggins et al. 1993; 37.9 ng ml⁻¹ Guinet et al. 2004). Antarctic phocids showed higher total cortisol concentrations (489 - 863 ng ml⁻¹) with high cortisol production rates (Liggins et al. 1993). The high levels of cortisol in Antarctic phocids are hypothesized to be an adaptation to prevent high-pressure nervous syndrome in phocids (Liggins et al. 1993), which tend to dive deeper than Steller sea lions (Merrick and Loughlin 1997; Loughlin et al. 2003).

TT3 levels in my study (0.53 - 1.05 ng ml⁻¹) were comparable to those reported for northern fur seals (0.63 ng ml⁻¹) (*Callorhinus ursinus*; St. Aubin 2001). Although TT4 levels in the Steller sea lions (4.0 - 14.0 ng ml⁻¹) were significantly lower than those for northern fur seals (28 ng ml⁻¹), similar ranges of TT4 were reported in harbour seals (Ashwell-Erickson et al. 1986; Oki and Atkinson 2004). Many of the TT4 levels in my study were lower than the detection limit of the assay kits (<10 ng ml⁻¹), which could be due to inappropriate specificity of the radioimmunoassay kit used or a result of the higher rate of T4 to T3 deiodination. A harbour seal study (Oki and Atkinson 2004) that used the same RIA kit I used in this study showed a similar range of TT4 concentrations, and also reported values lower than 10 ng ml⁻¹.

Thyroid hormones regulate metabolism, including changes through thermoregulation. Hence thyroid hormone levels are generally higher in younger animals (Leatherland and Ronald 1979; Englehardt and Ferguson 1980; Little 1991; Renouf and Brotea 1991; Stokkan et al. 1995; Boily 1996; Hall et al. 1998; Haulena et al. 1998; Woldstad and Jenssen 1999). The juvenile sea lions in my study had consistently lower TT3 and TT4 concentrations than the sub-adults (data not shown).

In general, lower thyroid hormone levels are thought to decrease metabolic rate so that animals can minimize severe winter energy requirements (Nagy et al. 1995). Seasonal fluctuations of thyroid hormones are also known to correspond to seasonal fluctuations in food intake, body mass, or body fat mass (Young 1984; Renouf and Noseworthy 1991). Unlike results from other studies (Nilssen et al. 1984; Young 1984; Ashwell-Erickson et al. 1986; Campbell and MacArthur 1998;

Ashutosh Dhanda and Kundu 2001; Oki and Atkinson 2004), neither initial TT3 nor TT4 values revealed dramatic seasonal fluctuations, despite observed variations in standard metabolic rate (Chapter II). Although I did not find any relationship between each of the thyroid hormones and metabolic rates, the TT3/TT4 ratio could play an important roll in seasonal fluctuations of metabolism in Steller sea lions.

Both cortisol and thyroid hormones have been suggested to play a role in the moulting process of phocid seals (Riviere et al. 1977; Ashwell-Erickson et al. 1986; John et al. 1987; Boily 1996, Gardiner and Hall 1997) although not all researchers agree (Renouf and Brotea 1991; Woldstatad and Jenssen 1999). Cortisol promotes hair loss and thyroid hormones stimulate hair growth (St. Aubin 2001). It is therefore expected that cortisol levels increase in the early stages of moult and that increased thyroid hormone levels follow towards the end of new hair growth period (Riviere et al. 1977; Ashwell-Erickson et al. 1986; Boily 1996, Gardiner and Hall 1997). In my study, cortisol levels were slightly elevated in the summer (May/June), approximately 1.5 months before the first signs of new hair growth (Chapter II).

Sea lions in my study did not show significant seasonal fluctuations in initial thyroid hormone levels, although TT3 levels insignificantly increased from prior to the moult (summer; 0.785 ± 0.120 ng ml⁻¹) to the end of the moult (fall; 0.794 ± 0.171 ng ml⁻¹), and TT4 levels decreased from 8.9 ± 2.5 ng ml⁻¹ to 8.3 ± 2.9 ng ml⁻¹ during the same time period. John et al. (1987) showed that, in harp seals, a high T3/T4 ratio was observed in the pre-molt phase and a low ratio in the post-molt phase, although this was not observed in captive harbor seals (Renouf and Brotea 1991). In my study, the lowest ratio occurred prior to the moult (0.094 in summer) and the highest in the finale moulting stages (0.116 in fall). These results indicate that deiodination (conversion of T4 to T3) increased over the course of new hair growth, which may suggest an increased demand for a biologically active form, T3, for new hair generations. The other possibility is that the rate of degradation of TT3 was lower. More frequent blood samplings and metabolic rate measurements are required to further investigate moulting physiology in Steller sea lions.

In my study, there were no seasonal fluctuations in initial leptin levels, and no

correlations between leptin levels and body mass or total body fat mass, as seen in some other mammals (Hissa et al. 1998; Backus et al. 2000; Mustonen et al. 2000; Martin et al. 2001; Sagawa et al. 2002). My observations counter leptin studies in rodents and humans, but are consistent with other leptin studies in pinnipeds (Rea and Nagy 2000; Ortiz et al. 2001a, 2001b; Arnould et al. 2002). It therefore appears that leptin is not likely a reliable indicator of body fat reserves in Steller sea lions.

The lack of significant seasonal fluctuations of cortisol, thyroid hormones or leptin in my study does not explain the highest body fat mass in spring, or lowest standard metabolic rate in summer (Chapter II). Further studies with measurements of free and reverse forms of hormones are required to shed greater light on the relationship of seasonal variations among hormones, body conditions and metabolism.

3.4.2 Seasonal effects of short-term low energy intake on serum hormone level changes

Steller sea lions responded differently to short-term low energy intake at different times of the year. Differences in changes in body mass (Chapter II) and hormone levels were observed in certain seasons suggesting that the effects of diet restriction on Steller sea lion health are seasonally dependent. Differences due to the type of diet (herring or pollock) suggest that food quality has an additional effect at certain times of the year.

Under restricted energy intake, body mass loss (Table 3.1; Chapter II), cortisol increase, TT3 decrease, and BUN increase were greatest in winter (Fig. 3.4). Conversely, body mass loss and cortisol increase were the least and BUN decrease was the greatest in summer. These data suggest that Steller sea lions were most susceptible to food restriction during colder seasons (non-breeding seasons), and that they were probably less sensitive in warmer seasons (breeding-seasons).

