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ORIGINAL PAPER

# Hormone changes indicate that winter is a critical period for food shortages in Steller sea lions

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**Abstract** Given that many marine mammals display seasonal energetic priorities, it is important to investigate whether the impact of unexpected food restriction differs during the year. Steller sea lions (*Eumetopias jubatus*) fed restricted diets for up to 9 days during spring, summer, fall, and winter lost an average of 10% of their initial body mass. We tracked changes in the levels of three hormones (cortisol, total thyroxine—TT4, total triiodothyronine—TT3) and one blood metabolite (blood urea nitrogen—BUN) following a food restriction in relation to season, body mass, body composition, and metabolism. Degree of changes in cortisol, TT3, and BUN after food restriction was significantly affected by season. The greatest changes in cortisol (+231%), BUN (+11.4%), TT4 (−23.3%), and TT3 (−35.6%) occurred in the winter (November/December) when rates of body mass loss were also greatest. Changes in cortisol levels were positively related to total body mass loss, while changes in TT3 levels were negatively related. While greater increases in BUN were related to greater rates of mass loss, the use of BUN levels as an indicator of metabolic state is complicated by the type and level of food intake. The observed changes in hormone levels support morphological data suggesting Steller sea lions may be more strongly impacted by short-term, reduced energy intake during winter than at other times of the year.

**Keywords** Nutritional stress · Sea lions · Hormones

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## Introduction

Many mammals with seasonal life cycles exhibit distinct, predictable changes in metabolic rates, body mass, and body condition (e.g., Ashwell-Erickson et al. 1986; Fitzgerald and McManus 2000; Rosen and Renouf 1998) in accordance with underlying rheostatic changes. Given such seasonal differences in energetic priorities, the effect of unpredicted disturbances on an animal's energy budget may have varying consequences depending on the time of the year (Nunes et al. 2002; Owen-Smith 1994; Rea et al. 2007). Kumagai et al. (2006) reported that captive Steller sea lions (*Eumetopias jubatus*) fed a restricted diet for up to 9 days in different seasons lost greater amounts of body mass in winter (November/December) and smaller amounts in summer (May/June) indicating that unpredicted food shortages would result in greater nutritional stress during winter. In addition, the source of this mass loss (measured as lean tissue or lipid stores) and its relationship to standard metabolic rates (SMR) differed between seasons. These results suggest a complex, seasonally dependent physiological response to unpredicted periods of food restriction.

Hormones serve as a mechanism for mediating seasonal changes in physiology, including altering and defending seasonal set-points in body mass, body composition, and metabolism (Adam and Mercer 2001; Armitage and Shulenberger 1972; Ward and Armitage 1981). This study examined seasonal differences in initial levels of three hormones (triiodothyronine, thyroxine, cortisol) and one metabolite [blood urea nitrogen (BUN)] in captive Steller sea lions, and the extent of changes in the levels of these substances during short-term, seasonal food restriction in relation to changes in body mass, body composition, and metabolism.

Cortisol is one of the major glucocorticoids, and increased cortisol levels indicate high levels of stress (Hunt et al. 2004; Mashburn and Atkinson 2004; St. Aubin 2001), such as when triggered by their role as a defensive mechanism against nutritional insults (Kitaysky et al. 1999, 2001). Similarly, BUN can indicate increased protein utilization and has been traditionally used as an indicator of nutritional stress in wildlife management (Harder and Kirkpatrick 1994). The principal thyroid hormones, triiodothyronine (T3) and its less metabolically active precursor thyroxine (T4), affect the metabolism of mammals and can alter growth rates (McNabb 1992).

Understanding the physiological impact of potential changes in prey availability on free-ranging animals at different times of the year is particularly pertinent for understanding the decline of numerous pinniped (Small et al. 2003; Towell et al. 2006; Winship and Trites 2006) and seabird species (Byrd and Dragoo 1997) in the North Pacific. Parallel decreases in populations have led to the hypothesis that these changes are linked to a broad ecosystem-wide change in available prey species (although see National Research Council 2003). The >80% decline of Steller sea lions in western Alaska (Trites and Larkin 1996) has led to its classification as "Endangered" in the United States, resulting in legislative changes to fishing practices, including seasonal restrictions designed to lessen the potential impact of fishing on sea lions during "critical periods" of the year. These "critical periods" have been primarily defined by presumed seasonal differences in energy requirements, but not the potential physiological impact of decreased prey availability on individual animals.

Previous experiments with captive pinnipeds have shown a wide variety of responses (both within and between studies) in body mass and metabolism to changes in prey quantity and/or composition (Kirsch et al. 2000; Kumagai et al. 2006; Rosen and Trites 2005; Trumble et al. 2003). Many of these discrepancies may be explained by seasonal changes in hormonal responses and bioenergetic priorities. The following paper examines the seasonal relationship between key hormones and major components of the animal's energy budget during periods of food restriction. Results will provide a clearer understanding of the underlying physiological responses and bioenergetic priorities at different times of the year, and will shed light on the ability of certain blood parameters to be used as indicators of nutritional status.

