Exxon Valdez Oil Spill
Restoration Project Annual Report

Recovery of Harbor Seals.
Phase II: Controlled Studies of Health and Diet

Restoration Project 98341
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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April 1999

Recovery of Harbor Seals. Phase II: Controlled Studies of Health and Diet

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Study History: This project grew out of a series of studies which began in FY93 as a Research Service Agreement with the Alaska Department of Fish and Game. In FY95 they were initiated as Restoration Project 95001, *Recovery of Harbor Seals from EVOS: Condition and Health Status* and were continued through Restoration Project 98001of the same title. A final report was issued in FY98 by Fadely, Castellini and Castellini, entitled *Recovery of Harbor Seals from EVOS: Condition and Health Status*. Publications arising from this work include: Castellini, J.M., H.J. Meiselman and M.A. Castellini. 1996. Understanding and interpreting hematocrit measurements in pinnipeds. *Marine Mammal Science* 12(2), 251-264; Zenteno-Savin, T. , M.A. Castellini, L.D. Rea, and B.S. Fadely. 1997. Plasma haptoglobin levels in threatened Alaskan pinniped populations. *Journal of Wildlife Diseases* 33(1), 64-71; Zenteno-Savin and M.A. Castellini. 1998. Plasma angiotensin II, arginine vasopressin and atrial natriuretic peptide in free ranging and captive seals and sea lions. *Comparative Biochemistry and Physiology.* 119C,1-6. Additional manuscripts are in preparation. Results from these initial studies led directly to the research proposed in Restoration Project 98341 – *Recovery of Harbor Seals. Phase II: Controlled Studies of Health and Diet.* The acclimation phase of this project has been completed and is the subject of this report. In addition, preliminary data have been collected from rehabilitated harbor seal pups and are included in this report. The first year of feeding trials and second year of rehabilitation studies are underway as Restoration Project 99341. This program is currently expected to continue into FY01 and will then be closed out with a Final Report to be prepared in FY01.

Abstract: The objective of this study is to quantify the impact of specific diets on the health and body condition of harbor seals. This includes measuring the effect of diet on health status biomarkers which have been monitored in animals in Prince William Sound and determining whether specific diets are nutritionally adequate to maintain health. In the first year of this project, eight harbor seals were brought to the Alaska SeaLife Center, assigned to one of two groups, and acclimated to their new environment and diets. During acclimation, morphometric measurements and blood sampling have occurred on a bi-weekly basis. Initial results indicate differences in several blood parameters between the two groups of seals being fed slightly different acclimation diets. Whether these differences are a result of diet should become apparent as seals progress through the feeding trials. Analysis of blood parameters and morphometric measurements of rehabilitated harbor seal pups reveal changes possibly indicative of several factors, including development, captivity, early weaning and disease or injury. Preliminary results show extreme perturbation in blood parameters of severely compromised individuals. Continuing these studies on injured or abandoned harbor seals through rehabilitation will enable more specific conclusions about the differences between healthy and unhealthy animals.

Key Words: Biomarker, blood chemistry, diet, *Exxon Valdez* oil spill, harbor seals, health, hematology, morphometrics, *Phoca vitulina*, physiology, pups, rehabilitation.

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

Citation:

Castellini, M.A., J.M. Castellini, and S.J. Trumble. 1999. Recovery of harbor seals. Phase II: controlled studies of health and diet, *Exxon Valdez* Oil Spill Restoration Project Annual Report (Restoration Project 98341), Alaska Department of Fish and Game, Habitat and Restoration Division, Anchorage, Alaska.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

EXECUTIVE SUMMARY

An underlying component of the ecosystem-based research approach supported by the Trustee Council has been the hypothesis that food limitation could be inhibiting the recovery of injured species in Prince William Sound. Inherent in this concept is the assumption that food stressed animals can be distinguished by population-wide surveys of critical health parameters. Following this approach, an extensive sampling effort by multiple projects established a series of biomarkers used to profile the health and body condition of wild populations of marine mammals inside Prince William Sound. Population health status and body condition indices were, and continue to be, developed and tested for a range of birds, sea otters and seals. Establishing such a series of population-wide health indicators is necessary, but not sufficient, to link their biological activity to known health problems or food limitation. This is because the variability of each indicator over time or under different feeding conditions in any one individual cannot be tested in the field. While we can establish the range of reference values for any particular indicator across a whole group of animals, we do not know how this indicator varies within any given animal under changing conditions of health or feeding status. This project seeks to provide just such a connection.

At the Alaska SeaLife Center in Seward, we have begun to test the variability of biomarkers under controlled conditions, in the same animals over time and under changing experimental conditions. Of particular interest is the effect of specific diets on harbor seal physiology. This study is designed to address the question of food limitation more completely, including the suggestion that certain prey items may not be nutritionally adequate. The major goals of the study are to quantify body condition, health and blood chemistry biomarker changes during a series of feeding trials and assess assimilation efficiency of seals fed different diets. Data are also being collected on harbor seals brought to the Alaska SeaLife Center for rehabilitation, as these animals often represent compromised individuals who might not otherwise survive.

The first year of this project was marked by preparation for the arrival of 8 adult harbor seals at the SeaLife Center and finalization of feeding regime protocols and analysis procedures. The seals arrived during April and early May 1998. Data were collected bi-weekly from each animal during acclimation to their new environment and baseline diet. This has resulted in an extensive database (6 – 8 complete sets of measurements from each of 8 seals) of baseline morphometric and blood values, including mass, lengths and girths, blubber depths (by ultrasound), blood chemistries and hematology. These data provide a health and condition profile for each animal as they began the first of 6 feeding trials in September 1998.

Upon arrival at the SeaLife Center, the seals were placed in one of two groups and each group was acclimated to a slightly different diet. Group A was fed exclusively herring and Group B was fed a mixed diet consisting of herring, squid and capelin. The feeding regime requires that seals be fed a maintenance diet and measurements confirm that, except for a small decrease in mass in July 1998, mass has remained relatively constant for each animal. Some seasonal fluctuation in mass is expected. Blood chemistry and hematological values were within the ranges expected for adult harbor seals. The two groups had several significant differences in blood parameters. The most striking of these differences included higher mean globulin, total protein and creatinine levles and lower triglyceride and blood urea nitrogen:creatinine values in Group B seals. Whether these differences are a result of the slightly different diets of the two groups or some other difference between the groups should become apparent as they enter

feeding trials and their diets are switched. Samples have been collected for biomarker analysis, however these analyses have not yet been completed.

A necessary aspect of understanding the effect of diet on health and physiology is a proper assessment of diet. While herring has a relatively high fat content and energy density, these values are variable according to season and fish size. As the seals entered the first of six planned feeding trials, compositional analysis revealed that herring in their diet had substantially higher lipid content (herring: 16.0 ± 1.5 % , $n = 5$; pollock: 5.0 ± 0.9 %, $n = 5$) and energy density (herring: 9.2 ± 0.8 kJ/g wet weight, $n = 10$; pollock: 5.2 ± 0.4 kJ/g wet weight, $n = 5$) than pollock. There has been no change in herring or pollock composition during 2 – 3 months of frozen storage, however analysis will continue to assess food quality changes during storage.

Three harbor seal pups were successfully rehabilitated at the Alaska SeaLife Center during 1998 (2 injured/unhealthy, 1 possibly abandoned). Each of the pups was monitored carefully including weekly morphometric measurements and blood samples. Blood and morphometric measurements were consistent with recovery, although all three seals exhibited decreasing hematocrit levels. While the number of individuals studied at this point is low, preliminary results show extreme perturbation in blood parameters of severely compromised individuals (seals which did not recover) as well as long-term changes in blood parameters and morphometrics as individuals recover and develop. A number of potential factors could contribute to the variability, including development, captivity, early weaning and disease or injury. All three recovering seals showed increases in cholesterol and globulin levels and decreases in gamma-glutamyl transpeptidase and triglyceride levels during their recovery. Continuing these studies on injured or abandoned harbor seals through rehabilitation will enable more specific conclusions about the differences between healthy and unhealthy animals.

The next phase of this project has begun and will continue during 1999, with seals progressing through a series of feeding trials in which their diet will be switched between herring and pollock. By the end of the study, each seal will have experienced each diet at each time of the year and data will be analyzed by a cross-over repeated measures approach. Data collection will continue to include bi-weekly blood sampling and morphometric measurements. During each feeding trial assimilation efficiency, metabolizable efficiency and mean retention times of different prey items are being assessed to determine if the seals are able to compensate physiologically for differences in food quality. The first of these experiments was conducted successfully in December 1998. Collection of samples from injured or unhealthy harbor seals sent to the Alaska SeaLife Center will continue, extending the database and making comparisons more valuable. The ability to collect data routinely and repeatedly from the same animals at the SeaLife Center has already resulted in extremely valuable data about the variability of these measurements within a normal individual. By extending these data to include shifts in diets over a variety of seasonal conditions, important questions about the nutritional physiology of harbor seals will be answered.

INTRODUCTION

An underlying component of the ecosystem-based approach supported by the *Exxon Valdez* Oil Spill Trustee Council has been the hypothesis that food limitation could be inhibiting the recovery of injured species within Prince William Sound (PWS). Inherent in this concept is the assumption that nutritionally stressed animals can be distinguished by population-wide surveys of critical health parameters. Following this approach, an extensive sampling effort by multiple projects established a series of biomarkers used to profile the health and body condition of wild populations of marine birds and mammals inside PWS (Romano et al. 1996, Fadely et al. 1998). On the basis of this wide-ranging effort, species specific reference range values for health parameters have been established and are being used to compare groups of animals temporally and spatially.

Establishing such a series of population-wide health indicators is necessary, but not sufficient, to link their biological activity to known health problems or food limitation. This is because the variability of each indicator over time or under different feeding conditions in any one individual cannot be tested in the field. We can establish the range of reference values for any particular indicator across a whole group of animals, but we do not know how this indicator varies within any given animal under changing conditions of health or feeding status. This type of information can only be obtained by recapturing an animal many times or by studying captive animals. Most comparative hematological values for captive harbor seals have been derived from a few studies with small sample sizes. While these studies have been useful for describing general health (Englehardt 1979, McConnell and Vaughan 1983, Bossart and Dierauf 1990), they were not designed to examine the variability of blood parameters in response to changing nutritional status at the population level, nor did they include biomarkers which have recently been measured in wild populations in PWS.

The Trustee Council has supported the population-monitoring component of health biomarkers for marine mammals within PWS. At the Alaska SeaLife Center (ASLC) in Seward, we are examining these biomarkers, as well as other blood and body condition parameters, under controlled conditions in the same animals over time. Of particular interest is the effect of specific diets as the animals undergo natural seasonal physiological changes. Experiments following the same conceptual framework have been carried out in Europe on harbor seals fed diets of fish that differed in contaminant loads (Ross et al. 1996). The investigators demonstrated a measurable decrease in immune function in seals fed contaminated fish. In this study we are not feeding contaminated fish, but rather fish of differing energy density (herring and pollock). In addition we are monitoring sick and injured animals that are at the ASLC for rehabilitation. These animals represent seals whose ability to survive in the wild has been compromised and they present a unique view into the biology of unhealthy animals that are under-represented in our field studies (Fadely et al. 1998).

