

Effects Of Food Deprivation On Blood Lipid Concentration And Composition In Steller Sea Lions (*eumetopias Jubatus*)

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EFFECTS OF FOOD DEPRIVATION ON BLOOD LIPID CONCENTRATION
AND COMPOSITION IN STELLER SEA LIONS (*Eumetopias jubatus*)

By

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A thesis submitted in partial fulfillment of the requirements
for the degree for Master of Science
in the Department of Biology
in the College of Arts and Sciences
at the University of Central Florida
Orlando, Florida

Summer Term 2005

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ABSTRACT

Steller sea lions, the largest Otariid, fast during their breeding season; during this time they refrain from ingesting food for a period of 12-43 days. Fasting, while undertaking an extremely energetically demanding activity (breeding and pupping), requires specific physiological adaptations. This study examined the physiological response to fasting of two age classes, juveniles and sub-adults, during the breeding and non-breeding seasons to determine how these animals utilize lipids and the pattern of fatty acid mobilization from lipid stores during fasting. Four juveniles and 5 sub-adults were fasted for one and two weeks, respectively, and blood samples were collected approximately every 3 days for lipid analysis. The concentrations of plasma non-esterified fatty acids (NEFA) were analyzed spectrophotometrically. Serum fatty acid composition was analyzed using gas chromatography (GC) and their individual weight percent (wt %) were correlated with their peak retention time and calculated using the area under each peak. Sixty-nine fatty acids were quantified from each sample. However, only those with concentrations above 0.2 wt. % were included in the analysis. Sub-adult samples were grouped on a percent mass loss basis (0%, 7-8% and 15% mass loss) to facilitate comparison with the juveniles. These data represent the total lipid fatty acid composition of each blood sample. Relative lipid concentration was calculated by multiplying the total lipid fatty acid compositional analysis (wt %) by the NEFA concentrations measured in that respective blood sample. Plasma NEFA concentrations in juvenile Steller sea lions ranged from 1.2 ± 0.51 mM to 3.7 ± 0.69 during fasting and was within the range of fasting phocids. Concentrations of NEFAs in the sub-adult

Steller sea lions ranged from 1.00 mM up to 9.70 mM and were generally higher than fasting phocids. The wt % of only one fatty acid (20:0) was significantly different between the breeding and non-breeding season in fasting juveniles. However, the wt % of seven fatty acids changed significantly during fasting in the juveniles and five of these were most significant in separating the beginning and end of the fasts using principal components analysis. In contrast, the wt % of 10 fatty acids were significantly different during the breeding and non-breeding season fasts of the sub-adults. Additionally, the wt % of 10 fatty acids changed significantly during fasting in the sub-adults and four of these (16:1n-7, 18:2n-6, 20:0, and 20:1n-9) were most significant in separating the beginning and end of the fasts using principal components analysis. These trends reveal the physiological differences between the juvenile and sub-adult Steller sea lions and suggest that the sub-adults may be better physiologically and metabolically adapted to fast than the juveniles in this study.

ACKNOWLEDGMENTS

First, I am thankful to my advisors, Dr. Lorrie Rea and Dr. Graham Worthy for their keen interest in fasting physiology and not giving up on me. To my committee members, Dr. Linda Walters and Dr. Jack Stout for I extend my gratitude for their time and thoughtful comments. Funding for this project was provided by University of Central Florida Biology Department, by the National Marine Mammal Laboratory, National Marine Fisheries Service, National Oceanic and Atmospheric Administration; by the National Academy of Sciences through a National Research Council Research Fellowship to Lorrie D. Rea; by the North Pacific Marine Science Foundation through the North Pacific Universities marine Mammal Research Consortium and by the National Fish and Wildlife Foundation through grant #98-244-008 to Lorrie D. Rea. This study was authorized under the Marine Mammal Protection Act Permit (#881-1443) held by the ASLC and by IACUC Protocols approved by both the ASLC (#98-007) , the University of Central Florida (#9807). Researchers and staff at the Vancouver Aquarium Marine Science Center and Alaska Sea Life Center assisted in the fasting trials for which I am grateful. I appreciate Dr. Sara Iverson and her lab staff for running the fatty acid analyses. I am indebted to my parents for their unending and unconditional support of my unconventional life and educational route. To Maggie, Murphy and Toby for their cold noses and warm hearts and making my life complete. Finally, special thanks to Shawn for encouraging me to finish.

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LIST OF ABBREVIATIONS

| | |
|--------|-------------------------------|
| ANOVA | Analysis of Variance |
| ASLC | Alaska Sea Life Center |
| BHT | Butylated Hydroxytoluene |
| FAME | Fatty Acid Methyl Ester |
| GC | Gas Chromatography |
| MANOVA | Multiple Analysis of Variance |
| MUFA | Monounsaturated Fatty Acid |
| NEFA | Non-Esterified Fatty Acid |
| PCA | Principle Components Analysis |
| PUFA | Polyunsaturated Fatty Acid |
| TLC | Thin Layer Chromotography |
| UCF | University of Central Florida |
| VA | Vancouver Aquarium |
| Wt. % | Weight Percent |

INTRODUCTION

The Steller sea lion (*Eumetopias jubatus*) is the largest animal in the Otariid family with the males growing as large as 1120 kg and the females growing up to 350 kg (Reeves et al. 1992). They are gregarious and polygynous with a harem-style breeding strategy. The males maintain breeding rookeries for 12-43 days and they remain ashore, fasting from food and water during this entire period (Bonner 1989). Females return to breeding rookeries around three days prior to parturition and usually have one pup. They remain on shore nursing their pup for approximately 9 days, at which time they also fast (Reeves et al. 1992). After this post-partum fast, the female returns to the sea to feed for about 1-3 days while the pup is left alone on shore fasting (Reeves et al. 1992). The female will then return to the rookery for 2 days to nurse its pup. This creates an alternate pattern of the mother fasting while at the rookery nursing and the pup fasting while its mom is at sea feeding (Reeves et al. 1992). The mother will forage longer as the pup grows and pups are usually weaned by their first birthday. Some pups may still nurse until 2 or 3 years old (Reeves et al. 1992).

The Steller sea lion population extends from the northern Pacific rim through the Aleutian Islands down the Alaskan coast south to central California. In the US population, two stocks exist and they are divided at 144° W longitude (Loughlin 1998, Trites and Donnelly 2003). The eastern stock extends from eastern Alaska to California

while the western stock extends from Prince William Sound, Alaska and west to Russia. Steller sea lions have been listed as threatened since 1990 under the Endangered Species Act and the western stock was listed as endangered in 1997. The Steller sea lion populations in the Gulf of Alaska and Aleutian Islands have been declining for several years while the eastern Steller sea lion population remains stable, leading to investigations into the possible cause of the decline (Loughlin 1998, Trites and Donnelly 2003).

Several hypotheses for this decline have been proposed. Some of these include: food limitation, redistribution, changed vital rates, pollution, predation, subsistence use, commercial harvest, disease, natural fluctuation, environmental changes, and commercial fishing (Loughlin 1998, Trites and Donnelly 2003). All factors may have contributed to the decline to some extent, however nutritional compromise is believed to be one of the most influential factors. Nutritional limitation could be due to commercial fisheries depleting the Steller sea lions' main diet source of gadids which includes walleye pollock, Pacific cod, saffron cod, Pacific tomcod, whiting and hake or altered prey distribution due to other factors (Trites and Donnelly 2003). It has also been suggested that juveniles may have been most affected since modeling has shown that a decline in the number of juvenile animals recruited into the adult breeding population could statistically account for the patterns of this decline (York 1994).

The hypothesis that limited or redistributed prey is a major cause of decline in the 1980's has been supported by several studies. Investigations have found indications of nutritional stress, depicted by reduced growth, when animals between 1-13 years of age

of the same population were compared about 10 years apart. Steller sea lions exhibited reduced growth in 1985-1986 compared to the same population in 1975-1978 (Calkins et al. 1998). It has been shown that food limitation can result in reduced body size in both marine and terrestrial mammals (Florant 1998). Hood and Ono (1997) concluded that maternal attendance decreased between 1973 and 1992. Comparing pup behavior between these years, they additionally concluded that pups spent less time suckling in 1992 than in 1973. This behavioral change is consistent with a reduction in prey availability or physical redistribution of prey such that nursing mothers must invest more time foraging and spends less time on the rookery nursing their pups. Newly weaned Steller sea lions may be additionally disadvantaged due to their limited diving capabilities stemming from their immature physiological and behavioral development (Merrick and Loughlin 1997, Richmond 2004). In contrast, Rea et al. (1998) found no indication of nutritional depression in pups less than one month old in the declining population in the 1990's indicating that young pups may be obtaining required nutrients even if time spent nursing is reduced.

This study addresses the physiological implications of food deprivation and the effect of the time of year in which food deprivation occurs. Steller sea lions naturally fast during the breeding season (May-July). In birds and mammals, fasting is generally comprised of three phases resulting in changes in metabolism which are reflected in blood chemistry changes, although animals spend different amounts of time in each phase and may not experience all three phases (Robin et al. 1988, Belkhou et al. 1991, Cherel et al. 1992). Glucose and protein usage characterize phase I, evident by an increase in

protein metabolic byproducts (blood urea nitrogen [BUN]) and low levels of lipid metabolic byproducts (ketone bodies). Phase II of fasting is characterized by protein sparing and increased lipid usage evident by an increase in ketone bodies and a decrease in BUN. Increasing BUN concentrations in latter stages of the fast indicates entrance into phase III. Using protein for energy becomes detrimental in this phase because muscle and organ tissue becomes the main protein source. Rea (2000) found Steller sea lions to be better adapted to fasting during the breeding season as compared to other times of the year based on transitions between these biochemical phases. Animals fasted during the breeding season began in phase I and rapidly switched to phase II. They remained in phase II for the remainder of the two-week fast. The animals fasted during non-breeding months followed a similar trend until the end of the fast where they entered phase III and reverted back to protein usage.

Other animals that undergo seasonal fasting also experience metabolic adaptations. Hibernating mammals, such as arctic ground squirrels (Ormseth et al. 1996), yellow-bellied marmots (Ward and Armitage 1981), and European brown bears (Hissa et al. 1998) undergo seasonal hyperphagia which increase the body reserves in preparation for long-term fasting. Penguins also experience a period of pre-fast hyperphagia in which they deposit extra adipose stores (170g of fatty acids per day) to rely on during breeding and reproduction (Groscolas 1990). It is unclear if Steller sea lions exhibit hyperphagia' however pinnipeds have the advantage of a high fat marine-based diet to rapidly build lipid stores in subcutaneous blubber, which they rely on during their seasonal fast.

These body lipid reserves prove to be essential in fasting, non-hibernating animals such as penguins and pinnipeds. Blubber stores are two-fold in their function: 1) to maintain thermoregulatory abilities, 2) to be used as energy stores. Pinnipeds and penguins that live on pack ice and in cold climates utilize blubber for its insulative properties and require a blubber layer for survival. Seals and penguins also rely on lipid stores for 94 and 93% of their energy, respectively, in order to spare protein during phase II of fasting (Nordøy and Blix 1985, Cherel et al. 1994). Seal pups typically depart rookeries prior to entering phase III of fasting (Castellini and Costa 1990, Rea 1995). In contrast, penguins often do not reach the sea until they have depleted lipid reserves (only 9% of body fat remaining) and have entered phase III of fasting (Groscolas 1986). Elephant seal pups usually depart rookeries after 2-3 months of a post-weaning fast with up to 50% body fat still remaining while adult elephant seals retain only around 25% body fat at the time of departure (Castellini and Costa 1990). With such large reserves of body fat remaining, some internal cue, other than total fat depletion, may be signaling these animals to return to sea to feed.

This cue to begin feeding again may be linked to depletion of specific individual fatty acids. For example, it is known that certain essential fatty acids are precursors to eicosanoids and specifically prostaglandins in terrestrial mammals. Prostaglandins and other eicosanoids have a wide range of functions such as thermoregulation and neural development, both of which are vital to pup development and survival (Mostafa et al. 1993, Florant 1998). Essential fatty acids, in particular n-3 and n-6 fatty acids, are sequestered in the adipose tissue while nursing or feeding and cannot be derived from de

novo synthesis or desaturation of existing fatty acids. Therefore, depletion of these or other individual fatty acids may be the determining factor in the need to feed.

Although lipid storage is vital, these resources are of little use as energy until they are mobilized from storage and circulated for metabolism. Fatty acid mobilization has been extensively studied in rats to determine the effect of molecular structure and chain length on mobilization rates of individual fatty acids. Under several different diets, feeding regimes and conditions (feeding or fasting), mobilization remained consistent based on three criteria: 1) chain length, 2) degree of saturation and 3) distance of double bond from the methyl end (Raclot and Groscolas 1993, 1995 and Raclot et al. 1995). Rates of fatty acid mobilization increased with increased unsaturation for a given chain length, increased with decreased chain length, and increased when double bonds were closer to the methyl end. Therefore, shorter chained, unsaturated, fatty acids with double bonds close to the methyl end had the highest mobilization rate, and thus are utilized first from storage. Although serum lipids must be compared to lipid stores to determine mobilization rates, knowledge of the biochemical process may assist in the determination of why specific fatty acids are present in serum during fasting.

Steller sea lions have shown significant biochemical seasonal adaptation to fasting. This study investigates whether there are seasonal difference in lipid utilization and patterns of circulating fatty acid composition during fasting in Steller sea lions. Lipid utilization is essential to long-term fasting and this study quantifies the concentration of non-esterified fatty acids (NEFA) to address the hypothesis that Steller sea lions accumulate NEFAs in circulation as the fast progresses. Additionally, a drop in NEFA

concentration would mark entrance into phase III which might be more likely to occur during the non-breeding period when animals are less likely to be adapted to undergo prolonged fasting in the wild. This study also considers the hypothesis that fatty acid composition of the serum lipids changes throughout fasting, possibly due to difference in mobilization rates of different fatty acids. I examined if there were any differences in the wt % contribution of specific fatty acids in serum lipids between the breeding and non-breeding season. Additionally, an index of the relative concentration of each fatty acid in the serum lipid was calculated by multiplying the NEFA concentration by the wt % of the individual fatty acids to determine the relative concentration of each individual fatty acid mobilized during each stage of fasting and season. The relative fatty acid concentration will indicate if the absolute amount of a fatty acid is reaching critically low levels based on overall blood lipid levels. The relative fatty acid concentration will allow us to test the hypothesis that a depletion of an individual fatty acid(s) may effect biochemical changes causing fasting animals to enter Phase III of fasting while still maintaining sufficient quantities of total body fat.

METHODS

Animal Sampling

This study included nine Steller sea lions, four females and five males (Table 1). A total of 16 fasts were conducted lasting from 7-14 days, limited by a maximum 15% body mass loss. Four of the sea lions were juveniles (1.5 – 2.5 years old) and five were sub-adults (2.5 – 5 years old). Due to a smaller body size, juveniles only fasted for seven days whereas the older animals were fasted up to 14 days. Thirteen of the fasts were completed at Vancouver Aquarium (VA), Vancouver, British Columbia, Canada and three were undertaken at Alaska Sea Life Center (ASLC), Seward, Alaska. Eight of the fasts were conducted during the breeding season (May – July) and the other eight were conducted during the non-breeding season (August – April).

All training and care was maintained under the routine protocols of the ASLC and VA. These animals were trained to accept ice cubes as rewards for completing tasks required of them, thus animals ingested fresh water throughout the study.

At the onset of the study, blood samples and body mass measurements were collected from each sea lion following an overnight fast. These data provided a control sample for each individual prior to prolonged fasting. Animals were held in a restraint cage during blood collection. The sea lions were acclimated to enter the restraint cage as

part of their normal husbandry behavior, and were manually restrained with the aid of chemical immobilization when deemed appropriate by the attending veterinarian.

Table 1. The dates and duration of fasting trials for each of the nine sea lions.

| Sea Lion | Sex | Fasting Season | Date of Fast | Length of Fast (days) | Age (years) |
|----------|-----|----------------|---------------|-----------------------|-------------|
| Woody | M | Breeding | June 1999 | 14 | 5 |
| | | Non-breeding | Nov/Dec 1996 | 13 | 2.5 |
| Kiska | F | Breeding | June 1999 | 11 | 5 |
| | | Non-breeding | Oct 1997 | 8 | 3.5 |
| Sugar | F | Breeding | May/June 1997 | 12 | 3 |
| | | Non-breeding | Nov/Dec 1998 | 10 | 4.5 |
| Adak | M | Breeding | May/June 1997 | 14 | 3 |
| Tag | M | Non-breeding | April 1997 | 9 | 3 |
| Sitka | F | Breeding | July 1999 | 7 | 2 |
| | | Non-breeding | March 1999 | 7 | 1.5 |
| Kodiak | M | Breeding | July 1999 | 7 | 2 |
| | | Non-breeding | March 1999 | 7 | 1.5 |
| Hazy | F | Breeding | July 1999 | 7 | 2 |
| | | Non-breeding | November 1999 | 7 | 2.5 |
| Timber | M | Breeding | July 1999 | 7 | 2 |
| | | Non-breeding | November 1999 | 7 | 2.5 |

Blood samples were collected from the caudal gluteal vein using 3.5 inch 18 gauge spinal needles and either collected directly into Vacutainers® blood collection tubes or transferred via a syringe. For the remainder of the fasting study, body mass was measured daily on a platform scale (± 0.1 kg) and blood samples were collected every 3 to 4 days. Blood samples were centrifuged, plasma or serum was removed, and stored frozen at -80°C in cryovials for later analysis.

To maintain optimal health for each animal during fasting, this study was discontinued if body mass loss exceeds 3% of an individual's body mass per day for 2 consecutive days or exceeded a total mass loss of 15% of initial body mass. This occurred five times throughout the study. This rate of mass loss has caused no adverse effects in previous studies and many pinniped species have been shown to lose between 20 and 30% of initial body mass during post-weaning, molting and breeding fasts (Boyd and Duck 1991, Baker et al. 1994, Rea 1995).

Blood Analysis

For this study, NEFA concentration and fatty acid composition of plasma lipids were quantified. Plasma concentrations of NEFAs were measured by spectrophotometric assays (NEFA-C kit, Wako Diagnostics, Richmond, Virginia). These assays were conducted at the laboratory facilities at ASLC and at University of Central Florida (UCF). Blood samples also provided data on plasma concentrations of glucose, blood urea nitrogen, total protein, ketone bodies, plasma water content and specific gravity and the hematocrit and hemoglobin content of whole blood for a concurrent study (Rea 2000).

These indices were used during the fast to determine state of physiological health of the sea lion.

Lipid Extraction

Preparation of serum samples for fatty acid composition analysis (separation, extraction, and methylation) was completed at UCF. Total lipids were extracted from approximately 1g of serum using 2:1 (vol/vol) chloroform-methanol with 0.01% BHT and NaCl according to the method of Folch et al. (1957) and modified by Iverson (1993). If the interface filled the lower phase after the initial centrifugation, 1 ml methanol was added and the sample was vortexed and re-centrifuged. After the upper phase was discarded, the lower phase was carefully removed from below the proteinacious interface. The lower phase containing chloroform and lipids was evaporated under nitrogen using N-EVAPORATOR (Organomation; NEVAP, Berlin, MA, USA), leaving only lipids in the tube. Nitrogen was used to prevent oxidation and further protection of the fatty acids was provided by the addition of BHT (0.01% v/wt) to the original chloroform-methanol solution.

Fatty acids in the lower phase were converted to fatty acid methyl esters (FAME) according to Morrison and Smith (1964) using 12% anhydrous boron trifluoride by weight in methanol (Fisher Scientific). When stored under nitrogen, FAME can be held long-term at -80°C with no deterioration from auto-oxidation. Samples were shipped on dry ice to Dr. Sara Iverson's laboratory at Dalhousie University, Halifax, NS, Canada for gas chromatography (GC) analysis. Duplicate analyses of FAME for identification and quantification were conducted using temperature-programmed GC on a Perkin Elmer

Autosystem II Capillary FID gas chromatograph fitted with a 30m x 0.25 mm id. column (Agilent CDB-23) coated with 50% cyanopropyl polysiloxane (0.25 film thickness) and linked to a computerized integration system (Turbochrome 4.1.2 software).

Thin Layer Chromatography

Separation of four lipid fractions: triglycerides, NEFA, cholesterol esters and phospholipids from the lipid extract were attempted using thin layer chromatography (TLC). The total lipid extract was applied to previously conditioned TLC plates coated with Silica Gel (Aldrich) and the plates were developed in a solvent solution of 80:20:2 (vol/vol/vol) hexane-diethyl ether-formic acid. After separation, the TLC plates were air dried and sprayed with primulin (Sigma) to better visualize the location of each fraction's band. The cholesterol esters, triglycerides, free fatty acids and phospholipid bands were identified using standards. These fractions were scraped onto glass wool and eluted from the silica gel with chloroform. The lipid fraction was eluted into glass tubes for preparation of FAME.

The above process yielded an insufficient quantity of fatty acids for GC analysis and results were unobtainable. Therefore an alternative technique was employed to attempt to preserve the maximum amount of fatty acids during the transesterification process.

The alternative technique involved separating lipid fractions using TLC exactly as above but a new method of transesterifying the lipid fractions was utilized. The eluted fractions suspended in chloroform were incubated under nitrogen with 1.5 ml methylene chloride and 3 ml Hilditch reagent (0.5 N H₂SO₄ in methanol) in the dark for 3-4 days.

After incubating, the fatty acids were rinsed with hexane three times and then dehydrated with NaSO₄. Nitrogen was used to evaporate remaining hexane. Unfortunately, this technique also yielded an insufficient quantity of fatty acids for GC analysis. Therefore, quantification of fatty acids in the 4 lipid fractions separated with TLC was unobtainable and no further results will follow.

Quantifying Fatty Acids

Turbochrome 4.1.2 integrated the fatty acid peaks in the chromatogram into a relative abundance format. The chromatogram consisted of several peaks, with the area under each peak representing the amount of the corresponding fatty acid that was mobilized through the column at the particular retention time at which the peak was displayed (Appendix A). The peaks were inspected to ensure a unified baseline and adjusted when necessary to manually separate any peaks that may have eluted together. This process also ensured accurate peak detection of each fatty acid. The area under each peak was directly correlated to the weight percent (wt %) of that particular fatty acid in the injected sample resulting in quantitative fatty acid composition data for each sample. Sixty-nine fatty acids were quantified from each sample however, only those with concentrations above 0.2 wt % were included in the analysis. An arc-sine transformation was utilized on all fatty acid analysis and the sub-adult samples were grouped on a % mass loss basis (0%, 7-8% and 15% mass loss) to facilitate comparison with the juveniles. These data represented the total lipid fatty acid composition of each blood sample.

