Exxon Valdez Oil Spill
Restoration Project Annual Report

Harbor Seal Recovery. Phase III: Effects of Diet on Lipid Metabolism and Health

Restoration Project 99441
Annual Report

This annual report has been prepared for peer review as part of the *Exxon Valdez* Oil Spill Trustee Council restoration program for the purpose of assessing project progress. Peer review comments have not been addressed in this annual report.

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Study History: This project was initiated in 1999 and continued in 2000 as a contract between the State of Alaska, Dept. of Fish and Game (Division of Habitat and Restoration) and Texas A&M University. The project will be completed in FY01 as Restoration Project 01441-BAA.

Abstract: The harbor seal (*Phoca vitulina richardsi*) population in Prince William Sound has not recovered and may continue to decline. An underlying hypothesis is that ecosystem-wide changes in food availability could be affecting harbor seal population recovery. To better understand the results from field studies of harbor seal health, body condition and feeding ecology, we need data for seals on diets that vary in nutritional composition. Working with the Alaska SeaLife Center (ASLC), we will determine how fatty acid profiles in the blubber of captive harbor seals change over time during controlled diets of herring and pollock. In addition, we will assess the aerobic capacity and lipid metabolism of skeletal muscle in harbor seals fed controlled diets and for wild harbor seals in Prince William Sound. The results will augment already funded investigations of diet and health to provide a more in depth understanding of the nutritional role and assessment of dietary fat for harbor seals.

Key Words: Diet, harbor seal, lipid, metabolism, Prince William Sound.

Project Data: (will be addressed in the final report)

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EXECUTIVE SUMMARY

Introduction. Understanding the feeding ecology and nutritional status of harbor seals is an essential component of ecosystem-based research on the recovery of species impacted by the Exxon Valdez oil spill in Prince William Sound. A new technique using fatty acid profiles of blubber can provide details on cumulative dietary history. It can also, in some cases, be used to determine foraging habitat. In pinnipeds, as with other carnivores and monogastric animals, dietary fatty acids generally remain intact through the digestion process and are deposited in adipose tissue with little or no modification (1). As a result, differences in the fatty acid composition of carnivore blubber can be used to infer dietary differences between individuals or populations and perhaps even species composition of the diet.

Previous research has shown that fatty acid signatures are significantly affected by spatial or temporal heterogeneity in habitat and food webs (1). In a study of harbor seal foraging ecology supported by the Restoration Program, Iverson, et al (2) were able to distinguish individual species of fish using fatty acid signatures. They also found fatty acid composition of these prey items to be correlated with body size as well as location within a study area. Hence, analysis of fatty acids in pinnipeds and their prey should provide details on the spatial scales of foraging and habitat use of both individuals and populations. Evaluating how harbor seal blubber fatty acids change with diet during controlled feeding studies where species composition of diet is known will improve the spatial and temporal interpretation of fatty acid profiles of wild seals whose diet composition is unknown.

Muscle condition and metabolic function can be used as indicators of the health status of marine mammals. Important indices of muscle function and health are aerobic capacity, the ability to store oxygen in the form of oxy-myoglobin and the size of lipid stores. In a preliminary study conducted by our laboratory (3), we observed that the volume density of mitochondria, myoglobin concentration and citrate synthase activity in the swimming muscles of harbor seals were elevated relative to terrestrial mammals and appeared to be an adaptation for aerobic metabolism during diving. One objective of this study is to study the effect of diet on the aerobic capacity, myoglobin concentration and lipid stores of skeletal muscles in harbor seals. In addition, we will measure the activities of citrate synthase and *B*-hydroxyacyl CoA dehydrogenase (an enzyme important for lipid metabolism) as indicators of aerobic capacity and the *B*-oxidation of fatty acids, respectively.

The harbor seal population in Prince William Sound has not recovered and may continue to decline. An underlying hypothesis is that ecosystem wide changes in food availability could be affecting harbor seal population recovery. To better understand the behavioral and physiological results obtained from field studies of harbor seal health, body condition and feeding ecology supported by the Restoration Program, we need comparable data for seals on diets that vary in nutritional composition. In 1998, a captive study was begun at the Alaska SeaLife Center to quantify the health effects of feeding several key Alaskan fish species to harbor seals. We will augment this study by examining changes in fatty acid profiles in seal blubber and muscle lipid content during controlled feeding studies where fish species composition is known. In addition, we will quantify the aerobic capacity and activities of enzymes that are crucial for muscle lipid metabolism and which may be affected by nutritional stress.