An additional component of nutritional studies of harbor seals relates to the "junk food" hypothesis which was proposed at a Sea Grant sponsored workshop in 1991 on whether or not food limitation could account for observed population declines (Alaska Sea Grant 1993). This thesis stated that while the biomass of pollock in Alaskan waters was sufficient to support marine mammal populations, the pollock were nutritionally poor compared to other less common species such as herring and capelin. Short-term studies of Steller sea lions suggested that the sea lions were unable to maintain mass on a diet consisting exclusively of pollock (A Trites, pers. comm.)

The metabolic demand of phocids varies throughout the year as a result of annual cycles (e.g. molting) (Ashwell-Erickson and Elsner 1981). We must be able to factor this variability into any nutritional limitations of the food itself. This project is designed to assess the nutritional needs of harbor seals over long periods and a variety of seasons and assess whether those needs can be met by pollock. Included in this design is a series of experiments in which assimilation efficiency, retention time and metabolizable energy are determined for each diet. Assimilation efficiency (AE), which is defined as the proportion of dry matter assimilated from a prey source, is influenced by food quality, meal size, feeding frequency and digestive passage rate (Robbins 1983, Lawson et al. 1998). Recent studies have suggested that AE is low when food quality is low (Brekke and Gabrielsen 1994, Mårtensson et al. 1994). However, conflicting results have been reported for harbor seals (Ashwell-Erickson and Elsner 1981) and northern fur seals (Miller 1978), while studies of California sea lions fed pollock did not show a significant decrease in AE with lower energy density food (Fadely et al. 1994).

At this point we have compiled baseline data for the 8 harbor seals participating in the feeding trials, including mass, lengths and girths, blubber depths (by ultrasound), clinical blood chemistry and hematology (6 – 8 complete sets of measurements per animal). Mass and morphometrics were stable on acclimation diets (Group A: herring, Group B: mixed diet) as the animals began the first feeding trial. There were some differences in blood chemistry between the two groups during acclimation, with animals fed a mixed diet having higher globulin, total protein and creatinine levels and lower blood urea nitrogen (BUN):creatinine and triglyceride levels. Whether these differences are a result of different diets or other differences between the two groups should become apparent as they progress through a series of feeding trials. Analyses of other biomarkers such as haptoglobin and erythrocyte sedimentation rate (ESR) are ongoing and the results will be reported in the next annual report.

Three harbor seal pups were successfully rehabilitated at the ASLC during 1998. Blood and morphometric measurements were consistent with recovery, although all three seals exhibited decreasing hematocrit levels. While the number of individuals studied at this point is low, preliminary results show extreme perturbations in blood parameters of severely compromised individuals (seals which did not recover) as well as long-term changes in blood parameters and morphometrics as individuals recover and develop. Analysis of these blood data suggest a number of potential factors that could contribute to the variability, including development, captivity, early weaning and disease or injury. All three recovering seals showed increases in cholesterol and globulin levels and decreases in gamma-glutamyl transpeptidase (GGT) and triglyceride levels during their recovery. Continuing these studies on injured or abandoned harbor seals through rehabilitation will enable more specific conclusions about the differences between healthy and unhealthy animals.

The next phase of this project has begun and will continue during 1999, with seals progressing through a series of feeding trials in which their diet will be switched between herring and pollock. By the end of the study, each seal will have experienced each diet at each time of the year and data will be analyzed by a cross-over repeated measures approach. Data collection continues to include bi-weekly blood sampling and morphometric measurements. Each feeding trial will include assimilation efficiency and retention time experiments, to determine if the seals are able to compensate physiologically for differences in food quality. The first of these experiments was conducted successfully in December 1998. Collection of samples from injured or unhealthy harbor seals sent to the ASLC will continue, extending the database and making comparisons more valuable.

OBJECTIVES

The objectives set forth for this multi-year project are:

1. Feed controlled diets of herring and pollock to harbor seals.
2. Quantify body condition, health, and blood chemistry biomarker changes in the seals during the feeding trials.
3. Assess the assimilation efficiency (AE) of the harbor seals fed different fish diets (i.e. the proportion of dry matter assimilated from a prey source).
4. Quantify seasonal, metabolic state and clinical health impacts on biomarkers and health indices.

We are not attempting to model the metabolic demands of harbor seals in the wild. The stresses and food requirements of wild populations are very different from captive animals. Instead, we are investigating the metabolic response to differing diets and the effect of these diets on blood chemistry and body condition of the animals. That is, we do not seek to model how many calories an animal may consume per month and apply that to field estimates of mass of fish consumed at sea. *We will quantify how biomarkers and other blood parameters change when an animal is fed different kinds of fish and compare those changes to observed patterns already collected from wild populations.*

METHODS

Preparation for Arrival of Seals at the Alaska SeaLife Center

In the months preceding the arrival of the harbor seals at the Alaska SeaLife Center, researchers prepared for moving the research program from the University of Alaska Fairbanks (UAF) to Seward, Alaska. Methods for analyzing assimilation efficiency, retention times and metabolizable energy were tested at UAF in collaboration with Dr. P. Barboza, Institute of Arctic Biology. Dr. Castellini traveled extensively to Seward, finally relocating on March 1, 1998 in order to oversee final arrangements for research as the ASLC prepared to receive animals and open to research. Other laboratory staff, including four graduate students and one research associate relocated themselves and research equipment to Seward between March and June 1998.

Seal Acclimation at the Alaska SeaLife Center

Eight adult captive harbor seals arrived at the ASLC during April and early May 1998. They were physically separated into two groups and each group was acclimated to a separate diet, similar to diets they had been fed previously. Group A was fed herring and group B was fed a mixed diet consisting of herring, squid and capelin (Table 1). The animals were allowed to acclimate to their new environment for approximately four months. This amount of time was extended from the three months originally anticipated. This allowed more time to ensure complete acclimation, as maintenance diets, training regimens and sampling protocols were adjusted. Final sampling protocols determined that diets should be switched in September, January and May to fit a matrix which would allow cross-over repeated measure analysis at the end of two years and correspond to natural seasonal cycles (winter/molting, spring, summer/breeding).

Animal Handling and Sample Collection

Morphometric measurements were performed as detailed in Frost et al. (1995) and Lewis (1995). The seals were trained to allow such measurements without restraint. The seals were weighed on a platform load cell scale (± 0.1 kg) and then held on a restraint board to allow collection of blood samples. Harbor seal pups were restrained without the use of a board. Blood

was sampled from the intervertebral extradural vein (Geraci and Smith 1975) using 2.5 – 3.5 in 18 G spinal needles (Monoject®, Sherwood Medical Co., St Louis, MO) into various blood collection tubes (Vacutainers®, Becton-Dickinson Vacutainer Systems, Rutherford, NJ). Adult seals were sampled bi-weekly whereas rehabilitated pups were sampled weekly.

Blood Chemistry and Hematology

For each adult seal, up to 20 mL of blood was collected for serum, 25 mL for plasma, and 10 mL in ethylenediaminetetraacetic acid (EDTA) tubes for measurement of complete blood counts (CBC) and sedimentation rate (ESR). Collection tubes were kept cool with ice or refrigerated until processed. They were usually processed within 30 minutes of collection. Blood hematocrit (% red blood cells by volume) was measured directly by microcentrifugation. Samples of whole blood (EDTA) were pipetted into Drabkin's reagent for hemoglobin analysis. Red blood cells were counted manually using whole blood (EDTA) diluted in a Unopette® and counted on a hemocytometer. Erythrocyte sedimentation rate (ESR) was determined by transferring a 1mL aliquot of whole blood (EDTA) into a tube with added isocitrate and analyzed on a HiChem mini-ves ESR analyzer. A separate 1 mL aliquot of whole blood (heparinized) was removed for whole blood water analysis. Blood was then centrifuged and plasma and serum were aliquoted into 1.2 mL cryogenic vials (Nalgene® Brand, Nalge Co., Rochester, NY) and frozen at -80°C for later laboratory analysis. One unfrozen aliquot of serum and 3ml of whole blood (EDTA) were kept refrigerated for chemical hematological analysis by the ASLC clinical laboratory.

Plasma samples were assessed for "standard" health indices (such as cholesterol level, salts, and enzymes characteristic of tissue damage) and also analyzed for indicators of dehydration (water content), malnutrition, and other stressors (haptoglobin). Standard panels that assay plasma sodium, potassium, chloride, phosphorus, blood urea nitrogen (BUN), creatinine, cholesterol, total bilirubin, total protein, albumin, globulin, alkaline phosphatase, glucose, lactate dehydrogenase (LDH), gamma-glutamyl transpeptidase (GGT), creatinine phosphokinase (CPK), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were performed by automated machine analysis at the ASLC using an IDEXX (ver-tex Model 8008) Analyzer. Additionally, concentrations of hemoglobin are determined using Drabkins reagent and performed in our laboratory. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin content (MCHC) were calculated from combinations of measured hematocrit, hemoglobin and red blood cell count (RBC) following Kerr (1989).

Statistical Analysis

Data are extremely preliminary at this point and the statistical analyses are limited to comparisons between mean blood chemistry parameters. Future analyses will include time series analysis of blood data and comparisons of feeding trials using cross-over repeated measures methods.

The feeding trial design incorporates a cross-over repeated measures approach and will allow statistical comparisons within any one group of seals between diet and season. A detailed matrix of the feeding schedule is shown below.

PERIOD	HERRING	POLLOCK	CONDITION
Sept-Dec 1998	Seals 1,2,3	Seals 4,5,6	Molting
Jan-April 1999	4,5,6	1,2,3	Spring
May-August 1999	1,2,3	4,5,6	Breeding
Sept-Dec 1999	4,5,6	1,2,3	Molting
Jan-April 2000	1,2,3	4,5,6	Spring
May-August 2000	4,5,6	1,2,3	Breeding

Two seals (7, 8) are in a separate feeding group. They are being fed a mixed diet of herring and pollock throughout the study. These animals undergo the same procedures as the animals on single prey diets.

This feeding matrix allows each group of seals to experience a different diet at similar physiologically relevant times of the year. Group 1, 2, 3, for example, was fed a herring diet during molting season in year one and will be fed a pollock diet during molting in year two. While all the harbor seals in this study were maintained previously on diets high in herring, they have all easily switched to experimental diets.

Proximate Analysis of Food

Individual batches of herring and pollock were subsampled (n = 5 for each) periodically (every 1 – 3 months during frozen storage) for proximate analysis. The samples were ground with a food grinder and further processed to a homogenous paste with a blender. For each fish, two samples of approximately 10 g each were frozen at -80°C and then freeze-dried to constant mass under vacuum (VirTis Freeze Dryer Model 5463) and the water content calculated on the basis of mass difference. Water content was verified by drying separate samples in a 80°C oven and also, in the case of one batch of herring, by freeze drying 5 whole fish. Lipid content and energy density were determined from freeze-dried samples and expressed as a per wet mass basis. Lipid content was determined as the mass difference after extraction of dry samples (initial mass 0.3 – 0.5g) for 24 hrs in a 2:1 chloroform-methanol mixture in a Soxhlet extraction apparatus. Energy density was determined by pelleting 0.3 – 0.6 g of dry sample and analyzing them in an adiabatic bomb calorimeter (Parr Co.).

The large size of pollock and the difficulty of grinding freeze-dried pollock were the main reasons for choosing to grind the fish prior to freeze drying. Tests were conducted on smaller fish (herring and capelin) to ensure that the two methods would result in comparable results. In this case, whole fish were freeze-dried to constant mass then ground in a coffee-grinder. Energy density was determined according to methods described above. Soxhlet analysis awaits specialized equipment that will allow analysis of the powdered fish.