## Materials and methods

### Study animals and manipulations

All procedures and protocols involving animals were conducted under the authority of University of British Columbia Animal Care Permit No. A98-0095. Study animals were held in an outdoor facility at the Vancouver Aquarium (British Columbia, Canada), and had previously participated in several research studies since being brought in as pups from northern Vancouver Island, British Columbia. The animals were regularly fed Pacific herring (*Clupea pallasi*; daily equivalent amounts of approximately 5–6% of an individual's body mass, fed over two daily meals at approx 0900 and 1430 h) with vitamin supplements (1 tablet per 3 kg food; 5M26 Vita-zu tablets, Purina Test Diets, Richmond, IN, USA), although they were familiar with a number of prey species.

The overall experimental protocol placed sea lions on restricted levels of food intake sufficient to decrease body mass 10–15% from initial values over a 9-day period. 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) (season independent variable). Data was collected on body mass, body composition, standard metabolism, and blood biochemistry (detailed below) and comparisons made between initial (pre-restriction) values at different seasons and between changes (pre- vs. post-restriction) due to food restriction during different seasons (repeated measures variable). In the original experiment the prey during the period of restriction alternated between either herring or pollock (Diet variable). Prey type only partly affected the degree and anatomical source of mass loss in interaction with seasonal changes (Kumagai et al. 2006), and given the perceived tangential nature of this variable to issues of hormonal control (and its impact on statistical power), data from both diet types were combined for the current analyses.

Seven captive female Steller sea lions were used in this study; five juveniles (2.5–3 years old), and two sub-adults (5.5–6 years old). During the approximately 3-month interval between trials (including the pre-restriction phase) the animals were fed normal herring diet ad libitum as previously detailed. During the period of restricted feeding, the animals were also fed two times per day, as per their regular ad libitum schedule.

Food intake during the period of restriction was the equivalent of 1–2.6% (depending on prey composition) of the sea lions' initial (day 1) body mass, and was carefully controlled so the animals received the same gross energy intake regardless of prey type (i.e., iso-caloric), based on the chemical composition of the prey (Kumagai et al. 2006). The sea lions were maintained on the restricted 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 (Fig. 1). We terminated one trial at 7 days when the sea lion reached the maximum mass loss (15%) dictated by the Animal Care Permit.

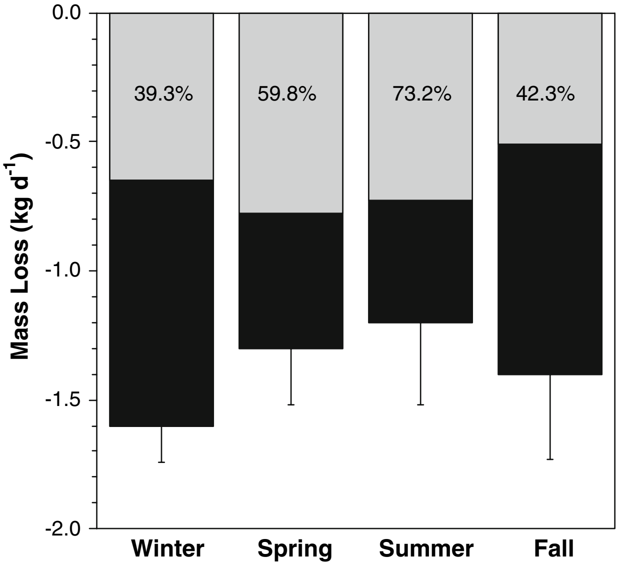

**Fig. 1** Mean ± standard deviations of rate of total body mass loss (larger, *dark bars*) during food restriction ($n = 7$ for each season; data from Kumagai et al. 2006). Mass loss was significantly greater in the winter trials ($P = 0.012$). The contribution of lipid mass loss as a proportion of total mass loss is also shown (*gray bars*)

## Data collection

Blood samples were obtained the morning of the first and last (ninth or tenth) day of each feeding trial, and were drawn before the first feed of the day (i.e., >16 h after the last meal). All of the hormones measured in this experiment can potentially exhibit diurnal fluctuations (Gardiner and Hall 1997; Horton et al. 2000; Mashburn and Atkinson 2004; Oki and Atkinson 2004). In our study, the majority of the blood samples were collected in the morning (>16 h after their last meal), with a few collected around noon. Blood for body composition and biochemistry analyses was drawn from the caudal gluteal vein of anesthetized animals (isoflurane administered under veterinary supervision) into collection tubes. Sera were separated using standard techniques (including running a centrifuge at 3,500 rpm for 5 min) and stored at −70°C.

