DIET QUALITY AND SEASON AFFECT PHYSIOLOGY AND ENERGETIC PRIORITIES OF CAPTIVE STELLER SEA LIONS DURING AND AFTER PERIODS OF NUTRITIONAL STRESS

by

TIPHAINE JEANNIARD DU DOT

DEUG Sciences de la Vie et de la Terre, Université de Tours, France, 1999
Licence Biologie des Organismes, Université Bordeaux 1, France, 2000
Maîtrise Biologie des Populations et Ecosystèmes, Université Bordeaux 1, France, 2001
DEA de Toxicologie Environnementale, Université de Paris V, France, 2002

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES

(Zoology)

THE UNIVERSITY OF BRITISH COLUMBIA
October, 2007

Abstract

The ability of animals to contend with unpredictable seasonal shifts in quality and quantity of prey has implications for the conservation of wildlife. Steller sea lions (*Eumetopias jubatus*) were subjected to different quantities and qualities of food to determine what physiological and endocrine responses would occur and whether they differed between season (summer and winter) or diet (high-lipid Pacific herring *Clupea pallasi* vs. low-lipid walleye pollock *Theragra chalcogramma*). Eight females were divided among two groups. One (Group H) were fed herring for 28 days (baseline), then received a reduced caloric intake for a subsequent 28 days (restriction) to induce a 15% loss of body mass. The second (Group P) were also fed herring during the baseline followed by a reduced isocaloric diet of pollock during the restriction. Both groups subsequently returned to their baseline intake of herring for a 28-day controlled re-feeding. The two groups of sea lions lost identical mass during restrictions independent of species eaten, but did differ in the type of internal energy reserve (protein vs. lipids) they predominantly used. Group H lost significantly more lipids and less lean mass than Group P in both seasons. In summer, Group H also increased activity levels and decreased thermoregulation capacity to optimize energy allocation. No such changes were observed for Group P whose capacity to adjust to the reduced caloric intake seemed to have been blocked by the pollock diet. During winter, the sea lions spared energy allocated to activity (especially Group H) and preserved thermoregulation capacity. Changes in body mass was negatively related to free cortisol and positively related to IGF-1 in winter, but only IGF-1 was related to changes in mass in summer when lean mass regulation seemed more important. Levels of IGF-1 were associated with changes in protein metabolism in both seasons for both groups, but changes in body condition were never explained by the measured metabolites or hormones. The capacity to compensate for mass loss was seasonally dependent with sea lions displaying compensatory growth (by restoring lipid stores) in winter but not in summer. Summer appears to be a more difficult season for sea lions to recover from mild nutritional stress. These physiological findings can be used to refine bioenergetic models needed for the conservation of Steller sea lion populations.

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Acknowledgements

I want to deeply thank Drs Andrew Trites and David Rosen for the wonderful work opportunity they provided me and for the guidance and help throughout this project. I learnt more from them than just research procedures. I am also grateful to my committee members Drs Patricia Schulte and Jeff Richards who brought their expertise and insights into this work. I could not forget the work of Pamela Rosenbaum who does not really want to see us leave the lab as much as she says! The funding was provided by the North Pacific Marine Science Foundation. Thanks also to the trainers at the Vancouver Aquarium – Billy Lasby, Nigel Waller, Gwyneth Shepard, Vance Mercer and Troy Neale – as well as Chad Nordstrom and Becky Barrick for taking care of all the technical parts of this project and making it possible. Thanks to Dr Dave Huff, Dr Marty Haulena and Danielle McLoughlin for the veterinary help during the procedures on the sea lions and obviously to Nuka, Izzy, Willo, Rogue, Tasu, Ashby, Mara and Yasha for their sometimes reluctant participation to the project! I am also grateful for the collaboration with Julie Richmond and Dr Alexander Kitaysky on hormones analyses. Big thanks to Marie Royerr, Pamela Allen, and Brian Bataille for their support during the statistical analysis phase. Our brainstorm sessions did pay off! It would not have been such a wonderful experience if I had not been surrounded by incredible people at the Marine Mammal Research Unit, Pamela Allen (best team, Pam!), Pamela Lestenkof, Michelle Marcotte, Aaron Keech, Luciano Dalla Rosa, Brian Battaile, Ruth Joy, Morgan Davis… Thanks also to Chiara Piroddi, for the great friendship and the fun we had looking for the perfect stone in all Vancouver when I needed a break! I could not forget to say a big "Merci" to my supportive family back home. And finally I want to dedicate this thesis to Erez, my pillar of strength behind the scene, who supported and encouraged me so much through these years. He deserves this thesis (almost!) as much as I do. At least now, he knows the difference between a seal and a sea lion!

Chapter 1: Introduction

Steller sea lions and the nutritional stress hypothesis

Steller sea lions range from the coast of California to northern Japan with the largest numbers residing along the Aleutian Islands, Alaska (Loughlin et al. 1992). The world population (282,000 animals in 1975) experienced a severe decline since the 1980s (Trites & Larkin 1996) to reach an estimated number of approximately 46,000 – 58,000 animals at the turn of the century (Pitcher et al. 2007). This decline was mainly experienced by the western portion of the population (western Alaska, west of 144°W, recently split into two distinct stocks, O'Corry-Crowe et al. 2006), which is listed as "endangered" under the US Endangered Species Act. The smaller eastern part of the population (east of 144°W) displayed a slow but steady increase of 3% per year since the 1980s and is currently listed as "threatened".

Nutritional stress is thought to be a contributing cause to the decline of the western population of Steller sea lions, based on several observed differences related to prey quality, quantity and prey diversity between the eastern and western populations (Alverson 1992, Merrick et al. 1997, Calkins et al. 1998, Trites & Donnelly 2003a). Rate of population decline could be related to both diet diversity and energy density of prey. Animals around Kodiak Island (western part of the population) switched from a diet containing a small proportion of gadids (32%) and a high (79%) proportion of fattier fish (salmonids and small schooling fish such as Pacific herring *Clupea pallasi*)— both high-energy fish —in the 1970s to a diet with a high proportion of gadids (85%) and a small amount of schooling fish and salmonids (37%) in the 1990s (Merrick et al. 1997). Portions of the population with the lowest diet diversity, mainly around the western Aleutian Islands, also experienced the highest rate of population decline (Merrick et al. 1997, Winship & Trites 2003, Trites et al. 2007a). Diet diversity and the proportion of high-energy fish in the diet of the eastern population are still high, which may explain why this population has not declined in numbers. A diet dominated by low-energy fish such as gadids could be deleterious to the health of sea lions and their population (Trites & Donnelly 2003a).

Mature and non-reproductive adult pinnipeds are capable of compensating for the low-energy of dominant prey items by increasing their intake of fish and invertebrates (Kirsch et al. 2000, Donnelly et al. 2003, Rosen & Trites 2004). However, parts of the population with higher energy demands (i.e., lactating females and growing young) may be unable to sufficiently increase consumption to cover their energy needs, resulting in lower birth or survival rates (York 1994, Pitcher et al. 1998, Donnelly et al. 2003, Holmes & York 2003, Rosen & Trites 2004, Pendleton et al. 2006).

Juveniles require a higher mass-specific energy intake than fully developed animals to undergo structural growth — around 17% of their body mass when consuming a mixed diet containing mainly low-energy fish (Winship & Trites 2003) — but their stomach and digestive capacity may not be sufficient to process such a high biomass intake (Rosen & Trites 2004). Problems in energy balance might thus arise when animals are unable to increase their fish intake to compensate for the low-energy content of some species of prey or when the biomass of fish available to the animals decreases (independent of the quality of the fish) due to fishing or environmental changes.

Energetic priorities

A decrease in quality of fish consumed and a decrease in fish intake can impede survival and reproduction when energy intake is insufficient to fulfill energy needs. An animal's energy budget is by definition balanced between energy output and the energy acquired through the food ingested. Energy output includes the energy needed for somatic growth, the storage of internal reserves, reproductive functions, thermoregulation needs, locomotion and basal metabolism (Lavigne et al. 1982). Energy allocated to support these functions will have to decrease to the same extent if an animal experiences a decrease in energy intake.

To offset potential energy deficits, pinnipeds can optimize energy extraction (Lawson et al. 1997, Trumble et al. 2003) or reduce energy expenditures. Production work can be reduced (i.e. reducing body growth, Stini 1969, Calkins et al. 1998) as can activity levels and time spent in thermally challenging environments (Limberger et al. 1986, Nash 1998). Other possibilities are to suppress or decrease reproductive functions, basal

metabolic rates and thermoregulation costs (Ohata & Miller 1977, Pitcher et al. 1998, Rosen & Trites 1999). The extent of changes in any one of these parameters reflects the energetic priorities of an animal.

Another potential energetic adjustment is for animals to start relying on their internal energy reserves to match energy input and output. Marine mammals possess a subcutaneous layer of lipids that provides thermoregulation and buoyancy and is a source of readily available energy (Patterson 1972). Depleting the blubber layer to provide energy could impair thermoregulation and buoyancy. Animals may also rely to some extent on their protein stores as an energy source. However, depleting protein stores may affect organ integrity, locomotion capacity (if proteins from muscles are oxidized) and thus foraging efficiency (Gòmez et al. 2000, Vaz 2003). Consequently, decisions regarding which tissue to predominantly utilize are likely to be linked to energetic priorities associated with maintaining essential physiological functions during nutritional stress (i.e., thermoregulation, activity, basal metabolism, and growth).

Differential bioenergetic decisions are likely to be highly variable and can be affected by factors such as season and quality of diet (Kirsch et al. 2000, Donnelly et al. 2003, Trumble et al. 2003, Kitts et al. 2004). For example, a diet of walleye pollock (*Theragra chalcogramma,* a low-energy gadid) results in a greater rate of lipid mass loss compared with a diet of high-quality fish when sea lions consume the same caloric intake (Rosen & Trites 2005, Kumagai et al. 2006). This effect of diet type can also differ between seasons, as shown by young sea lions that lost mass faster during winter than summer when subjected to identical food restrictions (Rea et al. 1999). This reflects the differential energy requirements of Steller sea lions (Winship et al. 2002).

Ultimately, partitioning the net energy intake among the principle physiological functions can affect the survival and reproductive capacity of animals in the wild. The degree to which vital rates are affected will depend on how optimal these energetic decisions are relative to environmental conditions. Assessing the effect quality of diet can have on the energetic strategies of animals at different times of the year therefore merits study to assess the potential impact these parameters may have on the fitness of individual animals.

Endocrine regulation

Changes in physiological processes are regulated by several integrated hormone systems. Investigating how season and diet quality differentially affect hormone levels in the body is needed to understand the mechanisms responsible for enacting the energetic strategies of Steller sea lions. Glucocorticoids, thyroid and somatotropic hormones are among the principal endocrine pathways regulating the energy budgets of mammals.

Glucocorticoids are associated with periods of stress, including nutritional stress (Sapolsky et al. 2000). They influence the behaviour and physiology of mammals which, although beneficial during emergency situations, can become deleterious during a period of chronic stress (Reeder & Kramer 2005, Kitaysky et al. 2006). Glucocorticoids are known to mobilize energy stores in the body and are related to depression of immune and reproductive systems on a long-term basis (St Aubin & Dierauf 2001, Romero 2002, Kitaysky et al. 2003, Wingfield 2003, Reeder & Kramer 2005). In Steller sea lions, levels of glucocorticoids increase during an acute food deprivation and are inversely related to changes in body mass and condition (Kumagai et al. 2006).

Thyroid hormones are involved in growth and development of young animals (Eales 1988, Kelly 2000, Choksi et al. 2003). They also play a role in regulating immune and reproduction systems as well as in determining the level of basal metabolism and metabolic thermogenesis through regulation at the cellular levels (Renouf & Noseworthy 1991, Silva 1995). Among pinnipeds, a decrease in thyroid hormones while nutritionally stressed has been linked to a decrease in metabolic rate as a means of minimizing energy expenditures (Young 1984, Ashwell-Erickson et al. 1986). Levels of thyroid hormones are also influenced by energy and nutrient intake levels (O'Brian et al. 1980, Serog et al. 1982, Eales 1988, Reinhardt et al. 1993) which differ between high- and low-energy fish.

The third principal endocrine pathway ─ somatotropic hormones ─ is involved in regulating energy balance and body size (mass and length) of animals (Breier 1999, Butler & Le Roith 2001). They mainly promote growth and development in young animals by regulating protein metabolism (anabolism/catabolism ratio) and bone growth (Breier 1999, Ortiz et al. 2003b). Levels of somatotropic hormones vary with nutritional status in terrestrial mammals and signal protein sparing during mass loss or enhance tissue

accretion during the recovery period (Hornick et al. 2000, Renaville et al. 2002). These hormones have seldom been studied in marine mammals (Ortiz et al. 2003b).

Changes in levels of glucocorticoids, somatotropic and thyroid hormones are all expected to reflect and explain physiological changes associated with changes in energy intake. These physiological processes include changes in lipid and protein metabolism and likely depend on diet and season. Differential utilization of energy reserves should also be reflected in the by-products of these catabolic routes in the blood. Non-estherified fatty acids and ketone bodies have been shown to accurately reflect lipid catabolism while measurements of nitrogen waste (i.e. blood urea nitrogen) reflect protein catabolism in fasting animals (Rea et al. 1998). However, these measurements may not be accurate for animals experiencing milder energy restrictions (resulting in milder catabolism) or when the nutrient composition of the food changes (Narayanan & Appleton 1980).

Compensatory growth

Studies on the nutritional physiology of marine mammals have mostly investigated how fasting or changes in diet affect the energy balance of animals during a period of nutritional restriction. However, differential effects of season and diet type are likely to affect how an animal recovers from nutritional stress. Recovery periods are likely an important phase for the future fitness of animals (Arendt 1997, Ali et al. 2003). Despite a large body of literature on the physiology of recovery for terrestrial mammals, only a few studies have examined the physiological responses of marine mammals during the post-deprivation period (Worthy & Lavigne 1983, Markussen et al. 1992b), and none have been conducted on Steller sea lions.

In general, animals have a tendency to recover from nutritional stress by displaying compensatory growth, which is defined as a period of high growth rate needed to restore body size lost during suboptimal conditions (Boersma & Wit 1997, Kelly & Van Horne 1997, Hornick et al. 1998, Wu et al. 2000, Ali et al. 2003, Bjorndal et al. 2003, Jespersen & Toft 2003). The extent of the compensation and the type of tissue deposited can impact the body composition of animals, which subsequently affects the fitness of an individual (Royle 2000, Victora et al. 2001, Alvarez & Nicieza 2005, Johnsson & Bohlin 2005,

2006). Compensatory growth can be highly beneficial in terms of survival (Nicieza & Metcalfe 1997), reproductive success, social performance (Arendt 1997), and ontogenic transitions (Ali et al. 2003) when bigger sizes are advantageous (Boltnev et al. 1998, Boness et al. 2002, Chambellant et al. 2003). However, compensatory growth can also be associated with a variety of longer-term costs, including higher risk of predation from increased foraging (McNamara & Houston 1990), developmental errors, tissue lesions (Ali et al. 2003), decreases in longevity (Hales & Ozanne 2003), and increased risk of disease (Metcalfe & Monaghan 2001).

Assessing the impacts of diet and season on the capacity of Steller sea lions to recover and compensate for suboptimal periods of nutrition can extend our understanding of the impacts of nutritional stress on the life history of these animals and ultimately on their populations.

Objectives

It is challenging to clearly establish connections between diet changes and population trends in the wild, given the extremely complex relationships between environmental parameters and the physiology, ecology, and behaviour of individual animals. Captive studies provide the opportunity to individually tease apart the effects of each parameter. The primary goal of our study on captive female Steller sea lions was to understand the impacts of season and diet quality on the energy balance and energetic priorities of the animals during periods of nutritional restriction. Our expectation was that body mass loss would be greater in winter than in summer when energy requirements are greater (Winship et al. 2002). Sea lions eating low-quality fish were also expected to lose body mass at the same rate as sea lions eating a reduced isocaloric diet of high-quality fish but to rely more heavily on lipid stores (Rosen & Trites 2005, Kumagai et al. 2006). The extent of lipid or protein catabolism and the resulting changes in body composition should theoretically lead to different energetic strategies of energy allocation between functions such as thermoregulatory capacity and levels of activity.

The second objective of our study was to assess the capacity of sea lions to compensate for periods of suboptimal conditions. Since compensatory growth is such a

ubiquitous phenomenon in the animal kingdom, we expected that Steller sea lions would greatly increase their growth rate to compensate for previous mass loss, and that recovery would be independent of the type of fish consumed during the energy restriction if mass loss was identical. We also expected compensation to be quicker in summer, since more energy should be available for growth compared to winter (Winship et al. 2002).

The third goal of our study was to assess how glucocorticoids, thyroid and somatotropic hormones regulate the physiological adjustments developed by animals eating high- or low-energy fish during nutritional stress and how these hormones contribute to the patterns of recovery after the restriction. Based on past studies of terrestrial mammals, glucocorticoids were expected to increase during the restriction and decrease during the recovery, while the thyroid and somatotropic hormones should display the reverse patterns (Hornick et al. 2000). Hormones related to lipid catabolism were expected to change more drastically for animals eating low-energy fish (who were predicted to lose more lipids) and hormones related to protein catabolism were expected to change more for animals eating high-energy fish. We also sought to investigate the validity of hormones and of blood metabolites as biomarkers for assessing nutritional status of sea lions.

This thesis consists of three major chapters. The first (Chapter 2) addresses the effects of season and quality of diet on the body mass and body composition of the Steller sea lions during periods of restriction and re-feeding. Chapter 3 discusses physiological and behavioural changes from a bioenergetic perspective. Finally Chapter 4 endocrine regulation and how it contributes to the observed changes. Each chapter is written as a stand-alone paper, which results in some necessary repetition of information between them.

Collectively, the body of research presented in the following pages extends our understanding of how changes in diet impact the physiology of Steller sea lions and how they interact with the time of the year. It also provides insight into how physiological and energetic decisions contribute to the capacity of sea lions to survive and reproduce and may help refine bioenergetic models needed for conservation of the endangered population of Steller sea lions.

References

Ali M, Nicieza A, Wootton RJ (2003) Compensatory growth in fishes: a response to growth depression. Fish and Fisheries 4:147-190

Alvarez D, Nicieza AG (2005) Compensatory response 'defends' energy levels but not growth trajectories in brown trout, *Salmo trutta L.* Proceedings of the Royal Society B-Biological Sciences 272:601-607

Alverson DL (1992) A review of commercial fisheries and the Steller sea lion (*Eumetopias jubatus*): the conflict arena. Reviews in Aquatic Sciences 6:203-256

Arendt JD (1997) Adaptive intrinsic growth rates: an integration across taxa. The Quarterly Review of Biology 72:149-177

Ashwell-Erickson S, Fay FH, Elsner R, Wartzok D (1986) Metabolic and hormonal correlates of molting and regeneration of pelage in Alaskan harbor and spotted seals (*Phoca vitulina* and *Phoca largha*). Canadian Journal of Zoology 64:1086-1094

Bjorndal KA, Bolten AB, Dellinger T, Delgado C, Martins HR (2003) Compensatory growth in oceanic loggerhead sea turtles: Response to a stochastic environment. Ecology 84:1237-1249

Boersma B, Wit JM (1997) Catch-up growth. Endocrine Reviews 18:646-661

Boltnev AI, York AE, Antonelis GA (1998) Northern fur seal young: interrelationships among birth size, growth, and survival. Canadian Journal of Zoology 76:843-854

Boness DJ, Clapham PJ, Mesnick SL (2002) Life history and reproductive strategies. In: Hoelzel AR (ed) Marine mammal biology: an evolutionary approach. Blackwell Science Publishing, Oxford ; Malden, MA, p 278-324

Breier BH (1999) Regulation of protein and energy metabolism by the somatotropic axis. Domestic Animal Endocrinology 17:209-218

Butler AA, Le Roith D (2001) Control of growth by the somatropic axis: Growth hormone and the insulin-like growth factors have related and independent roles. Annual Review of Physiology 63:141-164

Calkins DG, Becker EF, Pitcher KW (1998) Reduced body size of female Steller sea lions from a declining population in the Gulf of Alaska. Marine Mammal Science 14:232-244

Chambellant M, Beauplet G, Guinet C, Georges J-Y (2003) Long-term evaluation of pup growth and preweaning survival rates in subantarctic fur seals, *Arctocephalus tropicalis*, on Amsterdam Island. Canadian Journal of Zoology 81:1222-1232

Choksi NY, Jahnke GD, St Hilaire C, Shelby M (2003) Role of thyroid hormones in human and laboratory animal reproductive health. Birth Defects Research B-Developmental and Reproductive Toxicology 68:479-491

Donnelly CP, Trites AW, Kitts DD (2003) Possible effects of pollock and herring on the growth and reproductive success of Steller sea lions (*Eumetopias jubatus*): insights from feeding experiments using an alternative animal model, *Rattus norvegicus*. British Journal of Nutrition 89:71-82

Eales JG (1988) The Influence of nutritional state on thyroid function in various vertebrates. American Zoologist 28:351-362

Gòmez F, Galvan RR, Frenk S, Muñoz JC, Chavez R, Vasquez J (2000) Mortality in second and third degree malnutrition. Bulletin of the World Health Organization 78:1275-1280

Hales CN, Ozanne SE (2003) The dangerous road of catch-up growth. The Journal of Physiology 547:5-10

Holmes EE, York AE (2003) Using age structure to detect impacts on threatened populations: a case study with Steller sea lions. Conservation Biology 17:1794-1806

Hornick JL, Van Eenaeme C, Clinquart A, Diez M, Istasse L (1998) Different periods of feed restriction before compensatory growth in Belgian blue bulls: I. animal performance, nitrogen balance, meat characteristics, and fat composition. Journal of Animal Science 76:249-259

Hornick JL, Van Eenaeme C, Gerard O, Dufrasne I, Istasse L (2000) Mechanisms of reduced and compensatory growth. Domestic Animal Endocrinology 19:121-132

Jespersen LB, Toft S (2003) Compensatory growth following early nutritional stress in the wolf spider *Pardosa prativaga*. Functional Ecology 17:737-746

Johnsson JI, Bohlin T (2005) Compensatory growth for free? A field experiment on brown trout, *Salmo trutta*. Oikos 111:31-38

Johnsson JI, Bohlin T (2006) The cost of catching up: increased winter mortality following structural growth compensation in the wild. Proceedings of the Royal Society B-Biological Sciences 273:1281-1286

Kelly GS (2000) Peripheral metabolism of thyroid hormones: a review. Alternative Medicine Review 5:306-333

Kelly JF, Van Horne B (1997) Effects of food supplementation on the timing nest initiation in belted kingfishers. Ecology 78:2504

Kirsch PE, Iverson SJ, Bowen WD (2000) Effect of a low-fat diet on body composition and blubber fatty acids of captive juvenile harp seals (*Phoca groenlandica*). Physiological and Biochemical Zoology 73:45-59

Kitaysky AS, Kitaiskaia E, Piatt J, Wingfield JC (2003) Benefits and costs of increased levels of corticosterone in seabird chicks. Hormones and Behavior 43:140-149

Kitaysky AS, Kitaiskaia EV, Piatt JF, Wingfield JC (2006) A mechanistic link between chick diet and decline in seabirds? Proceedings of the Royal Society B-Biological Sciences 273:445-450

Kitts DD, Huynh MD, Hu C, Trites AW (2004) Seasonal variation in nutrient composition of Alaskan walleye pollock. Canadian Journal of Zoology 82:1408-1415

Kumagai S, Rosen DAS, Trites AW (2006) Body mass and composition responses to short-term low energy intake are seasonally dependent in Steller sea lions (*Eumetopias jubatus*). Journal of Comparative Physiology B 176:589-598

Lavigne DM, Barchard W, Innes S, Øritsland NA (1982) Pinniped bioenergetics. In: Mammals in the seas: Small cetaceans, seals, sirenians, and sea otters. FAO Fisheries Series 5, Rome, p 191-235

Lawson JW, Miller EH, Noseworthy E (1997) Variation in assimilation efficiency and digestive efficiency of captive harp seals (*Phoca groenlandica*) on different diets. Canadian Journal of Zoology 75:1285-1291

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

Loughlin TR, Perlov AS, Vladimirov VA (1992) Range-wide survey and estimation of total number of Steller sea lions in 1989. Marine Mammal Science 8:220-239

Markussen NH, Ryg M, Øritsland NA (1992) Metabolic rate and body composition of harbour seals, *Phoca vitulina*, during starvation and refeeding. Canadian Journal of Zoology 70:220-224

McNamara JM, Houston AI (1990) The value of fat reserves and the tradeoff between starvation and predation. Acta Biotheoretica 38:37-61

Merrick RL, Chumbley MK, Byrd GV (1997) Diet diversity of Steller sea lions (*Eumetopias jubatus*) and their population decline in Alaska: a potential relationship. Canadian Journal of Fisheries and Aquatic Sciences 54:1342-1348

Metcalfe NB, Monaghan P (2001) Compensation for a bad start: grow now, pay later? Trends in Ecology and Evolution 16:254-260

Narayanan S, Appleton HD (1980) Creatinine - a review. Clinical Chemistry 26:1119-1126

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

Nicieza AG, Metcalfe NB (1997) Growth compensation in juvenile Atlantic salmon: Responses to depressed temperature and food availability. Ecology 78:2385-2400

O'Brian JT, Bybee DE, Burman KD, Osburne RC, Ksiazek MR, Wartofsky L, Georges LP (1980) Thyroid hormone homeostasis in states of relative caloric deprivation. Metabolism-Clinical and Experimental 29:721-727

O'Corry-Crowe G, Taylor BL, Gelatt T, Loughlin TR, Bickham J, Basterretche M, Pitcher KW, DeMaster DP (2006) Demographic independence along ecosystem boundaries in Steller sea lions revealed by mtDNA analysis: implications for management of an endangered species. Canadian Journal of Zoology 84:1796-1809

Ohata CA, Miller LK (1977) Some temperature responses of northern fur seal (*Callorhinus ursinus*) pups. Journal of Mammalogy 58:438-440

Ortiz RM, Noren DP, Ortiz CL, Talamantes F (2003) GH and ghrelin increase with fasting in a naturally adapted species, the northern elephant seal (*Mirounga angustirostris*). Journal of Endocrinology 178:533-539

Patterson JH (1972) Thermoregulation in the harbour seal (phoca vitulina richardi) with particular reference to the insulative quality of the dry pelage. Bachelor of Science, Univeristy of British Columbia

Pendleton GW, Pitcher KW, Fritz LW, York AE, Raum-Suryan KL, Loughlin TR, Calkins DG, Hastings KK, Gelatt TS (2006) Survival of Steller sea lions in Alaska: a comparison of increasing and decreasing populations. Canadian Journal of Zoology 84:1163-1172

Pitcher KW, Calkins DG, Pendleton GW (1998) Reproductive performance of female Steller sea lions: an energetics-based reproductive strategy? Canadian Journal of Zoology 76:2075-2083

Pitcher KW, Olesiuk PF, Brown RF, Lowry MS, Jeffries SJ, Sease JL, Perryman WL, Stinchcomb CE, Lowry LF (2007) Abundance and distribution of the eastern North Pacific Steller sea lion (*Eumetopias jubatus)* population. Fishery Bulletin 105:102-115

Rea LD, Rosen DAS, Trites AW (1998) Blood chemistry and body mass changes during fasting in juvenile Steller sea lions (*Eumetopias jubatus*) Proceedings of the Comparative Nutrition Society, Banff, Canada, p 174-178

Rea LD, Rosen DAS, Trites AW (1999) Seasonal differences in adaptation to prolonged fasting in juvenile Steller sea lions (*Eumetopias jubatus*). Faseb Journal 13:A740-A740

Reeder DM, Kramer KM (2005) Stress in free-ranging mammals: Integrating physiology, ecology, and natural history. Journal of Mammalogy 86:225-235

Reinhardt W, Holtermann D, Benker G, Olbricht T, Jaspers C, Reinwein D (1993) Effect of small doses of iodine on thyroid function during caloric restriction in normal subjects. Hormone Research 39:132-137

Renaville R, Hammadi M, Portetelle D (2002) Role of the somatotropic axis in the mammalian metabolism. Domestic Animal Endocrinology 23:351-360

Renouf D, Noseworthy E (1991) Changes in food intake, mass, and fat accumulation in association with variations in thyroid hormone levels of harbour seals (*Phoca vitulina*). Canadian Journal of Zoology 69:2470-2479

Romero LM (2002) Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. General and Comparative Endocrinology 128:1-24

Rosen DA, Trites AW (2005) Examining the potential for nutritional stress in young Steller sea lions: physiological effects of prey composition. Journal of Comparative Physiology B 175:265-273

Rosen DAS, Trites AW (1999) Metabolic effects of low-energy diet on Steller sea lions, *Eumetopias jubatus*. Physiological and Biochemical Zoology 72:723-731

Rosen DAS, Trites AW (2004) Satiation and compensation for short-term changes in food quality and availability in young Steller sea lions (*Eumetopias jubatus*). Canadian Journal of Zoology 82:1061-1069

Royle N (2000) Overproduction in the lesser black-backed gull - can marginal chicks overcome the initial handicap of hatching asynchrony? Journal of Avian Biology 31:335-344

Sapolsky RM, Romero LM, Munck AU (2000) How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocrine Reviews 21:55-89

Serog P, Apfelbaum M, Autissier N, Baigts F, Brigant L, Ktorza A (1982) Effects of slimming and composition of diets on VO_2 and thyroid hormones in healthy subjects. American Journal of Clinical Nutrition 35:24-35

Silva JE (1995) Thyroid hormone control of thermogenesis and energy balance. Thyroid 5:481-492

St Aubin DJ, Dierauf LA (2001) Stress and marine mammals. In: Dierauf LA, Gulland FMD (eds) CRC Handbook of marine mammal medicine. CRC Press, Boca Raton, London, New York, Washigton DC, p 253-269

Stini WA (1969) Nutritional stress and growth - sex difference in adaptive response. American Journal of Physical Anthropology 31:417-426

Trites AW, Calkins DG, Winship AJ (2007) Diets of Steller sea lions (*Eumetopias jubatus)* in Southeast Alaska, 1993-1999. Fishery Bulletin 105:234-248

Trites AW, Donnelly CP (2003) The decline of Steller sea lions *Eumetopias jubatus* in Alaska: a review of the nutritional stress hypothesis. Mammal Review 33:3-28

Trites AW, Larkin PA (1996) Changes in the abundance of Steller sea lions (*Eumetopias jubatus*) in Alaska from 1956 to 1992: How many were there? Aquatic Mammals 22:153-166

Trumble SJ, Barboza PS, Castellini MA (2003) Digestive constraints on an aquatic carnivore: effects of feeding frequency and prey composition on harbor seals. Journal of Comparative Physiology B 173:501-509

Vaz M (2003) Undernutrition. In: Gibney MJ, Macdonald I, Roche HM (eds) Nutrition and Metabolism. Blackwell Publishing., Oxford ; Ames, Iowa, p 341-348

Victora CG, Barros FC, Horta BL, Martorell R (2001) Short-term benefits of catch-up growth for small-for-gestational-age infants. International Journal of Epidemiology 30:1325-1330

Wingfield JC (2003) Control of behavioural strategies for capricious environments. Animal Behaviour 66:807-815

Winship AJ, Trites AW (2003) Prey consumption of Steller sea lions (*Eumetopias jubatus*) off Alaska: How much prey do they require? Fishery Bulletin 101:147-167

Winship AJ, Trites AW, Rosen DAS (2002) A bioenergetic model for estimating the food requirements of Steller sea lions *Eumetopias jubatus* in Alaska, USA. Marine Ecology-Progress Series 229:291-312

Worthy GAJ, Lavigne DM (1983) Energetics of fasting and subsequent growth in weaned harp seal pups, *Phoca groenlandica*. Canadian Journal of Zoology 61:447-456

Wu L, Dong S, Wang T, Tian X (2000) Compensatory growth response following period of starvation in Chinese shrimp, *Penaues chinensis obsek*. Journal of Shellfish Research 19:717-722

York AE (1994) The population dynamics of northern sea lions, 1975-1985. Marine Mammal Science 10:38-51

Young RA (1984) Interrelationships between body weight, food consumption and plasma thyroid hormone concentration cycles in the woodchuck, *Marmota monax*. Comparative Biochemistry and Physiology A-Physiology 77:533-536

Chapter 2: Steller sea lions show diet-dependant changes in body composition during nutritional stress and recover more easily from mass loss in winter[1]

Summary

Controlled feeding experiments were undertaken with captive Steller sea lions (*Eumetopias jubatus*) to assess seasonal physiological responses of individual animals to reduced quantities and qualities of food. Eight animals were randomly divided into two experimental groups fed isocaloric diets: Group H ate Pacific herring (*Clupea pallasi*) throughout the experiment while Group P was switched to walleye pollock (*Theragra chalcogramma*) during a 28-day food restriction (after a 28-day baseline) and back to herring during a 28-day controlled re-feeding. Diet type did not impact the rates of body mass lost when food was restricted, but did influence the type of internal energy reserve (protein vs lipids) the sea lions predominantly used. In both summer and winter, Group H lost significantly more lipids and less lean mass than Group P that was fed pollock during the restriction phase. Lipid loss was also slightly lower in winter than in summer for both groups of sea lions. The response of Group H was consistent with the predicted pattern of nutritional stress physiology (i.e. protein sparing and utilization of lipid reserves). Group P lost a surprisingly high proportion of body protein while consuming restricted levels of pollock, which could lead to muscle impairment and vital organ failure on a long-term basis. When given increased amounts of herring during the controlled re-feeding phase, the capacity of both groups to compensate for the previous mass loss was found to depend on season and was independent of previous diet. All of the sea lions increased their rates of mass gain and returned to their pre-experimental weight during winter, but not during summer. Some intrinsic energetic plasticity related to seasonal adaptation to the environment may render winter an easier period to recover from nutritional stress.

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[1] A version of this chapter will be submitted for publication. Jeanniard du Dot T., Rosen, D.A. and Trites A.W. Steller sea lions show diet-dependant changes in body composition during nutritional stress and recover more easily from mass loss in winter.

Introduction

Animals routinely adjust their physiology and behaviour when faced with seasonally predictable or unpredictable shifts in nutrition and ecosystem conditions (Boyd 2002, McNab 2002). However, the range of adjustments an animal can make is often limited in scope and is insufficient to compensate for significant declines in energy intake. Determining how animals respond physiologically to changes in quantity and quality of diet, and whether these responses differ between seasons when the needs of animals are intrinsically different is essential to understanding how environmental changes affect the health and dynamics of populations.

Nutritional stress has been a leading hypothesis to explain the decline of several populations of marine mammals and sea birds in the North Pacific Ocean (Trites & Donnelly 2003a, Jodice et al. 2006). A number of studies have endeavoured to link observed changes in diets and body condition of the animals with population changes of birds and mammals (Merrick et al. 1997, Rea et al. 2000, Rosen & Trites 2000b, Donnelly et al. 2003, Romano et al. 2006). Some of them have found that birds and mammals cannot always fulfill their daily energy requirements when eating only low energy density prey even when fed to satiation. For example, black-legged kittiwakes (*Rissa tridactyla*) and tufted puffins (*Fratercula cirrhata*) fed to satiation on walleye pollock (*Theragra chalcogramma*) had reduced growth in mass and wing length compared to chicks fed high energy density fish such as Pacific herring (*Clupea pallasi*) and sand lance (*Ammodytes americanus*) (Romano et al. 2006). Similarly, harbour seals (*Phoca vitulina*) and Steller sea lions (*Eumetopias jubatus*) also fed to satiation on a pollock diet experienced mass loss and depletion of body fat stores (Kirsch et al. 2000, Rosen & Trites 2000b).