Relative Lipid Fatty Acid Concentration

During fasting, lipids were mobilized as free fatty acids bound to albumen protein, and rarely esterified to triglycerides or any other molecule. Although the total lipid analysis contained other lipid fractions such as phospholipids and cholesterol esters, these lipids are not typically mobilized and used for energy during fasting and therefore were assumed to contribute relatively little to the results of changes in fatty acid concentration. Since the thin layer chromatography analysis produced unsuccessful results in this study, plasma NEFA concentration was the closest measure of lipid concentration obtainable for this data set. To calculate an index of the relative concentration of each fatty acid in the blood I multiplied the total lipid fatty acid compositional analysis (wt %) by the NEFA concentrations measured in that respective blood sample. The relative fatty acid concentration indicated if the absolute amount of a fatty acid is reaching critically low levels based on overall plasma lipid levels.

Statistical Analysis

Juvenile and sub-adult samples were analyzed separately due to the different body mass and length of fast. NEFA concentrations were analyzed for statistical difference over the different stages of fasting using an ANOVA (SPSS 8.0 student version). NEFA concentrations of the breeding and non-breeding season were compared using a paired t-test. All relationships were determined to be significant at $p < 0.05$.

A paired t-test (SPSS 8.0 student version) compared seasonal differences in the juveniles and sub-adults for both the total lipid fatty acid composition and relative lipid concentration data. An ANOVA with a Bonferroni post-hoc test (SPSS 8.0 student

version) analyzed differences in fatty acid composition between stages of fasting for both the total lipid fatty acid composition and relative fatty acid concentration data. All groups were normally distributed with homogeneity of variances except for the sub-adults relative lipid content. This was corrected with a log transformation. All relationships were considered to be significantly different at $p < 0.05$.

Due to the large number of fatty acids, a data reduction technique known as principle components analysis (PCA) was employed. Principle component analysis was used as a data reduction technique to indicate which specific fatty acids were driving statistical changes. By graphing the eigenvectors calculated by PCA, samples that are similar group together where as samples that are dissimilar may group in a separate area of the graph. The fatty acids that have most influence in causing these samples to group have the largest (positive or negative) eigenvector for the axes that are graphed. Eigenvectors can be calculated for several axes however only the first two or three will be used in this analysis. The axes determine how much of the variation in the data is explained and the first two or three usually explain about 65-70% of the variation.

A MANOVA was used to determine if the eigenvectors of the first two axes significantly separated the samples used in the analysis (SPSS 8.0 student version).

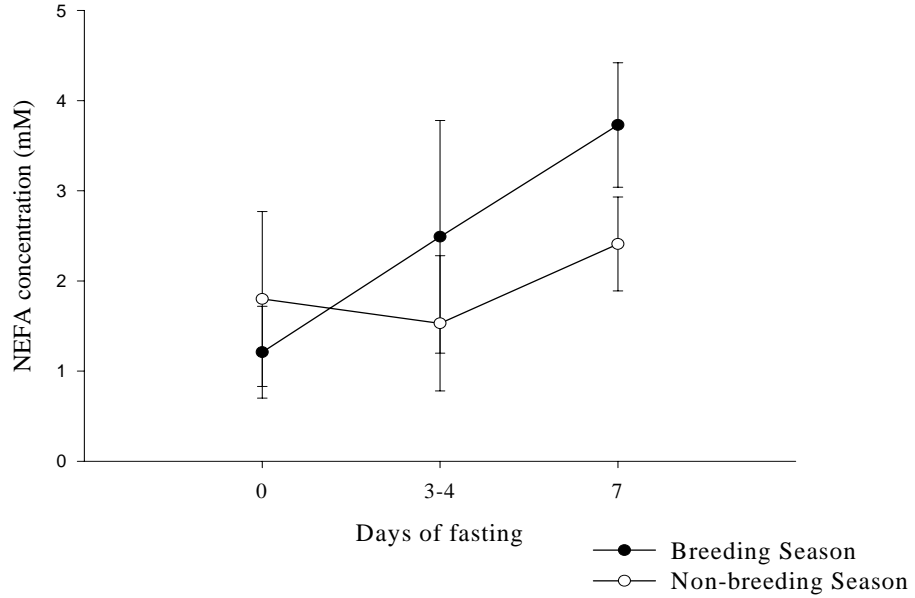
RESULTS

Lipid Concentration

Plasma NEFA concentrations were used as an indicator of total plasma lipid concentration. The plasma NEFA concentration in juveniles significantly increased during the breeding season fast from 1.2 ± 0.51 mM (mean \pm SD) after an overnight fast to 3.7 ± 0.69 mM after 7 days of fasting ($p < 0.05$; Fig. 1A). Amongst the juveniles, NEFA concentrations measured during the breeding season did not differ significantly from those measured during comparable days during the non-breeding season ($p = 0.8$). NEFA concentrations of the non-breeding season did not significantly differ ($p = 0.5$, Fig 1A) throughout the week of fasting.

Plasma NEFA concentration did not increase significantly during the breeding season in the sub-adults but did range from 1.3 ± 0.30 mM in after an overnight fast to 6.5 ± 3.67 mM after 12-14 days of fasting ($p = 0.1$, Fig. 1B). Additionally, plasma NEFA concentrations during the breeding season did not significantly differed from concentrations during the non-breeding season in the sub-adults ($p = 0.2$, Fig. 1B). Mean juvenile and sub-adult NEFA concentrations were within the same range of approximately 1.2 to 4 mM with the exception of the final stage of the sub-adults breeding season fast where higher concentrations were measured (Fig. 1B).

(A.)



(B.)

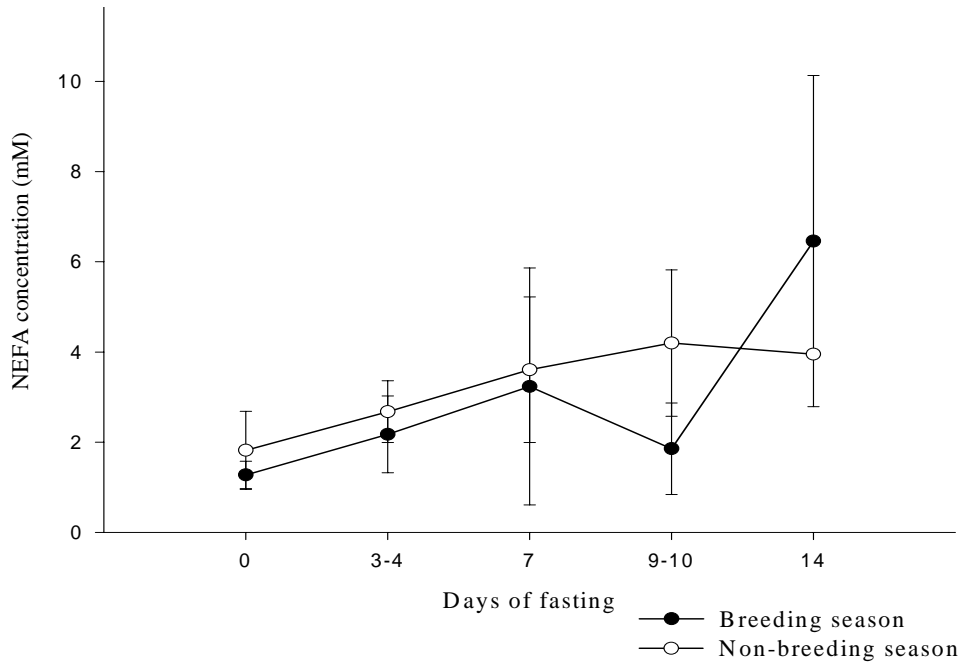


Figure 1. Plasma non-esterified fatty acid (NEFA) concentrations of juvenile (A) and sub-adult (B) Steller sea lions in both the breeding and non-breeding season fasts. Values are means \pm SD.

Total Lipid Fatty Acid Composition

Juveniles

Serum lipid fatty acid composition yielded a variety of trends during the fasting trials and when compared seasonally. Out of the 29 fatty acids that were present in concentrations above 0.2 wt % (see Appendix B), only 20:0 was significantly higher during the breeding season compared to the non-breeding season in fasting juvenile Steller sea lions (Table 2; $p=0.001$). This saturated fatty acid was 1.7 fold higher after an overnight fast and 1.8 fold higher at the end of the fast. Due to the similarity in composition between seasons, both seasons were combined to assess the affect of fasting on fatty acid composition of the serum lipids.

Table 2. The mean wt % of the fatty acid significantly different between the breeding and non-breeding seasons during fasting in juvenile Steller sea lions.

| Fatty acid wt % | Breeding Season | | | Non-Breeding Season | | |
|--------------------|-----------------|----------------------|------------------------|---------------------|----------------------|------------------------|
| | 0% Mass Loss | 7-8% Mass Loss | 14-15% Mass Loss | 0% Mass Loss | 7-8% Mass Loss | 14-15% Mass Loss |
| 20:0 | 0.2 | 0.34 | 0.26 | 0.35 | 0.36 | 0.46 |

Upon analysis of both of the seasons together, the mean wt % of 7 fatty acids changed significantly during fasting. These fatty acids were comprised of 2 MUFA and 5 polyunsaturated fatty acids (PUFA). The mean of a 16 carbon chain fatty acid, 16:1n-11, was higher after an overnight fast and decreased 1.3 fold during fasting ($p=0.02$; Table 3) and the mean of 16:2n-4 was 1.5 fold higher after an overnight fast than it was after the sea lions lost 7-8% body mass ($p=0.04$; Table 3).

The mean wt % of 18:1n-9 and of 18:2n-6 increased 1.2 fold ($p=0.02$) and 1.7 fold ($p=0.007$) respectively during fasting (Table 3). The mean wt % of 20:3n-6 increased 1.4 fold ($p=0.002$) from an overnight fast to the mid-fast sample and increased 1.7 fold ($p=0.004$) between the beginning and end of the fast (Table 3). The mean wt % of 20:5n-3 decreased 1.6 fold ($p<0.001$) during the first half of the fast and 2.0 fold ($p<0.001$) over the entire fast (Table 3).

Finally, the mean wt % 22:5n-3 decreased 1.2 fold ($p=0.05$) during the first half of the fast and 1.5 fold ($p<0.001$) during the entire fast (Table 3). This fatty acid, in addition to the 18 and 20 carbon chain length fatty acid represent 25% of the total long chained fatty acids and half of the PUFAs analyzed in fasting juveniles.

Table 3. The mean wt % of 7 fatty acids that significantly changed during fasting in juvenile Steller sea lions. Different letters denote significant changes for the given fatty acid.

| Fatty acid wt % | 0% Mass Loss | 7-8% Mass Loss | 14-15% Mass Loss |
|-----------------|--------------------|--------------------|--------------------|
| 16:1n-11 | 0.42 ^a | 0.34 ^a | 0.33 ^b |
| 16:2n-4 | 0.19 ^a | 0.13 ^b | 0.14 ^b |
| 18:1n-9 | 12.94 ^a | 13.60 ^a | 15.10 ^b |
| 18:2n-6 | 1.29 ^a | 1.88 ^b | 2.12 ^b |
| 20:3n-6 | 0.37 ^a | 0.50 ^b | 0.48 ^b |
| 20:5n-3 | 9.60 ^a | 5.91 ^b | 4.92 ^b |
| 22:5n-3 | 2.16 ^a | 1.74 ^b | 1.46 ^b |

Sub-adults

The mean wt % compositions of 10 fatty acids were significantly different between the breeding season and the non-breeding season fasts in sub-adults (Table 4). The mean of iso 16:0 was 1.7, 1.4 and 1.5 fold ($p=0.008$) higher in the beginning, middle and end of the non-breeding season fast, respectively, and the mean of 17:0 was 4.3, 3.0 and 1.9 fold ($p=0.003$) higher, in the non-breeding season (Table 4).

Of the 8 fatty acids identified in the 18 carbon chain length family, the wt % of 4 fatty acids were significantly different between seasons. The mean wt % of 18:1n-9 was 1.6, 1.3 and 1.3 fold ($p=0.001$) higher respectively in the beginning, middle and end of the non-breeding season fast and in the same fashion, the mean wt % of 18:1n-7 was 1.3, 1.1 and 1.2 fold ($p=0.004$) higher during non-breeding season (Table 4). The other MUFA, 18:1n-5, had mean wt %s 1.9, 2.7, and 1.9 fold ($p=0.02$) higher respectively in the beginning, middle, and end of the breeding season fast compared to the non-breeding season fast (Table 4). The mean wt % of 18:4n-3 was 1.8, 2.9 and 2.0 fold ($p=0.02$) higher during the breeding season in the same pattern as above in 18:1n-5 (Table 4).

The MUFA 20:1n-11 was 4.0, 4.1 and 2.8 fold ($p=0.009$) higher during the beginning, middle and end of the breeding season fast (Table 4). The mean wt % of 2 PUFAs, 20:3n-6 and 20:4n-6 were 1.6, 1.1 and 1.4 fold ($p=0.004$) and 1.6, 1.2 and 1.6 fold ($p=0.003$) higher respectively during the beginning middle and end of the non-breeding season fast (Table 4). Lastly, the mean wt % of 22:1n-11 was 2.0, 2.7 and 1.8 fold ($p=0.03$) higher during the breeding season fast (Table 4).

Table 4. The mean wt % of 10 fatty acids that significantly changed during fasting between the breeding and non-breeding season in sub-adult Steller sea lions. Each column in breeding season is significantly different from the corresponding % mass loss for the non-breeding season.

| Fatty acid wt % | Breeding Season | | | Non-Breeding Season | | |
|--------------------|-----------------|-------------------|---------------------|---------------------|-------------------|---------------------|
| | 0% Mass Loss | 7-8% Mass Loss | 14-15% Mass Loss | 0% Mass Loss | 7-8% Mass Loss | 14-15% Mass Loss |
| Iso 16:0 | 0.18 | 0.26 | 0.26 | 0.31 | 0.38 | 0.38 |
| 17:0 | 0.25 | 0.25 | 0.39 | 1.08 | 0.73 | 0.75 |
| 18:1n-9 | 12.14 | 16.85 | 16.73 | 19.41 | 21.08 | 21.85 |
| 18:1n-7 | 3.95 | 5.63 | 4.97 | 5.16 | 6.04 | 6.13 |
| 18:1n-5 | 0.27 | 0.30 | 0.30 | 0.15 | 0.11 | 0.16 |
| 18:4n-3 | 0.13 | 0.19 | 0.10 | 0.07 | 0.07 | 0.05 |
| 20:1n-11 | 2.02 | 2.17 | 1.98 | 0.50 | 0.53 | 0.71 |
| 20:3n-6 | 0.26 | 0.48 | 0.36 | 0.41 | 0.53 | 0.53 |
| 20:4n-6 | 9.14 | 13.20 | 9.20 | 14.31 | 16.21 | 15.03 |
| 22:1n-11 | 0.77 | 1.03 | 0.92 | 0.39 | 0.39 | 0.52 |

Of the 28 fatty acids analyzed, the means of 11 fatty acids were significantly different between the breeding and non-breeding season. Only 2 of these were saturates, while 6 were MUFAs and 3 were PUFAs. Out of the 11 fatty acids that differed between seasons, only 18:1n-7 also differed significantly during fasting. In addition, the wt % of 10 other fatty acids significantly changed during fasting (Table 5).

The mean wt % 14:0 increased 2.9 fold ($p=0.005$) during the first half of the fast and 3.1 fold ($p=0.003$) over the entire fast (Table 5). Likewise, the mean wt % of 16:1n-7 increased 2.0 fold ($p=0.008$) from the beginning to the end of the fast (Table 5). The mean wt % of 16:2n-4 decreased 2.1 fold ($p=0.009$) from the beginning of the fast until the sea lions experienced 7-8% mass loss then increased 1.9 fold ($p=0.03$) from the middle of the fast until they lost 15% of their mass (Table 5).

The saturate 18:0 decreased 1.3 fold ($p=0.004$) from the beginning to the end of the fasts while the MUFA 18:1n-7 increased 1.3 fold ($p=0.008$) during the first half of the both the breeding season and non-breeding season fasts (Table 5). The mean wt % of the PUFA 18:2n-6 increased 1.7 fold ($p<0.001$) over the course of the fast (Table 5).

The saturate 20:0 and the MUFA 20:1n-9 increased 1.8 fold ($p=0.003$) and 1.7 fold ($p=0.015$) respectively from the beginning to the end of the fast (Table 5). The mean wt % of 20:0 also increased 1.9 fold ($p=0.001$) from the beginning of the fast until the sea lions had a 7-8% mass loss (Table 5). The mean wt % of 20:5n-3 decreased 1.4 fold ($p=0.006$) during the first half of the fast and 1.6 fold ($p=0.001$) over the entire fast (Table 5).

The PUFA 22:5n-3 decreased 1.2 fold ($p=0.02$) during the first half of the fast and 1.3 fold ($p=0.002$) over the entire fast (Table 5). A wide range of fatty acids showed significant changes during the sub-adult fasts. In total, 3 saturated, 4 monounsaturated and 4 PUFAs changed comprising of 6 different chain length fatty acids.

Table 5. The mean wt % of 10 fatty acids that significantly changed during fasting in sub-adult Steller sea lions. Different letters denote significant changes for the given fatty acid.

| Fatty acid wt % (wt %) | 0% Mass Loss | 7-8% Mass Loss | 14-15% Mass Loss |
|---------------------------|--------------------|--------------------|--------------------|
| 14:0 | 0.33 ^a | 0.96 ^b | 1.04 ^b |
| 16:1n-7 | 1.67 ^a | 2.61 ^{ab} | 3.27 ^b |
| 16:2n-4 | 0.35 ^a | 0.17 ^b | 0.32 ^a |
| 18:0 | 13.32 ^a | 12.23 ^a | 10.36 ^b |
| 18:1n-7 | 4.64 ^a | 5.91 ^b | 5.59 ^a |
| 18:2n-6 | 1.17 ^a | 1.98 ^b | 2.01 ^b |
| 20:0 | 0.23 ^a | 0.45 ^b | 0.43 ^b |
| 20:1n-9 | 0.72 ^a | 1.00 ^b | 1.22 ^b |
| 20:5n-3 | 9.33 ^a | 6.59 ^b | 5.88 ^b |
| 22:5n-3 | 2.28 ^a | 1.83 ^b | 1.69 ^b |

Relative Fatty Acid Concentration

Juveniles

There were no seasonal differences in relative fatty acid concentrations calculated in the juveniles using the NEFA concentrations and the percent weight fatty acid composition data. However, the mean relative concentrations of 18 fatty acids changed significantly during fasting. Two saturated fatty acids 14:0 and 15:0 increased 1.8 fold ($p=0.05$) and 2.1 fold ($p=0.02$), respectively, from the beginning to the end of the fasts (Fig. 2).

An isomer of the saturated fatty acid 16:0 increased 2.6 fold ($p=0.02$) during fasting (Fig. 3) while 16:0, 16:1n-7, and 16:2n-4 increased 1.2 fold ($p=0.01$; Fig. 3), 2.4 fold ($p=0.01$) and 1.6 fold ($p=0.05$) (Fig. 4) respectively between the sample taken after an overnight fast and the final sample taken 7 days later after 14-15% mass lost in the sea lions. Mean concentrations of the MUFA and PUFA fatty acids, 16:1n-7 and 16:2n-4 increased 2.1 fold ($p=0.02$) and 1.8 fold ($p=0.01$) respectively between the beginning and half way through the fast after the sea lions lost 7-8% body mass (Fig. 4). The mean relative concentration of 17:0 increased 2.0 fold ($p=0.04$) from the beginning to the end of the fasts (Fig. 5). These 7 fatty acids comprise more than 75% of the middle chain length fatty acids and all of the saturated fatty acids and PUFA's in this range.

In the 18 carbon chain length family, the mean concentration of 4 fatty acids increased significantly. Three of these were MUFA, 18:1n-11, 18:1n-9 and 18:1n-7 increased 3.0 fold ($p=0.001$), 2.3 fold ($p=0.003$), and 2.3 fold ($p=0.006$) respectively

between the beginning and end of fasting (Fig. 5). Both 18:1n-11 and 18:1n-9 also significantly increased 2.1 fold ($p=0.007$) and 2.0 fold ($p=0.01$) respectively between the middle and the end of the fasts (Fig.5). One PUFA, 18:2n-6, increased 3.2 fold ($p=0.001$) from the beginning to the end of the fasts (Fig. 6). The increase between the middle and end of the fasts for 18:2n-6 was also significant as the mean concentration increased 2.1 fold ($p=0.02$) (Fig.6). Only 3 fatty acids (1 saturate, 2 MUFA and 1 PUFA) did not change significantly in the 18 carbon family.

Mean concentration of the saturate 20:0 increased 2.4 fold ($p=0.004$) from the beginning to the end of the fasts and increased 1.8 fold ($p=0.05$) from the middle to the end of the fasts (Fig. 7). Following the same pattern, the mean concentration of 2 MUFA fatty acids, 20:1n-11 and 20:1n-9 increased 2.3 fold ($p=0.002$) and 2.3 fold ($p=0.005$) respectively between the beginning and end of the fasts and 1.7 fold ($p=0.03$) and 1.9 fold ($p=0.03$) respectively between the middle and end of the fasts (Fig. 7). The mean concentration of 3 PUFAs in this family also increased significantly; 20:2n-6, 20:3n-6 and 20:4n-6 increased 2.3 fold ($p=0.009$), 2.1 fold ($p=0.003$) and 2.1 fold ($p=0.03$) respectively between the beginning and end of the fasts (Fig. 7). The concentration of 20:3n-6 also increased 1.9 fold ($p=0.05$) between the middle and the end of the fast (Fig. 7). These six 20 carbon chained fatty acids comprise of 75% of the fatty acids analyzed in this family, leaving only two PUFAs that did not significantly change (Fig. 6 & 7).

Of the 29 fatty acids included in this analysis the relative concentration of 18 increased significantly. Five of the fatty acids were saturates, 8 were MUFAs, and 5 were PUFAs. To summarize, the relative concentration of 5 fatty acids increased in the

serum more than 4 mM during fasting (Fig. 8). This includes 16:n11 18:1n-9, 18:1n-7, 18:2n-6 and 20:4n-6. The highest increase of 28 mM measured during fasting was for 20:4n-6.