Methods related to data on body mass, body composition, and metabolic rate are described previously in detail (Kumagai et al. 2006). Briefly, food intake (±0.01 kg $\text{day}^{-1}$) and body mass (±0.1 kg) were recorded daily. Body mass was measured each morning by having animals station on a platform scale before feeding. Body composition was determined at the start and end of each trial using standard deuterium dilution techniques (Reilly and Fedak 1991). After a background serum sample was obtained, labeled water (100.0 atom % D) was injected IM at a dose of 0.10–0.15 mg $\text{kg}^{-1}$. A second blood sample was obtained 2.0–2.5 h post-injection (sufficient for equilibration, D. A. S. Rosen unpublished data). Sera and dose samples were analyzed for isotope concentrations by Metabolic Solutions (Nashua, NH, USA). Water content was transformed into lipid and lean (i.e., non-lipid) body mass estimates using age-appropriate equations in Arnould et al. (1996). Although there are some suggestions that rapid acute mass loss can affect the hydration level of non-lipid components (and thus alter body water to composition equations, Fogelholm et al. 1997) we do not feel this was a factor in this study given the moderate rates of mass loss. SMR was measured prior to and on days 8–9 of the food restriction using gas calorimetry. Oxygen consumption was measured under standard conditions for growing animals (non-pregnant, thermoneutral, quiescent but awake, post-absorptive >16 h post-meal). Sea lions had been trained as pups to remain calm within the metabolic chamber, and behavior was monitored via video link. Animals were placed in the sealed respirometer for up to 60 min, and oxygen consumption and carbon dioxide production were monitored from a subsample of excurrent air using Sable Systems (Las Vegas, NV, USA) gas analyzers. Mass-corrected SMR was calculated to account for changes in body mass using the equation $\text{SMRc} = \text{SMR} \times \text{M}^{-0.65}$, based on observations that individual metabolic scaling is significantly lower than the interspecific scaling of $\text{M}^{-0.75}$ commonly applied (D. A. S. Rosen, unpublished data, Hunter 2005; McPhee et al. 2003).

## Laboratory and statistical analyses

Blood urea nitrogen was measured during routine clinical analyses at a commercial veterinary lab (Central Veterinary Laboratory, Langley, BC, Canada). Internal consistency was evaluated using duplicate plasma samples. Duplicate undiluted sera were tested for hormone concentrations by using a commercially available radioimmunoassay (RIA) for cortisol, total T3 (TT3), and total T4 (TT4) (Coat A Count, DPC, Los Angels, CA, USA). With the exception of TT4, 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%, $n = 8$). Inter-assay coefficients of variation for TT4 duplicate quality control samples were <5.0%. Cortisol levels were subsequently validated by independent analysis of ~50% of the samples by Dr. Sasha Kitaysky (University of Alaska).

Results are reported as mean ± standard deviation (SD). The effect of season (winter, spring, summer, fall) on initial variable levels or changes in levels after food restriction was tested via a repeated-measures, one-way ANOVA, using Fisher's post hoc comparisons between seasons (Statview, version 5.2, SAS Institute, Cary, NC, USA). One-group *t* tests were used to determine whether mean seasonal changes were statistically different from zero (no change). Results were considered significant at alpha = 0.05.

An initial analysis (Kumagai 2004) indicated no age-related differences in changes in body mass, body composition, or metabolism to food restriction that could not be explained by differences in body mass. Therefore, to maintain degrees of freedom, data from the two age classes were grouped together, and mass-corrected, mass-specific, or relative (percent) changes were analyzed as appropriate. Relative changes in variables after restriction were calculated as percent changes from pre-restriction levels, and data were arcsine transformed when required to increase normality of distribution. Linear regressions were used to determine the strength of the relationship between specific variables.

## Results

### Seasonal fluctuations in initial levels

There was no seasonal variation in the initial values of any of the blood parameters tested (cortisol, TT3, TT4, and BUN) ($P > 0.05$). As previously reported (Kumagai et al. 2006), there were no significant seasonal differences in pre-restriction body mass, while initial body lipid content varied seasonally, whether calculated as absolute lipid stores (kg; $F_{3,18} = 6.66$, $P = 0.003$) or percent body fat (as % total body mass; $F_{3,18} = 9.01$, $P < 0.001$). Both measures of lipid stores increased from winter (18.1 kg, 14.0%) to spring (25.7 kg, 19.6%), decreased from spring to summer (17.1 kg, 13.1%) and stayed constant from summer to fall (17.0 kg, 12.6%).

The pre-restriction SMR also varied throughout the year ($F_{3,17} = 27.0$, $P < 0.001$). Metabolism was constant from winter ($43.1 \pm 4.1$ MJ day$^{-1}$) to spring ($43.8 \pm 6.8$ MJ day$^{-1}$), decreased from spring to summer ($32.4 \pm 8.0$ MJ day$^{-1}$), and increased from summer to fall ($60.1 \pm 10.6$ MJ day$^{-1}$). These seasonal changes in SMR were marginally correlated to initial values of TT3 ($F_{1,25} = 4.26$, $P = 0.05$), but not TT4 hormones ($P > 0.05$) or body mass ($P > 0.05$).

### Summary of changes in morphology and metabolism after food restriction

As previously reported (Kumagai et al. 2006), body mass loss during the period of food restriction was greatest in winter (1.6 kg day$^{-1}$) compared to rates in spring (1.3 kg day$^{-1}$), summer (1.2 kg day$^{-1}$), or fall (1.4 kg day$^{-1}$). Similarly, there was a significant effect of season on the percent of total body mass loss due to changes in lipid mass ($F_{3,14} = 4.71$, $P = 0.017$) (Fig. 1). There were no consistent changes in SMR over the period of food restriction. Relative changes in SMR (calculated as percent change from initial values) for individual animals ranged from −32.4 to 41.7% (changes in SMRc ranged from −27.3 to 47.5%). There were no significant seasonal effects on percent changes in absolute or mass-corrected metabolism during the 8–9 days food restriction. Average (across animals) seasonal changes in absolute metabolism ranged from $-9.5 \pm 13.3\%$ (fall) to $+5.6 \pm 25.0\%$ (summer), but were overall not significantly different from zero (no change) in any season.