Objectives: The objectives of this study are:

1. Determine how fatty acids in the blubber of captive harbor seals change over time during controlled diets of herring and pollock.

2. Measure the content and composition of lipid in muscle of captive harbor seals fed controlled diets and for wild harbor seals in Prince William Sound.

3. Assess the aerobic capacity and lipid metabolism of skeletal muscle in harbor seals fed controlled diets and for wild harbor seals in Prince William Sound.

Two hypotheses will be tested:

1. Null hypothesis: Fatty acid profiles in the blubber of harbor seals are not affected by the fatty acid composition of the diet.

   Alternative hypothesis: Fatty acid profiles in the blubber of harbor seals will be directly affected by the fatty acid composition of the diet and will change as the diet is altered.

2. Null hypothesis: Mitochondrial volume density, myoglobin concentration, lipid content, and the enzymatic activities of citrate synthase and *B*-hydroxyacyl CoA dehydrogenase are not affected by diet.

   Alternative hypothesis: These variables of muscle condition and function are affected by changes in diet.

Methods. Eight harbor seals have been acquired by the ASLC for the feeding trials that began in September 1998. During the staggered feeding trials, the diet (either herring or pollock) will be changed every four months. Two additional control seals will receive a diet of half herring and half pollock throughout the study. During these dietary manipulations, we will obtain serial blubber samples every two months and muscle biopsies every four months from two sites on each animal.

Blubber samples will be analyzed for fatty acid signatures using gas-liquid chromatography. Individual fatty acids, expressed as weight percent of the total fatty acids, will be analyzed using classification and regression trees (CART), a non-parametric multivariate technique for classifying data. Muscle biopsies will be analyzed for citrate synthase activity, *B*-hydroxyacyl CoA dehydrogenase activity and myoglobin concentration. In addition, fixed muscle samples will be analyzed by electron microscopy for the volume density of mitochondria, myofibrils and lipid droplets. Samples from the main swimming muscles, blubber and splanchnic organs of 16 harbor seals will be obtained during BIOSAMPLING Program. They will be analyzed using the same techniques as the muscle samples.

Accomplishments for the first 18 months of the project. Feeding trials for eight harbor seals began in early September 1998 at the Alaska SeaLife Center. Six seals (two groups of three) received an alternating diet of either herring or pollock. To date, four feeding trials have been

completed and the fifth is ongoing. Six feeding trials are planned. A separate control group of two seals will receive a mixed diet of half herring and half pollock throughout the study.

At the mid-point and end of each feeding trial, blubber samples only were taken at two sites from each seal. Skeletal muscle samples were also taken at the end of each trail. Half of each muscle sample was placed in fixative, and the remainder along with the blubber samples were frozen at -70° C. The muscle samples were sent to the University of California at San Diego where analysis (% fiber type, volume density of lipid droplets and mitochondria, lipid enzyme activities, and myoglobin concentration) is underway. We have analyzed the samples from the mixed diet group for the volume density of mitochondria and lipid droplets per volume of muscle fiber. These animals have smaller volume densities of mitochondria but greater volume densities of lipid droplets than those previously measured in free-ranging harbor seals from Prince William sound, Alaska. The samples from the other trials are currently being processed and measured for the same parameters. The biochemical analysis has not begun, because we are waiting for the end of the feeding trials to analyze them in a single batch.

Blubber samples will be analyzed for fatty acid profiles at Texas A&M University. Analysis is underway for the first three feeding trials and should be completed this summer. The remainder of the samples will be analyzed after the feeding trials are finished in September 2000.

In June of 1999, we obtained extensive muscle, blubber and splanchnic organ samples from eight harbor seals through the BIOSAMPLING Program in Prince Williams Sound. These samples are under analysis at Texas A&M University and will form the basis for two Doctoral and one Masters dissertation. Preparations have been made for obtaining additional harbor seal samples from the BIOSAMPLING Program in June 2000. This collaborative effort with the Native community has been very successful for our program.

