Exxon Valdez Oil Spill
Restoration Project Final Report

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

Restoration Project 00341
Final Report

Michael A. Castellini
J. Margaret Castellini
Stephen J. Trumble

University of Alaska Fairbanks
School of Fisheries and Ocean Sciences
200 O'Neill Building
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Study History: This project grew out of a series of studies on the health of Prince William Sound harbor seals that began in FY93 as a Research Service Agreement with the Alaska Department of Fish and Game. In FY95 this assessment program was initiated as Restoration Project 95001 and a final report issued in FY98 (Fadely, Castellini and Castellini; *Recovery of Harbor Seals from EVOS: Condition and Health Status*). Multiple publications, reports and presentations were produced by that project. Results from those field studies led directly to the current Restoration Project 98341 – *Recovery of Harbor Seals. Phase II: Controlled Studies of Health and Diet*. This project was a two-year (1998-2000) controlled feeding study of herring and pollock to captive harbor seals at the Alaska SeaLife Center in Seward. Annual reports were issued in 1998 and 1999 under the same titles. Presentations of data from this project include: Castellini, J.M., S. Inglis, S. Trumble, and M.A. Castellini. Condition and health indices in rehabilitated harbor seal pups at the Alaska SeaLife Center. *13th Biennial Conference on the Biology of Marine Mammals.* 1999; Castellini, M.A. Seasonality in the Metabolism of Marine Mammals: From Blubber to biochemistry. *50th Arctic Science Conference*. AAAS. 1999; Mau, T.M., and M.A. Castellini. The effects of prey switching on erythrocyte fatty acids in the harbor seal. *FASEB Journal*. 14(4):A47. 2000; Castellini, M.A., J.M. Castellini, S. Trumble and T. Mau. How do dietary lipid levels impact the body condition and health of seals? *FASEB Journal.* 14(4):A440. 2000;Castellini, M.A. Using bio-electrical impedance to measure the body composition of seals and sea lions. *FASEB Journal.* 15(1): A90. 2001. Two Ph.D. projects will be completed in 2002 (Trumble and Mau) that will contain components of this project. The collection of data was closed out in 2001 and is summarized in this final report

Abstract: The goal of this study was to quantify the impact of specific diets on the health and body condition of harbor seals. This involved measuring the effect of diet on health status biomarkers which had been previously monitored in wild seals in Prince William Sound, determining whether specific diets were nutritionally adequate to maintain health in captive harbor seals and examining seals brought into the Alaska SeaLife Center for rehabilitation. In the first year of this project, eight harbor seals were brought to the ASLC, assigned to one of three groups, and acclimated to their new environment and diets. In September of 1998, the seals began two year feeding trials. They were fed either only pollock or herring or a mixed diet for 4 months at a time before switching diets to correct for seasonal changes in metabolism. They were weighed, measured and had blood taken every two weeks. At the end of each 4 month trial, their body condition was measured and they were placed into detailed digestion trials to assess their ability to process the different fish diets. In addition, each spring and summer, harbor seal pups brought to the ASLC for rehabilitation were monitored for changes in body condition and blood chemistry throughout recovery. The results suggest that there are a suite of blood chemistry variables that react to season and/or diet and also a group that are independent of diet or season. In addition, the body condition of the seals is strongly seasonally dependent such that all three diets (pollock, herring, mixed) showed some periods of body condition declines and other

periods of condition enhancement. Changes in digestive physiology indicate that animal age, diet and season all are factors that play into the variable response to different prey items. Thus, we conclude that simple measurements of prey "quality" (i.e., fat content) do not predict the body condition or health of the seals and that field assessments of harbor seal body condition and blood chemistry can be linked to diet and season. Analysis of blood parameters and morphometric measurements of rehabilitated harbor seal pups reveal changes indicative of several factors, including development, captivity, early weaning and disease or injury and provide validation for the use of statistical outliers to assess overall population health in the wild.

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

Project Data: *Description of data* - The data collected during this project include blood chemistries taken every two weeks for 2.5 years from 8 harbor seals at the ASLC in Seward. These blood data include values for hematology and veterinary chemistry. Additionally, body morphometric data were collected on the same schedule. Every four months, body condition values (percent fat and lean tissue) were collected. Proximate analysis of the prey items (pollock and herring) were measured. Digestive data for all seals were also collected every four months. Blood chemistries and morphometrics were taken every week for 15 rehabilitated harbor seal pups. *Format* - The aforementioned data are recorded on Microsoft EXCEL and ACCESS data sets. *Custodian* - Dr. Michael Castellini, Institute of Marine Science, University of Alaska Fairbanks, Fairbanks, AK 99775. Phone: (907) 474-6825; email: mikec@ims.uaf.edu

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EXECUTIVE SUMMARY

Harbor seal (*Phoca vitulina)* populations in Alaska have declined significantly over portions of their range, particularly in the Gulf of Alaska. Prince William Sound (PWS) populations, further impacted by the spill, continue to decrease. Previous work by our laboratory (EVOS project /001) demonstrated strong seasonal and regional variations in the body condition (morphometric and fat indices) and blood chemistry (hematology and clinical chemistry) of wild harbor seals throughout this region. While blood values are known to vary with disease (Bossart and Dierauf 1990, Roletto 1993), little is known of the specific effects of diet or season on these variables within an otherwise healthy population. Our previous field work was not able to verify if diet was a significant factor in the observed variation between populations or how season and diet might combine to produce variation. The current project (/341) was designed as a controlled laboratory experiment to determine how diet, season and health status impacts body condition and blood chemistry in harbor seals.

This final report summarizes work accomplished under Restoration Project /341 which was carried out at the Alaska SeaLife Center in Seward, Alaska from 1998 to 2000. The project was conducted with a group of eight resident adult harbor seals and 15 harbor seal pups brought in for rehabilitation.

Objectives

The objectives set forth in this multi-year project were:

1. Feed controlled diets of pollock and herring 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 different fish diets for harbor seals.
4. Quantify seasonal, metabolic state and clinical health impacts on biomarkers and health indices.

Objective 1: Feeding trials

Eight harbor seals were moved to the Alaska SeaLife Center in March/April of 1998 from other captive programs in North America and trained to be part of the scientific research program at the Center. The animals were both adults and young juveniles. From April to September of 1998, the animals were acclimated to feeding protocols, handling and blood sampling methods. In the fall of 1998, a repeated crossover feeding matrix was initiated that was designed to run for two years. In this design, the seals were assigned into three study sections that comprised a group of three adult seals, a group of three younger seals and a group of two adult seals. The two adult seals were fed a mixed diet of 50% pollock (*Theragra chalcogramma*) and 50% herring (*Clupea harengus*) for the entire two year study. The other two groups rotated from a diet of 100% pollock to 100% herring every four months with changeovers in September, January and May. These seasons were chosen to match the periods of breeding (spring), molting (fall) and winter. All the fish were from large, frozen batches and thus held constant throughout the trials. Proximate analyses of the fish were conducted throughout the two years to verify that the quality of the fish (based on lipid, protein, water content and calorimetry) remained constant. Herring had substantially higher lipid content (herring: 16.8 ± 2.2 %, wet mass, n = 104; pollock: 4.9 ± 1.1 %, wet mass, n = 34) and energy density (herring: 9.3 ± 0.8 kJ/g wet mass, n = 80; pollock:

4.9 ± 0.5 kJ/g wet mass, n = 35) than pollock. Fish were stored long-term during the feeding trials at -20º C. Storage time had no effect on gross water content, lipid content or energy density of either the herring or the pollock.