While the results have been acceptable, continued analysis of food quality will use a larger subsample (n = 10) for each type of fish to facilitate statistical analyses of results.

RESULTS

Captive Harbor Seals

Mass and Morphometric Measurements.-- The animals were placed on a maintenance diet designed to adjust feeding to maintain a constant mass. During acclimation each animal maintained a fairly constant mass, although each experienced a small decline in July 1998 (Figure 1). Mass stabilized as the animals approached the beginning of the first feeding trial. There were no significant changes in morphometric measurements during acclimation.

Blood chemistry and hematology.--Mean blood chemistry values for individual seals in Group A (herring diet) are presented in Table 2 while those for Group B (mixed diet) are presented in Table 3. Overall, 65% of the blood chemistry values were significantly different between the two groups. Seals in Group A had increased mean levels of BUN:creatinine, triglyceride, alkaline phosphatase, ALT, calcium, phosphorus, and albumin:globulin when compared to seals in Group B (Table 4). Those mean blood chemistry values found to be significantly higher for the Group B seals included total protein, globulin, creatinine, cholesterol, amylase, sodium, chloride, and GGT.

Mean hematological values for individual seals in Group A are presented in Table 5, while those for Group B are presented in Table 6. Overall, 5 of the 9 hematological values were significantly different between the two groups. Group A had significantly elevated Hct, WBC counts, lymphocyte/monocyte ratio, and platelet counts (Table 7). Data which have not been included here are still in the process of being analyzed and will be included in subsequent annual reports.

Rehabilitated Harbor Seals

Three harbor seal pups were successfully rehabilitated at the ASLC during 1998. Limited data are also available for two harbor seals brought to the ASLC which did not recover. The condition of each animal upon admission is presented in Table 8.

Mass and Morphometric Measurements.-- The three rehabilitated pups showed steady increases in mass during recovery and development (Figure 2a). The growth rates of the three recovered harbor seal pups ranged between 0.2 kg/d to 0.29 kg/d (Yukon, 0.29 kg/d; Denali, 0.20kg/d; Iliamna, 0.25 kg/d) while at the ASLC. Assuming an age of seven days upon arrival at the ASLC we estimated mass at age:

$$Mass = 5.9 + 0.293 * age\ (d)$$

Axillary girth growth rates ranged between 0.3cm/d to 0.5cm/d (Yukon, 0.5 cm/d; Denali, 0.3cm/d; Iliamna, 0.42cm/d) for the three pups (Figure 2b). We also estimated the growth rate of the axillary girth at age:

$$AG = 47.2 + 0.425 * age\ (d)$$

Blubber depth at three locations dorsally yielded similar site-specific maximum growth rates; hip, Yukon, 0.24mm/d; Denali, 0.26mm/d; Iliamna, 0.24mm/d; axillary, Yukon, 0.09mm/d, Denali, 0.05mm/d, Iliamna, 0.08mm/d; Mid, Yukon, 0.36mm/d, Denali, 0.31mm/d, Iliamna, 0.32mm/d.

Blood chemistry.-- Mean blood chemistry values for the three successful pups are presented in Table 9. It is interesting to note that most of the values are comparable to data collected from wild PWS harbor seal pups during June 1998 (analyzed at Fairbanks Memorial Hospital, Fairbanks, AK), however small sample sizes precluded statistical analysis (Table 9). Most of the apparent differences are in liver enzyme levels. Rehabilitated pups had persistently low creatinine compared both with wild PWS pups in 1998 and with healthy adult harbor seals at the ASLC (Table 4). The pups that did not recover had several blood chemistry values that were

markedly different from those of recovering animals (Table 10). Seal 98005 had five blood variables which were different, including a particularly high blood glucose value (331 mg/dL) just before death. Seal 98009, which was euthanized shortly after arrival at the ASLC had as many as 13 blood variables which were markedly different from those of the recovering seals. In recovering pups, several blood parameters changed over time, including hematocrit which decreased sharply in the first two weeks of life but continued to decrease more gradually, finally stabilizing in two of the animals at a low value of 34 – 36% (Figure 3a,b). Hemoglobin values tracked hematocrit and consequently MCHC remained constant (Figure 3c). Triglyceride values also decreased in the three recovering seals (Figure 4a,b), while cholesterol values increased (Figure 4c,d). Other changes include an increase in globulin values observed in the two youngest pups (Figure 5a,b) and a marked decrease in GGT which was quite high upon admission (Figure 5c,d). The third pup, Iliamna, had variable levels of globulin and a sharp drop in GGT that corresponded in time to treatment with the antibiotic trimethoprim sulfadiazine. The GGT levels increased within days of the cessation of antibiotic treatment. Hematological values for the rehabilitated seals are presented in Table 11.

Proximate Analysis of Food

Differences in food composition as the seals began the first feeding trial are presented in Table 12. As expected, herring had substantially higher lipid content and energy density than did pollock.

DISCUSSION

If we theorize that various health and body condition markers react in the field to ecosystem-wide changes in food availability and health (EVOS 98001, 98163 (Apex), 98347 (river otters)), then we should be able to quantify those changes in the laboratory under controlled conditions. The Alaska SeaLife Center has research harbor seals that are healthy and have been placed onto differing and specific diets. In addition, unhealthy seals are brought to the ASLC for rehabilitation. Both groups allow us to examine how various markers respond to food and health status. Experiments following the same conceptual framework have been carried out in Europe on harbor seals fed diets of fish that differed in contaminant loads (Ross et al. 1996). In those studies, seals fed contaminated fish showed measurable decreases in immune function. Studies of wild common seals have suggested a link between prey switching and changes in various blood parameters such as mean cell volume (MCV) and hemoglobin concentration (Thompson et al., 1997). Seals in this study are being fed fish of different energy densities (pollock and herring) rather than contaminated fish to monitor the effects of substantially different diets on health parameters. Additionally, seals brought to the ASLC for rehabilitation represent animals whose ability to survive in the wild has been compromised and they present a unique view into the biology of unhealthy animals that are under-represented in our field studies in PWS (Fadely et al. 1998). By monitoring a suite of health parameters in seals at the ASLC we will be able to show how these parameters, commonly measured in wild populations, vary within an individual (sick or healthy), how they are affected by diet, and whether these patterns are dependent on other variables such as season, age or gender.

At this point a database has been compiled containing baseline data from two groups of captive seals as they acclimated to their new environment at the ASLC (Table 1). These data provide a health and condition profile for each animal as they began the first of 6 feeding trials in September 1998. The feeding regime requires that seals are fed a maintenance diet and measurements confirm that, except for a small decrease in mass in July 1998, mass has remained

relatively constant for each animal (Figure 1). Some seasonal fluctuation in mass is expected (Ashwell-Erickson and Elsner 1981, Rosen and Renouf 1995). Blood chemistry and hematological values appear to be within the ranges expected for adult harbor seals (Bossart and Dierauf 1990) (Tables 2–3, 5–6), however, the two groups of seals had several significant differences in blood parameters (16 of 23 blood chemistry values and 5 of 9 hematological values) (Tables 4, 7). The most striking of these differences included higher mean globulin, total protein and creatinine levels and lower triglyceride and BUN:creatinine values in seals in Group B. These blood variables are associated with protein and lipid metabolism (DelGuidice et al. 1987, Schweigert 1993). Compared to Group A (herring diet), Group B was being fed a lower fat, mixed diet of herring, capelin and squid during acclimation. There are other differences between these two groups however, including age structure, so it would be premature to suggest that the differences in blood chemistry observed at this point are a result of different diets. It will be interesting to observe whether these variables change as the animals switch between low and high fat diets. Differences in some blood parameters may be a result of the small sample size (4 individuals in each group). However, future analyses will include time series analysis of blood data and comparisons of feeding trials using cross-over repeated measures methods. This approach will allow us to determine which parameters respond to changes in diet. Analysis of additional health biomarkers is ongoing and results will be presented in subsequent annual reports.

An additional component of these nutritional studies of harbor seals relates to the "junk food" hypothesis that was proposed at a Sea Grant sponsored workshop in 1991 on whether food limitation could account for observed population declines (Alaska SeaGrant 1993). This thesis stated that while the biomass of pollock in Alaskan water was sufficient to support marine mammal populations, the pollock were nutritionally poor compared to other less common species such herring and capelin. Short-term studies of Steller sea lions suggested that they were unable to maintain mass on a diet consisting exclusively of pollock (A. Trites, pers. comm.). The metabolic demand of phocids varies throughout the year as a result of annual cycles (e.g. molting) (Ashwell-Erickson and Elsner 1981). We must be able to factor this variability into any nutritional limitations of the food itself. In addition to monitoring health parameters such as blood chemistry, hematology and other biomarkers, this study is designed to assess the nutritional needs of harbor seals over long periods and a variety of seasons and assess whether those needs can be met by pollock. This includes measuring various physiological responses to changing diet, including body condition (morphometrics) and composition, and assimilation efficiency and metabolizable energy associated with different diets. These experiments started in September 1998 and preliminary results will be presented in subsequent annual reports. Preliminary proximate analysis of herring and pollock revealed the expected differences, with herring having higher lipid content and energy density than pollock (Table 12). Proximate analysis of food is continuing so that variability between batches of fish can be monitored.

Three harbor seal pups were successfully rehabilitated at the ASLC during 1998 (Table 8). Blood and morphometric measurements were generally consistent with recovery. The growth rates (mass and axial girth) for each of the rehabilitated pups followed a similar trend regardless of age at initial captivity (Figure 2). This may be partially explained by nutrient availability since maximum growth rates of young animals are established by genetically determined physiological limits to cellular metabolism and have evolved relative to selective pressures such as food and mortality (Ricklefs 1973). Estimated growth rates for pinniped neonates as a function of adult body mass are similar to results obtained from rehabilitated harbor seal pups at the ASLC

(Robbins 1993, 0.24 kg/d). These data will subsequently be used to estimate nutrition and growth requirements. Overall blubber depth increased throughout captivity for the harbor seal pups, and it is interesting that the rate of increase among selected blubber depth sites were similar (Figure 2). This may prove useful as a condition index for developing free-ranging and captive harbor seal pups. Blood chemistry parameters for captive harbor seal pups at the ASLC are comparable to data collected from wild PWS harbor seal pups during June 1998, however, the small sample sizes precluded statistical analysis (Table 9). Many of the apparent differences are in liver enzyme levels. Differences in these values may be misleading since there is high innate variability in liver enzyme levels and the PWS samples were analyzed in a different clinical laboratory (Fairbanks Memorial Hospital. Fairbanks, AK). Rehabilitated pups had persistently low creatinine levels compared both with wild PWS pups in 1998 and with healthy adult harbor seals at the ASLC (Table 4). Creatinine is a non-protein end product of creatine metabolism and may be directly proportional to an animal's body mass and glomerular filtration rate (Hayward et al. 1995). Creatinine values in the rehabilitated pups showed no trend as the animals developed and gained mass. While the number of individuals studied at this point is low, preliminary results show extreme perturbation in blood parameters of severely compromised individuals (seals which did not recover) (Table 10).