In my study, the impact of diet type (herring or pollock) depended on season. However, the nature of these effects was not always uniform across seasons. This highlights the complexity of understanding the effects of nutrition on Steller sea lion health. It is also likely that the effects of reduced energy intake masked some

potential differences due to diet given the low levels of food intake. It is also possible that diet quality-induced differences were not manifest or detectable over such a short experimental period.

After short-term restricted feeding in winter, cortisol levels in Steller sea lions were dramatically elevated (Fig. 3.4). Cortisol is required for free fatty acid mobilization during periods of tissue catabolism (Goodman and Knobil 1961), and elevated levels have been demonstrated in fasting (Ortiz et al. 2001a, 2001b, 2003a) and lactating (Englehardt and Ferguson, 1980; Guinet et al. 2004) pinnipeds. During starvation, corticosteroid-binding globulin, one of the major carrier-proteins for cortisol, increases, which may reduce cortisol clearance (Aron et al. 2001). It is not known whether the elevated cortisol levels in the Steller sea lions were the result of increased production or decreased clearance rates. However, it seems more likely that cortisol production increased because total protein levels in the circulation decreased during the trials (data not shown). Increased stress levels, measured by elevated cortisol levels, did not influence the changes in metabolic rate in Steller sea lions (Chapter II). Similarly, elevated cortisol concentrations did not result in elevated metabolic rates in grey seals (Boily 1996).

In my study, there was a negative correlation between cortisol and body mass, and total body fat (initial and final values were pooled for absolute and percentage), similar to those reported for post-weaning, fasting northern elephant seal pups (Ortiz et al. 2001a, 2003a) and lactating subantarctic fur seals (Guinet et al. 2004). The stronger negative correlation found in my study between body condition and cortisol levels suggests that cortisol informs the sea lion body of critical changes in body condition, i.e., low total body fat levels, as suggested by Ortiz et al. (2001a, 2001b, 2003a). If cortisol was an indicator of stress level in Steller sea lions, as in other mammals, my results could mean that Steller sea lions were under great stress from short-term low energy intake in winter due to an insufficient energy intake.

In my study, glucose levels did not change during periods of food restriction. Decreased food intake or fasting usually decreases glucose level (DelGiudice et al. 1987; Wolkers et al. 1994; Bossart et al. 2001), although glucose levels in post-weaning fasting harp seals were not different from those in feeding animals

(Worthy and Lavigne 1982). Fish contains almost no carbohydrates; therefore, fish eating mammals have to derive glucose from non-carbohydrate sources via gluconeogenesis. The observation that Steller sea lions in my study did not significantly change glucose levels in response to reduced food intake (and that glucose levels increased in some animals) suggests that the animals probably increased gluconeogenesis to maintain a constant supply of glucose to the vital organs (such as the brain), or decreased rate of glucose utilization. Since cortisol facilitates glucose synthesis from non-carbohydrate sources (Aron et al. 2001), the elevated cortisol observed in the present study may be crucial to maintaining glucose supply during energy restriction in Steller sea lions.

When glucose is depleted, animals turn to body fat reserves as their primary energy source, increasing lipolysis and decreasing BUN levels (Castellini and Rea 1992). This state is referred to as the second stage of fasting (Cherel et al. 1992). If fasting is prolonged, animals turn from body fat mass to lean tissues for energy, usually in the form of muscle. BUN levels are elevated during this third stage of fasting (Castellini and Rea 1992). The increase of BUN in the later stage of fasting is more likely due to increased body protein catabolism and decreased fat catabolism (Wolkers et al. 1994).

However, BUN levels in food-restricted animals are the product of both tissue catabolism and food protein digestion. The elevated levels of BUN during the winter and fall trials (Fig. 3.4) suggest that the sea lions catabolized more lean tissues for energy, likely to conserve their body fat for insulation during food restriction. This is supported by the elevated levels of cortisol during these periods, since cortisol facilitates gluconeogenesis from body proteins in order to maintain glucose supply. In contrast, Steller sea lions probably utilized body fat reserves as a primary energy source in the spring and summer. The greatest decrease of BUN in the summer herring-fed group supported the finding that body mass loss was comprised exclusively of total body fat loss in this group (Chapter II). Decreased BUN levels suggest that increased energy consumption and more efficient dietary protein metabolism (DelGiudice et al. 1992).

Small seasonal variations in thyroid hormone levels could have a large impact

on fat metabolism, even though thyroid hormone levels are very low in pinnipeds (Renouf and Noseworthy 1991). Steller sea lions decreased their thyroid hormone levels after short-term restricted diet intake, as has been shown to occur for deer during fasting (DelGiudice et al. 1987) and goats during restricted food intake (only T3 decreased; Gómez-Pastén et al. 1999). While both TT3 and TT4 levels increased in northern elephant seal pups during their post-weaning fasting this is believed to be due to decreased clearance levels from the circulation or decreased deiodination of T4, rather than increased production of thyroid hormones (Ortiz et al. 2001b, Ortiz 2003a). Deiodination from T4 to T3 is also affected by cortisol level (St. Aubin 2002). This may explain why I observed the greatest decrease in TT3 when the greatest increase was observed in winter.

Changes in TT3 may have a greater impact on the metabolism of Steller sea lions because TT3 is the biological active form of thyroid hormones. In my study, there was a trend for greater percent changes (-28%) in TT4 levels after feeding restricted amounts of herring compared to pollock (-9%). In contrast, changes in TT3 were similar in both diet groups (-15% and -12%, respectively), but were seasonally dependent, with the greatest decrease occurring in winter (-35.6%). This suggests that T3 production in the thyroid gland or deiodination from T4 to T3 produced enough TT3 to maintain seasonal metabolic activities, even though nutrition (prey quality) could influence the production of T4 in the thyroid gland. This was seen in some of the feeding groups whose TT3 levels increased while TT4 levels decreased. Campbell and MacArthur (1998) suggested that nutritional and energy intake may regulate metabolic rate via changing T4 levels in brown lemmings (*Lemmus sibiricus*). In my study, the TT3/TT4 ratio decreased in both groups in winter and increased in the both groups in summer and fall. Although the TT3/TT4 ratio may control seasonal variations in standard metabolic rate as explained earlier, it is unlikely that the thyroid hormone changes induced by reduced food intake control the metabolism in Steller sea lions.

