

Effects of Harbor Seal Metabolism on Stable Isotope Ratio Tracers

Project Number: 01371

Restoration Category: Research

Proposer: University of Alaska Fairbanks

Lead Trustee Agency: ADFG

Cooperating Agencies: None

Alaska SeaLife Center: yes

Duration: 3rd year, 3-year project

Cost FY 00: \$98,000

Cost FY 01: \$92,900

Cost FY 02: \$25.7 (Close-out, Report Writing)

Geographic Area: Prince William Sound/Gulf of Alaska

Injured Resource/Service: Harbor seals

ABSTRACT

A major concern when using stable isotope tracers in ecosystem studies is the fidelity with which isotope ratios are transferred up food chains. Use of specific habitats or prey cannot be assessed because geographic gradients in isotope ratios confound trophic effects and/or prey switching. To remove these problems, we developed complex analytical protocols to isolate amino acids from harbor seals which were pulse-labeled with ¹⁵N-amino acids. Subsequent samples of blood plasma and red blood cells over time allowed for estimation of nitrogen incorporation rates. The goal of the final year is to identify pathways of rapid versus slower turnover. Determination of habitat biomarkers will be investigated in year 3 of the project.

INTRODUCTION

Stable isotope ratios have become an essential tool in the study of living organisms and their physiology. The hazards of handling radioisotopes and severe protocol requirements when using live organisms have resulted in a steadily increasing shift to the use of stable isotopes as tracers for both human and animal subjects. Some usage such as the detection of *Helicobacter pylori* infections in ulcer patients are now routine and bringing stable isotope analysis to many hospitals as a standard method. In contrast to the employment of natural abundance techniques in the marine environment, most physiology experiments employ compounds enriched with ^{13}C or ^{15}N to enhance detectability and to follow the transfers to different metabolites within the organism. Improved lower limits of detectability and smaller sample size requirements now allow the use of stable isotopes where only radioisotopes would have worked in the past.

This proposal describes continuing experiments underway at the Alaska SeaLife Center (ASLC) and at the University of Alaska Fairbanks (UAF) to provide calibration and more detailed information on stable isotope transfers and fractionation in marine mammals (and perhaps sea birds in the future). This will enable better interpretation of natural abundance isotope data acquired in Prince William Sound and the adjacent Gulf of Alaska. Coordination with the studies of Dr. M. Castellini who is conducting feeding experiments and dietary studies at ASLC will lead to a thorough integration of efforts and optimization of the use of animal subjects in all years of the study. Year 1 has consisted of the refinement of analytical techniques isolating amino acids and will test for the presence of essential amino acids in harbor seals at ASLC. Succeeding years will focus on the search for biomarkers useful in identification of specific habitat usage and as indicators of the assimilation of various species of forage fishes.

Over the past two decades, isotope ratio analysis has emerged as a powerful tool in ecosystem research, both on the process scale and as a validation technique for large-scale ecosystem models (Michener and Schell, 1994). In relevant applications to this study, Saupé et al (1989) and Schell et al. (1989) described a geographic gradient in isotope ratios in biota across the Alaskan Beaufort Sea and the Bering–Chukchi seas and showed that this gradient could be applied to describing bowhead whale natural history. The isotopic gradient arises from the primary producers in the ecosystem and is passed up food chains to label consumers up to the top predators. Within each biome, there is reasonable fidelity to the $\delta^{13}\text{C}$ observed in the primary producers and a predictable increase in the $\delta^{15}\text{N}$ with each known increase in trophic level. However, among individuals of each taxon analyzed there are often large ranges in values, especially in the carbon isotope ratios.

A fundamental assumption in the employment of isotope ratios as natural tracers is that the amount of isotopic fractionation in the process of metabolizing food is known during the incorporation of assimilated components into the consumer. For marine mammals, these data are scarce and most of the ongoing work is based on the findings derived from terrestrial bird and mammal studies. The accurate interpretation of isotope ratio data on food webs and marine mammals depends completely on knowledge of fractionation effects arising from dietary sufficiency and composition. To date, we do not have this knowledge because it has become evident that there exist marked geographic gradients in isotope ratios in Prince William Sound and the Gulf of Alaska. This project is thus aimed at the goal of identifying specific biomarker molecules and acquiring accurate isotope fractionation data on harbor seals through controlled feeding and laboratory experiments. This project will be thoroughly integrated with ongoing

research on harbor seals at the ASLC and will be complementary to the physiological research projects in progress.