INTRODUCTION

Understanding the feeding ecology and nutritional status of harbor seals is an essential component of ecosystem-based research on the recovery of species impacted by the Exxon Valdez oil spill in Prince William Sound. Until recently, determinations of prey preferences for pinnipeds have been based on stomach content and fecal analyses, both of which can only yield information on the most recent meals and may be biased due to differential rates of passage of food items. A new technique using fatty acid profiles of blubber can provide details on cumulative dietary history. It can also, in some cases, be used to determine foraging habitat. In pinnipeds, as with other carnivores and monogastric animals, dietary fatty acids generally remain intact through the digestion process and are deposited in adipose tissue with little or no modification (1). As a result, differences in the fatty acid composition of carnivore blubber can be used to infer dietary differences between individuals or populations and perhaps even species composition of the diet.

Previous research has shown that fatty acid signatures are significantly affected by spatial or temporal heterogeneity in habitat and food webs (1). In a study of harbor seal foraging ecology (Project 117-BAA; Harbor seal blubber and lipids) supported by the Restoration Program, Iverson, et al (2) were able to distinguish individual species of fish using fatty acid signatures. They also found fatty acid composition of these prey items to be correlated with body size as well as location within a study area. Hence, analysis of fatty acids in pinnipeds and their prey should provide details on the spatial scales of foraging and habitat use of both individuals and populations. Evaluating how harbor seal blubber fatty acids change with diet during controlled feeding studies where species composition of diet is known will improve the spatial and temporal interpretation of fatty acid profiles of wild seals whose diet composition is unknown.

Muscle condition and metabolic function can be used as indicators of the health status of marine mammals. Important indices of muscle function and health are aerobic capacity, the ability to store oxygen in the form of oxy-myoglobin and the size of lipid stores. In a preliminary study conducted by our laboratory (3), we observed that the volume density of mitochondria, myoglobin concentration and citrate synthase activity in the swimming muscles of harbor seals were elevated relative to terrestrial mammals and appeared to be an adaptation for aerobic metabolism during diving. One objective of this study is to study the effect of diet on the aerobic capacity, myoglobin concentration and lipid stores of skeletal muscles in harbor seals. In addition, we will measure the activities of citrate synthase and *B*-hydroxyacyl CoA dehydrogenase (an enzyme important for lipid metabolism) as indicators of aerobic capacity and the *B*-oxidation of fatty acids, respectively.

The Restoration Program has supported the population monitoring component of health assessment, diving behavior and food preferences of harbor seals in Prince William Sound. Now, with controlled feeding studies of harbor seals underway at the Alaska SeaLife Center, we will continue our studies of the effects of diet on fatty acid signatures in blubber and the metabolic function of muscle, especially with regards to lipid. The results will improve our understanding of harbor seal feeding ecology and the effects of diet on health and metabolism.

NEED FOR THE PROJECT

A. Statement of Problem

The Restoration Program has supported three harbor seal studies in Prince William Sound (Project 001- Harbor seal condition and health status; Project 064- Monitoring habitat use and trophic interactions of harbor seals; Project 117-BAA- Harbor seal blubber and lipids). One objective of these studies has been to measure health and body condition indices related to metabolic alterations that might occur in animals that were food deprived. Although these studies collected much useful information, some researchers realized that controlled dietary studies were needed to more completely interpret field data. In 1997, the Restoration Program funded a captive study (Harbor Seal Recovery. Phase II: Controlled Studies of Health and Diet) at the Alaska SeaLife Center that will quantify the nutritional value of several key Alaskan fish species for harbor seals and will follow health indices over time in both healthy and rehabilitation animals. That project, which has been underway at the Alaska SeaLife Center for 18 months, will feed controlled diets of fish to harbor seals to examine changes in body condition, health, assimilation efficiency and blood chemistry biomarkers. Of particular interest will be the health and body condition effects of diets containing nutritionally poor (compared to herring) fish such as pollock, the so-called "junk food" hypothesis for explaining the decline of certain pinniped stocks. In this project, we will continue (four feeding Trials are completed and the fifth will be completed in May 2000) to take advantage of the controlled feeding studies at the Alaska SeaLife Center to examine the effects of diet on: 1) fatty acid markers in the blubber, 2) muscle condition and 3) lipid metabolism. In addition, we will use samples of blubber and muscle obtained by the BIOSAMPLING Program in Prince William Sound for comparison with captive seals fed known diets. This important work will augment already funded investigations of diet and health to provide a more in depth understanding of the nutritional role and assessment of dietary fat for harbor seals.