Leptin decreases energy intake and serves to regulate body fat in many mammals. Decreased leptin levels are usually observed during periods of energy restriction, given that leptin is secreted from adipose tissue and animals typically lose

body fat during these periods (Salfen et al. 2003; Fuglei et al. 2004). Decreases in leptin levels usually increase appetite, a phenomenon frequently observed in experimentally fasted animals (Horton et al. 2000; Wauters et al. 2000). One captive Steller sea lion study showed that leptin levels increased over the course of a short-term fasting regime in females, but decreased in males (Rea and Nagy 2000). Although I observed opposite responses between the two diet groups within each season, I was unable to find significant overall effects of diet types.

Pinnipeds usually depress metabolic rate during fasting to minimize energy expenditure (Markussen et al. 1992; Rosen and Trites 1999). Comparing the trend of changes (not statistically tested), serum leptin level was increased when standard metabolic rate was depressed (Chapter II), indicating traditional responses to food restriction: metabolic depression and appetite suppression. Conversely, some animals showed decreased levels of leptin with an elevated standard metabolic rate (Chapter II). It appears that increased leptin levels are a normal response during food restriction with a concurrent decrease in resting metabolic rate. Steller sea lions may resist unpredicted food shortages by increasing leptin levels so as not to feel "hungry". It has been shown that there is a negative feedback loop between leptin and glucocorticoids, such that glucocorticoids stimulate leptin gene expression in adipose cells (reviews in Houseknecht et al. 1998; Harris 2000; Wauters et al. 2000). Further studies on the relationships among leptin, cortisol and metabolic rate in Steller sea lions are required.

Many studies have shown seasonal variability in response or sensitivity of animals to leptin (Boyer et al. 1997; Clarke et al. 2000). It is possible that Steller sea lions have variable sensitivities to leptin at different times of the year, and that the function of leptin differs in sea lions from other mammalian species, as suggested by Arnould et al. (2002). It should also be noted that, even in humans, the relationship between leptin and lipid mass is very complex (Kronfeld-Schor et al., 2000; Domali and Messinis 2002). In my study, the concurrent decreases in metabolic rate and increases in leptin concentration may suggest that elevated levels of leptin could act as a fasting response initiator (although not statistically tested).

Ghrelin levels of the captive sea lions correlated negatively with body mass,

signifying that this hormone likely plays a similar role in Steller sea lions as reported for lab rodents and human (Ravussin et al. 2001; Tshcöp et al. 2001; Shiiya et al. 2002; Beck et al. 2002), whereby obese subjects have higher leptin levels and lower ghrelin levels than lean subjects (Tshcöp et al. 2001). This suggests that ghrelin levels should have also increased in response to body mass loss from the restricted energy intake (Asakawa et al. 2001; Melis et al. 2002) although ghrelin's role in increasing the food intake of an animal is relatively short-lived due to its brief half-life (1-2 hours; Wren et al. 2000; Tshcöp et al. 2001). However, out of the four complete pairs of samples, ghrelin levels increased in only one animal in the fall, while decreasing in the other three (Table 3.2).

A previous study of ghrelin levels in pinnipeds reported that northern elephant seal pups had increased ghrelin levels during a post-weaning fast, while experiencing decreased body and body fat mass (Ortiz et al. 2003b). Ghrelin injections have been shown to increase carbohydrate utilization and to decrease fat utilization and energy expenditure in lab rodents (Asakawa et al. 2001; Wren et al. 2001). Therefore, an increased ghrelin level during fasting likely represents a more energy efficient metabolism during starvation (Tshcöp et al. 2001). Since body fat reserves are primarily utilized during starvation (Cherel et al. 1992), decreased levels of ghrelin would assist in utilizing body fat and conserving lean tissue mass. Since only four serial samples (initial and end) were successfully obtained, no concrete conclusions could be made about the influence of the dietary trials in this study on the level of ghrelin in sea lions.

The lack of correlation between ghrelin and leptin levels raises further questions about the role of ghrelin in Steller sea lions. As with leptin — which appears to play a different role in Steller sea lions than in other mammals— the role of ghrelin could also be different. Ghrelin is known as a “meal initiator” hormone since levels increase around mealtime (Cummings et al. 2001; Wren et al. 2001; Cummings et al. 2002; Shiiya et al. 2002). Feeling “hungry” is probably not practical for a Steller sea lion when food intake is limited due to predictable changes in prey or foraging opportunities. Instead, increased leptin levels and decreased ghrelin levels could function to enhance survival mechanisms during prolonged

periods of food deprivation. Unfortunately, the data in my study are insufficient to clarify ghrelin endocrinology in Steller sea lions. However, future research should explore the role of leptin and ghrelin in Steller sea lions.

3.4.3 Ecological implications

Walleye pollock is one of the major winter prey items of Steller sea lions in western Alaska (Sinclair and Zeppelin 2002). It is also the second most abundant groundfish stock in the Gulf of Alaska and, therefore a major species targeted by fisheries (NMFS 1998). In the Bering Sea/Aleutian Islands region, the major pollock-fishing season was switched from spring/summer (April-September; 70-80% of the landing) to fall/winter (September-March; 35-65%) in 1987, in order to more effectively target roe-bearing fish (NMFS 1998). In the Gulf of Alaska, the peak pollock fishing season has been fall/winter since 1964, and 70% of all catches occurred in the fall/winter season even after the reduction of pollock biomass in the late 1980s (NMFS 1998). In relation to my study, these peak periods of pollock fishing season coincide with the time frame within which sea lions would experience the most severe impacts from reduced food intake.

The diet composition and average energy density of Steller sea lion prey are seasonally different (Sinclair and Zeppelin 2002; Winship and Trites 2003; Kitts et al. *in press*). Since the energy density of prey is lower in winter and spring, wild sea lions require greater amounts of fish to obtain sufficient energy for survival (Winship and Trites 2003). Although diet did not have a significant effect on total body fat mass changes in my study, total body fat mass loss constituted a higher proportion of body mass lost in the winter, spring, and fall seasons under a pollock diet than when feeding on herring. Given the thermal and energetic importance of their lipid layer, this diet-based effect may magnify the potential effects of insufficient prey on sea lion survival.