NEED FOR THE PROJECT

A. Statement of Problem

Harbor seals were undergoing an unexplained decline in numbers before the oil spill and the decline was further accelerated by the disaster. Since that time the population has not recovered and is still at a low level, although now perhaps finally stabilized. No definitive cause and effect relationships have been found for the decline or failure to recover. It is becoming increasingly evident, however, that change in the marine environment in the past two decades has altered the carrying capacity downward in the northern Gulf of Alaska and the effects are being felt to top of the food chains. Carbon isotope ratios in biota of the northern Pacific Ocean appear to have been declining for nearly twenty years (Schell, in preparation) and imply that a major decrease in productivity has occurred. Isotope ratios from wild seals also show changes over time in the isotope ratios but the interpretation requires knowledge of both the fractionation that occurs during assimilation and the natural variations arising from migratory movements. If one or more essential amino acids can be identified in the diet of seals, these would allow a conservative tracer independent of isotope fractionation effects arising from metabolism. There are almost no data regarding marine mammals on this subject and none on harbor seals. This study will undertake to follow both the “whole animal” carbon and nitrogen isotopic fractionation and the determination of specific biomarkers arising from diet that would allow clearer insight into dietary dependencies.

B. Rationale/Link to Restoration

Carbon isotope ratios serve as conservative tracers of energy supply between trophic levels (phytoplankton to zooplankton to fishes to top consumers). Seals, cetaceans, birds, etc. acquire the isotope ratios in proportion to the amount of food derived from each differing source. This, in turn, is reflected in the composition of body tissues and in keratinous tissues (claws, feathers, baleen, whiskers) as a temporal record when multiple sources of food are consumed over time and space. This allows the discerning of important habitats and food resources in animals such as harbor seals that seasonally migrate or undergo periods of hyper- and hypotrophy. Little is known, however, of the internal fractionation of isotopes that occurs in mammals during fasting and/or extended periods of suboptimal diets. Current experiments on the effects of differing diets on captive harbor seals conducted at the ASLC provide an ideal opportunity to enhance the physiological data gained by investigating the efficiency of amino acid transfers in diets and the presence of essential amino in pinnipeds.

Nitrogen isotope ratios reflect both the food sources and the trophic status of that animal. As nitrogen in food is consumed and assimilated by a consumer, the heavy isotope is enriched by approximately 3 ‰, with accompanying loss of the lighter isotope through excretion. The enrichment occurs with each trophic step and thus allows the construction of conceptual models and food webs and the assignment of relative trophic status to species for which dietary data are sparse. Hobson and Welch (1992) used isotope ratios to describe the trophic relationships of birds and mammals to the available prey species in the Canadian Arctic. Further extension to

benthos by Dunton et al. (1991) and to fishes (Vinette, 1992) has confirmed that the isotopic trends are evident across the entire food web. During fasting or starvation, nitrogen isotopes may be fractionated during transamination reactions leading to overall shifts in the average isotope ratios of the whole animal. Best and Schell (1996) observed, for example, that ^{15}N enrichment in southern right whales evidenced during winter breeding season in South African waters when carbon isotope ratios revealed that very little feeding occurred. Detailed interpretation of data from samples taken from wild seals requires that these effects be known.

C. Location

The research efforts are underway at the Alaska SeaLife Center and the University of Alaska Fairbanks. The instrumental analyses, specifically the development of the amino acid isolation protocols, has been conducted at UAF on samples collected during the dietary studies and sampling at ASLC by Dr. Castellini's group. We are now performing the isolations of both derivatized and free amino acids from seal samples and conducting the mass spectrometry.

COMMUNITY INVOLVEMENT AND TRADITIONAL ECOLOGICAL KNOWLEDGE

Much of the research will be conducted at the Alaska SeaLife Center and the Principal Investigator anticipates both community interaction and explanation of the research approach and usefulness at the site.