Changes in quantity and quality of prey have been shown to affect changes in amounts of lipids and proteins animals catabolize to meet energy demands, which in turn can affect survival and reproduction in marine mammals (Cherel et al. 1992 , Boltnev et al. 1998 , Pitcher et al. 1998, Donnelly et al. 2003, Rosen & Trites 2005). How animals respond physiologically to periods of nutritional restriction appears to vary with the time of the year in which the deficit occurs (Kitts et al. 2004, Sigler et al. 2004). In the case of Steller sea lions, total energy requirements are thought to be higher in winter than

summer, due primarily to higher foraging and thermoregulation costs associated with winter (Winship et al. 2002). Steller sea lions have also been shown to lose different amounts of lipid and protein tissues depending on the season they experience the nutritional stress (Rosen & Trites 2005, Kumagai et al. 2006). Consequently, seasonal variation in both the prey characteristics and requirements of Steller sea lions likely leads to seasonally adapted physiological states. The synergistic interactions between diet type, food intake level, and season need to be investigated to fully understand the potential impact that changes in diet can have on the life history of Steller sea lions and other species.

Many species of insects (Jespersen & Toft 2003), fish (Russell & Wootton 1992, Nicieza & Metcalfe 1997), reptiles (Bjorndal et al. 2003), birds (Heath & Randall 1985, Konarzewski et al. 1996) and mammals (Pitts 1986, Yambayamba et al. 1996) including humans (Boersma & Wit 1997) display enhanced growth rates following deleterious nutritional events to compensate for previous mass losses or cessation of growth. Most research on the recovery from deprivation has been performed on captive animals with a few observational studies confirming its occurrence in the wild (Bjorndal et al. 2003, Johnsson & Bohlin 2006). Despite the importance of the recovery phase to future fitness (Arendt 1997), it has rarely been investigated for large, top predators such as marine mammals (Markussen et al. 1992b).

The goal of our study was to provide a new perspective on the Nutritional Stress hypothesis by examining how captive Steller sea lions react physiologically to reduced amounts of different quality prey, and whether time of the year affects their ability to recover during a subsequent re-feeding period. The experiments were designed to place Steller sea lions in a negative energy balance in summer and winter using high and low energy density species of fish to determine the effect of diet type on their physiological response (in terms of body mass and composition) while nutritionally stressed, as well as their subsequent capacity to recover. Steller sea lions were expected to incur greater changes in body mass and condition on a low quality diet (independent of energy intake), especially in winter (Kumagai et al. 2006). They were also expected to be able to increase their growth rate above normal values during the recovery period, particularly during summer when energy demands are less.

Material and methods

Experimental design

Our study was conducted under permit No. A04-0169 of the University of British Columbia Animal Care Committee. The experiment was conducted twice to examine seasonal changes (summer June-August 2005 and winter January-March 2006) at the Vancouver Aquarium (BC, Canada) on eight female Steller sea lions reared and held in captivity. Five of the females were juveniles (2.5 to 3 years old) and three were sub-adults (5.5 to 6 years old) and all had participated in previous research experiments. The eight animals were randomly divided by age class into two experimental groups, Group H (3 juveniles and 1 sub-adult) and Group P (2 juveniles and 2 sub-adults). The experimental groups were kept identical and remained on the same diets for the two seasons.

The summer experiment consisted of 3 phases of 28 days each. The first phase was a baseline (B), during which all of the animals were fed their usual daily ration of Pacific herring (C*lupea pallasi*) ranging from 4.0-5.5% of their body mass. The level of food intake was controlled by the sea lion trainers and was determined by behavioural responses of the animals. They were given enough food to satisfy their appetites but not enough so that they would lose interest in their training.

After the baseline period, the animals started a 28-day restriction treatment (R) during which their energy intake was reduced by approximately 20-30%. Juveniles in both diet groups were given 260 kJ kg^{-1} d^{-1} of food and sub-adults received 230 kJ kg^{-1} d^{-1}. These values were designed to elicit a total mass loss not exceeding 15% of their initial body masses during the restriction, as per Animal Care protocols. The two diet groups were given the same "restricted" level of isocaloric diets, but differed in what type of fish they ate while nutritionally stressed. Group H was fed exclusively Pacific herring and Group P was fed solely walleye pollock. Whenever an animal did not eat its full ration, the leftover was carried forward and averaged over the remaining days of the treatment to keep intake consistent between the animals. Subsamples of the herring and pollock were analyzed for their proximate chemical composition. The analysis was performed by Northwest Labs (Surrey, British Columbia; soxhlet hexane extraction for lipids and total nitrogen estimates $\times$ 6.25 for proteins) and the gross energy contents of the fish were

calculated using the energy conversion factors provided by Schmidt-Nielsen (1997): 39.3 kJ g^{-1} for lipid and 18 kJ g^{-1} for protein.

The restriction phase was followed by a 28-day controlled re-feeding period (CR) during which each sea lion received the same diet and energy intake of Pacific herring that they received during the baseline phase. This allowed the efficiency of food utilization before and after nutritional stress to be directly compared.

The same experimental protocol was repeated during the winter, but an additional 2-week period called the Ad-libitum (LIB) treatment followed the controlled re-feeding phase. During this treatment, the sea lions were given as much Pacific herring as they demanded based on behavioural and training responses.

Data acquisition

Food intake (± 0.02 kg d^{-1}), energy intake (kJ d^{-1}) and body mass of each animal (± 0.1 kg) were recorded daily. The other parameters (see below) were measured at the end of the baseline (B4), in the middle (2 weeks) and at the end (4 weeks) of the restriction and controlled re-feeding (i.e. R2, R4, CR2, CR4), and at the end of the 2-week ad-libitum treatment in winter (LIB2). No vitamins or medicines were added to the diet at any time during the experiments. Based on the results from the summer controlled re-feeding, a decision was made to extend the recording of food intake, energy intake and body mass (and only these 3 variables) for an additional 14 days during the summer (similar to the ad-libitum period in the second experiment). To correct for intrinsic differences between sea lions, the rates of change in parameters were generally calculated in reference to the baseline phase. Cumulative body mass changes throughout the experiments were calculated in reference to the first measurement of the baseline phase. Levels of water intake can be considered directly related to diet intake (metabolic water) given that no animal was seen drinking freshwater during the trials.

Body composition, which measures the proportion of fat and fat-free tissues in the body, was measured every two weeks at B4, R2, R4, CR2, CR4 before the sea lions' first meal of the day (~ 18h overnight fast) using the deuterium (D_2O) dilution technique (previously validated on marine mammals, Costa 1987, Oftedal & Iverson 1987, Reilly &

Fedak 1990, Arnould et al. 1996, Bowen & Iverson 1998). Animals were anaesthetized with isoflurane under veterinarian supervision for each procedure. An initial 10 mL blood sample was drawn into a serum separator tube to assess the ^2H_2O background in the sea lions before a 0.10 – 0.15 g kg^{-1} dose of sterile ^2H_2O (99.9%) was injected intramuscularly. After an equilibration period of 2.0 to 2.5 h (previously validated on these animals, Rosen unpublished data) a second blood sample was taken. Sera were separated by centrifugation and stored at -70°C until analysis of the isotopes (including in the dose samples) was completed by Metabolic Solutions Inc. (Nashua, NH, USA). Animals were kept awake either in a dry run or in a close-contact cage between the two blood samples.

Total body water (TBW), total body fat (TBF) and lean body mass (LBM) were calculated using the equations Arnould et al. (1996) derived for Antarctic fur seals (*Arctocephalus gazella*), the only validation study available on female otariids. These equations did not significantly overestimate TBF of adult female fur seals which meant that no correction factor needed to be applied. Changes in the percent of TBF and changes in TBF and LBM as a percent of the total body mass loss were calculated with the baseline measurement as a reference point. Two measurements of the body composition were missing for one animal in Group H in the summer phases of R2 and CR2 because the animal exhibited unusual behaviours during the collection periods not conducive to anaesthetic procedures.

Body lengths (nose to tail) were measured opportunistically throughout the experiments while animals were lying ventral side down. Length changes were compared to the baseline point to account for any initial differences between the animals.

Statistical analysis

Linear mixed-effects (lme) models were used to analyze the repeated-measures body mass, body composition and morphometric data, using R 2.4.0 (nlme library from Pinheiro et al. 2006). This approach characterizes individual variation relative to the population mean while taking into account the correlation between repeated measurements on the same participant. The lme models were fitted using the Restricted Maximum Likelihood method and the "treatment" contrast matrix (Venables & Ripley 2002), such

that all comparisons within a variable were made in reference to the baseline measurement prior to the start of the restriction. The independence and the normality of the within-group residuals and random effects as well as homogeneity of variances were verified. Any autocorrelation or heterogeneity of variances of within-group residuals were corrected for each model run in our study as explained in Pinheiro and Bates (2000). For each analysis, the best model in terms of random effects factors, autocorrelation and homogeneity of variance corrections was determined by performing an ANOVA (F-test) on two models and by selecting the most parsimonious model based on Akaike's Information Criterion (AIC). Statistical significance for each parameter estimates was set at $\alpha = 0.05$.

The sample size of the body mass data consisted of 800 measurements for the summer and 789 for the winter. One animal was removed from the trial before the end of the ad-libitum period in the winter, but her dropout did not affect the results of the mixed-effects models (Mallinckrodt et al. 2001). Dependant variables tested were total body mass and total body mass rate of change.

The first set of analyses tested the effect of time (significant change of mass over time within each treatment, i.e. baseline, restriction, re-feeding), diet type (significant difference between the 2 diet groups), and age of animals on the change in total body mass stratified by treatment and season. Time, diet, age and the interactions between these covariates were considered fixed effects. The random effects were the individual variation of each animal from the mean effect (intercept). Other covariates were potentially added to this "animal" random effect (time, diet and/or age) depending on the best model fit. The second set of analyses investigated the differences in growth rates between the different treatments and the different diets stratified by season. The fixed effects were diet, treatment, time and the interactions between these terms and the random effects were determined according to the best model. The third set of analyses investigated effect of season stratified by diet and treatment. In this case, the fixed effects considered were time, season and the interaction between these terms. The random effects were the animals and potentially time and/or season depending on the best fit determined by ANOVA and AIC.

The sample size for the body composition data was 38 during the summer (2 missing data points), and 47 for the winter experiment (1 missing data point). The sample size for morphometric data was 117 in summer and 192 in winter. Dependant variables were length, changes in LBM and TBF as a percent of the total body mass loss (%) and changes in body condition (TBF%) compared to the baseline value. The first set of analyses investigated the diet effects on a two-week period between measurements. Fixed effects considered were diet, phase and their interactions. Random effects were individual animals and/or treatments, depending on the best fit (tested with ANOVA and AIC). Treatment (4-week restriction and 4-week recovery) was considered a random factor instead of the 2-week period between measurements to limit the relative number of parameters in the model (compared to the sample size) and avoid convergence problems. The second set of analyses tested if seasonal differences exist for each experimental groups stratified by phase. Fixed effects were diet and season and random effects were selected to obtain the best model. Finally, the significance of age was investigated for each season, during each phase between the two diets. Data values provided as means ± SE and all p values were extracted from the mixed effect models summaries, unless stated otherwise.

Results

Food intake

Proximate compositions of fish used in the experiments are listed in Table 2.1 and details of food intake levels are given in Table 2.2. Gross energy intakes (GEI) during the baseline ranged from 295.5 to 372.1 kJ kg^{-1} d^{-1} (mass specific gross energy intake) in the summer and from 260.4 to 453.1 kJ kg^{-1} d^{-1} in the winter. Food intake levels during the controlled re-feeding were set to be identical to those measured during the baseline phase in terms of type of fish (Pacific herring), energy, water, lipid and protein intakes.

All animals had a higher baseline energy intake during the winter than the summer (average increase +18.7 ± 3.6%) except sea lion F00YA (subadult from Group P) who consumed 11.9% less (-35.1 kJ kg^{-1} d^{-1}) than the other sea lions. Since energy intake during baseline periods varied seasonally, the average relative decrease during the

Table 2.1: Proximate composition of the fish used during summer 2005 and winter 2006 experiments. Values given are percent of wet weight.

Season	Herring (*Clupea pallasi*)			Pollock (*Theragra chalcogramma*)		
	Protein %	Lipid %	Water %	Protein %	Lipid %	Water %
Summer	16.3	11.2	68.9	14.7	7.1	74.8
Winter	16.5	12.18	68.3	15.1	4.35	76.8

restriction was greater in winter (-28.6 ± 2.6%) than in summer (-17.5 ± 1.8%, $p = 0.0002$). The differences in proximate composition between the herring and pollock (Table 2.1) meant that biomass of fish ingested, lipid, protein and water intakes differed between Group H and Group P for identical caloric intakes during the restriction (Table 2.2). These group differences were more accentuated during the winter trials due to a greater difference in fish composition.

Group H mass-specific food intake averaged 37.1 ± 2.3 g kg^{-1} d^{-1} (g of fish per kg of sea lion and per day) during the summer restriction and 34.6 ± 1.7 g kg^{-1} d^{-1} of fish during the winter restriction, while Group P ate approximately 28% more fish than Group H during summer and 65% more in the winter (47.7 ± 4.2 g kg^{-1} d^{-1} and 57.1 ± 7.4 g kg^{-1} d^{-1} respectively). Protein intake was greater and lipid intake was lower for Group P than for Group H in both seasons during the restriction (see Table 2.2). In addition, Group H obtained 23 – 27% less water while Group P had 10 – 20% more water from fish relative to the baseline levels, again with larger differences occurring during the winter.

During the ad-libitum treatment, the animals were offered as much fish (herring) as they wanted based on their interest. In summer, the sea lions ate approximately 44.2 ± 4.5% more herring than during the baseline and the controlled re-feeding (range 41.6 – 65.0%). All animals had a strong appetite and demonstrated "hunger" behaviours (more agitated, eager to work with trainers and asking for more food). In winter however, the range of food intake during the ad-libitum period compared to the baseline was wider (range -51.0 – 16.8%), with most animals showing a lack of interest in food. In this case, the amount of food fed to the animals was dropped not to reinforce uncooperative training behaviours which may result in mass loss during the ad-libitum phase in winter.

Table 2.2: Average daily gross energy (GEI), fish biomass, lipid, protein and water intakes during the different experimental treatments (B: baseline, R: restriction, CR: controlled re-feeding and LIB: ad-libitum) of summer 2005 and winter 2006 trials for Steller sea lions in Group H and Group P. Values in brackets represent the standard errors of the means.

Season	Treatment	Group H					Group P				
		GEI (kJ d^{-1})	Fish (kg d^{-1})	Lipid (kg d^{-1})	Protein (kg d-1)	Water (kg d^{-1})	GEI (kJ d^{-1})	Fish (kg d^{-1})	Lipid (kg d^{-1})	Protein (kg d^{-1})	Water (kg d^{-1})
Summer	B	35.43 (5.75)	4.83 (0.78)	0.54 (0.09)	0.79 (0.13)	3.50 (0.57)	40.73 (7.13)	5.55 (0.97)	0.62 (0.11)	0.90 (0.16)	4.02 (0.70)
	R	27.07 (3.92)	3.69 (0.53)	0.41 (0.06)	0.60 (0.09)	2.67 (0.39)	31.64 (4.64)	5.82 (0.85)	0.41 (0.06)	0.86 (0.13)	4.55 (0.67)
	CR	34.65 (6.07)	4.83 (0.78)	0.53 (0.09)	0.77 (0.13)	3.50 (0.57)	39.78 (6.74)	5.55 (0.97)	0.61 (0.10)	0.88 (0.15)	4.03 (0.71)
	LIB	48.08 (10.37)	6.55 (1.41)	0.73 (0.16)	1.07 (0.23)	4.75 (1.02)	56.17 (10.42)	7.65 (1.42)	0.86 (0.16)	1.25 (0.23)	5.55 (1.03)
Winter	B	47.59 (5.21)	6.13 (0.67)	0.75 (0.08)	1.01 (0.11)	4.37 (0.48)	47.53 (4.58)	6.13 (0.59)	0.75 (0.07)	1.01 (0.10)	4.37 (0.42)
	R	30.34 (3.78)	3.91 (0.49)	0.48 (0.06)	0.65 (0.08)	2.79 (0.35)	34.35 (3.94)	7.76 (0.89)	0.34 (0.04)	1.17 (0.13)	6.24 (0.72)
	CR	45.33 (5.71)	5.84 (0.74)	0.71 (0.09)	0.96 (0.12)	4.17 (0.52)	46.74 (4.66)	6.02 (0.60)	0.73 (0.07)	0.99 (0.10)	4.30 (0.43)
	LIB	30.26 (7.52)	3.90 (0.97)	0.47 (0.12)	0.64 (0.16)	2.39 (0.83)	47.90 (6.20)	6.17 (0.80)	0.75 (0.10	1.02 (0.13)	4.57 (0.48)

Changes in body mass

During the baseline phase, the sea lions' rates of mass gain ranged from 0 to 182 g d^{-1} (0 – 3.39% increase from the pre-baseline mass) in summer and from -236 to 143 g d^{-1} (-0.95 – 3.89%) in the winter (see Figures 2.1a and 2.1b). Both diet groups had similar changes in body mass in summer (p = 0.33) but Group P gained mass slightly slower in winter (p = 0.01). This difference was probably due to the "abnormal" mass loss of F00YA (-236 g d^{-1}) which lowered the overall average of the 4 animals in the group. Initial body masses at the beginning of the restriction ranged from 86.9 to 90.6 kg for juveniles and from 169.9 to 177.7 kg for sub-adults in summer, and from 99.80 to 106.8 kg for juveniles and 179.6 to 182.80 kg for subadults in winter.

After 28 days of restriction, Group H lost on average 10.11 ± 0.92% of their initial body mass while Group P lost 9.91 ± 0.63% during the summer. During winter, Group H lost 8.51 ± 1.95% and Group P lost 7.84 ± 1.1%. Rates of mass loss were generally linear and there were no seasonal differences (p = 0.42 for Group H, and p = 0.17 for Group P) or dietary differences (p = 0.78 in the summer and p = 0.55 in the winter).

During the subsequent controlled re-feeding in summer (when returned to the baseline diet), relative changes in mass ranged from -5.15 to 2.2% (-229 – 46 g d^{-1}), which were not statistically different from baseline rates (p = 0.71). Mass changes were identical for both diet groups (p = 0.07). Overall, the animals remained at the weight they reached at the end of the previous restriction throughout the controlled re-feeding (p = 0.24). Animals showed clear hunger behaviours even though their food intakes had returned to the baseline levels. During the ad-libitum period when food intake increased by approximately 45% over baseline/re-feeding levels, body mass increased by 7.96 ± 0.83% compared to the controlled re-feeding rate (336 – 943 g d^{-1}; p < 0.0001), with no difference between the two diet groups (p = 0.80). All the sea lions eventually reached their pre-experimental body mass, but only after the experiment had ended.

During the winter controlled re-feeding, relative mass gains increased by 7.29 ± 1.43% compared to the baseline levels (p < 0.0001). They ranged from 210 to 414 g d^{-1}, except for F00YA, who had a particularly low rate of mass gain (29 g d^{-1}) and was the
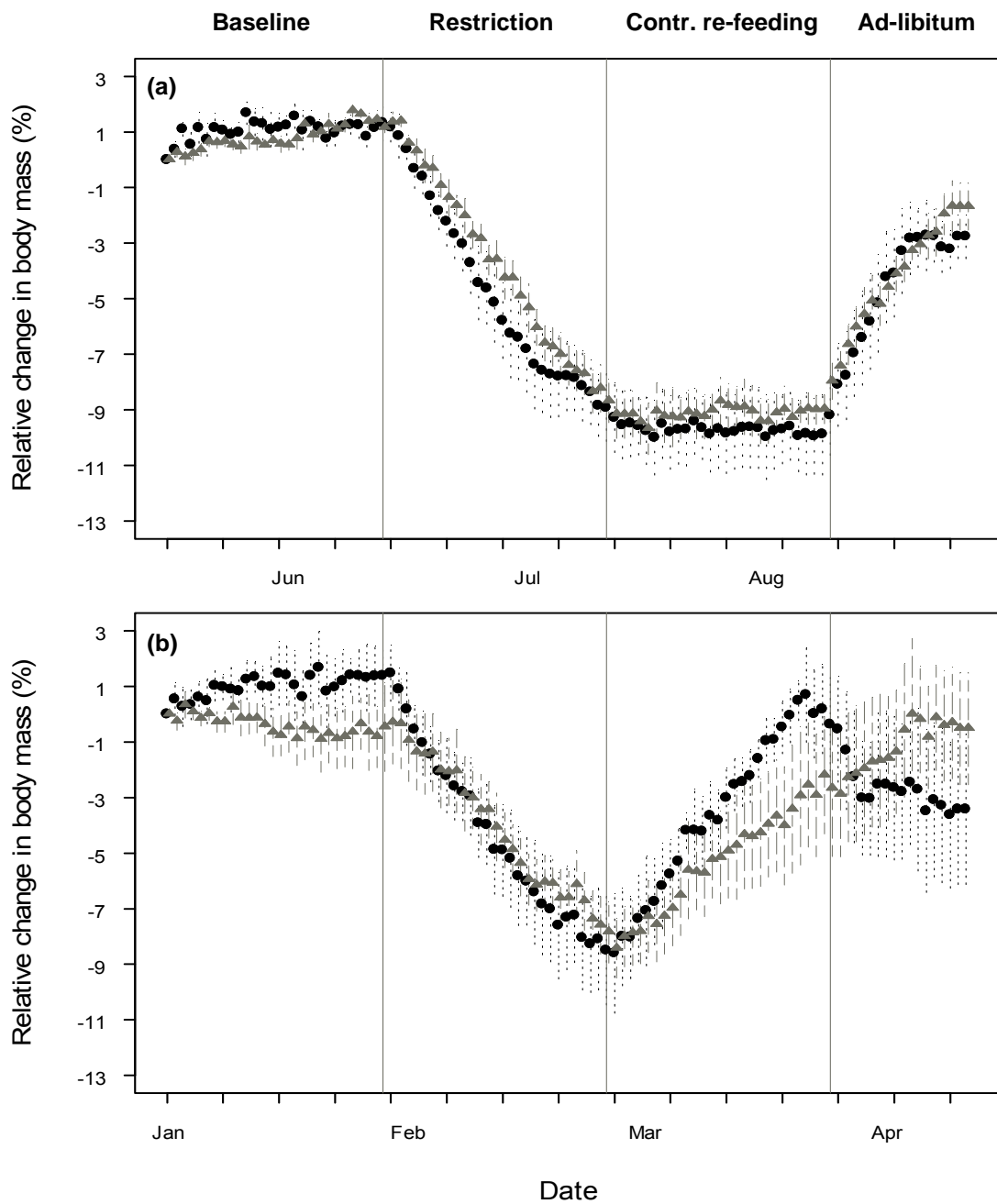
Figure 2.1: Mean ± SE changes in body mass as a percent of the initial pre-experimental body mass measurement for eight female Steller sea lions divided into 2 experimental groups (Group H in black circles and dotted lines and Group P in grey triangles and dashed lines), during 4 experimental phases of summer 2005 (a) and winter 2006 (b): Baseline, Restriction, Controlled re-feeding and Ad-Libitum.

same animal that had atypical changes in body mass during the other treatments of the experiment. The controlled re-feeding rates of mass gain did not differ significantly

between the two diet groups (p = 0.07), but were greater in winter than summer (p < 0.01). At the end of this treatment, most animals reached body masses that were close to their pre-experimental weights. Mass growth rates during the ad-libitum period depended on this intake/behaviour relationship and consequently varied highly. Some animals reached a plateau, some showed an increase, while others had decreased growth rates as a result of their uncooperative behaviour (Figure 2.1b). Age was never a factor significantly affecting rates of mass changes (all $p > 0.05$).

Changes in body composition

Changes in relative or absolute body composition indicated which type of tissue the animals preferentially catabolized while losing body mass or reacquired during subsequent re-feeding treatment. Age of the animals was found to have no significant effect on the changes in body composition at any time throughout the experiments or in any seasons (all $p > 0.05$). Group H started the summer experiment with an average TBF of 20.2 ± 1.6% (range 16.6 – 22.9%) which was comparable to Group P average of 16.9 ± 1.2% (range 14.7 – 19.7%; Wilcoxon rank-sum test, $p = 0.34$).

Changes in body composition during phases of mass loss were expressed as percent of the total mass loss, hence the average of total body fat (TBF) and lean body mass (LBM) losses always summed to 100% (of the total body mass loss; Figure 2.2). After 14 days of restriction in the summer, animals in Group H lost 144.6 ± 7.4% of their total body mass as TBF. This value exceeded 100%, which means that Group H gained lean body mass (LBM) despite losing overall body mass. After the full 28 days of restriction, 90.8 ± 21.2% of their body mass loss was from TBF and 9.2 ± 21.2% from LBM (Wilcoxon rank sum test, $p < 0.05$).

Group P lost significantly less TBF (as a percentage of the total mass loss) than Group H (-24.7 ± 45.8% after 14 days of restriction and -47.6 ± 12.6% and 28 days; both $p < 0.001$), therefore significantly more LBM (-75.3 ± 55.5% after 14 days and -53.3 ± 12.6% after 28 days ; both $p < 0.004$). Group H finished the summer restriction with an average TBF of 12.2 ± 1.4% of their total body mass similar to the 14.2 ±1.2% of Group P (Wilcoxon rank sum test, $p = 0.34$). The decrease in TBF as a percent of body mass (i.e.
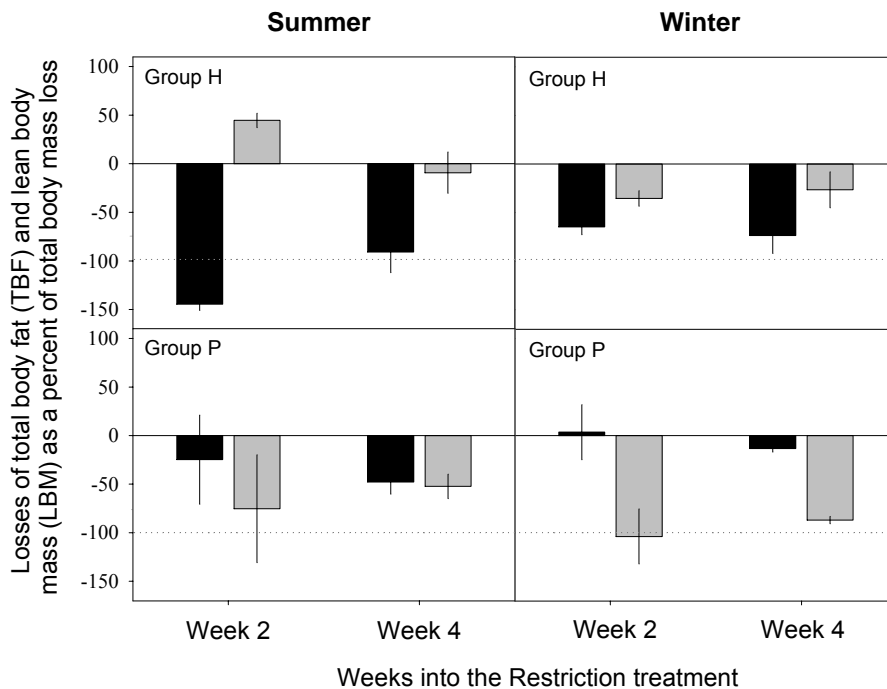
Figure 2.2: Mean ± SE loss of total body fat (black bars) and lean body mass (grey bars) as a percent of the total body mass lost at the end of the second and fourth week of the restriction treatment compared to the body composition at the baseline measurement for Group H and Group P during summer 2005 and winter 2006.

TBF%, changes in body condition compared to the initial baseline values) during the restriction was significantly greater for Group H than for Group P ($p = 0.01$; Figure 2.3).

In winter, animals in both diet groups started the experiment with the same percentages of total body fat (H = 20.2 ± 0.8% and P = 19.3 ± 1.4% of their body mass; Wilcoxon rank sum test, $p = 0.88$) which were also similar to summer values ($p = 0.68$). During the winter restriction, changes in body composition followed the trends observed in summer (test for seasonal differences: $p = 0.11$ for Group H and $p = 0.22$ for Group P), though both groups tended to spare more lipid in winter. Significantly more body mass was lost as TBF at 14 days by Group H (-64.6 ± 8.0%) than Group P (+3.6 ± 28.3%, $p < 0.001$), as well as at 28 days of food restriction (H = -73.4 ± 18.5% and P =-13.1 ± 3.7%; $p < 0.01$). Group P lost their body mass almost exclusively as lean body mass for the entire restriction period (-103.6 ± 28.3% after 14 days and -86.6 ± 3.7% after 28 days). This resulted in a decrease in body condition (TBF%) for Group H (Figure 2.3), but not
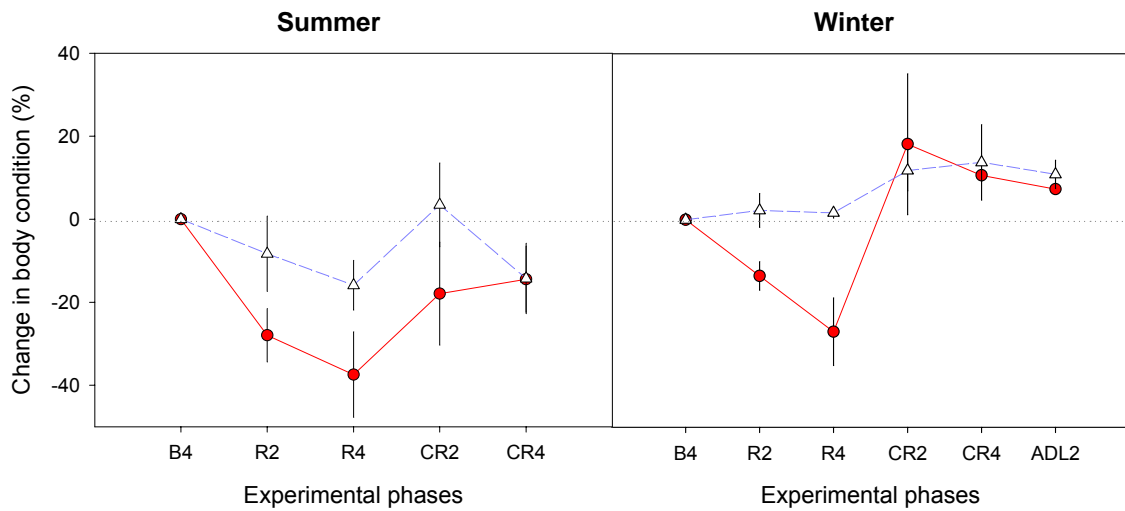
Figure 2.3: Mean ± SE changes in body condition (percentage of body fat in the body, TBF%) compared to the baseline measurements (B4) for animals in Group H (circles) and Group P (triangles) after 2 weeks and 4 weeks of energy restriction (R2 and R4) and 2 weeks and 4 weeks of controlled re-feeding (CR2 and CR4) for the summer and the winter experiments. In winter, an additional measurement was performed after 2 weeks of ad-libitum food (LIB2).

for Group P, even though the total body mass loss was the same. Sea lions in Group H finished the restriction with a relative body condition (14.5 ± 1.4%) significantly lower than the baseline value ($p < 0.01$). In contrast, body condition of Group P stayed stable throughout the food restriction (19.6 ± 1.4% at the end of the restriction, $p = 0.83$).

At the end of the controlled re-feeding phases in both winter and summer trials, the body composition of the sea lions was similar to the one measured at the end of the respective baseline ($p = 0.46$, $p = 0.89$). Group H's body condition (TBF%) increased from 12.3 ± 1.4% of the total body mass to 17.1 ± 1.9% at the end of the restriction ($p < 0.05$). Group P's body condition however did not significantly change (14.2 ± 0.6% of TBF at the end of controlled re-feeding compared to 14.2 ± 1.3% at the end of restriction, $p > 0.05$). During the winter controlled re-feeding, the increases in body mass of the two groups was mostly due to gains in body fat. This increase was particularly striking for Group H. As shown on Figure 2.3, the body condition of group H was -26.9 ± 8.1% lower than baseline levels at the end of the restriction but was actually greater than baseline levels (+18.2 ± 16.9%) two weeks into the controlled re-feeding. All animals finished the winter controlled re-feeding with a slightly higher proportion of TBF than before the

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Table 2.3: Mass and length of female Steller sea lions at the end of each experimental treatment: baseline (B4), restriction (R4), controlled re-feeding (CR4) and ad-libitum (LIB2). Animals of the same age belonging to experimental Groups H and P were pooled because diet was not a significant factor for any of these variables. Values in brackets represent the standard errors of the means.

Season	Age	Phase	Body mass (kg)	Body length (cm)
Summer	Juvenile	B4	89.22 (0.74)	165.87 (1.79)
		R4	79.56 (1.06)	165.60 (1.83)
		CR4	80.56 (1.15)	164.87 (1.80)
		LIB2	86.40 (0.80)	167.40 (1.44)
	Subadult	B4	171.40 (0.96)	212.44 (3.39)
		R4	156.17 (2.19)	213.33 (3.47)
		CR4	153.57 (2.40)	213.67 (3.76)
		LIB2	163.23 (3.11)	213.67 (3.56)
Winter	Juvenile	B4	103.88 (1.23)	173.07 (2.61)
		R4	94.76 (1.67)	171.40 (2.46)
		CR4	103.92 (1.49)	173.33 (1.47)
		LIB2	103.40 (2.36)	173.58 (1.50)
	Subadult	B4	181.47 (0.96)	218.56 (2.62)
		R4	169.20 (2.91)	220.22 (2.78)
		CR4	173.33 (0.88)	146.48 (4.15)
		LIB2	175.53 (3.73)	217.67 (2.83)

restriction for approximately the same body mass, even though the difference was not statistically significant ($p = 0.20$).

Morphometrics

In summer, length of the animals ranged from 161 to 171 cm for the juveniles and from 207 to 216 cm for the sub-adults at the baseline measurement (Table 2.3). In winter, length measurements ranged from 169 to 182 cm and 213 to 222 cm for the juveniles and the sub-adults respectively. The sea lions had a small (0.6 ± 0.2%) but statistically significant increase in length over the 3-month experiment during the summer (different

from 0, one sample *t* test $p = 0.017$) and but no significant length growth during winter (-0.3 ± 0.4%, one sample *t* test $p = 0.42$). Age of the sea lions did not influence the changes in length (all $p > 0.05$).

Discussion

Steller sea lions showed different physiological responses to reductions and subsequent increases in energy intake depending on season and the quality of diet consumed. The feeding experiments revealed that the rate of energy intake determined how quickly body mass changed during periods of nutritional stress, but the quality of prey consumed determined whether the internal energy source utilized during mass loss is predominantly lipids or proteins. The seasonal effect of nutritional stress was only apparent during the controlled re-feeding following the restriction, which enabled the sea lions to recover in winter but not in summer. Physiological adjustments observed from the captive study experiments extend our understanding of responses of Steller sea lions to nutritional stress and their consequences on the fitness of animals in the wild.

Body mass and composition changes during the nutritional restriction

Type of fish (herring or pollock) fed to the sea lions during the energy restriction did not affect the rate of mass loss. Rather, the loss of mass was explained by the degree of energy intake. The rates of mass loss observed during the energy restriction (around 10-15% of sea lions initial body mass) were consistent with the response of sea lions fed restricted diets of atka mackerel or Pacific herring for 29 days (Rosen & Trites 2005). These studies suggest that level of energy intake (20 – 30% decrease in our experiment) is the critical factor determining total mass loss rather than the type of fish, the protein/lipid ratio or the biomass intakes. However, Kumagai et al. (2006) subjected Steller sea lions to a more acute energy restriction (loss of 10 – 15% of initial body mass in 9 days) and found that those fed a submaintenance diet of walleye pollock lost body mass at a significantly higher rate than those fed Pacific herring in the summer and spring, and at a significantly lower rate in the fall. Consequently, effects of diet composition on total mass loss likely depend on the severity of the energy restriction, and are less obvious during milder restrictions such as those we imposed.