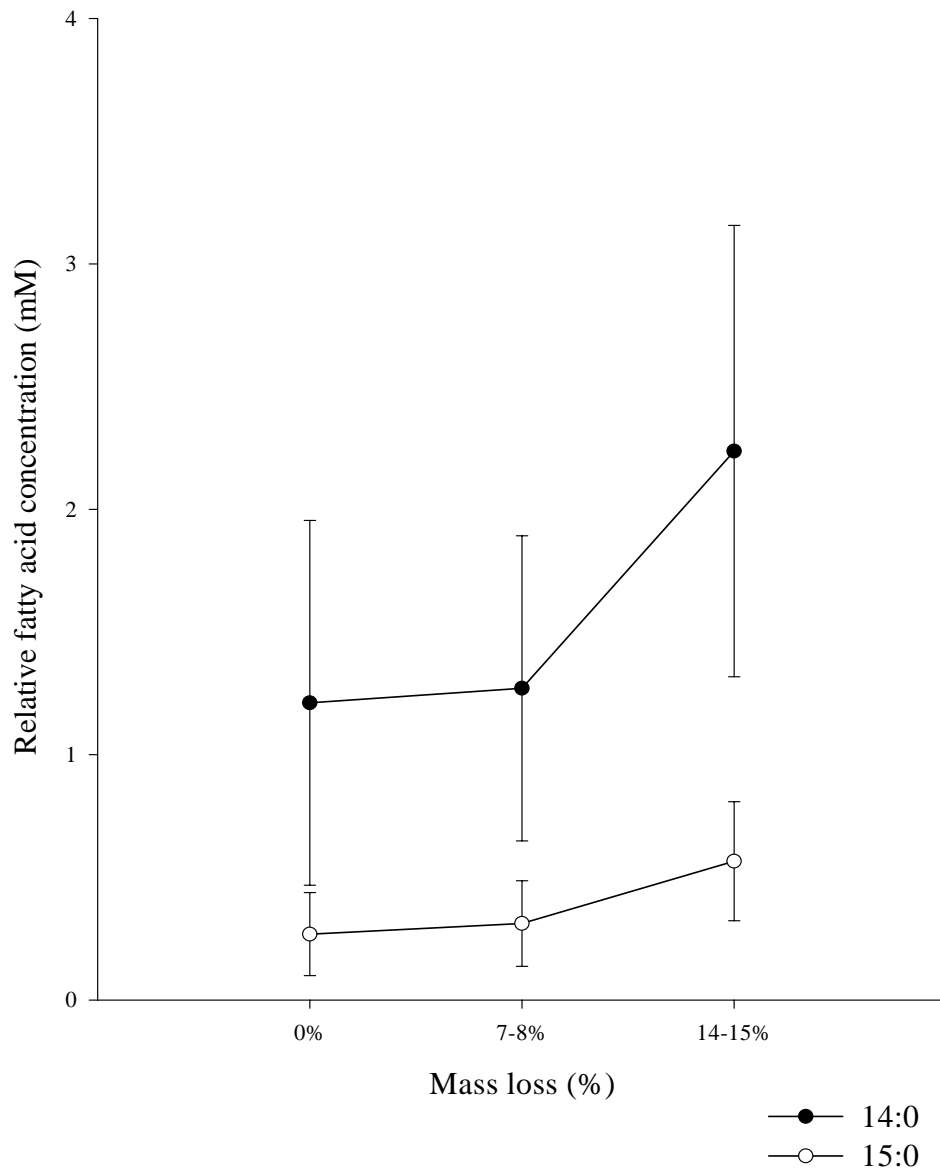


Figure 2. Changes in the mean relative fatty acid concentration of 14:0 and 15:0 during fasting in juvenile Steller sea lions. Breeding and non-breeding seasons were combined. Values represent means \pm SD.

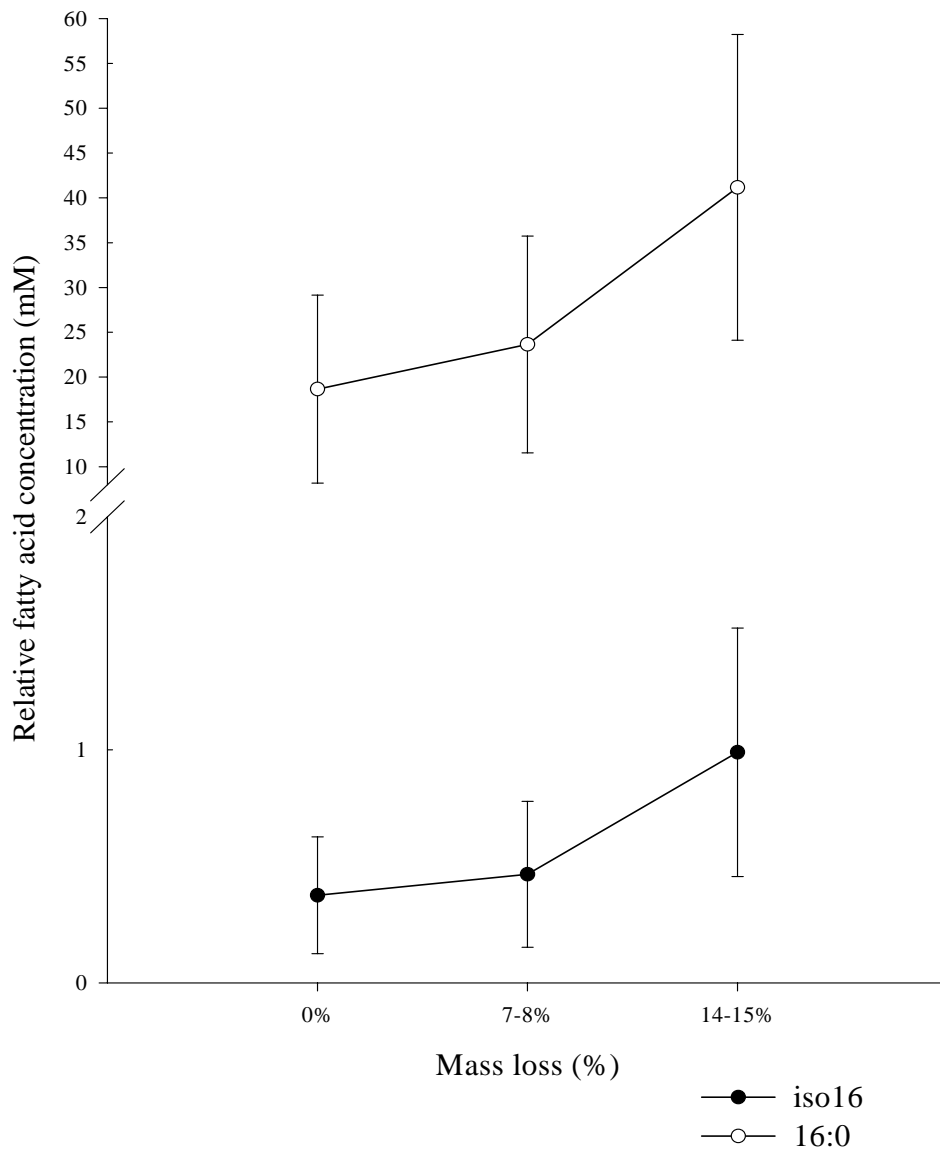


Figure 3. Changes in the mean relative fatty acid concentration of 16:0 and its isomer during fasting in juvenile Steller sea lions. Breeding and non-breeding seasons were combined because of no seasonal difference. Values represent means \pm SD.

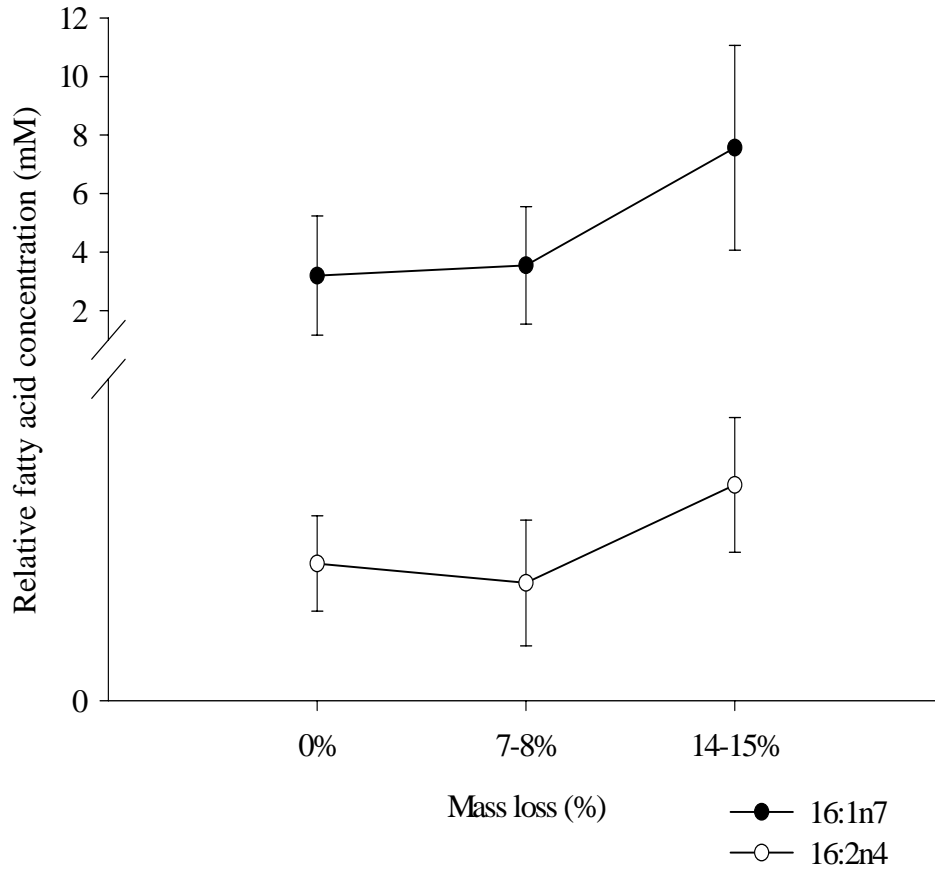


Figure 4. Changes in the mean relative fatty acid concentration of 16:1n-7 and 16:2n-4 during fasting in juvenile Steller sea lions. Breeding and non-breeding seasons were combined because of no seasonal difference. Values represent means \pm SD.

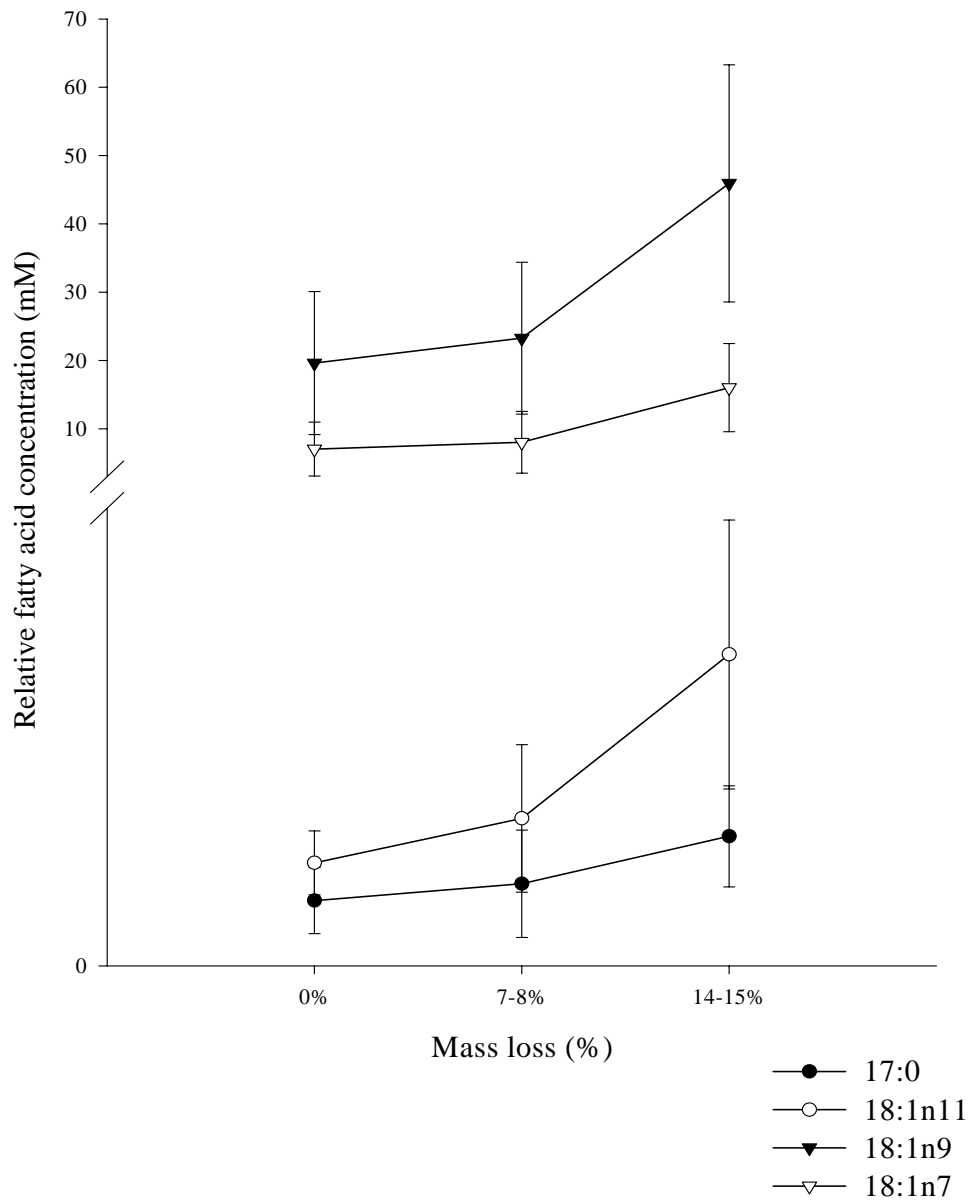


Figure 5. Changes in the mean relative fatty acid concentration 17:0, 18:1n-11, 18:1n-9, and 18:1n-7 during fasting in juvenile Steller sea lions. Breeding and non-breeding season were combined because of no seasonal difference. Values are means \pm SD.

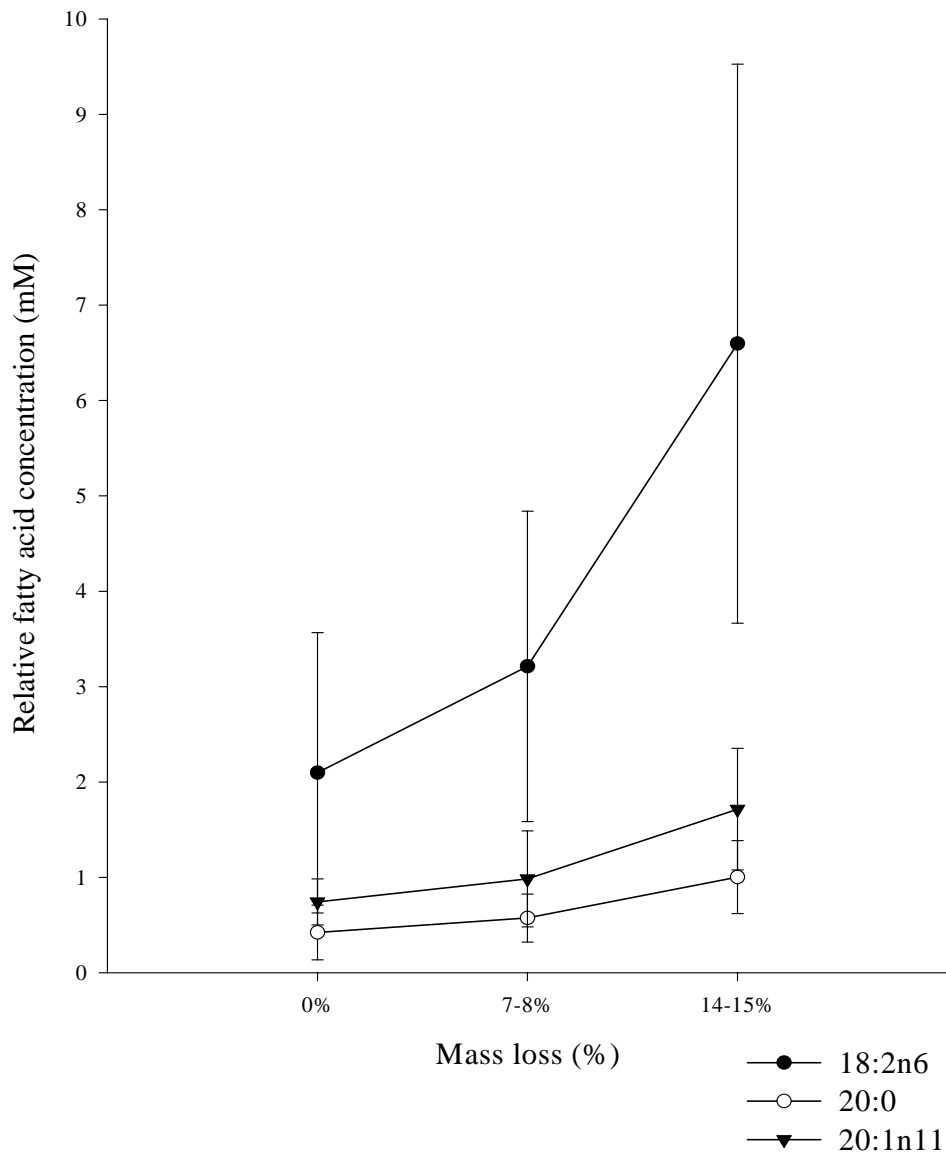


Figure 6. Changes in the mean relative fatty acid concentration of 18:2n-6, 20:0 and 20:1n-11 during fasting in juvenile Steller sea lions. Breeding and non-breeding seasons were combined because of no seasonal difference. Values represent means \pm SD.

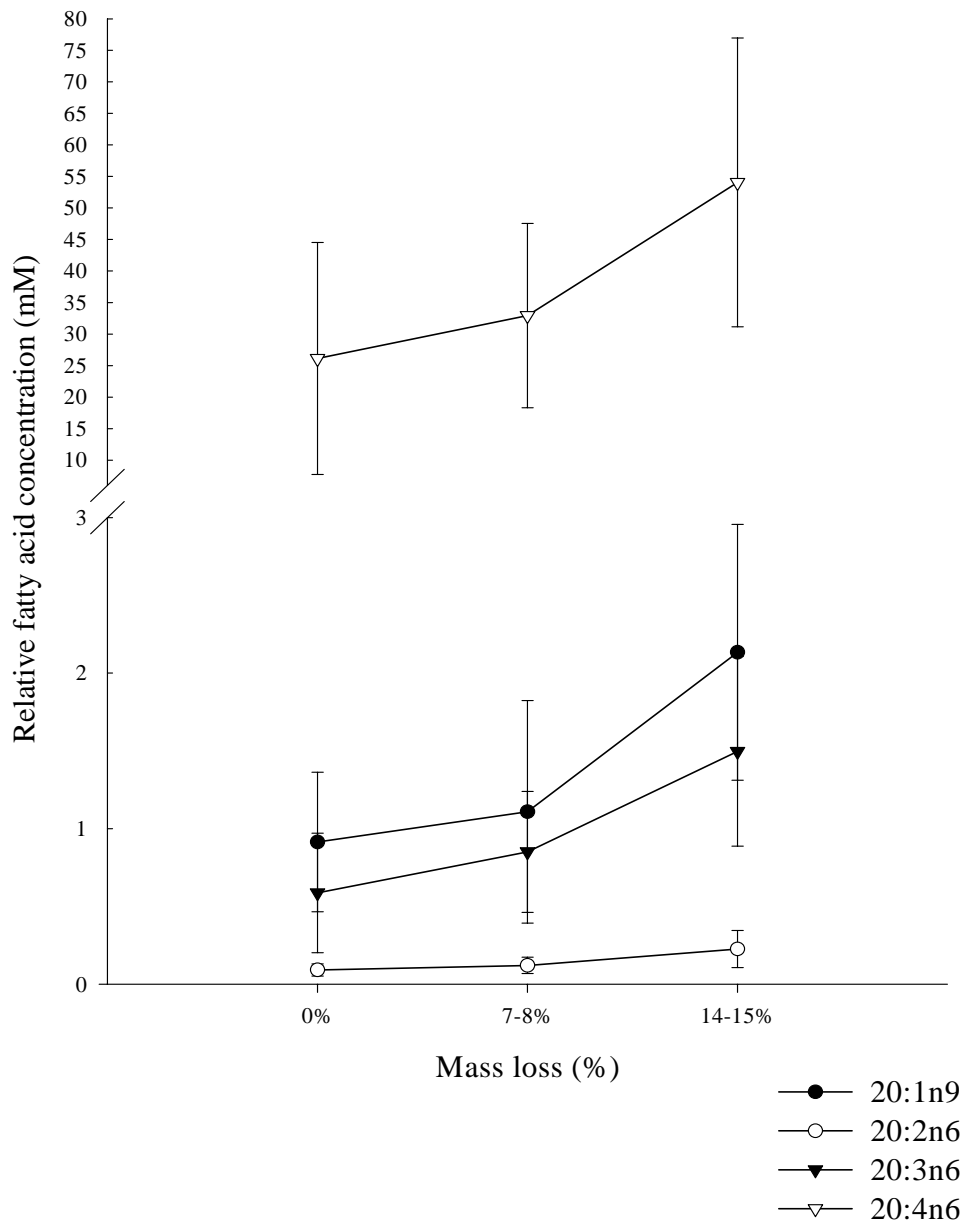


Figure 7. Changes in the mean relative fatty acid concentration of 20:1n-9, 20:2n-6, 20:3n-6 and 20:4n-6 during fasting in juvenile Steller sea lions. Breeding and non-breeding season were combined because of no seasonal difference. Values represent means \pm SD.