A number of long-term changes in blood parameters were observed as individuals recovered and developed. Hematocrit values decreased markedly in all three pups during rehabilitation (Figure 3a,b,). In the two youngest pups (2 – 10 days old upon admission), the initial sharp drop is possibly a developmental change typical of neonatal seals (Hall 1998). Hematocrit continued to decline however, when the seals were more than a month old. Hematocrit for two of the three seals was below 40% upon release back to the wild. While low hematocrit has been recorded in other studies of captive seals (Nielsen 1995), these values seem innordinately low. Hemoglobin concentrations tracked the changes in hematocrit, resulting in stable MCHC (Figure 3c). Triglyceride levels also decreased to extremely low levels and were generally low upon release (Figure 4a,b). Accompanying these decrease in triglycerides were marked increases in cholesterol (Figure 4c,d). By the time the pups were one month old, cholesterol levels were higher than those of the adult harbor seals at the ASLC (Table 4), but were similar to values for wild pups in PWS in June 1998 (Table 9). This suggests that changes in cholesterol in these pups were developmentally influenced. Globulin levels increased somewhat during rehabilitation (Figure 5a,b), while GGT values decreased fairly steadily in the two youngest pups (Figure 5c,d). The third, older pup (Iliamna) had a sharp drop in GGT levels that corresponded in time with treatment with the antibiotic trimethoprim sufadiazine. The GGT levels increased within days of the cessation of antibiotic treatment.

With such a small number of individuals, and since each individual had a different clinical history, drawing conclusions about the causes for these observed trends in blood values is not yet possible. A number of factors may contribute including development, captivity, diet, early weaning and disease or injury. It is interesting that most of these trends are apparent in all three seals and some seem more closely related to age than time in captivity (hematocrit and cholesterol). Continuing these studies on injured/sick or abandoned seals through rehabilitation will enable more specific conclusions about the differences between healthy and unhealthy animals.

CONCLUSIONS

Baseline data have been collected for the 8 harbor seals participating in feeding trials to assess the impact of diet on health biomarkers as well as other blood and body condition indicators. These data establish a context for comparison as the seals are switched between high and low fat diets (herring and pollock, respectively). Masses were stable as the seals began Feeding Trial 1. Whether the differences in blood parameters observed between the two groups were influenced by differences in acclimation diets will become clear as the feeding trials progress.

Blood and morphometric measurements of 3 successfully rehabilitated harbor seal pups were consistent with recovery, although all three seals exhibited decreasing hematocrit levels. While the numbers of individuals studied at this point is low, preliminary results show extreme perturbation in blood parameters of severely compromised individual as well as long term changes in blood indicators and morphometrics as individuals recover and develop. A number of factors could contribute to the variability, including development, captivity, diet, early weaning and disease or injury. Continuing these studies of injured/sick or abandoned harbor seals will enable more specific conclusions about the differences between healthy and unhealthy animals.

The next phase of this study has begun and will continue during 1999, with seals progressing through a series of feeding trials in which their diet will be switched between herring and pollock. Data collection continues to include bi-weekly blood sampling and morphometric measurements as well as experiments to assess body composition and assimilation efficiency. The first of these experiments was conducted successfully in December 1998. The ability to collect data routinely and repeatedly from the same animals at the ASLC has already resulted in extremely valuable data about the variability of these measurements within a normal individual. By extending these data to include shifts in diet over a variety of seasonal conditions, important questions about the nutritional physiology of harbor seals will be answered.

ACKNOWLEDGEMENTS

We thank the research and husbandry staff of the Alaska SeaLife Center: Susan Inglis, Jon Moreland, Pam Tuomi, Mille Gray, Dennis Christen, Nicole Neistadt, Lisa Corbin and Tara Rees. We also thank Tami Mau, Heather Ellingboe and Wendy Dunlap who were instrumental in this work. Bomb calorimetry was performed by Phyllis Shoemaker of the UAF Institute of Marine Science, Seward Marine Center.

Funding for 1998 was provided by the *Exxon Valdez* Oil Spill Trustee Council. Additional funding for graduate student stipends was provided by the Rasmuson Fisheries Research Center and Alaska Sea Grant. This research was conducted with authorization under the Marine Mammal Protection Act to MAC and both the University of Alaska Fairbanks and the Alaska SeaLife Center Institutional Animal Care and use Committees.

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Table 1. Grouping of adult harbor seals during acclimation from May – September 1998. Group A animals continued on a diet of herring and Group B animals switched to a diet of pollock for Feeding Trial 1. One animal from each group (Tina and Snapper) was placed on a mixed diet of herring and pollock for the entire feeding study.

	Name	*Age (years)*	*Sex*
Group A	Sydney	2	F
Herring Diet	Tina	7	F
	Pender	2	M
	Travis	2	M
Group B	Poco	23	F
Mixed Diet	Skeezix	23	F
	Snapper	15	M
	Cecil	14	M

Table 2. Serum chemistry values for Group A harbor seals, fed a herring diet during acclimation.

		ALB g/dL	*ALKP U/L*	*ALT U/L*	*AST U/L*	*AMYL U/L*	*Ca mg/dL*	*CHOL mg/dL*	*BUN mg/dL*	*CREA mg/dL*	*BUN: CREA*	*GLU mg/dL*	*PHOS mg/dL*
PENDER	*Mean*	**3.16**	**115**	**74**	**59**	**738**	**9.71**	**257.4**	**34.9**	**0.87**	**41.9**	**155.9**	**6.05**
	S.D.	0.26	42	24	22	291	0.39	31.5	8.4	0.15	15.0	12.1	0.96
	Min	2.82	68	31	38	408	9.30	216.0	27.1	0.62	25.1	143.3	4.82
	Max	3.54	218	121	105	114	10.30	313.0	49.0	1.10	77.6	183.5	7.39
	n	9	9	9	8	7	9	9	9	9	9	9	9
SYDNEY	*Mean*	**3.22**	**131**	**63**	**70**	**555**	**9.79**	**242.3**	**36.2**	**0.78**	**48.2**	**155.1**	**5.94**
	S.D.	0.25	41	24	34	222	0.37	34.8	9.0	0.15	16.6	10.3	1.36
	Min	2.88	92	30	26	335	9.24	198.9	24.2	0.57	30.0	138.0	3.30
	Max	3.60	204	97	127	864	10.50	289.3	49.0	1.10	84.9	167.0	7.78
	n	9	9	9	8	7	9	9	9	9	9	9	9
TRAVIS	*Mean*	**3.33**	**106**	**55**	**50**	**538**	**27.6**	**9.79**	**284.3**	**0.78**	**35.7**	**181.3**	**4.43**
	S.D.	0.28	27	24	14	189	3.0	0.38	41.6	0.08	5.3	26.1	0.80
	Min	2.97	66	15	34	315	24.5	9.53	195.2	0.67	26.9	154.6	3.30
	Max	3.70	149	83	74	776	31.6	10.59	317.5	0.91	42.6	236.0	5.47
	n	7	7	7	7	6	7	7	7	7	7	7	7
TINA	*Mean*	**3.58**	**79**	**32**	**46**	**859**	**9.57**	**287.6**	**29.2**	**1.15**	**25.7**	**176.4**	**5.90**
	S.D.	0.42	29	8	31	272	0.42	19.5	3.8	0.14	4.4	15.0	1.71
	Min	3.16	60	23	11	538	9.13	271.8	24.7	0.91	20.2	160.3	4.037
	Max	4.41	140	47	91	1200	10.31	328.5	36.8	1.36	30.6	174.5	9.23
	n	7	7	7	5	6	7	7	7	7	7	7	7

ALB – Albumin
ALKP – Alkaline phosphate
ALT – Alanine aminotransferase
AST – Aspartate aminotransferase
AMYL – Amylase
Ca – Calcium
CHOL – Cholesterol
BUN – Blood urea nitrogen
CREA – Creatinine
BUN:CREA – Blood urea nitrogen:creatinine
GLU – Glucose
PHOS – Phosphorous

Table 2 continued. Serum chemistry values for Group A harbor seals, fed a herring diet during acclimation.

		TBIL mg/dL	*TP g/dL*	*GLOB g/dL*	*ALB: GLOB*	*Na mEq/L*	*K mEq/L*	*Cl mEq/L*	*LDH U/L*	*TRIG mg/dL*	*CPK U/L*	*GGT U/L*
PENDER	*Mean*	**0.45**	**7.20**	**4.03**	**0.79**	**154.8**	**3.98**	**109.7**	**1496**	**39.3**	**309**	**20**
	S.D.	0.21	0.59	0.50	0.13	6.1	0.52	2.6	401	13.0	217	15
	Min	0.20	6.20	3.10	0.69	144.0	3.10	105.0	649	15.5	142	11
	Max	0.89	8.26	4.72	1.03	160.0	4.77	112.5	2063	58.3	755	56
	n	9	9	9	9	9	9	9	9	7	8	8
SYDNEY	*Mean*	**0.60**	**7.20**	**3.97**	**0.81**	**154.7**	**4.11**	**111.0**	**1986**	**58.4**	**938**	**18**
	S.D.	0.27	0.45	0.39	0.12	5.6	0.48	2.6	568	21.8	786	4
	Min	0.39	6.51	3.30	0.72	144.0	3.20	106.0	1365	37.5	93	12
	Max	1.16	7.76	4.49	1.09	158.9	4.68	114.0	3014	93.7	2036	22
	n	9	9	9	9	9	9	9	6	7	7	8
TRAVIS	*Mean*	**0.67**	**7.95**	**4.61**	**0.73**	**154.1**	**3.96**	**110.2**	**1762**	**39.7**	**326**	**20**
	S.D.	0.40	0.59	0.50	0.10	5.0	0.65	2.6	303	14.9	252	5
	Min	0.20	6.96	3.84	0.63	146.0	3.10	105.0	1338	23.2	59	11
	Max	1.50	8.70	5.10	0.90	158.6	5.15	113.8	2283	66.6	735	26
	n	7	7	7	7	7	7	7	7	6	7	7
TINA	*Mean*	**0.79**	**8.64**	**5.06**	**0.71**	**156.9**	**3.91**	**112.5**	**1973**	**56.6**	**1218**	**13**
	S.D.	0.43	0.39	0.16	0.09	4.9	0.45	2.5	511	41.3	101.5	3
	Min	0.46	8.25	4.88	0.61	145.9	3.28	108.9	1291	11.1	55	10
	Max	1.70	9.48	5.26	0.87	160.2	4.53	115.7	2700	133.3	2254	17
	n	7	7	7	7	7	7	7	6	7	6	6

TBIL – Total bilirubin
TP – Total protein
GLOB – Globulin
ALB:GLOB – Albumin:globulin
Na – Sodium
K – Potassium
Cl – Chloride
LDH – Lactate dehydrogenase
TRIG – Triglyceride
CPK – Creatine phosphokinase
GGT – Gamma-glutamyl transpeptidase

Table 3. Serum chemistry values for Group B harbor seals, fed a mixed diet during acclimation.