There was also no difference in rates of mass lost between the seasons. Some studies have reported that fasting pinnipeds lose mass quicker during the breeding season (summer) than at other times of the year (Rea et al. 2007b) while other studies reported greater losses during winter (Castellini & Rea 1992, Kumagai 2004, Rosen & Trites 2004). The lack of seasonal difference in our study is curious because baseline energy intakes were greater in winter than summer as commonly reported in pinnipeds (Kastelein et al. 1990, Renouf & Noseworthy 1991, Rosen & Renouf 1998, Kastelein et al. 2000), and as predicted by Steller sea lion bioenergetic models (Winship et al. 2002). This means that the relative decrease in energy intake of our study animals during the winter restriction (energy deficit) was greater than during the summer restriction, yet resulted in similar overall mass loss. While the energetic mechanisms that facilitated these seasonal differences are unclear, it suggests that Steller sea lions may more easily withstand a mild nutritional stress in winter than in summer.

The seasonally dependant physiological responses of Steller sea lions may be related to seasonal differences in biological and environmental parameters. Fish populations tend to be more dispersed and less predictable (Sigler et al. 2004, Womble et al. 2005) and to have lower intrinsic energy content in winter and spring than in summer and fall (Anthony et al. 2000, Kitts et al. 2004). Scarce fish in winter may have driven physiological adaptations towards conservative metabolic and physiological states, such that sea lions are more disposed to adjusting and sparing their body reserves in winter when facing a moderate deficit in energy intake. Maintaining body mass in winter may also be a greater priority for sea lions experiencing reduced energy intake than in summer for thermoregulation or reproductive purposes.

While energy intake drove the observed rates of body mass loss, quality of fish ingested determined the predominant energy source (lipid versus protein stores) used by the sea lions while losing mass. Typically, physiological reactions to nutritional stress involve a relatively short phase of mobilizing hepatic glycogen reserves followed by a phase of variable duration where lipids are the main energy source used to spare the core constituents of the body, and finally a phase where the lipid stores are depleted and replaced by protein catabolism as the energy source (Cahill et al. 1966, Klasing 1985, Waterlow 1986, Castellini & Rea 1992, Gibney et al. 2003, Roach et al. 2003). This

terminal phase ultimately leads to organ failure and death if better conditions are not rapidly restored. Consequently, the common pattern of fasting physiology is for animals to rely heavily on their readily metabolized lipid reserves as energy sources and spare their lean tissues as long as possible. This appears to have been the case for sea lions in Group H. Lipids are easily stored and depleted with no structural consequences. In addition, lipids are less necessary for thermoregulation (Rosen & Renouf 1997) during the summer which may have accounted for the almost complete reliance on lipid catabolism by Group H. Group P however, lost a large amount of lean body mass tissue which are the structural components of organs. This normally occurs only in the terminal phase of nutritional stress when the lipid stores are depleted. In our case however, lean tissue loss from Group P during the restriction reached 52% of their body mass loss in summer and 87% in winter.

Other studies have also shown marine mammals relying on their lean tissues at an unexpected rate while fasting. Harbour seals for example obtained 23% of their energy from protein catabolism (Markussen et al. 1992b) while 45% of the muscle mass accumulated by southern elephant seal pups during lactation was consumed during fasting (Bryden 1969). Bottlenose dolphins (*Tursiops truncatus*) and Pacific white sided dolphins (*Lagenorhynchus obliquidens*) also lost as much muscle as fat while fasting (Ridgway 1972). The sea lions in our experiment did not fast but were mildly deprived. It appears that the choice of their strategy of tissue catabolism was triggered by the type of diet fed to the sea lions. The pollock diet reduced or prevented the animals in Group P from sparing lean mass and core constituents, while animals in Group H were able to spare their lean tissues.

It is difficult to discriminate which components in the experimental diets (i.e., water content, fish biomass, and protein/lipid ratio) were responsible for the observed differences in tissue catabolism. The sea lions fed the herring diet experienced a decrease in fish biomass intake of up to 40% in winter in addition to a decrease in energy intake, which means that they also faced a direct 40-55% decrease in water intake through their food (the principal if not sole source of freshwater in pinnipeds, Depocas et al. 1971, Ortiz et al. 1978b, Keiver et al. 1984). While blood hematocrit, Na^+, and K^+ levels (data not shown) did not indicate any sign of dehydration, such a decrease in water intake may have

presented a homeostatic challenge thereby activating water saving mechanisms for animals in Group H but not in Group P. Group H may have decreased protein catabolism to conserve water by increasing the urine osmolality and/or by decreasing the amount of nitrogen waste they passed (Worthy 2001) as shown with fasting northern elephant seal pups (Adams & Costa 1993). Oxidation of fat would provide energy as well as a source of metabolic water to withstand long fasts with no access to water as catabolism of 1g of lipid produces 1.1g of metabolic water (Ortiz et al. 1978b). In contrast, Group P that ate pollock did not need to spare water or down-regulate their protein catabolism.

Another possible explanation for the observed diet-induced differences in body composition changes involves conflicting regulatory signals triggered by the low mass-specific energy content of the pollock diet — and thus the higher fish biomass intake for this group. Group P (eating low energy density fish) lost body mass while getting a "full" fish intake (relative to the biomass of herring consumed prior to the diet switch) but with a greater protein/lipid ratio. Conflicting signals between satiation regulation and energy balance regulation pathways could have occurred and shortcut the usual mobilization of lipid stores. Presence of a full chyme in the gastrointestinal tract for the sea lions fed pollock would have induced gastric distension and released satiation peptides (e.g., CCK, peptide YY) from the intestine wall (Cupples 2005). These satiation signals would also have interacted with adiposity and body weight hormones, such as insulin, leptin, and ghrelin. Leptin and insulin increase synergistically with satiation peptides, but are known to decrease during nutritional stress. In contrast, ghrelin decreases with satiation, but is known to be up regulated while losing weight (Cummings & Overduin 2007). Thus, opposite signals from the two regulatory pathways of these 3 hormones could have offset the lipid versus protein catabolism regulation and resulted in the observed differences in body composition.

Differences in protein/lipid ratio are a third possible explanation for the observed differences in body composition between the two groups of sea lions. It is interesting to note that the animals fed pollock during the restriction catabolized more proteins as energy resources, even though they were the nutrient in greatest supply in their diet. Pinniped diets are usually high in lipids and relatively poor in proteins compared to terrestrial carnivores (Boyd 2002). Switching to a low energy diet such as pollock results in an

elevated dietary protein load. Dietary protein content can affect amino-acid homeostasis, protein metabolism, body growth and food intake in mammals (Kerr & Easter 1995, Brosnan & Young 2003, Whang et al. 2003). It is commonly understood that a regulatory mechanism of protein anabolism/catabolism keeps the levels of free amino acids fairly constant (Peters & Harper 1985), but this mechanism can be overloaded, especially in non-adapted animals (Fujita et al. 1981).

An increase in protein intake through the diet may paradoxically lead to an increase in protein catabolism as a means to avoid excessive accumulation of some amino-acids (aromatic for example) in body tissues and a decrease of the availability of some amino-acids essential for growth as shown in rats (Moundras et al. 1993). Zhao et al. (2006) showed that harbour seals switched for 3 months from a high fat herring to a high protein pollock diet experienced a decrease in available essential amino-acids and an increase in protein catabolism. In our study, dietary protein did not vary significantly in Group P as a percent from the baseline diet (herring) to the restriction diet (pollock) since the amount of food intake was kept to a set level (the animals were not allowed to compensate for the low energy content of the food by increasing their intake). However, the protein/lipid ratio did change from 1.4 to 2.1 in the summer and from 1.3 to 3.4 in the winter (when the loss in lean body mass was the greatest). So even if the change in protein intake was not as drastic as in Zhao et al. (2006), higher relative protein intakes for Group P could have contributed, along with other regulatory mechanisms, to enhance body protein catabolism, and thereby explain the higher lean body mass loss for this experimental group.

While it is not clear which of the discussed mechanisms ultimately explains the different ratio of lipids and proteins mobilized by sea lions fed herring and pollock, it is clear that the inability of Group P to spare their core tissues would have ultimately resulted in much more deleterious consequences for the overall health of animals in this group than for those in Group H. The sea lions fed pollock lost between 7.7 and 13.4 kg of protein mass in winter, which represented a substantial loss of tissue. Major losses of protein mass during acute or chronic nutritional stress have been linked to reduced muscle strength and endurance (Vaz 2003) and could reduce the foraging efficiency of sea lions (further increasing energy debt, Rosen et al. in press). Skin lesions have also been

observed (Gòmez et al. 2000) which could increase their sensitivity to diseases. Ultimately, the loss of lean tissues leads to failures of vital organs (i.e., heart, liver, kidney and others). A pollock-dominant diet (i.e., high biomass with low energy intakes) may therefore be more problematic for Steller sea lions in the wild than a herring-dominant diet that would be consumed in lesser amounts to obtain the same gross energy intake.

Compensatory growth

The quality of diet during nutritional stress has been shown to impact the post-restriction recovery of a number of species (Wilson & Osbourn 1960), but in our case the lipid/protein ratio differences in the herring and pollock diet did not impact recovery. Sea lions in both groups ended their controlled recoveries with similar body masses and body compositions to each other. This means that recovery diets buffered the previously observed differences. However, it is difficult to distinguish whether this "buffering" resulted from the increased energy intake or was a result of the 'high quality' of the type of fish fed. Further studies are needed to tease apart the impact of these two variables on ability to recover.

The finding that Steller sea lions had greater difficulty recovering from nutritional stress in summer compared to winter was unexpected. Summer was presumed to be the easiest season to recover because energy requirements were believed to be lower than in winter (Winship et al. 2002). Instead, we found that winter was less challenging for sea lions to withstand nutritional stress, and was also the easiest time of year to recover from it (compared to summer). During the summer controlled re-feeding, the animals attained a growth rate that was no better than during the baseline and resulted in body masses that were similar to those at the end of the restriction (10 – 15% lower than during the baseline). They required substantially more energy to jump start their recovery (see Figure 2.1a). During the winter however, the recovery growth rate was significantly higher than before the restriction, which is typical of a compensatory growth episode (Ali et al. 2003).

Mechanisms that lead to compensatory growth following a nutritional stress episode are multifactorial and complex, and involve the type, quantity, quality, and content of diet during recovery (Heath & Randall 1985, Zubair & Leeson 1996, Boersma & Wit 1997, Ali et al. 2003). Most animals recovering from a food deprivation go through

a temporary phase of hyperphagia (i.e. an increase in appetite and in satiation level threshold leading to an energy intake that is well above normal, Wilson & Osbourn 1960, Miglavs & Jobling 1989, Zubair & Leeson 1996, Xie et al. 2001). This phase is thought to be needed to overcome energy-conserving physiological states and reactivate regulatory feedback loops while meeting the demands of enhanced rates of growth (Boersma & Wit 1997).

Baseline energy intakes were sufficient for sea lions to recover body mass during winter but were insufficient during summer even after a month of controlled re-feeding. It can be argued that this was due to the fact that the energy intake was higher during the winter controlled re-feeding than during the summer (based on higher baseline intakes). However, for both seasons food levels during the re-feeding periods were set at respective baseline levels during which the growth rates were similar between seasons. Hyperphagia needs were apparently not fulfilled during the summer until the ad-libitum phase when animals were allowed to eat as much food as they wanted (an energy intake increase of around 50% compared to the baseline). Only then did the sea lions start to recover and quickly reach their pre-experimental weights. If animals in the wild do not have access to this high energy intake phase in summer, they may stay at a suboptimal weight and be less fit in their environment for extended periods of time. However in winter, there was no need to increase the energy intake above baseline levels to overcome the mass loss caused by the nutritional stress.

Enhanced growth rates derived form the same relative energy intakes suggests that the conversion efficiency of food into body mass (FCE) increased in winter (compared to baseline) but did not change in summer. This implies that in winter some physiological or behavioural mechanisms allowed allocating a greater proportion of the energy intake to body mass growth. One possibility is that the sea lions extracted more energy and/or nutrients from their food (i.e. better digestive an/or nutrient conversion efficiencies, Wilson & Osbourn 1960, Reimers et al. 1993, Qian et al. 2000), but the fact that the sea lions ate the same fish and lost mass at the same rate following identical protocols suggests otherwise. Another possibility is that the energy-conserving mechanisms (such as metabolic depression) initiated during the restriction continued during the controlled re-feeding in the winter (Boyle et al. 1981) but not in summer.

It is also possible that losing more lean tissues in winter than summer could have contributed to the observed compensatory growth, given that lean tissues require greater maintenance energy than lipid mass. Protein turnover can reach up to 20% of the basal metabolic rate in adult humans (Waterlow 1995) and may be even higher in juvenile mammals compared to adults of the same species (Millward et al. 1976). Consequently, a greater loss of LBM in the winter could lead to a substantial decrease in the energy needed to maintain it, and thus a decrease in the basal metabolic rate that could carry forward in the first days of recovery (although greater losses of LBM for Group P did not lead to greater recovery than Group H in this season).

It is important to remember that compensatory growth can occur in terms of structural growth (tissues and bones, Royle 2000, Victora et al. 2001), body mass and condition, (energy status, Xie et al. 2001, Alvarez & Nicieza 2005, Johnsson & Bohlin 2005), or both (Johnsson & Bohlin 2006). In the case of Steller sea lions, the major body component deposited during the compensatory growth in winter was lipid, a non-structural restoration of energy status that is essential to regulate the costs of thermoregulation. Such a phenomenon has been observed in fish who favour the deposition of lipids during winter to increase the chances of over-wintering survival (Metcalfe & Monaghan 2001). Growth in terms of mobile reserves is not as costly to deposit (and to maintain) as is growth of bones and lean tissues (Pullar & Webster 1977, Webster 1981) and may thus be easier to gain than structural tissues.

During summer, restoring lipid mass may have been less of a priority compared to structural growth because thermoregulatory needs were minimal. The immature female Steller sea lions may have used their energy intake during the summer re-feeding phase to resume their structural growth (see morphometric data), which would have required more energy and would have been less efficient to deposit per unit of mass gained than fat mass (Pullar & Webster 1977, Webster 1981). Baseline levels of IGF-1 were also found to be higher in summer than winter (see Chapter 4), which is consistent with preferred structural growth in summer. Consequently, the seasonal difference in the occurrence of compensatory growth could have reflected different types of recovery between body energy status and structural growth between summer and winter.

The universal occurrence of compensatory growth within the animal kingdom implies that this phenomenon is beneficial. In terms of survival, pinnipeds of bigger size usually have greater foraging efficiency (Le Boeuf 1994), are better at avoiding predation and incur higher survival rates (Baker et al. 1994, Hall et al. 2001, Muelbert et al. 2003, Harding et al. 2005), and greater reproduction success and social dominance (Anderson & Fedak 1985, Boltnev et al. 1998, Boness et al. 2002, Chambellant et al. 2003). For marine mammals, a bigger size also infers energetic advantages and reduces costs associated with living in a highly thermally conductive environment (Kleiber 1975).

While compensatory growth happens to be universal, the fact that there has been no evolutionary selection for high growth rates implies that some costs are associated with them (Arendt 1997). Specifically, a hyperphagic response following nutritional stress would result in sea lions increasing their foraging effort and exposing themselves to greater predation risks (McNamara & Houston 1990, Jonsson et al. 1996, Gotthard 2000). This can be particularly risky for young and naïve animals (Johnsson & Bohlin 2006). Compensatory growth has also been associated with higher risks of developmental errors and tissue lesions (Ali et al. 2003), with increased risks to diseases (Metcalfe & Monaghan 2001) and with decreases in longevity (Hales & Ozanne 2003).

Whatever the mechanisms that explain the patterns of compensatory growth we observed, it seems that Steller sea lions recover more easily from an energy restriction in winter than in summer, meaning that restoring mass is a priority in this season to increase survival and reproduction success, two parameters which, in turn, shape the dynamics of the Steller sea lion population.

Conclusions

Steller sea lions that ate reduced amounts of energy from either pollock or herring lost equivalent amounts of body mass, but differed in how they mobilized their body reserves to compensate for the reduced energy intake. Those that ate pollock (a low-lipid, high-protein fish) lost their ability to mobilize their lipid energy source and incurred a sustained loss of lean tissue mass. In the long run, this strategy would have been more deleterious than the protein-sparing strategy adopted by the sea lions that ate herring (a high-lipid, low-protein fish). Time of the year did not impact the rate of mass loss while

nutritionally stressed (even though the energy deficit was relatively greater in winter than summer), but did determine the extent of the recovery when feeding conditions improved. Compensatory growth occurred during winter but not during summer which was both unexpected and of concern because of the impact that compensatory growth can have on the health and life history parameters of animals. Further research is needed to link changes in body mass and composition with changes in thermoregulation capacity, activity, basal metabolism, and reproductive ability to understand the impact on the immediate fitness of Steller sea lions and their ultimate effects on population dynamics.

References

Adams SH, Costa DP (1993) Water conservation and protein metabolism in northern elephant seal pups during the postweaning fast. Journal of Comparative Physiology [B] 163:367-373

Ali M, Nicieza A, Wootton RJ (2003) Compensatory growth in fishes: a response to growth depression. Fish and Fisheries 4:147-190

Alvarez D, Nicieza AG (2005) Compensatory response 'defends' energy levels but not growth trajectories in brown trout, *Salmo trutta L.* Proceedings of the Royal Society B-Biological Sciences 272:601-607

Anderson SS, Fedak MA (1985) Grey seal males: Energetic and behavioural links between size and sexual success. Animal Behavior 33:829-838

Anthony JA, Roby DD, Turco KR (2000) Lipid content and energy density of forage fishes from the northern Gulf of Alaska. Journal of Experimental Marine Biology and Ecology 248:53-78

Arendt JD (1997) Adaptive intrinsic growth rates: an integration across taxa. The Quarterly Review of Biology 72:149-177

Arnould JPY, Boyd IL, Speakman JR (1996) Measuring the body composition of Antarctic fur seals (*Arctocephalus gazella*): validation of hydrogen isotope dilution. Physiological Zoology 69:93-116

Baker JD, Fowler CW, Antonelis GA (1994) Body weight and growth of juvenile male northern fur seals, *Callorhinus ursinus*. Marine Mammal Science 10:151-162

Bjorndal KA, Bolten AB, Dellinger T, Delgado C, Martins HR (2003) Compensatory growth in oceanic loggerhead sea turtles: Response to a stochastic environment. Ecology 84:1237-1249

Boersma B, Wit JM (1997) Catch-up growth. Endocrine Reviews 18:646-661

Boltnev AI, York AE, Antonelis GA (1998) Northern fur seal young: interrelationships among birth size, growth, and survival. Canadian Journal of Zoology 76:843-854

Boness DJ, Clapham PJ, Mesnick SL (2002) Life history and reproductive strategies. In: Hoelzel AR (ed) Marine mammal biology: an evolutionary approach. Blackwell Science Publishing, Oxford ; Malden, MA, p 278-324

Bowen WD, Iverson SJ (1998) Estimation of total body water in pinnipeds using hydrogen-isotope dilution. Physiological Zoology 71:329-332

Boyd IL (2002) Energetics: consequences for fitness. In: Hoelzel AR (ed) Marine mammal biology: an evolutionary approach. Blackwell Sciences Publishing, Oxford, Malden, p 247-277

Boyle PC, Storlien LH, Harper AE, Keesey RE (1981) Oxygen consumption and locomotor activity during restricted feeding and realimentation. American Journal of Physiology 241:R392-397

Brosnan JT, Young VR (2003) Integration of metabolism 2: proteins and amino acids. In: Gibney MJ, Macdonald I, Roche HM (eds) Nutrition and Metabolism. Blackwell Publishing., Oxford ; Ames, Iowa, p 43-73

Bryden MM (1969) Growth of the southern elephant seal, *Mirounga leonina* (Linn.). Growth 33:69-82

Cahill GF, Herrera MG, Morgan AP, Soeldner JS, Steinke J, Levy PL, Reichard GA, Kipins DM (1966) Hormone-fuel interrelationships during fasting. Journal of Clinical Investigation 45:1751-1769

Castellini MA, Rea LD (1992) The biochemistry of natural fasting at its limits. Experientia 48:575-582

Chambellant M, Beauplet G, Guinet C, Georges J-Y (2003) Long-term evaluation of pup growth and preweaning survival rates in subantarctic fur seals, *Arctocephalus tropicalis*, on Amsterdam Island. Canadian Journal of Zoology 81:1222-1232

Cherel Y, Robin JP, Heitz A, Calgari C, Le Maho Y (1992) Relationships between lipid availability and protein utilization during prolonged fasting. Journal of Comparative Physiology B 162:305-313

Costa DP (1987) Isotopic method for quantifying material and energy intake of free-ranging marine mammals. In: Huntley AC, Costa DP, Worthy GAJ, Castellini MA (eds) Approaches to marine mammal energetics. Society for Marine Mammalogy. Allen Press, Lawrence, p 43-66

Cummings DE, Overduin J (2007) Gastrointestinal regulation of food intake. Journal of Clinical Investigation 117:13-23

Cupples WA (2005) Physiological regulation of food intake. American Journal of Physiology 288:R1438-R1443

Depocas F, Hart JS, Fisher HD (1971) Sea water drinking and water flux in starved and in fed harbor seals, *Phoca vitulina*. Canadian Journal of Physiological Pharmacology 49:53-62

Donnelly CP, Trites AW, Kitts DD (2003) Possible effects of pollock and herring on the growth and reproductive success of Steller sea lions (*Eumetopias jubatus*): insights from feeding experiments using an alternative animal model, *Rattus norvegicus*. British Journal of Nutrition 89:71-82

Fujita Y, Yamamoto T, Rikimaru T, Ebisawa H, Inoue G (1981) Effect of quality and quantity of dietary protein on free amino-acids in plasma and tissues of adult rats. Journal of Nutritional Science and Vitaminology 27:129-147

Gibney MJ, Macdonald I, Roche HM, Nutrition Society (Great Britain) (2003) Nutrition and metabolism. Blackwell Publishing., Oxford ; Ames, Iowa

Gòmez F, Galvan RR, Frenk S, Muñoz JC, Chavez R, Vasquez J (2000) Mortality in second and third degree malnutrition. Bulletin of the World Health Organization 78:1275-1280

Gotthard K (2000) Increased risk of predation as a cost of high growth rate: an experimental test in a butterfly. Journal of Animal Ecology 69:896-902

Hales CN, Ozanne SE (2003) The dangerous road of catch-up growth. The Journal of Physiology 547:5-10

Hall A, McConnell BJ, Barker RJ (2001) Factors affecting first-year survival in grey seals and their implications for life history strategy. Journal of Animal Ecology 70:138-149

Harding KC, Fujiwara M, Axberg Y, Harkonen T (2005) Mass-dependent energetics and survival in harbour seal pups. Functional Ecology 19:129-135

Heath RGM, Randall RM (1985) Growth of jackass penguin chicks (*Spheniscus demersus*) hand reared on different diets. Journal of Zoology 205:91-105

Jespersen LB, Toft S (2003) Compensatory growth following early nutritional stress in the wolf spider *Pardosa prativaga*. Functional Ecology 17:737-746

Jodice PGR, Roby DD, Turco KR, Suryan RM, Irons DB, Piatt JF, Shultz MT, Roseneau DG, Kettle AB, Anthony JA (2006) Assessing the nutritional stress hypothesis: relative influence of diet quantity and quality on seabird productivity. Marine Ecology-Progress Series 325:267-279

Johnsson JI, Bohlin T (2005) Compensatory growth for free? A field experiment on brown trout, *Salmo trutta*. Oikos 111:31-38

Johnsson JI, Bohlin T (2006) The cost of catching up: increased winter mortality following structural growth compensation in the wild. Proceedings of the Royal Society B-Biological Sciences 273:1281-1286

Jonsson E, Johnsson JI, Bjornsson BT (1996) Growth hormone increases predation exposure of rainbow trout. Proceedings of the Royal Society of London Series B-Biological Sciences 263:647-651

Kastelein R, Schooneman NM, Vaughan N, Wiepkema PR (2000) Food consumption and growth of California sea lions (*Zalophus californianus californianus*). Zoo Biology 19:143-159

Kastelein RA, Vaughan N, Wiepkema PR (1990) The food consumption of Steller sea lions (*Eumetopias jubatus*). Aquatic Mammals 15:137-144

Keiver KM, Ronald K, Beamish FWH (1984) Metabolizable energy requirements for maintenance and fecal and urinary losses of juvenile harp seals (*Phoca groenlandica*). Canadian Journal of Zoology 62:769-776

Kerr BJ, Easter RA (1995) Effects of feeding reduced protein, amino acid supplemented diets on nitrogen and energy balance in grower pigs. Journal of Animal Science 73:3000-3008

Kirsch PE, Iverson SJ, Bowen WD (2000) Effect of a low-fat diet on body composition and blubber fatty acids of captive juvenile harp seals (*Phoca groenlandica*). Physiological and Biochemical Zoology 73:45-59

Kitts DD, Huynh MD, Hu C, Trites AW (2004) Seasonal variation in nutrient composition of Alaskan walleye pollock. Canadian Journal of Zoology 82:1408-1415

Klasing KC (1985) Influence of stress on protein metabolism. In: Moberg GP (ed) Animal stress. American Physiological Society, Bethesda, Maryland, p 269-280

Kleiber M (1975) The fire of life: an introduction to animal energetics. R. E. Krieger Pub. Co., Huntington, N.Y.

Konarzewski M, Kowalczyk J, Swierubska T, Lewonczuk B (1996) Effects of long-term feed restriction, realimentation and overfeeding on growth of song thrush (*Turdus philomelos*) nestlings. Functional Ecology 10:97-105

Kumagai S (2004) Seasonal differences in physiology of captive Steller sea lions (*Eumetopias jubatus*) in response to short term low energy intake. MSc, University of British Columbia

Kumagai S, Rosen DAS, Trites AW (2006) Body mass and composition responses to short-term low energy intake are seasonally dependent in Steller sea lions (*Eumetopias jubatus*). Journal of Comparative Physiology B 176:589-598

Le Boeuf BJ (1994) Variation in the diving pattern of northern elephant seals with age, mass, sex, and reproductive condition. In: Le Boeuf BJ, Laws RM (eds) Elephant seals. University of California Press, Berkeley, p 237-252

Mallinckrodt CH, Clark WS, David SR (2001) Accounting for dropout bias using mixed-effects models. Journal of Biopharmaceutical Statistics 11: 9–21

Markussen NH, Ryg M, Øritsland NA (1992) Metabolic rate and body composition of harbour seals, *Phoca vitulina*, during starvation and refeeding. Canadian Journal of Zoology 70:220-224

McNab BK (2002) The physiological ecology of vertebrates: a view from energetics. Cornell University Press, Ithaca

McNamara JM, Houston AI (1990) The value of fat reserves and the tradeoff between starvation and predation. Acta Biotheoretica 38:37-61

Merrick RL, Chumbley MK, Byrd GV (1997) Diet diversity of Steller sea lions (*Eumetopias jubatus*) and their population decline in Alaska: a potential relationship. Canadian Journal of Fisheries and Aquatic Sciences 54:1342-1348

Metcalfe NB, Monaghan P (2001) Compensation for a bad start: grow now, pay later? Trends in Ecology and Evolution 16:254-260

Miglavs I, Jobling M (1989) The effects of feeding regime on proximate body composition and patterns of energy deposition in juvenile Arctic charr, *Salvelinus alpinus*. Journal of Fish Biology 35:1-11

Millward DJ, Garlick PJ, Reeds PJ (1976) Energy cost of growth. Proceedings of the Nutrition Society 35:339-349

Moundras C, Remesy C, Demigne C (1993) Dietary protein paradox - decrease of amino-acid availability induced by high protein diets. American Journal of Physiology 264:G1057-G1065

Muelbert MM, Bowen WD, Iverson SJ (2003) Weaning mass affects changes in body composition and food intake in harbour seal pups during the first month of independence. Physiological and Biochemical Zoology 76:418-427

Nicieza AG, Metcalfe NB (1997) Growth compensation in juvenile Atlantic salmon: Responses to depressed temperature and food availability. Ecology 78:2385-2400

Oftedal OT, Iverson SJ (1987) Hydrogen isotope methodology for measurement of milk intake and energetics of growth in suckling young. In: Huntley AD, Costa DP, Worthy GAJ, Castellini MA (eds) Approaches to Marine Mammal Energetics. Society for Marine Mammalogy, Lawrence, Kansas, p 67-96

Ortiz CL, Costa DP, Le Boeuf BJ (1978) Water and energy flux in elephant seal pups fasting under natural conditions. Physiological Zoology 51:166-178

Peters JC, Harper AE (1985) Adaptation of rats to diets containing different levels of protein - effects of food intake, plasma and brain amino acid concentrations and brain neurotransmitter metabolism. Journal of Nutrition 115:382-398

Pinheiro J, Bates D, DebRoy S, Sarkar D (2006) nlme: Linear and nonlinear mixed effects models

Pinheiro JC, Bates DM (2000) Mixed-Effects Models in S and S-PLUS. Springer-Verlag, New York

Pitcher KW, Calkins DG, Pendleton GW (1998) Reproductive performance of female Steller sea lions: an energetics-based reproductive strategy? Canadian Journal of Zoology 76:2075-2083

Pitts GC (1986) Cellular aspects of growth and catch-up growth in the rat : a reevaluation. Growth 50:419-436

Pullar JD, Webster AJF (1977) Energy cost of fat and protein deposition in rat. British Journal of Nutrition 37:355-363

Qian X, Cui Y, Xiong B, Yang Y (2000) Compensatory growth, feed utilization and activity in gibel carp, following food deprivation. Journal of Fish Biology 56:228-232

Rea LD, Rosen DAS, Trites AW (2000) Metabolic response to fasting in 6-week-old Steller sea lion pups (*Eumetopias jubatus*). Canadian Journal of Zoology 78:890-894

Rea LD, Rosen DAS, Trites AW (2007) Utilization of stored energy reserves during fasting varies by age and season in Steller sea lions. Canadian Journal of Zoology 85:190-200

Reilly JJ, Fedak MA (1990) Measurement of the body composition of living gray seals by hydrogen isotope dilution. American Physiological Society:885-891

Reimers E, Kjorrefjord AG, Stavostrand SM (1993) Compensatory growth and reduced maturation in second sea winter farmed Atlantic salmon following starvation in February and March. Journal of Fish Biology 43:805-810

Renouf D, Noseworthy E (1991) Changes in food intake, mass, and fat accumulation in association with variations in thyroid hormone levels of harbour seals (*Phoca vitulina*). Canadian Journal of Zoology 69:2470-2479

Ridgway SH (1972) Homeostasis in the aquatic environment. In: Ridgway SH (ed) Mammals of the sea: Biology and medicine. Charles C. Thomas, Springfield, Ill., p 590-747

Roach JO, Benyon S, Dominiczak M (2003) Metabolism and nutrition. 2d Ed. Elsevier Science Ltd, London

Romano MD, Piatt JF, Roby DD (2006) Testing the junk-food hypothesis on marine birds: effects of prey type on growth and development. Waterbirds 29:407-414

Rosen DA, Trites AW (2005) Examining the potential for nutritional stress in young Steller sea lions: physiological effects of prey composition. Journal of Comparative Physiology B 175:265-273

Rosen DA, Winship AJ, Hoopes LA (in press) Thermal and digestive constraints to foraging behavior in marine mammals. Philosophical Transactions, Royal Society of London B

Rosen DAS, Renouf D (1997) Seasonal changes in blubber distribution in Atlantic harbor seals: indications of thermodynamic considerations. Marine Mammal Science 13:229-240

Rosen DAS, Renouf D (1998) Correlates of seasonal changes in metabolism in Atlantic harbour seals (*Phoca vitulina concolor*). Canadian Journal of Zoology 76:1520-1528

Rosen DAS, Trites AW (2000) Pollock and the decline of Steller sea lions: testing the junk-food hypothesis. Canadian Journal of Zoology 78:1243-1250

Rosen DAS, Trites AW (2004) Satiation and compensation for short-term changes in food quality and availability in young Steller sea lions (*Eumetopias jubatus*). Canadian Journal of Zoology 82:1061-1069

Royle N (2000) Overproduction in the lesser black-backed gull - can marginal chicks overcome the initial handicap of hatching asynchrony? Journal of Avian Biology 31:335-344

Russell NG, Wootton RJ (1992) Appetite and growth compensation in the European minnow, *Phoxinus phoxinus* (Cyprinidae), following short periods of food restriction. Environmental Biology of Fishes 34:277-285

Schmidt-Nielsen K (1997) Animal physiology: adaptation and environment. Cambridge University Press, Cambridge, England ; New York, NY, USA

Sigler MF, Womble JN, Vollenweider JJ (2004) Availability to Steller sea lions (*Eumetopias jubatus*) of a seasonal prey resource: a prespawning aggregation of eulachon (*Thaleichthys pacificus*). Canadian Journal of Fisheries and Aquatic Sciences 61:1475-1484

Trites AW, Donnelly CP (2003) The decline of Steller sea lions *Eumetopias jubatus* in Alaska: a review of the nutritional stress hypothesis. Mammal Review 33:3-28

Vaz M (2003) Undernutrition. In: Gibney MJ, Macdonald I, Roche HM (eds) Nutrition and Metabolism. Blackwell Publishing., Oxford ; Ames, Iowa, p 341-348

Venables WN, Ripley BD (2002) Modern applied statistics with S. Springer, New York

Victora CG, Barros FC, Horta BL, Martorell R (2001) Short-term benefits of catch-up growth for small-for-gestational-age infants. International Journal of Epidemiology 30:1325-1330

Waterlow JC (1986) Metabolic adaptation to low intakes of energy and protein. Annual Review of Nutrition 6:495-526

Waterlow JC (1995) Whole body protein turnover in humans - past, present, and future. Annual Review of Nutrition 15:57-92

Webster AJF (1981) The energetic efficiency of metabolism. Proceedings, the Nutrition Society 40:121-128

Whang KY, Kim SW, Donovan SM, McKeith FK, Easter RA (2003) Effects of protein deprivation on subsequent growth performance, gain of body components, and protein requirements in growing pigs. Journal of Animal Science 81:705-716

Wilson PN, Osbourn DF (1960) Compensatory growth after undernutrition in mammals and birds. Biological Reviews 35:324-363

Winship AJ, Trites AW, Rosen DAS (2002) A bioenergetic model for estimating the food requirements of Steller sea lions *Eumetopias jubatus* in Alaska, USA. Marine Ecology-Progress Series 229:291-312

Womble JN, Willson MF, Sigler MF, Kelly BP, VanBlaricom GR (2005) Distribution of Steller sea lions *Eumetopias jubatus* in relation to spring-spawning fish in SE Alaska. Marine Ecology-Progress Series 294:271-282

Worthy GAJ (2001) Nutrition and energetics. In: Dierauf LA, Gulland FMD (eds) CRC Handbook of Marine Mammal Medicine. CRC Press, London, New York, Washington, p 791-827

Xie S, Zhu X, Cui Y, Wootton RJ, Lei W, Yang Y (2001) Compensatory growth in the gibel carp following feed deprivation: temporal patterns in growth, nutrient deposition, feed intake and body composition. Journal of Fish Biology 58:999-1009

Yambayamba ES, Price MA, Foxcroft GR (1996) Hormonal status, metabolic changes, and resting metabolic rate in beef heifers undergoing compensatory growth. Journal of Animal Science 74:57-69

Zhao L, Castellini MA, Schell DM (2006) Metabolic adjustments to varying protein intake in harbor seals (*Phoca vitulina*): evidence from serum free amino acids. Physiological and Biochemical Zoology 79:965-976

Zubair AK, Leeson S (1996) Compensatory growth in broiler chicken: a review. World's
Poultry Science 52:189-201

Chapter 3: A diet of low-energy fish prevents Steller sea lions from adjusting their physiologies and bioenergetic allocations during periods of nutritional stress[2]

Summary

Animals consume and store energy to cover the costs of maintenance (activity, thermoregulation and basal metabolism) and production (growth, energy storage and reproduction). How much energy an animal allocates to each of these functions likely has life history consequences and may be altered by season, quality and quantity of available prey. Two groups of female Steller sea lions (Group H and Group P, $n = 8$) were subjected to optimal and suboptimal nutritional conditions during winter and summer to determine how energy is allocated during nutritional stress and subsequent re-feeding. Both groups of sea lions consumed high-quality prey (Pacific herring) before and after periods of energy restriction during which Group H was offered less herring to cause a controlled weight loss and Group P received an isocaloric diet of walleye pollock (a low-quality prey). During summer, all animals compensated for the imposed energy deficit by releasing stored energy. In addition, sea lions in Group H increased their activity and decreased their thermoregulation capacity. During winter, both nutritionally stressed groups also catabolized stored energy but decreased the energy they allocated to maintenance functions by decreasing activity levels (especially Group H). All of the sea lions also preserved their thermoregulatory capacity in winter and restored their body lipid stores when given additional food in winter. However, no such change was noted during the summer re-feeding phase. Overall, quality of diet and time of year interacted to determine the strategies Steller sea lions used to allocate energy. Those fed restricted amounts of pollock did not optimize energy allocation as well as those fed herring. Consequently, a low-quality diet appears to block the intrinsic capacity of sea lions to adjust to nutritional stress and may ultimately reduce fitness of animals in the wild.