B. Rationale

The harbor seal population in Prince William Sound has not recovered and may continue to decline. An underlying hypothesis is that ecosystem wide changes in food availability could be affecting harbor seal population recovery. To better understand the behavioral and physiological results obtained from field studies of harbor seal health, body condition and feeding ecology supported by the Restoration Program, we need comparable data for seals on diets that vary in nutritional composition. In 1998, a captive study was begun at the Alaska SeaLife Center to quantify the health effects of feeding several key Alaskan fish species to harbor seals. We propose to augment this study by examining changes in fatty acid profiles in seal blubber and muscle lipid content during controlled feeding studies where fish species composition is known. In addition, we will quantify the aerobic capacity and activities of enzymes that are crucial for muscle lipid metabolism and which may be affected by nutritional stress.

C. Location

The experiments for this project will be conducted at the Alaska SeaLife Center in Seward. We will collaborate with existing projects that will examine the detailed metabolic alternations in stable isotope ratios (Schell/Project 170) and changes in body condition and health indices (Castellini/Project 341) in harbor seals that occur under different feeding regimes.

COMMUNITY INVOLVEMENT AND TRADITIONAL ECOLOGICAL KNOWLEDGE

Native communities have assisted Field studies of harbor seals in conjunction with the BIOSAMPLING program (Project 96244). We will continue that collaboration by analyzing samples of muscle, blubber and other tissues taken as part of subsistence hunting

PROJECT DESIGN

A. Objectives

1. Determine how fatty acids in the blubber of captive harbor seals change over time during controlled diets of herring and pollock.

2. Measure the content and composition of lipid in muscle of captive harbor seals fed controlled diets and for wild harbor seals in Prince William Sound.

3. Assess the aerobic capacity and lipid metabolism of skeletal muscle in harbor seals fed controlled diets and for wild harbor seals in Prince William Sound.

B. Methods

1. Hypotheses to be Tested.

1. Null hypothesis: Fatty acid profiles in the blubber of harbor seals are not affected by the fatty acid composition of the diet.

   Alternative hypothesis: Fatty acid profiles in the blubber of harbor seals will be directly affected by the fatty acid composition of the diet and will change as the diet is altered.

   Methodology: Feed controlled diets of different fish species to captive harbor seals. Assess temporal changes in the fatty acid composition of the blubber by taking serial biopsies. Compare with samples obtained from the BIOSAMPLING program of wild harbor seals in Prince William Sound.

2. Null hypothesis: Mitochondrial volume density, myoglobin concentration, lipid content, and the enzymatic activities of citrate synthase and *B*-hydroxyacyl CoA dehydrogenase are not affected by diet.

Alternative hypothesis: These variables of muscle condition and function are affected by changes in diet.

Methodology: Feed controlled diets of different fish species to captive harbor seals. Assess temporal changes in these variables by taking serial muscle biopsies. Compare with samples obtained from the BIOSAMPLING program of wild harbor seals in Prince William Sound.

2. Harbor Seal Feeding Trials Conducted at the Alaska SeaLife Center (ASLC).

Animals. Eight harbor seals have been acquired by the ASLC for the feeding trials that began in September 1998. Dr. Michael Castellini (Research Director at ASLC) developed dietary protocols for EVOS Project 99341. During the staggered feeding trials, the diet will be changed every four months. During these dietary manipulations, we will obtain serial blubber samples every two months and muscle biopsies every four months from two sites on each animal.

Design for Feeding Trials. A detailed matrix of the feeding schedule developed by Dr. Castellini is shown below. The procedure will use a cross-over repeated measures approach and will allow statistical comparisons within any one group of seals between diet and season. Statistical software (SYSTAT) will be used to analyze the cross-over method. However, there are several considerations that must be addressed using this matrix.

CROSS-OVER REPEATED MEASURES ANOVA FEEDING TRIALS
FOR HARBOR SEALS

Period	Herring	Pollock	Condition
Sept-Dec 1998	Seals A,B,C	Seals D,E,F	Molting
Jan-April 1999	D,E,F	A,B,C	Spring
May-Aug 1999	A,B,C	D,E,F	Breeding
Sept-Dec 1999	D,E,F	A,B,C	Molting
Jan-April 2000	A,B,C	D,E,F	Spring
May-Aug 2000	D,E,F	A,B,C	Breeding

This feeding matrix allows each group of seals to experience a different diet at similar physiologically relevant times of the year. Group A,B,C, for example, will receive a herring diet during the molting season in Year 1 and a high pollock diet in Year 2. After training during the summer of 1998, the seals accepted a pollock diet that was 100% pollock. Two additional control seals will receive a diet of half herring and half pollock throughout the study.