Pollock are typically found in the 100-200 m bottom depth and the 0-20 nautical mile distance range (Shima et al. 2002), but the distribution likely varies across season and location (NMFS 1998). Diving patterns of juvenile Steller sea lions are shallower and shorter (time and distance) than adults (Merrick and Loughlin

1997; Loughlin et al. 2003), suggesting that juveniles may expend more effort searching for food. If pollock were locally depleted by fishing or marine mammals in winter in the declining area, juvenile Steller sea lions would be the most vulnerable group at a time of year when they appear physiologically most susceptible to nutritional stress. This could provide a potential explanation for the higher mortality rate of juvenile groups during the dramatic population decline of Steller sea lions (York 1994).

3.5 Conclusion

Glucose was the only blood parameter measured that showed significant fluctuation throughout year, which likely corresponded with seasonal fluctuations in total body fat mass. Cortisol could play an important role in regulating the seasonal changes observed in body mass and total body fat. Cortisol is likely responsible for initiating hair loss, while thyroid hormones may promote new hair growth. Leptin did not appear to regulate or be an indicator of seasonal changes in body condition in Steller sea lions, unlike in lab rodents or humans.

The responses of blood parameters to restricted energy intake were seasonally dependent. This seasonal effect was especially prominent in cortisol, TT3 and BUN levels. All three showed greatest increased levels in winter, while the least increase of cortisol and the greatest decrease of BUN took place in the summer. These responses paralleled the greater rates of body mass loss observed in winter (Chapter II). These findings support the conclusions that Steller sea lions may be more susceptible to food restriction in the colder months (the non-breeding period) compared to the warmer months (the breeding and moulting period). Diet composition had little effect on changes in blood parameters as a whole. However, the type of prey (herring or pollock) appeared to have had season-specific effects on body condition and hormone levels. Decreased body fat reserves induced by decreased food intake possibly sent signals to the adrenal cortex to increase cortisol production. Increased level of cortisol decreased deiodination rate from T4 to T3 resulting decreased TT3 level. This, in turn, may have made Steller sea lions physiologically 'aware' of their poor condition. Elevated cortisol levels perhaps

facilitated gluconeogenesis by metabolizing lean tissue, which may have resulted in the elevated BUN. These responses could be the physiological set-point of body mass regulation that defends/maintains an appropriate body mass to prevent further body mass loss at the extreme condition. However, the function of hormones may differ at different times of the year, since these hormones are also associated with moulting in Steller sea lions. Although leptin and ghrelin did not appear to fulfill the same functions witnessed for other animals, they could possibly play a key role initiating a traditional fasting response in sea lions with increased leptin levels (and decreased ghrelin level) suppressing metabolism (metabolic depression).

3.6 References

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Chapter IV: The potential for anemia during restricted consumption of pollock by Steller sea lions (*Eumetopias jubatus*)

4.0 Summary

Steller sea lions (*Eumetopias jubatus*) were fed an iso-caloric and iso-iron content restricted diet of Pacific herring (*Clupea pallasii*) or walleye pollock (*Theragra chalcogramma*) for up to 9 days, four times per year, resulting in a loss of approximately 10% of their initial body mass. Changes in iron binding capacity total (IBCT) were significantly greater in the herring-fed group ($-30.0 \pm 6.1\%$) than in the pollock-fed group ($-20.2 \pm 10.2\%$), and the significantly greater decrease occurred in winter and spring ($\sim -30\%$) than in summer and fall ($\sim -23\%$). Iron saturation increased by $2.3 \pm 4.4\%$ in the herring-fed group, while it decreased by $3.8 \pm 4.1\%$ in the pollock-fed group ($P < 0.05$). Although not statistically significant, there was a trend that the pollock-fed animals lost a greater proportion of serum iron ($-33.1 \pm 18.5\%$) compared to those fed herring ($-15.1 \pm 26.3\%$). These results suggested a potential anemia from a reduced diet of pollock in Steller sea lions. Serum iron, IBCT, glucose, calcium, phosphorus, hematocrit and gamma glutamyltransferase showed consistency in changes of direction suggesting that these may serve as indicators of nutritional stress in Steller sea lions.

4.1 Introduction

Studies of the nutritional effects of alternative diets on Steller sea lions have focused primarily on the effect of macronutrient content (i.e., lipid concentration) on sea lion body mass and composition (e.g., Rosen and Trites 2000; Trumble et al. 2003). However, other dietary components may also affect fish-eating mammals (Geraci 1975). For example, mink kits fed gadid fish species showed symptoms collectively called "cotton fur", which were induced by anemia due to a decreased absorption of iron in the intestine (Helgebostad and Martinsons 1958; Stout et al. 1960). Thompson et al. (1997) also reported possible macrocytic anemia in wild harbour seals when they consumed a higher proportion of gadids. Such observations raise the possibility that the high proportion of gadids consumed by Steller sea lions in the Gulf of Alaska during the 1980s and 1990s (Sinclair and

Zeppelin 2002) may have negatively affected sea lion health and contributed to their population decline.

The following reports the results of a controlled feeding experiment with captive Steller sea lions that was designed to explore potential blood indicators of nutritional stress (including those attributable to prey composition) that might be indicative of specific health effects and that might be used to identify nutritional stress among wild sea lions. The study also considered how the effects of restricted food intake might be further compounded by the potential effects of iron deficiencies. Plasma iron concentrations were measured during the year long study, along with a broad suite of other clinical hematology and blood biochemistry parameters that are routinely measured by commercial laboratories to assess animal health.