PROJECT DESIGN

A. Objectives

The null hypotheses to be tested are as follows:

1. The isotope ratios of harbor seals accurately reflect diet under all conditions. Increased fractionation does not occur during periods of fasting or suboptimal feeding and does not affect either carbon or nitrogen isotope ratios in harbor seals.
2. There are no essential amino acids in harbor seals and their prey that can act as conservative markers of specific habitats of food sources or of specific prey species.

The objectives of this study are divided into three elements, which have been modified as the study progressed:

1. Year 2, now underway, consists of developing methods and protocols for the isolation of metabolites from harbor seal blood and tissue samples to be employed during the following controlled diet studies. The Institute of Marine Science has purchased a new GC-IRMS (gas chromatograph–isotope ratio mass spectrometer) that has been used to determine isotope ratios in the individual amino acids isolated from serum samples. These amino acids are separated using high performance liquid chromatography using semi-preparative columns and inorganic buffers. Testing for essential amino acids in harbor seals has been initiated using blood samples acquired from seals being used by Dr. Michael Castellini for food

assimilation efficiency studies. By feeding ^{15}N and ^{13}C -labeled glycine to the seals prior to blood sample collection, it will be evident if the label has been transaminated to amino acids and to what extent. If some amino acids remain unlabeled, the corresponding labeled amino acid will be administered to see if transamination occurs in the reverse direction. Conservative amino acids will be prime candidates for environmental biomarkers.

2. The second component will be a study of the effects of suboptimal versus optimal diet on the fractionation of carbon and nitrogen isotopes in harbor seals. Diets of known amount and composition (isotopic and energetic) are being fed to the seals at ASLC and blood samples are being monitored for composition and isotope ratios. Dr. M. Castellini is closely coordinating this research with studies of controlled diet/assimilation efficiencies in harbor seals so that minimal animal handling and sampling will be necessary. The first trial of the feeding study began in December 1998 and the second will commence in May 2000.
3. The third component was to determine source prey for isotopically distinct fatty acids or other metabolites. The identification of specific fatty acids that carry a conservative signal to top consumers (birds, cetaceans, fissipeds) would yield an extraordinarily valuable tool to follow food web transfers or to identify specific habitat importance. This aspect of the work has also been undertaken by study 00441 and we have shifted our emphasis to the nitrogen metabolism and amino acids. Protocol development for amino acid mass spectrometry has taken longer than anticipated and we are planning intense effort over the remaining time on this aspect. If time allows we will undertake fatty acid extractions and identifications during the final year. Many of the prey species samples are already archived and analysis can begin as soon as primary goals are attained.

B. Methods

Isotopic Analysis of Blood Protein Amino Acids

The proteins and free amino acids in blood serum samples from captive harbor seals and muscle protein from native harvested seals are hydrolyzed with 0.6 N HCl in sealed ampoules to free proteinaceous amino acids. This procedure provides several essential amino acids for mammals but if time permits we will use multiple procedures to optimize amino completeness, such as acid and basic hydrolysis. An investigation of the use of proteolytic enzymes is no longer being considered because of time constraints. Once isolated the free amino acids will be separated by HPLC (high performance liquid chromatography) either as derivatives of orthophthalaldehyde (OPA) or as underivatized amino acids. The latter procedure is preferable but will require modification of the HPLC system. This equipment for these modifications is on order and will be employed as they come online in late spring 2000. The aliquots with individual amino acids will be taken to dryness. These samples will then be run on an elemental analyzer coupled to the isotope ratio mass spectrometer and the nitrogen and carbon dioxide liberated in the elemental analyzer will be separated by gas chromatography and run individually in the IRMS. Preliminary data on seal amino acids are presented in the accompanying annual report.

In the past feeding experiment, we intravenously dosed 2 seals on different diets with ^{15}N -labeled glycine and the appearance of the label in the serum and red blood cells was followed over time in the total amino acid spectrum. This experiment has already demonstrated the in vivo appearance of the label and provided an approximate turnover time for free amino acids in the blood serum of the seals. Preliminary data are shown in the FY00 annual report. Samples of

the blood are currently being processed for individual amino acid analysis. Those amino acids remaining free of the label will be identified as probable essential amino acids derived solely from diet that would constitute conservative biomarkers. Mobilization and isotopic fractionation of these amino acids will be tested further in reverse dietary studies in summer 2000 wherein the labeled amino acid will be infused and the rate of transamination followed in feeding and fasting seals.