There were a number of factors that might influence morphology and health indices apart from those being investigated such as age, sex, activity level, social factors, temperature, light level, etc. This study was designed to allow interpretation of the data with respect to diet and season, in spite of the potential influence of these factors. This was achieved by the use of a repeated crossover design in the feeding trials. Such a design allowed each seal to function as it's own internal control and in the course of the two year trial each seal was exposed to the different dietary treatments in each of the three seasons. In this way it was possible to identify changes that were specific to diet or season and those that appeared to depend on both factors in spite of inter-animal variability (such as sex, age, activity, etc). The seals were all housed in large outdoor pools subject to ambient temperature and light, two variables that would be encompassed in any seasonal variations. The pools provided significant space for both swimming and haul-out, and while activity levels were not measured, the seals were generally active and housed in equal size groups. The fact of captivity itself could influence many of the indices we were monitoring, but since captivity was a condition shared by all seals in this study, any differences observed between experimental treatments would not be due to the fact of captivity.

Seals were fed 2-3 times per day to a level of satiation that would allow training methods to continue using routine animal handling protocols by the husbandry staff at the ASLC. Thus, the diets were not designed to be iso-caloric. This was an essential part of the experimental design – to allow the seals to adjust their intake naturally and examine if they could match intake to energy requirements. For training purposes, the animals were handled everyday at feeding times and trained to be weighed, measured and bled.

At each 4 month switchover time, the animals were measured for body condition (deuterium determination of water content) and then took part in a 48-72 hr digestion trial study. After the digestion trials, the seals were switched gradually to the next diet regime over a 2 week period at which time blood samples were again initiated.

Objective 1 summary. This objective was fully met. All eight animals completed the feeding trials except for one young seal that did not participate in Trial #5 because of an unrelated medical problem. This seal was assessed by the veterinary staff and returned to the study for subsequent trials. At the end of the trials in September 2000, all eight animals were returned to routine handling and feeding patterns established by the ASLC. The animals (except for the situation noted above) remained healthy on all three diets as assessed by the ASLC veterinarian throughout the experiment and participated in other simultaneous studies funded by both EVOS (Davis project /441 and Schell project /371) and other agencies.

Objective 2: Body condition, health, and blood chemistry changes during the feeding trials

Throughout the two year study, blood samples were collected every two weeks from each seal and masses were measured at least once per week. Over 400 blood samples were collected during the two year period. Length and girth values were taken approximately every two weeks. At the end of a diet switch-over at the four month mark, measurements of seal body condition

were made via total body water by deuterium dilution.

All blood samples were assayed for a suite of hematology and clinical chemistry parameters. Hematology included hematocrit and hemoglobin while clinical chemistry values included 24 different plasma or serum measurements for protein, lipid, carbohydrate, organ function, water content and osmolyte status.

The results from the blood and body condition examinations were matched to diet and season in order to test whether these two factors influenced blood chemistry or condition. Of the blood markers tested, five showed relationships to diet, 5 showed relationships to season and the remainder did not respond to either diet or season.

Body mass fluctuated based on the diet regime and season. Detailed analysis of each of the six feeding trials showed that seals could gain or lose mass on all three diets of just pollock, just herring or a mixed diet. Depending on their age and the season, the seals could either gain or lose body fat on herring, pollock and mixed diets. For example, in the winter/spring trial (2000), the young seals lost body mass and fat on pollock, but gained mass and fat in the next trial on pollock during the summer. During the winter/spring they gained mass but lost body fat on herring, while during the following summer they gained both mass and fat on herring. Thus, even though the fish quality was constant, the seals demonstrated an internal seasonal modulation of metabolism and/or intake that altered both their mass and their body condition. Therefore, body condition could not be predicted based on the food item alone.

Objective 2 summary. This objective was fully met. All seals were monitored for blood values, mass and body condition for the entire two years during all six feeding trials. The results indicate that there are suite of blood variables that respond to diet, some that respond to season and some that were not responsive to either diet or season. Neither changes body mass nor body condition were able to be predicted solely on the basis of diet.

Objective 3: Digestion trials

At each four-month diet regime switch, the seals were subjects in digestion feeding trials. This involved placing the seals in metabolic cages for 48-72 hours and feeding them controlled amounts of herring and pollock (depending on which diet regime they had just completed) at measured rates. The fish contained tracer chemicals that allowed us to calculate food passage rates. All feces and urine samples for the digestion experiments were collected and quantified.

Digestive efficiency, the proportion of ingested prey that is digested and absorbed and thus converted to usable energy, reflects the nutritional efficacy between an animal and its food resources. We measured food intake, mean retention time, and assimilation efficiency of herring or pollock diets in harbor seals fed once per day or four times per day. As with the results from the body condition studies, we found that values for digestive efficiency varied with diet and season as well as animal age and feeding pattern. Based upon mean retention time, the animals retained herring longer than pollock, probably to extract more of the higher lipid content from that species. However, digestive efficiency was altered by feeding rate such that animals fed 4x per day decreased their retention time in order to process the greater volume of food. Furthermore, the young harbor seals had generally higher retention times than the adults. Thus, the ability of the animals to process the two different food items was extremely plastic and responded to both the type of food and the amount of food.

Objective 3 summary. This objective was fully met. All seals participated in the detailed digestions trials for both years. Taken together with the evidence for seasonal shifts in metabolism, the data suggest that the seals respond to differing diets through a suite of physiological and biochemical adaptations that allow them to alter their digestive physiology as appropriate for the season, their age and their diet. Our data do not support the concept that digestive efficiency can be predicted based solely on the type of food item consumed.

Objective 4. Quantify seasonal, metabolic state and clinical health impacts on biomarkers and health indices

The combined data from the blood sampling, body condition and digestive efficiency trials indicate that harbor seals show biochemical, physiological and metabolic differences based on season, diet and age. However, they also demonstrate that the seals respond in a complex manner that cannot be predicted solely on diet. Thus, our data suggest that measuring any given variable (blood value, mass, body fat) in only one season or location in the field would not be sufficient to predict health status. Fadely et al. (1998) reached the same conclusion based upon the variance seen with season, location and age in the blood chemistry and body condition measurements of wild-caught harbor seals. We have provided the laboratory verification in this project that begins to describe the mechanisms by which that variability is maintained in the population.

The Fadely et al. (1998) EVOS report suggested that animals outside the normal distributions of health parameters might be important indicators of overall health status of populations of seals. In that report, we defined the concept of "outlier" theory that described how these animals could be used to monitor population health. Outliers could be a result of overt physiological problems or subclinical conditions that would not be readily apparent on the basis of a cursory external field exam. However, the sampling protocols in the wild could not select for sick or malnourished animals and there was some question as to how responsive blood chemistry variables might be to moderate or subclinical stress. Therefore, in the current project, we examined harbor seal pups that were brought to the ASLC for rehabilitation. We examined these pups for the same blood chemistry and body condition indices that we used in the captive feeding trial program. The assumption was that the occurrence of outliers would be more prevalent in these seals and thus validate the use of this analytical technique in assessing wild populations. If seals are dying in the field due to sickness or health related issues, then these pups should represent the animals that would not have survived had they not been brought in for treatment.