4.2 Methods

The basic protocol for this experiment was the same as outlined in Chapters II and III. In brief, seven captive female Steller sea lions were fed an iso-caloric sub-maintenance (20% of maintenance) diet of either Pacific herring or walleye pollock for up to 9 days, four times over the course of a year. Blood samples were obtained into EDTA and serum separator collection tubes on the first day of the experiment, and after the feeding trials were completed (9th or 10th day). Routine clinical analyses of 34 blood parameters were conducted at Central Veterinary Laboratory (Langley, British Columbia). The broad suite of parameters were chosen as they could be reliably measured in free-ranging sea lions using different commercial laboratories and (on an experimental level) their inclusion eliminated preconceptions about which parameters would respond to changes in food consumption. Iron content analyses of fish samples were conducted by Cantest (Burnaby, British Columbia).

Care was taken in the statistical analyses of the 34 blood parameters to minimize the likelihood of making a Type I error. Therefore, the consistency of the direction of changes was determined as the percentage of animals that increased or decreased their values of blood parameters after the feeding treatment in both diet types. A sign test was used to determine significant levels of consistency of changes.

All percentages were normalized using arc-sine transformations prior to the statistical analyses. Two way repeated measures ANOVAs were used to evaluate the effect of diet and season on changes from the initial parameter values (SAS, version 8.2, SAS institute, Care, NC). I selected the most parsimonious model and calculated post hoc tests of specific differences (SAS).

4.3 Results

4.3.1 Consistency of blood parameter changes

Of the 34 blood parameters measured, seven displayed significantly consistent changes during food restriction (Table 4.1). Phosphorous, glucose and calcium values consistently decreased during the trials, while gamma glutamyltransferase and hematocrit values consistently increased. Both total iron binding capacity (IBCT, a measure of the protein carrier, transferrin) and serum iron levels decreased after consuming restricted amounts of herring or pollock (Table 4.1). However, another measure of blood iron — serum percent iron saturation — did not change consistently during the trials. Given the hypothesized link between high gadid intake and anemia, these parameters were further examined in relation to each diet.

None of the other parameters changed consistently according to diet. That is, none of the blood parameters always increased while the sea lion ate pollock and always decreased while eating herring, or vice-versa.

4.3.2 Iron-related parameters

The pre-experimental values of serum iron were similar across seasons ($20.9 \pm 5.2 \mu\text{mol l}^{-1}$, $F_{3,18}=0.61$, $P=0.61$). During the trials, pollock-fed animals lost a greater proportion of serum iron ($-33.1 \pm 18.5\%$) compared to the herring-fed animals ($-15.1 \pm 26.3\%$; $F_{1,5}=6.29$, $P=0.054$; Fig.4.1). Season did not have a significant effect on iron concentrations ($F_{3,13}=0.89$, $P=0.47$).

There were significant seasonal differences in pre-experimental total iron binding capacity (IBCT) ($F_{3,18}=6.61$, $P=0.003$). These were highest in winter ($71.6 \pm 6.8 \mu\text{mol l}^{-1}$), followed by summer ($65.4 \pm 8.9 \mu\text{mol l}^{-1}$), spring ($62.1 \pm 6.6 \mu\text{mol l}^{-1}$).

l^{-1}), and fall ($60.3 \pm 5.1 \mu\text{mol l}^{-1}$). Changes in IBCT during the treatments were significantly greater in the herring-fed group ($-30.0 \pm 6.1\%$) than in the pollock-fed group ($-20.2 \pm 10.2\%$; $F_{1,5}=15.0$, $P=0.012$; Fig.4.1). There was also a seasonal component to changes in IBCT. Decreases in IBCT were greatest in winter ($-29.8 \pm 3.5\%$) and spring ($-30.1 \pm 11.1\%$), and lowest in summer ($-23.4 \pm 12.0\%$) and fall ($-19.9 \pm 5.1\%$; $F_{3,13}=4.13$, $P=0.029$; Fig. 4.2).

Pre-experimental values of percent iron saturation were similar across season ($24.8 \pm 5.5\%$, $F_{3,18}=1.08$, $P=0.38$). Iron saturation increased by $2.3 \pm 4.4\%$ in the herring-fed group and decreased $3.8 \pm 4.1\%$ in the group fed pollock ($F_{1,5}=13.6$, $P=0.014$; Fig.4.1). There was no seasonal effect on changes in iron saturation ($F_{3,13}=0.64$, $P=0.60$).

4.3.3 Iron intake

Mean proximate iron content was 0.18 mg g^{-1} wet weight for Pacific herring and 0.07 mg g^{-1} wet weight for walleye pollock. Since the herring-fed group consumed $1.3 \pm 0.25 \text{ kg}$ of fish and the pollock-fed group consumed $3.1 \pm 0.86 \text{ g}$ of fish, mean daily iron intakes were $228.4 \pm 43.8 \mu\text{g}$ in the herring-fed group and $219.2 \pm 60.0 \mu\text{g}$ in the pollock-fed group. There was no statistically significant difference in these iron intake levels ($t_{2,26}=0.469$, $P=0.643$).

Table 4.1 The consistency of changes in blood parameter values during the feeding treatment from the initial values (n=28; except for globulin, albumin/globulin ratio, sgot, serum iron, IBCT and iron saturation where n=27) and the mean (\pm SD) values of initial and final. The significance level ($\alpha=0.05$) was met when 70% of the trials exhibited a change in a consistent direction (shown in bold). Data are sorted by % increased.