		ALB g/dL	*ALKP U/L*	*ALT U/L*	*AST U/L*	*AMYL U/L*	*Ca mg/dL*	*CHOL mg/dL*	*BUN mg/dL*	*CREA mg/dL*	*BUN: CREA*	*GLU mg/dL*	*PHOS mg/dL*
CECIL	*Mean*	**3.05**	**37**	**30**	**42**	**623**	**8.98**	**259.4**	**28.3**	**1.21**	**24.4**	**143.2**	**4.65**
	S.D.	0.15	3	15	15	109	0.40	30.0	3.1	0.31	4.9	5.1	1.10
	Min	2.79	33	12	27	488	8.74	216.4	23.0	0.92	16.9	136.0	3.66
	Max	3.22	41	51	66	841	9.96	301.0	32.0	1.73	31.2	151.1	7.05
	n	8	8	7	7	8	8	8	8	8	8	8	8
POCO	*Mean*	**3.51**	**182**	**30**	**22**	**885**	**9.62**	**352.1**	**27.3**	**1.27**	**21.8**	**181.6**	**3.87**
	S.D.	0.27	28	21	11	207	0.35	55.4	4.0	0.18	4.1	16.8	0.79
	Min	3.17	141	10	9	546	9.19	284.4	22.9	1.05	16.9	150.4	2.67
	Max	4.00	219	64	41	1202	10.27	439.0	35.6	1.62	28.5	204.3	5.09
	n	8	8	7	7	8	8	8	8	8	8	8	8
SKEEZIX	*Mean*	**3.81**	**43**	**26**	**94**	**1124**	**9.75**	**369.0**	**31.9**	**1.28**	**25.0**	**194.4**	**5.84**
	S.D.	0.49	8	12	56	263	0.46	73.6	3.7	0.10	2.6	14.5	0.70
	Min	3.25	33	11	37	627	9.18	285.7	26.6	1.17	22.5	169.1	4.90
	Max	4.49	58	38	168	1393	10.36	470.0	36.7	1.44	29.74	210.8	6.62
	n	7	7	6	6	7	7	7	7	7	7	7	7
SNAPPER	*Mean*	**3.22**	**41**	**41**	**37**	**745**	**9.13**	**267.7**	**31.5**	**1.23**	**26.2**	**150.0**	**4.25**
	S.D.	0.17	2	12	12	197	0.35	16.8	1.8	0.21	4.7	6.8	1.05
	Min	2.99	38	28	26	491	8.74	245.6[a]	29.0	0.99	19.1	141.3	3.00
	Max	3.42	45	63	60	984	9.74	290.6	34.6	1.52	33.6	159.4	6.24
	n	8	8	7	7	8	8	8	8	8	8	8	8

ALB – Albumin
ALKP – Alkaline phosphate
ALT – Alanine aminotransferase
AST – Aspartate aminotransferase
AMYL – Amylase
Ca – Calcium
CHOL – Cholesterol
BUN – Blood urea nitrogen
CREA – Creatinine
BUN:CREA – Blood urea nitrogen:creatinine
GLU – Glucose
PHOS – Phosphorous

Table 3 continued. Serum chemistry values for Group B harbor seals, fed a mixed diet during acclimation.

		TBIL mg/dL	*TP g/dL*	*GLOB g/dL*	*ALB: GLOB*	*Na mEq/L*	*K mEq/L*	*Cl mEq/L*	*LDH U/L*	*TRIG mg/dL*	*CPK U/L*	*GGT U/L*
CECIL	*Mean*	**0.61**	**8.44**	**5.39**	**0.57**	**159.8**	**3.57**	**110.7**	**1572**	**29.7**	**835**	**19**
	S.D.	0.10	0.32	0.27	0.04	1.4	0.16	1.0	656	8.5	839	5
	Min	0.50	7.99	5.03	0.50	157.4	3.36	108.8	973	18.9	59	14
	Max	0.81	8.87	5.68	0.62	162.0	3.80	112.0	2708	41.8	2036	28
	n	8	8	8	8	8	8	8	7	8	7	8
POCO	*Mean*	**0.64**	**8.44**	**4.93**	**0.71**	**157.9**	**3.47**	**110.9**	**684**	**37.5**	**177**	**30**
	S.D.	0.17	0.36	0.24	0.07	3.8	0.19	1.7	78	9.5	205	20
	Min	0.42	7.92	4.47	0.63	148.9	3.28	107.9	617	24.4	19	13
	Max	0.87	9.02	5.32	0.83	160.4	3.76	113.6	844	55.6	576	66
	n	8	8	8	8	8	8	8	7	8	8	8
SKEEZIX	*Mean*	**0.88**	**9.16**	**5.35**	**0.72**	**162.0**	**4.09**	**107.7**	**2223**	**37.6**	**1542**	**30**
	S.D.	0.46	0.49	0.24	0.10	2.3	0.33	1.4	728	12.9	699	15
	Min	0.42	8.62	4.84	0.60	159.4	3.55	105.5	1259	25.5	352	14
	Max	1.76	10.03	5.54	0.88	164.7	4.51	109.3	2800	60.3	2036	55
	n	7	7	7	7	6	6	6	5	7	7	7
SNAPPER	*Mean*	**0.59**	**8.35**	**5.13**	**0.63**	**159.5**	**3.27**	**114.6**	**1166**	**32.0**	**455**	**23**
	S.D.	0.11	0.35	0.27	0.04	1.2	0.19	7.7	196	12.1	554	5
	Min	0.50	7.59	4.60	0.57	157.6	3.05	110.5	947	10.1	60	15
	Max	0.83	8.71	5.54	0.69	160.7	3.59	133.5	1499	49.4	1587	29
	n	8	8	8	8	8	8	8	7	8	8	8

TBIL – Total bilirubin
TP – Total protein
GLOB – Globulin
ALB:GLOB – Albumin:globulin
Na – Sodium
K – Potassium
Cl – Chloride
LDH – Lactate dehydrogenase
TRIG – Triglyceride
CPK – Creatine phosphokinase
GGT – Gamma-glutamyl transpeptidase

Table 4. Comparison of mean serum chemistry values between Group A (herring diet) and Group B (mixed diet).

		ALB g/dL	ALKP[a] U/L	ALT[b] U/L	AST U/L	AMYL U/L	Ca mg/dL	CHOL mg/dL	BUN[b] mg/dL	CREA mg/dL	BUN:[a] CREA	GLU mg/dL	PHOS mg/dL
Group A	*Mean*	**3.31**	**110**	**57**	**57**	**671**	**9.72**	**265.7**	**32.4**	**0.88**	**38.8**	**165.7**	**5.63**
Herring Diet	*S.D.*	0.33	39	26	26	268	0.38	36.6	7.6	0.19	14.4	19.5	1.35
	n	32	32	32	28	26	32	32	32	32	32	32	32
Group B	*Mean*	**3.38**	**77**	**32**	**47**	**835**	**9.35**	**310.2**	**29.7**	**1.24**	**24.3**	**166.4**	**4.61**
Mixed Diet	*S.D.*	0.40	65	16	38	264	0.49	67.2	3.7	0.21	4.3	24.2	1.14
	n	31	31	27	27	31	31	31	31	31	31	31	31
P value		***0.255***	***0.009***	***0.001***	***0.807***	***0.019***	***0.002***	***0.004***	***0.116***	***0.000***	***0.000***	***0.831***	***0.003***

		TP g/dL	TBIL[c] mg/dL	GLOB g/dL	ALB:[b] GLOB	Na[a] mE/L	K mE/L	Cl mE/L	LDH U/L	TRIG mg/dL	CPK U/L	GGT U/L
Group A	*Mean*	**7.67**	**0.61**	**4.36**	**0.77**	**155.1**	**4.00**	**110.7**	**1769**	**48.8**	**665**	**18**
Herring Diet	*S.D.*	0.77	0.33	0.60	0.11	5.5	0.51	2.7	470	25.9	714	8
	n	32	32	32	32	32	32	32	28	27	28	29
Group B	*Mean*	**8.58**	**0.67**	**5.19**	**0.65**	**159.6**	**3.56**	**111.2**	**1349**	**34.1**	**723**	**25**
Mixed Diet	*S.D.*	0.48	0.26	0.31	0.09	2.7	0.35	4.6	706	10.9	773	131
	n	31	31	31	31	30	30	30	28	27	28	30
P value		***0.000***	***0.714***	***0.000***	***0.002***	***0.005***	***0.002***	***0.047***	***0.120***	***0.009***	***0.767***	***0.002***

[a]data normalized using cube root transformation
[b]data normalized using square root transformation
[c]data normalized using log transformation

Table 5. Mean hematological values for Group A harbor seals, fed a herring diet during acclimation.

		Hct *%*	*Hb*[a] *g/dL*	*MCHC* *g/dL*	*WBC* *10^9/L*	*Gran* *10^9/L*	*% Gran*	*L/M* *10^9/L*	*%L/M*	*PLT* *10^9/L*
PENDER	*Mean*	**50.4**	**18.6**	**35.9**	**10.4**	**6.5**	**63**	**3.9**	**37**	**415**
	S.D.	2.6	0.7	0.7	0.9	0.7	2	0.3	2	55
	Min	47.5	17.5	34.7	9.2	5.7	59	3.5	34	348
	Max	55.0	19.3	36.6	11.5	7.6	66	4.5	41	513
	n	9	8	8	8	8	8	8	8	8
SYDNEY	*Mean*	**54.4**	**19.0**	**35.9**	**9.7**	**6.2**	**65**	**3.4**	**35**	**368**
	S.D.	5.3	3.1	1.7	1.5	1.2	9	1.0	9	148
	Min	46.0	14.6	32.2	7.8	4.8	56	1.5	17	59
	Max	60.0	22.8	36.8	12.6	7.7	83	4.9	44	518
	n	8	7	7	7	7	7	7	7	7
TRAVIS	*Mean*	**59.4**	**20.7**	**35.9**	**9.0**	**5.7**	**63**	**3.3**	**37**	**401**
	S.D.	2.1	1.4	1.0	0.5	0.7	7	0.7	7	58
	Min	57.0	18.6	33.8	8.4	4.7	55	2.5	27	320
	Max	62.5	22.3	36.7	10.0	6.7	73	4.5	45	480
	n	7	7	7	7	7	7	7	7	7
TINA	*Mean*	**52.4**	**19.6**	**36.2**	**8.8**	**5.5**	**63**	**3.3**	**37**	**395**
	S.D.	1.7	1.0	0.4	1.4	0.6	4	0.8	4	70
	Min	50.0	18.7	35.6	7.2	4.5	59	2.2	31	300
	Max	55.0	20.8	36.7	10.3	6.1	69	4.2	41	471
	n	7	6	6	6	6	6	6	6	6

[a]ASLC veterinary values
Hct - hematocrit
Hb - hemoglobin
MCHC - mean corpuscular hemoglobin content
WBC - white blood cell count
Gran - granulocytes
%Gran - Percent granulocyte
L/M - lymphocyte to monocyte ratio
%L/M - percent lymphocyte/monocyte
PLT - platelets

Table 6. Mean hematological values for Group B harbor seals, fed a mixed diet during acclimation.