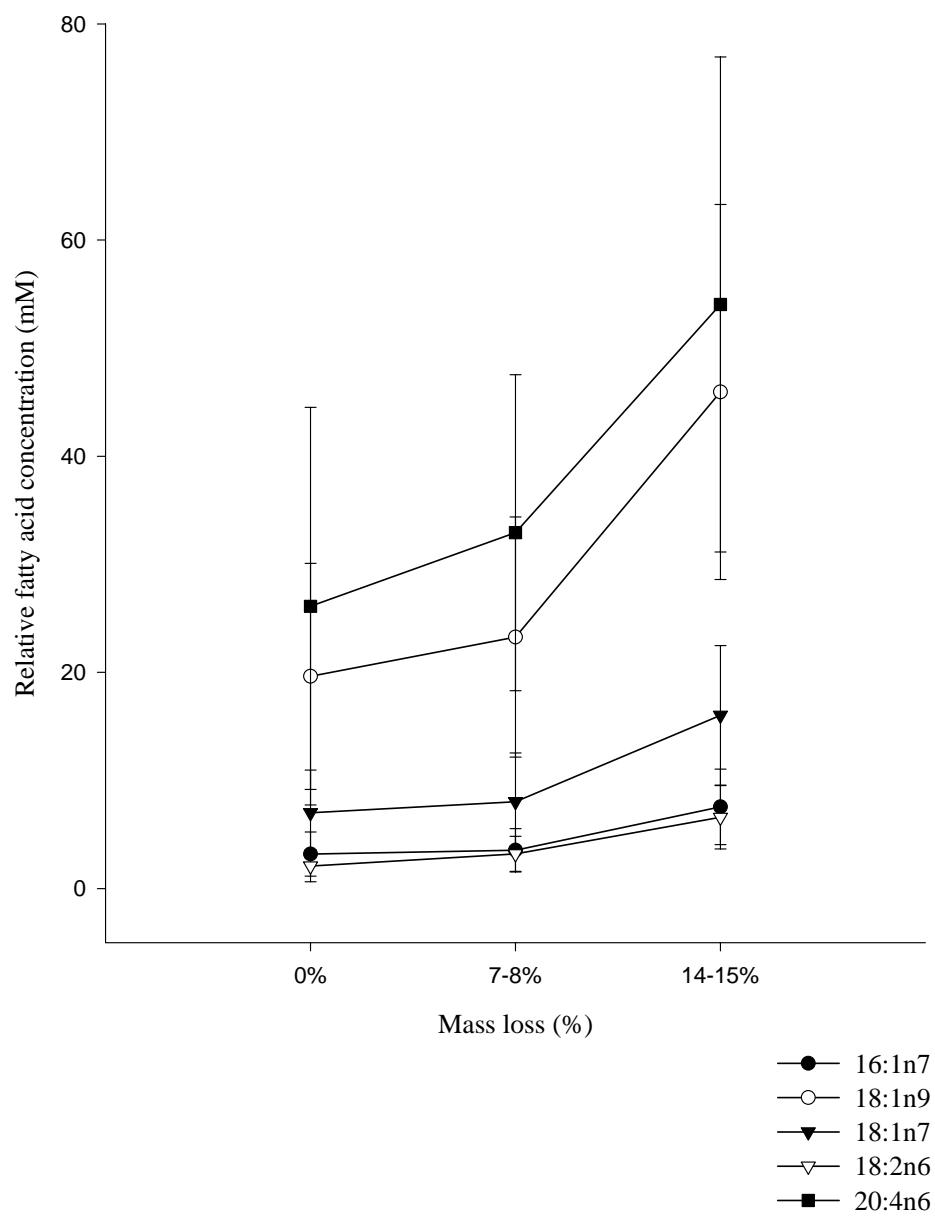


Figure 8. Mean relative fatty acid concentrations with the highest mean concentration changes in juvenile sea lions. Values represent means \pm SD.

Sub-adults

An index of the relative fatty acid concentration in the blood was calculated by multiplying the total lipid fatty acid compositional analysis (wt %) by the NEFA concentrations measured in that respective blood sample to determine the overall concentration of each individual fatty acid mobilized. The relative concentration of only one fatty acid differed significantly between the breeding and non-breeding season fasts. The relative concentration of 20:1n-11 was 2.8, 2.9 and 1.8 fold ($p=0.04$) higher during the beginning, middle and end respectively of the breeding season fast compared to the non-breeding season fast (Fig 9).

Although the relative concentration of only 1 fatty acid differed significantly between seasons, the concentration of 14 fatty acids changed significantly during fasting. The concentration of the 3 saturates 14:0, 15:0 and 16:0 increased 8.7 fold ($p=0.001$), 4.6 fold ($p=0.025$) and 4.8 fold ($p=0.002$) respectively from the beginning to the end of the fast (Fig. 10). In addition, 14:0 and 16:0 increased 4.7 fold ($p=0.015$) and 3.1 fold ($p=0.01$) respectively in just the first half of the fast (Fig. 10 and Fig. 11). The concentration of 16:1n-7 and 16:2n-4 increased 6.7 fold ($p=0.001$) and 3.9 fold ($p=0.03$) respectively over the entire fast and 16:1n-7 also increased 3.3 fold ($p=0.04$) just in the first half of the fasts (Fig. 11). These fatty acids represent over half of the fatty acids containing 14-17 carbons.

The mean relative concentrations of 18:1n-9 and 18:1n-7 increased 3.9 fold ($p=0.005$) and 3.8 fold ($p=0.004$) respectively from the beginning to the end of the fast

(Fig. 12). The concentration of 18:1n-7 increased 2.5 fold ($p=0.045$) from the beginning of the fast until the sea lions experienced a 7-8% mass loss (Fig. 12). One other 18 carbon chain length fatty acid, 18:2n-6 increased 3.3 fold ($p=0.01$) during the first half of the fast and 5.5 fold ($p<0.001$) over the entire fast (Fig. 12).

The saturate, 20:0, increased 3.5 fold ($p=0.01$) in the first half of the fast and 5.1 fold ($p<0.001$) over the entire fast (Fig. 13). The MUFAs 20:1n-11 and 20:1n-9 increased 3.0 fold ($p=0.02$) and 5.4 fold ($p=0.001$) respectively from the beginning to the end of the fast (Fig. 13). The PUFA 20:4n-6 increased 3.2 fold ($p=0.02$) during fasting (Fig. 13).

The relative concentration of 22:1n-11 and 22:6n-3 increased 4.1 fold ($p=0.03$) and 2.9 fold ($p=0.02$) respectively over the course of the fast (Fig. 14). These 14 fatty acids comprise of 4 saturates, 7 MUFAS and 3 PUFA from 6 different chain lengths.

To summarize 5 fatty acids increased greater than 20 mM during fasting (Fig 15). This includes 16:0, 18:1n-9, 18:1n-7, 20:4n-6 and 22:6n-3, 4 of which are long chained and 1 is saturated, for energy while in a nutrient-limited state.

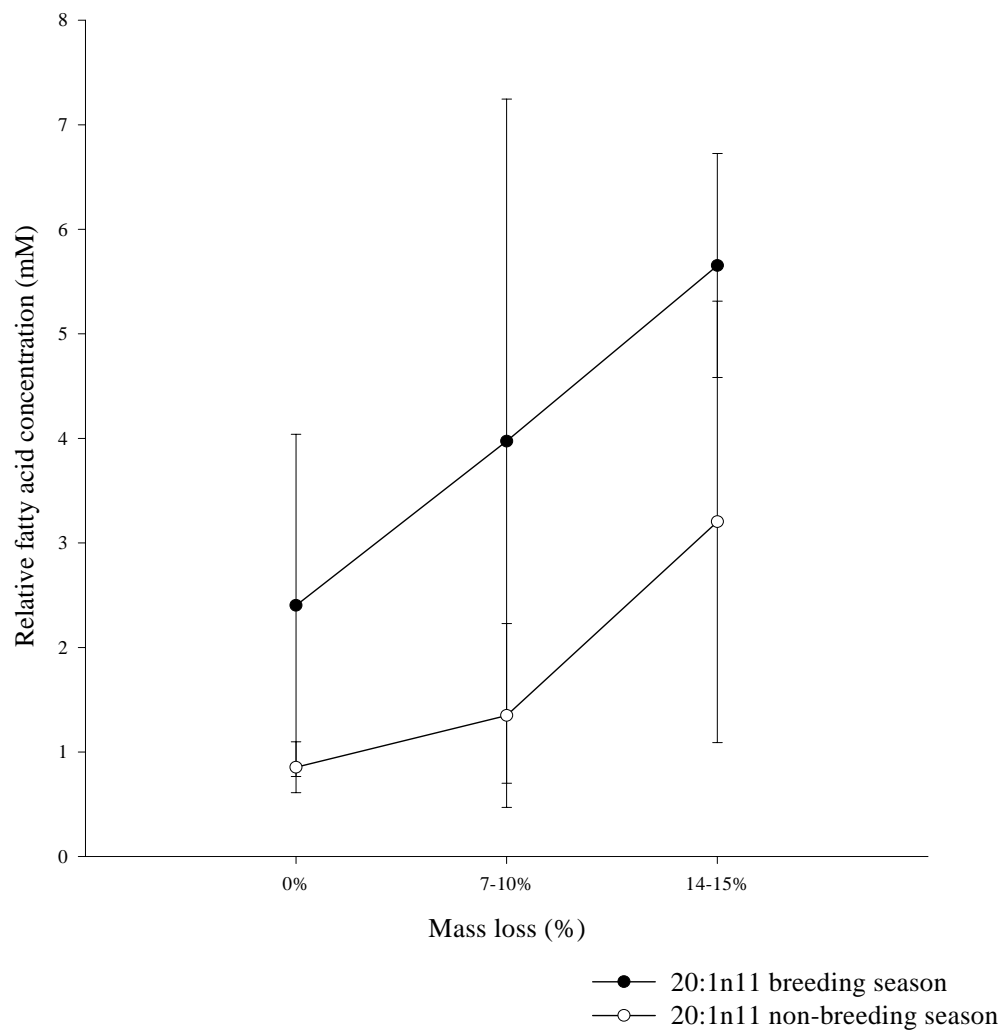


Figure 9. Changes in mean relative fatty acid composition of 20:n11 during breeding and non-breeding season fasting in sub-adult Steller sea lions. Values are means \pm SD.

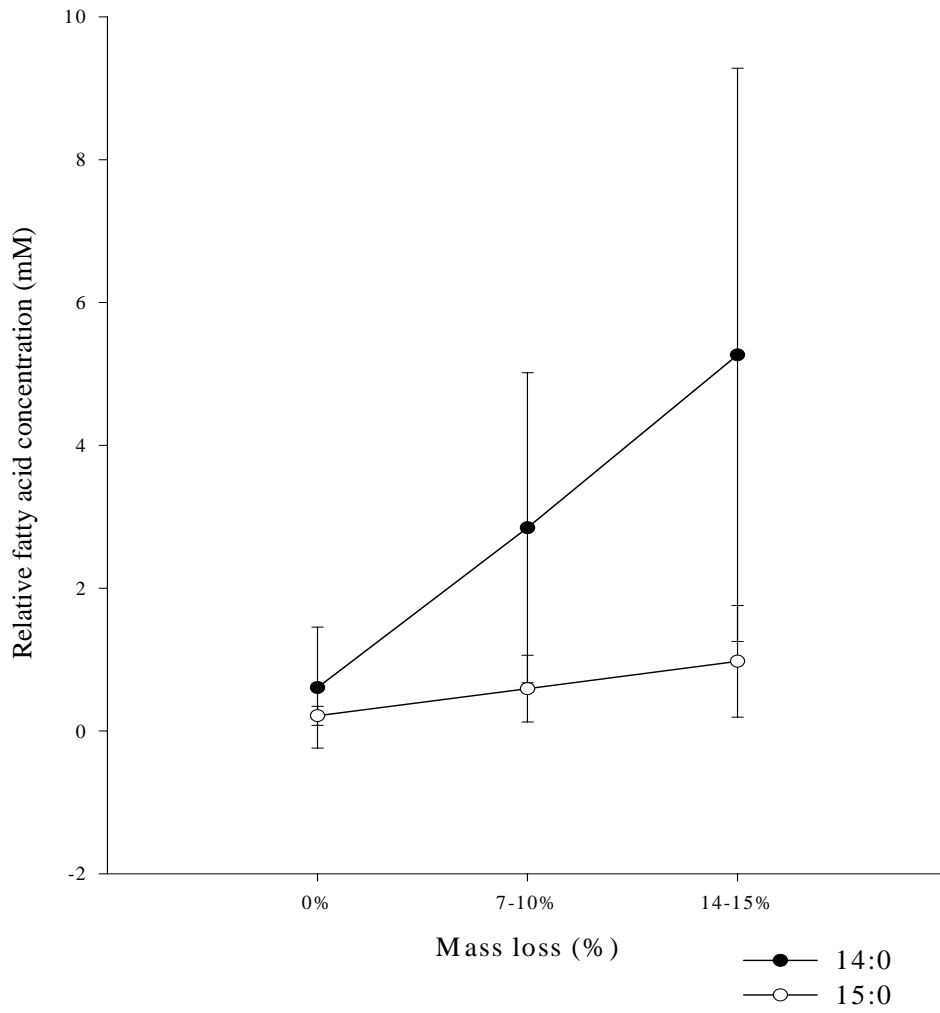


Figure 10. Changes in the mean relative fatty acid concentration of 14:0 and 15:0 during fasting in sub-adult Steller sea lions. Values represent means \pm SD.

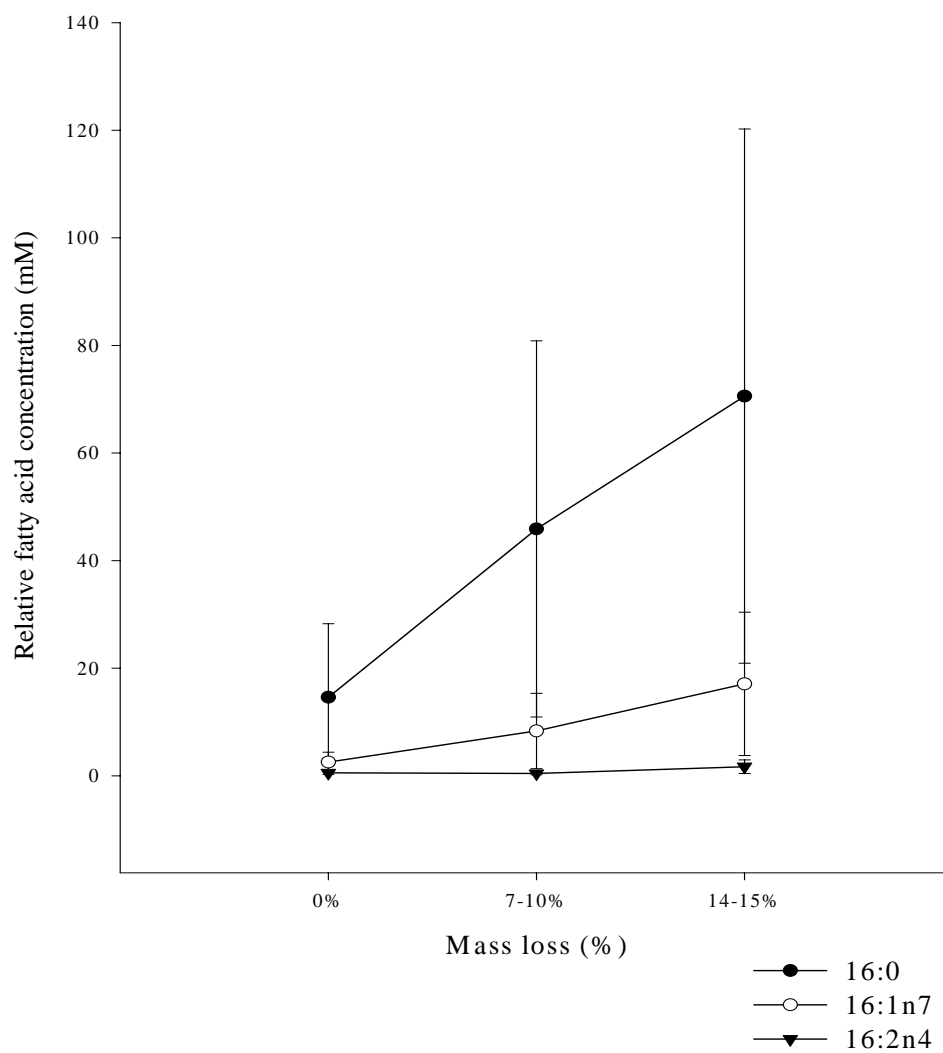


Figure 11. Changes in mean relative fatty acid concentration of 16:0, 16:1n-7 and 16:2n-4 during fasting in sub-adult Steller sea lions. Values represent means \pm SD.

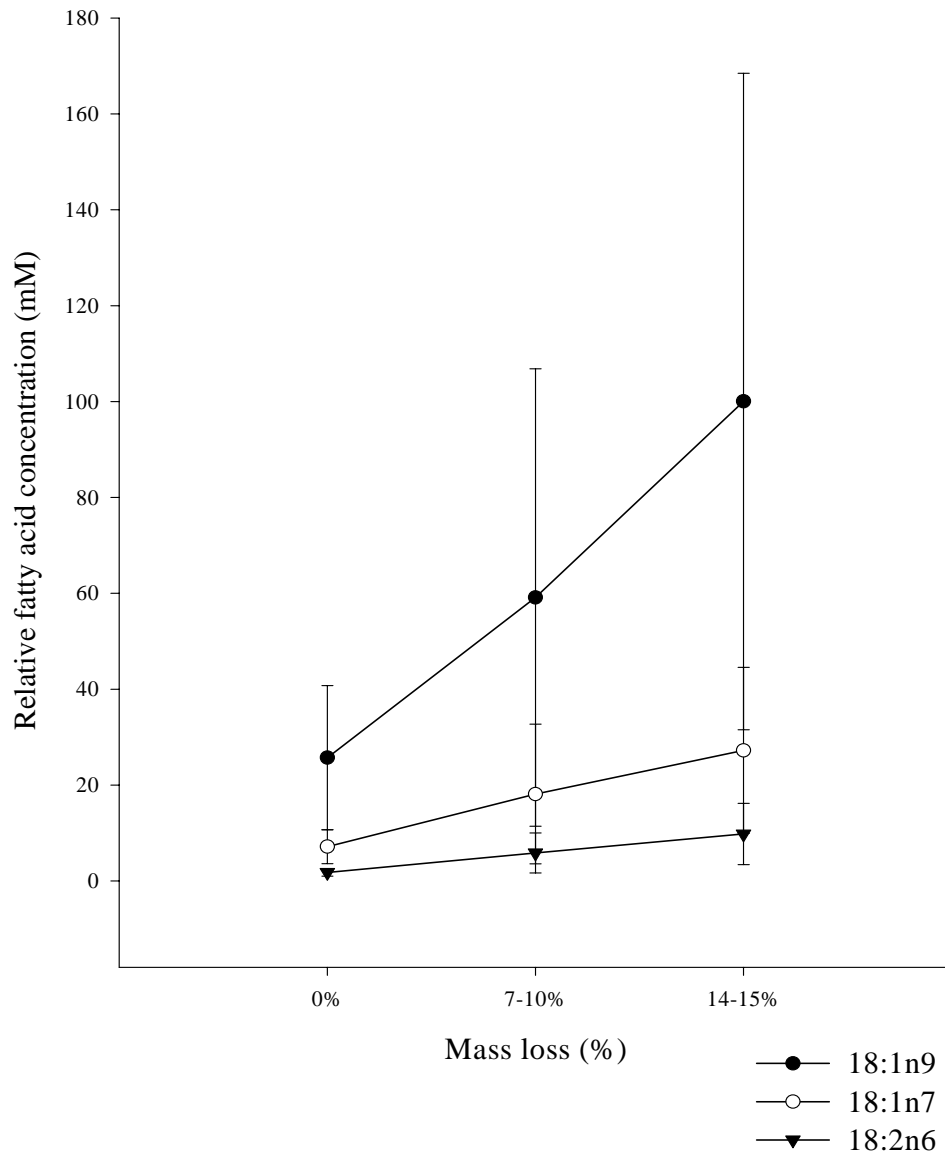


Figure 12. Changes in the mean relative fatty acid concentration of 18:1n-9, 18:1n-7 and 18:2n-6 during fasting in sub-adult Steller sea lions. Values represent means \pm SD.

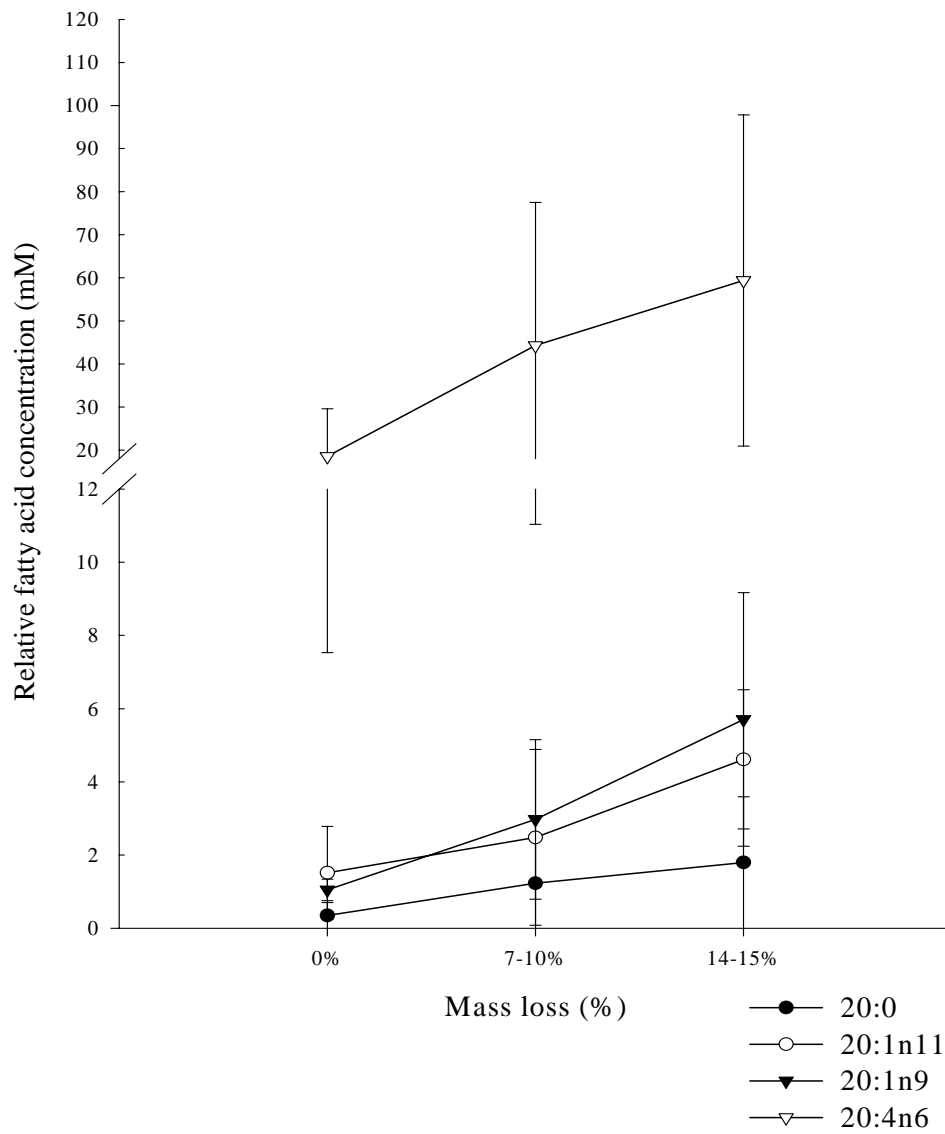


Figure 13. Changes in the mean relative fatty acid concentration of 20:0, 20:1n-11, 20:1n-9 and 20:4n-6 during fasting in sub-adult Steller sea lions. Values represent means \pm SD.