Isotope Fractionation During Fasting and Suboptimal Diets

Many marine mammals undergo periods of fasting or suboptimal diets such as during molt or reproduction. Nothing is known regarding the effects of these periods on the fractionation of either carbon or nitrogen isotopes in harbor seal tissues. The amino acid threonine, for example, has been shown to become very isotopically depleted in ^{15}N during starvation, with lesser effects on glycine and serine (Hare et al., 1991). In coordination with studies of dietary effects on blood hormones or other work requiring harbor seal blood samples at ASLC, we will analyze aliquots as described above for shifts in the isotope ratios. We have completed collection of blood samples from unlabeled seals (used as controls in the experiments), which showed shifts in the natural abundance of isotope ratios over the feeding experiment with constant diet. These physiologically induced shifts probably arise from mobilization of amino acids in molting or onset of breeding behavior. We plan to coordinate our summer 2000 sampling with that of Dr. Castellini. All procedures will be approved by the ASLC scientific committee and conducted as required by the IACUC (Institutional Animal Care and Use Committee) of the University of Alaska and ASLC.

This project will complete the sampling program in the next feeding trial beginning in May 2000. We do not anticipate the need for ASLC bench space or office space in FY 01. We do include funds for one trip for the PI and graduate student to the ASLC for finalization of sampling and any necessary clean up. The analytical work will be undertaken at UAF and the remainder of the project duration will be in Final Report preparation and the submission of manuscripts detailing our findings. We have already presented initial findings at the EVOS meeting in January 2000 and anticipate submission of more complete findings at the next EVOS meeting in 2001.

Sources of Essential Amino Acids in the Diets of Harbor Seals

We are fortunate in having a wide suite of potential prey samples derived from the Prince William Sound region and offshore Gulf of Alaska from past EVOS studies. Additional samples are also available from the Bering Sea region to allow geographic contrast in isotope ratios. The APEX program supported by EVOS will be a source of samples, as will other opportunistic cruises in the spill and control areas. Herring, sand lance, pollock and capelin will be special targets, given their importance in the food chains of Prince William Sound.

Ms. Liyang Zhao is the Ph.D. candidate is undertaking the experimentation on this project. Ms. Zhao has an exceptionally strong background in chemistry and has been undertaking the daunting task of developing the methodologies needed to isolate sufficient individual amino acids to provide an isotope ratio for each of the approximately ten essential amino acids. The task has required a triple approach – isolation of derivatized amino acids, isolation of free underivatized amino acids and an integrated GC-MS procedure that would eliminate the need for HPLC processing. To date, the first procedure offers the best results and cleanest separations but complicates the calculations of isotope ratios through the addition of the derivatizing carbon.

This requires back calculation of the apparent isotope ratio of the free amino acid. We hope to circumvent this obstacle in the next few months through the purchase of a larger semi-preparative scale HPLC with much larger capacity and an ion-exchange column. The costs for this instrumentation are being obtained from other sources.

The biochemical expertise and advisement of Ms Zhao are from her graduate committee of which the PI is the chair. Other members include:

Dr. Michael Castellini, Professor of Marine Science, has his background in biochemistry and is currently involved in studies of marine mammal nutrition at the ASLC.

Dr. Larry Duffy, Professor of Biochemistry and Chemistry, is the current Head of the Chemistry and Biochemistry Program.

Dr. Susan Henrichs, Professor of Marine Science, is a chemist specializing in the microbial biochemistry of amino acids in marine environments.

Dr. Bruce Finney, Professor of Marine Science is experienced with the environmental aspects of ocean chemistry and stable isotope methodology.

The above committee is assisting in experimental design and review of protocols as well as assist with scoping.

C. Cooperating Agencies, Contracts, and Other Agency Assistance

M. Castellini is concurrently working on Project 00341 for related work on blood hormones and food assimilation efficiency studies at the Alaska SeaLife Center. This project will be completely coordinated with his work to optimize sampling and mutual assistance.