A problem with cross-over ANOVA designs is that residual or carry-over effects from previous treatments can complicate the analysis. We correct for this with long test periods and phased cross-overs. That is, since each feeding trial will last for four months, several weeks of diet switching will be allowed. This provides the additional advantage of allowing us to study the

impact of the phased switch on blubber and muscle lipid content and composition, and on muscle lipid metabolism.

Blubber Biopsies. Blubber samples will be obtained through the full depth of blubber layer with a 6-mm punch biopsy inserted through a small incision in the skin. Samples will be immediately transferred to liquid nitrogen and stored at -70° C until analysis. Total lipids will be extracted in chloroform according to Folch et al. (3) as modified by Iverson (5). Fatty acid methyl esters (FAME) will be prepared from the purified lipid extracts using the Hilditch reagent (0.5 N H_2SO_4 in methanol). FAME for fish in the controlled diets will be obtained similarly from homogenates of individual food items. The methyl esters will be analyzed by temperature-programmed capillary gas-liquid chromatography. FAME will be identified and quantified using a combination of standard mixtures, including those identified using chromatography and an ion-trap mass detector. Individual fatty acids, expressed as weight percent of the total fatty acids, will be analyzed using classification and regression trees (CART) in S-plus (StatSci, Seattle), a non-parametric multivariate technique for classifying data. CART uses a series of algorithms to split data into groups as differently as possible, based on measures of deviance; the splitting continues in a tree-like form until a classification is made at a terminal node.

Muscle Biopsies. Two muscle samples of approximately 50 mg each will be collected with a 6 mm biopsy cannula (Depuy, Warsaw, Indiana) from both the swimming (*M. longissimus dorsi*) and non-swimming (*M. pectoralis*) muscles. Control samples will be collected from the *M. soleus*, a predominantly slow oxidative muscle, of laboratory rats (*Sprague Dawley*) euthanized by cervical dislocation after 2-3 min of carbon dioxide anesthesia. Muscle samples will be placed either into 2% glutaraldehyde fixative or frozen in liquid nitrogen immediately upon collection. Samples will remain in the fixative for a minimum of 48 hours but no longer then 14 days before being transferred and stored in 0.1 M cacodylate buffer pH 7.4. Frozen samples will be stored at -70 ° C until analysis for citrate synthase activity, *B*-hydroxyacyl CoA dehydrogenase activity and myoglobin concentration.

Electron Microscopy of Muscle Samples. Fixed muscle samples will be rinsed in cacodylate buffer and post-fixed for 2 hours in a 1% solution of osmium tetra oxide. They will be stained 'en bloc' with 2% uranyl acetate overnight in a refrigerator. After dehydration with increasing concentrations of ethanol (50-100%), they will be passed through propylene oxide and increasing concentrations of epoxy (50-100%). The samples are finally embedded in fresh epoxy and allowed to polymerize overnight at 60 ° C. Thick sections (1 mm) will be cut with a Leica Ultratome and stained with toulidine blue to determine fiber orientation. Ultrathin (50-70 nm), transverse sections will be cut and contrasted with lead citrate from four randomly chosen blocks per muscle. Micrographs will be taken with a Phillips 201 transmission electron microscope. The number of micrographs per muscle analyzed will range from 25 and 40, yielding relative standard errors of less than 10% in all muscles. Determination of the volume density of mitochondria, myofibrils and lipid droplets will be performed at a final magnification of x19,250 using standard point counting procedures (6, 7).

Citrate Synthase, B-hydroxyacyl CoA dehydrogenase and Myoglobin Assays of Muscle Samples. Frozen muscle samples will be weighed and then homogenized at 0° C in 1 ml of buffer containing 1 mmol L^{-1} EDTA, 2 mmol L^{-1} $MgCl_2$, and 75 mmol L^{-1} Tris-HCl, pH 7.6 at 25 ° C (8). The homogenates will be spun at 2,900 g for 30 minutes at 4° C. 500 ml from each supernatant will be