During the course of this study (1998 – 2000) 15 harbor seal pups were admitted to the ASLC rehabilitation facility and assessed by the staff veterinarian. Of those fifteen pups, 5 (Group A) were assessed as possibly undernourished or dehydrated with no further clinical symptoms; 5 (Group B) exhibited clinical symptoms indicative of moderate disease or injury from which they recovered and 5 (Group C) exhibited symptoms of severe disease or injury and either died or were euthanized. Seals from Group C had a much higher incidence of blood chemistry values outside of the normal reference ranges (outliers), than either Groups A or B. While most mean blood chemistry values were not statistically different between Group A and B, variability was often much higher in Group B as was the incidence of values falling outside normal ranges. These differences between Groups A and B often persisted for some time into recovery when the seals were no longer receiving veterinary treatment and no longer exhibited

external symptoms. While it may not be surprising that blood chemistry perturbation may precede external symptoms and may persist well beyond the time when symptoms or injuries are visible, blood chemistry values are often not the basis for determining the relative health of individuals or populations in the wild. In many studies where a random healthy sub-sample is desired, inclusion of specific individuals is often based on cursory external observation (eg. no lesions). Well established blood reference ranges, coupled with outlier analysis would provide a wider window through which to view population health, including animals with no obvious external symptoms and animals that have recently recovered.

Objective 4 summary. This objective was fully met. Seasonal and dietary impacts on health, body condition and digestive efficiency were described for the feeding trials. Seals at the ASLC for rehabilitation were examined for patterns between blood chemistry, growth, health and outlier theory to better link field studies with clinical and experimental results.

Conclusions

In this project we were able to successfully carry out a two year feeding trial with seals fed either 100% pollock or 100% herring. In our repeated crossover design, we corrected for season so that any given seal ate either pollock or herring in each season. Consequently, each animal served as it's own internal control, reducing differences caused by variability among individuals. These long-term trials (4 month) enabled us to achieve stable results within each period and therefore to measure biochemical and physiological adaptations to the diets. We found the seals remained healthy on all diets and that their responses varied by season, diet and age. Thus, the conclusions one would draw from a particular season or age (eg. adults in the summer) might be entirely different from the results from a different season or age (eg. adults in the winter or juveniles in either period). We conclude that it is not possible to predict body condition, energy balance and responses to diets based only on the type of fish fed.

We also demonstrated that blood chemistry responded to diet and season and our laboratory verifications can be used to better interpret the blood chemistry data available from field studies on harbor seals.

Digestion studies showed that the seals modified their retention time based on diet and feeding frequency to best extract energy from the various fish diets. Combined with the body condition work, this component of the project further supports the theory that the animals can adapt to differing diets and that seasonal aspects of metabolism are critical.

Finally, our work with rehabilitated pups demonstrates that blood chemistry can be used to identify outliers in populations of seals and that simple visual inspection of animals is not enough to assess health status.

We conclude that harbor seals respond to dietary shifts in a seasonally appropriate manner and that body condition, energy balance or "health" cannot be predicted solely on the basis of the type or quantity of fish consumed.

INTRODUCTION

OBJECTIVES

The objectives for this multi-year project were:

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.
4. Quantify seasonal, metabolic state and clinical health impacts on biomarkers and health indices.

An underlying component of the ecosystem-based approach supported by the *Exxon Valdez* Oil Spill Trustee Council has been the hypothesis that food quantity or quality limitations 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 (APEX program project, 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 necessarily 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 blood values for captive harbor seals have been derived from only a few studies. These studies (Englehardt 1979, McConnell and Vaughan 1983, Bossart and Dierauf 1990) 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 that 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 examined 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, de Swart et al., 1995). In our study we did not feed contaminated fish, but rather fish of differing energy density (herring and pollock). In addition we monitored harbor seals that were brought into 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 in Alaskan marine mammals (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.

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 was designed to assess the nutritional needs of harbor seals over long periods and different seasons. Included in this design was a series of experiments in which assimilation efficiency, retention time and metabolizable energy were 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).

We did not attempt 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 investigated the metabolic response to differing diets and the effect of these diets on blood chemistry and body condition of the animals. That is, we did 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.

There were a number of factors that might influence morphology and health indices apart from those being investigated such as age, sex, activity level, social factors, temperature, light level, etc. This study was designed to allow interpretation of the data with respect to diet and season, even with the potential influence of these factors. This was achieved by the use of a repeated crossover design in the feeding trials. Such a design allowed each seal to function as it's own internal control and in the course of the two-year trial each seal was exposed to the different dietary treatments in each of the three seasons. In this way it was possible to identify changes that were specific to diet or season and those that appeared to depend on both factors in spite of inter-animal variability (such as sex, age, activity, etc). The seals were all housed in large outdoor pools subject to ambient temperature and light, two variables that would be encompassed in any seasonal variations. The pools provided large spaces for both swimming and haul-out, and while activity levels were not measured, the seals were generally active and housed in equal size groups. The fact of captivity itself could influence many of the indices we were monitoring, but since captivity was a condition shared by all seals in this study (both resident and rehabilitated), any differences observed between experimental treatments would not be due to the fact of captivity.

METHODS

Animal handling and feeding schedule

In the spring of 1998, eight harbor seals were transferred to the ASLC from other holding facilities in North America as listed in the following table:

Name	Poco	Skeezix	Cecil	Snapper	Sydney	Tina	Pender	Travis
Age	23	23	15	14	8	7	2	2
Sex	M	F	M	F	F	F	M	M

All seals were housed in large outdoor pools with more than adequate space for swimming and haulout. During the spring and summer of 1998, they were acclimated to the new facility and trained to accept the handling methods necessary for this and other experiments. During this time, they were fed exclusively on herring. The seals were trained to be fed individually, so that animals on different diets could be held in the same tanks. Also, by individual training, it could be verified that each seal received only its own fish. The seals were under the constant supervision of the ASLC staff and veterinary services. They also took part in other projects, funded by both EVOS (Davis project /441 and Schell project /371) and other agencies. The seals were fed to a level of satiation as determined by the handlers to a point where they would still respond to training commands. The diets were not designed to be iso-caloric and the mass of food could vary throughout the experiment.

In September of 1998, the seals were divided into three study groups and the repeated crossover study design was initiated. As shown in the table below, this design allowed each seal to receive an exclusively herring or pollock diet in each of the three metabolically defined seasons. We defined three different periods for the seals: The breeding period from May to September, molting from September to January and the late winter/early spring period from January to May. While these periods reflect the timing of different physiological states in wild seals, they clearly overlap with physical seasons, encompassing differences in light and temperature. Any observed seasonal effects may be a result of a number of factors that are seasonally dependent, including physiological state, metabolic demand, light, temperature, activity, etc. As for individual variation among seals such as activity level, appetite, etc., the crossover nature of the experimental design allowed each seal to function as its own internal control, reducing the effects of such variability.

PERIOD	HERRING	POLLOCK	CONDITION
Sept 1998 - Jan 1999	**A**	**B**	**Season 1 (Molting)**
Jan - May 1999	**B**	**A**	**Season 2 (Spring)**
May - Sept 1999	**A**	**B**	**Season 3 (Breeding)**
Sept 1999 – Jan 2000	**B**	**A**	**Season 1 (Molting)**
Jan – May 2000	**A**	**B**	**Season 2 (Spring)**
May – Sept 2000	**B**	**A**	**Season 3 (Breeding)**

Group A seals included the younger animals, Pender, Sydney and Travis, while Group B seals included the older seals Cecil, Poco and Skeezix. Two additional seals (Tina and Snapper) were fed a mixed diet of 50% herring and 50% pollock throughout the study.