### Changes in serum profile after food restriction

#### *Cortisol*

Season significantly affected the observed absolute ($F_{3,18} = 4.45$, $P = 0.017$) and relative increases ($F_{3,18} = 4.49$, $P = 0.016$) in cortisol levels following food restriction (Table 1). The increases were greatest in the winter ($97.2 \pm 66.1$ ng ml$^{-1}$) compared to the spring ($13.3 \pm 22.0$ ng ml$^{-1}$), summer ($20.8 \pm 57.4$ ng ml$^{-1}$), or fall ($23.4 \pm 40.6$ ng ml$^{-1}$) (Fig. 2). While absolute cortisol levels were negatively related to total lipid mass after (but not before) periods of restriction ($F_{1,24} = 7.457$, $r^2 = 0.24$, $P = 0.012$) (Fig. 3a), greater increases in cortisol levels following food restriction were significantly related to greater rates of total mass loss ($F_{1,26} = 10.43$, $r^2 = 0.29$, $P = 0.003$) (Fig. 3b) but not rates of lipid loss ($P = 0.87$) or lean mass loss ($P = 0.09$).

#### *Thyroid hormones*

Average TT4 levels decreased following restriction during all seasons on both an absolute ($-1.73 \pm 0.44$ ng ml$^{-1}$) and relative ($-19.8 \pm 4.97\%$) basis. There were no significant seasonal differences in absolute ($P = 0.92$) or relative changes ($P = 0.72$) in TT4 levels. The TT3 levels of the sea lions generally decreased after the periods of restriction (Fig. 2), whether expressed as absolute changes or percent changes in relation to pre-restriction levels, with the exception of the fall trials. There was a significant seasonal effect on both absolute ($F_{3,15} = 3.35$, $P = 0.048$) and relative ($F_{3,15} = 4.54$, $P = 0.019$) changes in TT3 levels. Relative decreases in TT3 were significantly greater in the winter ($-35.6 \pm 31.9\%$) than the summer ($-12.4 \pm 30.0\%$; $t_{12} = 2.31$, $P = 0.040$), although not different from the spring ($-16.8 \pm 18.6\%$; $P = 0.08$) or fall ($+7.92 \pm 33.5\%$; $P = 0.56$).

A number of factors can affect levels of circulating TT3. Changes in TT3 levels were negatively related to rates of total mass loss ($F_{1,24} = 9.79$, $r^2 = 0.29$, $P = 0.022$) (Fig. 4a), but not rates of lipid loss ($P = 0.84$); that is greater rates of mass loss were associated with decreases in TT3 levels, while lower rates of mass loss were associated with increases in TT3 levels. There was a significant negative

**Table 1** Pre-restriction and post-restriction hormone levels for blood urea nitrogen (BUN, mmol $l^{-1}$), cortisol (Cort, ng $ml^{-1}$), total triiodothyronine (TT3, ng $ml^{-1}$), and total thyroxine (TT4, ng $ml^{-1}$). Data are presented by season and sea lion – the juveniles are designated a F00 prefix and the sub-adults a F97 prefix. The type of prey fed during the restriction period is also given. Details of concurrent changes in body mass, body composition, and standard metabolic rate are given in Table 1 of Kumagai et al, 2006