Introduction

Energy flux through a single organism follows the first law of thermodynamics which defines the conservation of mass and energy (Lucas 1992). This means that all of the energy acquired through food (energy intake) is exclusively partitioned between waste, production, and metabolic work (energy outputs). As such, an animal that does not consume sufficient energy to optimally satisfy its energy output will have to reduce and differentially reallocate energy among these variables. How animals prioritize the allocation of energy between the fundamental physiological functions when energy intake is reduced likely has consequences for reproduction and survival.

Marine mammals, such as Steller sea lions (*Eumetopias jubatus*), are known to experience predictable periods of decreased energy intake as a normal part of their life cycles (such as fasting during the breeding season). The balance between energy intake (i.e., consumption of prey) and the cost of existence are also affected by ecology and the environment in which the animals live (Prestrud 1991, Cuyler & Øritsland 1993, Patterson et al. 1998). For example, distribution patterns and nutritional quality of fish vary by season (Anthony et al. 2000, Kitts et al. 2004), as do animal energy requirements (Winship et al. 2002). In addition, age, sex, and reproductive status can alter energy budgets (Baldwin & Bywater 1984). Young animals for example require a higher mass-specific energy intake than adults to fulfill requirements for growth (Kleiber 1975). Animals are physiologically prepared for such predictable events, but may be less prepared to contend with unpredictable or extended changes in environmental conditions (Trillmich & Ono 1991, Trites & Donnelly 2003a, Soto et al. 2006). In either case, animals have no choice but to adjust their behaviour and decrease their physiological expenditures in proportion to the energy deficit they incur (Boyd 2002).

Pinnipeds can optimize energy retention by increasing digestive efficiency and reducing the energy lost as waste (Lawson et al. 1997, Trumble et al. 2003). Another set of strategies might entail reducing energy expenditures, including suppressing reproductive functions (Pitcher et al. 1998) or reducing production work (i.e., reducing body growth) (Stini 1969, Calkins et al. 1998). Reducing activity levels and time spent in thermally challenging environments is another strategy (Limberger et al. 1986, Nash

1998), as is decreasing basal metabolic rate or minimizing thermoregulation costs through social aggregation or optimal positioning of body parts (especially flippers) (Ohata & Miller 1977, Rosen & Trites 1999). Ultimately, all animals must determine how the net energy they ingest is partitioned between the principle physiological functions of growth, stored energy, reproduction, thermoregulation, voluntary activity, and basal maintenance (Lavigne et al. 1982), which can affect their capacity to survive and reproduce in the wild depending on how optimal these decisions are relative to environmental conditions.

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

Material and methods

Experimental design

All research was conducted under permit No. A04-0169 issued by the University of British Columbia Animal Care Committee, and followed the experimental design detailed in Chapter 2. In brief, experiments were conducted at the Vancouver Aquarium (BC, Canada) in the summer of 2005 (June to August) and winter of 2006 (January to March), on 8 female Steller sea lions. The animals were randomly divided into two groups — Group H and Group P.

Each seasonal experiment consisted of 3 phases of 28 days each and started with a baseline phase, during which all of the animals were fed their usual daily ration of Pacific herring (*Clupea pallasi*). The baseline phase was followed by a 28-day restriction treatment during which the two groups of sea lions received the same "restricted" level of gross energy intake (isocaloric diets intake between 230-260 kJ kg^{-1} d^{-1}) designed to elicit a total mass loss not exceeding 15% of the initial body mass over the restriction, as per
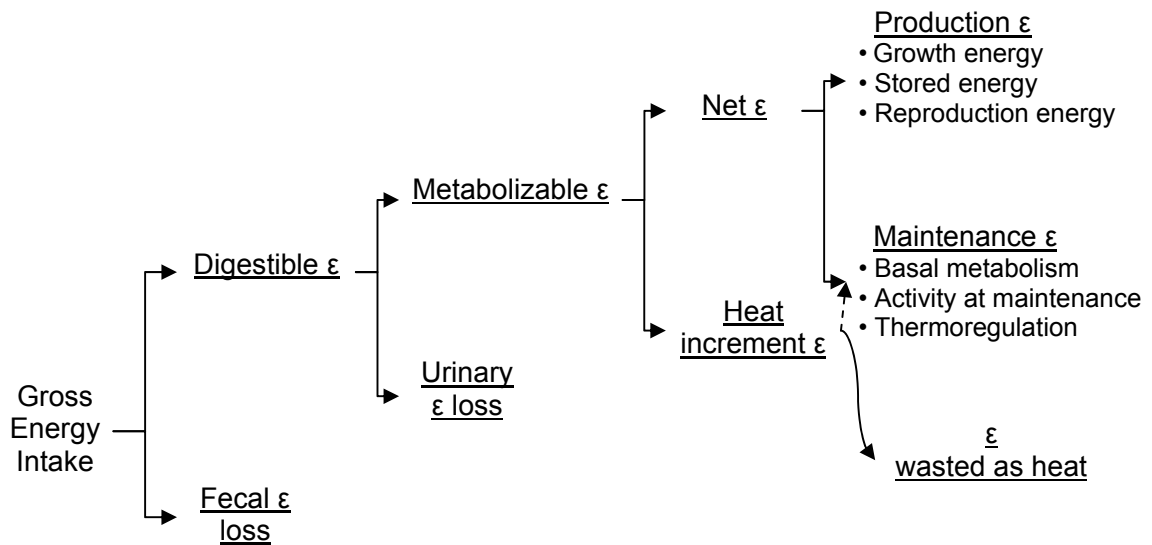
Figure 3.1: Schematic and simplified partitioning of the gross energy intake in an animal (based on Kleiber 1975, Lavigne et al. 1982). The symbol ε means energy.

Animal Care protocols. However, Group H was fed exclusively Pacific herring during the restriction while Group P was fed solely walleye pollock (*Theragra chalcogramma*). The restriction period was followed by a 28-day controlled re-feeding period during which each sea lion received the same initial intake of Pacific herring that they received during the baseline phase. An additional ad-libitum period of 2 weeks was added to the winter experiment to allow the animals as much food as they wanted based on behaviour and training procedures.

A subsample of the herring and pollock were analyzed for their proximate chemical composition. Gross energy content of the fish was calculated using the energy conversion factors provided by Schmidt-Nielsen (1997) — 39.3 kJ g^{-1} for lipid and 18 kJ g^{-1} for protein.

Bioenergetic partition

General partition of the energy budget. The energy budget of sea lions is, by definition, balanced between the energy ingested and the energy spent. It may be partitioned according to Eq. 1 derived from Figure 3.1:

$$GEI = GrE + StE + ReE + BME + AcE + ThE + FeE + UrE + HiE \qquad (1)$$

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where GEI is the gross energy intake, GrE the energy allocated to growth, StE the stored energy, ReE the energy used for reproduction (in our study, the animals used were non-pregnant, non-nursing female sea lions, thus ReE = 0), BME the basal metabolism energy, AcE the activity energy and ThE the energy for thermoregulation. FeE the energy lost in feces, UrE the energy lost in the urine, and HiE the energy lost as heat represent the part of gross energy lost through digestive processes. GrE, StE and ReE together represent the production energy (PE), and BME, AcE and ThE together comprise the maintenance energy (ME).

During the restriction phase, the animals obtained less energy through their food, meaning that they faced a deficit in energy intake compared to baseline levels. To determine which physiological functions Steller sea lions altered during this energy restriction and in which proportion, changes in net energy intake, production energy, and maintenance energy were estimated. The same types of calculations were performed for changes occurring during the controlled re-feeding when sea lions increased their energy intake compared to the previous restriction. In both cases, changes in energy intakes and allocation to physiological function were calculated for the first 2 weeks of each phase as well as for the whole 28-day periods (cumulative changes encompassing those measured during the first 2 weeks).

Estimation of the net energy intake (NE). The net energy intake is the energy readily available for production and maintenance processes, i.e. the gross energy intake from which the energy lost through feces, urine, and heat was removed. It was calculated according to the following equation:

$$NE = GEI - (FeE + UrE + HiE) = GEI \times \text{Digestive Efficiency} - (UrE + HiE) \quad (2)$$

Food intake ($\pm$ 0.025 kg d^{-1}) and energy intake (kJ d^{-1}) of the animals were recorded daily (Table 3.1) and were used to calculate the growth rates and gross energy intakes (GEI) of Steller sea lions over 2 and 4 weeks during the baseline, restriction and recovery phases (Table 3.2). They were also used to estimate changes in energy intake occurring between these treatments (deficit or increase). UrE, FeE and HiE were not directly measured; published estimates from Steller sea lions and other pinnipeds were used instead. Digestive efficiencies were considered to be 95.4% for herring and 93.9%

for pollock (Rosen & Trites 2000a). UrE in pinnipeds was estimated to be 7% of the digestible energy (Lavigne et al. 1982), which represents an average of different phocid values (Parsons 1977, Lavigne et al. 1982). HiE is difficult to separate experimentally from the heat increment of feeding (HIF) (Lavigne et al. 1982). In these calculations, HIF was used as an approximation of HiE. Based on data from Rosen et al. (1997), HIF energy lost was considered to be 12.5% of GEI during the baseline and the controlled re-feeding phases. During the restriction, HIF was assumed to be 10% of GEI for Group H and 12.5% for Group P. HIF in Steller sea lions is not recycled for thermoregulation and is totally lost as dissipated heat (Rosen & Trites 2003).

Estimation of production energy (PE). Production energy is by definition the portion of the net energy allocated to productive functions such as structural growth, storage of body energy reserves or reproductive functions (pregnancy, lactation, etc.). It is calculated as:

$$PE = GrE + StE + ReE \tag{3}$$

Changes in body composition were used to calculate changes in PE (ReE = 0 in our experiment). Body mass of each animal (± 0.1 kg) was recorded daily and body composition was measured at the end of the baseline and every two weeks till the end of the controlled re-feeding during the summer, and until the end of the ad-libitum phase in the winter. Briefly, this was done using the deuterium dilution method (see Chapter 2 for details) conducted before the sea lions' first meal of the day (~18h overnight fast). Two measurements of the body composition were missing for one animal in Group H during the summer trial.

Changes in body compartment (lipids versus lean tissues) were calculated from a common reference point (baseline for the restriction period and end of the restriction for the controlled re-feeding period). Energy content of proteins and lipids released during food restriction were calculated according to standard biochemical estimates (18 kJ g^{-1} and 39.3 kJ g^{-1}, Kleiber 1975). When increases in lipids or lean tissues occurred, additional costs of deposition were calculated using 2.38 kJ necessary per kJ of proteins deposited and 1.17 kJ per kJ of lipids (Roberts & Young 1988). These values are close to theoretical

values (2.37 kJ kJ^{-1} for proteins and 1.08 kJ kJ^{-1} for lipids) and within range of the values found experimentally in rats and pigs (Pullar & Webster 1977).

Estimation of the maintenance energy (ME). Maintenance energy (ME) comprises the energy allocated to physiological functions necessary to the life of the animals (that are not related to production) such as the energy needed for the basal maintenance of tissues and biochemical processes, for thermoregulation or voluntary activity as shown by:

$$ME = BME + AcE + ThE \qquad (4)$$

ME can also be calculated in reference to body composition changes and daily metabolic rate (DMR) data according to:

$$ME = DMR - HIF - DepE \qquad (5)$$

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

DMR was measured every two weeks concurrent with body composition measurements by enclosing the sea lions in a large metabolic chamber containing a small pool and with enough room for the animal to perform its daily routine for 22-24 hours. Both DMR and resting metabolic parameters (see below) were measured by open circuit respirometry. Fans were used to provide good air circulation in the chamber and the behaviour of the animals was recorded using a video camera. A thermometer measured the indoor temperature and a water system was installed to spray mist (when required) in the chamber to prevent the temperature from increasing too much compared to outside. After an adequate equilibration time, air was drawn from the metabolic chamber at a constant rate (using a Sable System International flow kit, model 500H Flow Generator and Controller) sufficient to keep levels of O_2 above 18.5% in the chambers (250-350 L min^{-1}). Desiccated outflow subsamples of O_2 and CO_2 concentrations were analyzed by Sable System FC-1B and CA-1B analyzers (Sable Systems, Salt Lake City, UT). Outside air baselines were taken before and after the metabolic rate recording to correct for any drift in the system. Analysers averaged 100 subsamples every 9 seconds for DMR measures,

and 1 sample per second for RMR measures. Data was recorded and analyzed using Sable Data Acquisition System.

During DMR measurements, the animals were fed twice a day through a feeding tube that was otherwise sealed. When the recording did not reach 24h, the data were extrapolated between the first and the last data point for the remaining hours using the Sable Data Acquisition software. Total daily metabolic rate was also split into day hours (from 6am to 8pm) and night hours (from 8pm to 6am) to assess diurnal differences. The mass-corrected metabolic rate was calculated using the scaling factor of 0.714 calculated by Hunter (2005) for marine mammals, which is lower than Kleiber's commonly used coefficient of 0.75. This single scaling coefficient corrects for mass differences between our experimental animals but not for age differences. Younger animals are on a steeper growth slope and should have a scaling coefficient different from older animals but the exact difference was impossible to estimate. To correct for initial differences between sea lions, rates of change compared to respective baseline measurement were calculated.

DMR data were converted into energy consumption (conversion: 20.2 kJ LO_2^{-1}) to be incorporated into Equ. 5. To estimate changes in ME allocation over 2 and 4 weeks periods, individual DMR measurements (measured on a 24h basis) were multiplied by 14 or 28 respectively based on the assumption that daily energy expenditures were consistent throughout the 2-week periods. The energy used to deposit tissues (DepE) was estimated as 0.17 kJ per kJ of lipids deposited and 1.38 kJ per kJ of deposited proteins respectively (Roberts & Young 1988). In these calculations the energy deposited was not taken into consideration, only the efficiency of deposition. HIF values were the same as those used to estimated NE.

Assumptions and approximations. A number of assumptions and gross approximations were invoked because the experimental design did not allow for all parameters to be calculated. First, changes in production energy — normally including somatic growth and storage of energy — were calculated solely from changes in body composition (estimated from deuterium dilution method). Cost of skeletal growth could not be teased apart from body composition measurements, but was probably not constant

between the seasons given that animals were growing more in terms of length during the summer (Chapter 2).

Second, energy wasted as heat was considered to only come from the heat increment of feeding and not from other metabolic mechanisms. In addition, all parameters used to calculate NE, HIF, digestive efficiency, and urinary excretion were considered to be fixed throughout the entire trials and the same for both seasons. However, these parameters are known to vary and adjust in response to changes in energy intake (Lawson et al. 1997, Trumble et al. 2003). In addition, these parameter estimates were taken from studies on otariids and phocids from different ages, nutritional conditions, fed different diets, thus on different experimental conditions than the present ones.

Measurements of DMR and body composition taken at the middle and at the end of each treatment were assumed to reflect changes between measurements over the 2-week periods. For body composition, this implies that the trend of deposition or mobilisation of body parts was constant throughout a given period between measurements. For the DMR data, it assumes that the animals behaved similarly outside of the metabolic chamber and expenditures were constant for 14 days in a row. DMR measurements were also taken a few days (1-3 days) following the body composition measurements. This means that maintenance and production energies considered in the same budget equation were estimated a few days apart and were thus potentially representing different physiological states.

In addition, only one DMR and body composition measurement was taken during the baseline periods, which makes changes occurring over a 2- or 4-week period during this treatment impossible to calculate. Since baseline rates of body mass changes did not show drastic variation on average, body growth (deposition of lipids and proteins) was assumed to be null during this treatment, which ignores the dynamics of growth processes.

Finally, the energetic cost of protein turnover was ignored in calculating production energy, although it is included in the DMR. When calculating the changes in DMR, protein turnover rate was assumed to be similar during all the phases (an attempt to directly measure this variable was unsuccessful) and was treated as such when calculating

the changes between phases. The model also did not consider the potential use of amino-acids for the production of lipids.

Qualitative energetic estimates

Thermoregulation energy (ThE), AcE, and BME were not directly measured and could not be estimated quantitatively to be incorporated into the energy budget equations (Eqs. 1 and 4), but respirometry measurements were performed to estimate qualitative changes in the energy allocated to these functions.

Thermoregulatory capacity. Thermoregulation data was estimated by measuring resting metabolic rate of animals encaged in a swimmill for 50 min with regulated water temperature, kept within 0.4°C of the 8° and 2°C target temperatures which represent the lower range of sea surface temperature in summer and winter for sea lions in the North Pacific (DFO 2007). Metabolic rates in waters of 8° or 2°C (MR_8 and MR_2) were measured on each animal every two weeks in the same week that the body composition and the DMR data were obtained. MR_8 and MR_2 were measured using similar respirometry methods as the DMR data (see above), with the exceptions that animals breathed into a small hermetically-sealed dome above the water level. The volume of the dome was small, so there was no need for an equilibration time. The air flow rate was lower than for the DMR (200 L min^{-1}) and 60 samples were averaged every second. Animals were post-absorptive and their activity levels were reduced and relatively consistent between and within animals Differences in oxygen consumption between the first 10 min and last 10 min of the trials were determined to estimate changes in thermoregulatory costs of the animals in water over time and were used as an index of the thermoregulatory capacity. The first 5 min of the recordings were ignored to avoid noise data (i.e. change in metabolic rate coming from the primary excitement of the animals just entering the swimmill). The following 10 min were considered the "baseline" for the metabolic rate (prior to thermal challenge).

Standard metabolic rate. Measurements of standard metabolic rate in air (SMR_A) were measured only during the baseline period in summer, but every two weeks in the same week as the other measurements during the entire experiment in winter. The animals were enclosed in a small sealed and dry metabolic chamber containing a fan for air

circulation and a video camera. The animals remained enclosed for 40 min in the chamber after a 5 min equilibration time. Respirometry method and measurement parameters used were similar to those described above (Thermoregulatory capacity). Measurements were performed on post-absorptive animals. The levels of activity in the chamber, monitored by video, varied between animals but were consistent throughout the experiment for the same animal. The rates of SMR_A change were calculated to buffer individual variability. The SMR_A values used in the calculations were taken for 20 minutes in the middle of the recording, to eliminate potential initial excitement and final impatience behaviours.

Exercise metabolism. Metabolism after exercise was measured to assess changes in fitness of the animals throughout the experimental conditions, and was performed only during the 2005 summer experiment. The sea lions were trained to run between two trainers as quickly as possible for 20 laps (total distance of 290 ± 10 m). To keep the animals interested, trainers rewarded them with small pieces of fish between every few laps. Prey ingestion was kept minimal in an attempt to limit the interference of oxygen consumption for digestion. The metabolic rates at the end of the running time were measured with animals in the swimmill (water set at 8°C) because the small volume of the respirometry dome allowed an immediate measurement to pick up the post-exercise peak of oxygen consumption. Oxygen debt after the exercise was determined by integrating the increase in oxygen consumption above the average baseline values. Time to reach baseline was assessed by finding two sequential VO_2 averages over a minute that were within a 5% margin of one another. Changes in oxygen debt compared to the baseline value were calculated to correct for initial individual variability. Trials when animals did not enter the dome right away at the end of the running time were discarded.

Statistical analyses

Our study followed a repeated measured design. Consequently, effects of diet, season and phase on dependant variables (DMR, SMR_A, $MR_{8\&2}$, etc) were estimated using mixed effect models. All of the models' assumptions were verified as per Pinheiro and Bates (2000) (see Chapter 2 for more details) and changes during the restrictions and the controlled re-feedings were compared to baseline measurements.

Sample size for DMR, RMR_8 and RMR_2 were 40 for the summer and 47 in winter. Exercise metabolism's sample size was 40. SMR_A's sample size was 8 in the summer (only baseline measurements) and 47 in the winter. One animal was removed from the trial before the end of the ad-libitum period in the winter, so one data point was missing for each parameter. The first set of analyses investigated the effect of diet type and experimental phase on the different dependant variables stratified by seasons. Fixed effects were diet, phase and the interaction between these parameters, and random effects were individual animals, diet and phase, depending on the best model fit estimated by ANOVA and AIC.

The second set of analyses investigated the within-group differences along the experimental time line stratified by diet and season. The fixed effect was phase, and the random effect the individual and the phase depending on the best fit determined by ANOVA and AIC. The third set of analyses investigated the season and phase differences stratified by diet groups. The fixed effects were season, phase and the interaction between these terms; and random effects were individual and potentially season depending on the best model fit. The significance of the relationship between SMR_A and the body composition of the animals was assessed using mixed effect models with SMR_A as the dependant variable, body condition as the fixed effect, and the animals as random effects. The analysis was performed on all the animals together and stratified by diet. All data values provided were means ± SE and all p values were extracted from the mixed effect models summaries, unless stated otherwise. Statistical significance of each parameters estimates was set at $\alpha = 0.05$.

The relative contribution of changes in PE and ME to compensate for the energy intake deficit during the restriction was statistically estimated using one-sample t-tests. The tests determined whether the proportions of contribution of PE and ME to energy intake change were different from 0 or 100%, stratified by diet group and season. To estimate the relative contribution of PE or ME to energy deficit, the hypotheses $H_0: \mu = 0$ % (no contribution) and $H_0': \mu = 100\%$ (total contribution) were tested with one-sample t-tests. The same procedures were performed to estimate the relative allocation of the increased (relative to restriction) energy intake during the controlled re-feeding to either a growth/storage of body energy reserves and/or to an increase in maintenance energy. All

data values provided are mean ± SE and statistical significance of each parameters estimates was set at α = 0.05.

Results

Bioenergetic partition

Energy intake, body mass and body composition. Experimental diet, body mass and body composition data are summarized in Table 3.1 (see also Chapter 2). In brief, body mass changes were similar between the two diet groups across both seasons and diet types. The extent of body mass loss reached approximately 10-15% of the baseline body mass during the restrictions. Both groups started the restrictions with similar body fat averages (see Table 3.1). During the summer restriction, animals in Group H lost exclusively body fat when losing mass, while Group P lost significantly less body fat and more lean mass than Group H. During winter, Group H lost again significantly more body fat and less lean mass than Group P both at 14 days and at 28 days of food deprivation, but both groups tended to spare lipids slightly more in winter compared to the summer restriction.

During the subsequent controlled re-feedings (when the sea lions returned to the baseline diet), the animals remained stable at the same mass they attained at the end of the restriction treatment in summer, but the body composition was homogenized between the two groups. During winter, animals displayed compensatory growth during the controlled re-feeding phase (mass gain rates increased by 7.29 ± 1.43% compared to baseline rates and was mostly achieved by gaining body fat). They finished the experiment with slightly more lipids than before the restriction. The individual changes in body mass and composition were translated into body energy (Table 3.1) and showed that energetic contribution of each compartment to the energy deficit during the restriction differed for both diet groups. The higher contribution of lipids to compensate for the deficit in energy intake by Group H led to a decrease in total body energy (from 1200 to 800 MJ in summer and from 1400 MJ to 1000 MJ in winter) and was significantly greater than Group P during the restriction phases in both seasons. In winter, Group P released exclusively lean mass to compensate for the energy intake deficit. This translated into a stable body energy throughout the restriction (around 1500 MJ) in this season, unlike Group H. During the

Table 3.1: Gross energy intake (GEI), body mass, composition and energy content measured during the different phases (B4, R4, CR4: fourth week of baseline, restriction and controlled re-feeding; and R2, CR2, ADL2 during the second week of same periods and ad-libitum) of the summer 2005 and winter 2006 experiments. Numbers in brackets represent the standard error of the means. The star and the italic numbers represent the measurements where one sub-adult animal was missing (ADL2 winter) which lowered the average.

Season	Diet group	Phase	GEI (kJ/d)	Body mass (kg)	Lipid mass (kg)	Lean mass (kg)	Body energy (MJ)
Summer	H	B4	35.43 (5.75)	108.88 (21.18)	22.48 (5.79)	86.39 (15.55)	1266.6 (294.7)
		R2	27.07 (3.92)	104.23 (20.03)	17.11 (4.99)	93.53 (18.25)	1089.2 (277.9)
		R4	27.07 (3.92)	98.65 (18.97)	12.03 (2.48)	86.62 (16.84)	859.6 (166.6)
		CR2	34.65 (6.07)	96.28 (17.63)	16.47 (2.64)	84.90 (18.04)	1025.0 (185.1)
		CR4	34.65 (6.07)	96.10 (17.12)	17.35 (5.36)	78.75 (11.77)	1031.6 (262.6)
	P	B4	40.73 (7.13)	131.35 (23.59)	22.39 (4.60)	108.96 (19.46)	1365.6 (260.5)
		R2	31.64 (4.64)	128.45 (23.60)	19.83 (4.10)	108.62 (19.66)	1264.2 (246.7)
		R4	31.64 (4.64)	120.40 (21.81)	17.82 (4.50)	102.58 (17.45)	1158.2 (252.9)
		CR2	39.78 (6.74)	118.10 (21.66)	19.98 (2.97)	98.12 (19.06)	1222.2 (195.3)
		CR4	39.78 (6.74)	118.33 (21.76)	16.68 (2.79)	101.65 (19.00)	1109.3 (194.5)
Winter	H	B4	47.59 (5.21)	121.70 (19.73)	24.94 (5.00)	96.76 (14.79)	1409.9 (261.5)
		R2	30.34 (3.78)	115.45 (18.81)	20.78 (4.18)	94.67 (14.80)	1238.0 (228.2)
		R4	30.34 (3.78)	110.50 (18.88)	16.22 (3.08)	94.28 (16.15)	1058.0 (187.4)
		CR2	45.33 (5.71)	114.85 (18.10)	25.19 (3.91)	89.66 (14.85)	1387.2 (210.1)
		CR4	45.33 (5.71)	119.50 (17.74)	26.89 (4.58)	92.61 (13.22)	1467.0 (238.1)
		*ADL2**	*30.26 (7.52)*	*98.00 (1.87)*	*20.65 (1.09)*	*77.35 (1.20)*	*1154.0 (44.5)*
	P	B4	47.53 (4.58)	143.60 (21.94)	27.49 (4.32)	116.11 (18.39)	1597.3 (240.0)
		R2	34.35 (3.94)	139.85 (21.76)	27.65 (4.94)	112.20 (17.49)	1585.6 (262.6)
		R4	34.35 (3.94)	134.30 (21.19)	26.31 (4.63)	107.99 (17.23)	1514.3 (249.0)
		CR2	46.74 (4.66)	136.25 (20.65)	28.97 (4.20)	107.28 (17.79)	1614.8 (223.1)
		CR4	46.74 (4.66)	139.75 (20.14)	30.27 (4.73)	109.48 (16.20)	1675.8 (246.9)
		ADL2	47.90 *(6.20)*	143.05 (21.13)	30.30 (4.36)	112.75 (17.13)	1691.7 (242.4)

controlled re-feeding in summer, body energy content of the animals remained at the level attained at the end of the restriction despite the increase in energy intake. In winter, both groups restored their pre-experimental body energy status by the end of the controlled re-feeding. Changes in body energy were then incorporated into the bioenergetic calculations (see Eqs. 1 and 3).

Daily metabolic rate. During summer, the sea lions in both diet groups started the experiment with similar mass-corrected daily metabolic rates (Wilcoxon rank sum test, *p* =

0.68), with an overall average of 1094.01 ± 79.35 kg$^{-0.714}$ d^{-1} (range 872 to 1577 kJ kg$^{-0.714}$ d^{-1}). The DMR of Group P stayed constant throughout the experiment, when considered either on a 24h basis or split into day or night hours (all $p > 0.30$, see Figure 3.2a). DMR of Group H however, increased by $18 \pm 3\%$ ($p = 0.003$) by the second week of the restriction, which lasted till the middle of the controlled re-feeding ($+19 \pm 6\%$, $p = 0.01$). DMR returned to the pre-restriction values at the end of the controlled re-feeding ($p = 0.22$).

Unlike Group P, the animals in Group H also displayed day/night cycle differences and the increase in total DMR was entirely due to an increase of metabolic rate during day hours (all $p < 0.001$ except for the last measurement at the end of the controlled re-feeding $p = 0.90$, see Figure 3.2b). The increase after 2 weeks of restriction reached $37 \pm 3\%$ of baseline levels (1492 ± 81 kJ kg$^{-0.714}$ d^{-1}), and stayed around 27-28% of baseline (in R4 and CR2) till it returned to initial values at the end of the controlled re-feeding (1106 ± 43 kJ kg$^{-0.714}$ d^{-1}). Metabolic rates at night however were constant throughout the study (all $p > 0.05$), with an average overall value of 784 ± 21 kJ kg$^{-0.714}$ d^{-1}. The differences in the daylight metabolism translated into Group H having significantly higher rates of change in DMR than Group P during the restriction both at 14 and 28 days ($p < 0.001$ and $p = 0.03$ respectively) and at 2 weeks into the controlled re-feeding ($p = 0.02$).

During winter, both diet groups started the experiment with similar mass-corrected daily metabolic rates (1235 ± 64 kJ kg$^{-0.714}$ d^{-1}, Wilcoxon rank sum test, $p = 0.34$). In both groups, the baseline averages were higher in winter than in summer (average difference = 141.15 ± 70.03 kg$^{-0.714}$ d^{-1}, $p = 0.002$). DMR of Group P during winter stayed stable most of the time, except at week 2 of the deprivation where a significant decrease occurred (-15 $\pm 5\%$ at 1073 ± 122 kJ kg$^{-0.714}$ d^{-1}, $p = 0.02$, see Figure 3.2c). Group P had similar changes in DMR in winter compared to summer when data was split into day and night hours ($p > 0.09$, see Figure 3.2d).

Changes in DMR of Group H were different in winter compared to summer (all $p < 0.001$). In winter, DMR averages were lower than the baseline levels for all subsequent measurements (all $p < 0.02$). This is true for the metabolic rate on a 24h basis, and also when data was split into day and night hours. It decreased by $23.60 \pm 2.90\%$ at the end of
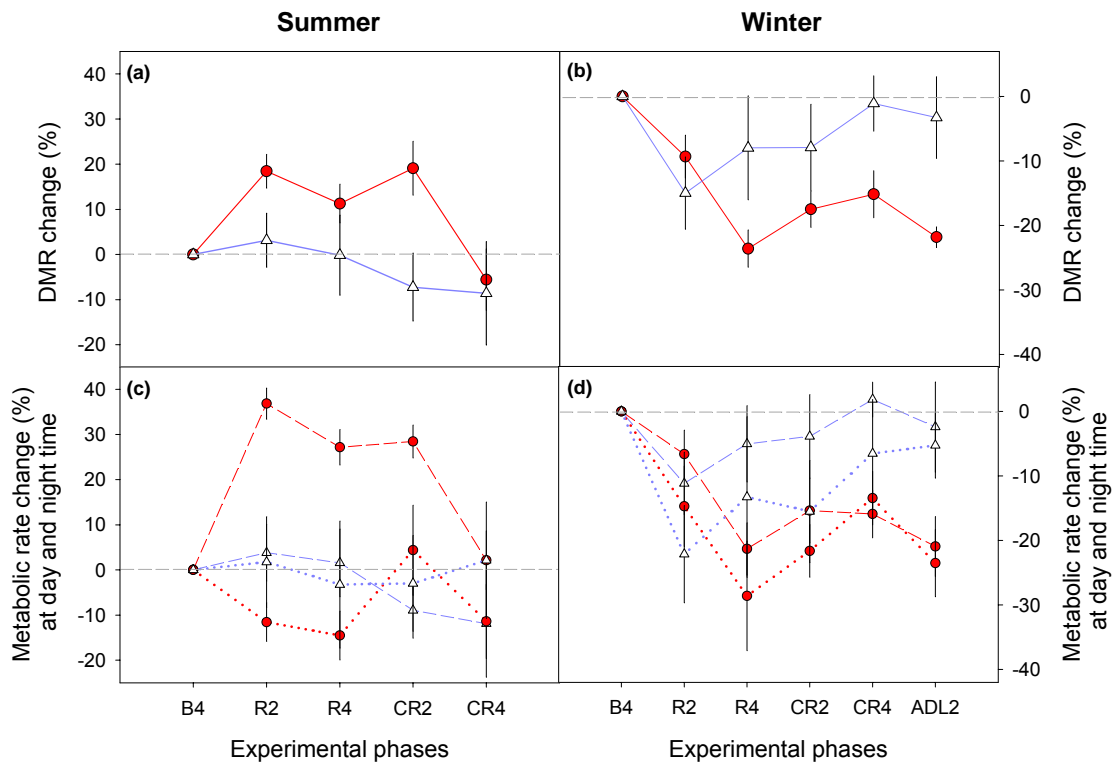
Figure 3.2: Mean ± SE changes in daily metabolic rates (DMR) compared to the baseline measurement (B4) at week 2 and 4 of the restriction R and the controlled re-feeding CR of sea lions in Group H (circles) or in Group P (triangles) in the summer (a, b) and in the winter (c, d) experiments. Panels a) and c) are DMR measured on a 24h basis and panels b) and d) show metabolic rates separated between day time (dashed lines, 6am-8pm,) and night time hours (dotted lines, 8pm-6am).

the restriction compared to the baseline (917 ± 40 kJ kg$^{-0.714}$ d^{-1}) by $15.15 \pm 3.65\%$ (1017 ± 38 kJ kg$^{-0.714}$ d^{-1}) at the end of the controlled re-feeding and by $21.82 \pm 1.61\%$ during the ad-libitum phase. Unlike summer time, Group H changes in 24 hr metabolism reflected similar changes in both daytime and night time metabolism.