This feeding matrix allowed 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 season 1 (molting) in year one and fed a pollock diet during season 1 in year two.

Proximate analysis of prey items

The feeding regime experiment was designed so that the food quality remained constant throughout the two-year period. The goal was to discern how diet type and season impacted the biology of the seals, therefore, it was essential that the quality of food did not change with time. Herring and pollock were purchased in large commercial batches and held at -20°C in the ASLC food storage freezer. The fish were packed in 40 pound, watertight boxes that were opened and thawed on the day of feeding. The freezer was regularly inspected by ASLC animal care staff and by the USDA for fish storage quality according to Federal guidelines.

Individual batches of herring and pollock were sub-sampled (n = 10 for each) periodically (at least once during each 4 month 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 an 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 on the basis of wet mass. Lipid content was determined as the mass difference after extraction of dry samples (initial mass 0.3 – 0.5g) for 24 hours in a 2:1 chloroform/methanol mixture in a Soxhlet extraction apparatus.

Energy density was determined with an adiabatic bomb calorimeter (Parr Co.) using pelleted dry samples (0.3 – 0.6 g).

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.

Several other batches of fish used as food at the ASLC were analyzed in addition to those used as food in this study. The batch numbers for fish used as food in the harbor seal feeding trials were H3, H4 and H6 (herring) and P2, P3 and P5 (pollock). Batch H3 was used only for a very short time, and none were saved for analysis.

Body condition

The harbor seals were trained to allow measurements of standard length, girth and mass without restraint. The animals were weighed on a platform load cell scale (± 0.1 kg). These morphometric measurements were made at a minimum of once every two weeks and many times more often depending on training schedules.

Once every four months, at the time of diet switching, the seals were measured for body condition assessment. This involved measurement of whole body water via deuterium dilution methods.

Body water determination. At the beginning of the determination, a blood sample was collected and then standard deuterium dilution techniques for the estimation of total body water (TBW) were utilized by injecting the seals (i.m.) with sterile, 99% enriched deuterated water. The syringes were weighed before and after injection to determine the administered dose. A post dose blood sample was collected after two hours and both samples were spun in a clinical centrifuge to prepare serum. The serum was frozen at -80°C and then shipped on dry ice to analytical facilities (Metabolic Solutions, Nashua, NH) for determination of enrichment. A sample of the injection dose was sent under separate cover. Dilution mathematics, assuming single pool distribution, were used to estimate the TBW from the enrichment of the serum samples. Because deuterium over-estimates TBW by about 4%, the calculated TBW was reduced by 4% to arrive at the estimated TBW for each animal (Metabolic Solutions Technical Paper #913).

Body condition (fat, lean tissue mass) and mass were analyzed using a suite of statistical tests (ANOVA, Kruskwall-Wallis ANOVA, ANCOVA and regression) as detailed in the individual results section. Significance was set a $p = 0.05$.

Blood sampling

Once every two weeks, the seals were either manually restrained on a custom made stretcher-board or, in some cases, the seals accepted blood sampling on a voluntary basis and would lie unrestrained while blood was taken. Blood was sampled from the intravertebral 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).

Blood Chemistry and Hematology. For each seal, up to 20 ml of blood was collected for serum, 25 ml for plasma, and 10 ml in ethylenediaminetetraacetic acid (EDTA) tubes for hematology. 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. 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.

Serum samples were assessed for standard clinical health indices and also analyzed for indicators of malnutrition and other stressors. 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 were determined using Drabkin's reagent and performed in our laboratory.

Digestion trials

Once every four months just prior to the diet switchover, the eight captive harbor seals were acclimated to individual indoor cages with 12 h light per day and temperatures ranging from 7^{o}-15^{o}C. Two cage sizes were used during this study, for the adult seals a larger 2.2m long x 1.1 m wide x 1.5m high while a smaller 1.8m long x 0.9 m wide x 1.2 m high was used for the sub-adult animals. Cages had mesh floors (8 cm^2) suspended over aluminum trays, which were used to collect excreta. Collection trays were positioned to allow urine to funnel to a collecting receptacle that assured separation from fecal material. Daily feed intake and excretion were measured over six periods (for each feeding frequency) of three days for caged seals with each on a particular diet approximately 90 days prior to caging. Previously frozen fish was provided at regular intervals during the four feedings per day whereas during the once per day feedings the seals were fed in the late mornings. Seals were allowed to consume as much fish as they wanted per feeding (maximum intake). Subsamples (5-10) of fish from each feed batch were used for later proximate analysis. Fecal samples were collected, when available, from collection trays every two hours throughout the experiment for up to 80 hours. All fecal samples collected were placed in plastic bags and stored frozen at –20°C until analyzed.

The influence of feeding frequency on mean retention time was assessed by feeding fish according to two protocols: once per day and four times per day for each digestion trial. Each seal was placed on a particular feeding frequency regime one week prior to caging. During each digestion trial seals were given a single oral dose of chromic oxide (Cr_20_3) and cobalt-ethylenediaminetetraacetic acid (Co-EDTA), which were used as markers of the digesta solid and liquid phases, respectively. These non-toxic passive markers have been widely used in food

passage studies in numerous species. Both markers were administered as a powder placed in gelatin capsules and placed in the opercular cavity or muscle of fish fed during the first morning meal of the feeding trial. Dose rates were 2500 mg of each marker per animal.

Fecal dry matter content was determined by freeze drying samples for 72 hours or until dry weights were consistent. Dried fecal samples were ground to a fine powder using either a mill grinder or mortar and pestle. Duplicate samples (0.25g) were assayed for Cr and Co using chemical digestion in a mixture of 70% v/v HNO_3 (1000 ml), 32 M H_2SO_4 (200 ml), 70% v/v $HCLO_4$ (343 ml) and deionized water (57 ml). Digestions were performed at 165^0C for 15 min followed by 315^0C for 35 min. Digests in duplicate were diluted with distilled, deionized water and assayed by atomic absorption spectrometry (Model 5000, Perkins Elmer, Norwalk, CN).

Statistics and data analysis. Values of intake and digestion were compared by ANOVA with repeated measures within each group for each feeding trial and feeding regime. An ANCOVA tested for the effects of two covariates, body mass and age, on mean retention time for all treatments. Least linear regressions were used to assess the relationship between mean mass and MRT values for each feeding trial. MRT data were standardized to body mass for comparison among individual seals. All percentage data were arcsine transformed prior to statistical analysis. Statistical significance was set at 0.05. Means are reported with 1 SE. Marker concentrations for each animal for each feeling trial and regime were plotted against time after dosing. Mean retention time was calculated by the equation:

$$MRT = \frac{\sum_{i=1}^{\mu} t_i c_i \cdot \Delta t_i}{\sum_{i=1}^{\mu} c_i \Delta t_i}$$

where c_i is the concentration of the marker in the $_i$th sample, collected at time t_i, over the time interval Δti from i=1 to μ.

Percent apparent digestibility was calculated by total balance method in which all feed ingested and feces produced were weighed and analyzed (Robbins 1993).

$$\text{Apparent Dry Matter Digestibility (\%)} = \frac{\text{Food intake} - \text{Fecal dry matter}}{\text{Food intake}} \times 100$$

Rehabilitation studies

Animal Handling and Sampling. Animal handling and morphometric sampling was the same as for the resident seals in the feeding study, except for a few minor variations. The pups

were manually held without the use of a board. The needles were smaller (1 – 2.5", 18 – 20G) and the total volume of blood drawn varied between 20 – 30 ml. Animals were sampled weekly. The approximate age and condition of the seals was assessed upon admission and subsequently throughout rehabilitation by P. Tuomi, DVM-ASLC. Sampling at the time of admission preceded formula feeding although it did not always precede rehydration since attempts were often made to rehydrate prior to transport.