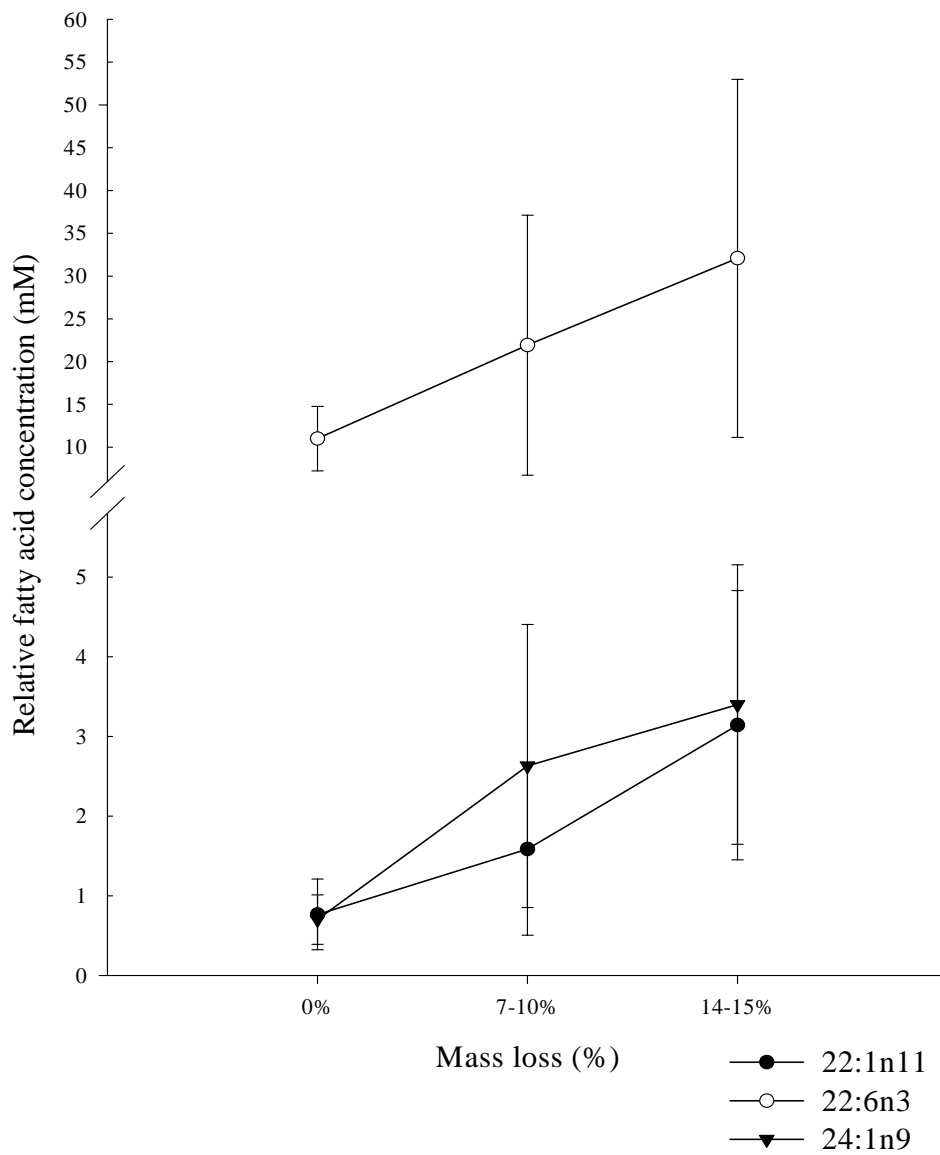


Figure 14. Changes in the mean relative fatty acid concentration of 22:1n-11, 22:6n-3 and 24:1n-9 during fasting in sub-adult Steller sea lions. Values represent means \pm SD.

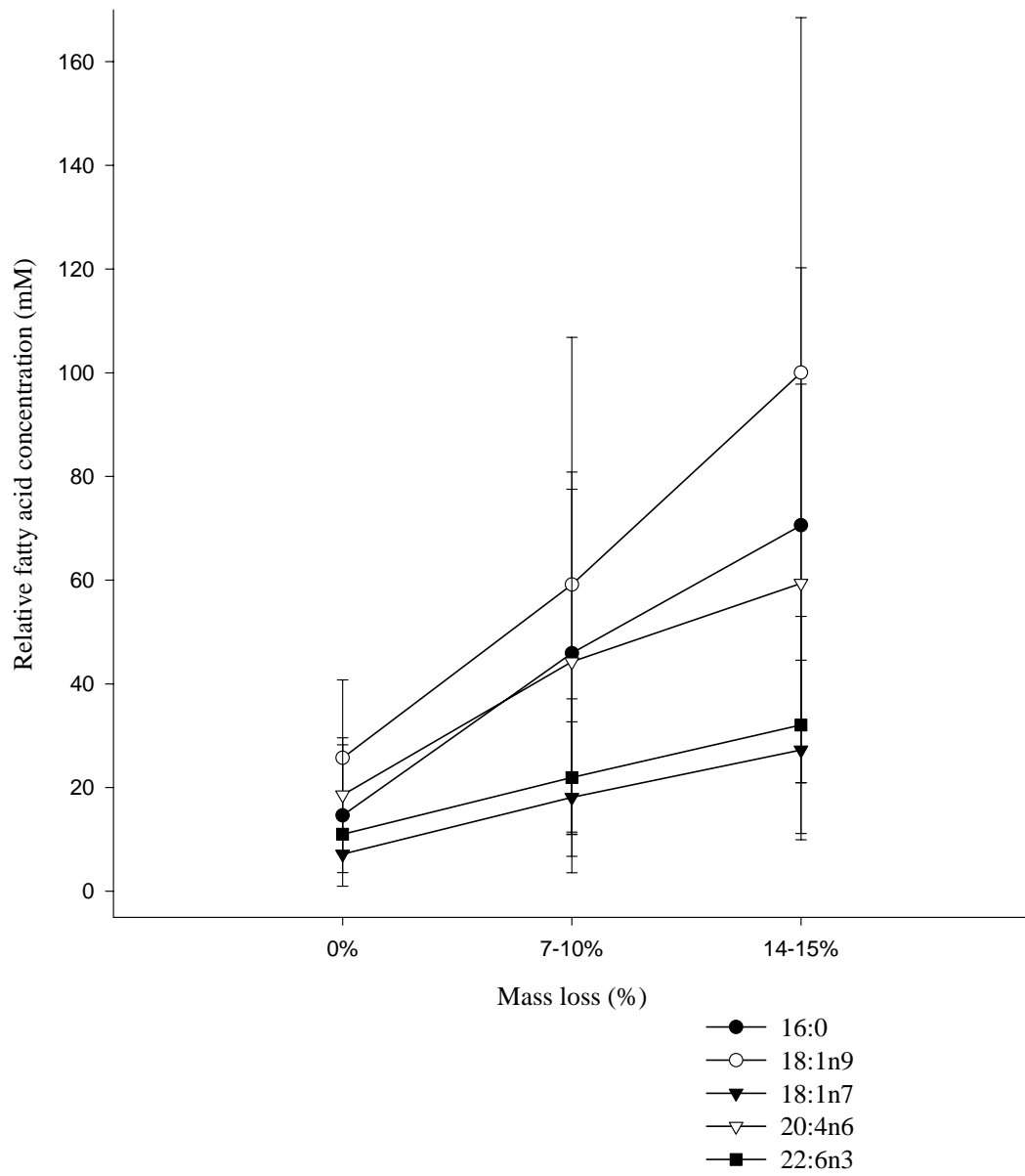


Figure 15. Relative fatty acid concentrations with the highest mean concentration changes in sub-adult sea lions. Values represent means \pm SD.

Principle Components Analysis

Juveniles

When comparing total lipid fatty acid composition changes during fasting in the juveniles, stage 1 of both the breeding and non-breeding season grouped in the upper half of the graph, whereas stage 3 grouped in the lower half (Fig. 16). The fatty acids affecting the majority of the separation along axis 2 are 16:1n-11, 18:1n-11, 18:1n-9, 20:3n-6, 20:4n-3 and 20:5n-3 (Table 6). Of these 6 fatty acids, the wt % of 4 (16:1n-11, 18:1n-9, 20:3n-6 and 20:5n-3) was also significantly different when tested with an ANOVA (Table 6). Of these 6 fatty acids, the relative concentration of 3 (18:1n-11, 18:1n-9 and 20:3n-6) also displayed the highest fold change during fasting. Additionally, the relative concentration of 3 (16:1n-11, 18:1n-9 and 20:3n-6) had the greatest overall relative concentration increase.

Axis one and two together significantly separate stage 1 and stage 3 of fasting for the juveniles and axis 2 additionally significantly separated stage 1 from stage 3 of fasting when tested with MANOVA. The first 2 axes explained 50% of the variation in the data. Although there was no significant difference within the total lipid composition along axis 1, there is some grouping of these data by age. With the exception of Timber's breeding season fast, the oldest sea lions are grouped on the left of the figure while the youngest sea lions are grouped on the right of the figure (Fig. 16). There was no significant difference between the breeding and non-breeding season.

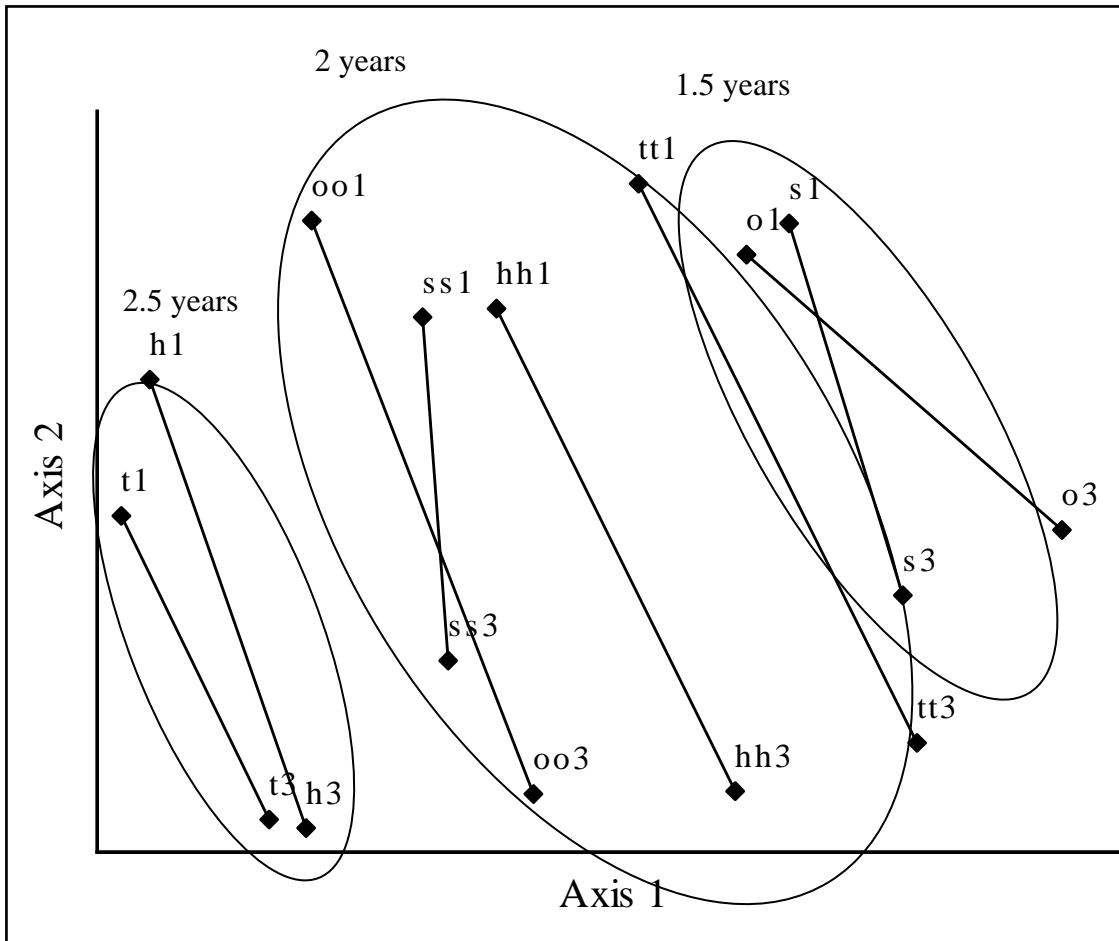


Figure 16. Principle components analysis comparing stages 1 and 3 of fasting in juvenile Steller sea lions. The two axes together explained 50% of the variation in the data and significantly separated stage 1 from stage 3 of fasting. The letters represent the individual sea lions (h=Hazy, o=Kodiak, s=Sitka, t=Timber). Double letters refer to breeding season samples and single letters refer to non-breeding season samples; numbers indicate stage of fasting.

Table 6. Eigenvectors for the first two axes for the beginning and the end of the juveniles' fasts

| Fatty Acid | Eigenvector for axis 1 | Eigenvector for axis 2 |
|------------|------------------------|------------------------|
| 14:0 | 0.089 | 0.037 |
| 15:0 | 0.336 | 0.012 |
| 16:0 | 0.317 | -0.056 |
| 16:1n-11 | 0.101 | 0.276 |
| 16:1n-7 | 0.189 | -0.186 |
| 16:2n-4 | -0.161 | 0.145 |
| 17:0 | 0.200 | 0.139 |
| 17:1 | 0.178 | 0.061 |
| 18:0 | 0.251 | 0.229 |
| 18:1n-11 | -0.143 | -0.285 |
| 18:1n-9 | 0.140 | -0.289 |
| 18:1n-7 | 0.238 | -0.175 |
| 18:1n-5 | 0.179 | 0.058 |
| 18:2n-6 | 0.281 | -0.251 |
| 20:0 | 0.093 | -0.120 |
| 20:1n-11 | -0.250 | -0.180 |
| 20:1n-9 | -0.068 | -0.187 |
| 20:2n-6 | -0.192 | -0.192 |
| 20:3n-6 | 0.198 | -0.272 |
| 20:4n-6 | 0.319 | 0.025 |
| 20:4n-3 | 0.141 | 0.275 |
| 20:5n-3 | -0.027 | 0.398 |
| 22:1n-11 | -0.246 | -0.046 |
| 22:5n-3 | -0.149 | 0.235 |
| 22:6n-3 | 0.022 | 0.205 |
| 24:1n-9 | 0.088 | -0.004 |

Sub-adults

A similar pattern occurred when comparing changes during fasting in the sub-adults, as seen in the juveniles. In this case, the final stage of fasting grouped at the top of the figure where as the first stage of fasting grouped at the bottom (Fig. 17). The fatty acids that expressed the greatest influence on the separation along axis 2 are 16:1n-11, 16:1n-7, 18:2n-6, 20:0, 20:1n-9, and 24:1n-9 (Table 7). Of these 6 fatty acids, the wt % of 4 (16:1n-7, 18:2n-6, 20:0, and 20:1n-9) changed significantly between the first and last stage of fasting for the sub-adults (Table 7). All fatty acids and their corresponding eigenvectors are listed in Table 3. The mean relative concentration of 4 of these fatty acids (16:1n-7, 18:2n-6, 20:0, and 20:1n-9) also display the highest fold change during fasting.

Axis 1 and 2 significantly explained 66% of the variation in separating stage 1 and stage 3 of fasting for the sub-adults when tested with a MANOVA. Although there was no significant difference within the total lipid composition along axis 1, there was some grouping of this data by age. The oldest sea lions are grouped on the right of the figure while the youngest sea lions are grouped on the left of the figure (Fig. 17). Again, there was no significant difference between the breeding and non-breeding season.

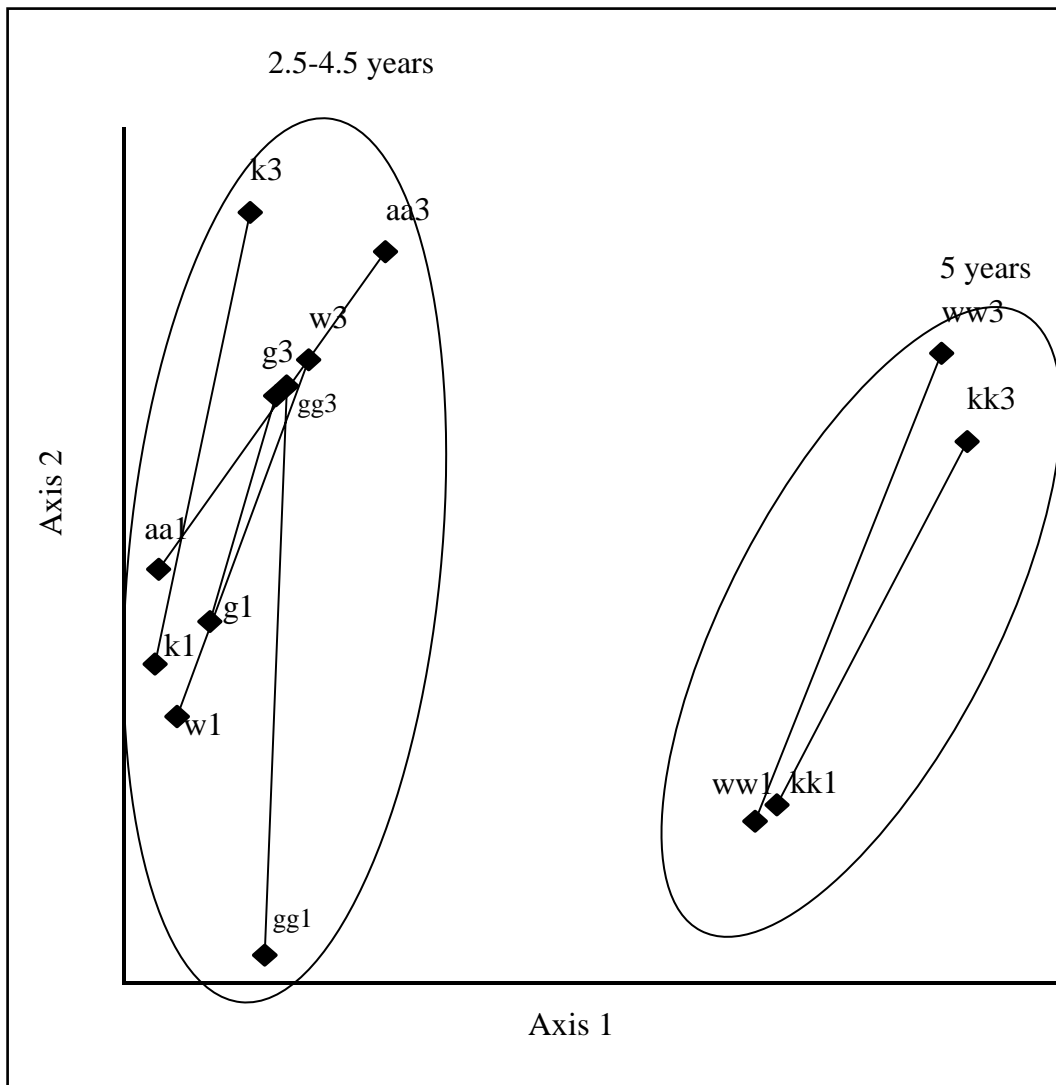


Figure 17. Principle components analysis comparing stages 1 and 3 of fasting in sub-adult Steller sea lions. The two axes together explained 66% of the variation in the data and significantly separated stage 1 from stage 3 of fasting. The letters represent the individual sea lions (a=Adak, g=Sugar, k=Kiska, w=Woody). Double letters refer to breeding season samples and single letters refer to non-breeding season samples; numbers indicate stage of fasting.

Table 7. Eigenvectors for the first two axes for the beginning and the end of the sub-adults' fasts.

| Fatty Acid | Eigenvector for axis 1 | Eigenvector for axis 2 |
|------------|------------------------|------------------------|
| 14:0 | 0.128 | 0.148 |
| 15:0 | -0.104 | 0.124 |
| 16:0 | -0.215 | 0.127 |
| 16:1n-11 | 0.105 | -0.124 |
| 16:1n-7 | -0.055 | 0.300 |
| 16:2n-4 | -0.202 | 0.150 |
| 17:0 | -0.266 | 0.110 |
| 17:1 | -0.131 | -0.103 |
| 18:0 | -0.256 | -0.205 |
| 18:1n-11 | 0.310 | -0.011 |
| 18:1n-9 | -0.235 | 0.245 |
| 18:1n-7 | -0.192 | 0.244 |
| 18:1n-5 | 0.270 | -0.023 |
| 18:2n-6 | -0.004 | 0.355 |
| 20:0 | 0.177 | 0.276 |
| 20:1n-11 | 0.307 | -0.033 |
| 20:1n-9 | 0.162 | 0.286 |
| 20:2n-6 | 0.178 | 0.184 |
| 20:3n-6 | -0.208 | 0.234 |
| 20:4n-6 | -0.298 | 0.068 |
| 20:4n-3 | -0.012 | 0.087 |
| 20:5n-3 | -0.195 | -0.232 |
| 22:1n-11 | 0.285 | 0.075 |
| 22:5n-3 | -0.134 | 0.222 |
| 22:6n-3 | 0.022 | -0.236 |
| 24:1n-9 | 0.071 | 0.289 |

DISCUSSION

Lipid Usage as an Energy Source During Fasting

Both juvenile and sub-adult Steller sea lions in this study relied heavily on lipids to provide energy while fasting. From a concurrent study on changes in body composition during fasting it was determined that the sub-adult Steller sea lions in this study lipid oxidation accounted for 92% of their energy expenditure during the breeding season fast but only 83% of their energy was derived from lipids during the non-breeding season fast (Rea, pers. comm.). Juvenile Steller sea lions derived 90% of their energy from lipid stores while fasting during the breeding season which is comparable to other fasting adapted animals. However, lipid oxidation provided 95% of the juveniles' energy while fasting during the non-breeding season (Rea, pers. comm.). The difference in lipid-based energy usage may be explained by the body condition of the different age classes. The sub-adult sea lions began fasting with 25% body fat during the breeding season and only 13% body fat during the non-breeding season (Rea, pers. comm.). In contrast, juveniles began fasting with more variable amounts of body fat resulting in a mean of 18% body fat during the breeding season and 19% body fat during the non-breeding season (Rea, pers. comm.). The sub-adults sea lions in this study were approaching sexual maturity (Christen et al. 2003) and may have been more

physiological prepared (body fat content and possibly hormonally) to undergo a seasonal fast. In contrast, it is likely that the juveniles, because of their young age and body size, were not yet physiologically prepared to undergo prolonged fasting periods.

In other bird and mammal species, lipids generally provide more than 90% of the energy used during fasting in animals that naturally undergo prolonged fasting (Cherel et al. 1998, Robin et al. 1988, Reilly 1991, Cherel et al. 1993, Nordøy et al. 1993, Cherel et al. 1995). However, in animals that do not normally fast, such as rats, lipid oxidation contributes only 83% of the energy during fasting (Belkhou et al. 1991). The sub-adult Steller sea lions oxidized lipids at the rate of fasting adapted animals during the breeding season but closer to the rate of non-adapted fasting animals during the non-breeding season (Belkhou et al. 1991).

Fasting adapted gray seals and harp seals derive 94% and 96% of their energy, respectively, from lipids during fasting (Reilly 1991, Nordøy et al. 1993). Harp seal pups begin their post-weaning fast relying 91% on lipid and as they enter Phase II this percentage increases to 96% (Nordøy et al. 1993). Additionally, Worthy and Lavigne (1983, 1987) found that blubber contributed 85% of the harp seals energy expenditure after about 2 weeks of fasting. Prior to this, harp seals relied more on core lipids and muscle (Worthy and Lavigne 1983). The juvenile Steller sea lions oxidized lipids at the rate of gray and harp seals during their non-breeding season fast. If their non-breeding season fast was prolonged, they would likely face severe lipid shortage and possibly starvation.

Other fasting adapted animals also rely heavily on lipid oxidation for energy during periods of food deprivation. Cherel et al. (1995) found that neutral lipids accounted for 91-92% of the body's fuel utilized by the European hedgehog during fasting whereas phospholipids and cholesterol together contributed less than 0.5%. Proteins accounted for only 8-9% of the hedgehogs' energy production. In fasting emperor penguins, lipids accounted for 96% of the energy mobilized during fasting (Robin et al. 1988). Similarly, the king penguin derived 94% of their energy from lipids during prolonged fasts (Cherel et al. 1993).

Lipids, and in particular NEFA and triglycerides, are an excellent energy source and easily mobilized from adipose tissue. Oxidizing NEFA and triglycerides spares vital phospholipids and cholesterol stores, which are important components of cellular membranes and precursors to hormones (Lands 1991). Castellini et al. (1987) found that NEFA were the primary energy source of fasting elephant seals (Fig. 18). Castellini et al. (1987) noted that the absolute levels of NEFA in elephant seal pups ranged from 1.82 mM up to 3.13 mM within different seals and were higher than any other marine mammal previously reported (see review in Castellini et al. 1987). However, plasma levels of NEFA in fasting adult elephant seal reached only 0.347mM. Nordøy and Blix (1991) reported grey seals with NEFA concentrations increasing from 1.09 to 2.45mM during a 52-day fast (Fig. 18). However, Nordøy et al. (1993) reported NEFA concentrations during a 30 day fast in harp seal pups (1.4 ± 0.2 mM to 3.4 ± 0.7 mM, Fig. 18) in the same range as the juvenile Steller sea lions (1.2 ± 0.51 mM to 3.7 ± 0.69 ; Fig. 18) after a 7 day fast. Concentrations of NEFAs in individual sub-adult Steller sea lions ranged from 1.00

mM up to 9.70 mM but averaged 1.3 ± 0.30 mM in after an overnight fast to 6.5 ± 3.67 mM (Fig 18). The 9.70 mM value was an exception although the concentrations were continually high (4.5-7.2 mM) and higher than reported in phocids during the latter stages of fasting. On the average, they continued to increase throughout fasting until the final stage of the non-breeding season where it began to level off. This may indicate the sea lions were approaching the end of the usable lipid reserves and entering phase III where they begin to resort back to utilizing protein stores.

The fasting duration in phocids can extend for up to 2 months whereas, female Steller sea lions generally feed while nursing their pups (Reeves et al. 1992). Although phocids generally have higher body fat compared to otariids (Bowen et al. 2002), preparing for an extended period without food intake requires adaptations, including metabolic rate depression (Castellini et al. 1987, Castellini and Costa 1990, Rea and Costa 1992, Mellish et al. 2000). Metabolic depression has been recorded in sub-adult Steller sea lions (Rosen and Trites 2002). However, because Steller sea lions are relatively leaner and undergo shorter bouts of fasting compared to phocids (Pitcher et al. 2000), they would be expected to have an overall higher mass specific metabolic rate and thus an increased rate of NEFA mobilization from adipose tissue stores. Rea and Costa (1992) reported that metabolic rate is most heavily influenced by lean body mass and overall is higher in leaner animals. Therefore it is possible that the increased NEFA concentration represents an increase in fatty acid mobilization from adipose tissue while overall fatty acid catabolism has declined due to a decreased metabolic rate.

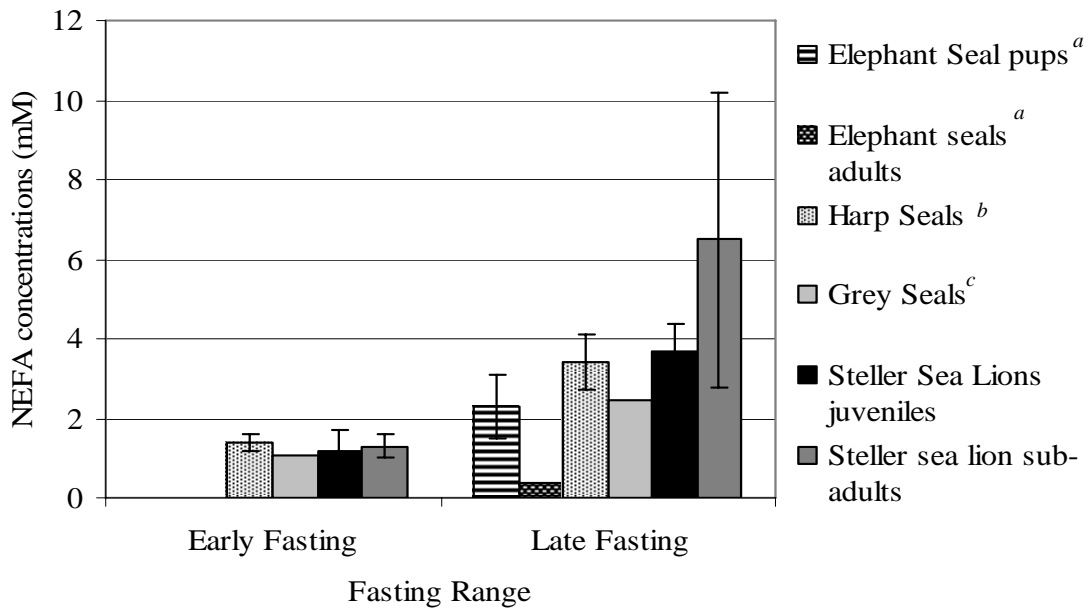


Figure 18. Mean non-esterified fatty acid (NEFA) concentrations of several phocids species compared to the juvenile and sub-adult Steller sea lions. *a*). Castellini et al. 1987, *b*). Nordøy et al. 1993, *c*). Nordøy and Blix 1991.

Lipid Compositional Changes During Fasting

Seasonal fatty acid changes were dramatically different between the juvenile and sub-adult Steller sea lions. The wt % of only one fatty acid was significantly different between seasons during fasting in the juvenile sea lions compared to ten fatty acids during the sub-adult sea lions breeding and non-breeding season fast. This trend may be the result of the initial body composition of the animals and the amount of lipids used during fasting. The mean juvenile body composition comprised of 18% and 19% body fat at the start of fasting and used 90% and 96% for energy while fasting during the breeding and non-breeding seasons, respectively (Rea, pers. comm.). Older sea lions may be more physiologically prepared for a breeding season fast due to the higher mean body fat content as a source of energy during fasting. The sub-adult's body composition consisted of 25% and 13% body fat at the start of fasting and they used 92% and 83% fat for energy during the breeding and non-breeding season fasts, respectively (Rea, pers. comm.). Sub-adults sea lions in this study were approaching sexual maturity and may have been better physiologically prepared to undergo a seasonal fast while the juveniles were not yet physiologically prepared to undergo a breeding season fast (Christen et al. 2003). The PCA analysis suggested subtly different responses at different ages even within each age class, and some of this variability may be related to the overall demand for growth in these different groups. It is expected that the sub-adults were growing more slowly than the juveniles. Additionally, the sub-adults may have been hormonally

influenced because in their natural environment were approaching breeding age and preparing to be able to fast (Christen et al. 2003).

It has been noted that lipid usage is not based on a “last in first out” pattern but based primarily on fatty acid structure (Raclot and Groscolas 1993, 1995). Fatty acids are selectively mobilized based on chain length, level of saturation and distance of the first unsaturated bond from the methyl end. The fatty acids that changed during fasting in this study represent only blood lipid concentrations and could be due to a combination of changes in mobilization from adipose tissue, *de novo* synthesis and utilization as an energy source. Relative lipid fatty acid concentration analysis yielded 2.5 times more fatty acids with significant concentration changes, than did the total lipid concentration analysis. Of the 18 fatty acids with significant relative lipid concentration changes, 72% were saturates or MUFAs and they all increased in the serum. In contrast, the percentage of long chained MUFAs increased in adipose tissue of fasting penguins, translating into preferential retention based on their molecular structure (Raclot and Groscolas 1993, 1995). Although the Steller sea lions are mobilizing saturated fatty acids as well as MUFAs they may be mobilizing these fatty acids to synthesize PUFAs. Serum fatty acid data analyzed in this study did not assess mobilization rate and therefore couldn't be directly compared to mobilization rates in other species or be used to confirm the predicted order of mobilization of fatty acid from body stores based on the chemical structure of the fatty acids. However some fatty acids changes in the juvenile and sub-adult Steller sea lions did coincide with the patterns of appearance seen in other fasting animals.

The wt % of seven fatty acids changed significantly during fasting in the juveniles and five of these (16:1n-11, 18:1n-9, 18:2n-6, 20:3n-6 and 20:5n-3) were most significant in separating the beginning and end of the fasts using principal components analysis. Additionally, the wt % of 10 fatty acids changed significantly during fasting in the sub-adults and four of these (16:1n-7, 18:2n-6, 20:0, and 20:1n-9) were most significant in separating the beginning and end of the fasts using principal components analysis. Of these fatty acids, 16:1n-7, 18:1n-9, 18:2n-6 and 20:5n-3 had some of the highest mobilization rates in fasting penguins (Groscolas 1990). Fasting rats also preferentially utilized fatty acids derived from 16:0 and 18:1 (Cunnane 1990). The relative concentration of derivatives from these two fatty acids increased significantly. During fasting in sea lions, 16:1n-7, 18:1n-9 and 18:1n-7 increased 4.4 mM, 26.3 mM and 9 mM respectively in the juveniles. Similarly, the relative concentration of 16:0, 18:1n-9 and 18:1n-7 increased 56 mM, 74.3 mM, 20.1 mM respectively during fasting in the sub-adults. The only fatty acid that had a comparable change was 20:4n-6 which increased 28 mM in the juveniles and 40.8 mM in the sub-adults and is usually preferentially retained in the liver (Cunnane 1990).

Three fatty acids, 20:1n-11, 20:1n-9 and 18:1n-7, were preferentially retained in the adipose tissue during fasting in penguins while they significantly increased in the serum of the fasting juvenile Steller sea lions (Groscolas 1986). In the same pattern, 20:5n-3 decreased in the juveniles but was preferentially released in penguins and in rats while fasting (Groscolas 1986, Raclot and Groscolas 1993, 1995). Raclot and Groscolas 1995 also reported a 90% loss of 20:5n-3 in fasted rats after 56% depletion of their total

fatty acids. The fact that this fatty acid also contributed to the separation from the beginning to the end of the fasts in the principle components analysis (PCA) demonstrates its importance. This fatty acid is an important component of cellular membranes and could be retained for this purpose.

Only one sub-adult Steller sea lion was able to withstand a 14 day fast during the non-breeding season before a 15% reduction in body mass. Two other sub-adults lasted only nine days until a 15% reduction in mass at which time fasting was terminated. The reduction in the wt % of 20:5n-3 may have been influential in physiological changes. This was one of the few fatty acids that decreased in wt % and was the fatty acid with the greatest wt % decrease in both the juveniles and sub-adults. The wt % of 20:5n-3 was 1.3 fold less on day 9 during the non-breeding season compared to the breeding season value for one of the sub-adults whose non-breeding season fast was terminated early. This same animal's wt % of 20:5n-3 also decreased 1.3 fold between day 9 and day 12 during the breeding season. If she continued to fast and lost 20:5n-3 at the same rate, she would have experienced a 2-fold decrease of this fatty acid and approached depletion. These trends could indicate a physiological response to nearing depletion of this valuable fatty acid, one essential to eicosanoid production. One role of eicosanoids is to regulate hypothalamic and pituitary hormones that are essential during the breeding season (Lands 1991).

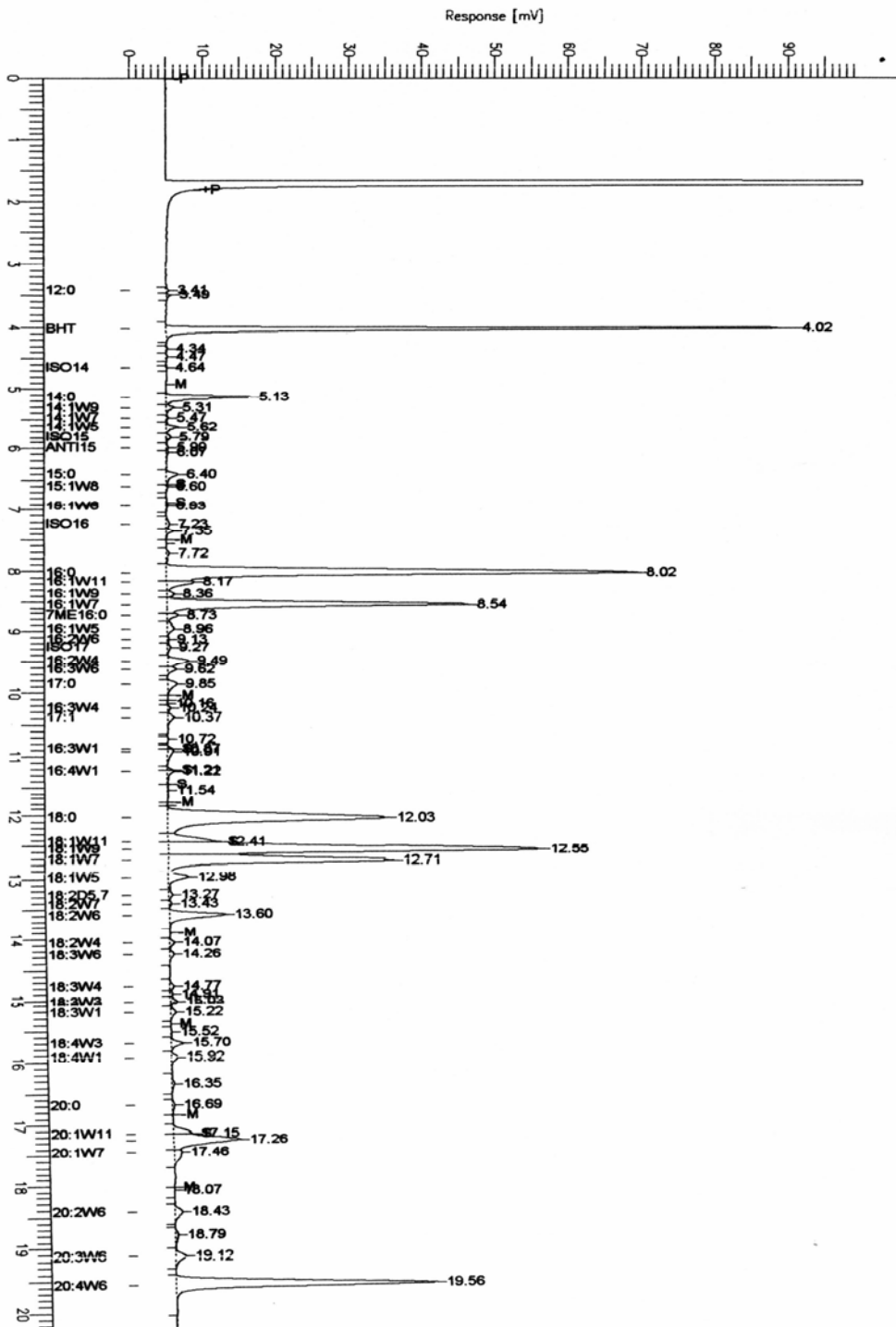
In addition, the fatty acid 20:5n-3 significantly decreased during fasting in both the juvenile and sub-adult Steller sea lions and was a significant component in the PCA for separating the beginning and the end of fasting in the sub-adults. Many other

mammals significantly rely on this fatty acid during fasting. In fasting rats, 90% of the initial mass of 20:5n-3 was depleted (Raclot and Groscolas 1995). Penguins and humans preferentially also utilized this fatty acid (Groscolas 1986, Raclot et al. 1997). The wt % of 20:5n-3 increased in plasma of fasting bears (Hissa et al. 1998) and Ballantyne et al. 1996 found this fatty acid to be highly represented in total plasma NEFA in migrating salmon. Finally, Rea et al. (1998) noted an increase in this fatty acid in plasma triglyceride of Weddell seal pups during their post-weaning fast. Reasons for the wt % of 20:5n-3 to decrease in the serum of fasting Steller sea lions are unclear but could stem from near depletion of this fatty acid. This fatty acid may help explain why some fasting adapted animals enter phase III of fasting before body lipid stores are depleted.

Entrance into phase III with sufficient lipid stores present may be hormonally driven. The fatty acid 20:5n-3 plays an essential role in hormone production and is a principal fatty acid in fasting animals. Therefore, maintaining lipid oxidation while fasting may be dependent on the availability of adequate concentrations of 20:5n-3. Critically low levels of 20:5n-3 may be the cue for fasting animals to begin to feed again.

APPENDIX A: EXAMPLE CHROMATOGRAM

Appendix A. Example chromatogram print out. Each peak indicated a separate fatty acid and the area under the peak is the percent weight of that fatty acid. The number above is the retention time.



