

# THE EFFECTS OF FOOD DEPRIVATION ON SERUM LIPID CONCENTRATION AND CONTENT IN STELLER SEA LIONS (*Eumetopias jubatus*)

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## Introduction

The western Alaska population of Steller sea lions has significantly declined over the past thirty-five years. A population estimate of 180,000 individuals in 1965 declined to a current estimate of 50,000. A widely accepted hypothesis for the cause of decline is from indirect competition with the commercial fishing industry. Analysis of Steller sea lion censuses have determined that decline is most evident in the juvenile portion of the population. This could be explained by a decrease in prey availability for juveniles which are physiologically and behaviorally limited in their ability to forage further and deeper for food. Although Steller sea lions naturally fast during their summer breeding season, they are not as biochemically adapted to handle food deprivation at other times of the year (Rea et al. 1999). This study addresses the physiological implications of food deprivation by analyzing the effects of fasting on serum lipid composition and content. Additionally, the breeding and non-breeding seasons were compared to determine if seasonality affects serum lipid composition and content.

## Methods

Nine Steller sea lions, 4 juveniles and 5 sub-adults, were used in 13 different fasting experiments lasting from 7 to 14 days. Eight of the fasts were conducted during the natural breeding season (May-July) and 5 were conducted throughout the rest of the year (non-breeding season). The length of the fast was dependent upon body size and fasting was discontinued once the animal lost 15% body mass. Blood samples were collected from the caudal gluteal or the flipper vein after an overnight fast and every 2 to 4 days thereafter. Samples were centrifuged and serum was stored at -80° until further analysis. Non-esterified fatty acid (NEFA) concentration was quantified using a spectrophotometric assay (NEFA-C kit, Wako Diagnostics, Richmond, Virginia). Additionally, total lipids contained in the serum were extracted using 2:1 (vol/vol) chloroform:methanol using a modified Folch et al. (1957) method. Fatty acids were converted to fatty acid methyl esters (FAME) according to Morrison and Smith (1964) using 12% boron trifluoride by weight in methanol. Fatty acid composition of FAME was determined by gas chromatography (GC). Duplicate analysis of FAME was performed using a temperature-programmed GC on a Perkin Elmer Autosystem II Capillary FID gas chromatograph fitted with a 30m x 0.25mm id. column coated with 50% cyanopropyl polysiloxane and linked to a computerized integration system (Turbochrome 4.1 software). Paired t-tests were used to compare NEFA concentrations in the breeding season to the non-breeding season. Additionally, a repeated measures ANOVA was employed to

determine any change in NEFA concentration during the fasting experiments. Samples were grouped into stages of fasting (stage 1 = day 1 of fast, stage 2 = day 3-4, stage 3 = day 6-7, stage 4 = day 10-12, stage 5 = 13-14). The 28 fatty acids that contributed to >0.2 weight percentages of the total were analyzed using principle components analysis (PCA) to determine if the fatty acid composition was changing throughout the fast. A MANOVA was used to determine if the scores (axis) developed in the PCA significantly explained the trends. In all cases, significance was measured at  $p < 0.05$ .

## Results

NEFA concentrations did not differ significantly between the breeding and non-breeding season ( $n=4$ ,  $p=0.06$ ). However, NEFA concentrations did increase in juveniles during the breeding season ( $n=4$ ,  $p=0.04$ ) (Figure 1) but not during their non-breeding season fasts ( $n=2$   $p=0.6$ ). Although the NEFA concentrations appear to increase during the breeding and non-breeding seasons fasts in sub-adults, this trend was not significant ( $n=3$ ,  $p=0.3$ ,  $n=3$ ,  $p=0.3$  respectively) (Figure 2).

PCA significantly identified a change between stage 1 and stage 3 in the juveniles ( $p=0.0006$ ) (Figure 3). Upon identifying a change from the beginning to the end of the fast, the first two stages were analyzed separately from the second two stages. This revealed no separation between stage 1 and stage 2 ( $p=0.7$ ). However, PCA did successfully separate stage 2 and stage 3 ( $p=0.02$ ) and separated the breeding and non-breeding season fasts ( $p=0.05$ ) (Fig. 3).

Stage 1 and stage 4/5 in the sub-adults were also significantly--separated by PCA ( $p=0.002$ ). Additionally, trends from stage 1 to stage 3 and from stage 3 to stage 4/5 were analyzed using PCA and a significant separation was found between stage 1 and stage 3 ( $p<0.001$ ) but not between stage 3 and stage 4/5 ( $p=0.07$ ).