Between group analyses showed a tendency for Group H to have lower DMRs than Group P starting at the end of the restriction (week 4, $p = 0.01$). It was also lower at the end of the controlled re-feeding (week 4, $p = 0.01$) and during the ad-libitum phase (week 2, $p < 0.01$) but not at week 2 of the controlled re-feeding (Group H's average: 991 ± 42 kJ kg$^{-0.714}$ d^{-1}, Group P's average: 1150 ± 87 kJ kg$^{-0.714}$ d^{-1}, $p = 0.13$). Individual DMR data was used to estimate the energy allocated to maintenance functions as described by Eq. 5.

Bioenergetic calculations. Bioenergetic calculations were performed using data on DMR, energy intake, and body composition. During the summer experiment, the calculated net energy deficit compared to the energy the sea lions were getting during the baseline phase averaged 121.5 ± 17.5 MJ during the first 14 days and 216.8 ± 46.3 MJ over the whole month for Group H and 111.1 ± 26.8 MJ during the first 2 weeks and 219.3 ± 62.2 MJ for the whole month for Group P. The decrease in energy intake triggered physiological adjustments regarding the repartition of energy between maintenance energy (ME, i.e. thermoregulation, voluntary activity and basal metabolism) and production energy (PE, i.e. growth and body energy storage; see Eqs. 1, 3 and 4). The energy allocated to ME during the summer restriction did not increase or decrease significantly (contribution to net energy deficit not significantly different from 0%) either after 2 or 4 weeks of energy restriction ($-5 \pm 31\%$ and $8 \pm 30\%$, $p = 0.87$ and $p = 0.81$) for animals in Group P.

Sea lions in Group H however increased the energy allocated to the maintenance energy after 2 weeks of restriction, which increased the calculated potential energy deficit by approximately 48% ($p = 0.03$) relative to changes in food intake alone (Table 3.2). After 4 weeks of deprivation, calculations for one animal out of four were the opposite of the others (F03RO). Consequently, with a low statistical power, the variability was too large to see any significant difference from 100% or 0% contribution of ME to the deficit (all $p > 0.2$). The bioenergetic calculations show that instead of a decrease in ME allocation, the compensation for the energy deficit in the summer was entirely covered by releasing stored energy after 2 or 4 weeks of deprivation for both diet groups (Table 3.2). The energy derived from releasing stored energy was equivalent to $166 \pm 41\%$ of the overall energy intake deficit for Group H and $126 \pm 35\%$, for Group P, neither of which were significantly different from 100% ($p = 0.20$ for Group H and $p = 0.50$ for Group P).

During the winter restriction, the net energy deficit resulting from a change in intake averaged 200.5 ± 21.1 MJ over the first 2 weeks and 401.0 ± 42.1 MJ over the whole month for Group H, and 150.0 ± 26.4 MJ over the first 2 weeks and 286.0 ± 57.5 MJ over the whole month for Group P. Group P significantly decreased its energy allocated to ME during restriction which was equivalent to $49 \pm 13\%$ of the energy intake deficit for the first two weeks of restriction (different from 0%, $p = 0.03$, and from 100%,

Table 3.2: Relative contribution of production energy (PE) and maintenance energy (ME) to the compensation of the energy deficit compared to the baseline during the restriction. Also shown is the percentage allocation of the energy gain compared to the restriction during the controlled re-feeding, during the first 2 weeks of the treatments or the entire 4 weeks. Values in brackets are the standard errors of the means with the letter "a" next to the mean percentage indicating values significantly different form 0% and the letter "b" values significantly different from 100%.

Diet group	Sampling time	Restriction				Controlled re-feeding			
		Summer		Winter		Summer		Winter	
		PE	ME	PE	ME	PE	ME	PE	ME
Group H	2 weeks	198 [a]	-48 [ab]	83 [a]	10 [b]	220 [a]	-31	182 [a]	-36 [b]
		(64)	(9)	(13)	(9)	(78)	(27)	(50)	(15)
	4 weeks	166 [a]	16	86 [a]	32 [ab]	67	-75	131 [a]	-19 [b]
		(41)	(51)	(24)	(7)	(54)	(135)	(17)	(14)
Group P	2 weeks	99	-5 [b]	-2 [b]	49 [ab]	203	-86 [ab]	113 [a]	-31 [b]
		(57)	(31)	(19)	(13)	(176)	(21)	(46)	(30)
	4 weeks	126 [a]	8 [b]	28 [ab]	40 [b]	-4 [b]	-71 [b]	157	-35 [b]
		(35)	(30)	(1)	(24)	(43)	(48)	(91)	(44)

N.B.: During the restriction, 100% means that the energy used by PE or ME was equal to the entire energy intake deficit. 0% means that none of the energy deficit was compensated for by a change in the PE or ME functions. A negative percent means that the energy allocated to PE or ME increased despite the energy restriction. A percent over 100% means that the energy expenditure for PE or ME decreased to a greater extent than the calculated energy deficit. During the controlled re-feeding, 100% means that the entire newly increased energy intake (compared to the restriction) is allocated to PE or ME. 0% means that no additional energy is allocated to PE or ME compared to the restriction allocation. A negative percentage means that the energy allocation to PE or ME decreased even though the energy intake increase. A percent over 100% means that more than the energy intake's increase is allocated to PE or ME

$p = 0.02$) and $40 \pm 24\%$ after 4 weeks. Group P also compensated for $28 \pm 1\%$ the energy deficit by releasing stored energy (contribution significantly lower than 100%, $p < 0.0001$) after 4 weeks of deprivation. Group H also significantly compensated $32 \pm 7\%$ of the energy deficit by decreasing the overall energy allocated to ME over the month period, but the contribution was significant only when calculated over the 4-week periods ($p = 0.01$), not over a 2-week period ($p = 0.36$). However, this group compensated for the majority of the energy intake deficit by releasing energy stored in their body. The calculated contribution for the first 2 weeks of restriction ($85 \pm 12\%$) or the whole month ($87 \pm 23\%$) are significantly different from 0 but not from 100% (both $p > 0.3$). Overall, the

contribution to the compensation of the energy intake deficit of the decrease in energy allocation to ME was significantly greater for Group P than for Group H after 2 weeks of restriction ($p = 0.05$) but not when the whole 4 weeks of restriction are taken into account ($p = 0.77$). The energy released from body stores was always greater for Group H than Group P (both $p < 0.05$) because of increased utilization of lipids which have a greater energy density.

In summer, the net increase in energy intake during the controlled re-feeding compared to the restriction averaged 181.5 ± 67.5 MJ for Group H and 199.6 ± 70.0 MJ for group P over the whole month. In winter, the energy intake "surplus" reached 370.0 ± 48.0 MJ for Group H and 240.2 ± 71.0 MJ for Group P over the month long controlled re-feeding. In summer, the change in allocated energy between ME and PE could not be determined due to high individual variability and low statistical power (neither different from 0% contribution nor from 100%). This was due to the different behaviour of one animal in each group compared to the others (F03RO and F00YA). In winter however, both diet groups allocated the majority of their net energy intake "surplus" compared to the restriction intake to PE (i.e. to energy storage in the body and/or growth). Group H allocated $130 \pm 16\%$ and Group P $155 \pm 88\%$ of the net energy difference to production energy (not different from 100%, $p > 0.15$ for both groups, see Table 3.2). Energy allocation to maintenance energy was not increased, either after 2 or 4 weeks of recovery for any of the groups ($19 \pm 28\%$ for Group H and $34 \pm 44\%$ for Group P, not different from 0% all $p > 0.2$).

During the ad-libitum period in winter, the inter-individual variability was too great to assess repartition of energy for Group P (values were not different from 0% and from 100%, all $p > 0.05$). However, Group H allocated approximately half the energy intake "surplus" to PE ($45 \pm 12\%$, significantly different from 0 and 100%, both $p < 0.05$), but no additional energy appeared to be directed to maintenance energy ($3 \pm 5\%$, not different from 0%, $p = 0.62$).

Qualitative energetic estimates

Thermoregulation capacity. During summer, average metabolic rates over the hour long trials during the baseline were similar between the two diet groups in water at
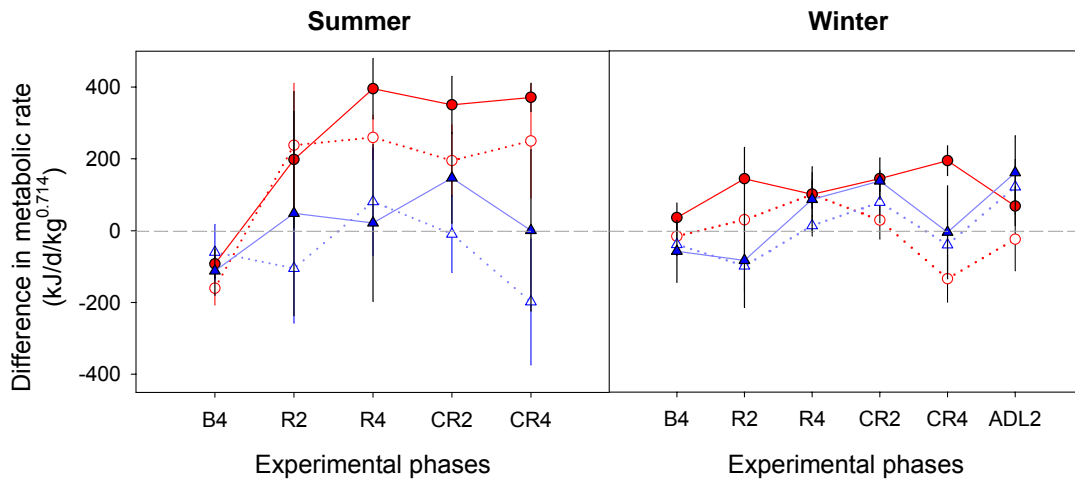
Figure 3.3: Mean ± SE metabolic rate differences between the beginning and the end of the measurement time after 45 min in water at 8°C (dotted lines) and 2°C (plain lines) for Group H (circles) and Group P (triangles) during the restriction (R2 and R4), the controlled re-feeding (CR2 and CR4) and the ad-libitum treatment (ADL2) in the summer 2005 and winter 2006 experiments.

8°C (average all animals pooled: 50.0 ± 1.2 kJ $kg^{-0.714} h^{-1}$) and at 2°C (48.9 ± 1.3 kJ $kg^{-0.714}$ h^{-1}; Wilcoxon rank sum test, $p = 0.68$ and $p = 0.34$ respectively). The baseline values for the average metabolic rates were also not higher for the 2°C temperature than for the 8°C (Wilcoxon rank sum test, $p = 0.44$). During the baseline phases, thermoregulatory costs (difference in oxygen consumption between the beginning and the end of the trial) decreased at both 8 °C (-4.60 ± 1.91 kJ $kg^{-0.714} h^{-1}$) and 2°C (-4.27 ± 1.52 kJ $kg^{-0.714} h^{-1}$), suggesting that metabolic expenditure was lower at the end of the thermal trial than at the start (Figure 3.3). However, Group H exhibited significant increases in thermoregulatory costs during the restriction and the controlled re-feeding treatments both at 8°C (all $p < 0.04$) and at 2°C (all $p < 0.002$). Thermoregulatory costs for Group H were 10.80 ± 2.59 kJ $kg^{-0.714} h^{-1}$ in the 8°C water at week 4 of the restriction, and 16.46 ± 3.50 kJ $kg^{-0.714} h^{-1}$ in water at 2°C. In contrast, Group P did not increase its thermoregulatory costs at either 8°C nor at 2°C (all $p > 0.2$) compared to the baseline values. When comparing both groups to one another, Group H increased its thermoregulatory costs significantly more than Group P in 2°C water, starting at week 4 of the restriction till the end of the controlled re-
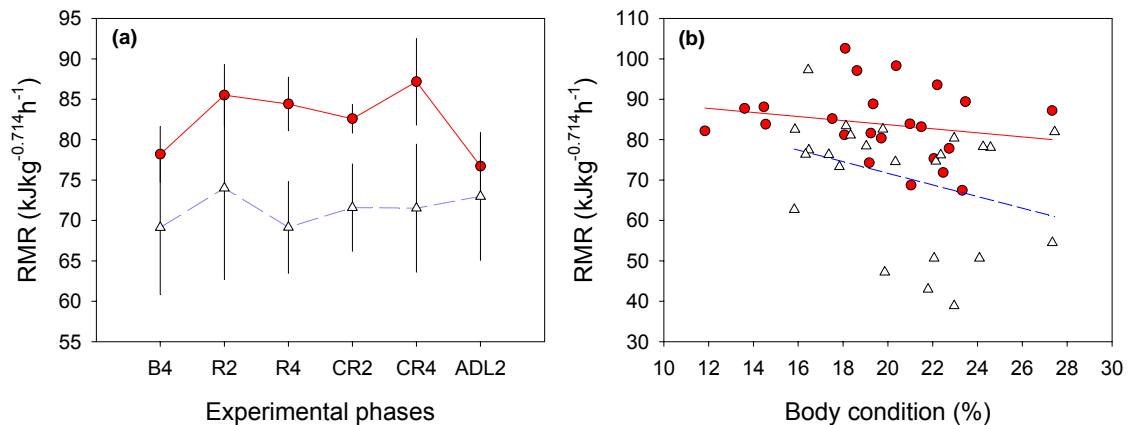
Figure 3.4: Standard metabolic rates (SMR) in air of (a) Group H (circles) and Group P (triangles) during the different experimental phases, and (b) related to the body condition (total body fat %) of the animals. SMR was measured throughout all the phases only during the winter experiment. The bars in (a) represent the standard error of the means. The lines in (b) represent the function from the linear mixed effects models run for Group H (plain line) and Group P (dashed line). Relationships fitted by linear mixed-effect models were not significant (Group H: p = 0.21; Group P: p = 0.88) and not different from one another (p = 0.52).

feeding (all p < 0.01). At 8°C, the between-group differences were significant only at the end of the controlled re-feeding (p < 0.01).

During winter, both experimental groups started the experiment with similar averaged metabolic rates over the hour of trial compared to one another and at both temperatures (Wilcoxon rank sum test, p = 0.34 at 8°C and p = 0.2 at 2°C). The average baseline metabolic rates at 8°C were 42.4 ± 2.08 kJ kg$^{-0.714}$ h^{-1} and 41.09 ± 1.97 kJ kg$^{-0.714}$ h^{-1} at 2°C, which were lower than the summer baseline values (p = 0.01 and p = 0.007). Thermoregulation capacity (difference in oxygen consumption between the beginning and the end of the trial) was constant during the baseline measurement (-1.17 ± 1.06 kJ kg$^{-0.714}$ h^{-1} at 8°C and -0.48 ± 1.99 kJ kg$^{-0.714}$ h^{-1} at 2°C; see Figure 3.3). Thermoregulation capacity during the other phases was similar between diet groups and similar to baseline measurements at all times (all p > 0.07). Thermoregulatory costs were 2.35 ± 1.47 kJ kg$^{-0.714}$ h^{-1} and 3.89 ± 1.92 kJ kg$^{-0.714}$ h^{-1} in waters of 8° and 2°C respectively at the end of the restriction, and 2.44 ± 2.47 kJ kg$^{-0.714}$ h^{-1} and 3.94 ± 3.06 kJ kg$^{-0.714}$ h^{-1} at the end of the ad-libitum period.

Standard metabolic rate. SMR_A was measured during the winter trial only. Baseline values averaged 73 ± 4 kJ kg-0.714 h-1 (range from 48 to 84 kJ kg-0.714 h-1, Figure 3.4a) with no differences between diet groups. SMRA during the following experimental phases were also identical between the two diet groups when expressed as either absolute values or as rates of changes compared to the baseline measurement (all $p > 0.08$). There were also no significant changes in SMR_A over time during the restriction (78.04 ± 4.55 kJ $kg^{-0.714}$ h^{-1}), the controlled re-feeding (79.34 ± 5.32 kJ $kg^{-0.714}$ h^{-1}), or the ad-libitum treatments (74.58 ± 4.27 kJ $kg^{-0.714}$ h^{-1}) (all $p > 0.06$). SMR_A was also not significantly correlated with body mass or body condition of the animals (all $p > 0.1$, Figure 3.4b).

Exercise metabolic rate. There were no diet difference in the baseline oxygen debt after a period of standard exercise (Wilcoxon rank sum test, $p = 0.68$), which averaged 4.10 ± 0.84 L (range from 2.41 to 8.23 L). However, there was an age difference, with the bigger animals having greater O_2 debts (average of 6.1 ± 1.3 L of O_2) than the smaller ones (average of 2.9 ± 0.1, Wilcoxon rank sum test, $p = 0.03$). Neither the diet nor the experimental phase had an impact on the O_2 debts of the animals, when expressed as absolute values or as rates of change compared to the baseline values (all $p > 0.1$). Finally, the time to reach the stable O_2 consumption rate after exercise ranged from 5 to 10 minutes. There was no age or diet differences observed and the experimental treatments did not impact the time to recover after exercising (all $p > 0.09$).

Discussion

Animals can employ a limited set of options to balance their energy budgets (Figure 3.1). The simplest is to adjust their digestive efficiencies to maintain a constant net energy intake during periods of nutritional stress. If this is insufficient, energy can be released from body stores and can be spared by reducing the energy allocated to physiological functions including activity, thermoregulation, or basal metabolism. Time of year and quality of diet may interplay with these processes and confound the energetic decisions that an animal might make while nutritionally stressed.

Our feeding experiments showed that Steller sea lions evoked different patterns of energy allocation in response to energy restrictions that were dependant on season and

quality of diet. Animals in both experimental groups compensated for the deficit we imposed by releasing stored energy from their bodies. However, animals fed herring also adjusted their thermoregulation capacities and activity levels relative to the seasonal conditions, while animals fed pollock did not appear to have the physiological ability to do so. During the re-feeding phases when energy intake was restored, the energetic priority of the sea lions was to restore energy status (by gaining lipid mass) in winter, but showed no clear pattern in summer.

Our calculations and measurements provide qualitative information on how Steller sea lions partitioned energy when faced with nutritional stress involving different types of prey at different times of year. The levels of net energy intake and metabolizable energy of our sea lions were within the same range of values reported for other pinnipeds (Parsons 1977, Miller 1978, Ashwell-Erickson & Elsner 1981, Lavigne et al. 1982, Keiver et al. 1984), which suggests that the urinary and fecal loss efficiencies, and the HIF proportion we used were good approximations. Levels of maintenance energy (ME, see Eq. 4) for our Steller sea lions were lower than values found for harbour seals (Markussen et al. 1990), but were within the range of those reported for grey seals (Ronald et al. 1984) and harp seals (Keiver et al. 1984). Consequently, although our estimates were approximate, the orders of magnitude were realistic and yielded reasonable approximations of bioenergetic partitioning for Steller sea lions.

Energetic priorities during summer

During the summer restriction, all animals (independent of diet type) relied exclusively on internal energy reserves to balance their budgets when facing an energy intake deficit. Depletion of internal energy reserves to counteract the net energy intake reduction usually happens when animals exhaust all of the energy conservation adjustments available to them. The range of adjustments include better digestive efficiencies, metabolic depression through behavioural adjustments to reduce activity or thermal costs, decrease in basal metabolism, and increase in foraging effort (Mrosovsky & Sherry 1980, Keiver et al. 1984, Worthy & Lavigne 1987, McCarter & McGee 1989, Nordøy et al. 1990, Oritsland 1990, Markussen et al. 1992a, Rosen & Trites 1999, 2002, Ali et al. 2003). Metabolic depression, defined here as a reduction in maintenance energy

(ME) coming from thermoregulation, activity and/or basal metabolism is usually the expected response of nutritionally stressed animals to save energy and thus reduce the amount of body components mobilized as observed in many marine mammals including Steller sea lions during nutritional stress (Worthy & Lavigne 1987, McCarter & McGee 1989, Nordøy et al. 1990, Markussen et al. 1992a, Rosen & Trites 1999, 2002).

During summer however, maintenance energy (ME) did not decrease significantly for any of the diet groups, meaning there was no overall evidence of energy expenditure sparing during the restriction from any of the functions included in ME. Counter intuitively, allocation to maintenance energy increased after 2 weeks of restriction for Group H as has been observed in other species during nutritional stress (Mrosovsky & Sherry 1980, Keiver et al. 1984, Ali et al. 2003). This increase in energy allocated to ME may have been why animals in Group H seemed to release more energy from their body than the actual gross energy deficit (198%, even though it was not significantly greater than 100% of the energy deficit, see Table 3.2).

Changes in maintenance energy (ME), particularly increases during nutritional stress, are generally thought to be associated with increases in activity (Mrosovsky & Sherry 1980, Keiver et al. 1984, Ali et al. 2003). Voluntary activity plays a major role in the energy budget of pinnipeds (Costa & Williams 1999). Immature Steller sea lions for example spend 40-75% of their time swimming (Winship et al. 2002), and mature animals spend 70-80% of this time in the water in winter which represents up to 65% of their daily energy expenditure (Winship et al. 2002). In our study, voluntary activity was not directly measured but the measured DMR provided indirect qualitative information about the changes in costs of activity compared to the baseline. It showed that DMR increased by about 35% during day light hours (20% on average on a 24h basis) for the animals fed herring during the energy restriction. These changes were likely due to an increase in activity since the animals were not thermally challenged in the chamber and the levels of changes were too high to be attributable only to basal metabolism (though we did not verify this).

Increases in voluntary activity during an energy restriction are typical of a "hunger response". Animals in Group H may therefore have increased their foraging effort

(activity) in an attempt to find more food to counteract the actual state of energy restriction (Rosen & Trites 2002). The captive animals in our study also appeared to alter their activity in a way that optimized their energy expenses against the probability to "catch" food since the increase in activity (foraging effort) was only seen during the day time when they usually received food from the trainers. However activity levels were decreased during the night hours when the probability for getting food was null. The overall significant increase in activity may explain why Group H increased maintenance energy. In contrast, the sea lions fed pollock did not display any significant change in activity levels, at least as assessed from the DMR data. This means that they did not initiate a "hunger response" in order to "optimize" the chances to get more food, nor did they decrease their activity levels to spare energy.

In conclusion, the experimental diets during the restriction had different impacts on the energetic priorities and strategies of our Steller sea lions, at least in terms of the energy allocation to activity levels and global maintenance energy (ME). Group H chose to increase activity as in a "hunger response" and Group P did not show any significant adjustments to the new energetic conditions which, if encountered in the wild, may reduce the chance of a sea lion terminating the nutritional stress associated with a pollock diet. It is important to keep in mind that the diets differed not only in terms of fish quality but also in terms of biomass intake. The animals in Group P were getting approximately twice as much fish as Group H for the same energy intake (and close to their baseline biomass). This difference in satiation levels (intrinsic consequence of a low-energy fish for isocaloric diets compared to high-energy fish, even in the wild) could explain why Group P did not display a "hunger response".

If the increase in activity observed in the laboratory for Group H translated into increased time in the water (as per increased foraging effort), it would have several secondary energetic implications (Rosen et al. in press) such as increasing the thermoregulation costs associated with spending more time in a thermally conductive environment looking for food. It is thus important to assess the changes in thermoregulatory capacity occurring during the restriction concomitantly with the changes in activity levels to estimate changes in maintenance energy levels. Thermoregulation costs of our sea lions with optimal body conditions were insignificant given the ability of

the animals to maintain constant and equal metabolism over an hour in water at 8° and 2°C. However, thermoregulation capacity decreased during the restriction while animals were losing mass, especially at the lower water temperatures for Group H. Unlike Group P, the animals fed herring had to increase their metabolic production of heat to stay warm after one hour in the water (no change in behaviour in the cage). Their depleted insulation did not seem to be sufficient after 15 or 28 days of losing mass to isolate the body core properly. Animals in Group P however did not have to increase their metabolic rates to thermoregulate after one hour in waters of 2° or 8°C.

It is noteworthy that the group of animals that relied the least on lipids to sustain their energy requirements (Group P) was able to maintain a constant thermoregulation capacity. Changes in production energy (PE — growth, energy storage) and maintenance energy (ME — thermoregulation, activity, basal metabolism) are not independent from one another. Substrates being used in metabolism provide insights into ways animals partition their use of different fuels especially when they have multiple roles. The subcutaneous lipid reserves provide sea lions with insulative capacity, which is especially critical in thermally conductive environments such as cold waters (Heath et al. 1977) in addition to being an energy reserve and a buoyancy factor (Beck et al. 2000, Hamilton et al. 2004). The animals fed herring (Group H) almost exclusively mobilized lipids as fuel to compensate for the energy intake deficit (Table 3.1, Chapter 2) which were probably mostly derived from the blubber layer. It is consequently not surprising that the thermoregulatory capacity of this group decreased. Animals in Group P on the other hand lost a significantly lower amount of lipids and greater amount of lean mass to compensate for the energy intake deficit (Table 3.1 and Chapter 2). The lower reliance of lipids for energy reserve allowed them to preserve a stable insulative capacity in cold water.

Group P did lose more lean tissues than Group H, and it is known that catabolism of proteins from muscle mass can affect organs integrity, and activity and foraging efficiencies (Vaz 2003). Consequently, a loss of energy reserves from the protein reserves could have lead to structural problems and could ultimately have a more drastic and permanent effect on the fitness of sea lions.

Metabolism during physical effort was measured to assess changes in fitness of the sea lions when energy intake was suboptimal. Animals losing body mass (especially lean tissues from muscles) or in a conservative physiological state when nutritionally stressed were expected to be less fit for strenuous physical activity and should have had a greater post-exercise oxygen debt during the restriction than before they were food restricted. However, results did not show any noticeable change in the capacity of sea lions to recover after a forced exercise. The animals did not increase their O_2 debt and they did not take longer to recover during the nutritional stress than during the baseline period. However, the few minutes of forced activity may not have been challenging enough to detect changes in fitness for animals that regularly spend hours (sometimes days) at a time foraging at sea, and we therefore cannot draw firm conclusions about changes in fitness relative to diets.

During the controlled re-feeding, the individual variability was too large to estimate allocation of energy towards production or maintenance. A closer inspection of data showed that one animal in each diet group yielded opposite results to the others. This combined with the low statistical power associated with a small sample size prevented drawing conclusions on the repartition of the energy allocations. Nevertheless there were strong clues among the data collected. They suggest that the animals probably did not allocate a significant amount of energy to production energy (PE) since they unexpectedly did not regain weight when returned to their baseline diet (see Table 3.1 and Chapter 2). Furthermore, the body composition did not provide clear indications of energy allocation to either lean or lipid mass.

Maintenance functions (at least activity and thermoregulation) did not change compared to the restriction period despite the increased energy intake (relative to restriction). Activity levels assessed from the DMR showed that Group H still displayed a "hunger response" two weeks into the controlled re-feeding even though their energy intake was higher. This indicates that the increased intake still did not lead to satiation. Thermoregulation capacity did not return to the initial baseline levels during the re-feeding period, suggesting that restoration of this function was not a priority in summer. Activity reached baseline levels only at the end of the month-long treatment. The animals in Group P however displayed no change in thermoregulation capacity compared to the

restriction or the baseline, once again showing a lack of adjustment to new energetic conditions.

Energetic priorities during winter

Energy allocation and the repercussion of the energy deficit on the physiological functions of Steller sea lions appeared different during winter than during the summer. Animals from both groups (H and P) still compensated for the energy deficit during the restriction by releasing stored energy (tissue catabolism), but the proportion of this contribution to the deficit was lower during winter. Unlike in summer, a significant part of the energy deficit was also compensated for by a decrease in maintenance energy (i.e., a metabolic depression resulting from a decrease in thermoregulation, activity and basal metabolism). Both diet groups showed this energy-sparing decrease in maintenance energy, which seemed to start earlier for Group P but was overall not greater than that of Group H over the month-long feeding restriction.

From the bioenergetic calculations it is apparent that the animals in Group P released a significantly lower amount of stored energy during winter than animals in Group H (Table 3.2) even though both groups were losing body mass at the same rate. This difference resulted from the tendency of Group P to rely almost exclusively on the lean mass tissues and barely on their lipid reserves to compensate for the energy deficit (Table 3.1), while animals in Group H lost significantly more lipid mass. As protein is less energetic than fat, approximately identical mass losses released a lower quantity of energy from the bodies of sea lions in Group P than Group H. The energetic strategy adopted by Group H seems to have been more efficient than that of Group P. In general, the typical physiological response of most animals to nutritional deprivation is to rely predominantly on lipids stores and only as a last resort on the lean tissues reserves (Castellini & Rea 1992). Thus, the atypical and more extreme response from Group P seems maladapted from an energetic point of view for the health of the animals.

Thermoregulation capacity was a priority during the winter for both experimental groups of sea lions. Pinnipeds have anatomical and adaptive mechanisms that allow them to minimize the amount of energy required for thermoregulation (Irving 1969, Blix & Steen 1979, Whittow 1987). During the colder winter season, the insulative layer of

blubber was thicker for all sea lions (measured by ultrasounds, data not shown) and both diet groups kept a greater proportion of lipid stores compared to the summer (Table 3.1 and Chapter 2). Average metabolic rates in 2° and 8°C water before the restriction (baseline measurement) were also lower in winter than in summer, which reflects better insulation during winter.

From a bioenergetic perspective, physiological and anatomical adjustments of animals related to insulation are adapted to conditions encountered in the natural environment for a given season. Ashwell-Erikson and Elsner (1981) for example found that the heat produced by basal metabolic rate in harbour and spotted seals were sufficient in summer and winter to maintain thermal homeostasis under natural conditions. In our study, sea lions from both groups did not need to increase their initial metabolic rate to stay warm in 2° and 8 °C water during and after the energy restriction. The range of water temperatures tested on our animals was closer to those encountered by Steller sea lions in the wild during winter (between 3° and 7°C). Our results therefore confirm that thermoregulation capacity is adjusted to the seasonal requirements (Ashwell-Erickson & Elsner 1981) and is a priority in winter.

Since the energy allocated to thermoregulation was stable during the winter energy restriction, the observed decrease in maintenance energy (ME) for the two groups must have derived from a decrease in voluntary activity and/or basal metabolism. During winter, both experimental groups showed lower activity levels — as estimated from the DMR data — during the restriction compared to the baseline measurement. This decrease in voluntary activity during nutritional stress has been observed in other animals, such as Mongolian gerbils (*Meriones unguiculatus*) that significantly decreased their locomotor activity during a 2-week food restriction (40% restriction, Karakas et al. 2006), or roach fish (*Rutilus rutilus*) that decreased their swimming activity by 50% when energetically deprived (van Dijk et al. 2002). In our study, the activity depression lasted longer for sea lions in Group H than for those in Group P, which indicates that the restricted pollock diet did not trigger extended energy saving as did the herring diet, nor was it sustained as long as needed (the restriction lasted 4 weeks and the adjustment only 2 weeks) which may lead to deleterious effects occurring earlier than for the other group over the long term.

It is interesting that Group H reacted to the energy restriction in opposite ways in winter and summer. Sea lions deprived in terms of both energy and biomass intake displayed a foraging response in the summer and a metabolic depression in the winter. The animals eating a high biomass of fish with a low-energy intake did not alter their activity levels in summer and altered it only slightly during the winter. This suggests that diet quality, biomass intake, and season are determining factors underlying the metabolic response of sea lions during nutritional stress. In our study, similar levels of decreased energy intake evoked three different strategies for sea lions eating different diets in different seasons. However, level of energy deficit can also affect the type of physiological reaction. It has been reported that fasting in Steller sea lions induces a depression in standard metabolic rates, while a 50-70% decrease in food intake (by weight) induces a foraging response (Rosen & Trites 2002). Consequently, it is important to consider the quantity and the quality of fish eaten as well as the level of energy deficit during the nutritional stress, and the season during which it occurs to understand and predict physiological responses.

The calculated overall decrease in allocation to maintenance energy (ME) seems to result more from a decrease in activity levels than thermoregulatory costs. Basal metabolism is the last function that can be adjusted to decrease ME (Lavigne et al. 1982, Dulloo & Jacquet 1998, Rosen & Trites 1999), which was not assessed per se in our study. One of the most important functions of basal metabolism relates to maintenance of the protein pool. Proteic tissues are constantly synthesised and degraded and require more energy for maintenance (constant turnover) than the less metabolically active lipid tissues (Buttery 1981). Welle and Nair (1990) showed that up to 20% of the resting metabolic changes in humans was due to protein turnover. In our experiment, the animals fed pollock lost up to 85% of their total mass loss as lean mass at the end of the restriction. Consequently, a loss of 10 kg of metabolically active tissue could lead to a decrease in the overall energy used to maintain the integrity of tissues, and to a greater extent for Group P.

Changes in basal metabolism mentioned above are speculative. We did measure metabolism in air in a small chamber, but the conditions of measurements did not fulfill Kleiber's requirements (1975) to obtain basal metabolic rate. Our experimental animals

were post-absorptive, but were growing and were not completely inactive in the metabolic chamber, which means that the measurements represented standard metabolic rate (SMR_A). During the restriction, SMR_A did not depart from the baseline values for any of the groups. Since activity levels (monitored in the metabolic chamber) were consistent per animal throughout the experiment, stable SMR_A may indicate that changes in basal metabolism were not significant enough to impact the standard metabolism, even for Group P who may have had a greater decrease in protein turnover. Consequently, the decrease in maintenance energy allocation was probably mostly due to a decrease in activity levels and to a lesser extent, if any, to changes in BMR.

During the winter controlled re-feeding, bioenergetic calculations showed that the majority of the increased energy intake was allocated to production energy (PE), while the allocation to maintenance energy (ME) did not change from the lower levels attained during the restriction. In winter, all of the animals regained mass when switched back to their baseline diet by greatly raising their growth rate (relative to baseline) and gaining almost exclusively lipid mass (Table 3.1 and Chapter 2). Such a differential gain of lipids compared to lean mass has already been observed in other animals (Xie et al. 2001). In our case, it likely represents an emphasis on maximizing energy stores in expectation of another stressful event (Metcalfe & Monaghan 2001) such as the upcoming summer breeding season when female Steller sea lions need to nurse their pups, which requires a greater energy output (lactation) combined with a broken feeding pattern (attendance to pup) (Winship et al. 2002). It may also be a strategy to restore the thermoregulation capacity as much as possible (allowing more energy to be spent on productive functions) (Guinet et al. 1998, Pitcher et al. 1998), although it remained constant during the experimental restriction.

Activity levels that were depressed during the restriction seemed to return to pre-restriction levels during the controlled re-feeding and the ad-libitum phase for the animals fed pollock. They stayed lower than baseline levels for those fed herring until the end of the trial. Synergistically, the delay in restoration of activity metabolism and in maintenance energy (ME) expenditures after a depression is known to help increase growth rates above their normal values after nutritional stress (see Chapter 2 and Condit & Ortiz 1987, Farbridge et al. 1992, Yambayamba et al. 1996, Ali et al. 2003). More energy

is available to be allocated to production since expenditures for maintenance energy are kept minimal. Consequently, the strategy of maintaining low activity levels during the controlled re-feeding phase adopted by Group H seemed to be energetically relevant and more optimal than the strategy invoked by Group P, although this may be difficult to accomplish in the wild.

Relevance of energetic strategies

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

Group H's strategy to compromise on thermoregulation capacity and to allocate energy to other more important functions probably reflected the fact that thermoregulatory needs are the lowest at this time of the year. Water temperature on the coast of British Columbia, the Gulf of Alaska and the Aleutian Islands ranges from $11° - 17°C$ at the surface and from $9° - 11°C$ at 40 m depth in summer (DFO 2007). Temperatures tested during the thermoregulation trials were lower than natural conditions in the summer, which means that even if Group H compromised on their thermoregulation capacity at 8° and 2°C, they may not have to compromise at warmer and perhaps more relevant water

temperatures. In addition, the animals were physically unable to move much during the metabolic measurements, and may not have been able to offset the thermoregulation needs by the heat released from activity as observed in the wild (Hind & Gurney 1997, Rosen et al. in press). An increase in foraging resulting in an increase in activity levels as observed for Group H could compensate for decreases in thermoregulation capacity. In winter however, sea temperatures in the North Pacific range from $4° – 10°C$ at the surface and from $3° – 8°C$ at 40 m depth (DFO 2007). Capacity to stay warm in water must be a priority at these temperatures. Poor heat management may force sea lions to increase the production of heat through metabolism and thus burn additional fuel. If lipids from the insulative layer of blubber are mobilized, the animal may find itself in a deleterious cycle of increasing energy demands (Rosen et al. in press).