One major difference between the handling of rehabilitated seal pups and the resident seals was housing. The pups were admitted to an inside quarantine facility with limited activity and no contact with other animals. This gradually changed with pups being given increasing access to water as they became bigger and stronger. Finally, once the initial quarantine requirements were fulfilled and the pups were past any medical crisis, they were moved to outside holding tanks with other rehabilitated pups. At this point, they were fed as a group and had to compete to some degree for food. This was all accomplished at the discretion of the rehabilitation and veterinary staff of the ASLC. It is possible that such changes in housing and social environment could affect some of the health parameters investigated in this study. We have attempted to take this into consideration.

Most harbor seal pups admitted to the ASLC for rehabilitation were less than 2 weeks old (Table1) and were initially fed formula. The composition of formula and ages of weaning varied among years and individuals. In 1998, formula was supplemented with vegetable oil, while in 1999 and 2000 fish oil was used. Vitamins (SeaTabs© or Mazuri Vita-Zu©) were given to the animals in all years, but B_{12} and iron were not added until July 2000. In 1998 pups were weaned quickly (2 – 4 days) and as soon as possible (approximately 2 weeks old), while in 1999 and 2000 seals were weaned later (3 – 6 weeks old) and more gradually (3 – 10 days). In the two later years, timing of weaning was controlled by the willingness of the pup to accept fish and was also delayed in some cases by health issues.

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, and manual measurements of hematocrit, red blood cell count and hemoglobin. Chemistry analyses included albumin (ALB), alkaline phosphatase (ALKP), alanine transferase (ALT), amylase (AMYL), aspartate aminotransferase (AST), blood urea nitrogen (BUN), creatinine (CREA), the ratio of BUN/Creatinine (BUN/CREA), calcium (Ca), cholesterol (CHOL), creatine phosphokinase (CK), gamma-glutamyl transaminase (GGT), glucose (GLU), phosphorous (PHOS), total bilirubin (TBIL), total protein (TP), globulin (GLOB), the ratio of albumin/globulin (ALB/GLOB), triglyceride (TRIG), sodium (Na), potassium (K), and chloride (Cl).

Plasma haptoglobin concentrations were determined using a colorimetric assay from Tri-Delta Diagnostics, Inc (Morris Plains, New Jersey 07950). The assay was validated by spiking harbor seal plasma with known concentrations of haptoglobin and by serial dilution of plasma samples with high concentrations of haptoglobin. In addition to samples collected from harbor seal pups undergoing rehabilitation, plasma haptoglobin concentrations were measured in 5 harbor seal pups sampled in Prince William Sound (PWS) in 1997.

Plasma Volume. Plasma volume was measured in six pups in 1999 and 2000 according to the methods of Zweens and Frankena (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. Plasma was separated and measured spectrophotometrically for Evans Blue concentration. 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. For the purposes of analysis, pups were assigned to one of three groups based upon relative health as assessed by the staff veterinarian at the ASLC. Of the 15 pups undergoing rehabilitation 5 were assessed as possibly undernourished or dehydrated upon admission to the ASLC, with no other clinical symptoms (Group A); 5 exhibited clinical symptoms indicative of moderate disease or injury from which they recovered (including one seal that appeared upon gross examination to be normal but exhibited a failure to thrive starting at 10 weeks old) (Group B); 5 exhibited symptoms of severe disease or injury and either died or were euthanized (Group C).

Data were analyzed both with respect to time and health status. Most seals from Group C died within 1 - 2 weeks of arriving at the ASLC, so analysis as a function of time was not possible. Statistical analyses were conducted using Systat 9.0. They included regression and correlation analyses. Temporal and health status effects were determined using Kruskal-Wallis analysis of variance (central tendency) and F-tests (variance). Although small sample size within each group limits statistical analyses, boxplots of blood chemistry values are presented to illustrate differences in variability between groups.

RESULTS

All differences reported were significant ($P < 0.05$), unless otherwise stated (ie. not significant). This includes reference to values having increased, decreased, being elevated, reduced etc. Apparent differences that were not tested or were not significant are pointed out within the text.

Proximate analysis of prey items

Compositional analysis revealed that herring used for this study had substantially higher lipid content (herring: 16.8 ± 2.2 %, wet mass, $n = 104$; pollock: 4.9 ± 1.1 %, wet mass, $n = 34$) and energy density (herring: 9.3 ± 0.8 kJ/g wet mass, $n = 80$; pollock: 4.9 ± 0.5 kJ/g wet mass, $n = 35$) than pollock. Fish were stored long-term during the feeding trials at -20º C. Storage time had no effect on gross lipid content (Figure 1) or energy density (Figure 2) of either the high fat herring or the lower fat pollock. A new batch of herring (H6) and pollock (P5) was used in the final feeding trial. These new batches of fish had the same lipid content and energy density as the previous batches (H4 and P3, respectively) (Figure 1-2).

As expected, energy density was strongly dependent on lipid content for both herring (Figure 3; $r = 0.963$, $r^2 = 0.927$ wet mass basis; $r = 0.902$, $r^2 = 0.811$ dry mass basis) and pollock (Figure 4; $r = 0.819$, r^2 0.666 wet mass basis; $r = 0.708$, $r^2 = 0.493$ dry mass basis). Lipid content (wet mass basis) was negatively correlated with water content in both herring (Figure 5a) and pollock (Figure 5b). This relationship was much stronger for herring (*Corr* = 0.966) than for

pollock (*Corr* = 0.793). Fish from other batches besides those fed to harbor seals were included in this analysis to provide a broader range of values.

When large (15 – 27 cm) and small (11 – 14 cm) herring were analyzed together, there was an apparent positive correlation between standard length and lipid content (Figure 6a; *Corr* = 0.707). This correlation existed only over a broad range of lengths – within each group (H5, H4 and H6) there was no correlation between standard length and lipid content. The large herring in this analysis were from batches H4 and H6 and were used in the harbor seal feeding trial. When a group of large roe herring (H99) was added to the analysis, the correlation between standard length and lipid content over a broad range of lengths disappeared (Figure 6b; r = 0.070). The roe herring had very low lipid content in spite of their large size (23 – 28 cm). H99 herring was not fed to the seals in the crossover diet study, but used here only for comparative proximate data.

Body condition

Mass changes

Mass from each seal was measured at least every two weeks during the duration of this experiment (Figure 7). This figure demonstrates two results that are essential to the interpretation of the data: First, there were periods of significant mass change in the animals that were on the mixed diets (Figure 7, panel C). Second, when animals were on a test pollock or herring diet (Panels A, B), mass change could occur within a single test period. For example, all three adult seals during the pollock trial of spring 99 (Panel B, trial 3), initially lost mass, then began to gain mass during the trial. Similarly, the juvenile seals (Panel A, trial 5) all initially gained mass on a herring diet, and then lost mass while still on herring. Thus, while there were significant differences in mean masses taken at the conclusion of each four month feeding trial for each seal (Tina, $F_{5,45} = 22.2$, p=0.000; Cecil, $F_{5,45} = 14.7$, p=0.000; Pender, $F_{4,36} = 15.1$, p=0.000; Poco, $F_{5,45} = 3.9$, p=0.006; Skeezix, $F_{5,45} = 7.05$, p=0.000; Sydney, $F_{5,45} = 5.35$, p=0.000; Travis, $F_{5,45} = 11.6$, p=0.000), the bi-weekly patterns were much more complex and demonstrated that an individual trial did not necessarily show a consistent pattern of mass gain or loss regardless of whether the seal was consuming pollock or herring.