## Discussion

It has been shown that Steller sea lions are more biochemically adapted to fast during the breeding season compared to other times of the year (Rea et al. 1999). The results of this study also suggest difference in lipid metabolism between season in some age classes. Although no difference of NEFA concentration was found between the breeding and non-breeding season, NEFA concentration did significantly increase during the breeding season of the juveniles and not during the non-breeding season. This supports the concept that Steller sea lions mainly rely on lipid stores for energy during fasting and are well adapted to do this during the breeding season. The sub-adults provided no support for this theory as their NEFA concentrations did not differ between season and did not significantly change over the fast.

In assessing the seasonal trends of serum fatty acid changes during fasting, the juveniles again experienced differences during the breeding and non-breeding seasons. In comparison of stage 2 and stage 3 of the juveniles' fasts, there was separation between the seasons. The variables that had the most influence on this separation were three

saturated fatty acids, 14:0, 15:0 and 16:0. The sub-adults showed no change in fatty acid composition between seasons but did change throughout the fasts. Further analysis is needed to determine if fatty acids are good indicators of seasonal adaptation to fasting. This study provides a preliminary foundation in which this trend may be more intensely studied. An increase in sample size and in age range would allow for increased comparison to determine the effects of food deprivation on the physiology of Steller sea lions.

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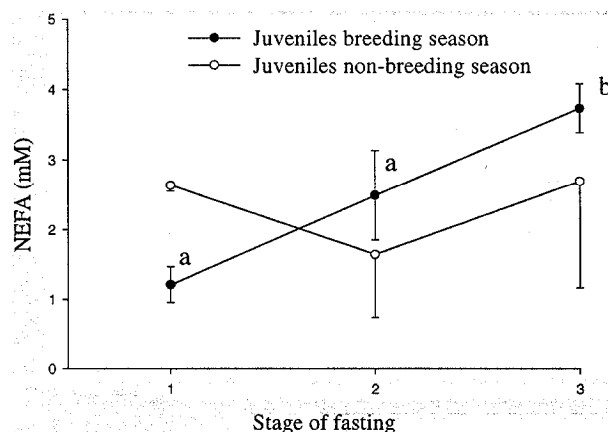


Figure 1. Non-esterified fatty acid concentration of the juveniles from their breeding and non-breeding season fasts. Values are means  $\pm$  SEM.

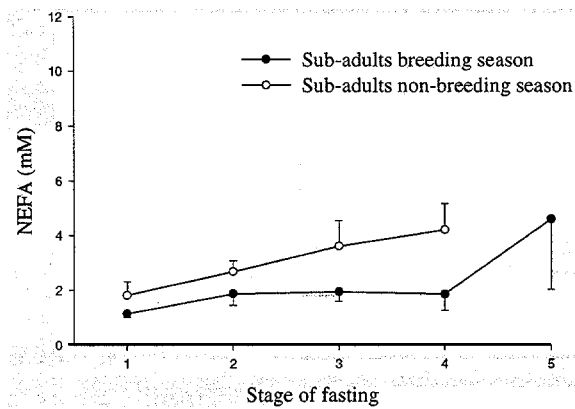


Figure 2. Non-esterified fatty acid concentration of the sub-adults from their breeding and non-breeding season fasts. Values are means  $\pm$  SEM.

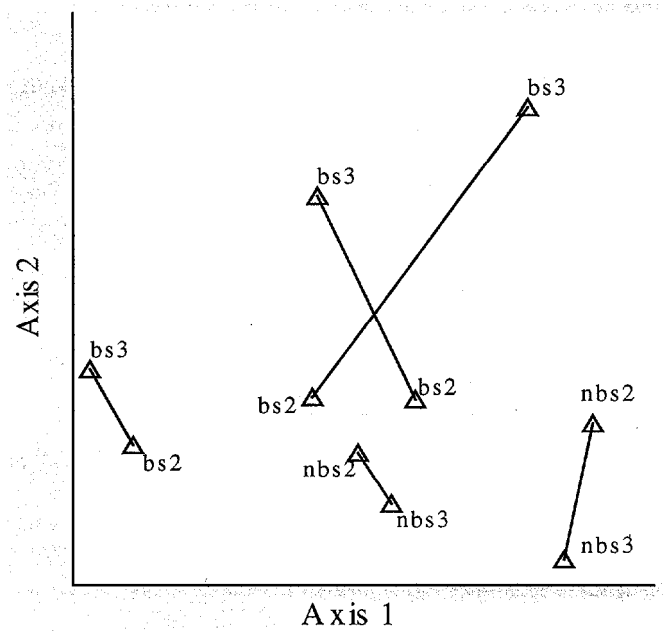


Figure 3. Principle components analysis on stage 2 and stage 3 for the breeding and non-breeding season fasts in juveniles. Plots of the first two axes (scores) reveal significant separation between the two stages. Vertical separation between the breeding season (bs) and non-breeding season (nbs) is also evident.