Energetic strategies of sea lions fed herring during both seasons appeared to adjust to prevailing environmental conditions. In contrast, the animals fed pollock did not alter their energetic allocation as much. Thermoregulation or activity levels stayed more or less stable regardless of the need to keep these functions optimal in a given season. For example, Group P kept a thermoregulation capacity constant during the summer restriction at 2°C even though these temperatures were not likely to be encountered in the wild in summer. Unlike the sea lions fed herring, animals fed pollock did not make clear adjustments to adequately respond to the energy deficits they encountered.

The strategy of Group P to not noticeably change their allocation of energy compared to baseline measurements and adjustments made by Group H suggests that a decrease in biomass intake in addition to a decrease in energy intake is needed to trigger relevant and adaptive responses to energy deficits. Contradictory signals related to a decrease in energy intake associated with a constant biomass intake such as for Group P during the restriction, seemed to offset the capacity of sea lions to optimally adjust to nutritional stress, independent of the season (see also Chapter 2). Physiological and behavioural adjustments under nutritional stress are mechanisms to optimally counteract the energy intake deficit and ultimately increase the fitness of animals in their natural environment. A full ration of low-energy fish such as pollock for sea lions in the wild may prevent their body from triggering physiological and behavioural mechanisms needed to adjust to low energy intake. The non-adjustments observed for Group P could thus result

in detrimental outcomes if the nutritional stress was carried over longer periods of time than the 28-day time frame of our study.

Conclusions

Our study provides qualitative information about the overall strategies that Steller sea lions employ under different nutritional conditions in captivity. We found that the energetic choices made by Steller sea lions were more affected by diet in summer than in winter. Animals consuming reduced amounts of high-energy fish (herring) showed physiological responses that were seasonally consistent with predicted responses and indicated optimization of energy partitioning between the different physiological functions. In contrast, a pollock diet seemed to prevent beneficial physiological adjustments from being made during the energy restriction. It is not clear what physiological processes explain these observed differences. Since balance between energy intake and expenditure is usually regulated by endocrine systems in response to environmental and internal signals, understanding the mechanistic behind the different physiological responses observed should consider assessing regulatory changes in metabolic and stress hormones during the different nutritional conditions tested.

References

Ali M, Nicieza A, Wootton RJ (2003) Compensatory growth in fishes: a response to growth depression. Fish and Fisheries 4:147-190

Anthony JA, Roby DD, Turco KR (2000) Lipid content and energy density of forage fishes from the northern Gulf of Alaska. Journal of Experimental Marine Biology and Ecology 248:53-78

Ashwell-Erickson S, Elsner R (1981) The energy cost of free existence for Bering Sea harbor and spotted seals. In: Hood DW, Calder JA (eds) The Eastern Bering Sea Shelf: Oceanography and Resources. University of Washington Press, Seattle, p 869-899

Baldwin RL, Bywater AC (1984) Nutritional energetics of animals. Annual Review of Nutrition 4:101-114

Beck CA, Bowen D, Iverson SI (2000) Seasonal changes in buoyancy and diving behaviour of adult grey seals. Journal of Experimental Biology 203:2323-2330

Blix AS, Steen JB (1979) Temperature regulation in newborn polar homeotherms. Physiological Reviews 59:285-304

Boyd IL (2002) Energetics: consequences for fitness. In: Hoelzel AR (ed) Marine mammal biology: an evolutionary approach. Blackwell Sciences Publishing, Oxford, Malden, p 247-277

Buttery PJ (1981) Protein turnover in animals. Tropical Animal Production 6:204-213

Calkins DG, Becker EF, Pitcher KW (1998) Reduced body size of female Steller sea lions from a declining population in the Gulf of Alaska. Marine Mammal Science 14:232-244

Castellini MA, Rea LD (1992) The biochemistry of natural fasting at its limits. Experientia 48:575-582

Condit R, Ortiz CL (1987) The physiological transition from fasting to feeding in weaned elephant seal pups. Marine Mammal Science 3:207-219

Costa DP, Williams TM (1999) Marine mammals energetics. In: Reynolds JE, Rommel SA (eds) Biology of marine mammals. Smithsonian Institution Press, Washington, D.C., p 176-217

Cuyler LC, Øritsland NA (1993) Metabolic strategies for winter survival by Svalbard reindeer. Canadian Journal of Zoology 71:1787-1792

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

Dulloo AG, Jacquet J (1998) Adaptive reduction in basal metabolic rate in response to food deprivation in humans: a role for feedback signals from fat stores. American Journal of Clinical Nutrition 68:599-606

Farbridge KJ, Flett PA, Leatherland JF (1992) Temporal effects of restricted diet and compensatory increased dietary intake on thyroid function, plasma growth hormone levels and tissue lipid reserves of rainbow trout *Oncorhynchus mykiss*. Aquaculture 104:157-174

Guinet C, Roux JP, Bonnet M, Mison V (1998) Effect of body size, body mass, and body condition on reproduction of female South African fur seals (*Arctocephalus pusillus*) in Namibia. Canadian Journal of Zoology 76:1418-1424

Hamilton JL, Dillaman RM, McLellan WA, Pabst DA (2004) Structural fiber reinforcement of keel blubber in harbor porpoise (*Phocoena phocoena*). Journal of Morphology 261:105-117

Heath ME, McGinnis SM, Alcorn D (1977) Comparative thermoregulation of suckling and weaned pups of the northern elephant seal, *Mirounga angustirostris*. Comparative Biochemistry and Physiology 57:203-206

Hind AT, Gurney WSC (1997) The metabolic cost of swimming in marine homeotherms. Journal of Experimental Biology 200:531-542

Hunter AMJ (2005) A multiple regression model for predicting the energy requirements of marine mammals. MSc., University of British Columbia

Irving L (1969) Temperature regulation in marine mammals. In: Andersen HT (ed) The biology of marine mammals. Academic Press, Inc., New York, USA

Karakas A, Serin E, Gunduz B (2006) Food restriction affects locomotor activity in Mongolian gerbils (*Meriones unguiculatus*). Turkish Journal of Biology 30:23-28

Keiver KM, Ronald K, Beamish FWH (1984) Metabolizable energy requirements for maintenance and fecal and urinary losses of juvenile harp seals (*Phoca groenlandica*). Canadian Journal of Zoology 62:769-776

Kitts DD, Huynh MD, Hu C, Trites AW (2004) Seasonal variation in nutrient composition of Alaskan walleye pollock. Canadian Journal of Zoology 82:1408-1415

Kleiber M (1975) The fire of life: an introduction to animal energetics. R. E. Krieger Pub. Co., Huntington, N.Y.

Lavigne DM, Barchard W, Innes S, Øritsland NA (1982) Pinniped bioenergetics. In: Mammals in the seas: Small cetaceans, seals, sirenians, and sea otters. FAO Fisheries Series 5, Rome, p 191-235

Lawson JW, Miller EH, Noseworthy E (1997) Variation in assimilation efficiency and digestive efficiency of captive harp seals (*Phoca groenlandica*) on different diets. Canadian Journal of Zoology 75:1285-1291

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

Lucas A (1992) Bioénergétique des animaux aquatiques. Masson, Paris

Markussen NH, Ryg M, Oritsland NA (1992) Metabolic rate and body composition of harbour seals, *Phoca vitulina*, during starvation and refeeding. Canadian Journal of Zoology 70:220-224

Markussen NH, Ryg M, Øritsland NA (1990) Energy requirements for maintenance and growth of captive harbour seals, *Phoca vitulina*. Canadian Journal of Zoology 68:423-426

McCarter RJ, McGee JR (1989) Transient reduction of metabolic rate by food restriction. American Journal of Physiology 257:E175-E179

Metcalfe NB, Monaghan P (2001) Compensation for a bad start: grow now, pay later? Trends in Ecology and Evolution 16:254-260

Miller LK (1978) Energetics of the northern fur seal in relation to climate and food resources of the Bering Sea. Report No. MMC-75/08, U.S. Marine Mammal Commission

Mrosovsky N, Sherry DF (1980) Animal anorexias. Science 207:837-842

Nash LT (1998) Vertical clingers and sleepers: seasonal influences on the activities and substrate use of *Lepilemur leucopus* at Beza Mahafaly Special Reserve, Madagascar. Folia Primatologica 69:204-217

Nordøy ES, Ingebretsen OC, Blix AS (1990) Depressed metabolism and low protein catabolism in fasting grey seal pups. Acta Physiologica Scandinavia 139:361-369

Ohata CA, Miller LK (1977) Some temperature responses of northern fur seal (*Callorhinus ursinus*) pups. Journal of Mammalogy 58:438-440

Oritsland NA (1990) Starvation survival and body composition in mammals with particular reference to Homo sapiens. Bulletin of Mathematical Biology 52:643-655

Parsons JL (1977) Metabolic studies on ringed seals (*Phoca hispida*). M.Sc., University of Guelph

Patterson BR, Benjamin LK, Messier F (1998) Prey switching and feeding habits of eastern coyotes in relation to snowshoe hare and white-tailed deer densities. Canadian Journal of Zoology 76:1885-1897

Pinheiro JC, Bates DM (2000) Mixed-Effects Models in S and S-PLUS. Springer-Verlag, New York

Pitcher KW, Calkins DG, Pendleton GW (1998) Reproductive performance of female Steller sea lions: an energetics-based reproductive strategy? Canadian Journal of Zoology 76:2075-2083

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

Pullar JD, Webster AJF (1977) Energy cost of fat and protein deposition in rat. British Journal of Nutrition 37:355-363

Roberts SB, Young VR (1988) Energy costs of fat and protein deposition in the human infant. American Journal of Clinical Nutrition 48:951-955

Ronald K, Keiver KM, Beamish FWH, Frank R (1984) Energy requirements for maintenance and fecal and urinary losses of the grey seal (*Halichoerus grypus*). Canadian Journal of Zoology 62:1101-1105

Rosen DA, Winship AJ, Hoopes LA (in press) Thermal and digestive constraints to foraging behavior in marine mammals. Philosophical Transactions, Royal Society of London B

Rosen DAS, Renouf D (1997) Seasonal changes in blubber distribution in Atlantic harbor seals: indications of thermodynamic considerations. Marine Mammal Science 13:229-240

Rosen DAS, Trites AW (1999) Metabolic effects of low-energy diet on Steller sea lions, *Eumetopias jubatus*. Physiological and Biochemical Zoology 72:723-731

Rosen DAS, Trites AW (2000) Digestive efficiency and dry-matter digestibility in Steller sea lions fed herring, pollock, squid, and salmon. Canadian Journal of Zoology 78:234-239

Rosen DAS, Trites AW (2002) Changes in metabolism in response to fasting and food restriction in the Steller sea lion (*Eumetopias jubatus*). Comparative Biochemistry and Physiology B 132:389-399

Rosen DAS, Trites AW (2003) No evidence for bioenergetic interaction between digestion and thermoregulation in Steller sea lions *Eumetopias jubatus*. Physiological and Biochemical Zoology 76:899-906

Schmidt-Nielsen K (1997) Animal physiology: adaptation and environment. Cambridge University Press, Cambridge, England ; New York, NY, USA

Sigler MF, Womble JN, Vollenweider JJ (2004) Availability to Steller sea lions (*Eumetopias jubatus*) of a seasonal prey resource: a prespawning aggregation of eulachon (*Thaleichthys pacificus*). Canadian Journal of Fisheries and Aquatic Sciences 61:1475-1484

Soto KH, Trites AW, Arias-Schreiber M (2006) Changes in diet and maternal attendance of South American sea lions indicate changes in the marine environment and prey abundance. Marine Ecology-Progress Series 312:277-290

Stini WA (1969) Nutritional stress and growth - sex difference in adaptive response. American Journal of Physical Anthropology 31:417-426

Trillmich F, Ono K (eds) (1991) Pinnipeds and El Niño: responses to environmental stress. Springer-Verlag, New York

Trites AW, Donnelly CP (2003) The decline of Steller sea lions *Eumetopias jubatus* in Alaska: a review of the nutritional stress hypothesis. Mammal Review 33:3-28

Trumble SJ, Barboza PS, Castellini MA (2003) Digestive constraints on an aquatic carnivore: effects of feeding frequency and prey composition on harbor seals. Journal of Comparative Physiology B 173:501-509

van Dijk PLM, Staaks G, Hardewig I (2002) The effect of fasting and refeeding on temperature preference, activity and growth of roach, *Rutilus rutilus*. Oecologia 130:496-504

Vaz M (2003) Undernutrition. In: Gibney MJ, Macdonald I, Roche HM (eds) Nutrition and Metabolism. Blackwell Publishing., Oxford ; Ames, Iowa, p 341-348

Welle S, Nair KS (1990) Relationship of resting metabolic rate to body composition and protein turnover. American Journal of Physiology 258:E990-998

Whittow GC (1987) Thermoregulatory adaptations in marine mammals: interacting effects of exercise and body mass. A review. Marine Mammal Science 3:220-241

Winship AJ, Trites AW, Rosen DAS (2002) A bioenergetic model for estimating the food requirements of Steller sea lions *Eumetopias jubatus* in Alaska, USA. Marine Ecology-Progress Series 229:291-312

Womble JN, Willson MF, Sigler MF, Kelly BP, VanBlaricom GR (2005) Distribution of Steller sea lions *Eumetopias jubatus* in relation to spring-spawning fish in SE Alaska. Marine Ecology-Progress Series 294:271-282

Worthy GAJ, Lavigne DM (1987) Mass loss, metabolic rate, and energy utilization by harp and gray seal pups during the postweaning fast. Physiological Zoology 60:352-364

Xie S, Zhu X, Cui Y, Wootton RJ, Lei W, Yang Y (2001) Compensatory growth in the gibel carp following feed deprivation: temporal patterns in growth, nutrient deposition, feed intake and body composition. Journal of Fish Biology 58:999-1009

Yambayamba ES, Price MA, Foxcroft GR (1996) Hormonal status, metabolic changes, and resting metabolic rate in beef heifers undergoing compensatory growth. Journal of Animal Science 74:57-69

Chapter 4: Changes in blood metabolites and hormones do not reflect nutritional status of Steller sea lions during and after periods of nutritional stress[3]

Summary

Physiological alterations due to changes in energy balance are under tight endocrine regulation. Glucocorticoid (cortisol), thyroid (T3, T4) and somatotropic (IGF-1, GH) hormones contribute to the regulation of physiological responses during nutritional stress and subsequent re-feeding. Hormone levels should explain differential changes in metabolic pathways which should also be reflected by levels of blood metabolites (NEFA, β-HBA, and BUN/creatinine). Changes in hormonal regulation were studied in eight captive female Steller sea lions in parallel with changes in body mass, body composition, and energetic priorities in summer and winter. Accuracy of blood metabolites and hormones as indicators of body composition change was also investigated. During a 28-day energy restriction, one group of four sea lions was fed only Pacific herring (Group H) and another group was fed an isocaloric diet of walleye pollock (Group P). Both groups were fed their pre-experimental diet of herring during a subsequent 28-day recovery phase. Animals lost mass at the same rate during the energy restriction in both seasons, but Group H lost more lipids and Group P lost more proteins. Overall, free cortisol and IGF-1 regulated changes in body mass in winter but only IGF-1 was significantly involved in summer, indicating a predominant regulation of mass change through lean mass in summer and a tighter regulation of lipids in winter. Differences in utilization of internal energy stores (lipid vs protein) were not reflected by changes in blood metabolites nor were they related to levels of glucocorticoid or thyroid hormones, while IGF-1 significantly regulated changes in lean mass in both seasons. Overall, changes in body condition (percent fat in the body) were not explained by any of the hormones measured, raising concerns regarding their accuracy as indicators of nutritional status for Steller sea lions.

Introduction

Natural habitats are neither constant nor completely predictable, and animals have to continuously adjust to these environmental variations (Wingfield & Kitaysky 2002). Regulation of energy intake and expenditure is an essential part of these adjustments, and is linked to the survival and reproductive efficiency of animals in their natural environment (Lavigne et al. 1982). When quantity or quality of food is insufficient, it results in physiological and behavioural modifications to rebalance the energy budget (Stini 1969, Lawson et al. 1997, Boyd 2002, Trumble et al. 2003). Ultimately these physiological changes are regulated via complex, interconnected endocrine systems.

Our study investigated the hormonal response to undernutrition in Steller sea lions whose population has declined since the 1980s, potentially due to a decrease in diet quality and quantity (Trites & Donnelly 2003b). Steller sea lions have been shown to display different physiological responses to nutritional stress depending on the quality of fish (Chapters 2 and Rosen & Trites 2005, Kumagai et al. 2006). In our study, sea lions fed a suboptimal diet of pollock lost predominantly proteins after 2 weeks of restriction, while the animals fed herring lost predominantly lipids (Chapter 2), and the extent of these losses varied seasonally. These differences in tissue use during an energy restriction should result from a differential regulation by hormones of lipid and protein metabolism. Determining how changes in hormone levels are influenced by external factors such as diet type, age and season during nutritional stress can help to understand the regulatory mechanisms behind changes in body composition and partitioning of the energy budget.

Endocrine response to nutritional stress primarily (but not exclusively) involves the somatotropic, glucocorticoid, and thyroid hormones (Hornick et al. 2000, Robson et al. 2002). Hormones from the somatotropic axis, (growth hormone GH, and insulin-like growth factor 1 IGF-1) are involved in protein and lipid metabolism as well as mineral metabolism and bone growth (Breier 1999, Butler & Le Roith 2001). During a negative energy balance, GH usually increases and IGF-1 decreases in association with a cessation of growth, a sparing of proteins and an increase in lipolysis in terrestrial vertebrates (Hornick et al. 2000). Thyroid hormones (T3, T4) are secreted by the hypothalamo-pituitary-thyroid axis and are essential for growth, metabolism, and thermogenesis in

mammals (Kelly 2000). During nutritional stress, the levels of thyroid hormones usually drop in terrestrial mammals, helping to decrease overall energy expenditures through reduction in metabolic rates (Buonomo & Baile 1991, Yambayamba et al. 1996). Finally, glucocorticoids (cortisol, free cortisol) are known to be involved in stress responses, and correlate with unpredictable life situations like energy intake shortages (Reeder & Kramer 2005). Food deprivation is usually associated with an elevation of these hormones, which have been found to increase lipid metabolism, and modify development and behaviour of birds and mammals (Ortiz et al. 2001b, Kitaysky et al. 2003).

While these three groups of hormones regulate lipid or protein metabolism, changes in these catabolic routes should also be reflected in blood levels of relevant metabolite by-products. If changes in blood metabolites are found to be accurate markers of lipid and protein catabolism, they could be used as tools to assess nutritional status of sea lions in different environmental conditions. In fasting animals, levels of non-estherified fatty acids (NEFA) and ketone bodies (β-HBA) are usually representative of lipid catabolism while levels of nitrogen wastes in the blood (BUN) reflect changes in protein catabolism (Rea et al. 2000, Mellish & Iverson 2001). However, these parameters may not accurately reflect the source of tissue catabolism during a milder energy restriction (when the rate of mass loss is lower than during a fast) and will be additionally affected by levels of dietary protein and lipid intake (Narayanan & Appleton 1980, Ramsay et al. 1991).

Interpreting the relationship between hormones and nutritional stress is complex. Metabolism and hormone levels of Steller sea lions naturally vary throughout the year independent of nutritional stress (Thissen et al. 1994, Romero 2002, Wingfield & Kitaysky 2002, Kumagai 2004, Myers et al. 2006, Mashburn & Atkinson 2007) and in response to seasonally-dependant energetic priorities (Winship et al. 2002). In addition, nutritional restrictions are thought to be more stressful for young animals and endocrine regulatory systems vary with age (Engelhardt & Ferguson 1980, Little 1991, Hall et al. 1998, Haulena et al. 1998, Myers et al. 2006). Consequently, it is essential to use controlled studies to tease apart the impact of each parameter (diet quality, level of energy intake, season, and age of the animal) on the endocrine systems to understand the resulting physiological responses.

The primary goal of our study was to investigate the impact of season, age, and type of diet on the endocrine regulation of Steller sea lions that display different physiological adjustments during nutritional stress. Hormone measurements are discussed in reference to changes in body mass, body composition, and energetic priorities observed during our study (Chapters 2 and 3). Accuracy of blood metabolite and hormones as tools to evaluate nutritional status of the animals was also assessed. Defining the relationship between the endocrine system and metabolic and energetic status could expand our understanding of how Steller sea lions make energetic adjustments.

Material and methods

Experimental design

Our study was conducted with the approval of the University of British Columbia Animal Care Committee, under permit No. A04-0169 and all experimental procedures are detailed in Chapter 2. Briefly, experiments were conducted at the Vancouver Aquarium (BC, Canada) in the summer 2005 and in winter 2006, on 8 female Steller sea lions (five 3-year old and three 5-year old animals). The 8 animals were randomly assigned to one of the two experimental groups, Group H or Group P, which were kept identical for the two seasons.

The experiments consisted of three phases, starting with a 28-day baseline (B) during which all the animals were fed their usual daily ration of Pacific herring (*Clupea pallasi*). Next, the sea lions were placed on a 28-day restriction treatment (R) during which the energy intake was reduced by approximately 20-30%. The two diet groups were given the same restricted level of isocaloric diets but Group H was fed exclusively Pacific herring and Group P was fed only walleye pollock (*Theragra chalcogramma*). The restriction was followed by a 28-day controlled re-feeding (CR) period during which each sea lion received the same diet and energy intake of Pacific herring that they received during the baseline phase.

Data acquisition

Methodologies for measurements of food intake ($\pm$ 0.025 kg d-1), energy intake (kJ d-1), body mass ($\pm$ 0.1 kg), and body composition are described in detail in Chapter 2.

Blood samples were taken from sea lions in the morning before the first meal of the day (~18h overnight fast) and every two weeks concurrent with the measurement of body composition (deuterium dilution method). Procedures were performed while animals were under anaesthesia (isoflurane) and with veterinary supervision. Blood samples were collected into serum separator tubes and EDTA tubes (plasma) and were immediately centrifuged (3500 rpm for 5 min). Sera and plasma were then stored at -70°C until analyses. Thyroid hormones (total thyroxine TT4, total triiodothyronine TT3 and free thyroxine fT4) and blood metabolites (BUN, creatinine, NEFA and β-HBA) levels were measured at the Central Veterinary Laboratory (Langley, BC, Canada). The BUN/creatinine ratio was used for the analyses instead of BUN to control for the impact of protein load in the diet on the BUN levels. GH, IGF-1, cortisol, and free cortisol were analysed by radioimmunoassay validated for Steller sea lions by Richmond et al. (2006) and Kitaysky et al. (2001).

Statistical analyses

Effects of diet, season, age and experimental phase on changes in levels of hormones and metabolites compared to the baseline measurement were estimated using mixed effect models (R 2.4.0 and nlme library, Pinheiro et al. 2006). All models' assumptions were verified as explained in Pinheiro and Bates (2000) (see Chapter 2 for more details).

Sample size for all hormones and blood metabolites was 40 in the summer and 47 in the winter. The first set of analyses investigated the effects of diet type and experimental phases on the changes in hormone and metabolite levels stratified by seasons. Fixed effects were diet, phase, and the interaction between these parameters, and random effects were individual animals and diet and/or phase, depending on the best model fit estimated by ANOVA and AIC. The second set of analyses investigated the effect of age and experimental phases on the changes in hormone and metabolite levels. If

age and diet were not significant parameters, all animals were pooled and only the impact of the experimental phases stratified by season was tested. In this case, the fixed effect was phase and the random effects were the individuals. The third set of analyses investigated the seasonal differences in the levels and changes of hormone and metabolite levels during different phases, stratified by diet, age, or not stratified at all depending on the results of the previous tests. The fixed effects were season, phase, and the interaction between these terms and random effects were the individuals and potentially season depending on the best model fit.

Finally, the relationship between levels of hormones or metabolites and mass, body composition, and daily or standard metabolic rates (DMR or SMR_A) of the animals were analyzed using linear mixed effect models. Relationships between potentially inter-connected hormone pathways were also analyzed. Fixed effects were mass, body fat expressed in kg (TBF) or as a percent of the total body mass (TBF%), DMR or SMRA (in winter only) or other hormones measured, stratified or not by diet or age group. Random effects were the individuals. All data values provided are means ± SE and all p values were extracted from the mixed effect models summaries, unless stated otherwise. Statistical significance of each parameter estimate was set at $\alpha = 0.05$.

Results

Body mass, body composition and metabolic rates

The body mass and body composition data are detailed in Chapter 2 and summarized in Table 4.1. In brief, body mass changes were always similar between the two diet groups. During the restriction, mass loss for both groups averaged 10-15% of their initial body mass. In summer, animals in Group H lost all of the body mass from body fat and conserved their lean tissues while Group P lost significantly less body fat and more lean tissues (all $p < 0.01$). In winter lipid loss was slightly lower within each group than in summer, but Group H again lost significantly more body fat and less lean mass than Group P which lost its body mass almost exclusively as lean body mass. During the subsequent controlled re-feeding (when back on the baseline diet) in summer, the animals remained stable at the same mass as at the end of the restriction treatment ($p = 0.24$)

Table 4.1: Body mass, body condition, gross energy intake, and fish biomass intake of eight female Steller sea lions in diet groups H and P in summer 2005 and winter 2006. Measurements were taken at the end of the baseline (4th week, B4) and at week 2 and 4 of the restriction (R2 and R4) and of the controlled re-feeding (CR2 and CR4). The rates of change are calculated in reference to levels at the end of the baseline (B4). Numbers in brackets represent the standard errors of the means.

Season	Diet group	Phase	Mass (kg)	Mass change (%)	Body condition (%fat)	Body condition change (%)	Gross energy intake (kJ/d)	Fish biomass intake (kg/d)
Summer	H	B4	108.88 (21.18)	/	20.10 (1.65)	/	35.43 (5.75)	4.83 (0.78)
		R2	110.63 (23.24)	-4.53 (0.19)	14.83 (1.13)	-27.94 (6.43)	27.07 (3.92)	3.69 (0.53)
		R4	98.65 (18.97)	-9.29 (1.10)	12.23 (1.46)	-37.43 (10.32)	27.07 (3.92)	3.69 (0.53)
		CR2	101.37 (20.68)	-12.26 (0.83)	16.59 (0.66)	-17.92 (12.40)	34.65 (6.07)	4.83 (0.78)
		CR4	96.10 (17.12)	-11.06 (1.44)	17.06 (1.89)	-14.45 (7.95)	34.65 (6.07)	4.83 (0.78)
	P	B4	131.35 (23.59)	/	16.96 (1.20)	/	40.73 (7.13)	5.55 (0.97)
		R2	128.45 (23.60)	-2.45 (0.55)	15.28 (0.77)	-8.33 (9.09)	31.64 (4.64)	5.82 (0.85)
		R4	120.40 (21.81)	-8.42 (0.61)	14.22 (1.28)	-15.92 (5.99)	31.64 (4.64)	5.82 (0.85)
		CR2	118.10 (21.66)	-10.29 (0.69)	17.43 (1.89)	-13.47 (10.10)	39.78 (6.74)	5.55 (0.97)
		CR4	118.33 (21.76)	-10.14 (0.70)	14.26 (0.58)	-14.27 (8.48)	39.78 (6.74)	5.55 (0.97)
Winter	H	B4	121.70 (19.73)	/	20.21 (0.86)	/	47.59 (5.21)	6.13 (0.67)
		R2	115.45 (18.81)	-5.17 (0.22)	17.79 (1.14)	-13.56 (3.45)	30.34 (3.78)	3.91 (0.49)
		R4	110.50 (18.88)	-9.51 (0.85)	14.65 (1.44)	-26.98 (8.15)	30.34 (3.78)	3.91 (0.49)
		CR2	114.85 (18.10)	-5.46 (0.73)	22.14 (2.04)	18.20 (16.98)	45.33 (5.71)	5.84 (0.74)
		CR4	119.50 (17.74)	-1.27 (1.40)	22.34 (0.71)	10.69 (1.50)	45.33 (5.71)	5.84 (0.74)
	P	B4	143.60 (21.94)	/	19.32 (1.43)	/	47.53 (4.58)	6.13 (0.59)
		R2	139.85 (21.76)	-2.74 (0.65)	19.74 (1.66)	2.23 (4.08)	34.35 (3.94)	7.76 (0.89)
		R4	134.30 (21.19)	-6.68 (1.14)	19.63 (1.46)	1.65 (1.24)	34.35 (3.94)	7.76 (0.89)
		CR2	136.25 (20.65)	-5.04 (1.16)	21.74 (2.31)	11.86 (4.89)	46.74 (4.66)	6.02 (0.60)
		CR4	139.75 (20.14)	-2.26 (1.06)	21.76 (1.57)	13.82 (9.09)	46.74 (4.66)	6.02 (0.60)

Table 4.2: Serum concentrations of BUN/creatinine ratio, NEFA, β-HBA, total T4, free T4, and total T3 of eight female Steller sea lions in diet groups H and P. Measurements were taken at the end of the baseline (4th week, B4) and at week 2 and 4 of the restriction (R2 and R4) and of the controlled re-feeding (CR2 and CR4) of the summer 2005 and winter 2006 experiments. Numbers in brackets represent the standard error of the means. <DL means that levels in serum were lower than detection limits of the measurements kits.

Season	Group	Phase	BUN/creatinine	NEFA (mM)	β-HBA (mM)	TT4 (nM)	fT4 (pM)	TT3 (nM)
Summer	H	B4	100.9 (2.9)	1.02 (0.29)	0.14 (0.07)	13.50 (1.44)	26.95 (3.16)	1.03 (0.11)
		R2	94.8 (4.4)	0.58 (0.07)	0.05 (0.01)	14.50 (2.47)	24.47 (3.73)	0.75 (0.13)
		R4	93.0 (9.1)	0.43 (0.07)	0.00 (0.00)	10.75 (1.55)	18.11 (2.04)	0.75 (0.09)
		CR2	116. 8 (6.8)	0.58 (0.07)	0.04 (0.01)	12.75 (2.32)	27.84 (4.41)	0.68 (0.05)
		CR4	102.8 (9.3)	0.35 (0.05)	0.02 (0.01)	10.75 (1.31)	21.80 (1.43)	0.78 (0.09)
	P	B4	92.2 (5.8)	0.47 (0.07)	0.03 (0.01)	14.50 (0.87)	24.56 (4.34)	1.00 (0.18)
		R2	88.0 (8.5)	0.39 (0.13)	0.04 (0.02)	16.75 (1.93)	23.25 (3.60)	0.90 (0.16)
		R4	95.9 (10.6)	0.78 (0.27)	0.06 (0.04)	12.75 (1.55)	25.18 (2.47)	0.48 (0.09)
		CR2	102.9 (5.0)	0.48 (0.17)	0.05 (0.02)	13.00 (1.08)	30.20 (4.67)	0.60 (0.07)
		CR4	133.9 (26.2)	0.69 (0.27)	0.05 (0.03)	13.25 (2.32)	27.96 (2.34)	0.70 (0.15)
Winter	H	B4	95.7 (8.5)	0.45 (0.10)	0.02 (0.01)	12.25 (1.89)	18.37 (2.28)	0.68 (0.09)
		R2	89.1 (7.4)	0.67 (0.16)	0.01 (0.01)	10.75 (1.93)	27.23 (2.18)	1.15 (0.12)
		R4	105.4 (3.9)	0.69 (0.23)	<DL	7.50 (2.87)	20.82 (5.99)	0.75 (0.18)
		CR2	107.1 (7.7)	0.62 (0.15)	0.01 (0.01)	11.75 (1.38)	21.60 (1.56)	0.88 (0.09)
		CR4	114.2 (8.1)	0.58 (0.19)	<DL	12.00 (1.87)	29.14 (2.31)	0.95 (0.10)
	P	B4	90.12 (5.9)	0.39 (0.07)	<DL	13.00 (1.68)	25.37 (1.35)	1.15 (0.05)
		R2	112.9 (6.6)	0.66 (0.13)	0.01 (0.01)	7.75 (1.80)	24.78 (1.92)	0.78 (0.09)
		R4	126.2 (2.9)	0.62 (0.12)	<DL	11.25 (1.38)	30.84 (3.27)	0.55 (0.05)
		CR2	104.5 (9.2)	0.51 (0.05)	<DL	12.25 (1.49)	26.41 (2.31)	0.98 (0.03)
		CR4	95.4 (6.0)	0.50 (0.11)	<DL	12.50 (1.55)	28.56 (2.12)	0.75 (0.03)

but the body composition of the animals went back to the pre-experimental level. In winter, the rates of mass gain during the controlled re-feeding were greater (+7.29 ± 1.43%) than the rate during the baseline ($p < 0.0001$) which was accomplished mostly by gaining body fat. The age of the animals was found to have no significant effect on the changes in body composition at any time throughout the experiments or in any season (all $p > 0.05$).

Daily (DMR) and standard (SMR$_A$) metabolic rate measurements and data are detailed in Chapter 3. In brief, DMR of Group P stayed constant throughout the summer experiments (all $p > 0.30$) while DMR of Group H increased during the summer restriction ($p = 0.003$) till the middle of the controlled re-feeding ($p = 0.01$) and returned to the pre-restriction values at the end of the experiment ($p = 0.22$). In winter, DMR of Group P stayed stable most of the time, except for a 20% decrease at week 2 of the restriction ($p = 0.02$). However, DMR of Group H decreased by 15-30% throughout the restriction and controlled re-feeding in winter (all $p < 0.02$). SMR$_A$ was only measured during the winter with no significant between-groups and within-group (compared to the baseline) differences (all $p > 0.06$).

Blood metabolites

In summer, neither diet nor age influenced the changes in BUN/creatinine during the restriction or the controlled re-feeding (all $p > 0.05$, Table 4.2). When all the animals were pooled together, BUN/creatinine ratios were higher than baseline levels only in the middle of the controlled re-feeding (109.88 ± 4.71 compared to baseline level: 96.57 ± 3.43, $p = 0.01$). In winter however, diet had a significant impact. Animals in Group P displayed a significant increase in BUN/creatinine ratio during the restriction (28% increase after 2 weeks of restriction $p = 0.05$ and 42% after 4 weeks $p = 0.005$) and a subsequent decrease during the controlled re-feeding back to baseline levels, while Group H levels stayed constant throughout the experiment (all $p > 0.05$). Absolute BUN/creatinine ratios were significantly higher for Group P relative to Group H during the winter restriction (both $p < 0.03$). Changes in BUN/creatinine levels were not related to changes in lean body mass ($p > 0.05$).

Table 4.3: Serum concentrations of total and free cortisol, IGF-1 and GH of eight female Steller sea lions during the summer 2005 and winter 2006 experiments. Measurements were taken at the end of the baseline (4th week, B4) and at week 2 and 4 of the restriction (R2 and R4) and of the controlled re-feeding (CR2 and CR4). The averages are given per age groups and numbers in brackets represent the standard error of the means.