SCHEDULE

A. Measurable Project Tasks for FY 01 (October 1, 2000–September 30, 2001) and Project Milestones and Endpoints

FY 01

- October - February: Continue amino acid analyses on samples acquired from final feeding trials, summer 2000. These will constitute reverse labeling with phenylalanine and valine to study transamination efficiencies of essential amino acids.
- March - July: Continue analytical work, prepare prey samples for analysis. Write manuscripts, outline Final Report.
- August–September: Begin Final Report, submit manuscripts, clean up data gaps, outline needs for future work.

C. Completion Date

This project will be completed by April 2002.

PUBLICATIONS AND REPORTS

Results of this project will be made available via the following:

Annual Reports: These reports will detail progress and preliminary findings and notable achievements. The annual report due April 2000 (18-month progress) is submitted with this proposal. The next report will be the Final Report as scheduled below.

Final Report: A Final Report will be provided. Technical results in this report will be shared with EVOS collaborators and assistance provided as opportune during the experiments. Preliminary exchange of findings will be conducted with EVOS investigators and the scientific community via professional meetings and informal communications.

Peer-reviewed publications: Over the course of this study peer-reviewed publications will be generated for the open literature based on the scientific findings. These publications will be generated by the PI and graduate students as first author publications when the primary focus is on the findings produced by the isotopic techniques or as second author publications when the isotope work is a minor part of other scientific results resulting from feeding experiments conducted by colleagues.

Papers at scientific society meetings: We request support for travel to appropriate scientific meetings for dissemination of results and interaction with colleagues. It is anticipated that the PI and a graduate student will attend the Society for Marine Mammalogy and/or the American Society for Limnology and Oceanography meetings.

Public lectures: Interaction with the public will arise through formal and informal presentation of results as part of ongoing public participation in the work at ASLC. Synthesis meetings designed to explain the findings will be presented at meetings coordinated by ASLC or EVOS and open to the public. Informal presentation of results will occur through interaction with interested members of the public, press and scientific community. Classroom instruction will also involve integration of findings into the presentation of educational material.

PROFESSIONAL CONFERENCES

The results of this project will be communicated at appropriate meetings. The biennial meeting of the Society for Marine Mammalogy or the American Society for Limnology and Oceanography (ASLO) is typical for this type of presentation, as are specific workshops and meetings emphasizing application of isotope techniques to biological problems. The next opportunity will be the annual meeting of ASLO or ad hoc meeting on marine mammals in 2001.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

Resources and Services – We have been fortunate to have full coordination and assistance of the ASLC staff and Dr. Michael Castellini for the animal handling requirements of this project. The infusions of amino acids, coordination with known diets and sample handling have been very efficient and helpful. This has allowed full time effort on the difficult and complex analytical aspects at UAF and assured high quality samples for our work. The final label infusions,

feeding, and blood collection will occur in FY00 (summer 2000) and we will spend the remaining program resources on completion of the analytical work and publication of results. No ASLC bench or office space will be needed in FY01.

PROPOSED PRINCIPAL INVESTIGATOR

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PRINCIPAL INVESTIGATOR

D. M. Schell has been involved in stable isotope studies for over 25 years. His research has included natural abundance tracer studies and enrichment experiments. His work on bowhead whales and geographic gradients in stable isotope ratios has been published and extended to the assessment of ecosystem carrying capacity in the Bering Sea and to the assessment of trophic dynamics and feeding of harbor seals in the EVOS region.

Dr. Schell oversees the Stable Isotope Ratio Mass Spectrometry Facility on the UAF campus. This consists of three working instruments, which are dedicated to specific elements, as demand requires. A Europa automated continuous flow system will be used for most samples but back-up analytical capability is available. A new HPLC is being ordered from other sources that have the ability to handle separations of larger quantities of amino acids and will be available for this project. As PI, Schell will oversee the Quality Assurance/Quality Control aspects of this project. Protocols for sampling for mass spectrometry have been established and working standards are cross-calibrated with other nationally recognized laboratories.

OTHER KEY PERSONNEL

Machine operations are the responsibility of Norma Haubenstock, mass spectrometry technician. She is well trained and has more than 11 years experience with mass spectrometers. She will oversee laboratory operations, assist in sample preparation, and archive all isotope data. Ph.D. student Liying Zhao is responsible for the amino acid identification and separation, sample preparation for mass spectrometry and for synthesis of data in cooperation with the PI.