APPENDIX B: STELLER SEA LION FATTY ACID DATA

Appendix B. Steller sea lion fatty acid data.

| | | 14:00 | 15:00 | Iso16 | 16:00 | 16:1n-11 |
|-------|-----------|-------|-------|-------|--------|----------|
| Adak | 29-May-96 | 0.975 | 0.265 | 0.245 | 14 | 0.37 |
| Adak | 1-Jun-96 | 0.865 | 0.155 | 0.205 | 14.635 | 0.36 |
| Adak | 5-Jun-96 | 0.94 | 0.21 | 0.38 | 15.52 | 0.27 |
| Adak | 12-Jun-96 | 1.46 | 0.245 | 0.305 | 15.785 | 0.34 |
| Woody | 20-Nov-96 | 0.79 | 0.15 | 0.315 | 14.28 | 0.335 |
| Woody | 23-Nov-96 | 0.89 | 0.17 | 0.43 | 14.27 | 0.33 |
| Woody | 27-Nov-96 | 0.875 | 0.18 | 0.26 | 14.335 | 0.32 |
| Woody | 29-Nov-96 | 0.715 | 0.17 | 0.395 | 13.93 | 0.35 |
| Woody | 3-Dec-96 | 1.045 | 0.19 | 0.415 | 13.415 | 0.345 |
| Woody | 23-Jun-99 | 1.285 | 0.195 | 0.195 | 13.905 | 0.38 |
| Woody | 26-Jun-99 | 1.305 | 0.225 | 0.345 | 17.045 | 0.415 |
| Woody | 29-Jun-99 | 1.2 | 0.215 | 0.35 | 16.26 | 0.405 |
| Woody | 2-Jul-99 | 0.845 | 0.19 | 0.21 | 12.455 | 0.385 |
| Woody | 7-Jul-99 | 0.83 | 0.14 | 0.185 | 11.26 | 0.325 |
| Tag | 12-Apr-97 | 0.79 | 0.175 | 0.35 | 15.255 | 0.34 |
| Tag | 16-Apr-97 | 0.72 | 0.17 | 0.195 | 16.195 | 0.365 |
| Tag | 18-Apr-97 | 0.8 | 0.18 | 0.4 | 15.35 | 0.32 |
| Tag | 21-Apr-97 | 0.895 | 0.175 | 0.41 | 15.28 | 0.345 |
| Sugar | 28-May-97 | 0.55 | 0.13 | 0.135 | 12.17 | 0.305 |
| Sugar | 30-May-97 | 0.67 | 0.155 | 0.295 | 14.58 | 0.345 |
| Sugar | 2-Jun-97 | 0.73 | 0.165 | 0.165 | 15.67 | 0.365 |
| Sugar | 5-Jun-97 | 0.78 | 0.33 | 0.3 | 15.05 | 0.29 |
| Sugar | 9-Jun-97 | 1.195 | 0.245 | 0.365 | 16.195 | 0.37 |
| Kiska | 21-Oct-97 | 0.715 | 0.2 | 0.26 | 13.685 | 0.355 |
| Kiska | 24-Oct-97 | 0.75 | 0.27 | 0.34 | 14.22 | 0.22 |
| Kiska | 27-Oct-97 | 0.55 | 0.22 | 0.48 | 14.62 | 0.28 |
| Kiska | 29-Oct-97 | 0.925 | 0.165 | 0.345 | 14.34 | 0.235 |
| Kiska | 31-May-99 | 1.08 | 0.15 | 0.2 | 10.97 | 0.39 |
| Kiska | 3-Jun-99 | 1.94 | 0.245 | 0.13 | 17.795 | 0.415 |
| Kiska | 6-Jun-99 | 1.65 | 0.2 | 0.275 | 14.47 | 0.51 |
| Kiska | 9-Jun-99 | 1.125 | 0.19 | 0.31 | 14.81 | 0.535 |
| Kiska | 11-Jun-99 | 1.225 | 0.17 | 0.23 | 11.395 | 0.375 |

| | | 16:1n-7 | 16:2n-4 | 17:00 | 18:00 | 18:1n-13 |
|-------|-----------|---------|---------|-------|--------|----------|
| Adak | 29-May-96 | 3.115 | 0.455 | 1.06 | 13.51 | 0.01 |
| Adak | 1-Jun-96 | 2.675 | 0.1 | 0.175 | 12.805 | 0.83 |
| Adak | 5-Jun-96 | 2.92 | 0.08 | 0.69 | 12.08 | 0 |
| Adak | 12-Jun-96 | 4.205 | 0.405 | 0.695 | 10.23 | 0.06 |
| Woody | 20-Nov-96 | 2.17 | 0.385 | 1.045 | 14.25 | 0.19 |
| Woody | 23-Nov-96 | 2.33 | 0.14 | 0.93 | 14.36 | 0 |
| Woody | 27-Nov-96 | 2.76 | 0.18 | 0.26 | 12.305 | 0.135 |
| Woody | 29-Nov-96 | 2.43 | 0.155 | 0.205 | 12.14 | 0.185 |
| Woody | 3-Dec-96 | 3.155 | 0.425 | 0.915 | 11.205 | 0.185 |
| Woody | 23-Jun-99 | 1.845 | 0.26 | 0.295 | 11.115 | 0.705 |
| Woody | 26-Jun-99 | 1.92 | 0.255 | 0.32 | 12.45 | 0.67 |
| Woody | 29-Jun-99 | 2.015 | 0.25 | 0.295 | 11.375 | 0.63 |
| Woody | 2-Jul-99 | 1.99 | 0.255 | 0.215 | 8.595 | 0.58 |
| Woody | 7-Jul-99 | 2.2 | 0.205 | 0.19 | 7.27 | 0.445 |
| Tag | 12-Apr-97 | 2.125 | 0.435 | 1.04 | 14.055 | 0.18 |
| Tag | 16-Apr-97 | 2.34 | 0.135 | 0.21 | 13.095 | 0.615 |
| Tag | 18-Apr-97 | 3.03 | 0.17 | 0.95 | 11.35 | 0 |
| Tag | 21-Apr-97 | 3.345 | 0.45 | 1.02 | 11.36 | 0.145 |
| Sugar | 28-May-97 | 1.66 | 0.17 | 0.18 | 14.3 | 0.82 |
| Sugar | 30-May-97 | 2.115 | 0.125 | 0.2 | 15.74 | 0.2 |
| Sugar | 2-Jun-97 | 2.325 | 0.12 | 0.175 | 13.16 | 0.645 |
| Sugar | 5-Jun-97 | 2.6 | 0.1 | 0.66 | 12.01 | 0 |
| Sugar | 9-Jun-97 | 4.155 | 0.33 | 0.79 | 11.265 | 0.055 |
| Kiska | 21-Oct-97 | 2.18 | 0.395 | 1.155 | 14.365 | 0.22 |
| Kiska | 24-Oct-97 | 2.03 | 0.11 | 0.94 | 14.08 | 0 |
| Kiska | 27-Oct-97 | 1.98 | 0.13 | 0.98 | 13.04 | 0 |
| Kiska | 29-Oct-97 | 3.22 | 0.49 | 1.02 | 11.88 | |
| Kiska | 31-May-99 | 1.7 | 0.36 | 0.27 | 11.62 | 0.68 |
| Kiska | 3-Jun-99 | 2.805 | 0.24 | 0.36 | 16.52 | 0.485 |
| Kiska | 6-Jun-99 | 3.235 | 0.26 | 0.265 | 12.33 | 0.805 |
| Kiska | 9-Jun-99 | 2.905 | 0.3 | 0.265 | 11.805 | 0.74 |
| Kiska | 11-Jun-99 | 3.36 | 0.235 | 0.195 | 8.375 | 0.44 |

| | | 18:1n-11 | 18:1n-9 | 18:1n-7 | 18:1n-5 | 18:2n-6 |
|-------|-----------|----------|---------|---------|---------|---------|
| Adak | 29-May-96 | 0.2 | 19.74 | 5.165 | 0.135 | 1.355 |
| Adak | 1-Jun-96 | 0.4 | 20.23 | 5.57 | 0.11 | 1.635 |
| Adak | 5-Jun-96 | 1.08 | 20.5 | 6.38 | 0.14 | 1.89 |
| Adak | 12-Jun-96 | 0.375 | 22.915 | 5.85 | 0.135 | 2.14 |
| Woody | 20-Nov-96 | 0.515 | 19.505 | 5.065 | 0.12 | 1.12 |
| Woody | 23-Nov-96 | 1 | 18.9 | 5.88 | 0.09 | 1.4 |
| Woody | 27-Nov-96 | 0.57 | 22.065 | 6.06 | 0.13 | 1.83 |
| Woody | 29-Nov-96 | 0.575 | 20.97 | 6.34 | 0.125 | 1.94 |
| Woody | 3-Dec-96 | 0.655 | 21.975 | 7 | 0.21 | 2.235 |
| Woody | 23-Jun-99 | 1.44 | 10.665 | 3.87 | 0.375 | 1.055 |
| Woody | 26-Jun-99 | 1.785 | 14.865 | 6.01 | 0.425 | 1.825 |
| Woody | 29-Jun-99 | 1.825 | 17.02 | 6.12 | 0.37 | 2.22 |
| Woody | 2-Jul-99 | 1.655 | 13.785 | 4.835 | 0.34 | 1.705 |
| Woody | 7-Jul-99 | 1.74 | 15.14 | 4.77 | 0.245 | 1.89 |
| Tag | 12-Apr-97 | 0.315 | 18.63 | 5.27 | 0.175 | 1.4 |
| Tag | 16-Apr-97 | 0.455 | 19.45 | 5.99 | 0.145 | 1.815 |
| Tag | 18-Apr-97 | 0.75 | 21.09 | 6.11 | 0.09 | 1.95 |
| Tag | 21-Apr-97 | 0.465 | 21.4 | 6.185 | 0.21 | 1.985 |
| Sugar | 28-May-97 | 0.415 | 14.81 | 4.745 | 0.095 | 0.95 |
| Sugar | 30-May-97 | 0.335 | 17.74 | 5.595 | 0.13 | 1.545 |
| Sugar | 2-Jun-97 | 0.34 | 19.105 | 5.7 | 0.185 | 1.84 |
| Sugar | 5-Jun-97 | 0.83 | 18.09 | 6.3 | 0.24 | 1.98 |
| Sugar | 9-Jun-97 | 0.475 | 21.29 | 5.765 | 0.13 | 2.095 |
| Kiska | 21-Oct-97 | 0.425 | 20.1 | 5.13 | 0.14 | 1.23 |
| Kiska | 24-Oct-97 | 0.87 | 18.93 | 5.79 | 0.11 | 1.69 |
| Kiska | 27-Oct-97 | 1.17 | 20.09 | 5.94 | 0.11 | 2.08 |
| Kiska | 29-Oct-97 | 0.555 | 23.19 | 5.86 | 0.135 | 2.23 |
| Kiska | 31-May-99 | 1.37 | 10.94 | 3.23 | 0.34 | 1.09 |
| Kiska | 3-Jun-99 | 1.57 | 12.975 | 4.12 | 0.3 | 1.845 |
| Kiska | 6-Jun-99 | 1.97 | 14.415 | 5.075 | 0.34 | 2.065 |
| Kiska | 9-Jun-99 | 1.84 | 16.34 | 5.945 | 0.335 | 2.405 |
| Kiska | 11-Jun-99 | 1.975 | 13.75 | 4.38 | 0.53 | 1.765 |

| | | 18:4n-3 | 20:00 | 20:1n-11 | 20:1n-9 | 20:2n-6 |
|-------|-----------|---------|-------|----------|---------|---------|
| Adak | 29-May-96 | 0.1 | 0.27 | 0.52 | 0.745 | 0.115 |
| Adak | 1-Jun-96 | 0.075 | 0.57 | 0.395 | 0.665 | 0.095 |
| Adak | 5-Jun-96 | 0.14 | 0.35 | 0.19 | 0.85 | 0.24 |
| Adak | 12-Jun-96 | 0.17 | 0.4 | 0.84 | 1.43 | 0.11 |
| Woody | 20-Nov-96 | 0.085 | 0.215 | 0.405 | 0.565 | 0.08 |
| Woody | 23-Nov-96 | 0.05 | 0.22 | 0.56 | 0.26 | 0.09 |
| Woody | 27-Nov-96 | 0.07 | 0.455 | 0.645 | 0.795 | 0 |
| Woody | 29-Nov-96 | 0.045 | 0.695 | 0.475 | 0.69 | 0.095 |
| Woody | 3-Dec-96 | 0.065 | 0.315 | 0.855 | 1.16 | 0.14 |
| Woody | 23-Jun-99 | 0.135 | 0.26 | 2.52 | 0.725 | 0.145 |
| Woody | 26-Jun-99 | 0.09 | 0.315 | 3.145 | 1.075 | 0.22 |
| Woody | 29-Jun-99 | 0.055 | 0.535 | 3.08 | 1.145 | 0.25 |
| Woody | 2-Jul-99 | 0.045 | 0.455 | 2.6 | 1.17 | 0.25 |
| Woody | 7-Jul-99 | 0.045 | 0.54 | 2.76 | 1.32 | 0.285 |
| Tag | 12-Apr-97 | 0.065 | 0.235 | 0.635 | 0.8 | 0.13 |
| Tag | 16-Apr-97 | 0.045 | 0.475 | 0.485 | 0.87 | 0.115 |
| Tag | 18-Apr-97 | 0.07 | 0.34 | 0.52 | 1.11 | 0.18 |
| Tag | 21-Apr-97 | 0.05 | 0.33 | 0.725 | 1.06 | 0.14 |
| Sugar | 28-May-97 | 0.1 | 0.145 | 0.52 | 0.64 | 0 |
| Sugar | 30-May-97 | 0.05 | 0.295 | 0.49 | 0.355 | 0.075 |
| Sugar | 2-Jun-97 | 0.145 | 0.55 | 0.35 | 0.67 | 0.075 |
| Sugar | 5-Jun-97 | 0.28 | 0.51 | 0.28 | 0.68 | 0.91 |
| Sugar | 9-Jun-97 | 0.125 | 0.27 | 0.645 | 1.045 | 0.105 |
| Kiska | 21-Oct-97 | 0.055 | 0.22 | 0.46 | 0.69 | 0.1 |
| Kiska | 24-Oct-97 | 0.1 | 0.33 | 0.3 | 0.92 | 0.05 |
| Kiska | 27-Oct-97 | 0.06 | 0.48 | 0.42 | 0.63 | 0.26 |
| Kiska | 29-Oct-97 | 0.06 | 0.38 | 0.92 | 1.29 | 0.13 |
| Kiska | 31-May-99 | 0.14 | 0.29 | 3.03 | 0.88 | 0.12 |
| Kiska | 3-Jun-99 | 0.28 | 0.68 | 2.62 | 1.095 | 0.16 |
| Kiska | 6-Jun-99 | 0.375 | 0.455 | 3.075 | 1.8 | 0.19 |
| Kiska | 9-Jun-99 | 0.31 | 0.545 | 2.23 | 1.765 | 0.195 |
| Kiska | 11-Jun-99 | 0.135 | 0.365 | 2.545 | 1.715 | 0.13 |

| | | 20:3n-6 | 20:4n-6 | 20:4n-3 | 20:5n-3 | 22:1n-11 |
|-------|-----------|---------|---------|---------|---------|----------|
| Adak | 29-May-96 | 0.475 | 12.505 | 0.26 | 10.18 | 0.34 |
| Adak | 1-Jun-96 | 0.515 | 15.005 | 0.135 | 8.445 | 0.465 |
| Adak | 5-Jun-96 | 0.47 | 14.68 | 0.18 | 6.78 | 0.31 |
| Adak | 12-Jun-96 | 0.42 | 11.41 | 0.22 | 5.705 | 0.81 |
| Woody | 20-Nov-96 | 0.35 | 14.62 | 0.205 | 11.325 | 0.375 |
| Woody | 23-Nov-96 | 0.45 | 16.83 | 0.17 | 8.93 | 0.24 |
| Woody | 27-Nov-96 | 0.44 | 15.91 | 0.19 | 7.865 | 0.55 |
| Woody | 29-Nov-96 | 0.475 | 16.495 | 0.195 | 8.305 | 0.37 |
| Woody | 3-Dec-96 | 0.47 | 13.165 | 0.29 | 7.315 | 0.6 |
| Woody | 23-Jun-99 | 0.165 | 7.285 | 0.135 | 6.39 | 0.895 |
| Woody | 26-Jun-99 | 0.265 | 11.16 | 0.14 | 6.305 | 1.31 |
| Woody | 29-Jun-99 | 0.325 | 12.2 | 0.13 | 5.645 | 1.17 |
| Woody | 2-Jul-99 | 0.275 | 8.78 | 0.13 | 3.75 | 0.88 |
| Woody | 7-Jul-99 | 0.31 | 7.58 | 0.14 | 3.835 | 0.995 |
| Tag | 12-Apr-97 | 0.435 | 13.98 | 0.22 | 9.7 | 0.435 |
| Tag | 16-Apr-97 | 0.54 | 15.575 | 0.13 | 6.7 | 0.535 |
| Tag | 18-Apr-97 | 0.47 | 15.1 | 0.16 | 6.54 | 0.35 |
| Tag | 21-Apr-97 | 0.48 | 13.535 | 0.21 | 6.355 | 0.515 |
| Sugar | 28-May-97 | 0.325 | 11.71 | 0.115 | 8.68 | 0.15 |
| Sugar | 30-May-97 | 0.51 | 15.84 | 0.14 | 8.93 | 0.295 |
| Sugar | 2-Jun-97 | 0.485 | 16.135 | 0.13 | 7.57 | 0.39 |
| Sugar | 5-Jun-97 | 0.64 | 14.27 | 0.45 | 6.75 | 0.65 |
| Sugar | 9-Jun-97 | 0.445 | 12.24 | 0.22 | 6.975 | 0.49 |
| Kiska | 21-Oct-97 | 0.45 | 14.315 | 0.235 | 10.025 | 0.345 |
| Kiska | 24-Oct-97 | 0.66 | 17.02 | 0.11 | 6.54 | 0.22 |
| Kiska | 27-Oct-97 | 0.69 | 17.63 | 0.11 | 5.74 | 0.26 |
| Kiska | 29-Oct-97 | 0.625 | 15.06 | 0.195 | 5.195 | 0.665 |
| Kiska | 31-May-99 | 0.29 | 8.41 | 0.3 | 9.02 | 1.27 |
| Kiska | 3-Jun-99 | 0.465 | 10.52 | 0.33 | 7.105 | 1.335 |
| Kiska | 6-Jun-99 | 0.625 | 11.275 | 0.32 | 6 | 1.515 |
| Kiska | 9-Jun-99 | 0.61 | 12.01 | 0.27 | 6.32 | 0.95 |
| Kiska | 11-Jun-99 | 0.335 | 7.79 | 0.275 | 4.755 | 1.27 |

| | | 22:5n-3 | 22:6n-3 | 24:1n-9 |
|-------|-----------|---------|---------|---------|
| Adak | 29-May-96 | 2.635 | 6.92 | 0.615 |
| Adak | 1-Jun-96 | 2.005 | 6.505 | 1.375 |
| Adak | 5-Jun-96 | 1.8 | 6.72 | 0.83 |
| Adak | 12-Jun-96 | 2.01 | 7.24 | 0.78 |
| Woody | 20-Nov-96 | 2.13 | 6.77 | 0.425 |
| Woody | 23-Nov-96 | 1.88 | 6.79 | 0.63 |
| Woody | 27-Nov-96 | 1.585 | 6.23 | 0.865 |
| Woody | 29-Nov-96 | 1.63 | 6.4 | 1.36 |
| Woody | 3-Dec-96 | 2.015 | 6.615 | 0.525 |
| Woody | 23-Jun-99 | 2.61 | 7.985 | 0.405 |
| Woody | 26-Jun-99 | 2.975 | 9.875 | 0.52 |
| Woody | 29-Jun-99 | 2.495 | 9.055 | 0.865 |
| Woody | 2-Jul-99 | 1.87 | 7.075 | 0.765 |
| Woody | 7-Jul-99 | 1.605 | 5.895 | 0.94 |
| Tag | 12-Apr-97 | 2.11 | 7.81 | 0.58 |
| Tag | 16-Apr-97 | 1.76 | 7.555 | 0.985 |
| Tag | 18-Apr-97 | 1.78 | 7.47 | 0.77 |
| Tag | 21-Apr-97 | 1.88 | 7.35 | 0.57 |
| Sugar | 28-May-97 | 2.275 | 7.445 | 0.275 |
| Sugar | 30-May-97 | 2.31 | 8.17 | 0.835 |
| Sugar | 2-Jun-97 | 1.795 | 7.08 | 1.24 |
| Sugar | 5-Jun-97 | 1.58 | 7.28 | 0.55 |
| Sugar | 9-Jun-97 | 1.705 | 7.095 | 0.56 |
| Kiska | 21-Oct-97 | 2.3 | 6.755 | 0.45 |
| Kiska | 24-Oct-97 | 1.76 | 6.49 | 0.78 |
| Kiska | 27-Oct-97 | 1.44 | 5.78 | 1.1 |
| Kiska | 29-Oct-97 | 1.64 | 5.725 | 0.725 |
| Kiska | 31-May-99 | 1.9 | 8.43 | 0.49 |
| Kiska | 3-Jun-99 | 1.54 | 8.49 | 0.82 |
| Kiska | 6-Jun-99 | 1.925 | 9.775 | 0.715 |
| Kiska | 9-Jun-99 | 1.725 | 9.095 | 0.79 |
| Kiska | 11-Jun-99 | 1.39 | 6.71 | 0.505 |

| | | 14:00 | 15:00 | Iso16 | 16:00 | 16:1w11 |
|--------|-----------|-------|-------|--------|---------|---------|
| Sitka | 14-Jul-99 | 0.645 | 0.16 | 0.16 | 11.61 | 0.345 |
| Sitka | 17-Jul-99 | 0.705 | 0.17 | 0.315 | 13.65 | 0.4 |
| Sitka | 21-Jul-99 | 0.445 | 0.155 | 0.335 | 11.845 | 0.25 |
| Sitka | 12-Mar-99 | 0.895 | 0.21 | 0.23 | 13.395 | 0.45 |
| Sitka | 16-Mar-99 | 0.735 | 0.205 | 0.405 | 15.195 | 0.31 |
| Sitka | 19-Mar-99 | 0.845 | 0.21 | 0.355 | 15.375 | 0.395 |
| Kodiak | 14-Jul-99 | 0.6 | 0.145 | 0.15 | 10.6 | 0.36 |
| Kodiak | 17-Jul-99 | 0.71 | 0.185 | 0.21 | 15.09 | 0.33 |
| Kodiak | 12-Mar-99 | 0.84 | 0.18 | 0.33 | 12.03 | 0.47 |
| Kodiak | 16-Mar-99 | 0.64 | 0.18 | 0.24 | 13.41 | 0.395 |
| Kodiak | 19-Mar-99 | 0.755 | 0.21 | 0.1875 | 15.4075 | 0.34 |
| Timber | 9-Jul-99 | 0.78 | 0.205 | 0.36 | 14.285 | 0.43 |
| Timber | 14-Jul-99 | | | | | |
| Timber | 16-Jul-99 | 0.86 | 0.22 | 0.305 | 15.39 | 0.425 |
| Timber | 5-Nov-99 | 0.705 | 0.145 | 0.22 | 11.095 | 0.355 |
| Timber | 9-Nov-99 | 0.785 | 0.15 | 0.295 | 12.38 | 0.35 |
| Timber | 12-Nov-99 | 0.765 | 0.15 | 0.325 | 12.37 | 0.28 |
| Hazy | 9-Jul-99 | 0.92 | 0.18 | 0.265 | 13.21 | 0.505 |
| Hazy | 13-Jul-99 | 0.79 | 0.2 | 0.255 | 14.18 | 0.325 |
| Hazy | 16-Jul-99 | 0.67 | 0.19 | 0.255 | 13.21 | 0.345 |
| Hazy | 5-Nov-99 | 0.785 | 0.14 | 0.195 | 10.96 | 0.405 |
| Hazy | 9-Nov-99 | 0.745 | 0.145 | 0.115 | 11.065 | 0.29 |
| Hazy | 12-Nov-99 | 0.96 | 0.165 | 0.265 | 11.87 | 0.335 |

| | | 16:1w7 | 16:2w4 | 17:00 | 18:00 | 18:1w13 |
|--------|-----------|--------|--------|-------|---------|---------|
| Sitka | 14-Jul-99 | 1.755 | 0.14 | 0.275 | 14.955 | 0.16 |
| Sitka | 17-Jul-99 | 2.125 | 0.115 | 0.255 | 16.59 | 0.525 |
| Sitka | 21-Jul-99 | 1.655 | 0.12 | 0.24 | 12.485 | 0.07 |
| Sitka | 12-Mar-99 | 2.395 | 0.135 | 0.275 | 14.805 | 0.11 |
| Sitka | 16-Mar-99 | 2.105 | 0.11 | 0.345 | 17.865 | 0.15 |
| Sitka | 19-Mar-99 | 2.46 | 0.105 | 0.27 | 14.28 | 0.39 |
| Kodiak | 14-Jul-99 | 1.52 | 0.16 | 0.26 | 15.225 | 0.2 |
| Kodiak | 17-Jul-99 | 1.77 | 0.09 | 0.345 | 20.64 | 0.19 |
| Kodiak | 12-Mar-99 | 2.265 | 0.135 | 0.26 | 16.34 | 0.485 |
| Kodiak | 16-Mar-99 | 1.815 | 0.11 | 0.265 | 16.355 | 0.29 |
| Kodiak | 19-Mar-99 | 2.23 | 0.12 | 0.35 | 15.8875 | 0.0975 |
| Timber | 9-Jul-99 | 2.035 | 0.315 | 0.395 | 17.31 | 0.16 |
| Timber | 14-Jul-99 | | | | | |
| Timber | 16-Jul-99 | 3.485 | 0.12 | 0.295 | 15.17 | 0.115 |
| Timber | 5-Nov-99 | 1.79 | 0.22 | 0.2 | 11.805 | 0.315 |
| Timber | 9-Nov-99 | 1.975 | 0.155 | 0.21 | 11.77 | 0.24 |
| Timber | 12-Nov-99 | 2.075 | 0.16 | 0.22 | 11.14 | 0.205 |
| Hazy | 9-Jul-99 | 2.43 | 0.17 | 0.255 | 16.475 | 1.075 |
| Hazy | 13-Jul-99 | 2.45 | 0.15 | 0.37 | 18.085 | 0.105 |
| Hazy | 16-Jul-99 | 2.42 | 0.165 | 0.305 | 15.945 | 0.115 |
| Hazy | 5-Nov-99 | 1.86 | 0.215 | 0.305 | 11.85 | 0.32 |
| Hazy | 9-Nov-99 | 1.765 | 0.155 | 0.205 | 12.25 | 0.165 |
| Hazy | 12-Nov-99 | 2.73 | 0.19 | 0.23 | 10.815 | 0.185 |