Season	Age	Phase	Total cortisol (ng/ml)	Free cortisol (ng/ml)	IGF-1 (ng/ml)	GH (ng/ml)
Summer	Juvenile	B4	93.70 (5.13)	27.12 (4.32)	333.39 (22.56)	/
		R2	102.61 (4.57)	52.18 (4.51)	211.18 (16.02)	/
		R4	104.17 (3.04)	49.68 (3.65)	131.30 (12.80)	/
		CR2	110.67 (4.66)	55.48 (5.22)	133.87 (23.63)	/
		CR4	109.56 (8.34)	47.00 (9.78)	130.75 (21.88)	/
	Subadult	B4	68.58 (5.00)	6.00 (2.48)	288.25 (69 .04)	/
		R2	91.75 (15.05)	39.67 (10.90)	261.80 (52.84)	/
		R4	85.98 (0.83)	28.10 (0.40)	206.59 (19.04)	/
		CR2	80.11 (7.82)	16.98 (5.56)	219.07 (28.16)	/
		CR4	90.15 (10.53)	23.87 (8.94)	213.29 (19.57)	/
Winter	Juvenile	B4	82.91 (6.51)	12.91 (4.86)	134.67 (13.62)	2.18 (0.43)
		R2	103.32 (4.10)	34.51 (5.93)	65.46 (4.11)	6.09 (1.74)
		R4	114.91 (6.10)	51.75 (6.38)	61.10 (8.80)	3.40 (0.82)
		CR2	109.79 (7.29)	41.69 (5.75)	81.84 (13.39)	4.58 (1.21)
		CR4	102.26 (4.75)	33.77 (2.13)	94.22 (5.72)	4.22 (0.67)
	Subadult	B4	78.03 (6.37)	10.39 (4.67)	143.41 (23.97)	5.94 (1.00)
		R2	97.16 (7.75)	26.18 (2.03)	111.42 (17.75)	2.15 (0.40)
		R4	91.89 (6.51)	30.72 (12.12)	94.84 (21.41)	4.77 (1.93)
		CR2	102.59 (9.89)	32.14 (12.37)	109.79 (39.24)	4.46 (0.70)
		CR4	99.69 (9.50)	32.25 (15.38)	87.96 (20.55)	2.96 (1.06)

Baseline levels of NEFA and β-HBA were not different between diet groups or age groups in either season (all $p > 0.1$; see Table 4.2). When animals were pooled together, the experimental treatments did not trigger any significant changes in levels of NEFA or β-HBA in summer (all $p > 0.1$). In winter however, NEFA levels increased during the restriction (60-75% increase, $p = 0.03$) and decreased back to baseline levels during the controlled re-feeding ($p = 0.22$). Overall, β-HBA levels were too close to the detection
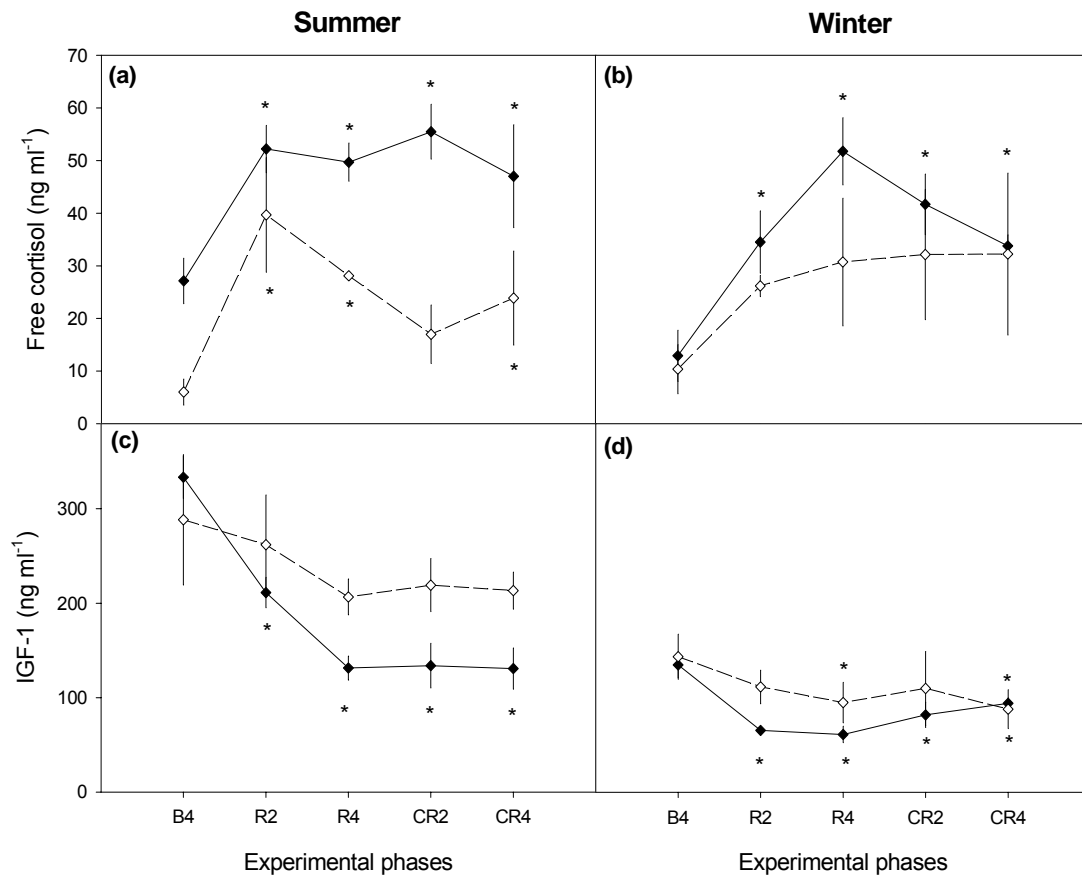
Figure 4.1: Mean ± SE serum concentration of free cortisol and IGF-1 in eight female Steller sea lions segregated by age class (filled diamonds: juveniles and open diamonds: subadults) measured at the end of the baseline (B4), and after 2 and 4 weeks of a restriction (R2 & R4) and a controlled re-feeding (CR2 & CR4) period. The two graphs on the left (a & c) represent the data collected during the summer 2005 experiment and the two graphs on the right (b & d) during the winter 2006 experiment. The asterisks indicate significant within-group differences compared to the respective B4 measurement.

limit of the measurement kits to evaluate changes during the winter restriction and recovery accurately, so these data were discarded. Changes in neither NEFA nor β-HBA were related to changes in body fat ($p > 0.07$).

Glucocorticoids

Experimental diet was never a factor influencing absolute or changes in stress hormone levels during the experiments (all $p > 0.1$). In summer, relative changes in cortisol and in free cortisol levels were also similar for both age groups during the

restriction and the controlled re-feeding (even though juveniles started the experiments with higher baseline levels). When animals were pooled together, levels of cortisol were significantly elevated by 20% and levels of free cortisol were approximately three times higher during both the restriction and the controlled re-feeding compared to the baseline levels, (all $p < 0.04$; Table 4.3 and Figure 4.1).

In winter, cortisol levels increased significantly during the restriction treatment for the juveniles (25-40% increase, all $p < 0.01$, Table 4.3) but changes were not significant for subadults (all $p > 0.05$). Elevations in cortisol above baseline levels were only significant in the middle of the controlled re-feeding for the subadults ($p = 0.05$, otherwise all $p > 0.08$). Overall, juveniles had higher cortisol levels than subadults at the end of the restriction (juveniles: 114.91 ± 6.10 ng ml^{-1}, subadults: 91.89 ± 6.51 ng ml^{-1}, $p = 0.04$).

Juvneiles showed a 5-fold increase in levels of free cortisol during winter restriction (all $p < 0.001$; Figure 4.1) compared to baseline levels. During the controlled re-feeding, levels tended to decrease slightly but were still higher than the initial pre-experimental values (all $p > 0.01$). Free cortisol levels for the subadults tended to slightly increase throughout the winter experiment as well but changes were not significant (all $p > 0.1$). Free cortisol levels were higher for the juveniles compared to the subadults only at the end of the restriction (juveniles: 51.75 ± 638 ng ml^{-1}, subadults: 30.72 ± 12.12 ng ml^{-1}, $p = 0.05$).

Levels of cortisol and free cortisol were negatively related to changes in body mass of the sea lions in winter (all $p < 0.001$) but not in summer either when all animals were pooled (all $p > 0.1$) or segregated by age class (all $p > 0.09$). In winter, both levels of cortisol and free cortisol were negatively related to changes in body mass for the juveniles ($p < 0.001$), but only levels of free cortisol were related for subadults ($p = 0.01$). Cortisol and free cortisol were also never related to changes in body condition in either season (TBF%, all $p > 0.1$). Finally, NEFA was related to free cortisol levels in winter (all $p < 0.02$) but not in summer (all $p > 0.4$).
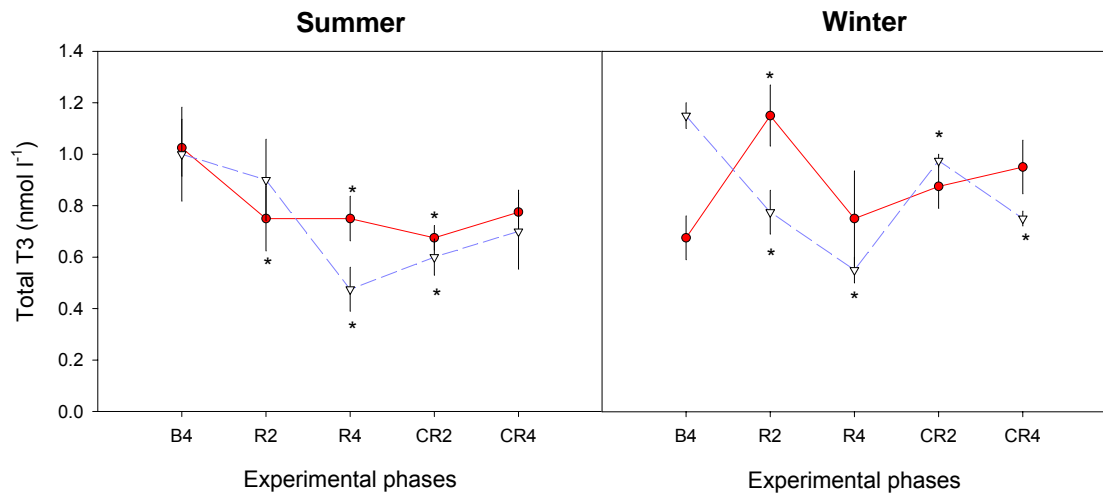
Figure 4.2: Mean ± SE serum concentration of total T3 in eight female Steller sea lions segregated by diet groups (circles: Group H, triangles: Group P) in summer 2005 (a) and winter 2006 (b). Measurements were taken at the end of the baseline (4th week, B4) and at week 2 and 4 of the restriction (R) and of the controlled re-feeding (CR). The asterisks indicate significant within-group differences compared to respective B4 measurement.

Thyroid hormones

In summer, relative changes in levels of TT3 during the restriction and the controlled re-feeding were similar for both diet and age groups (all $p > 0.2$; Table 4.2 and Figure 4.2). When all animals were pooled, levels of TT3 were significantly lower than baseline levels from the end of the restriction (-35%) until the end of the experiment (all $p < 0.02$). In winter however, diet (but not age) significantly affected changes in TT3 levels during the restriction (Figure 4.2). TT3 levels of Group P decreased during the restriction compared to the respective baseline value (-50% at the end of the restriction) and increased slightly during the controlled re-feeding, although they remained lower than baseline levels (-35% at the end of the controlled re-feeding, all $p < 0.03$). TT3 levels of Group H were generally stable compared to the baseline (all $p > 0.08$), except for a temporary increase (78%) in the middle of the restriction ($p > 0.01$), when TT3 levels were higher for Group H than for Group P ($p = 0.04$).

Neither diet type nor age influenced changes in TT4 and fT4 levels during the restriction and controlled re-feeding during any season (all $p > 0.09$, Table 4.2). When all
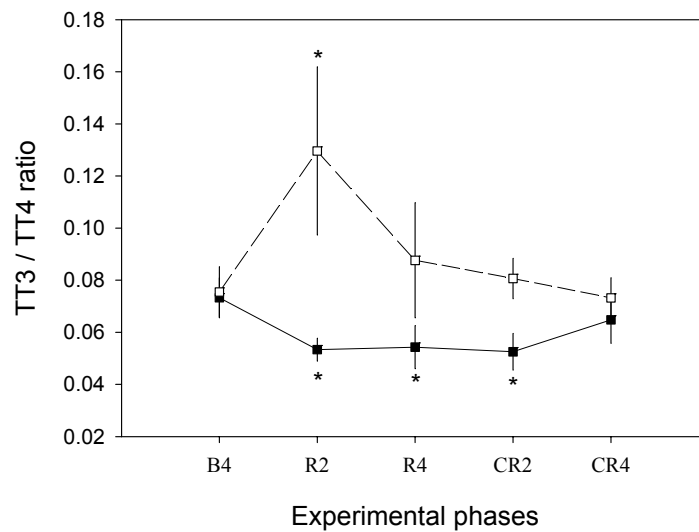
Figure 4.3: Mean ± SE changes in ratio of total T3 (TT3) / total T4 (TT4) in eight female Steller sea lions in summer 2005 (filled squares) and winter 2006 (open squares). Measurements were taken at the end of the baseline (4th week, B4) and at week 2 and 4 of the restriction (R2 & R4) and of the controlled re-feeding (CR2 & CR4). Asterisks indicate the within-group differences compared to the respective B4 measurements.

the animals were pooled, levels of TT4 and fT4 were not affected by energy restriction or by the subsequent recovery in the summer (all $p > 0.2$) and stayed stable near the baseline values (TT4: 14.00 ± 0.80 nM and fT4: 25.75 ± 2.52 pM). In winter, fT4 levels remained again constant (approximately 21.87 ± 1.81 pM) but TT4 concentrations decreased significantly during the restriction (approximately 25%, $p = 0.03$ at R2 and $p = 0.04$ at R4) before returning to baseline levels during the controlled re-feeding (all $p > 0.05$).

Changes in TT3/TT4 ratios were not affected by diet or age in either season (all $p > 0.5$; Figure 4.3). When all animals were pooled together, the TT3/TT4 ratio decreased significantly during the restriction in summer (0.06 ± 0.01, $p < 0.002$) and returned to baseline levels only for the last measurement at the end of the controlled re-feeding ($p = 0.27$). In winter, levels of TT3/TT4 significantly increased in the middle of the restriction ($p = 0.03$) but returned to baseline levels at the end of the restriction and during the controlled re-feeding (all $p > 0.3$). Neither TT3, TT4, nor fT4 showed a significant relationship with standard metabolic rate in air (SRM$_A$) or daily metabolic rate (DMR, all $p > 0.05$ in both seasons).
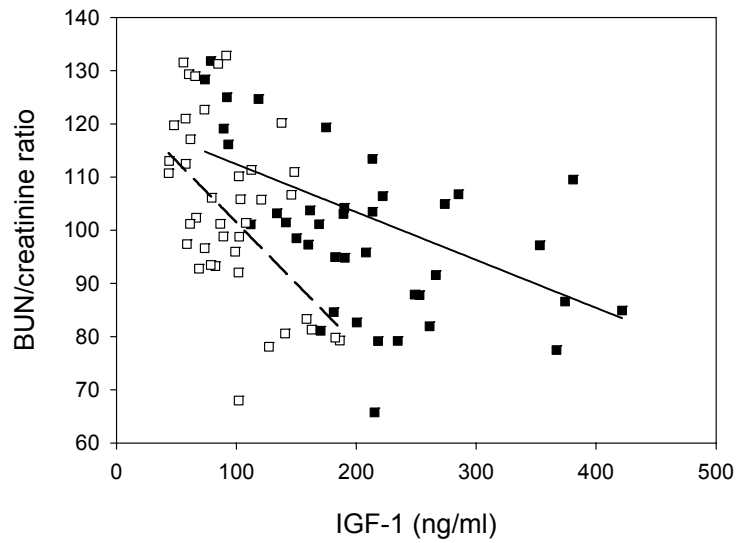
Figure 4.4: Relationship between the blood urea nitrogen (BUN)/creatinine ratio and serum concentration of IGF-1 in eight female Steller sea lions in summer 2005 (filled squares) and winter 2006 (open squares). The regression lines were fitted using linear mixed-effects models and are both significant (both p < 0.003).

Figure 4.5: Relationships between serum concentrations of IGF-1 and (a) free cortisol and (b) total T4 in eight female Steller sea lions in summer 2005 (filled squares) and winter 2006 (open squares). The regressions were fitted using linear mixed-effects models and are all significant (all p < 0.02).

Insulin-like growth factor-1

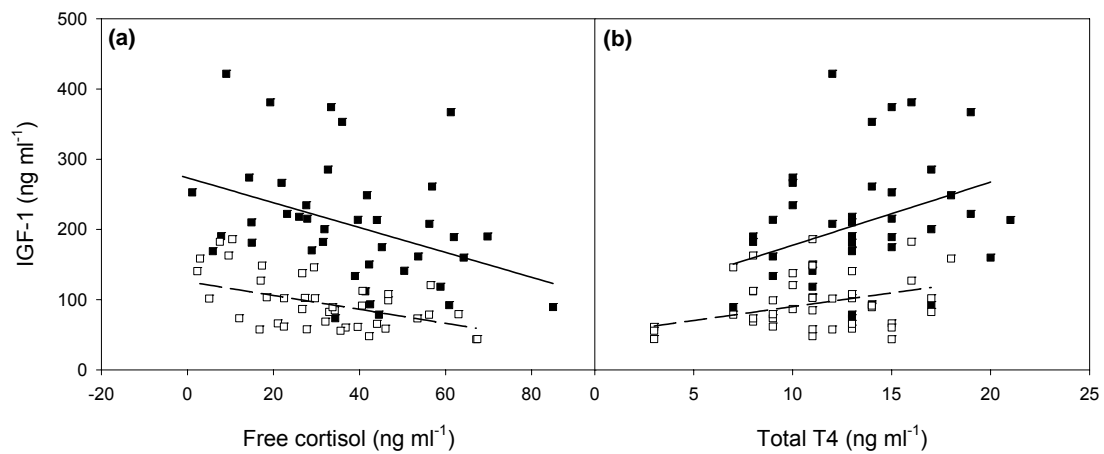Absolute levels of IGF-1 in each phase were overall higher in summer (316.46 ± 27.58 ng ml^{-1}) than in winter (137.95 ± 11.42 ng ml^{-1}, p = 0.0001; Table 4.2), and the relative changes during the restrictions and the re-feeding phases were more pronounced in summer as well (Figure 4.1). Diet quality never affected changes in IGF-1 levels in either season (all p > 0.1).

In summer, changes in IGF-1 during the restriction and the subsequent controlled re-feeding were significantly more pronounced for the juveniles than for the subadults (all p < 0.0001; Figure 4.1c). IGF-1 levels for juveniles decreased throughout the experiment (60% decreases at the end of the restriction and throughout the re-feeding, all p < 0.0001), while for the subadults the observed slight decrease was not significant (baseline value: 288.25 ± 69.04 ng ml^{-1}, all p > 0.07). As a result, absolute levels of IGF-1 were lower for juveniles than subadults at the end of the restriction and during the controlled re-feeding (131.30 ± 22.56 ng ml^{-1}, all p < 0.05).

In winter, both juveniles and subadults decreased their levels of IGF-1 overall compared to the baseline (all p < 0.002). However, changes in IGF-1 from baseline happened sooner (middle of restriction, p = 0.06) for the juveniles than for the subadults (end of the restriction, p < 0.002, Figure 4.1). In terms of absolute value, juveniles showed a lower concentration of IGF-1 than subadults only in the middle of the restriction (juveniles: 65.46 ± 4.11 ng ml^{-1}, subadults: 111.42 ± 17.75 ng ml^{-1}, p = 0.01; other phases: p > 0.13).

GH data are only available from the winter experiment (Table 4.2). Neither diet nor age impacted levels of GH hormones (all p > 0.1). GH levels did not change during the restriction and the controlled re-feeding when all animals were pooled together (all p > 0.05).

IGF-1 was significantly related to body mass in both summer and winter (all p = 0.0001). Changes in levels of IGF-1 were also significantly related to changes in lean body mass in both seasons (p < 0.001) but was never related to body condition (TBF%, all p > 0.08). Changes in BUN/creatinine were significantly negatively related to changes in IGF-1 levels both in summer (p < 0.002) and in winter (p < 0.001; Figure 4.4). Finally,

IGF-1 was significantly negatively related to free cortisol concentration in both seasons (both $p < 0.01$), and positively related to TT3 and TT4 in summer (both $p < 0.02$) and to TT4 in winter ($p < 0.005$; Figure 4.5). Levels of GH were not related to any of the aforementioned body condition, hormone, or metabolite variables (all $p > 0.5$).

Discussion

Levels of thyroid, somatotropic, and stress hormones in mammals and birds vary with energy intake (quality and quantity of food), sex, and age as well as external parameters such as season and temperature (Eales 1988, Kitaysky et al. 2001, Romero 2002, Oki & Atkinson 2004, Reeder & Kramer 2005, Myers et al. 2006). These hormones regulate lipid and protein metabolism to optimize fitness of the animals during suboptimal conditions (Ward & Armitage 1981). Changes in metabolic pathways may in turn be reflected by levels of catabolic metabolites (NEFA and β-HBA for lipids and BUN/creatinine for proteins). Our results indicate that changes in glucocorticoids and somatotropic hormones were similar to those observed in terrestrial mammals during energy restrictions and that both sets of hormones were involved in regulation of body mass change in winter. In summer however, only IGF-1 appeared to be involved in regulating body mass. Regulation of lean mass changes was strongly driven by IGF-1 in both seasons but none of the hormones or metabolites measured related to changes in body condition of the sea lions. Thyroid hormones were also not directly involved in observed changes in body mass or metabolism.

Endocrine changes during mild nutritional stress

Changes in glucocorticoid levels represent a major endocrine response to stressors including nutritional stress in mammals and birds (Ortiz et al. 1978a, Bergendahl et al. 1996). Levels of cortisol usually increase during periods of energy restriction (Engelhardt & Ferguson 1980, Ortiz et al. 2001a, Ortiz et al. 2001b, Ortiz et al. 2003a, Guinet et al. 2004) to help mobilize lipid stores for energy requirements. The two somatotropic hormones, GH and IGF-1, are the major inducers of growth in terrestrial mammals (Lupu et al. 2001) and are mostly involved in protein metabolism by increasing protein synthesis and reducing protein degradation (Breier 1999, Butler & Le Roith 2001, Renaville et al.

2002). GH is typically up-regulated during nutritional stress in mammals including humans (in terms of amplitude rather than in frequency of peaks, Mosier 1986, Yambayamba et al. 1996, Ortiz et al. 2003b), while IGF-1 levels decrease to enhance conservation of energy through a decrease in lean and bone tissue synthesis and to direct the utilization of available nutrients as fuel to restore homeostasis rather than for tissue production (Van Den Brande 1986, Smith et al. 1995, Breier 1999, Renaville et al. 2002, Ali et al. 2003, Ortiz et al. 2003b).

Changes in glucocorticoids and IGF-1 in our sea lions were as expected (i.e. an increase in cortisol and free cortisol levels and a decrease of IGF-1 during nutritional stress). GH levels did not show an increase, but this could be due to the pulsating pattern of secretion of this hormone from the pituitary gland masking changes in discrete measurements. In winter, levels of IGF-1, cortisol, and free cortisol were all related to changes in body mass during the restriction, indicating a cooperative regulation of body homeostasis in this season (Goodman & Knobil 1961, Ashwell-Erickson et al. 1986). Regulation of body size (both lean and lipid mass) is likely to be important in winter when energetic and thermoregulatory requirements are higher (Winship et al. 2002). In summer however, glucocorticoids were not significantly involved in regulation of body mass changes, while IGF-1 was. This indicates that the regulation of lean mass by IGF-1 is a priority in summer (see also Chapters 2 and 3) and that the degree of involvement of glucocorticoids in the overall stress response decreases when the priority to maintain lipid mass for thermoregulation is lower such as in summer.

Relative changes in body mass were never age- or season-dependent but levels of both glucocorticoids and somatotropic hormones were different between age groups and sometimes per season. Decreases in IGF-1 were greater for juvenile sea lions compared to subadults in summer. The somatotropic axis is likely more active and responsive to changes in energy balance in younger animals since their growth rate is higher. Greater endocrine responses seem to be necessary for juveniles to preserve lean mass to the same level as subadults during periods of energy restriction in summer, since changes in body mass and body composition were not age dependant. The fact that levels of IGF-1 were always higher in summer than winter also indicates that structural growth may occur preferentially in summer during optimal nutritional conditions. A greater decrease in IGF-

1 also indicates that this system is more responsive to energy restriction in summer than winter for the same resulting changes in body mass in both seasons.

Changes in free cortisol were similar between juveniles and subadults in summer but absolute levels of free cortisol were higher for juveniles at all time (see also Boily 1996, Gardiner & Hall 1997). This indicates that the nutritional restriction was not more stressful for juveniles in summer but that younger animals require higher absolute levels of stress hormones to perform the same metabolic adjustments as older animals in this season. In winter, juvenile sea lions expressed a more intense stress response compared to older animals during the restriction although the different responses were associated with similar body changes. Consequently, younger animals may require greater changes in cortisol hormones than older ones in winter as part of natural response to stress.

Although an increase in glucocorticoids is part of natural defence response, the long term high levels of stress hormones can be detrimental, particularly for development and cognitive capacities of young animals. Kitaysky et al. (2006) showed that chicks of marine birds exposed to high corticosterone levels from nutritional stress in early life stages had poorer learning and cognitive capacities leading to lower foraging efficiencies. If wild Steller sea lions experience mild restriction on a chronic basis at an early age resulting in high levels of stress hormones then growth, diving capacity, foraging efficiency, and immune system could be impaired (St Aubin & Dierauf 2001, Kitaysky et al. 2003, Reeder & Kramer 2005). Consequently, survival of new cohorts could decrease, particularly as nutritional stress is more likely to hit young animals who require a higher mass-specific energy intake than older ones (Winship et al. 2002).

Thyroid hormones regulate growth and development in mammals (Choksi et al. 2003) and play an important role in basal metabolic rate and metabolic thermogenesis (Renouf & Noseworthy 1991, Silva 1995, Haulena et al. 1998). Energy restriction usually results in a decrease in thyroid activity, which serves to decrease basal metabolism to save energy (Azizi et al. 1979, Eales 1988, Kelly 2000, Diez et al. 2004). Consequently, thyroid hormone levels were expected to decrease during the energy restriction.

Basal metabolism was never directly measured in our study, so effects of changes in thyroid hormone levels were not directly assessable. However, changes in daily

metabolic rates (DMR) and standard metabolic rates (SMR$_A$) were measured (SMR$_A$ only in winter, see Chapter 3 for more details). Unlike in other pinnipeds and woodchucks (Young 1984, Ashwell-Erickson et al. 1986, Boily 1996), none of the thyroid hormones were correlated to the metabolic rates measured, indicating that changes in thyroid hormones were not major enough to impact our metabolic rate measurements. This does not however mean that energy expenditure and basal metabolic rate did not decrease at all. When animals were pooled together, deiodination of T4 into T3 (as observed from TT3/TT4 data) was down regulated in summer, reflecting a decrease in the anabolic activity of the thyroid hormones. Levels of TT3 also decreased in summer when all animals were pooled (even though the decrease was weak). These two changes may have helped lower the general energy expenditure during periods of energy restriction. The temporary increase in TT3/TT4 levels in winter (especially for Group H) is more surprising, especially since the animals were shown to decrease their energy allocated to maintenance functions in this season (see Chapter 3), and is probably due to the temporary increase in TT3 from Group H during the restriction.

Changes in thyroid hormones were overall less obvious and pronounced than anticipated and did not vary by age in our sea lions (Riviere et al. 1977, Engelhardt & Ferguson 1980, Ashwell-Erickson et al. 1986, Little 1991, St Aubin et al. 1996, Haulena et al. 1998), which suggests that they may not be predominant in the regulation of energy expenditure during mild energy restrictions. However, we did not measure free T3 (fT3), the most metabolically active hormone and reverse T3 (rT3) which plays an important role in the regulation of thyroid hormone activity during nutritional stress (O'Brian et al. 1980, Kelly 2000). Changes in their levels could show a more pronounced role of thyroid hormones in Steller sea lions energy regulation than our results indicate.

Indication of metabolic changes between experimental groups

Quality of diet had a significant impact on the type of tissue reserve used during the energy restriction in Steller sea lions. Group H consistently lost more lipids (and exclusively lipids in summer) and less protein than Group P (who lost exclusively protein in winter) as measured by the deuterium dilution method. It is thus expected that the observed differences between major catabolic routes (lipid or protein oxidation) would be

reflected in the blood metabolite levels, i.e. higher BUN/creatinine for Group P, and higher circulating NEFA and β-HBA levels for Group H, as well as different levels of glucocorticoids and somatotropic hormones.

Despite mass loss and body composition changes, none of the metabolites measured reflected the difference in physiological response between the diet groups. Levels of NEFA for the eight animals pooled indicated that lipid catabolism occurred during the restriction and decreased during the controlled re-feeding. However, it failed to detect the difference in intensity of lipid catabolism between Group H and P. Levels of β-HBA were also not useful in detecting metabolic changes. However, we performed measurements of absolute circulating levels of those metabolites which integrate rates of synthesis, utilization by tissues (if any), and clearance from the body. If these processes vary simultaneously with each other, overall levels can stay virtually stable despite changes in physiological function.

In winter, the BUN/creatinine ratio, which reflects a change in protein metabolism increased significantly more for Group P than Group H, due to a decrease in creatinine rather than an increase in BUN. Creatinine levels are independent of level of diuresis and of protein in the diet but dependant on the muscle mass in the animals (Narayanan & Appleton 1980). A decrease in creatinine levels for Group P may reflect the high rate of protein loss (between 80 and 100% of the total body mass loss) from muscles in this season (see Chapter 2). It seems, however, that this parameter was able to detect only particularly major changes in protein catabolism and may not be useful in more subtle cases.

In previous studies, significant changes in blood metabolites were found to accurately reflect the levels of protein and lipid mass changes during a complete fast when the physiological changes are drastic (Harlow 1981, Rea et al. 1998, 2000, Arnould et al. 2001, Mellish & Iverson 2001). The difference in the degree of nutritional restriction is the most likely explanation for the lack of response in blood metabolites in our experiment compared to other studies. During a moderate restriction resulting in a mass loss of 10-15 % of body mass (which may be more realistic of Steller sea lions' nutritional situation in

the wild), these blood parameters are not sensitive indicators of metabolic and nutritional states.

Somatotropic hormones act as a preservation agent of body protein during periods of energy restriction (Breier 1999). Consequently, increases in the action of somatotropic hormones were expected to be greater in animals in Group H who lost a lower amount of lean mass compared to Group P. There was, however, no difference between changes in levels of GH or IGF-1 for Group P and Group H. When all the animals were pooled together (and thus the statistical power increased), IGF-1 was significantly correlated to changes in lean mass both in winter and summer. This indicates that IGF-1 regulated the amount of lean mass lost (or gained) during the different nutritional phases in Steller sea lions. The fact that IGF-1 levels were also significantly related to BUN/creatinine ratio confirms that a decrease of this hormone results in a higher protein degradation.

Stress hormones are usually correlated to changes in body condition (percent of lipid in the body) in marine mammals, with metabolically active cortisol (free cortisol) mobilizing energy stores through lipolysis of adipocytes during nutritional stress (Ortiz et al. 2001a). Consequently, cortisol and free cortisol were expected to increase slightly more in Group H (which lost more lipids) than Group P (see Chapter 2). However, results showed that stress hormone levels were not related to changes in body condition in our animals, unlike previous findings on Northern elephant seal pups, lactating Antarctic fur seals, and Steller sea lions (Ortiz et al. 2001b, Ortiz et al. 2003a, Guinet et al. 2004, Kumagai 2004). Ortiz et al. (2001b) argued that levels of cortisol represent a critical signal to inform the animal of changes in body condition. This may be the case during acute nutritional restrictions (especially fasting), but not in milder ones such as in our experiment. Glucocorticoids were thus more affected by energy intake levels than composition of the food or changes in sea lion body composition (Kitaysky et al., Kumagai 2004).

Although free cortisol was not related to variation in body condition, it was related to levels in NEFA in winter, suggesting that cortisol was somewhat involved in the lipid metabolism in our animals in this season (Ortiz et al. 2003b). The strong relationships in winter between free cortisol and NEFA, and between free cortisol and mass change in

winter indicate that body size and lipid metabolism were under a tighter control during this season when thermoregulatory function is more essential (Heath et al. 1977, Rosen & Renouf 1997).

Thyroid hormones are also known to be involved in lipid metabolism during nutritional stress in marine mammals (Renouf & Noseworthy 1991, Haulena et al. 1998) and to regulate metabolic thermogenesis by activating thermogenic mechanisms such as the Na/K-ATPase or Ca^{2+} cycling in muscles (Silva 1995). The only between-diet group difference in thyroid hormone levels is a differential change in TT3 in winter when regulation of the body temperature is particularly important for marine mammals. A decrease in TT3 for animals in Group P may imply a greater decrease in oxidation of lipid reserves but also a decrease in metabolic thermogenesis. This sparing of the insulative layer of blubber may allow decreasing metabolic thermogenesis (and thus energy expenditure) without compromising on thermoregulatory capacity. In contrast, thyroid hormone levels for animals fed herring (Group H) may have increased or stayed constant to conserve the metabolic thermogenesis capacity, necessary since the loss of lipids was greater than for Group P potentially resulting in lower blubber insulation (Chapter 2).

Traditionally, assessing health and nutritional status of marine mammals that rely on a large reserve of lipids to survive in their environments requires a marker able to detect relative changes in body condition (i.e., proportion of lipid/lean mass in the body), which none of the measured hormones was able to do. IGF-1 seems a reliable marker of changes in protein metabolism, but no clear marker of regulation of lipid metabolism emerged from our data. Regulation of lipid catabolism could actually come from the cooperation of several hormonal systems collectively causing the observed changes even though individual effects were not significant.

Leptin and ghrelin are two other hormones likely involved in lipid catabolism regulation. They are two antagonistic hormones related to both appetite and nutritional status through regulation of lipids (Weigle et al. 1997, Geary 2004). Leptin is negatively and ghrelin is positively correlated to lipid mass and both are known to vary in opposite direction during a food restriction and in relation to satiation (Cummings & Overduin 2007). In our study, the experimental diets differed in composition as well as in biomass

of fish fed daily for the same caloric intake. Group P (which was fed lower quality fish) received twice as much biomass of food as Group H (see Chapter 2). The integration of these different signals of satiation and mass loss may have resulted in higher leptin/ghrelin ratios for Group H and a greater signal of lipid oxidation than Group P during the restrictions. This assumes that leptin and ghrelin play the same role in satiation and lipid metabolism in Steller sea lions as they do in human and rodents which may not be the case (Rea & Nagy 2000, Nieminen et al. 2001, Arnould et al. 2002, Ortiz et al. 2003b). In any case, assessing changes in body condition in Steller sea lions may require integrating several additional hormones related to lipid and protein metabolism.

Endocrine changes during the controlled re-feeding

The endocrine changes that occur during the energy restriction periods set up the hormonal state at the beginning of the re-feeding phase. These altered levels likely modify the capacity of organisms to recover from nutritional stress (Mosier 1986, Boersma & Wit 1997, Hornick et al. 2000). In our study, the sea lions displayed a compensatory growth post-restriction in winter but not in summer (see Chapter 2). The hormonal control of compensatory growth is well documented in domestic animals and humans (Mosier 1986, Boersma & Wit 1997, Hornick et al. 2000) and involves cooperative changes mostly from somatotropic hormones, but also from glucocorticoids and thyroid hormones (Boersma & Wit 1997).

Compensatory growth usually involves an increase in the number of GH receptors especially in the liver upon re-feeding (i.e., a cessation of the GH resistance started during the restriction, Perez-Sanchez & Le Bail 1999, Hornick et al. 2000). This induces an important increase in production of IGF-1 (especially if GH levels were elevated during the restriction). Simultaneous high levels of GH and IGF-1 during the re-feeding may stop the utilization of nutrients for energy metabolism and increase overall anabolism, structural growth, or energy storage (Mosier 1986, Van Den Brande 1986, Boersma & Wit 1997).

In our study, levels of IGF-1 remained low during the summer re-feeding period when the mass of the animals stayed stable at a low level. On the other hand, in winter levels of IGF-1 increased back towards baseline values upon re-feeding, when a

compensatory growth was observed. This correlation between changes in IGF-1 levels and the occurrence of compensatory growth corroborates the hypothesis that somatotropic hormones play a major role in the capacity to recover after nutritional stress in Steller sea lions (Hornick et al. 2000).

During the winter controlled re-feeding period, IGF-1 and somatotropic hormones both increased and levels of stress hormones decreased. The collaboration between the increase in energy intake and the signals from different endocrine systems to increase tissue synthesis provoked a spurt of growth in the sea lions in winter, in the same way described for terrestrial mammals (Hornick et al. 2000). The clear priority for sea lions to replete their lipid stores over lean tissue mass during this season was not, however, explainable through changes in these hormones. Whatever the mechanisms, it seems there is a priority for Steller sea lions to regain their optimal mass in winter compared to summer, potentially for reproductive or thermoregulatory reasons (Chapter 2 and Kleiber 1975, Pitcher et al. 1998).

It is unclear why IGF-1 levels increased in winter but not in summer. Hormone levels are known to vary seasonally (Thissen et al. 1994, Romero 2002, Myers et al. 2006) and absolute concentrations of IGF-1 were higher in summer than in winter during all experimental phases. This could indicate that structural growth is seasonally dependant and greater in summer. In this case, the seasonal difference in compensatory growth could result from a priority to resume structural growth (to the detriment of body energy content) in summer and a priority for energy accumulation in winter (see Chapters 2 and 3). Higher absolute concentrations in summer versus winter for the same body changes could also mean that higher hormone levels are required in summer for the same degree of response to re-feeding, and that this seasonal intrinsic resistance to somatotropic hormones could at least partly explain why the sea lions were unable to restore their body mass during the summer.

However, the somatotropic axis itself is affected by several other metabolic hormones, including glucocorticoids and thyroid hormones. High levels of glucocorticoids have been associated with a resistance to GH metabolic effects and stimulation of IGF-1 secretion (Butler & Le Roith 2001), but their general effects on the somatotropic axis

remain unclear (Renaville et al. 2002). In summer, levels of free cortisol remained high and levels of thyroid hormones remained low during the controlled re-feeding while they returned to their baseline levels in winter. The negative relationship observed in our study between free cortisol and IGF-1 suggests that the low growth response to the increase in nutrients in summer could come from the observed inhibitory effect of glucocorticoids on IGF-1 production and energy storage through insulin resistance (Sapolsky et al. 2000, Reeder & Kramer 2005). Thyroid hormones also stimulate the production of GH at the pituitary level (Boersma & Wit 1997, Robson et al. 2002), so their low levels could contribute to the low synthesis of IGF-1 and subsequent lack of mass gain upon re-feeding.

In summer, the mass loss initiated during the restriction ceased even though the hormone levels stayed stable during the controlled re-feeding at the same levels as at the end of the restriction. This means that under different levels of energy intake (restriction and re-feeding) the same levels of hormones triggered different levels of catabolic responses and body mass changes. During the restriction, levels of hormones signalled a mass loss, while the same hormonal state was related to a cessation of mass loss during the controlled re-feeding. The newly available nutrients during the controlled re-feeding period were not sufficient to elicit hormonal changes to return to pre-experimental body mass but may have been responsible for a difference in tissue sensitivity to the hormones triggering an end to mass loss (Wingfield & Kitaysky 2002).

Conclusions

All the hormones discussed above are intricately related to one another to create a complex web that regulates nutritional and energetic status (Robson et al. 2002). Hormone levels provide only a reductive snapshot of the energetic regulation by hormones changing in a very complex manner (Eales 1988). Their actions are also subject to changes in sensitivity at the organ level, either on a seasonal basis as part of the natural life cycle (Romero 2002) or as a security control in case of chronic elevated hormone levels (St Aubin & Dierauf 2001). Consequently, it is extremely difficult to assess the dominant regulatory systems and the endocrine changes related to the physiological responses observed. The results of our study show that, except for levels of IGF-1 involved in lean

mass regulation, the measured hormones and metabolites cannot be considered reliable biomarkers to assess the body condition and nutritional status of Steller sea lions during a mild nutritional stress such as in our study. These energetic priorities either reflected the regulatory response to another set of hormones (e.g., leptin and ghrelin), or were due to a complex interaction of factors beyond the simple absolute concentrations of individual hormones in the blood.

References

Ali M, Nicieza A, Wootton RJ (2003) Compensatory growth in fishes: a response to growth depression. Fish and Fisheries 4:147-190

Arnould JP, Green JA, Rawlins DR (2001) Fasting metabolism in Antarctic fur seal (*Arctocephalus gazella*) pups. Comparative Biochemistry and Physiology A 129:829-841

Arnould JPY, Morris MJ, Rawlins DR, Boyd IL (2002) Variation in plasma leptin levels in response to fasting in Antarctic fur seals (*Arctocephalus gazella*). Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology 172:27-34

Ashwell-Erickson S, Fay FH, Elsner R, Wartzok D (1986) Metabolic and hormonal correlates of molting and regeneration of pelage in Alaskan harbor and spotted seals (*Phoca vitulina* and *Phoca largha*). Canadian Journal of Zoology 64:1086-1094

Azizi F, Mannix JE, Howard D, Nelson RA (1979) Effect of winter sleep on pituitary thyroid axis in American black bear. American Journal of Physiology 237:E227-E230

Bergendahl M, Vance ML, Iranmanesh A, Thorner MO, Veldhuis JD (1996) Fasting as a metabolic stress paradigm selectively amplifies cortisol secretory burst mass and delays the time of maximal nyctohemeral cortisol concentrations in healthy men. Journal of Clinical Endocrinology and Metabolism 81:692-699

Boersma B, Wit JM (1997) Catch-up growth. Endocrine Reviews 18:646-661

Boily P (1996) Metabolic and hormonal changes during the molt of captive gray seals (*Halichoerus grypus*). American Journal of Physiology 39:R1051-R1058

Boyd IL (2002) Energetics: consequences for fitness. In: Hoelzel AR (ed) Marine mammal biology: an evolutionary approach. Blackwell Sciences Publishing, Oxford ; Malden, MA, p 247-277

Breier BH (1999) Regulation of protein and energy metabolism by the somatotropic axis. Domestic Animal Endocrinology 17:209-218

Buonomo FC, Baile CA (1991) Influence of nutritional deprivation on insulin-like growth factor-I, somatotropin, and metabolic hormones in swine. Journal of Animal Science 69:755-760

Butler AA, Le Roith D (2001) Control of growth by the somatropic axis: Growth hormone and the insulin-like growth factors have related and independent roles. Annual Review of Physiology 63:141-164

Choksi NY, Jahnke GD, St Hilaire C, Shelby M (2003) Role of thyroid hormones in human and laboratory animal reproductive health. Birth Defects Research B-Developmental and Reproductive Toxicology 68:479-491

Cummings DE, Overduin J (2007) Gastrointestinal regulation of food intake. Journal of Clinical Investigation 117:13-23

Diez M, Michaux C, Jeusette I, Baldwin P, Istasse L, Biourge V (2004) Evolution of blood parameters during weight loss in experimental obese Beagle dogs. Journal of Animal Physiology and Animal Nutrition 88:166-171

Eales JG (1988) The Influence of nutritional state on thyroid function in various vertebrates. American Zoologist 28:351-362

Engelhardt FR, Ferguson JM (1980) Adaptive hormone changes in harp seals, *phoca groenlandica*, and gray seals, *Halichoerus grypus*, during the postnatal period. General and Comparative Endocrinology 40:434-445

Gardiner KJ, Hall AJ (1997) Diel and annual variation in plasma cortisol concentrations among wild and captive harbor seals (*Phoca vitulina*). Canadian Journal of Zoology 75:1773-1780

Geary N (2004) Endocrine controls of eating: CCK, leptin, and ghrelin. Physiology & Behavior 81:719-733

Goodman HM, Knobil E (1961) Some endocrine factors in regulation of fatty acids mobilization during fasting. American Journal of Physiology 201:1-3

Guinet C, Servera N, Mangin S, Georges JY, Lacroix A (2004) Change in plasma cortisol and metabolites during the attendance period ashore in fasting lactating subantarctic fur seals. Comparative Biochemistry and Physiology A-Molecular & Integrative Physiology 137:523-531

Hall AJ, Green NJ, Jones KC, Pomeroy PP, Harwood J (1998) Thyroid hormones as biomarkers in grey seals. Marine Pollution Bulletin 36:424-428

Harlow HJ (1981) Metabolic adaptations to prolonged food deprivation by the American badger *Taxidea taxus*. Physiological Zoology 54:276-284

Haulena M, St Aubin DJ, Duignan PJ (1998) Thyroid hormone dynamics during the nursing period in harbour seals, *Phoca vitulina*. Canadian Journal of Zoology 76:48-55

Heath ME, McGinnis SM, Alcorn D (1977) Comparative thermoregulation of suckling and weaned pups of the northern elephant seal, *Mirounga angustirostris*. Comparative Biochemistry and Physiology 57:203-206

Hornick JL, Van Eenaeme C, Gerard O, Dufrasne I, Istasse L (2000) Mechanisms of reduced and compensatory growth. Domestic Animal Endocrinology 19:121-132

Kelly GS (2000) Peripheral metabolism of thyroid hormones: a review. Alternative Medicine Review 5:306-333

Kitaysky AS, Kitaiskaia E, Piatt J, Wingfield JC (2003) Benefits and costs of increased levels of corticosterone in seabird chicks. Hormones and Behavior 43:140-149

Kitaysky AS, Kitaiskaia EV, Piatt JF, Wingfield JC (2006) A mechanistic link between chick diet and decline in seabirds? Proceedings of the Royal Society B-Biological Sciences 273:445-450

Kitaysky AS, Springer AM, Wingfield JC When, where and why Steller sea lions experience physiological stress - Evidence form stress hormones and diet quality.

Kitaysky AS, Wingfield JC, Piatt JF (2001) Corticosterone facilitates begging and affects resource allocation in the black-legged kittiwake. Behavioral Ecology 12:619-625

Kleiber M (1975) The fire of life: an introduction to animal energetics. R. E. Krieger Pub. Co., Huntington, N.Y.

Kumagai S (2004) Seasonal differences in physiology of captive Steller sea lions (*Eumetopias jubatus*) in response to short term low energy intake. MSc, University of British Columbia

Kumagai S, Rosen DAS, Trites AW (2006) Body mass and composition responses to short-term low energy intake are seasonally dependent in Steller sea lions (*Eumetopias jubatus*). Journal of Comparative Physiology B 176:589-598

Lavigne DM, Barchard W, Innes S, Øritsland NA (1982) Pinniped bioenergetics. In: Mammals in the seas: Small cetaceans, seals, sirenians, and sea otters. FAO Fisheries Series 5, Rome, p 191-235

Lawson JW, Miller EH, Noseworthy E (1997) Variation in assimilation efficiency and digestive efficiency of captive harp seals (*Phoca groenlandica*) on different diets. Canadian Journal of Zoology 75:1285-1291

Little GJ (1991) Thyroid morphology and function and its role in thermoregulation in the newborn southern elephant seal (*Mirounga leonina*) at Macquarie Island. Journal of Anatomy 176:55-69

Lupu F, Terwilliger JD, Lee K, Segre GV, Efstratiadis A (2001) Roles of growth hormone and insulin-like growth factor 1 in mouse postnatal growth. Developmental Biology 229:141-162

Mashburn KL, Atkinson S (2007) Seasonal and predator influences on adrenal function in adult Steller sea lions: Gender matters. General and Comparative Endocrinology 150:246-252

Mellish JE, Iverson SJ (2001) Blood metabolites as indicators of nutrient utilization in fasting, lactating phocid seals: does depletion of nutrient reserves terminate lactation? Canadian Journal of Zoology 79:303-311

Mosier HD, Jr. (1986) The control of catch-up growth. Acta Endocrinologica Supplements 279:1-7

Myers MJ, Rea LD, Atkinson S (2006) The effects of age, season and geographic region on thyroid hormones in Steller sea lions (*Eumetopias jubatus*). Comparative Biochemistry and Physiology A-Molecular & Integrative Physiology 145:90-98

Narayanan S, Appleton HD (1980) Creatinine - a review. Clinical Chemistry 26:1119-1126

Nieminen P, Asikainen J, Hyvarinen H (2001) Effects of seasonality and fasting on the plasma leptin and thyroxin levels of the raccoon dog (*Nyctereutes procyonoides*) and the blue fox (*Alopex lagopus*). Journal of Experimental Zoology 289:109-118

O'Brian JT, Bybee DE, Burman KD, Osburne RC, Ksiazek MR, Wartofsky L, Georges LP (1980) Thyroid hormone homeostasis in states of relative caloric deprivation. Metabolism-Clinical and Experimental 29:721-727

Oki C, Atkinson S (2004) Diurnal patterns of cortisol and thyroid hormones in the harbor seal (*Phoca vitulina*) during summer and winter seasons. General and Comparative Endocrinology 136:289-297

Ortiz CL, Costa D, Boeuf BJL (1978) Water and energy flux in elephant seal pups fasting under natural conditions. Physiological Zoology 51:166-178

Ortiz RM, Houser DS, Wade CE, Ortiz CL (2003a) Hormonal changes associated with the transition between nursing and natural fasting in northern elephant seals (*Mirounga angustirostris*). General and Comparative Endocrinology 130:78-83

Ortiz RM, Noren DP, Litz B, Ortiz CL (2001a) A new perspective on adiposity in a naturally obese mammal. American Journal of Physiology 281:E1347-1351

Ortiz RM, Noren DP, Ortiz CL, Talamantes F (2003b) GH and ghrelin increase with fasting in a naturally adapted species, the northern elephant seal (*Mirounga angustirostris*). Journal of Endocrinology 178:533-539

Ortiz RM, Wade CE, Ortiz CL (2001b) Effects of prolonged fasting on plasma cortisol and TH in postweaned northern elephant seal pups. American Journal of Physiology 280:R790-R795

Perez-Sanchez J, Le Bail PY (1999) Growth hormone axis as marker of nutritional status and growth performance in fish. Aquaculture 177:117-128

Pinheiro J, Bates D, DebRoy S, Sarkar D (2006) nlme: Linear and nonlinear mixed effects models

Pinheiro JC, Bates DM (2000) Mixed-Effects Models in S and S-PLUS. Springer-Verlag, New York

Pitcher KW, Calkins DG, Pendleton GW (1998) Reproductive performance of female Steller sea lions: an energetics-based reproductive strategy? Canadian Journal of Zoology 76:2075-2083

Ramsay MA, Nelson RA, Stirling I (1991) Seasonal changes in the ratio of serum urea to creatinine in feeding and fasting polar bears. Canadian Journal of Zoology 69:298-302

Rea LD, Nagy TR (2000) Changes in serum leptin levels during fasting and food limitation in Steller sea lions (*Eumetopias jubatus*). Proceedings of the Comparative Nutrition Society, Monterey, California, USA, p 171-175

Rea LD, Rosen DAS, Trites AW (1998) Blood chemistry and body mass changes during fasting in juvenile Steller sea lions (*Eumetopias jubatus*) Proceedings of the Comparative Nutrition Society, Banff, Canada, p 174-178

Rea LD, Rosen DAS, Trites AW (2000) Metabolic response to fasting in 6-week-old Steller sea lion pups (*Eumetopias jubatus*). Canadian Journal of Zoology 78:890-894

Reeder DM, Kramer KM (2005) Stress in free-ranging mammals: Integrating physiology, ecology, and natural history. Journal of Mammalogy 86:225-235

Renaville R, Hammadi M, Portetelle D (2002) Role of the somatotropic axis in the mammalian metabolism. Domestic Animal Endocrinology 23:351-360

Renouf D, Noseworthy E (1991) Changes in food intake, mass, and fat accumulation in association with variations in thyroid hormone levels of harbour seals (*Phoca vitulina*). Canadian Journal of Zoology 69:2470-2479

Richmond JP, Jeanniard du Dot T, Rosen DA, Zinn SA (2006) Effect of prey composition on the endocrine response to nutritional restriction and re-alimentation in Steller sea lions (*Eumetopias jubatus*). Proceedings of the Comparative Nutrition Society, Keystone, Colorado, USA, p 136-141

Riviere JE, Engelhardt FR, Solomon J (1977) The relationship of thyroxine and cortisol to the moult of the harbor seal, *Phoca vitulina*. General and Comparative Endocrinology 31:398-401

Robson H, Siebler T, Shalet SM, Williams GR (2002) Interactions between GH, IGF-I, glucocorticoids, and thyroid hormones during skeletal growth. Pediatric Research 52:137-147

Romero LM (2002) Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. General and Comparative Endocrinology 128:1-24

Rosen DA, Trites AW (2005) Examining the potential for nutritional stress in young Steller sea lions: physiological effects of prey composition. Journal of Comparative Physiology B 175:265-273

Rosen DAS, Renouf D (1997) Seasonal changes in blubber distribution in Atlantic harbor seals: indications of thermodynamic considerations. Marine Mammal Science 13:229-240

Sapolsky RM, Romero LM, Munck AU (2000) How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocrine Reviews 21:55-89

Silva JE (1995) Thyroid hormone control of thermogenesis and energy balance. Thyroid 5:481-492

Smith WJ, Underwood LE, Clemmons DR (1995) Effects of caloric or protein restriction on insulin-like growth factor-I (IGF-I) and IGF-binding proteins in children and adults. Journal of Clinical Endocrinology and Metabolism 80:443-449

St Aubin DJ, Dierauf LA (2001) Stress and marine mammals. In: Dierauf LA, Gulland FMD (eds) CRC Handbook of marine mammal medicine. CRC Press, Boca Raton, London, New York, Washigton DC, p 253-269

St Aubin DJ, Ridgway SH, Wells RS, Rhinehart H (1996) Dolphin thyroid and adrenal hormones: Circulating levels in wild and semidomesticated *Tursiops truncatus*, and influence of sex, age, and season. Marine Mammal Science 12:1-13

Stini WA (1969) Nutritional stress and growth - sex difference in adaptive response. American Journal of Physical Anthropology 31:417-426

Thissen JP, Ketelslegers JM, Underwood LE (1994) Nutritional regulation of the insulin-like growth factors. Endocrine Reviews 15:80-101

Trites AW, Donnelly CP (2003) The decline of Steller sea lions *Eumetopias jubatus* in Alaska: a review of the nutritional stress hypothesis. Mammal Review 33:3-28

Trumble SJ, Barboza PS, Castellini MA (2003) Digestive constraints on an aquatic carnivore: effects of feeding frequency and prey composition on harbor seals. Journal of Comparative Physiology B 173:501-509

Van Den Brande JL (1986) Catch-up growth. Possible mechanisms. Acta Endocrinologica Supplements 279:13-23

Ward JM, Armitage KB (1981) Circannual rhythms of food consumption, body mass, and metabolism in yellow bellied marmots. Comparative Biochemistry and Physiology A-Physiology 69:621-626

Weigle DS, Duell PB, Connor WE, Steiner RA, Soules MR, Kuijper JL (1997) Effect of fasting, refeeding, and dietary fat restriction on plasma leptin levels. Journal of Clinical Endocrinology and Metabolism 82:561-565

Wingfield JC, Kitaysky AS (2002) Endocrine responses to unpredictable environmental events: Stress or anti-stress hormones? Integrative and Comparative Biology 42:600-609

Winship AJ, Trites AW, Rosen DAS (2002) A bioenergetic model for estimating the food requirements of Steller sea lions *Eumetopias jubatus* in Alaska, USA. Marine Ecology-Progress Series 229:291-312

Yambayamba ES, Price MA, Foxcroft GR (1996) Hormonal status, metabolic changes, and resting metabolic rate in beef heifers undergoing compensatory growth. Journal of Animal Science 74:57-69

Young RA (1984) Interrelationships between body weight, food consumption and plasma thyroid hormone concentration cycles in the woodchuck, *Marmota monax*. Comparative Biochemistry and Physiology A-Physiology 77:533-536

Chapter 5: Conclusions

The goal of our study was to determine how season and quality of diet impact the physiology and energetic priorities of captive female Steller sea lions during nutritional stress and subsequent periods of recovery. To do so, we measured changes over two 3-month experiments (summer and winter) in body mass and body composition as well as in energy allocated to different physiological functions including maintenance functions (metabolism, thermoregulation capacity, and activity levels). Effects of season and quality of diet on the endocrine system were also investigated to understand how these parameters interact with hormones relative to the observed physiological decisions. The ability of hormone and metabolite concentrations to indicate body composition changes was also determined.

Summary of findings

Overall, our study confirmed that season and quality of diet interact to influence the physiological responses of Steller sea lions that experience nutritional stress. Levels of energy intake controlled the rates of total mass loss, but the quality of diet determined the proportional catabolism of lipids and protein tissues as well as the relative energy sea lions allocated to physiological functions such as thermoregulation and activity. In our study, the pollock diet led to the sea lions using more lean tissues as energy resources compared to the higher rate of lipid use of sea lions that ate the isocaloric herring diets. This effect was seen in both seasons.

In response to energy restriction, captive sea lions eating herring also adjusted portions of their energy budget in accordance with environmental conditions they would experience in the wild, while animals consuming pollock did not. More specifically, the animals eating herring increased their activity levels and decreased their thermoregulation capacity in summer when water temperatures and the likelihood of finding fish in the wild are expected to be the highest. They also showed a sustained decrease in activity levels while maintaining a stable thermoregulation capacity in winter when greater body insulation was needed and when the distribution of prey in their natural environment may lower their foraging efficiency. The estimated thermoregulation capacity and activity

levels of sea lions in Group P remained relatively constant throughout both experiments except in winter when their activity levels decreased during the first two weeks of energy restriction. The fact that animals eating low-energy fish have a lower capacity to adjust their energy budget to changes in levels of energy intake may shorten the duration of their resistance to mild nutritional stress.

In our study, it was unclear to what extent physiological changes were regulated by glucocorticoids, somatotropic, and thyroid hormones. Levels of free cortisol and IGF-1 were related to body mass of the sea lions in winter, but only IGF-1 was significantly involved in mass regulation in summer, indicating that regulation of body mass through lean mass may be a priority in this season. Overall, IGF-1 regulated changes in lean mass observed in the sea lions but none of the hormones measured were a good indicator of changes in body condition (proportion of lipids versus lean mass in the body) of the animals. Thyroid hormone results were not associated with any observed changes in metabolism, and blood metabolites (non-estherified fatty acids, ketone bodies and blood urea nitrogen/creatinine ratio) also did not accurately reflect levels of lipid or protein catabolism of the sea lions. Consequently, even though protein metabolism can be assessed from levels of IGF-1, all of the measured blood parameters failed to accurately reflect physiological status of Steller sea lions that were mildly nutritionally stressed.

Steller sea lions displayed seasonally dependent responses to nutritional stress. It appeared easier for them to resist nutritional stress and to recover from it in winter than in summer, which was contrary to predictions derived from existing bioenergetic models (Winship et al. 2002). Specifically, the sea lions lost mass at the same rate in winter as in summer during the restriction even though the energy intake deficit (compared to baseline levels) was greater. Sea lions adjusted their energy budgets so that the relatively greater nutritional stress they incurred in winter had a lower relative impact compared to summer. This appears to have been achieved through behavioural plasticity and changes in activity levels. Sea lions in both experimental groups also displayed compensatory growth in winter when their activity levels remained low after the restriction but were unable to do so in summer.

Steller sea lions can alter their behaviours depending on the season and their diet to buffer the effects of different environmental conditions. These adjustments were more visible during the periods of energy restriction and were surprisingly driven by the quality of diet ingested rather than by a decrease in energy intake. Our results showed that a decrease in fish intake was needed in addition to the decrease in energy intake to trigger behavioural and physiological adjustments even though body mass losses were similar. Consequently, levels of satiation are likely to be important parameters related to physiological and behavioural adjustments to changes in diet.

It is interesting to note that adjustments made by the sea lions during the restriction and controlled re-feeding periods were also visible during the pre-restriction phases in summer and winter. Energy intakes during baseline phases were determined by the behavioural interaction between animals and trainers (i.e., whether the animals showed an interest in food) and resulted in greater baseline intakes in winter than in summer as commonly observed in pinnipeds. However, rates of mass gain were similar during the baseline in both seasons. This implies that the additional energy ingested during the winter baseline was allocated to functions other than growth, such as thermoregulation (even though blubber insulation is thought to be adapted to the season) or activity levels. Physiological and behavioural plasticity may thus buffer the seasonal differences in environmental conditions and energy requirements outside of periods of nutritional stress.

Results from Chapters 2, 3, and 4 showed that summer is a more sensitive season for nutritionally stressed Steller sea lions than is winter. This finding runs contrary to expectations. Our data provide evidence that sea lions can not only withstand nutritional stress better in winter, but they can also recover from suboptimal conditions more easily during winter as well. In addition, our study confirmed that a low-energy diet reduces the capacity to behaviourally and physiologically adjust to environmental conditions, potentially due to the intrinsic contradictory signals between energy intake and satiation resulting from low energy-density diets. Finally, combining the observed changes in body composition and hormonal regulation indicates that growth of body structure was a priority in summer and that increasing energy storage was a priority in winter when additional efforts were made to preserve lipid stores and thermoregulation capacity. These seasonal differences in type of body compartment synthesized could explain why sea lions

displayed a compensatory growth in winter but not in summer, since it is faster to replenish lipid stores than to build core structures.

Future studies

Compensatory growth merits further study because of the impact it has on the health and life parameters of animals. Studies on compensatory growth on marine mammals are lacking and may contribute to better understanding the population trends of Steller sea lions and other species. Reasons behind the seasonal difference in capacity of Steller sea lions to recover from nutritional stress remain unclear and should be addressed and clarified in future studies that incorporate season as a co-factor. To be relevant to conditions of wild animals, it could also be interesting to assess the impact of a low-quality fish diet on the capacity of sea lions to compensate. Compensatory growth usually requires a period of high-energy input which may not be feasible if animals only have access to low-energy fish and have limited digestive capacity.

Blood parameters such as blood metabolites or hormones have been widely used to assess physiological states of animals during a fast (Costa & Ortiz 1982, Arnould et al. 2001, Mellish & Iverson 2001, Guinet et al. 2004). However these parameters seem to be less accurate during mild restrictions such as in our study. It would thus be useful to develop and validate a reliable blood parameter that could be easily collected from sea lions to assess their physiological state and overall body condition. It is also important to keep in mind that changes in endocrine pathways regulating physiology and behaviour occur at different levels in the body through hormone concentrations in the blood, bioavailability and degradation rates, and receptors affinity and availability. Consequently, any attempt to understand the endocrine regulation of energy budgets and body condition of Steller sea lions should incorporate measurements of receptors and binding proteins. A wider range of hormones (e.g., leptin, ghrelin) and other relevant hormonal forms (such as free T3 and reverse T3 in the case of thyroid hormones) should also be included in future studies.

The difference in lipid/protein content between low- and high-energy diets has been proposed to explain the deleterious effects of a pollock diet on marine mammals and

sea birds. However, different levels of satiation that high- and low-energy fish provide for similar energy intakes could be the key parameter to explain differences in behavioural and physiological adjustments in Steller sea lions. Future research should investigate the effects of different levels of satiation on endocrine signals and the nutritional status of Steller sea lions to better understand how low-energy diets impact fitness of these animals.

Strength and weaknesses

Studies investigating the impact of changes in diet on the physiology and fitness of animals are strengthened when the animal is considered in its entirety. The holistic approach we attempted provides a wide scope of understanding of the overall response of individual sea lions at different levels: physiological, bioenergetic, endocrine and behavioural. Integrating the adjustments made at each of these levels allows for a more accurate and thorough analysis of how sea lions react to environmental changes and the potential consequences it has on their survival and reproductive capacity.

Captive studies provide a unique opportunity to acquire data that are difficult or impossible to collect in the wild. They also provide a controlled setting to tease apart the effects of intricate factors. The simple, yet realistic experimental design of our study reflected the hypothesis that sea lions from the declining western populations may be mildly restricted on a chronic basis while consuming predominantly low-energy fish compared to those in the increasing eastern population that are consuming fattier fish. Consequently a 20-30% decrease of energy intake when animals are switched from high to low-energy pollock during a 28-day period is a reasonable approximation of what is thought to have occurred in the wild rather than acute experimental restrictions.

An intrinsic consequence of simplified studies in a controlled environment is that the animals are not subject to the multi-factorial conditions that their wild counterparts encounter. Wild animals are likely to consume more than a single species of fish as in our study, although sea lions from the western population have been primarily (94.5%) consuming low-energy fish (Sinclair & Zeppelin 2002, Trites et al. 2007b). As such, using a single species of low-energy prey or a mixture of low-energy species should yield similar results.

Foraging and activity expenses are unquestionably lower for sea lions in captivity and could lead to significant differences in bioenergetic decisions made by animals. In terms of bioenergetic calculations, a number of model parameters were based on assumptions, and yielded estimates of net energy and maintenance energy that were within the range of what were found in other studies (see Chapter 3). Overall, results of the feeding experiments represented qualitative changes for sea lions during the restriction and subsequent re-feeding. They showed that sea lions (even though captive) significantly altered their levels of activity (increase or decrease depending on the season) to optimize energy expenditures for foraging in relation to the probability of finding fish under the conditions they would have seasonally encountered in the wild. Given that physiological reactions should be similar among Steller sea lions regardless of whether they are captive or wild animals, reliable conclusions can be drawn about the physiological effects of season, quality of diet and how they interact to result in the physiological and behavioural changes observed for Steller sea lions.

Sample size was both a strength and a weakness of our study. On one hand, the eight female Steller sea lions that took part in our experiments represented a relatively large sample for a captive study on large marine mammals. On the other hand, a sample size of eight can seem low from a statistical perspective. This was partially overcome by having a simple study design and employing repeated measures on each animal to increase the statistical power. The significant differences found despite the low sample size are thus likely to be realistic. Overall, our study provided valuable information to further understand nutritional physiology of Steller sea lions under different diet and season conditions, although important caveats should be kept in mind when extrapolating our results to wild populations.

Implications

Our results have implications for management of wild Steller sea lions. Nutritional stress has been commonly assumed to be more strenuous during winter than summer (Merrick & Loughlin 1997, Winship et al. 2002). However, our results showed not only that sea lions withstand periods of mild nutritional stress more easily in winter but they also are able to recover more easily from it compared to the summer.

Steller sea lions appeared to need a large increase in energy intake in summer to recover from any loss of body mass, otherwise they may remain in a suboptimal state in the summer that will ultimately decrease their fitness. Increasing energy intake post-restriction via a hyperphagic response would require a sea lion to increase its foraging effort, which means that young and naïve animals might expose themselves to a greater risk of predation in summer compared to winter. Such large increases in energy intake may also be impossible to attain for juvenile animals that have a limited digestive capacity, particularly if their diet during the hyperphagic response is mainly composed of low-energy density fish. Consequently, summer should be considered as a more critical period of the year for Steller sea lions than winter, especially for survival of juveniles eating primarily low-energy fish. Management decisions should also consider the greater need of sea lions to substantially increase their energy intake to recover after a mild energy restriction in summer and the resulting potential greater conflicts that might occur with fisheries.

Proportion of fat in the body (TBF%), also called body condition, has long been used as an index to assess health of pinnipeds (Costa et al. 1989, Gales & Renouf 1994, Rea et al. 2007a). However, our results showed that while sea lions eating pollock were losing mass at the same rate as animals eating herring, their body conditions stayed stable or slightly improved due to a predominant loss of lean tissues, especially in winter. Sustained protein loss can be more deleterious on a chronic basis than loss of lipid tissue and yet it was not detectable by traditional measurements of body condition. Percent of body fat as an index of the body condition of animals may thus not be the best indicator of their health — at least not for Steller sea lions that have a predominant diet of low-energy fish. Information on the body condition of Steller sea lions in populations with predominantly low-energy fish diets may thus not be representative of their health, and should be taken into account in future field studies, especially for the western population.

Our results deepen the understanding of the consequences that nutritional stress may have on the fitness of Steller sea lions and provides interesting new results on the seasonal effect that a diet of low-energy fish can have on sea lions. They show that a diet of pollock may have subtle effects beyond the rate of mass loss that prevent sea lions from optimally adjusting their physiologies to compensate for suboptimal environmental

conditions leading to nutritional stress. Chances of survival may be lower for animals faced with chronic shortage of low-energy prey compared to animals eating high-energy fish. Ultimately, our findings should help to refine bioenergetic models needed to assist with the management and the conservation of Steller sea lions.

References

Arnould JP, Green JA, Rawlins DR (2001) Fasting metabolism in Antarctic fur seal (*Arctocephalus gazella*) pups. Comparative Biochemistry and Physiology A 129:829-841

Costa DP, Croxall JP, Duck CD (1989) Foraging energetics of Antarctic fur seals in relation to changes in prey availability. Ecology 70:596-606

Costa DP, Ortiz CL (1982) Blood chemistry homeostasis during prolonged fasting in the northern elephant seal. American Journal of Physiology 242:R591-595

Gales R, Renouf D (1994) Assessment of body condition of harp seals. Polar Biology 14:381-387

Guinet C, Servera N, Mangin S, Georges JY, Lacroix A (2004) Change in plasma cortisol and metabolites during the attendance period ashore in fasting lactating subantarctic fur seals. Comparative Biochemistry and Physiology A-Molecular & Integrative Physiology 137:523-531

Mellish JE, Iverson SJ (2001) Blood metabolites as indicators of nutrient utilization in fasting, lactating phocid seals: does depletion of nutrient reserves terminate lactation? Canadian Journal of Zoology 79:303-311

Merrick RL, Loughlin TR (1997) Foraging behavior of adult female and young-of-year Steller sea lions in Alaskan waters. Canadian Journal of Zoology 75:776-786

Rea LD, Rosen DA, Trites AW (2007) Utilization of stored energy reserves during fasting varies by age and season in Steller sea lions. Canadian Journal of Zoology 85:190-200

Sinclair EH, Zeppelin TK (2002) Seasonal and spatial differences in diet in the western stock of Steller sea lions (*Eumetopias jubatus*). Journal of Mammalogy 83:973-990

Trites AW, Miller AJ, Maschner HDG, Alexander MA, Bograd SJ, Calder JA, Capotondi A, Coyle KO, Di Lorenzo E, Finney BP, Gregr EJ, Grosch CE, Hare SR, Hunt GL, Jahncke J, Kachel NB, Kim HJ, Ladd C, Mantua NJ, Marzban C, Maslowski W, Mendelssohn R, Neilson DJ, Okkonen SR, Overland JE, Reedy-Maschner KL, Royer TC, Schwing FB, Wang JXL, Winship AJ (2007) Bottom-up forcing and the

decline of Steller sea lions (*Eumetopias jubatus*) in Alaska: assessing the ocean climate hypothesis. Fisheries Oceanography 16:46-67

Winship AJ, Trites AW, Rosen DAS (2002) A bioenergetic model for estimating the food requirements of Steller sea lions *Eumetopias jubatus* in Alaska, USA. Marine Ecology-Progress Series 229:291-312