Figure 8 is a series of graphs showing the absolute mass change (closed symbols) and percent mass change (open symbols) normalized to the initial mass at the start of the trials (Sept 98) for all eight seals. A cursory review of this graph would suggest that the pollock diets caused animals to lose mass most of the time. However, the animals on the mixed diets also showed patterns of mass loss and gain. Furthermore, the circled areas of each of the 6 graphs for the test diets represent episodes where seals eating pollock appeared to lose substantial amounts of mass. Every one of these mass loss events for all animals followed a period of extreme mass gain on herring (> 30% for juveniles and >20% for adults). In 2 of the 6 cases (Pender and Travis), the period of mass loss on pollock continued even after the seals were switched back to herring, implying that pollock *per se* was not driving mass loss.

Body condition

At the end of each 4 month feeding trial, total body water was determined by deuterium dilution. From the total body water, the body fat was calculated. Figures 9 is a series of graphs showing the body mass change (closed diamond), fat mass change (open square) and percent fat

mass change (triangle) normalized to the initial mass and fat conditions at the start of the trials (Sept 98) for all eight seals As with the results of total mass (Figure 8), the two mixed diet seals (Tina, Snapper) showed periods of fat mass change that were independent of diet types or changes. Furthermore, there were no consistent patterns of fat balance change seen in the other seals on either the pollock or herring diets. For example, the adult seal Skeezix did not change in mass or fat balance during the second feeding trial on herring in the spring of 1999. At the same time, the adult Cecil gained body mass, did not change total body fat and therefore dropped in percent body fat in the same period.

There were also adult vs juvenile differences in fat balance control during the trials. Figure 10 is a regression plot of how fat mass change was dependent on total mass change. While increases in fat mass were correlated with increases in body mass, the relationship was tighter for the juveniles than the adults. This means that these two groups of animals did not gain fat mass in the same manner when they were gaining total body mass.

For juveniles, both diet and season appeared to affect mass changes, but not fat mass and the effect of diet on mass did not occur in every season. In season 2 (Jan-May), there was a general trend toward mass loss on both diets. The largest increases in mass occurred in Season 3 (May-Sept), which was accompanied by relative increases in fat mass on both diets. Body composition in juveniles appeared to not be significantly affected by diet or season.

For adults, these results were different. There was a significant effect of diet on mass gain, but this was season specific. Season 1 (Sept-Jan) was marked by a general increase in mass and fat mass on all diets and overall, there was a significant effect of season on body composition with no apparent effect of diet.

All of these results are summarized in Figure 11, which shows the change in body mass, and body fat for both the adult and juvenile seals for all six trials broken down by season. It is immediately clear from this figure that adults and juveniles responded differently to the feeding trials and that there was no consistent pattern based on diet.

Statistical testing. All of the graphical comparisons shown in the results for body condition (Figures 7-11) were tested for statistical significance as follows:

A. In juveniles, total body mass change was affected by both diet and season when each factor was analyzed independently (KW p= 0.033, p=0.011 respectively) and in combination (ANOVA p=0.003, p=0.002)

B. In juveniles, diet and season had no impact on fat mass change (ANOVA p=0.358, p=0.052), percent body fat (ANOVA p= 0.246, p=0.511) or normalized percent body fat (ANOVA p=0.444, p=0.648).

C. For adults, total body mass change was affected by diet, while fat mass change was not (KW p=0.27, p=0.172); fat mass was affect by season while body mass change was not (KW p=0.024, p=0.478).

D. When diet and season were combined, total body mass was impacted by diet but not season (ANOVA p=0.010, p= 0.462) while fat mass was affected by both diet and season (ANOVA, p=0.022, p=0.007) for adults.

E. Adult changes in body composition were affected by season but not by diet (ANOVA, p=0.001, p=0.240)

Summary of body condition results:

1.The effects of diet and season on mass and fat mass are inter-related.

2. Mass and fat mass change are affected differently by diet and season.

3. Body composition (% body fat) of adults is sensitive to season, but not diet. Body composition of juveniles is not sensitive to either season or diet.

4. The combined effects of diet and season are different between adults and juveniles.

We conclude that it was not possible to predict body condition in the seals based solely on the type of fish fed during the feeding trial.

Blood patterns

The goal of this component of the experiment was to ascertain which blood parameters changed with season and/or diet in harbor seals. It was not designed to assess the mechanisms by which these changes could occur. That is, the experimental protocols were not configured to examine the biochemical or physiological mechanisms by which a certain blood chemistry or hematology could be impacted by diet or season.

Figure 12 shows an example of blood markers from a single seal (Cecil) that changed with diet (AST), season (Hb) and one that did not change (Na) in response to either variable. In this figure, there is a clear dietary signal with the enzyme AST such that it was low when the animals were fed pollock and increased when fed herring. Similarly, hemoglobin (Hb) changed with season so that it was lowest during fall/molting. Finally, in panel 3, the osmolyte sodium (Na) showed no clear response to either diet or season.

All blood chemistry and hematology values were tested using ANOVA with repeated crossover methods. Table 2 shows results for ALT and AST which were then repeated for all blood values to create the summary Table 3. This table lists the blood parameters that changed with diet or season during the two year feeding trials.

Of the five blood variables that changed with season in this study all except albumin also exhibited significant seasonal differences in our previous field samples from animals collected in spring and fall (Fadely et al. 1998). Because our original field work was not able to discern different diets in the seals, it is possible that what appeared to be seasonal changes in the field may in fact have been dietary changes associated with season. Of the five blood variables that responded to diet in this captive study, creatinine, ALT and GGT were found to be also seasonally dependent in the field studies. It is possible that the seasonal dependence in the field was a result in seasonal shifts in diet between spring and fall.

Digestion trials

Digestion trials were conducted every four months for the two-year period lasting from September 1998 to September 2000. A total of 594 scat samples were collected from these experiments and used in assimilation and retention time analysis.

Percent changes in mass calculated for each seal during each caged feeding trial (feeding once/day and four times/day) are shown in Figure 13.

All fecal samples collected during this study had high water content, ranging from 39 to 83% (mean = 64%, SE = 14%). The concentrations of chromium and cobalt, used as solid and

liquid phase markers, respectively, were plotted in relation to the time from dosing (Figure 14). The MRT was calculated for both the solid and liquid phase markers and ranged from 18.4 h (SE=3.5) to 29.4 h (SE=4.6) for herring during once per day feeding to 15.4 h (SE=2.9) to 27.1 h (SE=2.1) for herring fed four times per day (Figure 15). MRT appeared to be mass dependent during all seasons (Figure16).

Pooled MRT seasonal values during the once per day feeding produced significantly greater values for pollock during summer when compared with fall and winter values (p=0.028, Figure17). During the four feeding per day regime fall pollock values were significantly lower than values found in summer and winter (p=0.005, Figure 17). An ANCOVA with age as a covariate revealed a significantly greater MRT for herring in sub-adults for each feeding frequency ($F_{2,14}$=4.12, p=0.016, Figure 18). No difference was detected in pollock with respect to age.