		Hct *%*	*Hb*[a] *g/dL*	*MCHC* *g/dL*	*WBC* *10^9/L*	*Gran* *10^9/L*	*% Gran*	*L/M* *10^9/L*	*%L/M*	*PLT* *10^9/L*
CECIL	*Mean*	**50.8**	**18.9**	**36.3**	**8.8**	**5.8**	**65**	**3.1**	**35**	**385**
	S.D.	4.4	1.3	0.5	1.1	1.1	8	0.6	8	77
	Min	43.0	16.0	35.5	7.8	4.0	51	2.4	27	273
	Max	56.0	20.2	36.8	11.0	7.6	73	4.0	49	485
	n	8	8	8	8	8	8	8	8	8
POCO	*Mean*	**52.8**	**19.5**	**36.7**	**8.4**	**5.7**	**68**	**2.7**	**32**	**320**
	S.D.	2.3	0.6	0.1	0.9	1.1	7	0.5	7	40
	Min	49.5	18.9	36.4	6.8	4.3	56	1.9	22	250
	Max	56.5	20.7	36.8	9.4	6.8	78	3.4	44	365
	n	8	8	8	8	8	8	8	8	8
SKEEZIX	*Mean*	**53.8**	**19.6**	**36.7**	**9.0**	**5.7**	**63**	**3.3**	**37**	**300**
	S.D.	1.6	0.9	0.1	0.6	1.1	11	1.0	11	64
	Min	52.0	18.3	36.6	8.2	4.6	53	1.7	19	179
	Max	56.0	20.4	36.8	10.2	7.3	81	4.4	47	356
	n	7	7	7	7	7	7	7	7	7
SNAPPER	*Mean*	**49.1**	**18.0**	**36.7**	**7.7**	**5.6**	**72**	**2.1**	**28**	**269**
	S.D.	3.2	1.0	0.1	1.3	1.1	3	0.3	3	34
	Min	45.0	17.0	36.6	6.4	4.4	67	1.8	24	209
	Max	54.5	20.1	36.8	10.1	7.7	76	2.6	33	313
	n	8	8	8	8	8	8	8	8	8

[a] ASLC veterinary values

Hct - hematocrit
Hb - hemoglobin
MCHC - mean corpuscular hemoglobin content
WBC - white blood cell count
Gran - granulocytes
%Gran - Percent granulocyte
L/M - lymphocyte to monocyte ratio
%L/M - percent lymphocyte/monocyte
PLT - platelets

Table 7. Comparison of mean hematological values between Group A (herring) and Group B (mixed diet) harbor seals.

		Hct[a] *%*	*Hb*[b] *g/dL*	*MCHC g/dL*	*WBC* 10^9*/L*	*Gran* 10^9*/L*	*%Gran*[a]	*L/M* 10^9*/L*	*%L/M*[a]	*PLT* 10^9*/L*
GROUP A	*Mean*	**54.1**	**19.5**	**36.0**	**9.5**	**6.0**	**63**	**3.5**	**37**	**395**
	S.D.	3.8	0.9	0.1	0.7	0.5	1	0.3	1	20
	Min	46.0	14.6	32.2	7.2	4.5	55	1.5	17	59
	Max	62.5	22.8	36.8	12.6	7.7	83	4.9	44	518
	n	4	4	4	4	4	4	4	4	4
GROUP B	*Mean*	**51.6**	**19.0**	**36.6**	**8.5**	**5.7**	**67**	**2.8**	**33**	**318**
	S.D.	2.1	0.7	0.2	0.6	0.1	4	0.5	4	49
	Min	43.0	16.0	35.5	6.4	4.0	51	1.7	19	179
	Max	56.5	20.7	36.8	11.0	7.7	81	4.4	49	485
	n	4	4	4	4	4	4	4	4	4
P values		*0.011*	*0.353*	*0.006*	*0.001*	*0.165*	*0.088*	*0.001*	*0.09*	*0.002*
ALL ADULTS	*Mean*	**52.9**	**19.2**	**36.3**	**9.0**	**5.8**	**65**	**3.1**	**35**	**357**
	S.D.	3.2	0.8	0.4	0.8	0.3	3	0.5	3	53
	n	8	8	8	8	8	8	8	8	8

[a] data transformed using arcsine transformation
[b] ASLC veterinary values

Hct - hematocrit
Hb - hemoglobin
MCHC - mean corpuscular hemoglobin content
WBC - white blood cell count
Gran - granulocytes
%Gran - Percent granulocyte
L/M - lymphocyte to monocyte ratio
%L/M - percent lymphocyte/monocyte
PLT - platelets

Table 8. Admission information for harbor seal pups brought to the Alaska SeaLife Center for rehabilitation.

	Identification	*Sex*	*Admission Date*	*Approximate Age at Admission*	*Condition at Admission*	*Date of Release/Death*
Recovered and Released	Yukon (98004)	M	5-25-98	3 days	No apparent injury[a], good condition	8-11-98
	Denali (98006)	F	6-17-98	7 – 10 days	Abandoned[b], dehydrated, thin	8-11-98
	Iliamna (98009)	F	7-10-98	3 weeks	Injured, laceration, pus, thin (9kg)	10-3-98
Died	98005	M	5-28-98	10 – 12 days	Injured, propeller laceration	6-5-98
	98009	F	8-11-98	2 months	Sick, hypothermic, distended abdomen, granulating laceration	8-11-98[c]

[a]picked up by tourist
[b]observed 48 hours
[c]euthanized

Table 9. Serum chemistry values for harbor seal pups brought to the Alaska SeaLife Center for rehabilitation in 1998. Values are compared to data collected from harbor seal pups in Prince William Sound (PWS) in June 1998.

		ALB g/dL	*ALKP U/L*	*ALT U/L*	*AST U/L*	*AMYL U/L*	*Ca mg/dL*	*CHOL mg/dL*	*BUN mg/dL*	*CREA mg/dL*	*BUN: CREA*	*GLU mg/dL*	*PHOS mg/dL*
YUKON	*Mean*	**3.19**	**377**	**62**	**62**	**402**	**10.32**	**307.9**	**34.3**	**0.39**	**88.4**	**168.6**	**7.97**
	S.D.	0.43	70	26	26	46	0.43	71.2	8.4	0.04	23.9	9.6	1.08
	Min	2.90	303	23	38	315	9.79	159.7	26.6	0.32	66.8	151.1	5.90
	Max	4.27	564	121	135	480	11.36	384.9	51.6	0.47	135.8	186.0	9.43
	n	12	12	12	12	12	12	12	12	12	12	12	12
DENALI	*Mean*	**3.05**	**220**	**100**	**57**	**415**	**9.88**	**329.4**	**36.2**	**0.30**	**120.4**	**163.0**	**6.49**
	S.D.	0.08	47	67	28	14	0.25	49.1	8.2	0.02	27.4	13.6	0.56
	Min	2.95	150	47	18	387	9.55	219.1	28.7	0.27	95.7	149.2	5.65
	Max	3.14	293	265	114	431	10.28	387.8	53.9	0.35	168.4	190.2	7.54
	n	9	9	9	9	9	9	9	9	9	9	9	9
ILIAMNA	*Mean*	**2.91**	**238**	**81**	**101**	**378**	**10.10**	**373.4**	**28.3**	**0.33**	**86.1**	**168.2**	**6.56**
	S.D.	0.16	83	55	82	129	0.45	59.9	7.8	0.04	25.0	12.7	1.05
	Min	2.64	87	13	27	265	9.38	279.6	17.6	0.27	53.1	147.1	5.04
	Max	3.22	362	189	298	622	11.14	470.6	40.0	0.39	125.0	189.4	7.75
	n												
PWS 98[a]	*Mean*	**3.4**	**276**	**33**	**82**		**10.5**	**342**	**32**	**0.8**	**41.3**	**166**	**6.4**
	S.D.	0.1	144	10	19		0.3	78	7	0.1	8.6	24	1.0
	n	14	14	14	14		14	14	14	14	14	14	14

[a]analyzed at Fairbanks Memorial Hospital

ALB – Albumin
ALKP – Alkaline phosphate
ALT – Alanine aminotransferase
AST – Aspartate aminotransferase
AMYL – Amylase
Ca – Calcium
CHOL – Cholesterol
BUN – Blood urea nitrogen
CREA – Creatinine
BUN:CREA – Blood urea nitrogen:creatinine
GLU – Glucose
PHOS – Phosphorous

Table 9 continued. Serum chemistry values for harbor seal pups brought to the Alaska SeaLife Center for rehabilitation in 1998. Values are compared to data collected from harbor seal pups in Prince William Sound (PWS) in June 1998.

		TBIL mg/dL	*TP g/dL*	*GLOB g/dL*	*ALB: GLOB*	*Na mEq/L*	*K mEq/L*	*Cl mEq/L*	*LDH U/L*	*TRIG mg/dL*	*CPK U/L*	*GGT U/L*
YUKON	*Mean*	**0.55**	**6.50**	**3.31**	**0.99**	**153.8**	**4.61**	**108.5**	**3107**	**44.2**	**309**	**30**
	S.D.	0.25	0.42	0.47	0.27	2.2	0.46	3.7	1550	50.1	392	5
	Min	0.38	5.81	2.58	0.78	148.0	3.70	100.6	2353	5.6	82	23
	Max	1.20	6.96	3.89	1.66	157.5	5.27	112.4	6615	187.1	1446	39
	n	12	12	12	12	12	12	12	7	11	12	12
DENALI	*Mean*	**0.81**	**6.91**	**3.86**	**0.79**	**154.2**	**4.62**	**111.3**	**2517**	**45.2**	**218**	**37**
	S.D.	0.67	0.40	0.34	0.06	1.7	0.33	3.1	296	21.6	260	7
	Min	0.50	6.19	3.23	0.73	151.8	4.09	103.6	2151	9.6	95	31
	Max	2.59	7.42	4.28	0.91	156.3	5.15	114.7	2834	78.7	907	519
	n	9	9	9	9	9	9	9	7	9	9	9
ILIAMNA	*Mean*	**0.67**	**7.49**	**4.58**	**0.64**	**154.8**	**4.64**	**114.9**	**2189**	**30.7**	**280**	**31**
	S.D.	0.08	0.39	0.37	0.07	1.6	0.40	13.6	243	22.0	484	11
	Min	0.55	6.78	3.98	0.56	151.9	3.97	109.3	1821	0.0	49	16
	Max	0.81	8.30	5.33	0.79	157.6	5.26	155.8	2568	81.0	1710	49
	n	12	12	12	12	11	11	11	11	12	11	12
PWS 98[a]	*Mean*	**0.5**	**6.6**	**3.2**	**1.07**	**145**	**3.6**	**108**	**3071**		**806**	**20**
	S.D.	0.2	0.3	0.2	0.07	2	0.3	2	458		578	10
	n	14	14	14	14	14	14	14	14		14	14

[a]analyzed at Fairbanks Memorial Hospital
TBIL – Total bilirubin
TP – Total protein
GLOB – Globulin
ALB:GLOB – Albumin:globulin
Na – Sodium
K – Potassium
Cl – Chloride
LDH – Lactate dehydrogenase
TRIG – Triglyceride
CPK – Creatine phosphokinase
GGT – Gamma-glutamyl transpeptidase

Table 10. Comparisons between serum chemistry values of pups which were successfully rehabilitated at the Alaska SeaLife Center during 1998 and pups that did not recover.

		ALB g/dL	*ALKP U/L*	*ALT U/L*	*CHOL mg/dL*	*BUN mg/dL*	*CREA mg/dL*	*BUN/ CREA*	*GLU mg/dL*	*TP g/dL*	*ALB: GLOB*	*K MEq/L*	*TRIG mg/dL*	*GGT U/L*
Recovering	*Yukon*	3.19	377	62	307.9	34.3	0.39	88.4	168.6	6.50	0.99	4.61	44.2	30
	Denali	3.05	220	100	329.4	36.2	0.30	*120.4*	163.0	6.91	0.79	4.62	45.2	37
	Iliamna	2.91	238	81	373.4	28.3	0.33	86.1	168.2	7.49	0.64	4.64	30.7	31
Died	*98005*	3.34	**148**	123	255.6 **209.6**[a]	24.9	*0.28*	92.5	**240.7** **331.0**[a]	**6.17**	1.18	**4.37** **3.94**[a]	89.6	49.5
	98009	**1.79**	**41**	**10**	**452.6**	**99.2**	**0.66**	**150.3**	**109.2**	**5.90**	**0.44**	**6.25**	**334.4**	**67**

[a] sampled 3 days prior to death

ALB – Albumin
ALKP – Alkaline phosphate
ALT – Alanine transferase
CHOL – Cholesterol
BUN – Blood urea nitrogen
CREA – Creatinine
BUN:CREA – Blood urea nitrogen:creatinine
GLU – Glucose
TP – Total protein
ALB:GLOB – Albumin:globulin
K – Potassium
TRIG – Triglyceride
GGT – Gamma-glutamyl transpeptidase

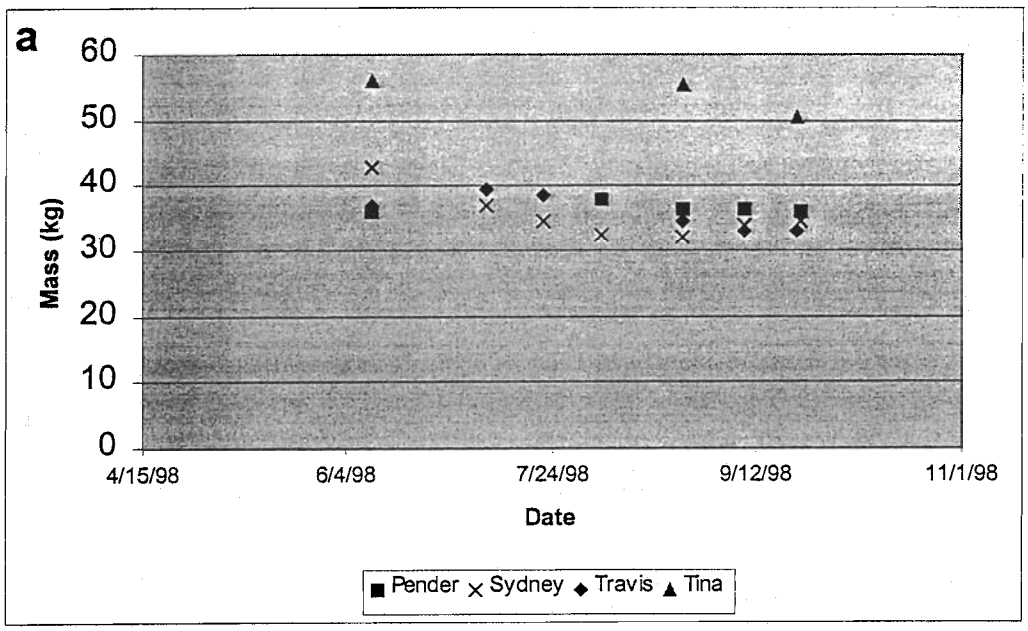
Table 11. Hematological values from harbor seal pups brought to the Alaska SeaLife Center for rehabilitation. Animals 98005 and 98009 did not recover.

		Hct *%*	*Hb*[a] *g/dL*	*MCHC* *g/dL*	*WBC* *10^9/L*	*Gran* *10^9/L*	*% Gran*	*L/M* *10^9/L*	*%L/M*	*PLT* *10^9/L*
YUKON	*Mean*	**40.8**	**13.7**	**33.9**	**12.7**	**6.9**	**70**	**2.9**	**30**	**679**
	S.D.	4.5	1.6	0.5	8.7	1.3	3	0.3	3	137
	Min	36.0	12.4	32.9	8.4	5.7	65	2.5	26	390
	Max	47.0	16.7	34.6	35.7	9.8	74		35	828
	n	9	9	9	8	9	9	9	9	9
DENALI	*Mean*	**54.8**	**18.7**	**33.9**	**11.0**	**8.0**	**72**	**3.0**	**28**	**602**
	S.D.	4.9	1.7	1.3	2.4	2.6	10	1.2	10	241
	Min	49.0	17.1	31.0	8.2	5.9	64	0.6	4	28
	Max	66.5	22.1	35.4	14.6	12.8	96	4.4	36	870
	n	9	9	9	9	9	9	9	9	9
ILIAMNA	*Mean*	**37.5**	**12.2**	**32.3**	**14.2**	**11.8**	**82**	**2.4**	**18**	**867**
	S.D.	4.9	1.4	1.0	5.0	4.5	10	1.6	10	171
	Min	34.0	10.8	31.0	8.6	6.2	70	0.6	3	620
	Max	51.0	15.7	33.9	25.4	19.8	97	5.6	30	1151
	n	11	11	11	11	11	11	11	11	11
98005	*Mean*	**57.8**	**20.4**	**35.3**	**13.6**	**10.9**	**78**	**2.7**	**23**	**305**
	Min	51.0	17.6	34.5	11.7	6.9	59	0.6	4	110
	Max	64.5	23.2	36.0	15.4	14.8	96	4.8	41	499
	n	2	2	2	2	2	2	2	2	2
98009		**58.5**	**19.3**	**36.6**	**10.5**	**9.9**	**94**	**0.6**	**6**	**43**

[a] ASLC veterinary values
Hct - hematocrit
Hb - hemoglobin
MCHC - mean corpuscular hemoglobin content
WBC - white blood cell count
%Gran - Percent granulocyte
L/M - lymphocyte to monocyte ratio
%L/M - percent lymphocyte/monocyte
PLT - platelets
Gran - granulocytes

Table 12. Composition of herring and pollock being fed to harbor seals in Feeding Trial 1.

		H_2O *%*	*Lipid* *% wet mass*	*Energy Density* *kJ/g wet mass*
Herring	*Mean*	**66.0**	**16.0**	**9.2**
	S.D.	1.5	1.5	0.8
	n	10	5	10
Pollock	*Mean*	**76.9**	**5.1**	**5.3**
	S.D.	1.3	1.1	0.4
	n	5	5	5



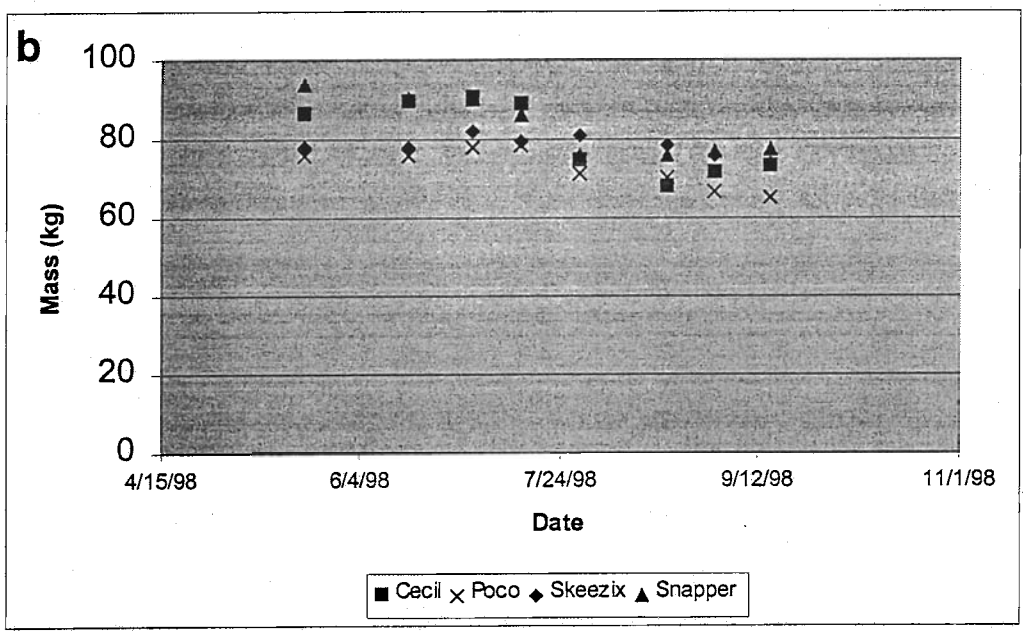


Figure 1. Change in mass of harbor seals acclimating to the Alaska SeaLife Center. ***a.*** Group A seals fed a herring diet during acclimation. ***b.*** Group B seals fed a mixed diet during acclimation.

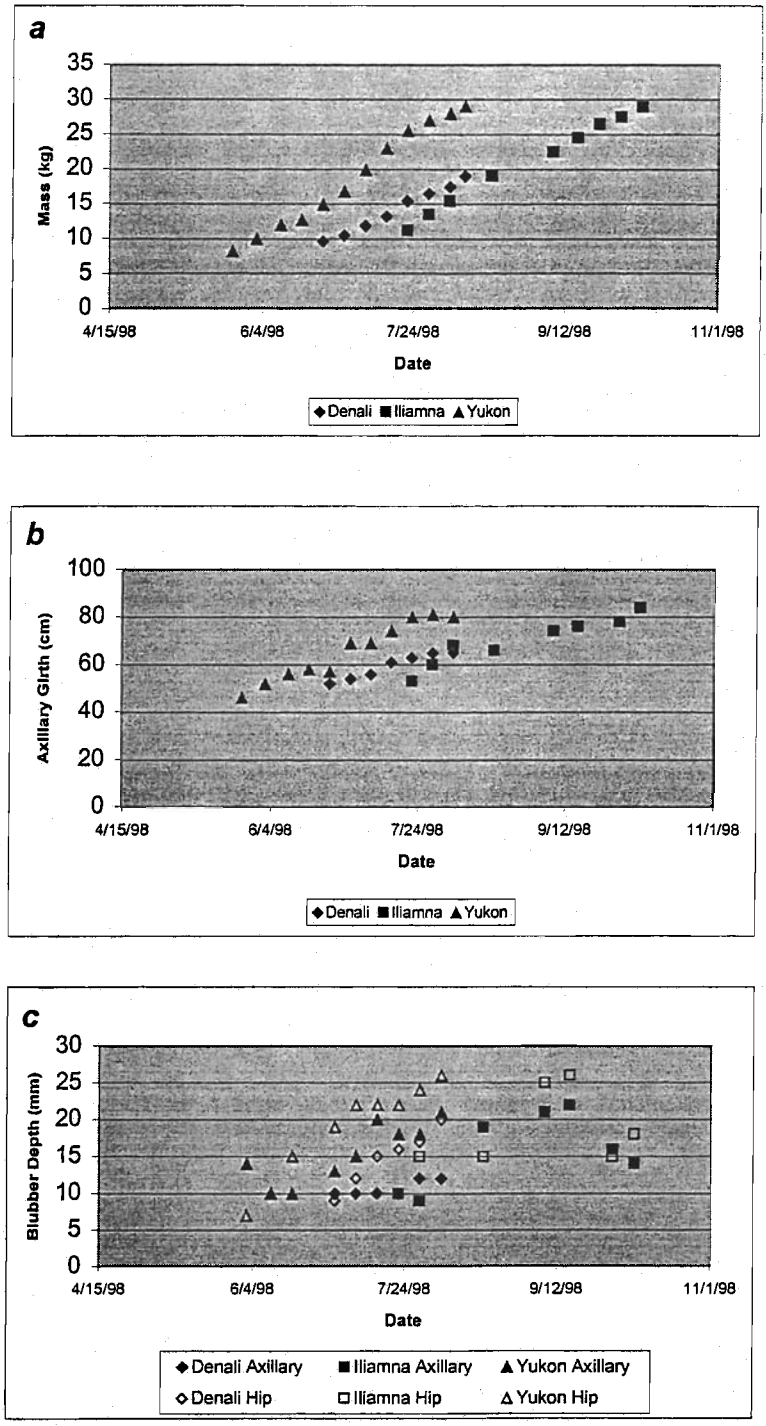


Figure 2. Morphometric measurements of wild harbor seals pups during rehabilitation at the Alaska SeaLife Center. ***a.*** mass, ***b.*** axillary girth, ***c.*** blubber depth.

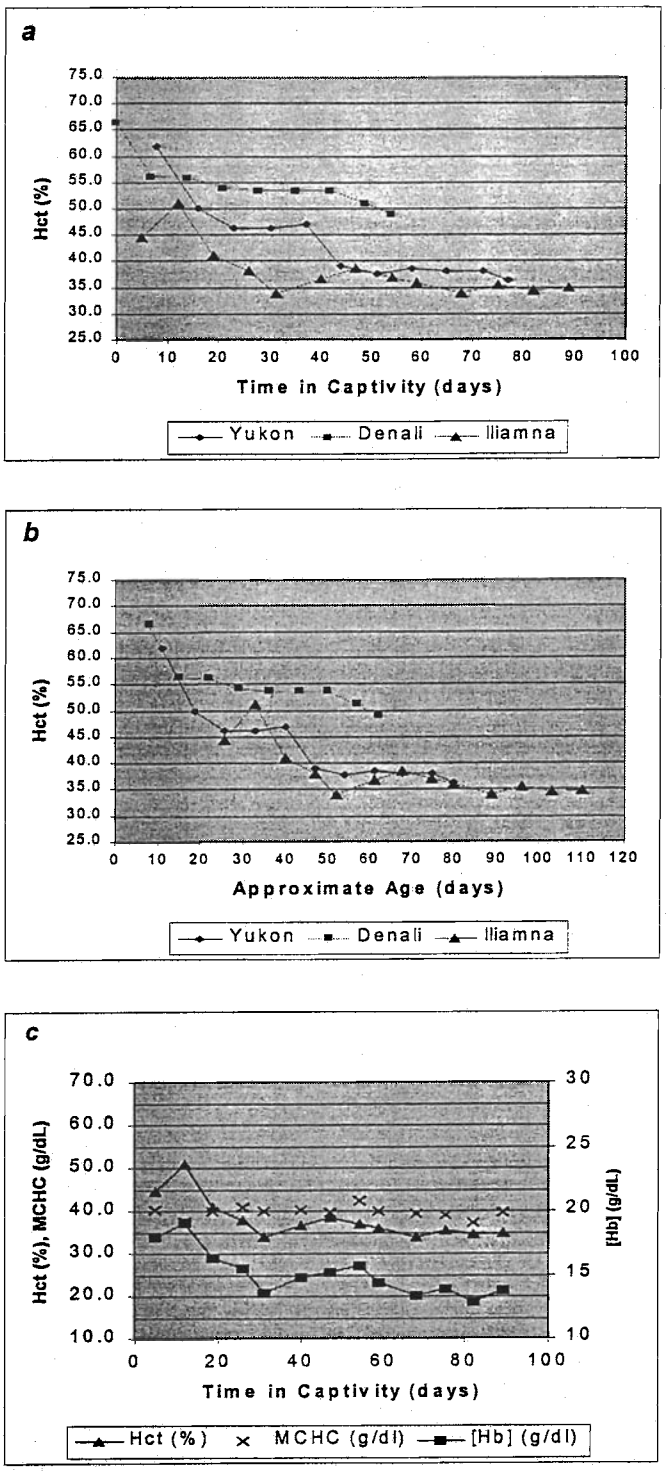


Figure 3. Hematocrit and hemoglobin values for wild harbor seal pups during rehabilitation at the Alaska SeaLife Center. *a.* hematocrit expressed as a function of time in captivity. *b.* hematocrit as a function of estimated age. *c.* relationship between hematocrit, hemoglobin concentration, and mean cell hemoglobin (MCHC) in one pup (Iliamna).

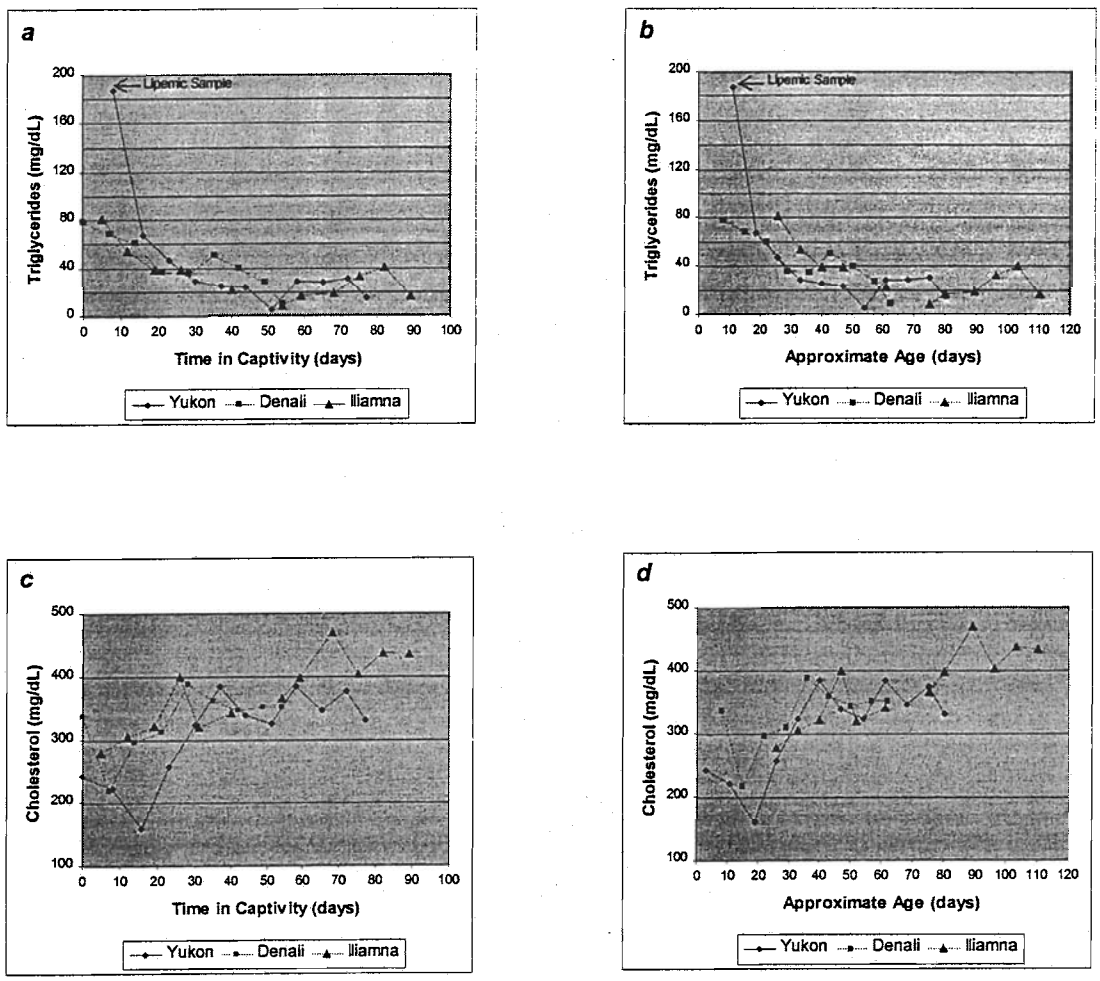


Figure 4. Changes in serum triglyceride and cholesterol levels in wild harbor seal pups during rehabilitation at the Alaska SeaLife Center. ***a.*** triglyceride levels expressed as a function of time in captivity, ***b.*** triglyceride levels expressed as a function of estimated age, ***c.*** cholesterol levels expressed as a function of time in captivity, ***d.*** cholesterol levels expressed as a function of estimated age.

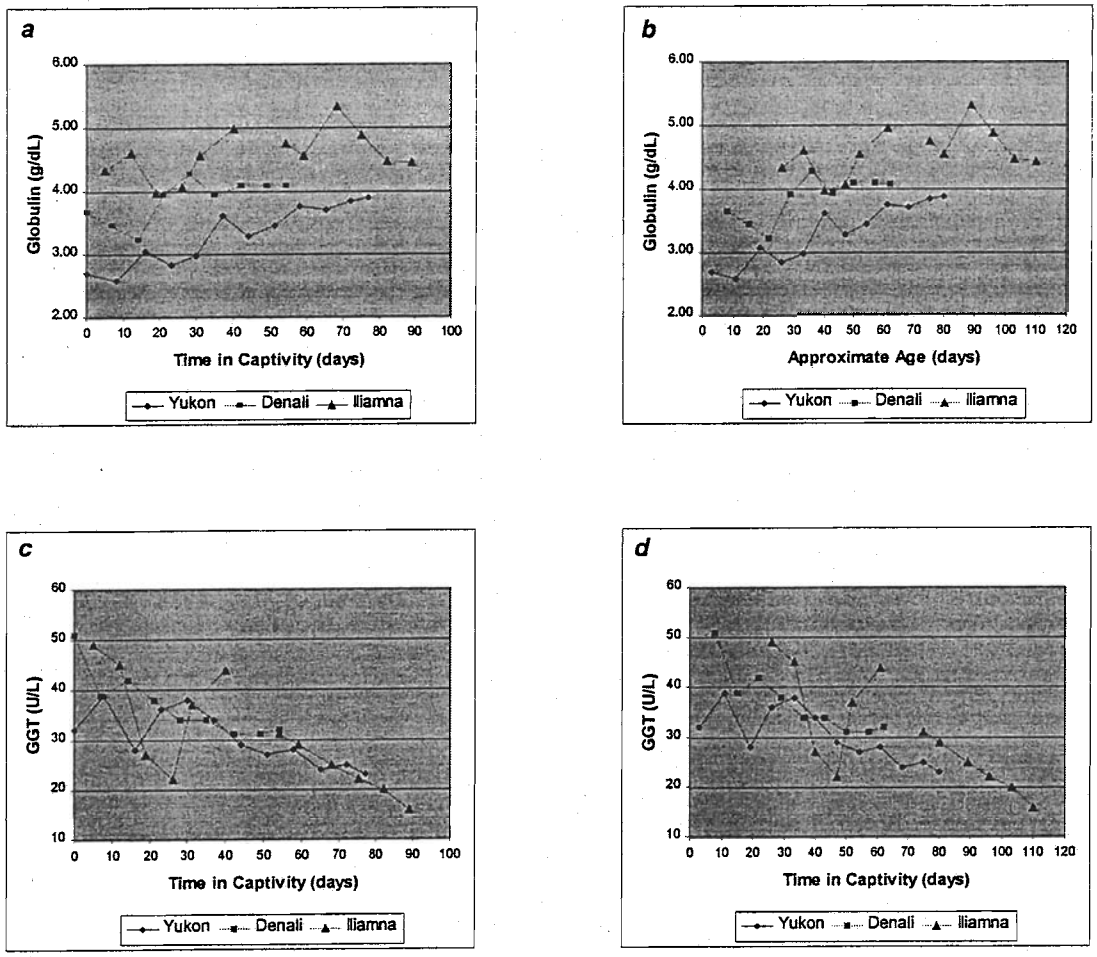


Figure 5. Changes in serum globulin and gammaglobulin transferase (GGT) levels in wild harbor seal pups during rehabilitation at the Alaska SeaLife Center. ***a.*** globulin levels expressed as a function of time in captivity, ***b.*** globulin levels expressed as a function of estimated age, ***c.*** GGT levels expressed as a function of time in captivity, ***d.*** GGT levels expressed as a function of approximated age.