	Decreased (%)	Increased (%)	Initial	Final
Iron binding capacity total (IBCT) ($\mu\text{mol/l}$)	100.0	0.0	64.9 \pm 7.9	48.0 \pm 6.2
Serum iron ($\mu\text{mol/l}$)	81.5	11.1	20.9 \pm 5.2	15.3 \pm 4.1
Phosphorus (mmol/l)	82.1	17.9	2.1 \pm 0.3	1.9 \pm 0.2
Mean platelet volume (fl)	14.3	17.9	9.5 \pm 1.2	9.5 \pm 1.5
Calcium (mmol/l)	78.6	21.4	2.4 \pm 0.1	2.3 \pm 0.1
Glucose^a (mmol/l)	75.0	21.4	6.7 \pm 0.7	6.24 \pm 0.4
Albumin (g/l)	57.1	21.4	41.3 \pm 1.5	40.2 \pm 1.5
Globulin (g/l)	59.3	22.2	33.1 \pm 2.9	32.0 \pm 2.6
Mean corpuscular volume (MCV) (fl)	35.7	28.6	103.0 \pm 5.4	102.9 \pm 4.8
Albumin/globulin ratio	25.0	28.6	1.3 \pm 0.1	1.3 \pm 0.1
Alkaline phosphatase (ALP) (i μ /l)	64.3	32.1	88.9 \pm 27.4	77.8 \pm 16.5
Blood urea nitrogen (BUN) ^a (mmol/l)	64.3	35.7	8.0 \pm 1.0	7.6 \pm 1.7
White cell count (WBC) (10 ⁹ /l)	57.1	35.7	5.5 \pm 1.4	5.5 \pm 1.5
Iron saturation (%)	51.9	37.0	24.8 \pm 5.5	24.0 \pm 5.6
Platelet count (10 ⁹ count)	60.7	39.3	324.9 \pm 75.7	311.2 \pm 59.7
Creatinine ($\mu\text{mol/l}$)	57.1	39.3	90.4 \pm 11.2	89.4 \pm 17.4
Mean corpuscular hemoglobin concentration (MCHC) (g/l)	53.6	39.3	345.5 \pm 5.2	344.5 \pm 5.5
Mean corpuscular hemoglobin (MCH) (pg)	53.6	42.9	35.6 \pm 1.8	35.4 \pm 1.8
Carbon dioxide (mmol/l)	42.9	42.9	27.3 \pm 1.6	27.2 \pm 2.9
Potassium (mmol/l)	28.6	46.4	3.7 \pm 0.2	3.7 \pm 0.2
Anion gap	39.3	50.0	15.9 \pm 1.7	16.5 \pm 2.5
Creatinine phosphokinase (i μ /l)	46.4	53.6	192.5 \pm 189.6	410.9 \pm 726.2
Hemoglobin (g/l)	42.9	53.6	152.3 \pm 14.5	153.6 \pm 13.9
Sgpt (alt) (i μ /l)	42.9	57.1	72.3 \pm 17.5	79.3 \pm 27.3
Calculated osmolality (mmol/kg)	35.7	57.1	297.4 \pm 3.9	298.7 \pm 3.8
Sgot (ast) (i μ /l)	40.7	59.3	11.3 \pm 5.6	16.8 \pm 15.4
Red blood cell distribution width	39.3	60.7	16.8 \pm 1.4	17.0 \pm 1.6
Total protein (g/l)	35.7	60.7	74.4 \pm 2.7	72.3 \pm 2.6
Chloride (mmol/l)	25.0	60.7	109.0 \pm 2.1	109.6 \pm 2.6
Red cell count (RBC) 10 ¹² /l	35.7	64.3	4.3 \pm 0.3	4.3 \pm 0.3
Sodium (mmol/l)	17.9	64.3	148.3 \pm 1.9	149.4 \pm 2.0
Bilirubin total ($\mu\text{mol/l}$)	14.3	64.3	3.5 \pm 1.0	4.3 \pm 1.1
Hematocrit (l/l)	28.6	71.4	0.44 \pm 0.04	0.45 \pm 0.04
Gamma glutamyltransferase (iμ/l)	10.7	85.7	89.0 \pm 21.9	101.3 \pm 28.7

a=data were shown in Chapter III.

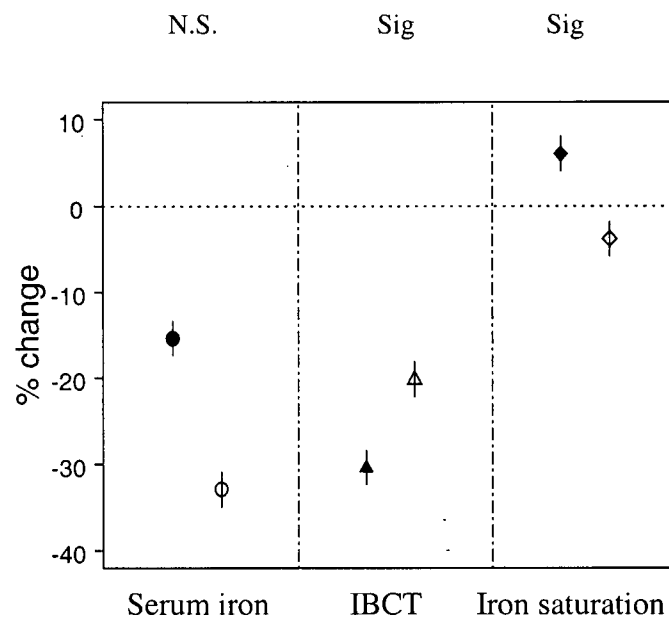


Fig. 4.1 Mean \pm SEM percent changes in serum iron levels (circle), IBCT (triangle), and percent iron saturation (diamond) for the herring-fed group (closed: $n=15$) and the pollock fed group (open: $n=12$). There was a significant effect of diet ($P<0.05$) on IBCT and iron saturation.

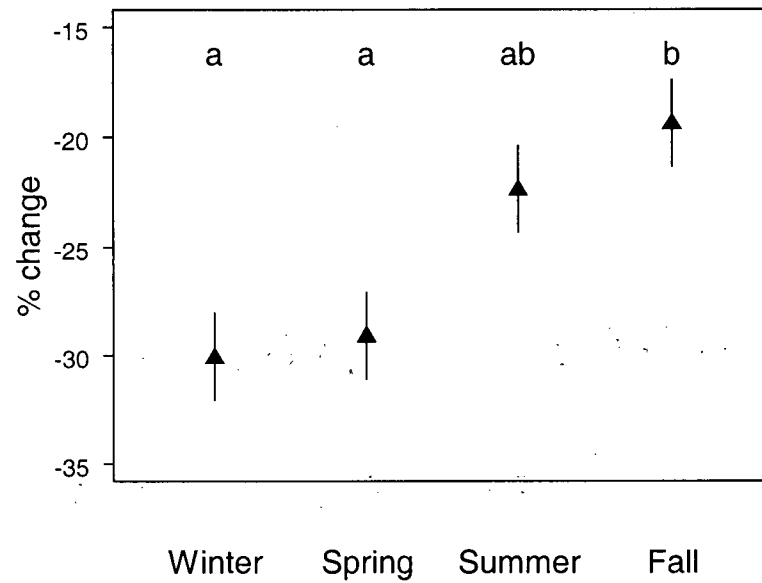


Fig. 4.2 Mean \pm SEM percent changes in IBCT in winter, spring, summer and fall. Sample size = 7 for each season except for winter where $n=6$. There was a significant effect of season ($P<0.05$), with different letters indicating significant differences.