Apparent dry matter digestibility was greater for herring than pollock (herring, mean=92.0%, SE=2.4; pollock, mean=88.5, SE=2.7) for either feeding frequencies (1x/d, mean=91.3, SE=2.6, p=0.004, 4x/d, mean=87.2, SE=1.8, p=0.005).

Rehabilitation studies

Mass and Morphometric Measurements. Rehabilitated seals exhibited positive growth in mass, standard length, axillary girth and axillary blubber depth (Figure 19). Growth was 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. Growth rate appeared unrelated to relative differences in health, in that there was no statistical difference between Group A and Group B. Growth rates for all pups, whether fed formula or fish, were very much slower that those of wild seals (Trumble and Castellini 2001). Three pups experienced a marked increase in growth rate (mass) at some point after weaning. This rapid growth occurred in only one pup per year and coincided with the time when the seals were housed in groups rather than individually.

Hematology. Throughout 1998 and most of 1999, white blood cell (WBC) counts were measured at the ASLC using only an automated blood analyzer (IDEXX). Starting sporadically in 1999 and routinely in 2000, WBCs were also counted manually. Total WBC counts and granulocyte counts measured using an automatic blood analyzer (IDEXX) were positively correlated with those measured manually using blood smears stained with Diff-Quik (Figure 20 **a.** WBC counts, *Corr* = 0.716**, *b.*** granulocytes, *Corr* = 0.729). Automatic and manual measurement of % granulocytes were not correlated (Figure 21, *Corr* = 0.427). The lack of manual counts in 1998 and 1999 precluded manual counts being used for comparison of age or health-related changes in WBC. Since automated and manual WBC counts and granulocyte counts were positively correlated, we proceeded to analyze changes in these values using the automated counts. Due to the lack of correlation between automated and manual measurements of % granulocytes we did not use these automated counts in our analyses.

The healthy seals from Group A exhibited a decrease in WBC counts with age (Figure 22: Kruskal-Wallis, *P* = 0.007). The WBC counts of seals in Group C were variable. However WBC counts measured just prior to death in at least three of these animals (PV98005, PV00001, PV00004) were elevated (> 2 S.D. from comparably aged seals in Group A, Figure 23).White blood cell and granulocyte counts were transiently elevated in two seals from Group B – Iliamna

(PV98008) and S.K. (PV99008) (Figure 22). Both individuals exhibited external symptoms of severe infection at the time that the WBC counts were elevated

Hematocrit decreased dramatically in most pups during the course of rehabilitation. This decrease appeared to occur in two phases – a sharp initial drop right after admission to values ranging from 45 – 57%, followed by a later, more gradual decline to values as low as 34 – 42% (Figure 24, Kruskall-Wallis one-way analysis of variance $P = 0.007$). There was no significant influence of year of rehabilitation (Figure 24a) or health status (Group C excluded) (Figure 24*b*) on these changes in hematocrit. Whole blood hemoglobin concentrations were highly correlated with hematocrit values (Figure 25*a*, $r = 0.965$, $r^2 = 0.931$). In contrast, mean cell hemoglobin concentrations (MCHC) remained unchanged throughout the hematocrit decline (Figure 25*b*). In most cases, red blood cell (RBC) counts tracked hematocrit changes (Figure 26). In some individuals, the secondary hematocrit decline was accompanied by an apparent initial increase in mean cell volume (MCV), followed by a dramatic decrease (Figure 26*a,b*). At the lowest hematocrit values, MCV was highly variable (Figure 26*c,d*). The secondary decline in hematocrit appeared to be associated in some cases with a surge in growth rate. In many instances the secondary hematocrit decrease was accompanied by an apparently increased growth rate as measured either by a volume index (S.L. X Girth^2) or girth (Figure 27). The influences of growth parameters such as volume index on hematocrit were significant (ANCOVA, volume index, $P = 0.000$; age, $P = 0.137$). Girth and mass were also found to co-vary with hematocrit.

To better understand the hematocrit changes in the pups, blood volume measurements were made in 1999 and 2000. Absolute plasma volume (l) increased with age, while relative plasma volume (%) declined (Figure 28). Absolute plasma volume was more closely correlated to mass (*Corr* = 0.85) than to age (*Corr* = 0.61). Conversely, relative plasma volume was more closely related to age (*Corr* = -0.82) than to mass (*Corr* = -0.69). Knowing plasma volume and hematocrit, it is possible to determine total blood volume. Having determined total blood volume, it is possible to determine the volume of the RBC pool (Total blood volume – Plasma blood volume). Figure 32 illustrates the changes in plasma volume and RBC pool volume as a function of age. As the seals aged and gained mass, plasma volume increased. In the earlier phase of recovery, the RBC pool also increased as mass increased (except for Kali). However, the RBC pool failed to increase in the later phase of rehabilitation, in spite of significant growth (Fig 29).

Blood chemistry. Blood chemistry values for harbor seals upon admission are presented in Table 4. Data are also presented that represent only admission values for seals less than 2 weeks old. Only ALT and GGT values were found to be statistically different between Group A and Group B (< 2 weeks old). However, even within Group A, many blood chemistry values fell outside the normal range of values for harbor seal pups in the Gulf of Alaska (Trumble and Castellini 2001). Any statistical comparison of these values is confounded by small sample size, high variability and differences in ages between seals admitted to the ASLC (< 2 weeks old) and those normally sampled in the wild (2 – 6 weeks old). Also included in Table 4 are chemistry data from a single seal from Group C (PV98009, 2 months old) that was euthanized on the day of admission, as representative of an animal that was critically ill at the time of admission. Many serum chemistry values from PV98009 fell well beyond the minimum or maximum values for Group A seals at admission (bold) and/or Group A seals of comparable age (asterisk). In most cases these values were also outside the range of values for wild harbor seals from the Gulf of

Alaska (i.e. albumin, albumin/globulin, BUN, BUN/creatinine, GGT, phosphorous, bilirubin, and potassium, see Trumble and Castellini 2002).

Several blood chemistry values were found to vary in seals from Group A as a function of age, including AST, GGT, cholesterol, triglyceride, creatinine, BUN/creatinine, glucose and potassium (Figure 30, Table 4, Kruskal-Wallis $P < 0.05$). In some cases, such as AST, cholesterol, triglyceride, and potassium, the biggest differences occurred between pre- (2 weeks old) and post-weaning (> 6 weeks old), although it is not always clear that weaning played any specific role since other developmental changes may be temporally overlaid. Other variables appeared to change over time (e.g. GGT and glucose), or after weaning (e.g. creatinine, BUN/creatinine).

The specific effect of weaning on blood variables is difficult to determine. Cholesterol however, appeared to be influenced by diet. Values from seals in Group A fell sharply after being admitted to the ASLC and remained low until weaning occurred, at which time they increased (Figure 31). The lower values corresponded closely to when the seals were being fed formula (initial samples were collected before feeding was begun). The subsequent increase appeared to be more closely linked to weaning than to age.

Many blood chemistry values from Groups A and B varied significantly as a function of health status (Figure 32**,** Table 5**,** Kruskal-Wallis $P < 0.05$), even after 6 – 7 weeks of age, when most seals from Group B no longer displayed any outward symptoms of injury or illness. At 6 – 7 weeks old, Group B seals had significantly higher potassium values. Group B seals had significantly lower alkaline phosphatase and creatinine values at 9 – 10 weeks old and significantly lower phosphorous values at 12 – 13 weeks old. Mean values for seals from Group C just prior to death are presented for comparison. Statistical comparison is not possible because of the variability in age of the seals when they died (5 – 60 days old). However, many variables appear different with respect to mean value or variability in seals just prior to death (Group C) than in the relatively healthy recovering seals in Group A.