| | | 18:1w11 | 18:1w9 | 18:1w7 | 18:1w5 | 18:2w6 |
|--------|-----------|---------|---------|--------|--------|--------|
| Sitka | 14-Jul-99 | 0.39 | 11.36 | 4.125 | 0.19 | 1.365 |
| Sitka | 17-Jul-99 | 0.505 | 14.635 | 5.21 | 0.225 | 2.43 |
| Sitka | 21-Jul-99 | 0.415 | 12.435 | 4.395 | 0.125 | 2.075 |
| Sitka | 12-Mar-99 | 0.265 | 13.275 | 4.635 | 0.125 | 1.685 |
| Sitka | 16-Mar-99 | 0.425 | 12.43 | 5.04 | 0.24 | 1.98 |
| Sitka | 19-Mar-99 | 0.355 | 14.705 | 5.47 | 0.14 | 2.305 |
| Kodiak | 14-Jul-99 | 0.35 | 10.525 | 3.96 | 0.13 | 1.045 |
| Kodiak | 17-Jul-99 | 0.525 | 12.365 | 5 | 0.12 | 1.84 |
| Kodiak | 12-Mar-99 | 0.295 | 13.155 | 4.94 | 0.145 | 1.535 |
| Kodiak | 16-Mar-99 | 0.265 | 13.875 | 5.155 | 0.095 | 2.145 |
| Kodiak | 19-Mar-99 | 0.4825 | 15.9375 | 5.33 | 0.2325 | 2.4125 |
| Timber | 9-Jul-99 | 0.535 | 13.085 | 4.86 | 0.175 | 1.485 |
| Timber | 14-Jul-99 | | | | | |
| Timber | 16-Jul-99 | 0.86 | 16.985 | 5.965 | 0.22 | 2.43 |
| Timber | 5-Nov-99 | 0.76 | 14.5 | 4.305 | 0.13 | 0.95 |
| Timber | 9-Nov-99 | 0.73 | 14.46 | 2.355 | 0.155 | 1.475 |
| Timber | 12-Nov-99 | 0.82 | 14.195 | 4.6 | 0.13 | 1.63 |
| Hazy | 9-Jul-99 | 0.515 | 13.715 | 5.465 | 0.26 | 1.36 |
| Hazy | 13-Jul-99 | 0.59 | 15.03 | 5.205 | 0.22 | 1.98 |
| Hazy | 16-Jul-99 | 0.775 | 17.135 | 6.215 | 0.145 | 2.385 |
| Hazy | 5-Nov-99 | 0.83 | 13.9 | 4.21 | 0.16 | 0.86 |
| Hazy | 9-Nov-99 | 0.7 | 12.435 | 4.145 | 0.115 | 1.305 |
| Hazy | 12-Nov-99 | 0.915 | 15.05 | 4.87 | 0.12 | 1.62 |

| | | 18:4w3 | 20:00 | 20:1w11 | 20:1w9 | 20:2w6 |
|--------|-----------|--------|-------|---------|--------|--------|
| Sitka | 14-Jul-99 | 0.105 | 0.195 | 0.48 | 0.545 | 0.06 |
| Sitka | 17-Jul-99 | 0.105 | 0.245 | 0.48 | 0.71 | 0.06 |
| Sitka | 21-Jul-99 | 0.025 | 0.23 | 0.395 | 0.525 | 0.06 |
| Sitka | 12-Mar-99 | 0.205 | 0.355 | 0.33 | 0.445 | 0.045 |
| Sitka | 16-Mar-99 | 0.12 | 0.22 | 0.435 | 0.64 | 0.06 |
| Sitka | 19-Mar-99 | 0.135 | 0.42 | 0.405 | 0.66 | 0.05 |
| Kodiak | 14-Jul-99 | 0.09 | 0.18 | 0.425 | 0.57 | 0.05 |
| Kodiak | 17-Jul-99 | 0.08 | 0.405 | 0.525 | 0.645 | 0.095 |
| Kodiak | 12-Mar-99 | 0.2 | 0.295 | 0.335 | 0.595 | 0.06 |
| Kodiak | 16-Mar-99 | 0.05 | 0.4 | 0.31 | 0.365 | 0.05 |
| Kodiak | 19-Mar-99 | 0.06 | 0.545 | 0.3825 | 0.515 | 0.0325 |
| Timber | 9-Jul-99 | 0.09 | 0.265 | 0.535 | 0.63 | 0.06 |
| Timber | 14-Jul-99 | | | | | |
| Timber | 16-Jul-99 | 0.195 | 0.195 | 0.625 | 0.77 | 0.06 |
| Timber | 5-Nov-99 | 0.06 | 0.35 | 0.825 | 0.63 | 0.095 |
| Timber | 9-Nov-99 | 0.045 | 0.41 | 0.825 | 0.69 | 0.085 |
| Timber | 12-Nov-99 | 0.055 | 0.365 | 0.83 | 0.725 | 0.08 |
| Hazy | 9-Jul-99 | 0.13 | 0.16 | 0.635 | 0.94 | 0.07 |
| Hazy | 13-Jul-99 | 0.15 | 0.355 | 0.63 | 0.825 | 0.065 |
| Hazy | 16-Jul-99 | 0.05 | 0.355 | 0.555 | 0.795 | 0.105 |
| Hazy | 5-Nov-99 | 0.075 | 0.385 | 0.825 | 0.63 | 0.065 |
| Hazy | 9-Nov-99 | 0.03 | 0.395 | 0.755 | 0.375 | 0.08 |
| Hazy | 12-Nov-99 | 0.1 | 0.495 | 0.86 | 0.895 | 0.085 |

| | | 20:3w6 | 20:4w6 | 20:4w3 | 20:5w3 | 22:1w11 |
|--------|-----------|--------|---------|--------|--------|---------|
| Sitka | 14-Jul-99 | 0.4 | 15.2 | 0.2 | 8.375 | 0.44 |
| Sitka | 17-Jul-99 | 0.65 | 21.325 | 0.175 | 6.545 | 0.39 |
| Sitka | 21-Jul-99 | 0.455 | 16.8 | 0.12 | 3.98 | 0.29 |
| Sitka | 12-Mar-99 | 0.435 | 20.25 | 0.27 | 11.19 | 0.425 |
| Sitka | 16-Mar-99 | 0.51 | 21.32 | 0.205 | 6.305 | 0.375 |
| Sitka | 19-Mar-99 | 0.475 | 21.165 | 0.165 | 6.125 | 0.485 |
| Kodiak | 14-Jul-99 | 0.305 | 15.13 | 0.18 | 8.87 | 0.395 |
| Kodiak | 17-Jul-99 | 0.455 | 20.54 | 0.135 | 5.71 | 0.52 |
| Kodiak | 12-Mar-99 | 0.445 | 21.09 | 0.25 | 10.355 | 0.42 |
| Kodiak | 16-Mar-99 | 0.555 | 24.67 | 0.15 | 6.98 | 0.195 |
| Kodiak | 19-Mar-99 | 0.4725 | 21.8975 | 0.16 | 5.7375 | 0.265 |
| Timber | 9-Jul-99 | 0.38 | 15.645 | 0.21 | 10.42 | 0.465 |
| Timber | 14-Jul-99 | | | | | |
| Timber | 16-Jul-99 | 0.535 | 17.05 | 0.22 | 5.825 | 0.355 |
| Timber | 5-Nov-99 | 0.36 | 13.375 | 0.17 | 8.97 | 0.65 |
| Timber | 9-Nov-99 | 0.465 | 16.32 | 0.12 | 5.71 | 0.83 |
| Timber | 12-Nov-99 | 0.47 | 14.59 | 0.13 | 4.6 | 0.755 |
| Hazy | 9-Jul-99 | 0.34 | 15.46 | 0.19 | 9.32 | 0.64 |
| Hazy | 13-Jul-99 | 0.5 | 17.71 | 0.16 | 5.225 | 0.615 |
| Hazy | 16-Jul-99 | 0.58 | 18.355 | 0.155 | 4.46 | 0.33 |
| Hazy | 5-Nov-99 | 0.275 | 13.29 | 0.16 | 9.295 | 0.75 |
| Hazy | 9-Nov-99 | 0.39 | 15.52 | 0.11 | 4.905 | 0.725 |
| Hazy | 12-Nov-99 | 0.405 | 15.395 | 0.14 | 4.5 | 0.88 |

| | | <u>22:5w3</u> | <u>22:6w3</u> | <u>24:1w9</u> |
|--------|-----------|---------------|---------------|---------------|
| Sitka | 14-Jul-99 | 1.745 | 7.27 | 0.48 |
| Sitka | 17-Jul-99 | 1.72 | 7.2 | 0.51 |
| Sitka | 21-Jul-99 | 1.215 | 5.375 | 0.51 |
| Sitka | 12-Mar-99 | 1.835 | 7.86 | 0.87 |
| Sitka | 16-Mar-99 | 1.665 | 8.265 | 0.465 |
| Sitka | 19-Mar-99 | 1.395 | 7.53 | 0.865 |
| Kodiak | 14-Jul-99 | 1.9 | 6.67 | 0.49 |
| Kodiak | 17-Jul-99 | 1.735 | 6.275 | 0.885 |
| Kodiak | 12-Mar-99 | 1.885 | 7.13 | 0.85 |
| Kodiak | 16-Mar-99 | 1.425 | 6.525 | 1.06 |
| Kodiak | 19-Mar-99 | 1.2 | 5.7325 | 1.1025 |
| Timber | 9-Jul-99 | 2.325 | 8.55 | 0.645 |
| Timber | 14-Jul-99 | | | |
| Timber | 16-Jul-99 | 1.58 | 6.495 | 0.405 |
| Timber | 5-Nov-99 | 2.33 | 6.485 | 0.765 |
| Timber | 9-Nov-99 | 1.67 | 6.175 | 0.78 |
| Timber | 12-Nov-99 | 1.455 | 5.75 | 0.69 |
| Hazy | 9-Jul-99 | 2.735 | 9.885 | 0.405 |
| Hazy | 13-Jul-99 | 2.115 | 8.895 | 0.675 |
| Hazy | 16-Jul-99 | 1.985 | 8.63 | 0.68 |
| Hazy | 5-Nov-99 | 2.55 | 7.75 | 0.925 |
| Hazy | 9-Nov-99 | 1.865 | 7.27 | 0.765 |
| Hazy | 12-Nov-99 | 1.655 | 7.1 | 0.89 |

LIST OF REFERENCES

- Baker J.D., C.W. Fowler, and G.A. Antonelis. 1994. Mass change in fasting immature male northern fur seals. *Canadian Journal Zoology*. 72: 326-329.
- Ballantyne, J.S., F. Mercure, M.F. Gerrits, G. Van Der Kraak, S. McKinley, D.W. Martens, S.G. Hinch and R.E. Diewert. 1996. Plasma nonesterified fatty acid profiles in the male and female sockeye salmon, *Oncorhynchus nerka*, during the spawning migration. *Canadian Journal of Fisheries and Aquatic Science* 53: 1418-1426.
- Belkhou, R., Y. Cherel, A. Heitz, J.P. Robin, and Y. LeMaho. 1991. Energy contribution of proteins and lipids during prolonged fasting in the rat. *Nutrition Research* 11: 365-374.
- Bonner, W. N. 1989. The natural history of seals. Christopher Helm, London, Great Britain.
- Bowen, W.D., C.A. Beck and D.A. Austin. 2002. Pinniped Ecology. Pages 911-920 *In* W.F. Perrin, B. Würsig and J.G.M. Thewissen (eds.). "Encyclopedia of Marine Mammals". Academic Press, San Diego, California.
- Boyd and Duck. 1991. Mass changes and metabolism in territorial male Antarctic fur seals (*Arctocephalus gazella*). *Physiological Zoology*. 64: 375-392.
- Calkins, D.G., E.F. Becker, and K.W. Pitcher. 1998. Reduced body size of female Steller sea lions from a declining population in the Gulf of Alaska. *Marine Mammal Science*. 14: 232-244.
- Castellini, M.A. and D.P. Costa. 1990. Relationships between plasma ketones and fasting duration in neonatal elephant seals. *American Journal of Physiology*. 259: R1086-R1089.
- Castellini, M.A., D.P. Costa, and A.C. Huntley. 1987. Fatty acid metabolism in fasting elephant seal pups. *Journal of Comparative Physiology*. B 157: 445-449.
- Cherel, Y., B. El Omari, Y. LeMaho, and M. Saboureau. 1995. Protein and lipid utilization during fasting with shallow and deep hypothermia in the European

- hedgehog (*Erinaceus europaeus*). *Journal of Comparative Physiology. B* 164: 653-658.
- Cherel, Y., F. Freby, J. Gilles, J.P. Robin. 1993. Comparative fuel metabolism in Gentoo and King penguins: adaptation to brief versus prolonged fasting. *Polar Biology*. 13: 263-269.
- Cherel, Y., J. Gilles, Y. Handrich and Y. Le Maho. 1995. Nutrient reserves dynamics and energetics during long-term fasting in the King penguin (*Aptenodytes patagonicus*). *Journal of Zoology London*. 234: 1-12.
- Cherel, Y., J.P. Robin, and Y. Le Maho. 1988. Physiology and biochemistry of long-term fasting in birds. *Canadian Journal of Zoology*. 66: 159-166.
- Christen, D., K. Mashburn, C. Stephens, J. Mellish, and S. Atkinson. 2003. Monitoring a male Steller sea lion into adulthood. The 15th Biennial Conference on the Biology of Marine Mammals, 14-19 December, Greensboro, North Carolina.
- Cunnane, S.C. 1990. Differential utilization of long chain fatty acids during fasting-induced triacylglycerol depletion. III. Comparison of n-3 and n-6 fatty acids in rat plasma and liver. *Biochemistry and Biophysics Acta*. 1036: 64-70.
- Florant G.L. 1998. Lipid metabolism in hibernators: The importance of essential fatty acids. *American Zoology*. 38: 331-340.
- Folch, J., S.M. Lee, and G.H. Sloane-Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissue. *Journal of Biological Chemistry*. 226: 497-506.
- Groscolas, R. 1986. Changes in body mass body temperature, and plasma fuel levels during the natural breeding fasting in male and female Emperor penguins, *Aptenodytes forsteri*. *Journal of Comparative Physiology*. 156: 521-527.
- Groscolas, R. 1990. Metabolic adaptations to fasting in Emperor and King Penguins. Pages 269-296 *In* L. Davis and J. Darby (eds.). *Penguin Biology*. Academic Press, New York.
- Hissa, R., E. Hohtola, T. Tuomala-Saramaki, T. Laine, and H. Kallio. 1998. Seasonal changes in fatty acids and leptin contents in the plasma of the European brown bear (*Ursus arctos arctos*). *Annual Zoology Fennici* 35: 215-224.
- Hood, W.R. and K.A. Ono. 1997. Variation in maternal attendance patterns and pup behaviour in a declining population of Steller sea lions (*Eumetopias jubatus*). *Canadian Journal of Zoology*. 75: 1241-1246.

- Iverson, S.J. 1993. Milk secretion in marine mammals in relation to foraging: can milk fatty acids predict diet? Symposium of the Zoological Society of London 66:211-214.
- Lands, W.E.M. 1991. Biosynthesis of prostaglandins. Annual Review of Nutrition 11:41-60.
- Lands, W.E.M. 1992. Biochemistry and physiology of n-3 fatty acids. Federation of American Societies for Experimental Biology Journal 6: 2530-2536.
- Loughlin, T.R. 1998. The Steller sea lion: a declining species. Biosphere Conservation 1: 91-98.
- Mellish, J.E, S.J. Iverson, and W.D. Bowen. 2000. Metabolic compensation during high energy output in fasting, lactating gray seals (*Halichoerus grypus*): metabolic ceilings revisited. Proceeding of the Royal Society of London B 267: 1245-1251.
- Merrick, R.L. and T.R. Loughlin. 1997. Foraging behavior of adult female and young-of-the-year steller sea lions in Alaskan waters. Canadian Journal of Zoology. 75: 776-786.
- Morrison, W.R. and L.M. Smith. 1964. Preparation of fatty acid methyl esters and dimethyl acetals from lipids with boron fluoride-methanol. Journal Lipid Research. 5: 600-608.
- Mostafa, N., D.C. Everett, S.C. Chou, P.A. Kong, G.L. Florant, and R.A. Coleman. 1993. Seasonal changes in critical enzymes of lipogenesis and triacylglycerol synthesis in the marmot (*Marmota flaviventris*). Journal of Comparative Physiology B163: 463-469.
- Nordøy, E.S., A. Aakvaag, and T.S. Larsen. 1993. Metabolic Adaptations to fasting in harp seal pups. Physiological Zoology 66: 926-945.
- Nordøy, E.S. and A.S. Blix. 1985. Energy sources in fasting grey seal pups evaluated with computed tomography. American Journal of Physiology. 249: R471-R476.
- Nordøy, E.S. and A.S. Blix. 1991. Glucose and ketone body turnover in fasting grey seal pups. Acta Physiology Scandinavia. 141: 565-571.
- Ormseth, O.A., M. Nicolson, M.S. Pelleymounter and B.B. Boyer. 1996. Leptin inhibits prehibernation hyperphagia and reduces body weight in Arctic ground squirrels. American Journal Physiology. 271: R1775-R1779.
- Pitcher, K.W., D.G. Calkins and G.W. Pedleton. 2000. Steller sea lion body condition indices. Marine Mammal Science 16: 427-436.

- Raclot, T. and R. Groscolas. 1993. Differential mobilization of white adipose tissue fatty acids according to chain length, unsaturation, and positional isomerism. *Journal of Lipid Research* 34: 1515-1526.
- Raclot, T. and R. Groscolas. 1995. Selective mobilization of adipose tissue fatty acids during energy depletion in the rats. *Journal of Lipid Research* 36: 2164-2173.
- Raclot, T., D. Langin, M. Lafontan, and R. Groscolas. 1997. Selective release of human adipocyte fatty acids according to molecular structure. *Biochemistry* 324: 911-915.
- Raclot, T., E. Mioskowski, A.C.Bach, and R. Groscolas. 1995. Selectivity of fatty acid mobilization: a general metabolic feature of adipose tissue. *American Journal of Physiology*. 269: R1060-R1067.
- Rea, L.D. 1995. Prolonged fasting in pinnipeds. Ph.D. thesis. University of Alaska at Fairbanks, Alaska 135pp.
- Rea, L.D. 2000. Seasonal Changes in blood chemistry and lipid metabolism in Steller sea lions. Final report to the National Fish and Wildlife Foundation upon completion of Grant project # 98-244-008.
- Rea, L.D., M.A. Castellini, B.S. Fadely, and T.R. Loughlin. 1998. Health status of young Alaskan steller sea lion pups (*Eumetopias jubatus*) as indicated by blood chemistry and hematology. *Comparative Biochemistry And Physiology. A.* 120: 617-623.
- Rea, L.D. and D.P. Costa. 1992. Changes in standard metabolism during long-term fasting in northern elephant seal pups (*Mirounga angustirostris*). *Physiological Zoology* 65: 97-111.
- Rea, L.D., R. Groscolas, E. Mioskowski and M. Castellini. 1997. Changes in plasma fatty acids indicate changes in nutritional status in developing Weddell seal pups. *Polar Biology* 18: 351-357.
- Reeves, R.R., B.S. Stewart, P.J. and S. Leatherwood. 1992. The Sierra Club handbook of seals and sirenians. Sierra Club Books, San Francisco, pp.359.
- Reilly, J.J. 1991. Adaptations to prolonged fasting in free-living weaned gray seal pups. *American Journal of Physiology*. 260: R267-R272.
- Richmond, J.P., J.M. Burns, L.D. Rea, and K.L. Mashburn. 2005. Postnatal ontogeny of erythropoietin and hematology in free-ranging Steller sea lions (*Eumetopias jubatus*). *General and Comparative Endocrinology* 141: 240-247.

- Robin, J.P., M. Frain, C. Sardet, R. Groscolas, and Y. LeMaho. 1988. Protein and lipid utilization during long-term fasting in emperor penguins. *American Journal of Physiology*. 254: R61-R68.
- Rosen, D.A.S. and A.W. Trites. 2002. Changes in metabolism in response to fasting and food restriction in the Steller sea lion (*Eumetopias jubatus*). *Comparative Biochemistry and Physiology Part B* 132: 389-399.
- Trites, A.W. and C.P. Donnelly. 2003. The decline of Steller sea lions *Eumetopias jubatus* in Alaska: a review of the nutritional stress hypothesis. *Mammal Review* 33: 3-28.
- Ward, J. M. and K.B. Armitage. 1981. Circannual rhythms of food consumption, body mass, and metabolism in yellow-bellied marmots. *Comparative Biochemistry and Physiology*.A.69: 621-626.
- Worthy, G.A.J. and D.M. Lavigne. 1983. Energetics of fasting and subsequent growth in weaned harp seal pups, *Phoca groenlandica*. *Canadian Journal of Zoology* 61: 447-456.
- Worthy, G.A.J. and D.M. Lavigne. 1987. Mass loss, metabolic rate and energy utilization by harp and gray seal pups during the postweaning fast. *Physiological Zoology*. 60: 352-364.
- York, A. 1994. The population dynamics of Northern Sea Lion 1975-1985. *Marine Mammal Science* 10: 38-51.