prepared for myoglobin assay and the rest will be used for the analysis of citrate synthase. Citrate synthase and *B*-hydroxyacyl CoA dehydrogenase will be assayed on a Beckman DU series 64 spectrophotometer according to the method of Reed et al. (1994). Assay temperature will be maintained at 37 ° C using a constant temperature water bath and a water-jacketed cuvette holder. The assay conditions for citrate synthase (CS; EC 4.1.3.7) will be 50 mmol L^{-1} imidazole, 0.25 mmol L^{-1} 5,5-dithiobis (nitrobenzoic acid, DTNB), 0.4 mmol L^{-1} acetyl CoA, and 0.5 mmol L^{-1} oxaloacetate, at pH 7.5; DA_{412}, e_{412} = 13.6 (8). For *B*-hydroxyacyl CoA dehydrogenase (HAD; EC 1.1.1.35), the assay conditions will be 50 mmol L^{-1} imidazole, 1 mmol L^{-1} EDTA, 0.1 mmol L^{-1} acetoacetyl CoA, and 0.15 mmol L^{-1} NADH, pH 7.0 at 37° C; DA_{340}, e_{340} = 6.22 (9). Enzyme activities (mmol min^{-1} g^{-1} wet mass muscle) will be calculated from the rate of change in absorbance at the maximum linear slope. Myoglobin will be assayed according to the method of Reynarfarje (1963) with the following modifications. A portion (500 ml) of the supernatant is further diluted with 1 ml of phosphate buffer (0.04 M, pH 6.6). The resulting mixture is centrifuged for 50 min at 28,000 g at 4°C. The supernatant is bubbled with carbon monoxide for three min. Spectrophotometric absorbance will be measured at 538 and 568 nm, and the concentration of myoglobin in milligrams g^{-1} wet mass of muscle will be calculated as:

$$(Abs_{538} - Abs_{568}) \times 5.865 [(1.5/0.5) \times (\text{mass of sample})]$$

Statistical Analysis. Results will be expressed as the mean ± one standard error. We will use a cross-over repeated measures approach that will allow statistical comparisons within any one group of seals between diet and season. Statistical software (SYSTAT) will be used to analyze the cross-over method. The relative proportions of fatty acids from blubber samples of seals in the controlled feeding study will be used as a basis for generating tree-based models (using S-Plus; StatSci, Seattle) of groups or classes of samples such that new samples (obtained via BIOSAMPLING) can be compared with the modeled classes to decide their membership, i.e. obtain a classification of their "diet". Similarly, classification and regression trees will be used to screen the set of prey fatty acids and choose a subset of those fatty acids which can be used to classify the "diets" of seals based the patterns of fatty acid proportions in their blubber.

3. Blubber and Muscle Samples Obtained from the BIOSAMPLING Program in Prince William Sound.

Samples from the main swimming muscles, blubber and splanchnic organs of 16 harbor seals will be obtained during BIOSAMPLING Program. The entire muscle will be removed and weighed, and three transverse sub-samples will be taken along the muscle bundle. Each sub-sample of the swimming muscle will be precisely labeled for its orientation and location within the animal. These will then be further sub-sampled along points on a circular grid using a stainless steel borer, averaging 35 samples per muscle section. Cores of tissues weighing 200 and 300 mg will be removed for assay. A spectrophotometric technique will be used to determine myoglobin, citrate synthase, and *B*-hydroxyacyl CoA dehydrogenase concentration (see above for details). Detailed contour maps and statistical tests for all concentrations will be made using a PC based program S-Plus (Stat-Sci, Seattle). Blubber samples will also be obtained from the same approximate anatomical location as on animals used in the captive studies and stored frozen at -70 ° C. Blubber samples will be analyzed according to the protocols described in Section 2 of this proposal. Samples will also be taken from the liver, kidneys, stomach, small

intestine, diaphragm and brain. They will be analyzed using the same techniques as the muscle samples.

ACCOMPLISHMENTS FOR THE FIRST 18 MONTHS (OCT. 1998 TO MARCH 2000)

Feeding trials for eight harbor seals began in early September 1998 at the Alaska SeaLife Center. Six seals (two groups of three) received an alternating diet of either herring or pollock. To date, four feeding trials have been completed and the fifth is ongoing. Six feeding trials are planned. A separate control group of two seals will receive a mixed diet of half herring and half pollock throughout the study.