LITERATURE CITED

Best, P.B. and D. M. Schell 1996. Stable isotopes in southern right whale (*Eubalaena australis*) baleen as indicators of seasonal movements, feeding and growth. *Mar. Biol.* 124:483-494

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Schell, D.M., S.M. Saupe and N. Haubenstock. 1989. Bowhead whale (*Balaena mysticetus*) growth and feeding as estimated by techniques. *Mar. Biol.* 103: 433–443.

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2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2000 - September 30, 2001

Budget Category:	Authorized FY 2000	Proposed FY 2001						
Personnel		\$0.0						
Travel		\$0.0						
Contractual		\$86.8						
Commodities		\$0.0						
Equipment		\$0.0	LONG RANGE FUNDING REQUIREMENTS					
Subtotal		\$86.8			Estimated 2002	Estimated		
General Administration		\$6.1						
Project Total		\$92.9			\$25.7			
Full-time Equivalentents (FTE)		1.4						
Dollar amounts are shown in thousands of dollars.								
Other Resources								
Comments:								

FY01

Prepared:

Project Number: 01371
 Project Title: Effects of Harbor Seal Metabolism on Stable
 Isotope Ratio Tracers
 Agency: Alaska Department of Fish and Game

Budget Category:	Authorized FY 2000	Proposed FY 2001					

2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2000 - September 30, 2001

Personnel		\$53.1					
Travel		\$4.9					
Contractual		\$8.2					
Commodities		\$3.2					
Equipment		\$0.0					
Subtotal		\$69.4	LONG RANGE FUNDING REQUIREMENTS				
Indirect		\$17.4			Estimated 2002	Estimated	
Project Total		\$86.8			\$24.0		
Full-time Equivalents (FTE)		1.4					
Other Resources							

Dollar amounts are shown in thousands of dollars.

Comments:

The indirect rate is 25% TDC, as negotiated by the *Exxon Valdez* Oil Spill Trustee Council with the University of Alaska.

Student personnel costs include resident tuition of \$3,006 per year.

FY01

Project Number: 01371
 Project Title: Effects of Harbor Seal Metabolism on Stable Isotope Ratio Tracers
 Name: Donald M. Schell

Prepared:

Personnel Costs:		Months Budgeted	Monthly Costs	Overtime
Name	Position Description			
Donald M. Schell (PI, Director, IMS; Professor)		1.7	11.8	
Norma Haubenstock, Technician		3.0	5.2	

2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2000 - September 30, 2001

Ph.D. Student (TBA)			12.0	1.5	
Subtotal			16.7	18.5	0.0
Personnel Total					
Travel Costs:					
	Description	Ticket Price	Round Trips	Total Days	Daily Per Diem
	Fairbanks to national meeting	1.2	1	8	0.1
	Fairbanks to Anchorage (EVOS meeting)	0.3	1	5	0.1
	Fairbanks to Seward	0.8	2	7	0.1
Travel Total					

FY01

Project Number: 01371
 Project Title: Effects of Harbor Seal Metabolism on Stable Isotope Ratio Tracers
 Name: Donald M. Schell

Prepared:

Contractual Costs:	
Description	
Mass spectrometry (400 samples @ \$18/sample)	
Final report preparation, page charges	
Communications, photocopying	3 of 5

2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2000 - September 30, 2001

		Contractual Total
Commodities Costs:		
Description		
HPLC column, chemicals, expendables		
Project supplies (computer software)		
		Commodities Total

FY01

Prepared:

Project Number: 01371
 Project Title: Effects of Harbor Seal Metabolism on Stable
 Isotope Ratio Tracers
 Name: Donald M. Schell

2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2000 - September 30, 2001

New Equipment Purchases:		Number of Units	Unit Price	
Description				
Those purchases associated with replacement equipment should be indicated by placement of an R.			New Equipment Total	
Existing Equipment Usage:		Number of Units		
Description				

FY01

Project Number: 01371
 Project Title: Effects of Harbor Seal Metabolism on Stable Isotope Ratio Tracers
 Name: Donald M. Schell

Prepared: