Exxon Valdez Oil Spill
Restoration Project Annual Report

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

Restoration Project 99341
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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Recovery of Harbor Seals.
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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.* Baseline data for seals participating in this study, as well as data from the first year of rehabilitation monitoring were presented in the first annual report, Castellini, M.A., J.M. Castellini and S.J. Trumble. 1999. *Recovery of Harbor Seals. Phase II. Controlled Studies of Diet and Health.* Restoration Project 98341. The first of two years of feeding trials has now been completed and the preliminary results are the subject of this report. In addition, two seasons of data have been collected from rehabilitated harbor seal pups and are included in this report. Presentations arising from this work include: Castellini, J.M., S.D. Inglis, S.J. Trumble and M.A. Castellini, *Condition and health indices in rehabilitated harbor seal pups at the Alaska SeaLife Center,* presented at the 13th Marine Mammal Conference in Maui, Hawaii in November 1999; Castellini, M.A., J.M. Castellini, S. Trumble and T. Mau, *Do dietary lipid levels impact the body condition and health of seals?*, presented at the Experimental Biology 2000 Conference in San Diego, California in April 2000; Mau, T.L. and M.A. Castellini, *The effects of prey switching on erythrocyte fatty acids in the harbor seal,* presented at the Experimental Biology 2000 Conference in San Diego, California in April 2000. The second year of feeding trials and third year of rehabilitation studies are underway as Restoration Project 00341. 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 second year of this project, six harbor seals underwent a series of feeding trials during which they alternated between diets of herring or pollock. Two additional seals were maintained, as controls, on a mixed diet of herring and pollock. Morphometric measurements and blood sampling have occurred bi-weekly. Initial results indicate differences in body condition and blood parameters

between the two groups of seals being fed different diets. Whether these differences are a result of diet should become apparent as seals progress through the feeding trials. Morphometric and blood chemistry/hematology measurements of rehabilitated harbor seal pups reveal changes possibly indicative of 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, body composition, diet, *Exxon Valdez* oil spill, harbor seals, health, hematology, morphometrics, *Phoca vitulina*, pups, rehabilitation,

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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 are testing 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.

During the second year of this project, three of six diet manipulations were completed. Three seals began in September of 1998 with a diet of herring (Group A), three with a diet of pollock (Group B) and two were maintained, as controls, on a mixed diet of herring and pollock throughout the entire project. At the end of each four month period the diet of the seals was switched to the alternate prey item. By the end of two years, each seal will have been fed each prey item during each season (September – January, January – May, May – September). Morphometric and blood data are collected bi-weekly from each animal. 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. Total body water, a proxy for lean mass, is measured at the point at which diets are switched. Temporal changes in body composition are being monitored. Cross-over repeated measures analysis will be applied to the data at the end of the feeding trials to determine the specific effects of diet.

The feeding regime requires that seals be fed a maintenance diet, although some seasonal fluctuation in mass was expected. Preliminary results suggest that changes in mass and percent body fat are not a straightforward result of dietary regime and that there is a strong seasonal component. During fall (first trial, September – January) seals on all diets gained mass, while only those eating pollock or a mixed diet increased percent body fat. The same seals gained much more mass while eating herring and lost mass while eating pollock in the third trial (May – September). Both groups appear to have experienced a decrease in percent body fat during the summer. Results varied seasonally for seals eating a mixed diet for all three trials. We must

stress the preliminary nature of these results since the study has not ended and other variables such as age might also affect these data.

Several blood enzymes and metabolites appear to change in response to diet, some with a potential seasonal component. These include, but may not be limited to, alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transpeptidase and creatinine. Most hematological variables were fairly constant, although hematocrit and hemoglobin values appear to be influenced by season, with no apparent effect of diet. Assimilation experiments are still in the early phase of analysis, although preliminary results suggest increased mean retention time on a pollock diet. Statistically, the effects of diet, season and age cannot be separated until the end of the six trials, so while these data are interesting, they are preliminary and, by no means conclusive. Samples have been collected for other biomarker analyses, which are currently underway.

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. Compositional analysis revealed that herring used for this study had substantially higher lipid content (herring: 16.8 ± 2.2 %, wet mass, $n = 85$; pollock: 4.9 ± 1.1 %, wet mass, $n = 25$) and energy density (herring: 9.5 ± 0.9 kJ/g wet mass, $n = 40$; pollock: 5.1 ± 0.5 kJ/g wet mass, $n = 25$) than pollock. There has been no change in herring or pollock composition during 12 months of frozen storage, however analysis will continue to assess food quality changes during storage or as new batches of fish are introduced.

Seven harbor seal pups were successfully rehabilitated at the Alaska SeaLife Center during 1998 and 1999. Each of the pups was monitored carefully including weekly morphometric measurements and blood samples. Blood and morphometric measurements were consistent with recovery, although all the pups were significantly underweight for several weeks past weaning and hematocrit decreased substantially in five seals. 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. Pups had low cholesterol values when formula-fed, and decreases in gamma-glutamyl transpeptidase and triglyceride levels during their recovery. Percent granulocyte levels were good indicators of exposure to infection. Continuing these studies on injured or abandoned harbor seals through rehabilitation will enable more specific conclusions about the differences between healthy and unhealthy animals.

This project will continue during 2000, with seals progressing through the second year of feeding trials. 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. 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 an 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 harbor seals 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. 2000). 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 and completed the first year of feeding trials. Data include bi-weekly measurement of mass, length and girths, blubber depths (by ultrasound), clinical blood chemistry and hematology. The baseline data were presented in the previous annual report (Castellini, et al. 1999). Results from the first year of feeding trails are the subject of this report.

Preliminary results suggest that changes in mass and percent body fat are not a straightforward result of dietary regime and that there is a strong seasonal component. During fall (first trial, September – January) seals on all diets gained mass, while only those eating pollock or a mixed diet increased percent body fat. The same seals gained much more mass while eating herring and lost mass while eating pollock in the third trial (May – September). Both groups appear to have experienced a decrease in percent body fat during the summer. Results varied seasonally for seals eating a mixed diet for all three trials. We must stress the preliminary nature of these results since the study has not ended and other variables such as age might affect these data.

Blood chemistry and hematological values were within the ranges expected for adult harbor seals. Several blood enzymes and metabolites appear to change in response to diet, some with a potential seasonal component. These include, but may not be limited to, alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transpeptidase and creatinine. Most hematological variables were fairly constant, although hematocrit and hemoglobin values appear to be influenced by season, with no apparent effect of diet. Assimilation experiments are still in the early phase of analysis, although preliminary results suggest increased mean retention time on a pollock diet. Statistically, the effects of diet, season and age cannot be separated until the end of the six trials, so while these data are interesting, they are preliminary and, by no means conclusive. Samples have been collected for other biomarker analyses, which are currently underway.

Seven harbor seal pups were successfully rehabilitated at the Alaska SeaLife Center during 1998 and 1999. Two additional pups were more seriously injured and did not recover. Each of the pups was monitored carefully including weekly morphometric measurements and blood samples. Blood and morphometric measurements were consistent with recovery, although all the pups were significantly underweight for several weeks past weaning and there was a substantial decrease in hematocrit in five seals. 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. Pups had low cholesterol values when formula-fed, and decreases in gamma-glutamyl transpeptidase and triglyceride levels during their recovery. Percent granulocyte levels were good indicators of exposure to infection. Continuing these studies on injured or abandoned harbor seals through rehabilitation will enable more specific conclusions about the differences between healthy and unhealthy animals.

This project will continue during 2000, with seals progressing through the second year of feeding trials. 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. 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 an 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.

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

Feeding Trials

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. Blood was sampled from the intervertebral extradural vein (Geraci and Smith 1975) using 2.5 – 3.5" 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.

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 and 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).

Assimilation Efficiency.-- Once the seals had been established on a specific diet during each feeding trial, they participated in two feeding experiments to quantify assimilation efficiency and metabolizable energy (ME). Each seal was fed a diet of the specific prey item(s), keeping other variables such as meal size and feeding frequency constant. In the first experimental regime, feeding frequency was four times a day. In the second regime feeding frequency was once a day. The design and interpretation of feeding experiments takes into account the potential effects of seasonal variation in AE and ME and this is discussed below in the feeding trial design using staggered schedules.

For all animals, dietary prey and fecal samples were quantified, freeze-dried and analyzed for energy (kJ/g), nitrogen, total lipid, and ash. In order to determine the passage of digesta (mean retention time), time of feeding and sampling were recorded. Bomb calorimetry is being used to determine energy density, nitrogen (protein) concentration is being determined using a carbon–nitrogen auto-analyzer, total lipid by Soxhlet extraction and ash by muffle furnace combustion.

To determine digestibility of food absorbed in the digestive tract of seals, inert markers such as chromic oxide and cobalt-EDTA were added to the diets and subsequently assayed in fecal samples. These inert markers, along with naturally occurring manganese (Mn^{2+}) levels, are used to determine assimilation efficiency and compared with the digestibility results of a total

balance trial. These markers have been used in pinniped AE studies (Fadely et al. 1990, Fadely et al. 1994) where dry matter digestibility has been calculated. Chromium, cobalt and Mn^{2+} concentrations are assessed using atomic absorption spectrophotometry (Fadely et al, 1990). The tissue samples are extracted in Seward and analyzed by staff in Fairbanks.

Total Body Water.-- Body composition was assessed by whole body bio-impedance (BIA) (Gales et al. 1994) and labeled water techniques (Bowen and Iverson 1998). Dilution of labeled water allows calculation of the change in the amount of body fat and lean tissue relative to the total change in body mass. A precisely measured mass of heavy water (deuterated water, D_2O) is injected into the seal and allowed to equilibrate with the total body water for two hours. A blood sample is drawn and the concentration of D_2O is measured. The amount of total water in the animal is calculated, and from that, the amount of fat and lean tissue is derived. This is a routine procedure for body water and body composition determination and we have used it previously in pinnipeds (Davis et al. 1996). Samples were analyzed at *Metabolic Solutions*, an analytical facility in New Hampshire.

Statistical Analysis.-- Data are extremely preliminary at this point and the analyses are limited to descriptive temporal relationships (trends). Future analyses will include time series analysis of data and comparisons of feeding trials using cross-over repeated measures methods. This 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 1998 - Jan 1999	A	B	Molting
Jan - May 1999	B	A	Spring
May - Sept 1999	A	B	Breeding
Sept 1999 – Jan 2000	B	A	Molting
Jan – May 2000	A	B	Spring
May – Sept 2000	B	A	Breeding

Sampling began in mid-September 1998 and diet switches occur mid-month, every 4 months. Group A seals include Pender, Sydney and Travis, while Group B seals include Cecil, Poco and Skeezix. Two additional seals (Tina and Snapper) 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 A 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.

Proximate Analysis of Food.-- Individual batches of herring and pollock were subsampled (n = 10 for each) periodically (at least once during each feeding trial) for proximate

analysis. The samples were processed with a food grinder and food processor. 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 produce comparable results. In this case, whole fish were freeze-dried to constant mass then ground. Energy density was determined according to methods described above. Soxhlet analysis awaits specialized equipment that will allow analysis of the powdered fish.

Rehabilitated Harbor Seals

Animal Handling and Sampling.-- Animal handling and morphometric sampling was the same as for adults (see above) except for a few minor variations. Since all the rehabilitated seals were pups, they were manually restrained without the use of a restraint board. The needles were smaller (1 – 2.5", 18 – 20G) and the total volume of blood drawn varied between 20 – 30 mls. Animals were sampled weekly. The condition of the seals was assessed upon admission and subsequently throughout rehabilitation by P. Tuomi, DVM. Sampling at the time of admission preceded formula feeding.

Blood Chemistry and Hematology.-- Blood processing was the same as for adults, although some variables were not measured. Analyses included standard chemistry panel, standard hematological panel, ESR (when blood volume permitted), and manual measurements of hematocrit, red blood cell count and hemoglobin.

Plasma Volume.-- Plasma volume was measured in three pups in 1999 according to the methods of Zweens and Franena (1981) using the plasma dye, Evans Blue. After the initial blood draw, Evans Blue was injected to a predicted concentration of 3mg/ml plasma. Samples were drawn into heparinized tubes 10 and 20 minutes after injection and plasma was separated. The calculated concentration of Evans Blue at the time of injection was used to calculate plasma volume. Total blood volume and the volume of the red blood cell pool were calculated using hematocrit values obtained from the samples. These measurements were repeated several weeks later, allowing determination of plasma volume before and after hematocrit had begun to decrease.

Statistics.-- Statistical analyses were conducted using Systat 8.0. They included regression and correlation analyses. Temporal changes were determined using non-parametric statistics, in particular Friedman analysis of variance.

RESULTS

Feeding Trials

Mass and Morphometric Measurements.--Analyses have been completed for the first year of the feeding trials (Trials 1 – 3). Results show that the changes in mass or percent body fat are not a straightforward result of dietary regime and that there is a strong seasonal component.

During the molt and post-molt season (Trial 1, September 1998 – January 1999), seals on all three diets (herring, pollock and mixed diets) gained mass (Fig. 1). On both the mixed and pollock diets, the seals also experienced an increase in percent body fat, while seals eating herring had a small, possibly insignificant decrease in percent body fat (-1%). It must be re-emphasized that we are not able to run the full statistical treatment on these data until the entire experiment is finished. The data presented here are simply trends over the first year. From January – May 1999 seals eating pollock lost mass while those eating herring or a mixed diet gained mass (Fig 2). However, once again, percent body fat may have actually decreased in seals eating herring. The animals on other diets exhibited either no changes or increases in percent body fat. This leads to an interesting situation in which the animals eating pollock lost total mass while increasing percent body fat. From June – September 1999 (breeding and post-breeding in the wild) seals on the herring diet gained mass but had no change in percent body fat (Fig 3). The seals eating pollock lost mass and experienced decreased percent body fat while the seals eating a mixed diet gained mass but had decreased percent body fat.

Blood chemistry and hematology.-- Data presented in this report represent 312 blood samples (n = 8 seals, approximately 39 samples/seal) taken from June 1998 to November 1999. Mean blood chemistry values for individual seals during periods of herring, pollock and mixed diets are presented in Tables 1 and 2. Due to ongoing blood collection, statistics were limited to determining significance among herring and pollock blood chemistry values for individual seals. While there are few significant differences between overall mean pollock and herring blood chemistry values for individual harbor seals, trends would indicate potential dietary (eg. Figs. 4, 5) or seasonal (eg. Figs. 6, 7) influences. Other variables appear unaffected by either season or diet (eg. Fig. 8, 9). Variables that are potentially affected by diet include creatinine, BUN/creatinine, GGT, AST, ALT and possibly cholesterol and alkaline phosphatase. Variables that are potentially affected by season include chloride, hemoglobin, hematocrit, globulin, albumin, MCHC and, possibly, triglyceride and alkaline phosphatase. Blood variables that do not appear to be affected by diet or season include Na^+, K^+, amylase, protein, total bilirubin and, possibly, cholesterol, calcium and phosphorus. Mean hematology values for herring and pollock feeding trials for individual seals are presented in Table 3. Mean erythrocyte sedimentation rate (ESR) for harbor seals at the ASLC appears to be a function of both age and diet (Fig. 10). Seals fed pollock had higher ESR values than when fed herring. Statistically, the effects of diet, season and age cannot be separated until the end of the six trials, so while these data are suggestive and interesting, they are preliminary. Data that have not been included here are still in the process of being analyzed and will be included in subsequent reports.

Assimilation Efficiency.-- Assimilation experiments are still in the early phase of analysis, although preliminary results suggest increased mean retention time on a pollock diet (Fig. 11). Analyses are ongoing and results will be presented in subsequent reports.

Rehabilitated Harbor Seals

Seven harbor seal pups were successfully rehabilitated at the ASLC during 1998 and 1999 (three in 1998 and four in 1999). 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 4.

Mass and Morphometric Measurements.-- Rehabilitated seals exhibited positive growth according to all parameters including mass, standard length, axillary girth and axillary blubber depth (Fig. 12). Growth was fairly steady, although each animal exhibited periods of relatively

faster or slower growth. There were no differences in growth patterns associated with the year of rehabilitation, in spite of differences in formula and weaning strategy between years.

Hematology.-- Several hematological parameters changed during rehabilitation and development. These included Hct, Hb, WBC, granulocytes and less obvious changes in numbers and proportions of leukocytes and monocytes (Tables 5 and 6). There were gaps in measurements of differential cell counts in 1998, so statistical comparisons were limited to data from 1999 (Table 6). From 2 – 12 weeks of age, pups exhibited decreases in white blood cell counts, granulocytes and, to a lesser degree, leukocyctes and monocytes. This small sample of 4 seals included one that developed a severe gastrointestinal infection within a week of admission to the ASLC. This animal had high WBC (29.1 x 10^9/L) and granulocyte values (20.1 x 10^9/L) at two weeks of age, driving the mean and standard deviations for those values quite high.

While small size still restricts statistical comparison of clinically sick or injured pups from abandoned pups (underweight and dehydrated – otherwise, apparently healthy), percentage of granulocytes appears to be a good indicator of exposure to infection. In most rehabilitated pups this value ranged from about 60 – 70% (Table 5) and the mean value for wild harbor seal pups was 59 ± 10 (Small et al. 1999). In pups exposed to infection, percentage of granulocytes ranged from 78 – 97%, including two animals which did not survive (Fig. 13).

Hematocrit decreased significantly during the time the pups were at the ASLC. This decrease occurred in two phases (Fig 14). During the first 2 – 3 weeks of age, hematocrit dropped dramatically. This was followed by stabilization and then, further decrease in hematocrit to values as low as 34 – 40%. Statistical analysis confirms that the decreases that occurred between 6 – 12 weeks of age were significant (Table 6). Hemoglobin concentrations tracked hematocrit changes, while MCHC remained relatively constant (Fig. 15). The initial drop in hematocrit is typical of neonatal development and further results will focus on the second phase of hematocrit changes. Sudden drops in hematocrit appear to be associated with growth spurts, and the relationship between the rate of hematocrit change and the rate of growth has a significant negative slope (Fig. 16). This relationship is somewhat confounded by the fact that increased growth rate did not necessarily result in decreasing hematocrit, although rapid decreases in hematocrit were always associated with rapid growth. In addition, there may be some lag between increased growth and decreasing hematocrit. The decrease in hematocrit was typically associated with initial increases followed by sudden decreases in MCV (Fig. 17). Plasma volume measurements indicate that plasma volume increased as mass increased. However, after about 50 – 60 days old, the volume of the pups' red blood cell pool did not increase in spite of increases in plasma volume and mass (Fig. 18).

Blood chemistry.-- Mean blood chemistry values that varied over time are presented in Tables 7 and 8. Of 23 variables measured, 13 exhibited some temporal effect. These included liver enzymes (ALT, AST, GGT), globulin, protein, cholesterol, creatinine, triglycerides, bilirubin, phosphorous and chloride. Cholesterol values were similar to values obtained from wild harbor seal pups from PWS, but dropped dramatically during formula-feeding (Fig 19). They returned to normal values once the pups had been weaned. Triglyceride values were high and variable upon admission. They decreased progressively, finally stabilizing at about 9 – 12 weeks old. Data from non-survivors illustrate the fact that many blood chemistry variables are seriously altered in animals that are compromised (Fig. 19, Castellini, et al. 1999). PV98005 had normal cholestorol and low triglyceride values (pre-weaning at time of sample), while PV98009 had high cholesterol and exceedingly high triglyceride values right before death.

Liver enzymes were quite variable. Serum ALT was very high and variable upon admission, decreased rapidly and increased after weaning, while AST was lowest during formula-feeding, increasing thereafter (Table 7). Serum GGT values were extremely high and variable upon admission and decreased progressively during recovery and growth (Fig 20). GGT values during the first several weeks of rehabilitation and in non-survivors appeared higher than values obtained from wild harbor seal pups from PWS. Creatinine values were lower than values obtained for similarly aged wild pups in PWS. A plot of creatinine as a function of mass produced a U-shaped curve (Fig 21). Creatinine, a metabolite of muscle was negatively correlated with mass during the first several weeks of life. Creatinine became positively correlated with mass at about the same time that hematocrit values were decreasing.

These results are preliminary. Further statistical analyses will include, but not be limited to, comparisons of year and, hopefully, comparison of sick and injured pups to those which were abandoned (underweight and dehydrated). We anticipate an increased sample size during the third and final year of this project, which will strengthen statistical analyses. Further results will be included in subsequent reports.

Proximate Analysis of Food

Compositional analysis revealed that herring used for this study had substantially higher lipid content (herring: 16.8 ± 2.2 %, wet mass, $n = 85$; pollock: 4.9 ± 1.1 %, wet mass, $n = 25$) and energy density (herring: 9.5 ± 0.9 kJ/g wet mass, $n = 40$; pollock: 5.1 ± 0.5 kJ/g wet mass, $n = 25$) than pollock. Storage time had no effect on water, lipid or energy content of herring or pollock. (Fig 22).

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, potentially 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 (Castellini et al. 1999) and the first of two years of controlled dietary regimes has been concluded. Data pertaining to

this first year of feeding trials are the subject of this report. They are, however, preliminary. Given the cross-over nature of this experimental design, statistical analysis of the specific effects of diet cannot be determined at this time. There are trends apparent that suggest the influence of diet or season. Some of these trends are described in this report and will be assessed more rigorously at the end of the experimental protocol.

An integral component of this feeding study is an assessment of how the different diets affect body composition. By body composition, we mean the change in the amount of body fat and lean tissue relative to the total change in body mass through a trial. This is determined by the use of labeled water techniques as described in the methods section. Some seasonal fluctuation in mass was expected (Ashwell-Erickson and Elsner 1981, Rosen and Renouf 1995) and did, in fact, occur (Figs. 1 – 3). Preliminary results suggest that changes in mass and percent body fat are not a straightforward result of dietary regime and that there is a strong seasonal component. During fall (first trial, September – January) seals on all diets gained mass, while only those eating pollock or a mixed diet increased percent body fat. The same seals gained much more mass while eating herring and lost mass while eating pollock in the third trial (May – September). Both groups appear to have experienced a decrease in percent body fat during the summer. Results varied seasonally for seals eating a mixed diet for all three trials. It is apparent that feeding trials in seals must correct for seasonality. A seal fed pollock in January does not have the same physiological result as the same seal fed pollock in August. More importantly, it is not appropriate to compare results from seals fed herring in January with those of seals fed pollock in August. An interesting situation arose in the second feeding trail (February – May) in which seals eating pollock experienced a decrease in mass and a simultaneous increase in percent body fat (Fig. 2). This would suggest that they lost lean tissue during this trial. Very few studies have examined how seals control lean tissue (protein) metabolism. However, the experiments by Schell (Restoration Project 00371) are examining amino acid turnover in these same seals and may shed some light on how they regulate protein turnover. There were also instances when seals gained mass while percent body fat remained unchanged or decreased, suggesting overall increases in lean tissue (Fig. 1, 3) and a possible growth phase.

Blood chemistry and hematological values appear to be within the ranges expected for adult harbor seals (Bossart and Dierauf 1990) (Tables 1 - 3). While there are few significant differences between overall mean pollock and herring blood chemistry values for individual harbor seals, trends would indicate potential dietary (eg. Figs. 4, 5) or seasonal (eg. Figs. 6, 7) influences. Other variables appear unaffected by either season or diet (eg. Fig. 8, 9). Variables that are potentially affected by diet include creatinine, BUN/creatinine, GGT, AST, ALT and possibly cholesterol and alkaline phosphatase. Variables that are potentially affected by season include chloride, hemoglobin, hematocrit, globulin, albumin, MCHC and, possibly, triglyceride and alkaline phosphatase. Many of these blood variables are associated with protein and lipid metabolism (DelGuidice et al. 1987, Schweigert 1993). Interestingly, some of these variables were different between the two groups during acclimation (Castellini et al. 1999) and these relationships will be considered when the final statistics are compiled. Blood variables that do not appear to be affected by diet or season include Na^+, K^+, amylase, protein, total bilirubin and, possibly, cholesterol, calcium and phosphorus. Mean erythrocyte sedimentation rate (ESR) for harbor seals at the ASLC appears to be a function of both age and diet (Fig. 10). Seals fed pollock had higher ESR values than when fed herring. Statistically, the effects of diet, season and age cannot be separated until the end of the six trials, so while these data are suggestive and interesting, they are preliminary. The relationships between these blood variables and water

balance, molting energetics, growth, and fat and protein metabolism will be pursued more fully once the study is concluded and we can more clearly assess the specific effects of diet and season. Analysis of additional health biomarkers is ongoing and results will be presented in subsequent 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 not only body condition (morphometrics) and composition, but also assimilation efficiency and metabolizable energy associated with different diets. Assimilation experiments are still in the early phase of analysis, although preliminary results suggest increased mean retention time on a pollock diet (Fig. 11). Analyses are ongoing and results will be presented in subsequent reports. Proximate analysis of herring and pollock revealed the expected differences, with herring having higher lipid content and energy density than pollock (Fig. 22). Gross composition of the fish has not changed during frozen storage. Proximate analysis of food is continuing so that variability between batches of fish can be monitored. Analysis of nitrogen content will also be included in future reports.

Seven harbor seal pups were successfully rehabilitated at the ASLC during 1998 and 1999. Two additional pups were more seriously injured and did not recover. The condition of the seals upon admission to the ASLC is presented in Table 4. Morphometric measurements were consistent with recovery, although all the pups were significantly underweight for several weeks past weaning (Fig. 12). Two to six week old wild pups sampled in PWS from 1997-99 had masses ranging from 18 – 37 kg (Small et al. 1999). Similarly aged rehabilitated pups weighed from 8.5 – 17 kg, and most were still less than 25 kg at 11 weeks of age. The effect of this delayed growth on other aspects of development is unknown. There were no differences in growth patterns associated with the year of rehabilitation, in spite of differences in formula and weaning strategy between years (Table 9).

Several hematological values changed during the course of rehabilitation. These included Hct, Hb (Table 5), WBC and granulocytes (Table 6). These values may be influenced by a number of factors including normal development, early weaning, captivity, or injury and disease. While the small sample size restricts statistical comparison of clinically sick or injured pups from abandoned pups (underweight and dehydrated – otherwise, apparently healthy) the percentage of granulocytes appears to be a good indicator of exposure to infection. In the pups exposed to infection this value ranged from 78 – 97%, compared to 60 –70 % for non-exposed pups (Fig 13) and 59 ± 10 for wild pups (Small et al. 1999). In the group of seals exposed to infection, the pups with the lowest peak in percent granulocytes (S.K.) received treatment immediately, while the others were exposed for an undetermined length of time. This variable may be particularly

valuable for monitoring seals in the wild since it does not appear to be correlated to age in young pups and elevated values may persist for some time after the infection has resolved (see Iliamna, Fig. 13). This would provide a bigger window for detecting such exposure.

Hematocrit decreased significantly during the time the pups were at the ASLC. This decrease occurred in two phases (Fig. 14). The initial sharp decrease is probably a developmental change typical of neonatal seals (Hall 1998). After a brief period of stabilization, however, hematocrit continued to decline significantly to values as low as 34 – 40%. While low hematocrit has been recorded in other studies of captive seals (Nielsen 1995), these very low values are of concern. Hemoglobin concentrations tracked hematocrit values, while MCHC remained relatively constant (Fig. 15). This is consistent with the fact that supplementary iron failed to reverse the hematocrit trend. There is evidence that the decreases in hematocrit are associated with increases in growth rate (Fig. 16) as well as shifts in MCV (Fig. 17). Measurements of plasma volume show that plasma volume increased as mass increased. However, after the pups reached 50 – 60 days old the volume of the red blood cell pool did not increase with increasing mass (Fig. 18). Taken together, these data suggest a shift in red blood cell population which should be further explored. They also indicate a possible competition for resources (particularly protein or iron) between the production of muscle mass and the need to produce red blood cells. Interestingly, it is during this later phase of development that creatinine values become strongly correlated to mass, suggesting increased muscle growth (Fig. 21). While the data are extremely limited at this time, it is possible that hematocrit is affected by delayed growth in these pups. These relationships bear further investigation.

A number of serum chemistry values varied throughout the course of rehabilitation. Of 23 variables measured, 13 exhibited some temporal effect. These included liver enzymes (ALT, AST, GGT), globulin, protein, cholesterol, creatinine, triglyceride, bilirubin, phosphorous and chloride. As with hematological values, these may be affected by normal development, early weaning, captivity and injury or disease. Low cholesterol values appear to be a result of formula-feeding (Fig. 19). Values upon admission (and before introduction to supplemented formula) were similar to those obtained from wild seals from PWS. They decreased significantly while the seals were fed formula and increased back to original levels once the pups were weaned. Interestingly, the low values were not affected by the year of sampling even though formula in 1998 was supplemented with vegetable oil, while the formula in 1999 was supplemented with salmon oil. Triglyceride values were high and variable upon admission, finally stabilizing at fairly low values at about 9 – 12 weeks old. Data from non-survivors illustrates the fact that many blood chemistry variables are seriously altered in animals that are compromised (Fig. 19, Castellini et al. 1999). PV98005 had normal cholesterol values (formula-fed), but quite low triglyceride levels considering his age (<3 weeks old), while PV98009 had high cholesterol values and exceedingly high triglyceride values right before death. These are only two of a number of variables which were outside normal ranges, as discussed in the previous annual report (Castellini et al. 1999). Liver enzymes were quite variable. In particular, serum GGT values were extremely high and variable upon admission and decreased progressively throughout rehabilitation (Fig. 20). GGT values during the first several weeks of rehabilitation and in non-survivors appeared higher than values obtained from similarly-aged wild harbor seal pups from PWS. This bears further investigation, since high GGT values have been observed in other injured species in PWS (Near-Shore Vertebrate Project \\025). It is unclear whether stress can affect GGT and the stresses on the pups in this study were from a variety of sources, including injury, infection, undernourishment, early weaning and stress associated with initial captivity. As

stated earlier, creatinine became positively correlated with mass at about the same time that hematocrit was decreasing. Prior to that the relationship between creatinine and mass had a significantly negative slope (Fig. 21). Creatinine is a non-protein end product of creatine metabolism and may be directly proportional to an animal's body mass (muscle mass) and glomerular filtration rate (Hayward et al. 1995).

These results are preliminary. Further statistical analysis will include, but not be limited to, comparisons of year and, hopefully, comparisons of sick and injured pups to those which were abandoned (underweight and dehydrated). We anticipate an increased sample size during the third and final year of this project, which will strengthen statistical analyses. Further results will be included in subsequent reports.

CONCLUSIONS

The first year of feeding trials in this two year experiment has been concluded. Analyses are preliminary, and given the cross-over nature of the experimental design, conclusions are inappropriate at this time. However, a number of trends have been identified which will be further explored once the full experimental protocol has been completed.

Changes in mass and body composition are not a straigthforward result of diet and appear to have a strong seasonal component. The relative importance of diet and season should become more apparent as the study progresses. Trends indicate a potential dietary and/or seasonal effect on a number of blood chemistry and hematological variables. These trends will continue to be assessed as the study continues. Assimilation efficiency experiments are still in the early phases of analysis, although preliminary results suggest increased mean retention time in seals eating pollock.

Data from seven rehabilitated harbor seal pups were consistent with recovery, although all seals experienced delayed growth and decreasing hematocrit levels. Whether these two observations are related requires further investigation. Data suggest that red cell production may have to compete with muscle growth in these seals. There were several long-term changes in blood chemistry and hematological values. A number of factors may contribute to the variability, including development, early weaning, captivity and disease or injury. Increased sample size should allow more definitive conclusions about the factors affecting some of the variables. It is apparent, however, that percentage of granulocytes is a good indicator of exposure to infection and that formula-feeding results in reduced cholesterol levels.

The final phase of this experiment is ongoing and will continue through September 2001. Data collection will continue to include bi-weekly blood sampling and morphometric measurements as well as experiments to assess body composition and assimilation efficiency. These measurements will conclude at the end of September 2000, followed by sample and data analysis. In addition, harbor seals admitted to the ASLC for rehabilitation during 2000 will continue to be monitored. 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 individuals. These data are now being extended to include shifts in diet over a variety of seasonal conditions, answering important questions about the nutritional physiology of harbor seals.

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Table 1. Overall serum chemistry values for captive harbor seals at the Alaska SeaLife Center 1998-1999. Group B: approximately 22 samples during pollock regimes and 17 samples during herring regimes; approximately 39 total samples/seal. H = herring diet, P = pollock diet and Mixed = pollock/herring diet. Values represent Mean (± S.D.). A t-test was performed to detect differences between diet values for each seal. Bold indicates a statistical significance (0.05).

	Skeezix		***Cecil***		***Poco***		***Snapper***
Variable	***H***	***P***	***H***	***P***	***H***	***P***	***MIXED***
Sodium (mmol/L)	157.0 (4.0)	157.8 (3.7)	159.5 (5.8)	158.1 (4.0)	160.1 (2.6)	157.4 (4.2)	154.8 (6.0)
Potassium (mmol/L)	3.8 (0.4)	3.8 (0.4)	3.9 (0.4)	3.8 (0.4)	3.7 (0.4)	3.6 (0.3)	3.7 (0.3)
Chloride (mmol/L)	112.8 (1.9)	112.1 (1.8)	111.2 (1.7)	112.1 (5.8)	113.3 (1.2)	114.2 (7.3)	112.1 (1.1)
Glucose (mg/dL)	177.6 (22.1)	192.3 (35.5)	150.4 (14.5)	149.2 (12.1)	165.1 (16.4)	181.2 (40.4)	163.5 (12.4)
Phosphorus (mg/dL)	5.15 (1.7)	5.8 (0.9)	5.7 (0.6)	6.0 (0.8)	5.0 (0.8)	4.8 (0.7)	5.3 (1.0)
Calcium (mg/dL)	9.1 (0.3)	9.2 (0.3)	8.8 (0.3)	8.8 (0.3)	9.0 (0.2)	9.1 (0.3)	8.8 (0.2)
BUN (mg/dL)	36.8 (4.3)	36.8 (6.0)	32.7 (5.9)	37.2 (7.4)	34.3 (3.9)	34.4 (5.4)	33.9 (4.7)
Creatinine (mg/dL)	1.2 (0.3)	1.0 (0.2)	1.1 (0.1)	0.97 (0.2)	1.0 (0.2)	0.9 (0.2)	0.9 (0.1)
BUN:Creatine	32.1 (7.7)	36.7 (9.0)	**30.7 (7.3)**	**40.0 (12.6)**	35.8 (6.5)	41.0 (10.4)	37.5 (5.0)
Cholesterol (mg/dL)	292.5 (32.5)	267.0 (28.5)	286.0 (35.5)	259.5 (21.1)	272.6 (25.2)	254.0 (26.3)	251.6 (12.9)
Total Bilirubin (mg/dL)	0.7 (0.3)	0.8 (0.2)	0.57 (0.23)	0.65 (0.15)	0.7 (0.3)	0.8 (0.3)	0.4 (0.1)
Globulin (g/L)	4.8 (0.4)	5.0 (0.4)	4.8 (0.5)	5.1 (0.6)	4.3 (0.4)	4.6 (0.4)	4.7 (0.1)
Albumin (g/L)	3.4 (0.5)	3.4 (0.3)	3.1 (0.2)	3.1 (0.4)	3.5 (0.3)	3.5 (0.7)	2.9 (0.1)
Albumin:Globulin	0.8 (0.1)	0.7 (0.1)	0.6 (0.1)	0.6 (0.1)	0.8 (0.1)	0.7 (0.1)	0.6 (0.03)
Alkaline Phosphatase	44.3 (3.6)	45.3 (7.4)	36.6 (5.8)	37.1 (4.3)	203.2 (41.8)	207.8 (41.0)	43.3 (10.5)
AST (iu/L)	57.2 (25.7)	68.7 (31.0)	45.0 (18.4)	60.8 (32.7)	**36.6 (13.8)**	**60.8 (32.3)**	46.5 (10.4)
ALT (iu/L)	41.5 (14.6)	45.6 (24.7)	20.5 (12.6)	29.3 (10.9)	37.6 (12.5)	55.6 (25.6)	30.3 (10.5)
CPK (iu/L)	536.9 (558)	392.7 (570)	563.8 (945)	436.3 (575)	169.3 (356)	310.6 (600)	444.8 (533.9)
GGT (iu/L)	24.2 (5.1)	25.7 (6.5)	**13.9 (2.8)**	**18.3 (3.3)**	16.3 (4.2)	19.7 (5.3)	17.3 (1.7)
LDH (iu/L)	1183 (354)	1545 (596)	1253 (606)	1651 (613)	854 (243)	1112 (495)	1037 (366)
Triglycerides (mg/dL)	30.3 (10.6)	28.5 (17.9)	18.2 (15.3)	25.8 (14.2)	32.9 (18.6)	38.7 (18.5)	33.0 (20.7)

Table 2. Overall serum chemistry values for captive harbor seals at the Alaska SeaLife Center 1998-1999. Group A: approximately 22 samples during herring regimes and 17 samples during pollock regimes; approximately 39 total samples/seal. H = herring diet, P = pollock diet and Mixed = pollock/herring diet. Values represent Mean (± S.D.). A t-test was performed to detect differences between diet values for each seal. Bold indicates a statistical significance (0.05).

	Sydney		***Pender***		***Travis***		***Tina***
Variable	***H***	***P***	***H***	***P***	***H***	***P***	***MIXED***
Sodium (mmol/L)	153.4 (10.0)	157.9 (3.0)	154.5 (3.8)	154.2 (3.7)	155.0 (8.1)	153.4 (2.3)	154.6 (9.8)
Potassium (mmol/L)	4.3 (0.3)	4.2 (0.3)	4.0 (0.3)	3.8 (0.4)	4.2 (0.3)	4.1 (0.2)	3.8 (0.3)
Chloride (mmol/L)	113.6 (5.6)	113.5 (1.6)	**110.5 (1.5)**	**113.0 (1.4)**	106.1(24.6)	100.2 (33.6)	111.9 (1.4)
Glucose (mg/dL)	171.9 (20.1)	145.9 (46.6)	162.8 (9.0)	163.6 (14.3)	173.5 (10.1)	167.0 (10.6)	201.7 (37.5)
Phosphorus (mg/dL)	5.9 (1.0)	5.1 (1.0)	**7.2 (1.8)**	**5.8 (1.0)**	5.0 (1.0)	5.6 (1.0)	4.8 (1.3)
Calcium (mg/dL)	9.3 (0.3)	9.2 (0.2)	9.6 (0.3)	9.1 (0.2)	9.4 (0.3)	9.4 (0.3)	9.4 (0.4)
BUN (mg/dL)	36.1 (6.5)	39.1 (3.9)	33.1 (7.6)	35.5 (4.9)	32.6 (6.7)	34.8 (5.0)	35.4 (4.2)
Creatinine (mg/dL)	0.9 (0.2)	0.8 (0.1)	0.85 (0.2)	0.8 (0.1)	0.85 (0.1)	0.8 (0.1)	1.3 (0.3)
BUN:Creatine	42.4 (8.6)	49.7 (11.5)	39.8 (8.4)	43.8 (8.4)	38.6 (6.7)	46.0 (7.8)	29.1 (5.8)
Cholesterol (mg/dL)	235.3 (27.3)	258.4 (21.7)	205.8 (27.5)	223.0 (47.7)	288.0 (29.2)	318.3 (28.8)	301.8 (32.5)
Total Bilirubin (mg/dL)	0.8 (0.3)	0.8 (0.3)	0.5 (0.2)	0.5 (0.2)	0.75 (0.1)	0.8 (0.3)	0.8 (0.2)
Globulin (g/L)	3.9 (0.5)	3.8 (0.4)	3.5 (0.5)	3.5 (0.3)	4.4 (0.6)	4.2 (0.3)	4.5 (0.5)
Albumin (g/L)	3.5 (0.4)	3.4 (0.3)	3.1 (0.2)	3.1 (0.2)	3.3 (0.3)	3.2 (0.2)	3.4 (0.3)
Albumin:Globulin	0.9 (0.2)	0.9 (0.2)	0.9 (0.1)	0.9 (0.1)	0.8 (0.1)	0.8 (0.1)	0.8 (0.1)
Alkaline Phosphatase [a]	85.8 (11.3)	78.7 (30.4)	99.4 (23.8)	99.6 (71.2)	91.8 (17.0)	77.4 (22.4)	68.2 (8.8)
AST (iu/L)	83.0 (25.6)	79.7 (47.6)	**64.1 (20.8)**	**200.9 (181)**	95.5 (42.5)	86.5 (60.9)	70.6 (30.6)
ALT (iu/L)	62.5 (22.7)	61.7 (26.8)	**75.8 (38.3)**	**223.7 (353)**	75.9 (32.2)	87.5 (20.0)	46.0 925.2)
CPK (iu/L)	998 (703)	311 (310)	253.1(217.4)	350.5 (550)	630.7 (701)	301.5 (550)	739.4 (798.6)
GGT (iu/L)	15.4 (5.5)	19.1 (4.5)	12.2 (6.6)	21.1 (10.2)	20.1 (6.7)	26.4 (7.7)	14.7 (7.7)
LDH (iu/L)	2115 (450)	1785 (295)	1814 (163)	2038 (422)	2137 (533)	1765 (480)	1948 (617)
Triglycerides (mg/dL)	36.4 (14.7)	51.3 (11.6)	**32.1 (6.0)**	**18.6 (11.0)**	33.6 (10.0)	31.7 (11.0)	56.9 (13.1)

Table 3. Overall blood hematology values for captive harbor seals at the Alaska SeaLife Center 1998-1999. Group B: approximately 22 samples during pollock regimes and 17 samples during herring regimes; Group A: approximately 22 samples during herring regimes and 17 samples during pollock regimes; approximately 39 total samples/seal. H = herring diet, P = pollock diet, and Mixed = herring/pollock diet. Values represent Mean (± S.D.). A t-test was performed to detect differences between diet values for each seal. Bold indicates a statistical significance (0.05).

	Skeezix		***Cecil***		***Poco***		***Snapper***
Variable	***H***	***P***	***H***	***P***	***H***	***P***	***MIXED***
Hematocrit (%)	53.0 (3.9)	51.5 (4.4)	52.3 (3.2)	49.2 (3.7)	53.7 (3.2)	50.8 (2.5)	49.9 (3.30)
Hemoglobin (g/dL)	22.0 (2.2)	21.5 (2.1)	21.6 (2.3)	19.6 (2.70)	22.1 (1.8)	20.7 (1.8)	20.2 (2.1)
MCHC (g/dL)	41.5 (1.9)	41.8 (3.5)	41.2 (2.8)	39.7 (3.1)	41.3 (1.8)	40.8 (2.2)	40.4 (3.0)
MCV	103.5 (14.1)	101.2 (13.5)	100.9 (10.4)	103.9 (12.5)	106.7 (13.8)	107.1 (10.9)	108.4 (14.0)
WBC (10^9/L)	9.1 (0.6)	9.2 (1.6)	8.6 (1.0)	9.1 (2.0)	7.6 (0.9)	7.9 (0.8)	7.6 (1.30
%Granulocytes	61.8 (3.5)	60.8 (4.7)	70.5 (4.2)	67.4 (7.3)	70.6 (1.8)	67.5 (4.9)	72.8 (1.9)
%L/M	38.2 (3.5)	39.2 (4.7)	29.5 (4.2)	32.6 (7.3)	29.4 (1.8)	32.5 (4.9)	25.4 (5.7)
Platelets (10^9/L)	347.3 (122)	341.6 (85.2)	339.2 (105)	393.3 (72.9)	289.7 (64.0)	334.6 (50.3)	269.9 (53.1)
RBC Counts (10^6)	5.2 (0.63)	5.13 (0.48)	5.23 (0.58)	4.8 (0.7)	5.1 (0.67)	4.8 (0.49)	4.7 (0.63)
	Sydney		***Pender***		***Travis***		***Tina***
Variable	***H***	***P***	***H***	***P***	***H***	***P***	***MIXED***
Hematocrit (%)	57.1 (3.7)	60.5 (2.2)	46.4 (4.7)	49.7 (4.5)	54.2 (4.1)	57.2 (5.0)	53.8 (3.7)
Hemoglobin (g/dL)	23.1 (2.7)	26.5 (2.1)	18.7 (1.7)	20.6 (3.2)	22.3 (1.9)	**23.1 (2.9)**	**21.8 (1.6)**
MCHC (g/dL)	40.4 (3.6)	42.1 (2.9)	40.4 (2.9)	41.3 (3.8)	40.8 (2.5)	40.3 (2.0)	40.6 (3.5)
MCV	119.1 (20.6)	117.1 (13.2)	106.9 (14.3)	103.1 (12.8)	106.4 (12.3)	103.6 (10.8)	102.6 (12.6)
WBC (10^9/L)	9.9 (1.5)	9.6 (0.9)	10.7 (1.3)	10.2 (1.6)	9.6 (1.1)	8.9 (1.4)	8.4 (1.2)
%Granulocytes	66.8 (3.8)	65.4 (4.2)	66.7 (5.0)	67.8 (3.8)	67.9 (3.9)	66.0 (3.8)	66.9 (5.9)
%L/M	33.2 (3.8)	33.7 (4.2)	33.3 (5.0)	32.2 (3.8)	32.1 (3.9)	34.0 (3.9)	33.0 (5.9)
Platelets (10^9/L)	413 (54.7)	373.4 (67.4)	449.3 (59.2)	405.7 (109)	371.4 (70)	359.8 (59.3)	357.9 (65.8)
RBC Counts (10^6)	4.9 (0.6)	5.2 (0.57)	4.4 (0.5)	0.49 (0.7)	5.2 (0.62)	5.6 (0.6)	5.3 (0.5)

Table 4. Admission information for harbor seal pups brought to the Alaska SeaLife Center for rehabilitation during 1998 and 1999. Condition was assessed by the ASLC veterinarian (P. Tuomi, DVM).

	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, dehydrated, thin	8-11-98
	Iliamna (98009)	F	7-10-98	3 weeks	Injured, laceration, pus, thin	10-3-98
	Kenai (99004)	M	5-20-99	7-10 days	Thin	9-23-99
	Mackenzie (99007)	F	6-14-99	3 days	Thin, dehydrated, laceration	9-23-99
	S.K. (99008)	F	6-22-99	7-10 days	Dehydrated, infection within 1 week	9-23-99
	Iggy (99009)	M	6-30-99	10 days	Dehydrated	9-23-99
Died	98005	M	5-28-98	10 – 12 days	Injured, laceration, infection within 1 week	6-5-98
	98009	F	8-11-98	2 months	Sick, hypothermic, distended abdomen, granulating laceration	8-11-98[b]

[a]picked up by tourist
[b]euthanized

Table 5. Mean hematological values for rehabilitated harbor seal pups during recover and development. Gaps in data from 1998 prevented statistical analysis of white blood cell parameters. Data for 1999 are presented separately Statistical differences were determined by Friedman's analysis of variance.

1998-99 Pups		**Approx. Age (days)**	**ESR**	**Hct (%)**	**Hb (mg/dL)**	**MCHC (mg/dL)**	**WBC ($x10^9$/L)**	**Grans ($x10^9$/L)**	**Grans (%)**	**L+M ($x10^9$/L)**	**L+M (%)**
Admission	**Mean**	**6**	**1**	**65.8**	**25.7**	**39.6**	**12.5**	**7.8**	**63.5**	**4.6**	**36.5**
(approx. 1 wk old)	S.D.	3	0	5.2	1.6	0.7	0.6	0.8	4.0	0.4	4.0
	n	6	4	6	6	6	4	4	4	4	4
Pre-Weaning	**Mean**	**14**	**1**	**56.6**	**22.5**	**39.8**	**16.9**	**11.2**	**65.8**	**5.6**	**34.3**
(approx. 2 wks old)	S.D.	2	0	3.5	1.0	1.6	8.2	5.9	3.2	2.3	3.2
	n	6	4	5	5	5	4	4	4	4	4
Post-Weaning	**Mean**	**40**	**1**	**49.6**	**19.4**	**39.7**	**10.3**	**7.7**	**74.4**	**2.6**	**25.6**
(approx. 6 wks old)	S.D.	2	1	4.9	1.9	0.7	1.4	1.9	9.8	1.0	9.8
	n	7	7	7	6	6	7	7	7	7	7
(approx. 9 wks old)	**Mean**	**63**	**7**	**46.2**	**17.7**	**38.1**	**11.1**	**8.3**	**72.0**	**2.7**	**28.0**
	S.D.	2	15	6.3	1.9	2.4	3.9	4.8	11.2	1.0	11.2
	n	7	7	7	7	7	7	7	7	7	7
(approx. 12 wks old)	**Mean**	**88**	**11**	**41.1**	**16.0**	**39.0**	**10.7**	**7.8**	**71.7**	**2.9**	**28.3**
	S.D.	4	23	5.9	2.1	0.9	2.4	2.7	12.3	1.5	12.3
	n	6	6	6	6	6	6	6	6	6	6
From 1 – 12 wks old [1]	*p* =			*0.001*	*0.001*		*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
From 6 – 12 wks old [2]	*p* =			*0.011*	*0.006*		*N/A*	*N/A*	*N/A*	*N/A*	*N/A*

[1] n = 5 (PV98004, PV99004, PV99007, PV99008, PV99009) [2] n = 6 (PV98004, PV98008, PV99004, PV99007, PV99008, PV99009)

ESR – Erythrocyte sedimentation rate
Hct – Hematocrit
Hb – Hemoglobin
MCHC – Mean cell hemoglobin content
WBC – White blood cells
Grans – Granulocytes
L+M – Leukocytes and Monocytes

Table 6: Mean hematological values for rehabilitated harbor seal pups during recover and development in 1999. Statistical differences were determined by Friedman's analysis of variance. Gaps in data prevented inclusion of 1 week old pups in statistical analyses.

1999 Pups		**Approx. Age (days)**	**ESR**	**Hct (%)**	**Hb (mg/dL)**	**MCHC (mg/dL)**	**WBC ($x10^9$/L)**	**Grans ($x10^9$/L)**	**Grans (%)**	**L+M ($x10^9$/L)**	**L+M (%)**
Admission	**Mean**	**7**	**1**	**65.8**	**25.8**	**39.9**	**12.6**	**8.0**	**63.3**	**4.6**	**36.7**
(approx. 1 wk old)	S.D.	3	0	6.7	2.1	0.6	0.6	1.0	4.9	0.5	4.9
	n	4	3	4	4	4	3	3	3	3	3
Pre-Weaning	**Mean**	**15**	**1**	**58.0**	**22.7**	**39.1**	**16.9**	**11.2**	**65.8**	**5.7**	**34.3**
(approx. 2 wks old)	S.D.	2	0	1.9	1.1	0.9	8.2	5.9	3.2	2.3	3.2
	n	4	3	4	4	4	4	4	4	4	4
Post-Weaning	**Mean**	**39**	**1**	**51.4**	**20.3**	**39.5**	**10.7**	**7.6**	**71.0**	**3.1**	**29.0**
(approx. 6 wks old)	S.D.	2	0	3.5	1.3	0.8	1.0	1.2	5.2	0.4	5.2
	n	4	4	4	4	4	4	4	4	4	4
(approx. 9 wks old)	**Mean**	**64**	**1**	**49.8**	**18.6**	**36.8**	**9.8**	**6.6**	**67.0**	**3.2**	**33.0**
	S.D.	2	1	3.2	0.7	2.6	1.0	0.7	1.4	0.4	1.4
	n	4	4	4	4	4	4	4	4	4	4
(approx. 12 wks old)	**Mean**	**90**	**2**	**44.1**	**17.0**	**38.5**	**10.6**	**7.0**	**66.0**	**3.6**	**34.0**
	S.D.	4	0	4.7	1.9	0.4	2.4	1.6	5.1	1.0	5.1
	n	6	4	4	4	4	4	4	4	4	4
From 2 – 12 wks old [1]	*p* =			*0.026*	*0.017*	*0.068*	*0.026*	*0.023*		*0.058*	*0.087*
From 6 – 12 wks old [1]	*p* =			*0.039*	*0.039*						

[1] n = 4 (PV99004, PV99007, PV99008, PV99009) [2] n = 6 (PV98004, PV98008, PV99004, PV99007, PV99008, PV99009)

ESR – Erythrocyte sedimentation rate
Hct – Hematocrit
Hb – Hemoglobin
MCHC – Mean cell hemoglobin content
WBC – White blood cells
Grans – Granulocytes
L+M – Leukocytes and Monocytes

Table 7: Mean serum enzyme and protein values that varied over time in rehabilitated harbor seal pups during recovery and development. Statistical differences were determined by Friedman's analysis of variance.

1998-99 Pups		**Approx. Age (days)**	**ALT (U/L)**	**AST (U/L)**	**GGT (U/L)**	**Globulin (g/dL)**	**ALB/ GLOB**	**Total Protein (g/dL)**
Admission	**Mean**	**6**	**246**	**85**	**56**	**2.71**	**1.41**	**6.44**
(approx. 1 wk old)	S.D.	3	238	30	29	0.48	0.34	0.31
	n	6	6	6	6	6	6	6
Pre-Weaning	**Mean**	**14**	**57**	**38**	**45**	**2.72**	**1.28**	**6.11**
(approx. 2 wks old)	S.D.	2	44	16	15	0.38	0.26	0.50
	n	6	6	6	6	6	6	6
Post-Weaning	**Mean**	**40**	**54**	**65**	**31**	**3.31**	**0.99**	**6.46**
(approx. 6 wks old)	S.D.	2	16	19	4	0.58	0.23	0.50
	n	7	7	7	7	7	7	7
(approx. 9 wks old)	**Mean**	**63**	**91**	**101**	**28**	**3.46**	**1.00**	**6.72**
	S.D.	2	23	29	8	0.87	0.28	0.69
	n	7	7	6	7	7	7	7
(approx. 12 wks old)	**Mean**	**88**	**73**	**86**	**21**	**3.56**	**0.97**	**6.79**
	S.D.	4	22	27	4	0.97	0.27	0.84
	n	6	6	6	6	6	6	6
From 1 – 12 wks old [1]	*p* =		*0.024*	*0.037*	*0.008*	*0.004*	*0.027*	*0.054*
From 1 – 9 wks old [2]	*p* =		*0.006*	*0.021*	*0.012*	*0.003*	*0.043*	*0.133*
From 6 – 12 wks old [3]	*p* =				*0.034*			*0.016*

[1] n = 5 (PV98004, PV99004, PV99007, PV99008, PV99009) [2] n = 6 (PV98004, PV98006, PV99004, PV99007, PV99008, PV99009)
[3] n = 6 (PV98004, PV98008, PV99004, PV99007, PV99008, PV99009)
ALT – Alanine aminotransferase GGT – Gamma-glutamyl transpeptidase
AST – Aspartate aminotransferase ALB/GLOB – Albumin/Globulin

Table 8: Mean serum metabolite and electrolyte values that varied over time in rehabilitated harbor seal pups during recover and development. Statistical differences were determined by Friedman's analysis of variance.

1998-99 Pups		**Approx. Age (days)**	**CHOL (mg/dL)**	**Creatinine (mg/dL)**	**BUN/ Creatinine**	**TRIG (mg/dL)**	**TBIL (mg/dL)**	**PHOS (mg/dL)**	**Cl (mM)**
Admission	**Mean**	**6**	**324.8**	**0.39**	**116.6**	**106.9**	**2.90**	**7.12**	**106.2**
(approx. 1 wk old)	S.D.	3	67.5	0.06	59.1	30.6	1.73	1.13	3.9
	n	6	6	6	6	6	6	6	6
Pre-Weaning	**Mean**	**14**	**225.2**	**0.37**	**101.8**	**91.1**	**0.89**	**6.95**	**110.7**
(approx. 2 wks old)	S.D.	2	22.8	0.06	20.7	49.5	0.24	1.41	2.3
	n	6	6	6	6	6	6	6	6
Post-Weaning	**Mean**	**40**	**304.2**	**0.32**	**114.9**	**41.4**	**0.66**	**6.55**	**111.9**
(approx. 6 wks old)	S.D.	2	60.9	0.05	25.0	10.6	0.13	1.02	2.5
	n	7	7	7	7	7	7	7	7
(approx. 9 wks old)	**Mean**	**63**	**316.1**	**0.33**	**89.2**	**33.1**	**0.68**	**7.31**	**112.3**
	S.D.	2	46.0	0.04	12.3	15.0	0.15	1.13	1.2
	n	7	7	7	7	7	7	7	7
(approx. 12 wks old)	**Mean**	**88**	**319.2**	**0.43**	**64.8**	**36.0**	**0.67**	**8.09**	**111.5**
	S.D.	4	78.9	0.03	8.1	16.2	0.13	0.54	1.5
	n	6	6	6	6	6	6	6	6
From 1 – 12 wks old [1]	*p* =		*0.023*	*0.011*		*0.011*	*0.005*	*0.081*	*0.111*
From 1 – 12 wks old [2]	*p* =		*0.024*	*0.063*		*0.004*	*0.007*		*0.038*
From 6 – 12 wks old [3]	*p* =			*0.011*	*0.030*	*0.021*		*0.009*	

[1] n = 5 (PV98004, PV99004, PV99007, PV99008, PV99009) [2] n = 6 (PV98004, PV98006, PV99004, PV99007, PV99008, PV99009)
[3] n = 6 (PV98004, PV98008, PV99004, PV99007, PV99008, PV99009)
CHOL – Cholesterol
BUN/Creatinine – Blood urea nitrogen/Creatinine
TRIG – Triglyceride
TBIL – Total bilirubin
PHOS – Phosphorous
Cl – Chloride

Table 9. Differences between formula and timing of weaning for rehabilitated harbor seal pups at the ASLC between 1998 and 1999.

Differences in Feeding/Weaning	
1998	**1999**
Formula contained vegetable oil	Formula contained salmon oil
Weaned early (< 2 weeks old)	Weaned later (2 – 4 weeks old)
Weaned abruptly (2 – 4 days)	Weaned gradually (4 – 6 days)

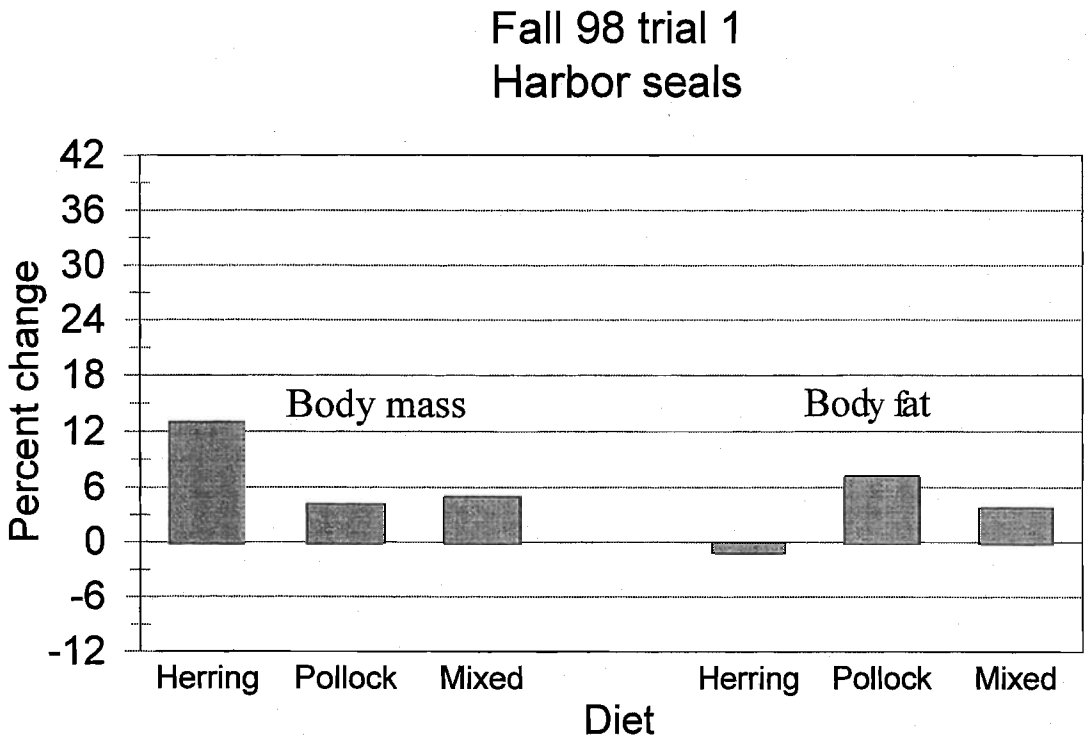


Figure 1. Change in body mass (%) and body fat (%) during feeding trial 1, September, 1998 to January, 1999.

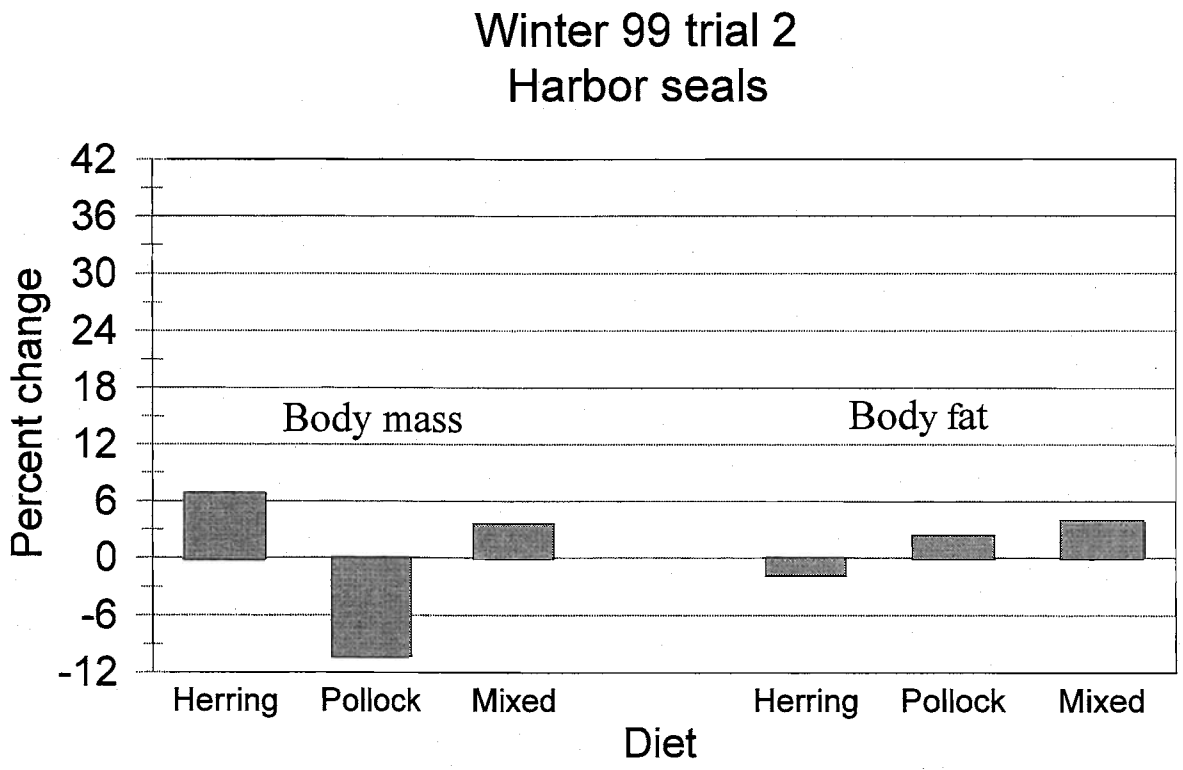


Figure 2. Change in body mass (%) and body fat (%) during feeding trial 2, January to May, 1999.

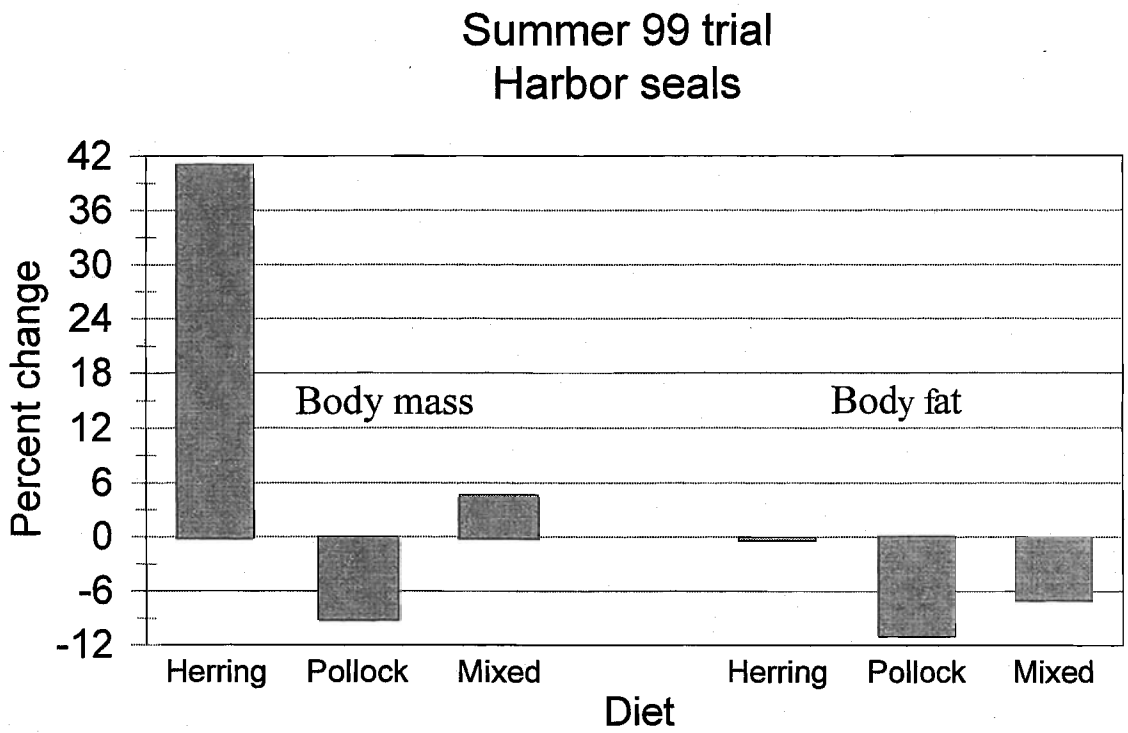


Figure 3. Change in body mass (%) and body fat (%) during feeding trial 3, May to September, 1999.

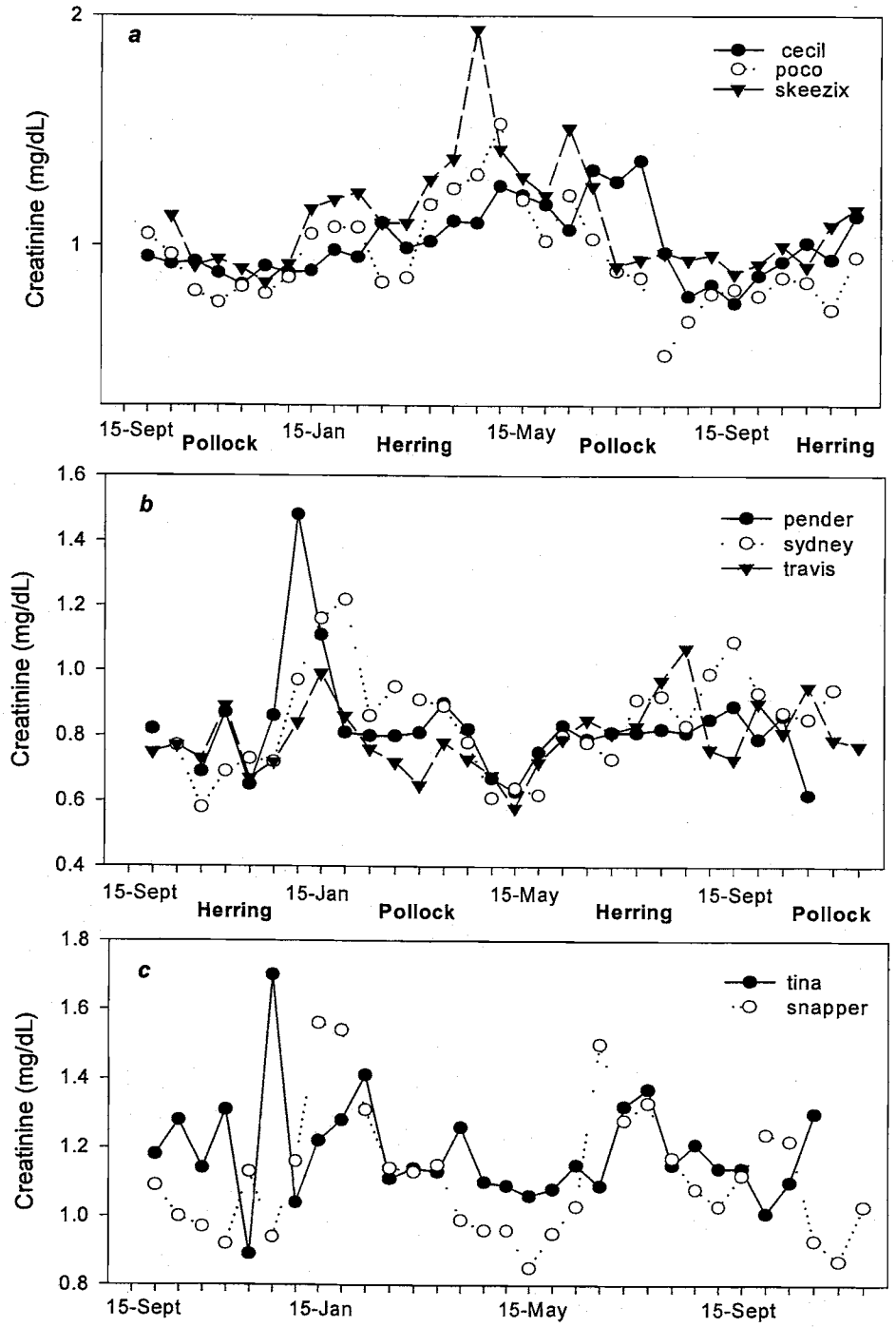


Figure 4. Trends in serum creatinine values for captive harbor seals fed herring or pollock at the ASLC during 1998 – 1999. ***a.*** Group B, ***b.*** Group A, ***c.*** continuous mixed diet

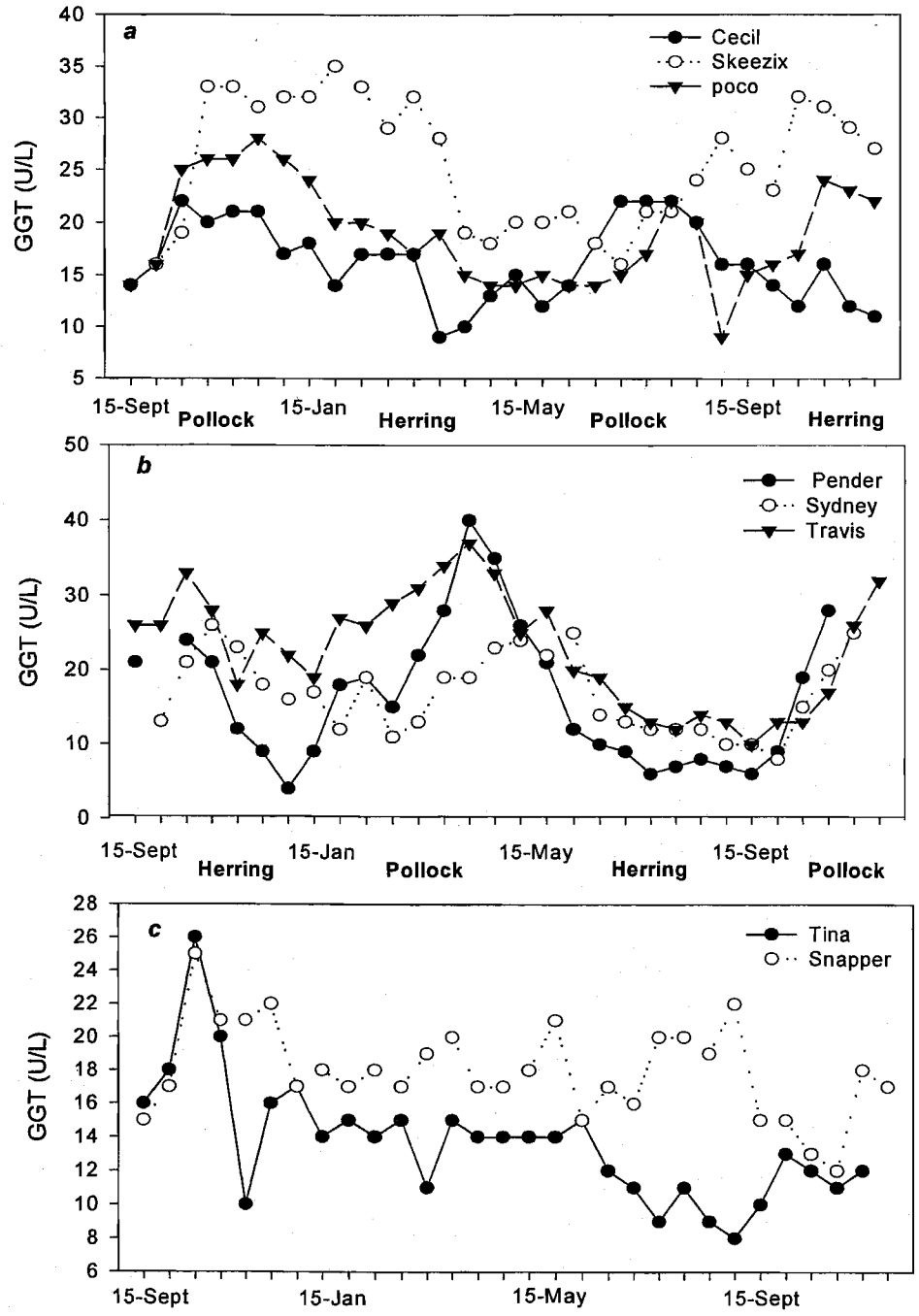


Figure 5. Trends in serum GGT values for captive harbor seals fed herring or pollock at the ASLC during 1998 – 1999. ***a.*** Group B, ***b.*** Group A, ***c.*** continuous mixed diet

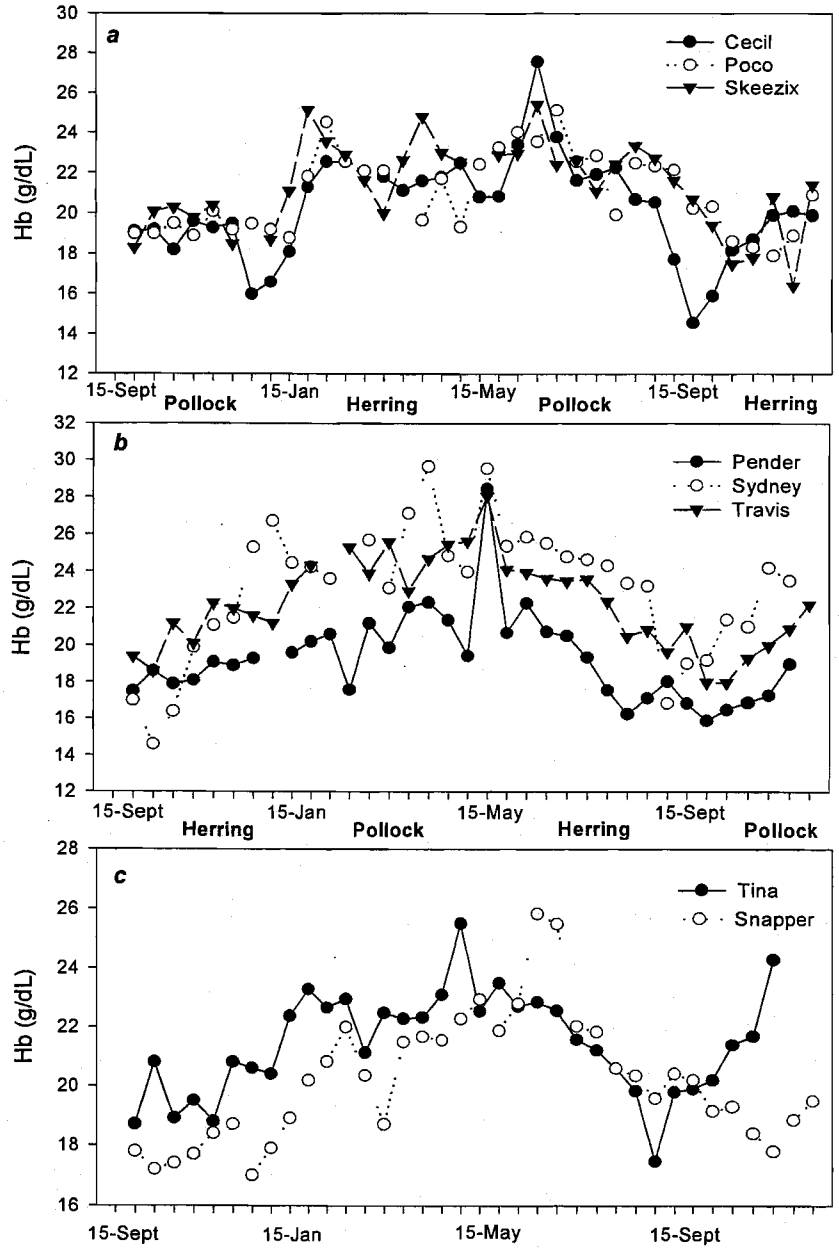


Figure 6. Trends in hemoglobin values for captive harbor seals fed herring or pollock at the ASLC during 1998 - 1999. ***a.*** Group B, ***b.*** Group A, ***c.*** continuous mixed diet

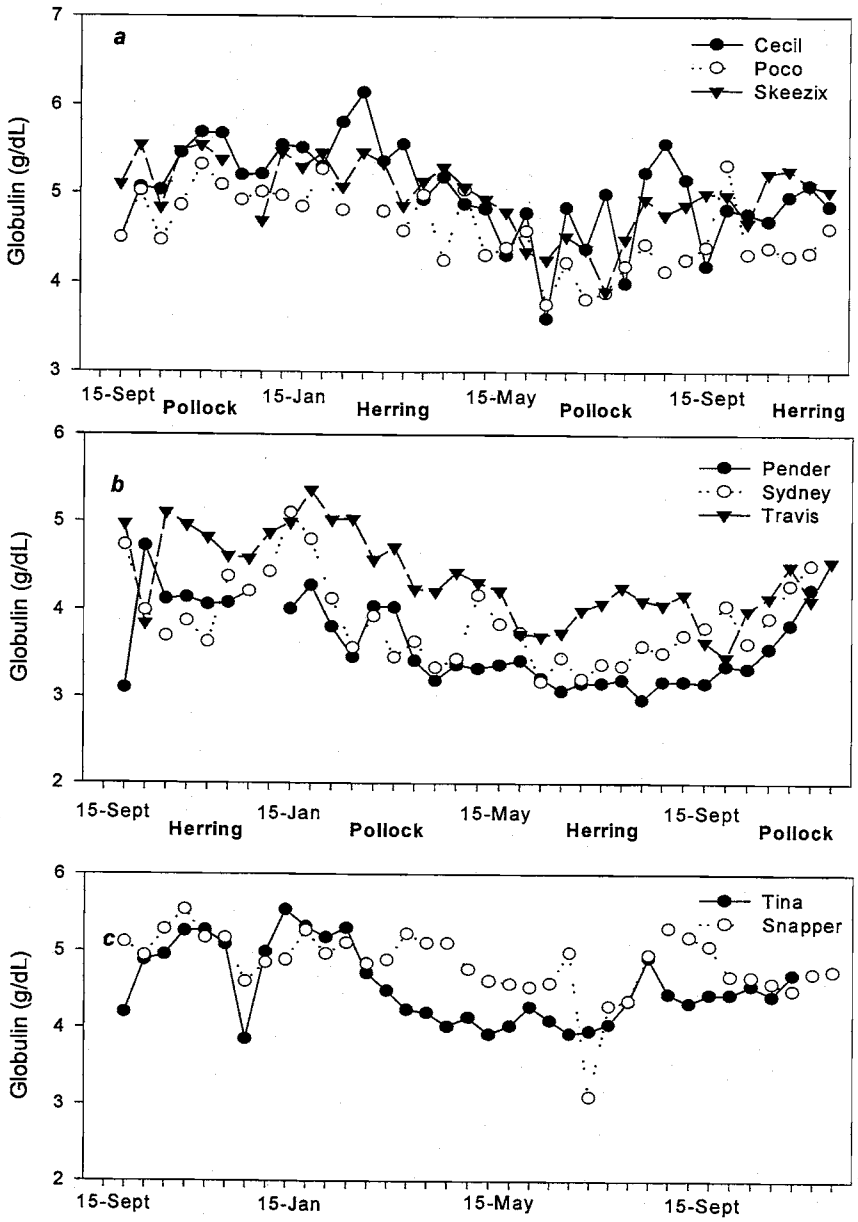


Figure 7. Trends in serum globulin values for captive harbor seals blood fed herring or pollock at the ASLC during 1998 - 1999. ***a.*** Group B, ***b.*** Group A, ***c.*** continuous mixed diet

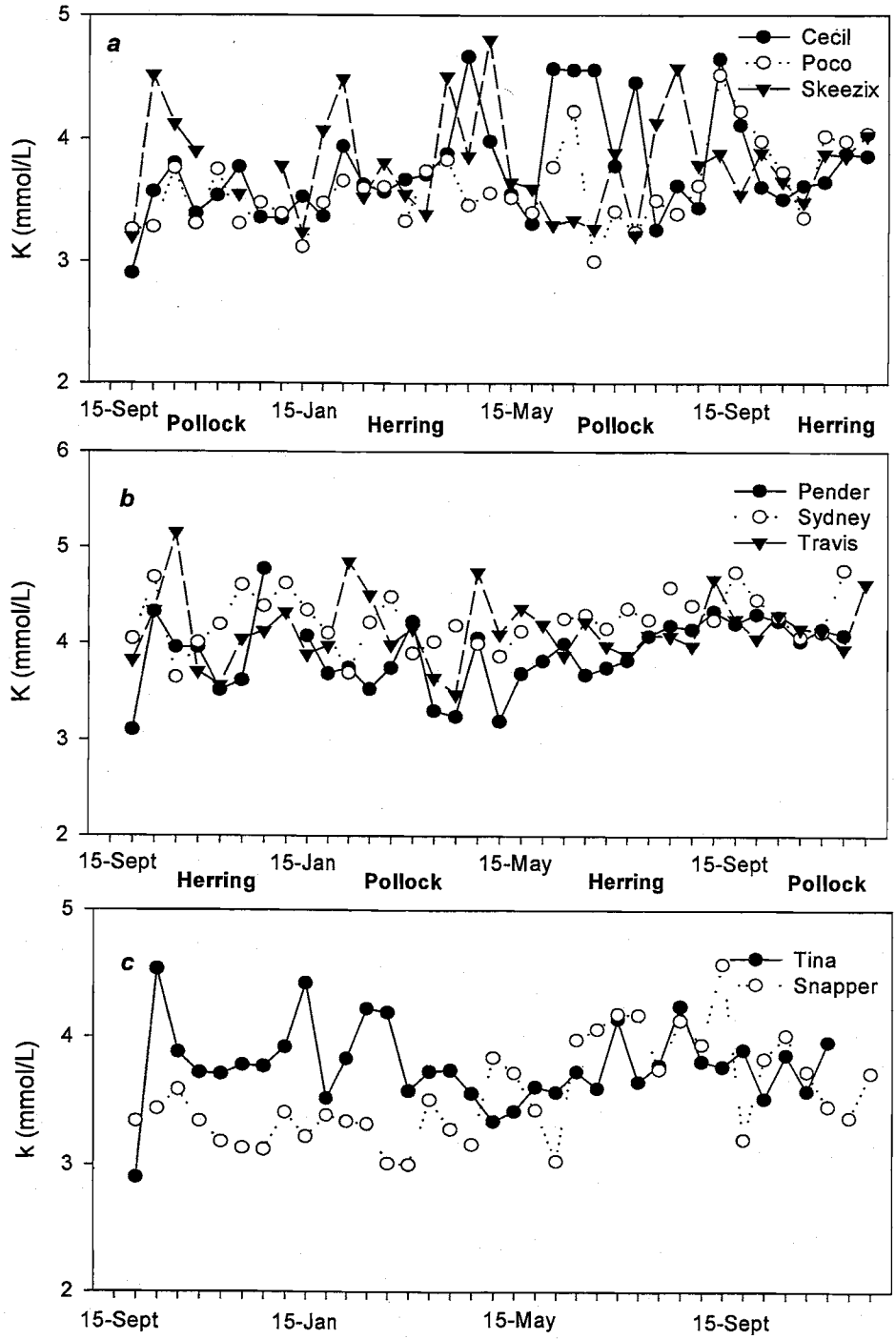


Figure 8. Trends in serum potassium values for captive harbor seals fed herring or pollock at the ASLC during 1998 - 1999. ***a.*** Group B, ***b.*** Group A, ***c.*** continuous mixed diet

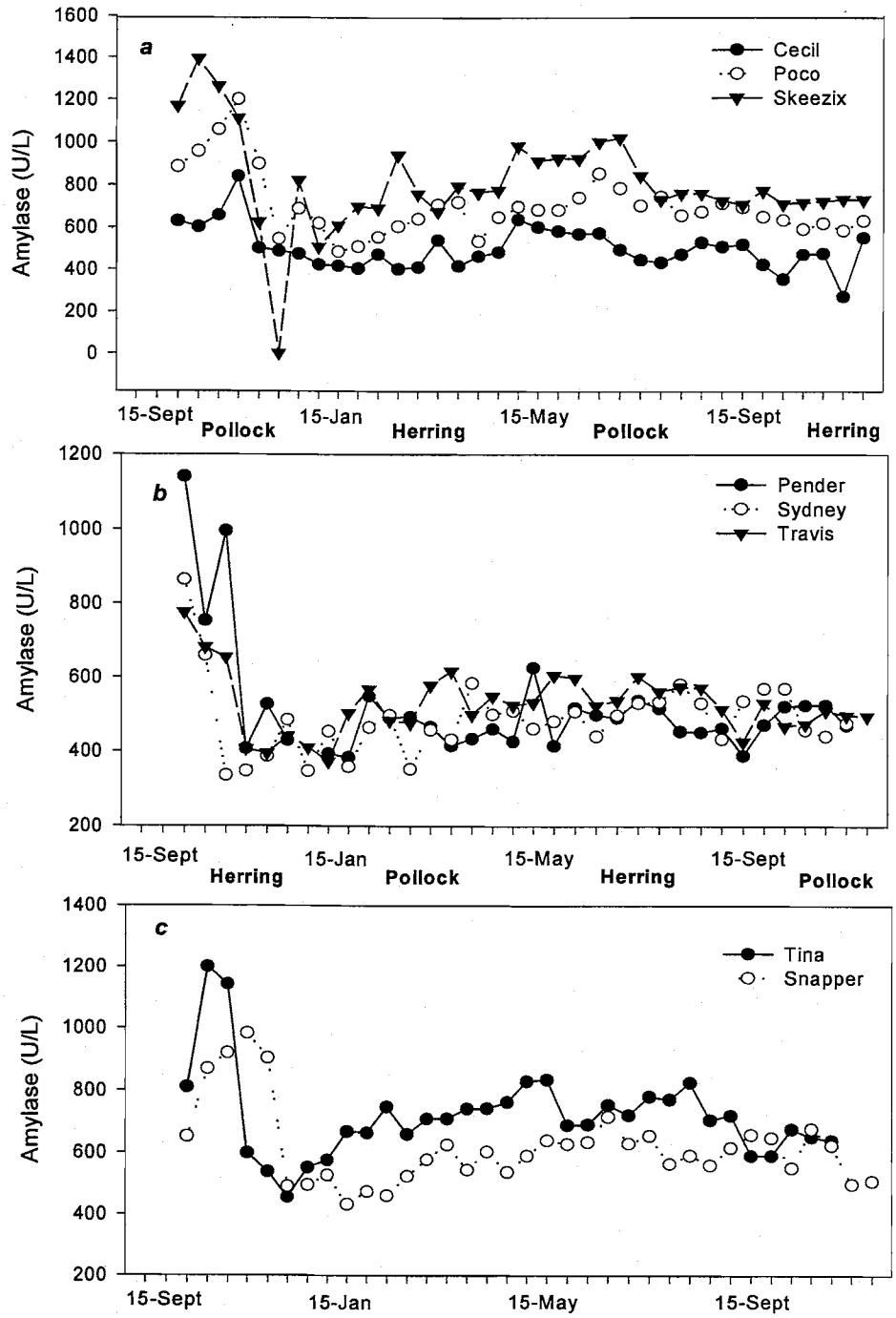


Figure 9. Trends in serum amylase values for captive harbor seals fed herring or pollock at the ASLC during 1998 -1999. ***a.*** Group B, ***b.*** Group A, ***c.*** continuous mixed diet

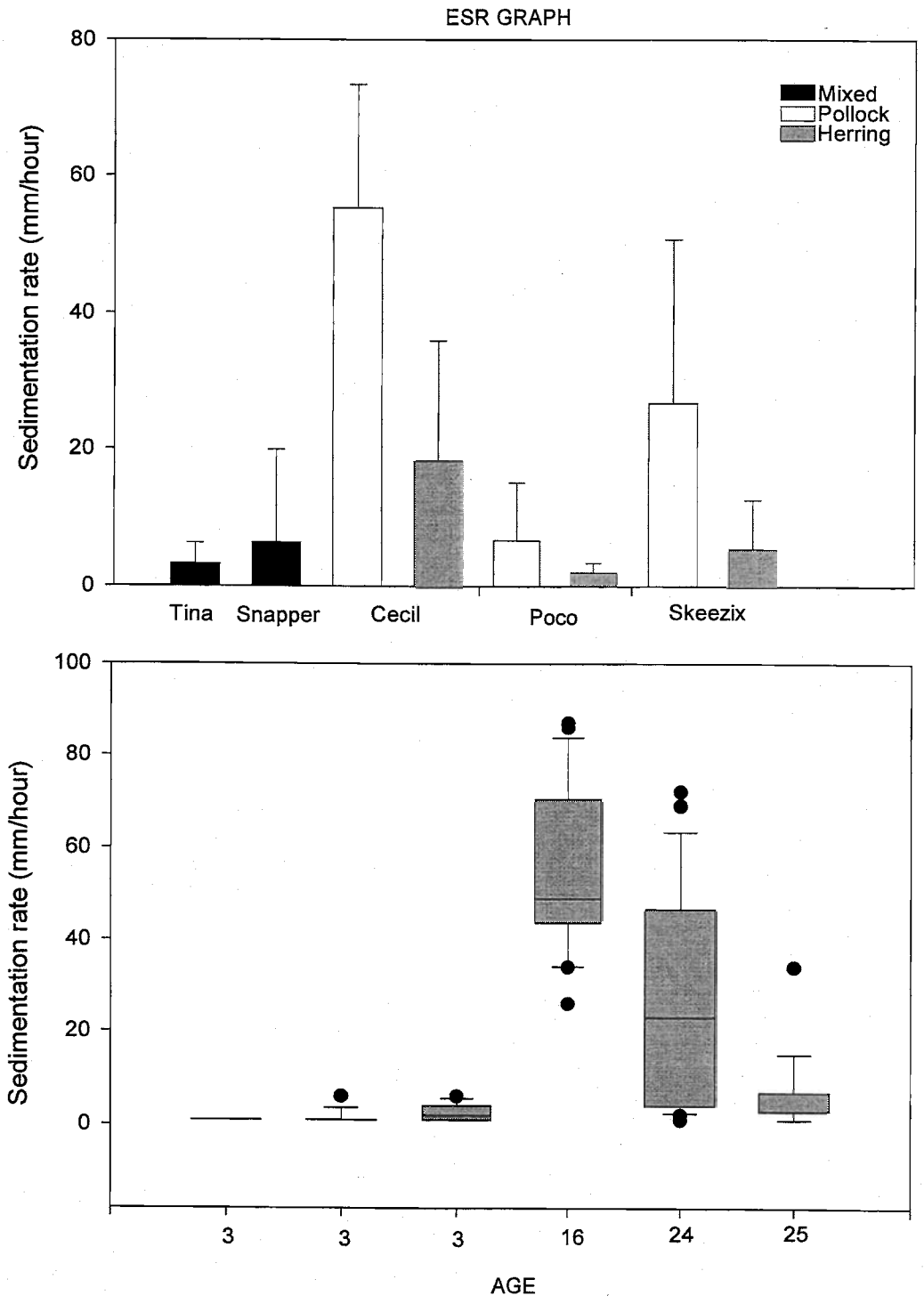


Figure 10. Erythrocyte sedimentation rate (ESR) values for harbor seals at the ASLC during 1998-1999. ***a.*** differences between pollock, herring, and mixed diets ***b.*** ESR as a function of age in all captive harbor seals.

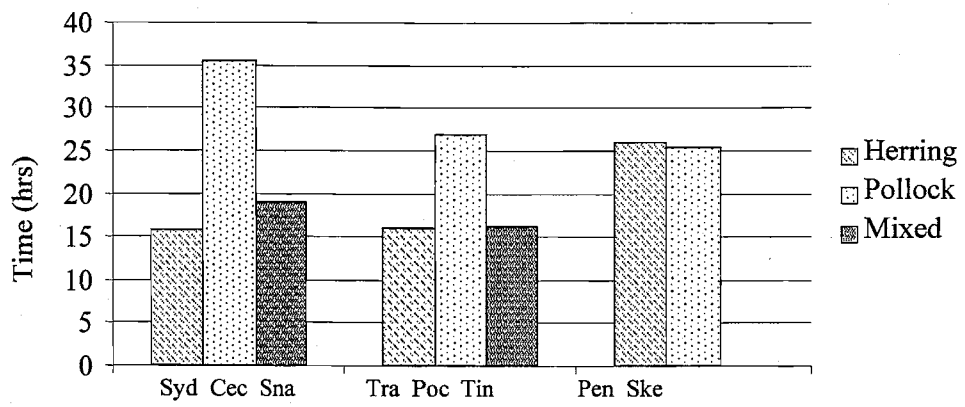


Figure 11. Mean retention times for captive seals (Feeding Trial #1) at the ASLC. Syd = Sydney, Cec = Cecil, Sna = Snapper, Tra = Travis, Tin = Tina, Pen = Pender, Ske = Skeezix

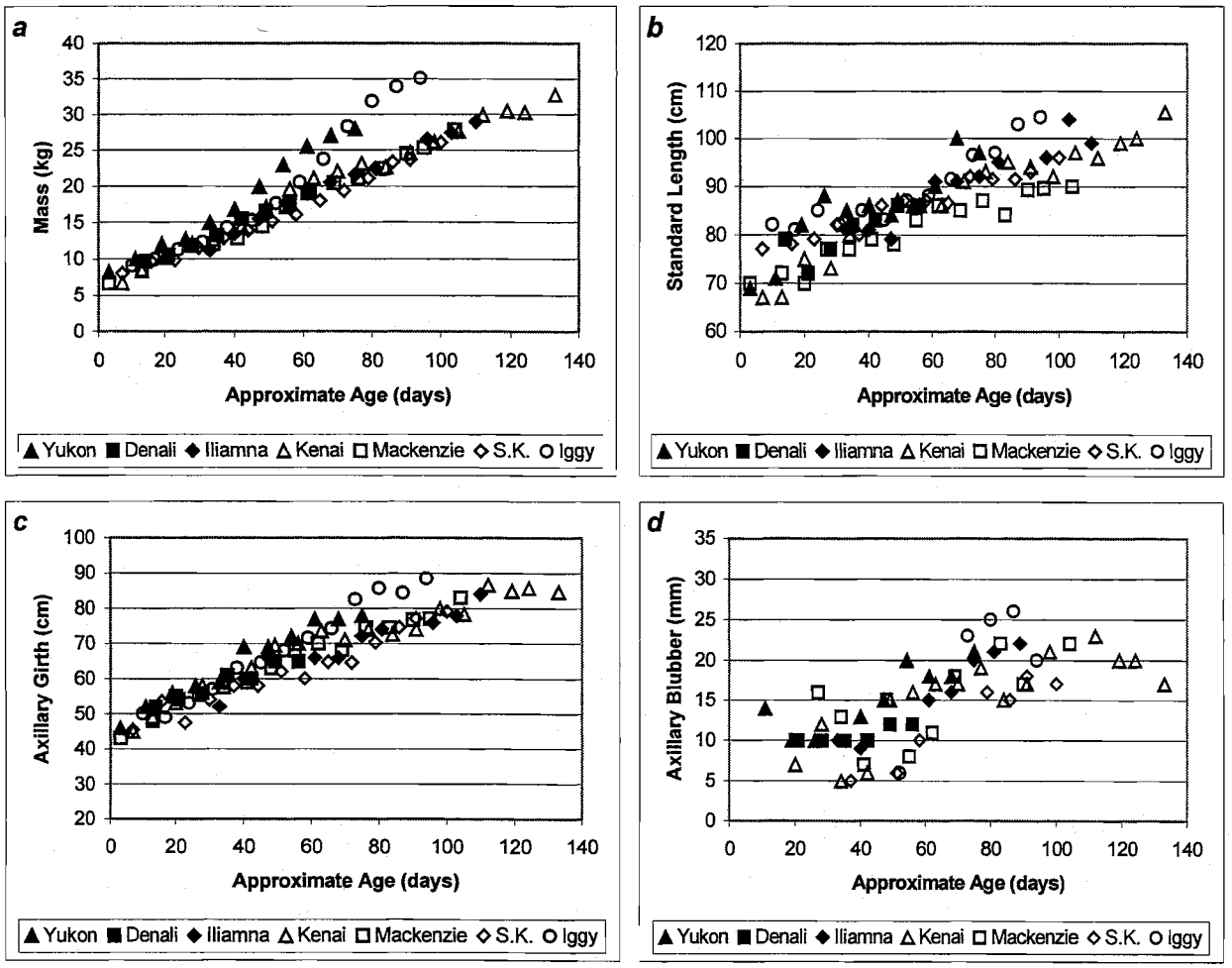


Figure 12. Growth rates of rehabilitated harbor seal pups from 1998 (closed symbols) and 1999 (open symbols) ***a.*** mass, ***b.*** standard length, ***c.*** axillary girth, ***d.*** axillary blubber depth

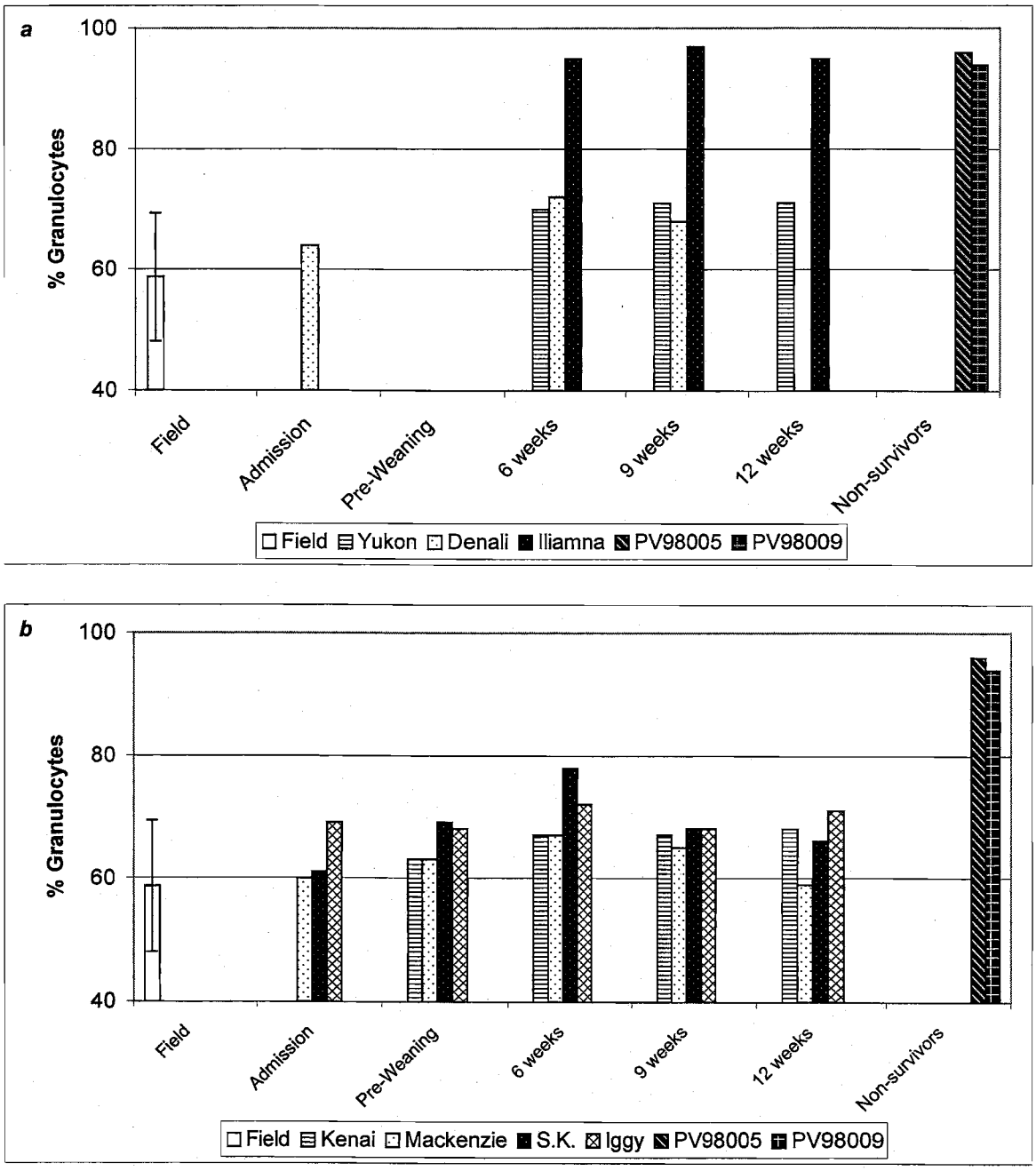


Figure 13. Differential percentage of granulocytes in blood samples taken from harbor seal pups rehabilitated at the ASLC. Data from wild harbor seal pups (Prince William Sound, 1997-99, n = 115, from Small et al. 1999) and two non-survivors from 1998 have been included on both graphs for comparison. Bars with black backgrounds represent animals known to be exposed to infection. ***a.*** 1998, ***b.*** 1999

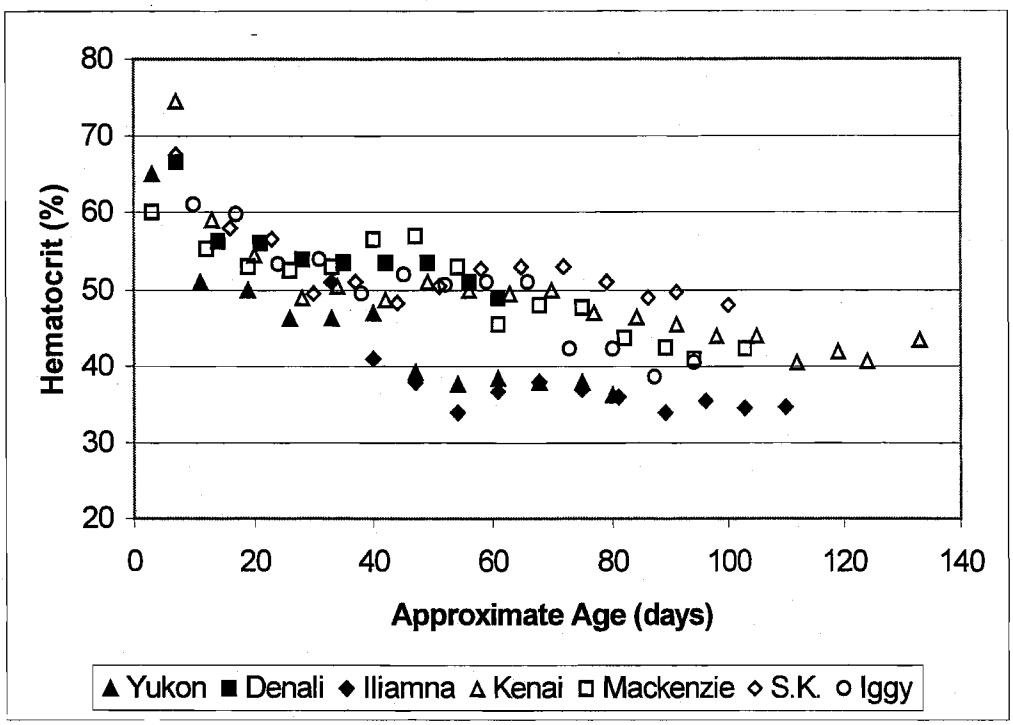


Figure 14. Changes in hematocrit in rehabilitated harbor seal pups at the ASLC as a function of estimated age from 1998 (closed symbols) and 1999 (open symbols).

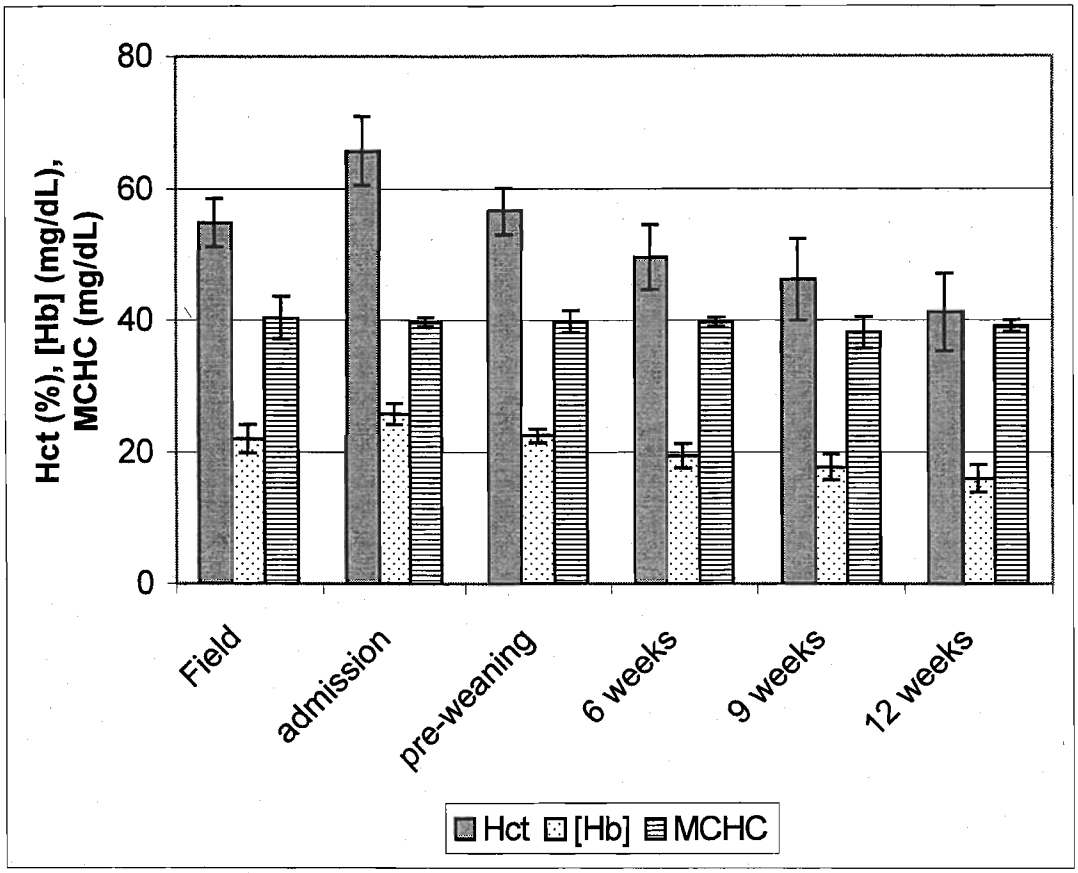


Figure 15. Mean hematocrit, hemoglobin and MCHC as a function of age for harbor seal pups rehabilitated at the ASLC during 1998 and 1999. Values representing admission were from seals approximately 1 week old (n = 6) and those representing pre-weaning were from seals approximately 2 weeks old (n = 5). Pups from 6 – 9 weeks old were post-weaning (n = 7, weeks 6 and 9; n = 6, week 12). Field samples were taken from wild pups (2 – 6 weeks old) in Prince William Sound during June of 1997-99 (n = 120) (from Small et al. 1999).

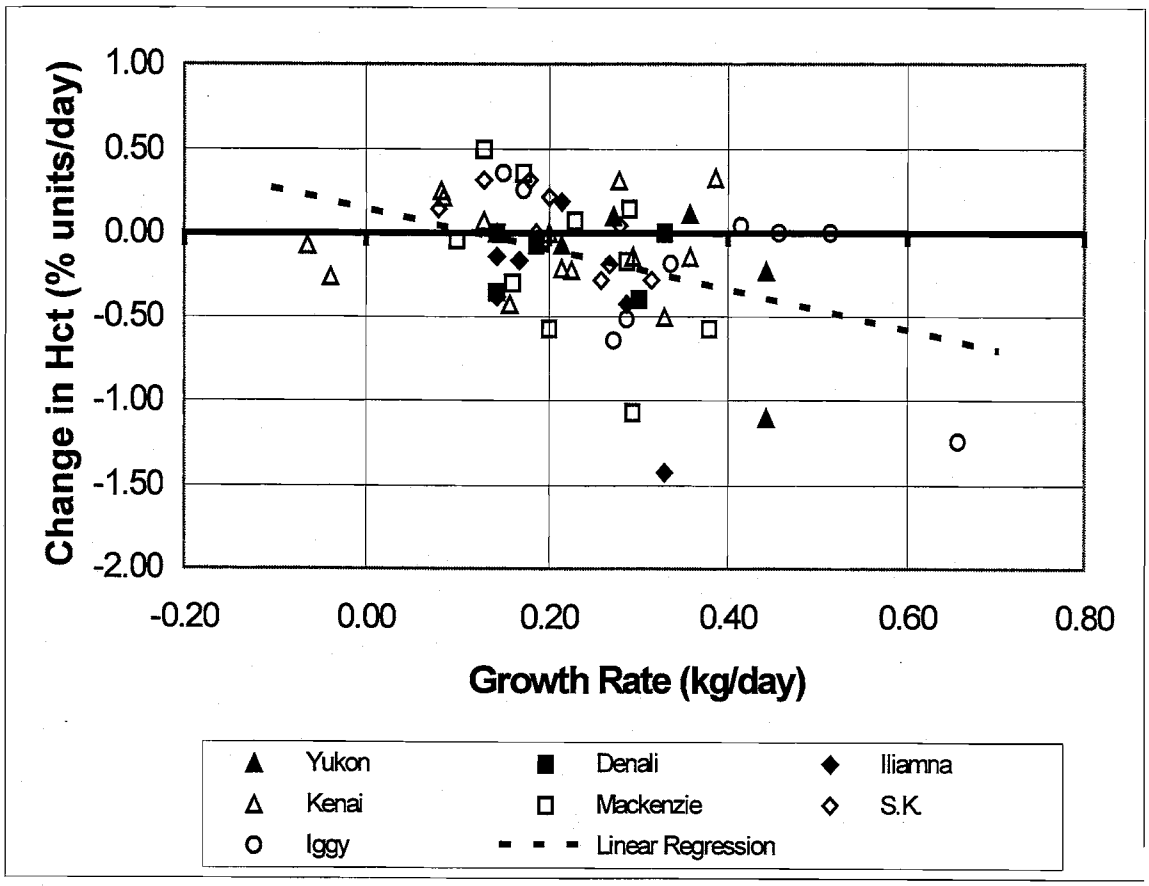


Figure 16. Relationship between growth rate and rate of hematocrit change in harbor seal pups rehabilitated at the ASLC during 1998 (closed symbols) and 1999 (open symbols).
$r^2 = 0.13$, Slope = -1.223, $p = 0.002$.

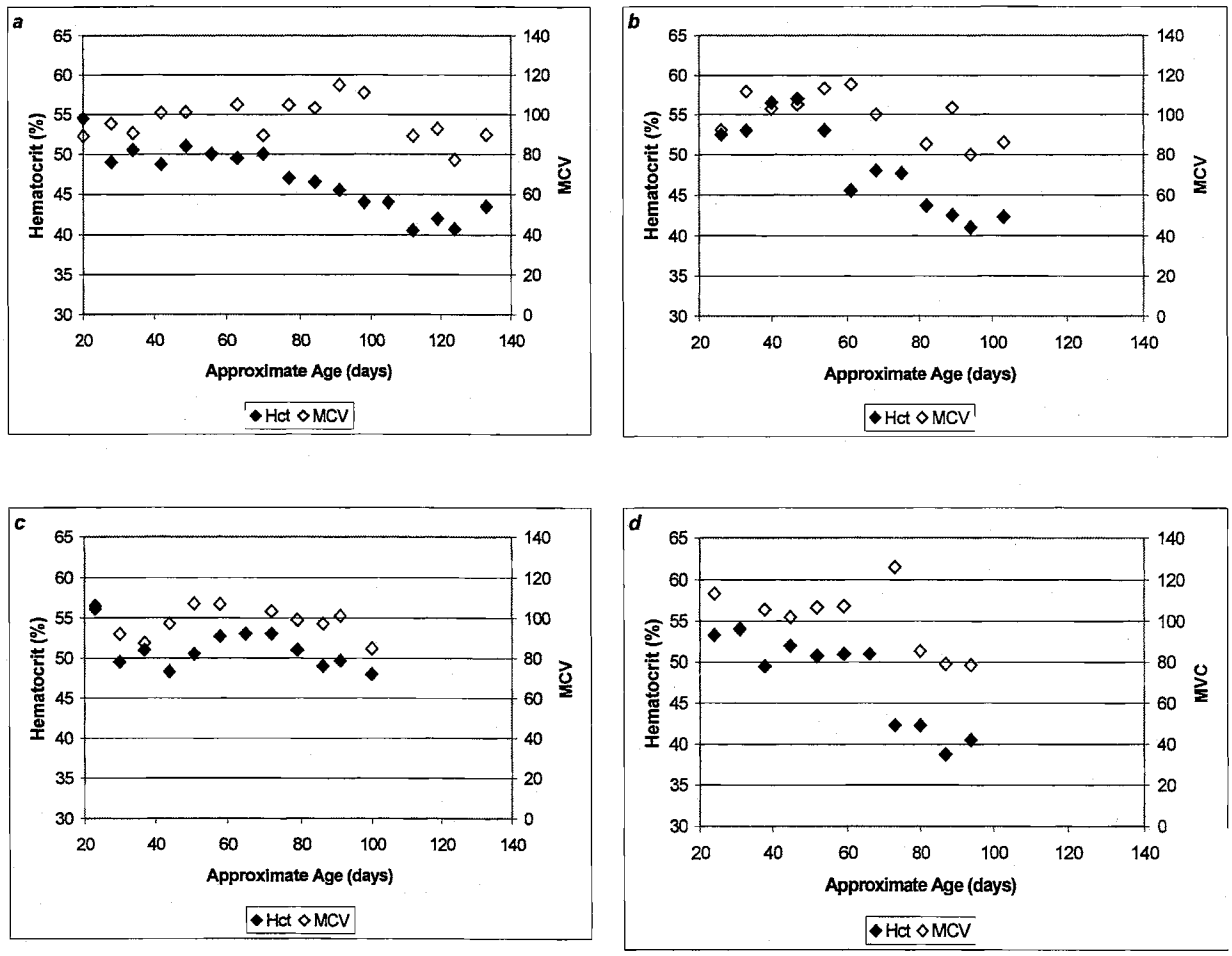


Figure 17. Hematocrit and MCV as a function of estimated age in individual harbor seals rehabilitated at the ASLC during 1999. ***a.*** PV99004, ***b.*** PV99007, ***c.*** PV99008, ***d.*** PV99009

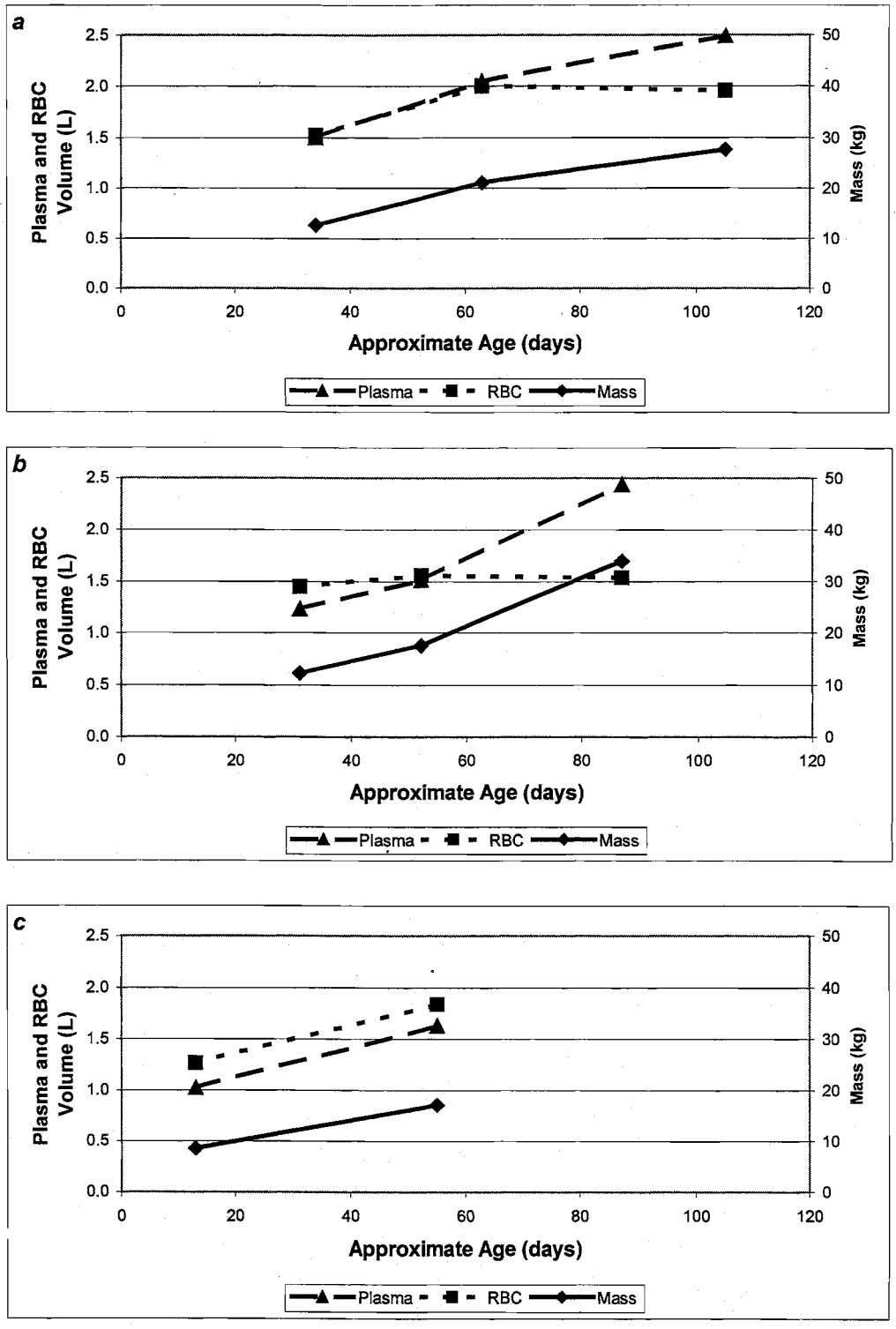
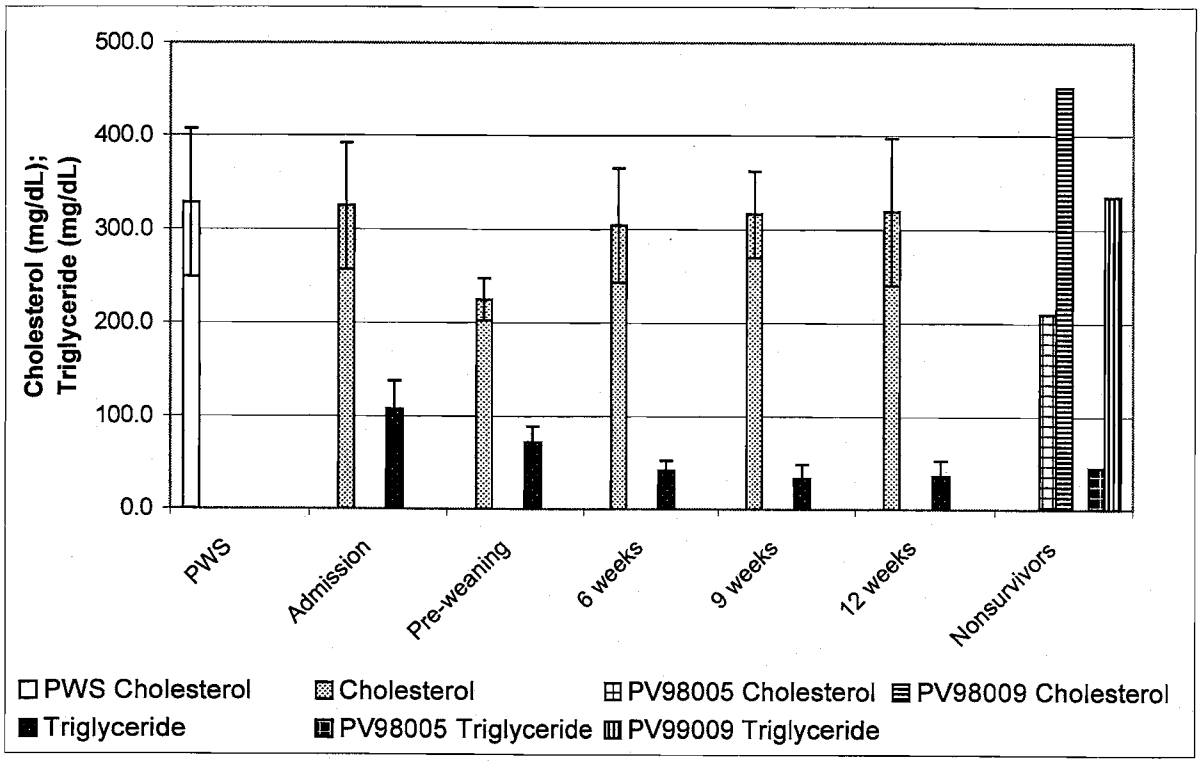
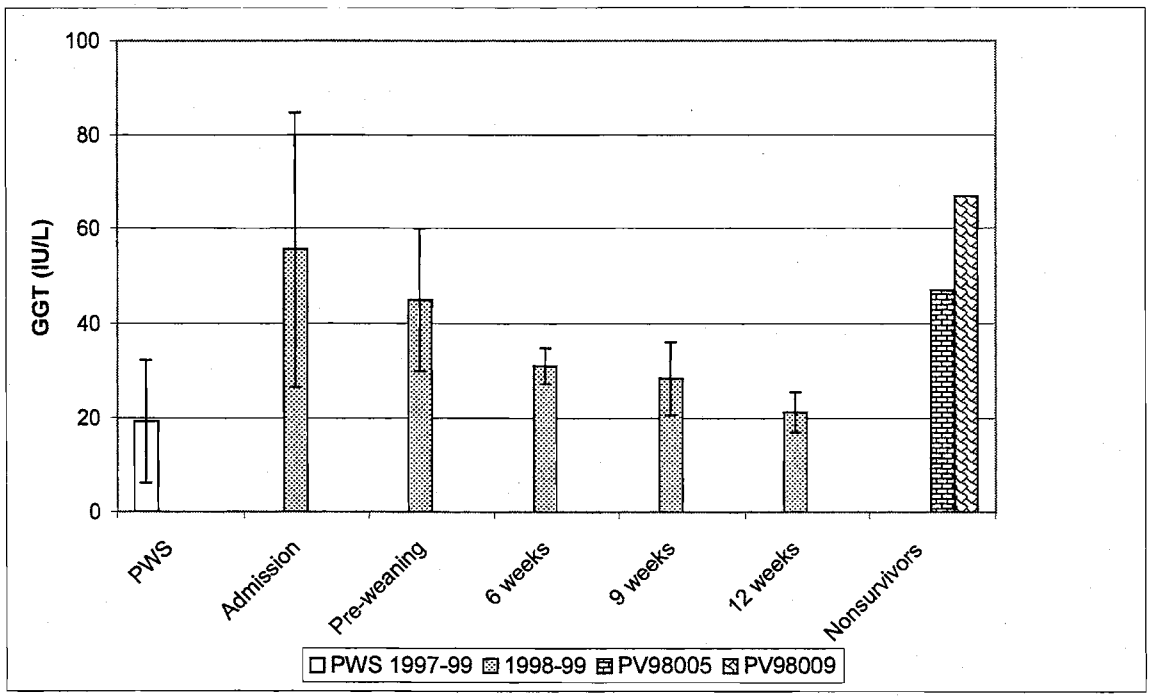


Figure 18. Plasma volume, estimated volume of red blood cell pool and mass as a function of estimated age in 3 harbor seal pups rehabilitated at the ASLC in 1999 ***a.*** PV99004 (Kenai), ***b.*** PV99009 (Iggy), ***c.*** PV99007 (Mackenzie)



Data for PWS pups taken from Small et al. 1999.

Figure 19. Serum cholesterol and triglyceride values from harbor seal pups rehabilitated at the ASLC during 1998-99. Data from wild harbor seal pups (PWS, 1997-99, n = 120) and two non-survivors from 1998 have been included for comparison. Values representing admission were from seals approximately 1 week old (n = 6) and those representing pre-weaning were from seals approximately 2 weeks old (n = 6). Pups from 6 –9 weeks old were post weaning (n = 6-7).



Data for PWS pups taken from Small et al. 1999.

Figure 20. Serum gamma-glutamyl transpeptidase values from harbor seal pups rehabilitated at the ASLC during 1998-1999. Data from wild harbor seal pups (PWS, 1997-99, n =120) and two non-survivors from 1998 have been included for comparison. Values representing admission were from seals approximately 1 week old (n = 6) and those representing pre-weaning were from seals approximately 2 weeks old (n = 6). Pups from 6 —9 weeks old were post weaning (n = 6-7).

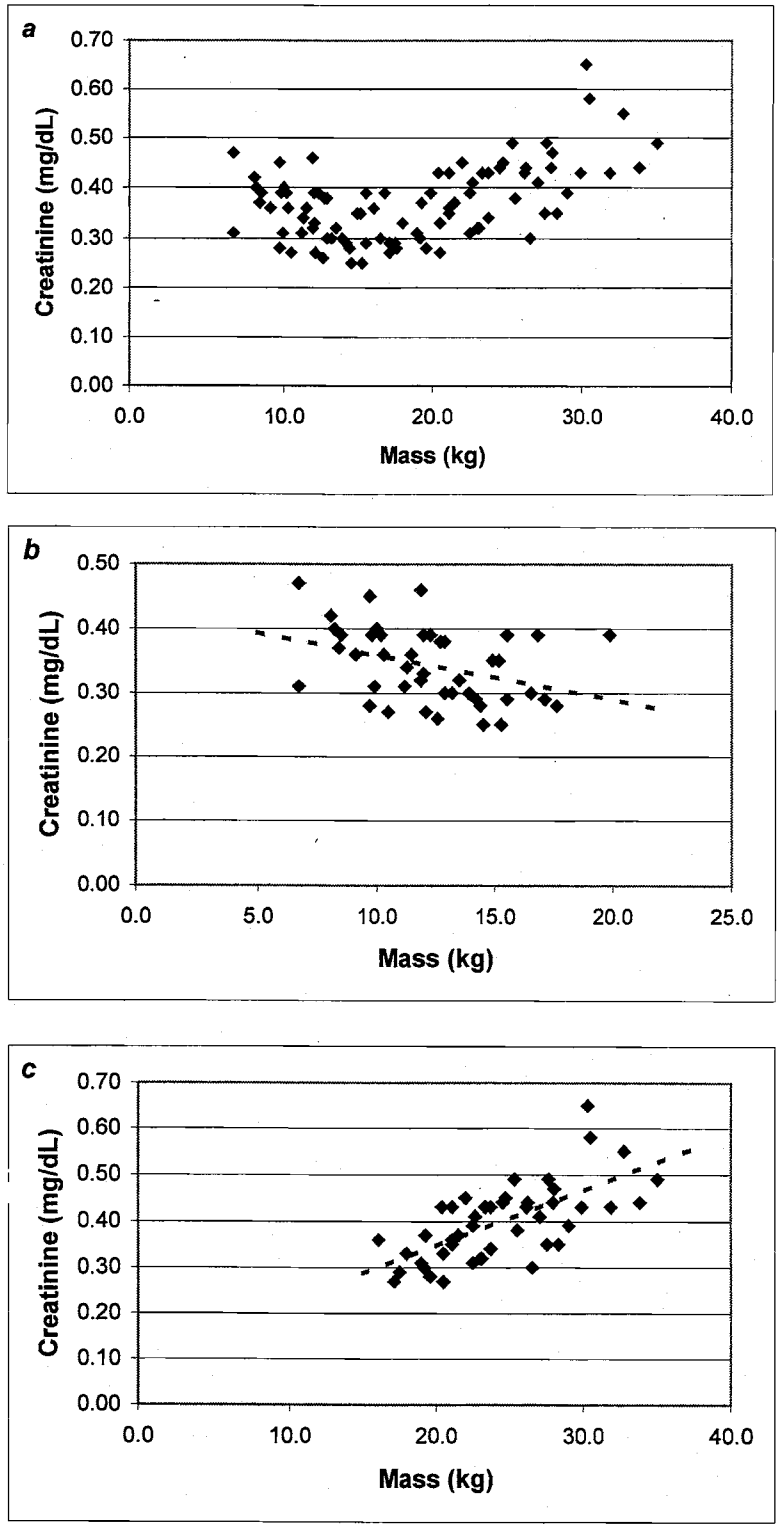


Figure 21. Serum creatinine levels as a function of mass. ***a.*** all values, ***b.*** values from seals less than 8 weeks old, $r^2 = 0.14$, $p = 0.01$ ***c.*** values from seals more than 8 weeks old, $r^2 = 0.45$, $p < 0.001$.

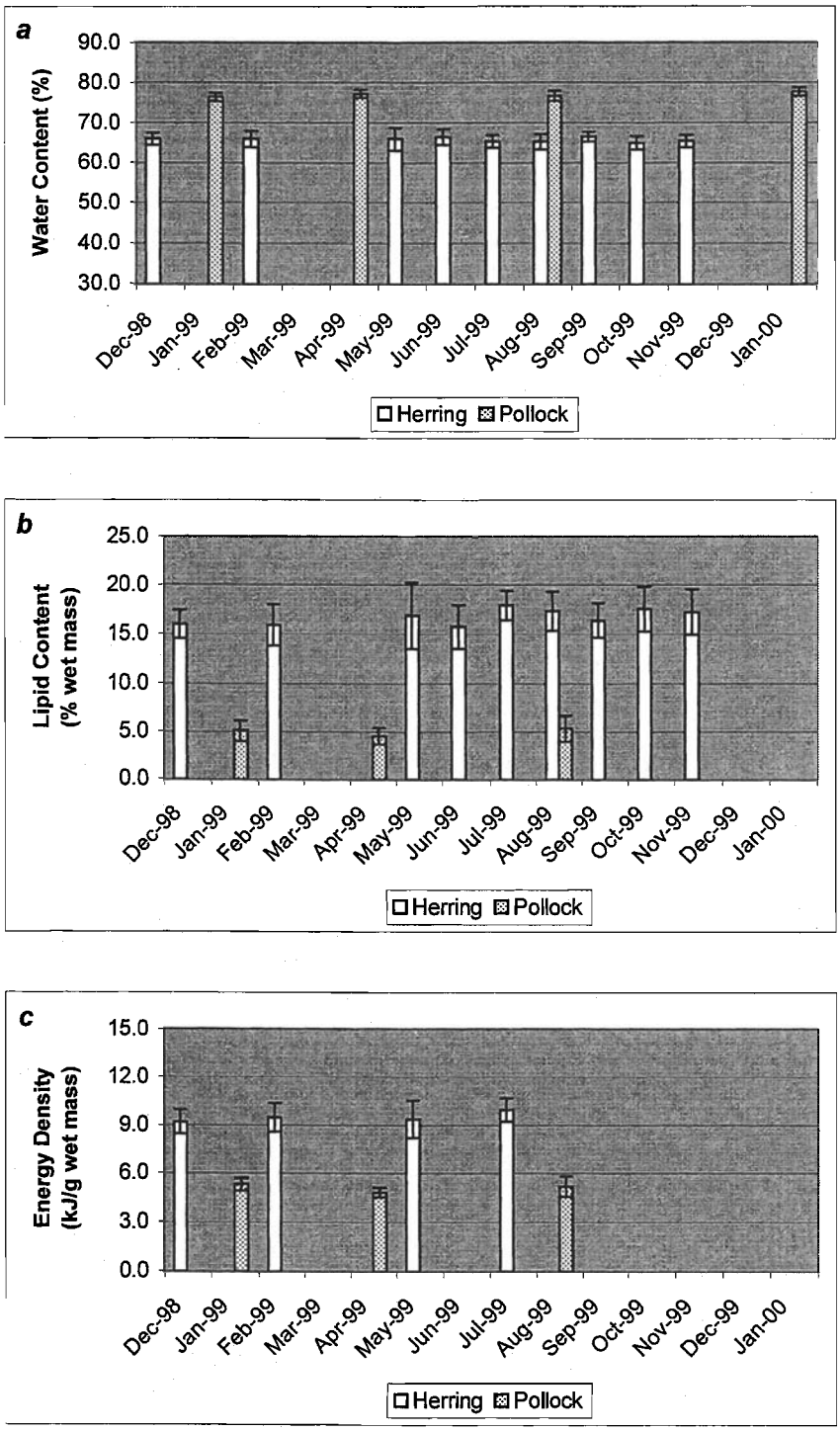


Figure 22. Proximate composition of herring and pollock fed to harbor seals during diet experiments. Fish were re-analyzed to determine the effect of long-term frozen storage. ***a.*** water content, ***b.*** lipid content (wet mass basis), ***c.*** energy density (wet mass basis)