At the mid-point and end of each feeding trial, blubber samples only were taken at two sites from each seal. Skeletal muscle samples were also taken at the end of each trail. Half of each muscle sample was placed in fixative, and the remainder along with the blubber samples were frozen at -70^{o} C. The muscle samples were sent to the University of California at San Diego where analysis (% fiber type, volume density of lipid droplets and mitochondria, lipid enzyme activities, and myoglobin concentration) is underway. We have analyzed the samples from the mixed diet group for the volume density of mitochondria and lipid droplets per volume of muscle fiber. These animals have smaller volume densities of mitochondria but greater volume densities of lipid droplets than those previously measured in free-ranging harbor seals from Prince William sound, Alaska. The samples from the other trials are currently being processed and measured for the same parameters. The biochemical analysis has not begun, because we are waiting for the end of the feeding trials to analyze them in a single batch.

Blubber samples will be analyzed for fatty acid profiles at Texas A&M University. Analysis is underway for the first three feeding trials and should be completed this summer. The remainder of the samples will be analyzed after the feeding trials are finished in September 2000.

In June of 1999, we obtained extensive muscle, blubber and splanchnic organ samples from eight harbor seals through the BIOSAMPLING Program in Prince Williams Sound. These samples are under analysis at Texas A&M University and will form the basis for two Doctoral and one Masters dissertation. Preparations have been made for obtaining additional harbor seal samples from the BIOSAMPLING Program in June 2000. This collaborative effort with the Native community has been very successful for our program.

SCHEDULE

A. Measurable Project Tasks for FY 99 (October 1, 1998 - September 30, 1999), FY 00 (October 1, 1999 - September 30, 2000) and FY 01 (October 1, 2000 - March 31, 2001)

Each feeding trial will take four months beginning in September, 1998.

1998	
September	Set up fatty acid analysis and muscle lipid and enzyme analysis
September-December	Trial 1 of staggered feeding protocol at ASLC. Obtain blubber and muscle biopsies. Status- completed on schedule.

1999	
January-April	Trial 2 of staggered feeding protocol. Obtain blubber and muscle samples. Status- completed on schedule.
May-August	Trial 3 of staggered feeding protocol. Obtain blubber and muscle samples. Status- completed on schedule. Obtain blubber and muscle samples from wild harbor seals in Prince William Sound in conjunction with BIOSAMPLING Program. Status- completed on schedule.
September-December	Trial 4 of staggered feeding protocol at ASLC. Obtain blubber and muscle biopsies. Status- completed on schedule.
2000	
January-April	Trial 5 of staggered feeding protocol. Obtain blubber and muscle samples. Status- underway and on schedule.
May-August	Trial 6 of staggered feeding protocol. Obtain blubber and muscle samples. Planned and on schedule Obtain blubber and muscle samples from wild harbor seals in Prince William Sound in conjunction with BIOSAMPLING Program. Planned and on schedule.
September-December	Complete analysis of blubber and muscle samples.
2001	
January-September	Analyze data and prepare Final Report. Prepare and submit manuscripts. Two manuscripts are anticipated at this time.

PROJECT MILESTONES

FY 99:	Obtain blubber and muscle samples during first four feeding studies at ASLC and the BIOSAMPLING Program in Prince William Sound.
FY 00:	Continue to obtain blubber and muscle samples during feeding studies at ASLC; obtain blubber and muscle samples from seals in Prince William Sound in conjunction with BIOSAMPLING Program.
FY 01	Complete analysis and prepare Final Report and manuscripts by September.

PUBLICATIONS AND REPORTS

Since this is a new project, there are no current publications from the proposed research. However, the results from a preliminary study of the aerobic capacity and lipid content of muscles from harbor seals in Prince William Sound were be published in the Journal of Applied

Physiology in April 1999. We do not anticipate any referred articles in FY 00. However, by FY 2001 most of the data will be analyzed and manuscripts in preparation. Because samples will continue to be collected through September 2000, we request an additional year (Oct. 2000 to September 2001) to complete data analysis and prepare the Final Report and manuscripts. We anticipate at least two publications by 2001 on the effects of diet on fatty acids in blubber and the aerobic capacity and lipid metabolism in harbor seal muscle.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

We are working in close coordination with Dr. Michael Castellini (PI on Harbor Seal Recovery. Phase II: Controlled Studies of Health and Diet) and staff at the Alaska SeaLife Center (see attached letter). Dr. Castellini is supervising the controlled diet studies. We have coordinated our blubber and muscle samples with the veterinary staff at ASLC. Samples obtained from the BIOSAMPLING program will be coordinated with Ms. Monica Riedel of the Alaska Native

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