| Season | SSL | Diet | BUN init | BUN final | Cort init | Cort final | TT3 init | TT3 final | TT4 init | T4 final |
|---|---|---|---|---|---|---|---|---|---|---|
| Winter | F97HA | Pollock | 7.7 | 7.1 | 32.30 | 72.25 | 0.861 | 0.619 | 10.18 | 6.82 |
| | F97SI | Herring | 7.2 | 7.0 | 76.81 | 87.85 | n/a | 0.559 | 11.66 | 9.89 |
| | F00BO | Herring | 7.7 | 10.0 | 57.94 | 146.49 | 0.710 | 0.437 | 7.11 | 6.32 |
| | F00ED | Herring | 9.4 | 13.5 | 91.37 | 293.87 | 0.619 | 0.233 | 7.37 | 3.62 |
| | F00NU | Pollock | 7.9 | 7.5 | 48.52 | 126.50 | 0.671 | 0.149 | 4.56 | 5.02 |
| | F00TS | Pollock | 7.3 | 10.0 | 22.17 | 182.26 | 0.794 | 0.645 | 4.24 | 3.13 |
| | F00YA | Herring | 9.5 | 8.1 | 78.45 | 178.39 | 0.525 | 0.586 | 12.95 | 8.23 |
| Spring | F97HA | Herring | 6.9 | 5.9 | 80.81 | 112.01 | 0.904 | 0.625 | 8.20 | 6.93 |
| | F97SI | Pollock | 7.8 | 7.0 | 79.00 | 85.34 | 0.905 | n/a | 9.22 | 7.15 |
| | F00BO | Pollock | 8.9 | 6.7 | 70.31 | 58.55 | 0.525 | 0.484 | 7.67 | 7.39 |
| | F00ED | Herring | 7.9 | 6.1 | 70.65 | 70.46 | 0.719 | 0.562 | 6.39 | 4.12 |
| | F00NU | Herring | 8.2 | 7.6 | 30.75 | 77.48 | 0.596 | 0.410 | 6.17 | 4.94 |
| | F00TS | Herring | 7.8 | 7.0 | 25.41 | 52.93 | 0.832 | 0.615 | 4.65 | 3.59 |
| | F00YA | Pollock | 9.0 | 7.6 | 42.60 | 35.92 | 0.684 | 0.799 | 13.96 | 15.02 |
| Summer | F97HA | Pollock | 10.1 | 10.3 | 134.38 | 253.60 | 0.961 | 0.534 | 10.82 | 4.06 |
| | F97SI | Herring | 10.3 | 6.3 | 93.29 | 57.39 | 0.844 | 0.832 | 8.00 | 5.21 |
| | F00BO | Herring | 7.5 | 5.0 | 34.74 | 77.28 | 0.645 | 0.731 | 6.95 | 7.29 |
| | F00ED | Herring | 9.0 | 6.8 | 115.43 | 68.44 | 0.755 | 0.807 | 8.47 | 5.74 |
| | F00NU | Pollock | 7.0 | 7.4 | 65.79 | 122.91 | 0.875 | 0.323 | 8.40 | 9.55 |
| | F00TS | Pollock | 7.8 | 7.2 | 32.90 | 38.81 | 0.785 | 0.887 | 6.13 | 4.62 |
| | F00YA | Herring | 7.5 | 5.7 | 81.34 | 85.19 | 0.632 | 0.562 | 13.62 | 10.64 |
| Fall | F97HA | Herring | 6.6 | 6.7 | 103.96 | 85.20 | 0.893 | 0.779 | 9.18 | 5.30 |
| | F97SI | Pollock | 7.7 | 7.9 | 59.84 | 58.35 | 0.715 | 0.557 | 9.53 | 6.62 |
| | F00BO | Pollock | 6.7 | 7.4 | 51.19 | 65.32 | 0.661 | 1.068 | 7.11 | 11.73 |
| | F00ED | Herring | 8.3 | 8.8 | 61.46 | 68.46 | 1.048 | 0.763 | 5.42 | 2.80 |
| | F00NU | Herring | 6.9 | 7.0 | 80.56 | 151.69 | 0.571 | 0.618 | 10.16 | 3.96 |
| | F00TS | Herring | 7.2 | 7.0 | 44.15 | 133.75 | 0.947 | 0.984 | 4.02 | 3.79 |
| | F00YA | Pollock | 7.0 | 6.8 | 79.23 | 81.10 | 0.726 | 1.044 | 12.43 | 12.56 |

linear relationship between changes in cortisol and changes in TT3 ($r^2 = 0.17$, $P = 0.040$) but there was no significant relationship between relative changes in TT3 and TT4 ($P = 0.089$).

Counter to predictions, changes in TT3 were negatively related to both changes in SMR ($F_{1,23} = 4.49$, $r^2 = 0.17$, $P = 0.043$) and changes in mass-specific metabolic rates ($F_{1,23} = 8.27$, $r^2 = 0.26$, $P = 0.009$) (Fig. 4b). The relationship between TT3 and SMR is complicated by the fact that both may be affected by concurrent changes in mass or other hormones such as cortisol (which are themselves related to changes in body mass). An attempt to unravel these complexities using a forward stepwise regression revealed that variation in changes in TT3 levels could primarily be explained by changes in body mass ($r^2 = 0.28$) and secondarily by changes in SMR (total $r^2 = 0.53$), but not by changes in cortisol.

### *Blood urea nitrogen*

There were significant seasonal effects on changes in absolute ($F_{3,18} = 4.74$, $P = 0.013$) and relative ($F_{3,18} = 5.13$, $P = 0.009$) BUN levels (Fig. 2). For both measures, the changes in BUN levels in the winter (relative changes = 11.4 ± 24.4%) were significantly different from the spring (−15.1 ± 6.6) and summer (−17.2 ± 17.4%), but not the fall (2.34 ± 4.73%). Greater changes in relative (and absolute) BUN levels were related to greater rates of non-lipid mass loss ($F_{1,27} = 5.204$, $P = 0.031$) and total mass loss ($F_{1,27} = 10.517$, $P = 0.0032$). Further analysis

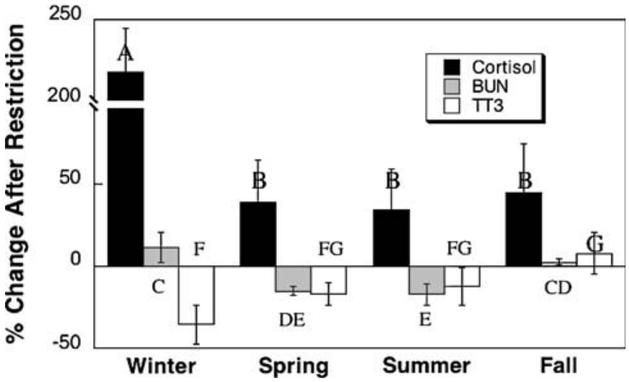

**Fig. 2** Mean percent changes in cortisol, BUN, and TT3 after restricted feeding within each season. Note *y*-axis discontinuity to accommodate substantial change in cortisol levels. *Letters* denote average seasonal changes that are statistically different from each other within each hormone

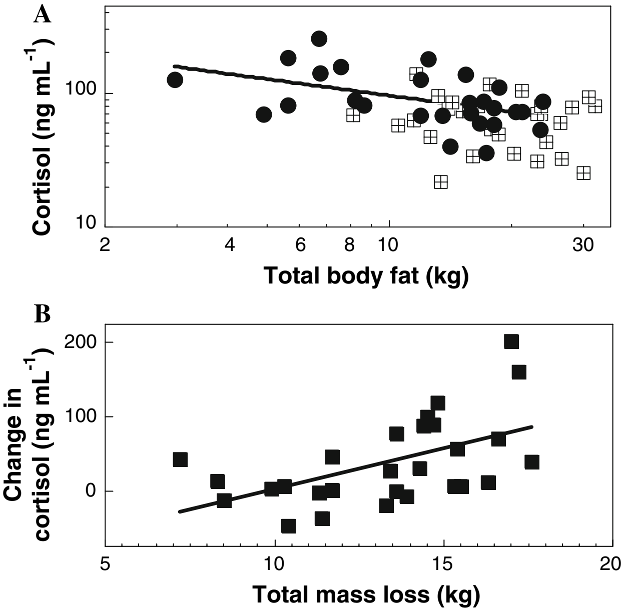

**Fig. 3** Relationship between circulating cortisol levels and total body fat (**a**). Cortisol levels were negatively related to total body fat after periods of restriction (*circles*; $r^2 = 0.24$), but not before periods of restriction (*squares*). Further analysis (**b**) revealed that changes in cortisol levels were positively related to rates of total mass loss ($r^2 = 0.29$), but not rates of lipid loss

revealed that the decreases in BUN were only significantly different from zero during the spring ($t_6 = 6.00$, $P = 0.001$) and summer trials ($t_6 = 2.498$, $P = 0.040$).

## Discussion

Given that many mammals display seasonal bioenergetic priorities induced by changes in energy intake or expenditures (e.g., Campbell and MacArthur 1998; Lehmer and Van Horne 2001; Naess 1998; Poulle et al. 1995; Rosen and Renouf 1998) it is pertinent to investigate whether the impact of unexpected food perturbations differs during the year. Previously, we reported that captive Steller sea lions subject to short-term periods of food restriction exhibited different rates of body mass loss depending on the season (Kumagai et al. 2006), suggesting that the overall physiological effects of diet restriction on Steller sea lion health are similarly seasonally dependent. However, while statistically significant, it was unclear whether these differences in mass loss were biologically important. Therefore, we examined changes in hormones and blood metabolites to discern the physiological relevance of seasonal differences in mass loss.

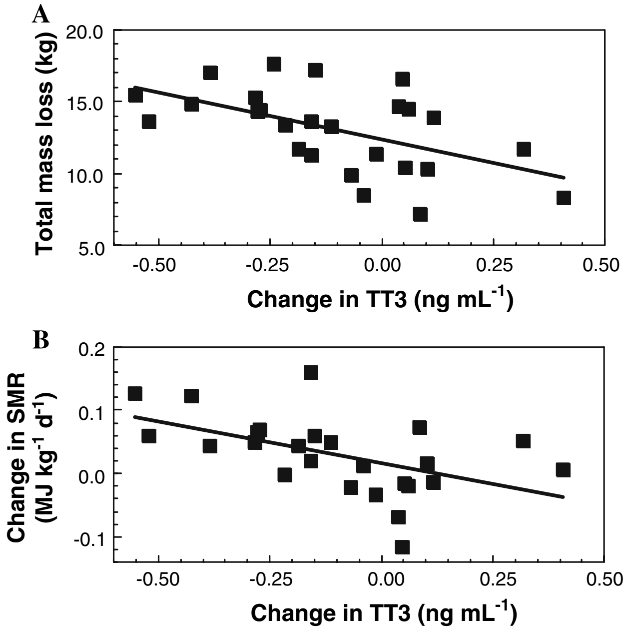

**Fig. 4** The relationship between changes in TT3 concentrations and changes in mass-specific metabolic rates and body mass. Changes in TT3 levels were negatively related to rates of total mass loss ($r^2 = 0.29$) (**a**). Although there was a positive relationship between initial (pre-restriction) absolute levels of TT3 and metabolism, there was a surprising negative relationship between changes in these two parameters ($r^2 = 0.26$) (**b**)

Cortisol is the principal glucocorticoid in marine mammals, and it is usually elevated in response to stress (St. Aubin 2001), such as may result from restricted energy intake or increased mass loss (Fair and Becker 2000; Suzuki et al. 1998). While the observed seasonal differences in rate of mass loss appeared small (e.g., winter 1.6 kg day$^{-1}$ vs. summer 1.2 kg day$^{-1}$), cortisol levels in the Steller sea lions were dramatically elevated after short-term restricted feeding in winter (218%) compared to increases observed in the other seasons (~40%). This pattern, together with the mass loss data, clearly supports the notion that similar levels of food restriction resulted in greater physiological reaction in the winter.

However, variation in cortisol levels is part of the natural energy balance system, assisting in instigating foraging bouts (Angelier et al. 2007) and facilitating glucose synthesis from non-carbohydrate sources (Aron et al. 2004). Cortisol is required for free fatty acid mobilization during periods of tissue catabolism (Goodman and Knobil 1961), and elevated levels have been demonstrated in naturally fasting (Ortiz et al. 2001a, b, 2003a) and lactating (Engelhardt and Ferguson 1980; Guinet et al. 2004) pinnipeds that primarily mobilize lipid stores to meet energy demands. Ortiz et al. (2001a, b, 2003a) suggested that cortisol informs the animal of critical changes in body condition (i.e., body fat levels) in relation to seasonal set-points; however this may be more true under conditions of natural fasting. It is true that, in our study, post-restriction cortisol levels were negatively related to total body lipids, so that animals with less absolute lipid mass had higher circulating levels. However, greater increases in cortisol levels following food restriction were significantly related to higher rates of total mass loss but not rates of lipid loss. This suggests the aforementioned post-restriction cortisol/lipid mass relationship was a byproduct of lower lipid stores primarily resulting from higher rates of total mass loss. In other words, while an overall increase in cortisol levels during food restriction facilitated increased fatty acid mobilization, the higher winter levels were more likely an indicator of greater (potential) physiological stress due to higher rates of mass loss. In addition, there is growing evidence that sea lions are more likely to conserve their insulative blubber layer and thermoregulatory capacity in the winter when potential thermoregulatory costs are highest (this study, Jeanniard du Dot 2007; Rosen and Trites 2005), which would also increase cortisol levels.

BUN levels have also often been used as a hematological indicator of nutritional stress in wild animal management (Constable et al. 1998; DelGuidice et al. 1987; Dunbar et al. 1999; Harder and Kirkpatrick 1994). The premise is that, when glucose is depleted, animals turn to body fat reserves as their primary energy source (Phase II fasting, Cherel et al. 1992), increasing lipolysis and decreasing BUN levels (Castellini and Rea 1992). If fasting is prolonged, animals switch from lipolysis to increased body protein catabolism for energy (Phase III) (Wolkers et al. 1994), leading to increased BUN levels (Castellini and Rea 1992).

The greater increases in BUN levels observed during the winter and fall trials (Fig. 2) agree with the observation that more lean tissues were catabolized for energy during these periods (Fig. 1), likely to conserve hypodermal lipid stores for insulation during food restriction. It is also consistent with the elevated levels of cortisol during these periods, since cortisol facilitates gluconeogenesis from body proteins in order to maintain glucose supply. However, interpretation of circulating BUN levels in food-restricted animals is more complicated than in fasting animals, as the concentrations are the net result of both tissue catabolism and food protein digestion (including protein quantity and quality, and feeding frequency). Nor is the relationship a simple one; while there was no statistical relationship overall between changes in BUN levels and changes in protein intake (baseline vs. restriction), changes in BUN levels for sea lions consuming lower-protein diet (herring) were strongly related to the amount of lean mass lost, while no such relationship was seen for sea lions consuming higher-protein diet (pollock) during the restriction period (Kumagai 2004). This complexity highlights the dangers of relying on BUN levels to evaluate animal health without considering levels or patterns of protein intake (Mongeau et al. 1995; Whang and Easter 2000). In addition, certain species show clear seasonal differences in baseline BUN levels between different “healthy” populations (Constable et al. 1998; Trumble et al. 2006), making inferences about nutritional status using cross-sectional data tenuous. Finally, the effect of nutritional and health status on BUN levels in pinnipeds has largely concentrated on changes associated with natural (or extended) fasting periods in pups (Costa and Ortiz 1982; Hubbard 1968; Rea et al. 2000). Although most studies confirm the relationship between BUN levels and the stage of fasting, the details of the response can be species-specific (Roletto 1993). BUN levels are more meaningful when used in concert with indicators of lipid metabolism (such as ketone concentrations), particularly in reference to fasting physiology where emphasis may be placed on either lipid or protein metabolism (Castellini et al. 1993; Rea et al. 1998).

Small variations in thyroid hormone levels can have a large impact on protein and fat metabolism rates, even though thyroid hormone levels are generally very low in pinnipeds (Renouf and Noseworthy 1991). In general, animals adapted to periods of food restriction tend to exhibit metabolic depression, characterized by a decrease in SMR invoked by decreasing free thyroid hormones, which decreases energy deficits and effectively increases survival time. Previous experiments have demonstrated metabolic depression in captive Steller sea lions under a variety of (although not all) restricted feeding regimes (Rosen and Trites 2002, 2005). The Steller sea lions in this study exhibited decreased thyroid hormone levels after short-term restricted diet intake, as has been shown to occur for deer during fasting (DelGuidice et al. 1987) and goats during restricted food intake (only T3 decreased, Gomez-Pasten et al. 1999). Relative decreases in TT3 were significantly greater in the winter than the summer, consistent with the increased rates of mass loss in the winter. The relationship between changes in TT3 and body mass is a mirror image of the results observed for cortisol levels. As a result, there was a significant linear relationship between changes in cortisol and changes in TT3. Although triiodothyronine levels are affected by cortisol concentrations through the regulation of deiodination rate from T4 to T3 (St. Aubin 2002), our statistical analysis implies that the main contributor to changes in T3 levels were changes in body mass, which similarly affected changes in cortisol levels.