4.4 Discussion

4.4.1 Signs of nutritional stress in Steller sea lions

Differences induced by diet type were observed during the feeding trials in two iron-related parameters, IBTC and percent iron saturation, but not in the serum iron level. Serum iron level decreased more in the pollock-fed group than in the herring-fed group, although total dietary iron intake was similar between the two. Iron circulating in the body is typically bound to hemoglobin, myoglobin, cytochrome proteins, or protein carriers (transferrin, lactoferrin, ferritin), with only small amounts remaining in their free form (Bossart et al. 2001). Iron is transferred to the liver and spleen for storage, or transported to the site of hemoglobin formation (bone marrow) (Smith 1997). Chronic iron deficiency is indicated by decreased levels of serum iron, IBCT (a measurement of transferrin), and percent iron saturation (the percentage of circulating iron bound to transferrin; Mroz 2003).

It is unclear whether the Steller sea lions in my experiment experienced iron deficiency anemia. While RBC, hemoglobin, and hematocrit levels increased, and MCV, MCH, MCHC decreased, none showed statistically consistent changes (data not shown). The hydration status of the animal is critical in such an evaluation because anemia can be masked by dehydration (Bossart et al. 2001). Moderate dehydration was suspected and could have potentially masked signs of an iron deficiency in these Steller sea lions. Hemoconcentration is often associated with lower food intake (Harlow and Seal 1981; Knick et al. 1993). Although hemoglobin is often considered a primary parameter for assessing iron deficiency anemia, signs of iron deficiency only gradually appear in hemoglobin (Smith 1997).

A longer-term (30 d) restricted diet study found that sea lions consistently increased hematocrit, hemoglobin, red cell count, and gamma glutamyltransferase while they decreased alkaline phosphatase, phosphorus, platelet count, white cell count and serum iron (Rosen et al. 2004). I observed similar consistency in serum iron, phosphorus, hematocrit and gamma glutamyltransferase (Table 4.1). This therefore suggests that these four parameters could be useful for diagnosing inadequate food intake in Steller sea lions.

A number of previous studies on nutritional stress have reported various

blood parameters in Steller sea lions. The majorities of these studies have been on pups and have focused on hematological parameters (WBC, RBC, hematocrit, hemoglobin, etc.; Calkins and Goodwin 1988; Bishop and Morado 1995; Castellini et al. 1993; Rea et al. 1998; Rea et al. 2000) or energy utilization parameters (e.g., ketone, non-esterified fatty acids, glucose, BUN; Castellini et al. 1993; Rea et al. 1998; Rea et al. 2000). However, none of these studies reported serum irons, phosphorous, or gamma glutamyltransferase. Future studies of nutritional stress should consider including these three parameters, as well as measures of hydration state given its effect on other hematological parameters.

4.4.2 The possible effects of a reduced pollock diet on Steller sea lions

It is not clear why the diet-related differences in iron-related parameters occurred amongst the sea lions. Although pollock has a lower iron content than herring, the iso-caloric nature of the experimental diets resulted in a similar intake of iron between the two groups (herring = $228.4 \pm 43.8 \mu\text{g}$, pollock = $219.2 \pm 60.0 \mu\text{g}$). One possible explanation could be related to the differences in the amounts of protein ingested from the two diets. The sea lions received 0.21 g d^{-1} in the herring-fed trials, compared to 0.53 g d^{-1} during the pollock-fed trials. Decreased protein intake during the herring-fed trials may have resulted in decreased IBCT levels given that protein plays an important role in transporting iron (Campos et al. 1996).

Another possible explanation for my findings is that the type of iron (heme or non-heme) or bioavailability of iron may have differed between herring and pollock. Heme iron is generally abundant in fish, and is more easily absorbed into the body than non-heme iron (whose absorption is influenced by other ingredients in the diet; Smith 1997). If the type of iron differed between pollock and herring the different diets could have triggered a different rate of iron absorption or metabolism in the body, resulting in the observed changes.

In a study on mink, a diet high in raw gadid fish (codfish and whiting) resulted in anemia, and caused a symptom known as "cotton fur" in mink kits, where they developed a drab, white color and flimsy hair texture (Helgebostad and Martinsons 1958; Stout et al. 1960). Iron is required in higher concentrations than

normal to support growing fur (Rouvinen et al. 1998). Feeding on raw gadid fish decreased iron absorption in the intestine of anemic mink, which could be negated by boiling the fish prior to feeding (Harvre et al. 1967). Subsequent studies showed that the trimethylamine oxide (TMAO) found in raw fish precipitates iron-forming insoluble crystalline ferric oxide-hydroxides, which prevents iron from being absorbed into the lumen of the intestine (Ender 1972; Ender et al. 1972; Ender and Helgebostad 1972). TMAO levels in fish vary across seasons and regions, and are found in higher concentrations in colder waters (Raymond 1998). However, a high composition of TMAO in the diet did not necessarily induce cotton fur in mink (Rouvinen et al. 1998), and the reason why boiling the fish prior to ingestion reduced anemia in mink remains unknown (Ender et al. 1972).

It is unclear whether gadid diets inevitably trigger the malabsorption of iron, since the amounts of TMAO in gadids and clupeids are intermediate (Ender and Halgebostad 1972). Even among fish from the same gadoid family, the level of reaction with TMAO varies due to the different iron types in fish. Spinelli and Koury (1981) showed that heme iron (Fe^{++}), which is abundant in Pacific whiting muscle, reacted with TMAO, while non-heme iron (Fe^{+++}), which is abundant in the muscle of rockfish and Dover sole, did not.

Based largely on the aforementioned mink studies, Geraci (1975) stressed the possibility of anemia in marine mammals feeding on high amounts of gadids. Data on serum iron levels in pinnipeds are limited compared to other parameters (Vallyathan et al. 1969; Kuiken 1985). Kuiken (1985) published a short note mentioning that the iron level of harbour seals fed mainly plaice and flounder was significantly lower than when fed on mackerel. However, no detailed information on the study design or values was presented. The lack of information on circulating iron levels in pinnipeds makes difficult to assess whether sea lions experienced iron deficiency. For clinical purposes, the Vancouver Aquarium Marine Science Centre uses values provided by Central Veterinary Laboratory (Surrey, British Columbia) where the range for serum iron is between 12 and 48 $\mu\text{mol l}^{-1}$ (reference values for IBCT is not provided). Of the 27 samples in our study (pre- and post-experimental samples), 5 post-experimental samples were lower than 12 $\mu\text{mol l}^{-1}$. However,

these low serum iron levels were not concurrent with any decreased levels of hematological parameters.

A study of wild harbour seals showed that RBC, hemoglobin, and MCHC levels were lower in a year characterized by high gadid prey intake (compared to a high clupeid year), suggesting a link between macrocytic anemia and gadid consumption (Thompson et al. 1997). However, Trites and Donnelly (2003) questioned whether iron deficiency could be related to a gadid diet, since a TMAO-induced iron deficiency usually occurred when mink were fed frozen, and not fresh fish. A 6-week feeding trial of previously frozen pollock on three Steller sea lions showed no significant change in hematological and iron related parameters ensuing from the diet (Azana 2002). It is therefore possible that my finding on animals feeding on reduced amounts of pollock could have been influenced by a combination of the restricted amount and the fact they were frozen. Further research is required to determine the influence of fresh fish on iron metabolism.

Reduced iron intake can lead to decreased nutrient absorption in the stomach due to decreased stomach acid secretion (Smith 1997). Animals are known to lose weight and increase metabolic rate under an iron deficient diet (Henderson et al., 1986; Stangl and Kirchgessner 1998; Lewis et al. 2001; Yamagishi et al., 2000). They also experience elevated glucose levels. Steller sea lions in my study did not always increase their metabolic rate or glucose level (Chapter II and III). A lack of iron is of particular concern during pregnancy, as it can cause decreased maternal body mass and a decreased growth rate in the offspring (Lewis et al. 2001). Therefore, the possible role of iron deficiency should not be dismissed as a potential factor in the decline of Steller sea lions.

4.5 Conclusion

Iso-caloric and iso-iron content diets of herring or pollock revealed significant differences in IBCT and percent iron saturation changes caused by diet type. Although hematological parameters did not show typical clinical signs of iron deficiency, significant changes due to diet type in iron related parameters suggest a possible iron deficiency for Steller sea lions feeding on reduced amounts of pollock. Several additional blood parameters altered consistently during periods of nutritional stress. Changes in these parameters may serve as indicators of nutritional stress in Steller sea lions, and may help clarify the underlying physiological processes.

4.6 References

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Chapter V: Conclusion

Overall, my study revealed seasonal variations in body condition and standard metabolic rate in captive female Steller sea lions. None of the hormones I measured were likely responsible for these seasonal variations; however, cortisol and thyroid hormones could likely play an important role in moulting. Body mass was a good indicator of body fat mass, but was not a good primary indicator of body condition when the "condition" of animals was known (pre- or post- feeding experiment). Serum cortisol levels showed a negative relationship with body fat mass suggesting that cortisol is a more dependable indicator of body fat mass and play an important role in body fat regulation in Steller sea lions. The mean ghrelin level in Steller sea lions correlated negatively with body mass but not with serum leptin.

Feeding Steller sea lions restricted iso-caloric amounts of Pacific herring or walleye pollock for 8-9 days, four times a year, resulted in them losing an average of 10.1% of their initial body mass while on both experimental diets. The rate of body mass loss was seasonally dependent rather than diet dependent, being higher in winter ($1.6 \pm 0.14 \text{ kg d}^{-1}$), and lower in summer ($1.2 \pm 0.32 \text{ kg d}^{-1}$). Metabolic depression was not always observed during the trials despite the constant loss of body mass. Changes in cortisol, triiodothyronine and blood urea nitrogen (BUN) were also seasonally dependent. Over the course of the trials, serum levels of cortisol ($217.6 \pm 231.1 \%$) and BUN ($11.4 \pm 24.4 \%$) increased and total triiodothyronine ($-35.6 \pm 31.9 \%$) decreased the most in winter.

My findings support the hypothesis that restricted energy intake at different times of the year differently affects Steller sea lions, and that diet type (herring or pollock) may have seasonally-specific effects on body mass and composition. Steller sea lions may be more severely impacted by reduced energy intake in winter than other times of the year.

The sea lions fed herring lost a significantly greater proportion of iron binding capacity than those fed pollock ($-30.0 \pm 6.1 \%$ and $-20.2 \pm 10.2 \%$, respectively). A significantly greater decrease of iron binding capacity total

occurred in winter ($-29.8 \pm 3.5\%$) and spring ($-30.1 \pm 11.1\%$) compared to summer ($-23.4 \pm 12.0\%$) and fall ($-19.99 \pm 5.1\%$). Iron saturation increased in the herring-fed group ($2.3 \pm 4.4\%$) and decreased in the group fed pollock ($3.8 \pm 4.1\%$). These results suggested a potential iron deficiency anemia from a reduced diet of pollock in Steller sea lions. Serum iron, phosphorus, hematocrit and gamma glutamyltransferase showed consistent changes during food restriction, suggesting that these may serve as indicators of nutritional stress in Steller sea lions.

My study was not without limitations given that it relied on a small longitudinal sample size. As with all captive animal studies, care should be taken before extrapolating the short-term changes I observed in individual physiologies to parameters that would directly affect populations. Some of the techniques I employed (e.g., hormone assays, deuterium dilutions etc.) were developed for domestic species and have not been validated for sea lions. Lastly, using thawed herring and pollock may have influenced my results, especially in iron deficiency anemia.

Despite some of the shortcomings associated with captive feeding trials, my results showed the importance of season when considering the relationship between nutrition and health in Steller sea lions. Future studies should be conducted on young animals that consider Steller sea lion seasonal life cycles. Cortisol should receive further research consideration given that it may regulate body fat and appears to be a novel measure of stress levels in Steller sea lions. Micronutrients as well as macronutrients, should also be taken into consideration since they could have greater impact on sea lion health.