It is well-documented that decreased T3 concentrations should lead to decreased metabolic rates (although see Campbell and MacArthur 1998). However, in our study, lower TT3 levels were associated with increases in metabolism, and vice versa. The disparity in our results can be the result of one or more factors. The first possibility is that food restriction induced physiologically significant changes in behavior that, while not apparent to observers, effectively increased the rates of oxygen consumption during the measures of "standard" metabolic rate (i.e., animals were not "resting"). Second, we measured total circulating T3 (and T4) concentrations, whereas the biologically active free forms (unbound to proteins) make up only a small proportion of total hormone levels (in humans fT3 = 0.3%, fT4 = 0.03%), and the consistency of the relationship between total and free thyroid levels is unclear; future studies should obviously concentrate on the unbound hormone component. Third, this study measured absolute levels of circulating thyroid hormones, which may not be indicative of (the more biologically relevant) rates of hormone utilization or clearance rates. For example, an increase in TT3 and TT4 levels was observed in northern elephant seal pups during their post-weaning fasting, 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. 2003a).

Overall, restricted energy intake during the winter resulted in the greatest decreases in body mass and TT3 and the greatest increases in cortisol and BUN, and the opposite results in summer. This suggests that the Steller sea lions had a greater physiological reaction to food restriction during colder seasons (non-breeding seasons), and that they were probably less impacted in warmer seasons (breeding seasons). This does not necessarily mean that they were in greater nutritional stress per se during the winter under the specific conditions of food restriction of this study. The responses demonstrated during the course of the study might have been within the norm of physiological adaptations, and probably also reflect changes in energetic priorities (i.e., maintaining thermoregulatory capacity in winter, Jeanniard du Dot 2007; Rosen et al. 2007). However, the ability to adapt is (temporally and quantitatively) finite; since the responses were clearly greater in the winter (including rates of mass loss which could have not been sustained) it is reasonable to suggest that unpredictable periods of restricted food intake in the winter have a greater potential to inflict nutritional stress and impact sea lion health.

It should also be noted that while the hormones we studied are known to directly affect mass loss, body composition, and metabolism, they are by no means the only hormones that can be affected by food restriction. Two key hormones that may be involved in the seasonal regulation of physiological adjustments to food restriction are ghrelin and leptin, which both regulate energy balance primarily through altering appetite (Fuglei et al. 2004; Ortiz et al. 2003b; Reidy and Webber 2000; Tschöp et al. 2000). Food deprivation generally results in increases in circulating ghrelin levels (Toshinai et al. 2001) serving to suppress energy expenditure and fat mobilization from adipose tissue in mammals (Tschöp et al. 2000). In contrast, leptin is primarily synthesized and secreted into circulation by adipose cells (Doyon et al. 2001), so that decreasing leptin levels are associated with decreasing body fat during periods of food restriction (Fuglei et al. 2004; Salfen et al. 2003), resulting in increases in appetite and sometimes higher metabolic rates (aka, "foraging response") (Horton et al. 2000; Wauters et al. 2000).

While we tried to investigate the seasonal relationship between body condition and these two hormones after restriction, we had poor results using commercial kits (specifically, Ghrelin Canine RIA kit; Phoenix Pharmaceuticals Inc., Belmont, CA, USA; and Multi-species Leptin RIA kit, Linco Research Inc., St Charles, MO, USA). For ghrelin, we managed to successfully process (i.e., intra-assay coefficient of variation <10%) only 18 samples. Since only four 4 sets of matched (pre-, post-restriction) samples were successfully obtained, no concrete conclusions could be made about the influence of the dietary restrictions on the level of ghrelin in sea lions. However, it is very telling that in the three sets where ghrelin levels decreased from 3 to 30% the animals lost significant amounts of body lipid, while in the single set where ghrelin levels increased 13% the animal actually gained lipid mass while losing body mass.

A similar preliminary analysis was performed with leptin. Unexpectedly, there was an overall increase in leptin levels within each season, and there were no correlations between leptin levels and body mass or total body fat mass. Although these results are counter to results from other mammals (Backus et al. 2000; Martin et al. 2001; Mustonen et al. 2000; Sagawa et al. 2002), they are consistent with other leptin studies in pinnipeds (Arnould et al. 2002; Gurun et al. 2001; Ortiz et al. 2001a, b; Rea and Nagy 2000). However, this response was exceedingly variable, partly because some samples returned questionably low leptin levels.

### Ecological implications

There have been concerns that commercial fisheries might be competing with Steller sea lions for food, and therefore contributing to the observed population decline (National Research Council 2003). 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 a major species targeted by fisheries (NMFS 1998). Our study suggests that sea lions may be more physiologically susceptible to short, severe reductions in prey during the winter, which coincides with the timing of the pollock roe fishery. This would also correspond to the time of the year when average energy densities of pollock are lower (Kitts et al. 2004) thereby requiring ingestion of greater amounts of pollock to yield the same net energy intake levels, and is the time when feeding trips by sea lions are generally longer (suggesting greater foraging effort) (Loughlin et al. 2003; Pitcher et al. 2005; Trites et al. 2006). Thus, while competition between fisheries and sea lions is still a contentious hypothesis, the potential effects of such interactions would appear to be greater in the winter. Hence, our data can contribute to refining current fisheries legislation by incorporating the results of empirical experiments. They also highlight important physiological variables and control mechanisms that may help measure and identify nutritional stress and its underlying causes among populations of wild Steller sea lions.

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