There were a number of blood chemistry variables that were not significantly different on the basis of central tendency (Kruskal-Wallis analysis), but displayed a significantly greater variability in the Group B seals than in those from Group A. This was based on a combination of factors including significantly different variances between Group A and Group B (F-test $P < 0.05$), the presence of outliers in Group B, and values from Group B that were beyond the normal range for wild harbor seal pups in the Gulf of Alaska (Trumble and Castellini 2002). At 6 – 7 weeks old distribution differences between Group A and Group B occurred for ALT, calcium, BUN, BUN/creatinine, and GGT (Figure 33). This pattern persisted for some variables (BUN, BUN/creatinine, and GGT) through 9 – 10 weeks old and expanded to include AST and total protein (Figure 34). By 12 – 13 weeks old, differences in distribution of BUN, BUN/creatinine and total protein persisted, with the addition of differences between ALKP, creatinine, globulin and cholesterol (Figure 35).

Gamma-glutamyl transaminase values were highly variable in seals upon admission. GGT values were significantly different between Group A and B only at admission. From 2 – 13 weeks old, GGT values decreased in both groups of seals (Figure 30, Figure 36). While there was no statistical difference between mean GGT values between Groups A and B during this time there were significant differences in variance from 2 - 9 weeks old (Figure 36). These were the result of elevated values for 3 of the 5 seals in Group B during recovery. Figure 37 presents GGT values for seals from Group C just prior to death, relative to the other seals undergoing

rehabilitation (Groups A and B). There is extremely high variability in these values, with 2 of the 5 seals experiencing extremely high GGT values immediately preceding death. For one individual (PV00001), these high GGT values had persisted for 3 weeks prior to death and had been accompanied by no outward symptoms of injury or illness. The necropsy on this seal revealed that it had drowned.

Plasma haptoglobin concentrations were highly variable in harbor seal pups from Group A compared with samples collected from wild pups in Prince William Sound (PWS) in 1997 (Figure 38a,b). There was no obvious effect of age on these values. Median values were generally less than 50mg/100ml and maximum values never exceeded 200mg/100ml. Plasma haptoglobin concentrations were considerably more variable in seals from Groups B and C than from Group A (Figure 41c). In both Groups B and C, median values were generally above 100mg/100ml and maximum values ranged from 300 – 600mg/100ml. In Group C pups just prior to death, the median haptoglobin concentration was greater than 400mg/100ml.

DISCUSSION

Proximate analysis of prey items

Proximate analysis revealed that lipid content was approximately 3.5 times higher in the herring than in the pollock used in this study but indicated no change in gross lipid content or energy density throughout 18 months of frozen storage (Figures 1-2). This analysis does not address the potential for changes in specific fatty acids, essential amino acids or vitamins which might occur over such a prolonged storage time (Englehardt and Geraci 1978, Worthy 1990).

In spite of a strong positive correlation between lipid content and energy density (Figures 3-4), energy density was only 1.8 times higher in herring than pollock (Figure 2). This is indicative of the higher contribution of protein to energy density in pollock than in herring. A very large difference in lipid content between herring and pollock translated to a much smaller difference in actual calories consumed. This is important when considering the role of diet in seal energetics, emphasizing that the role of protein should not be overlooked.

Lipid content (wet mass basis) and water content were inversely related in both herring and pollock, although the relationship was much stronger for herring (Figure 5; herring *Corr* = -0.966,; pollock *Corr* = -0.793). Only the high fat herring (H4 and H6) was used as food in order to ensure the greatest possible difference between the herring and pollock diets.

The range of values for water and lipid content was much larger for herring than for pollock. In fact, while herring is widely regarded as a “high fat” food, in many instances, lipid content for herring was within the range of values observed for pollock. This is due to factors such as fish size and season of capture. It has been documented that lipid content increases with age class in herring (Paul et al. 1998, Payne et al. 1999). In fact, many of the lower lipid values occurred in fish from a batch of small herring (H5) used to feed birds. When these small herring (11 – 14 cm) were analyzed with the larger fish used as food in this study (H4 and H6, 15 – 27 cm), there was a positive correlation between standard length and lipid content (Figure 6a). Within each group (H5, H4 and H6), however, there was no correlation between standard length and lipid content. Therefore, the relationship between standard length and lipid content is only valid over a large range of standard lengths, indicative of different age classes. When another group of large herring was added to the analysis (roe herring, H99, 23 – 28 cm), the correlation between standard length and lipid content disappeared (Figure 6b). The roe herring was caught in

the spring of 1999 after over-wintering. These large, mature fish had lipid values as low as much younger, immature fish. While herring certainly have the potential to have higher fat content than pollock, much depends on the size of fish eaten and the season of capture. Herring are truly only "high fat" fish if they are large enough and if caught at certain seasons (Paul et al. 1998, Payne et al. 1999). The herring used as food in this study was captured in November of 1998 (Prince William Sound, H4) and 2000 (Petersburg, H6). Another batch of herring that was not used as food in this study was caught in Prince William Sound in November of 1997 (H1), but had very low lipid content (Figure 5). This is surprising, given the season of capture. Unfortunately the sample size was very small (n = 3), but it may suggest that in addition to seasonal effects, there are significant yearly or local differences in lipid content in herring in the Gulf of Alaska. The pollock used in this study were quite large (23 – 45 cm), and probably represent the high end of lipid content for this species (Payne et al. 1999). There was no correlation between standard length and lipid content in pollock in this study, but that may simply be because the size range investigated was too narrow.

Quality of fish is obviously a consideration when feeding captive seals. This is particularly true when a carefully controlled diet is required. Herring exhibit a wide variability in quality as assessed by lipid content or energy density. It is not enough to assume that herring have high fat content just because they are large or were caught in the fall or early winter. It is essential to monitor quality by measuring either lipid or energy density. Given the tight correlation in this study between water content and lipid content in herring from many different batches (representing different sizes, seasons, years), it may be possible to estimate lipid content by measuring water content. Measurement of water content is simple and inexpensive. Just as quality of diet is important in feeding captive seals, the potential variability in herring quality in the wild must not be overlooked when assessing population changes on the basis of high or low fat diets. Herring can be both.

Body condition

The results from the body condition studies over the two years and six feeding trials demonstrate that broad statements as to the "quality" of herring vs. pollock as food items for seals are not justified. There are clear differences in both the direction and magnitude of the response to the diets based on the season and the age of the seals.

First, let us consider the older seals (Group B). These were mature animals that were not growing and did not have any reproductive activity. Therefore, the energy requirements for growth and pup-rearing were eliminated from this group. If we had conducted our study only during the summer months (Season 3; June through September, Figure 11), we would have concluded that pollock was "bad" for these animals and herring was "good" because older seals appeared to lose mass and body fat on pollock and gain mass and body fat on herring. This is the type of response that has generated the "junk food" hypothesis that pollock is bad for marine mammals. However, during the fall (Season 1, Figure 11), these same seals gained body mass and fat on both herring and pollock. If we had done only one experiment, during the fall with older animals, we would have concluded that diet made no difference to their body condition, a conclusion exactly opposite that of the summer study. Finally, during the winter period (Season 2, Figure 11), these older seals lose body fat on pollock, showed no change in body fat on herring and gained mass on herring, but not pollock. If we had done our study only during the

winter, we would have concluded that herring was good to gain mass, but neither diet would prevent a decline in body fat. Thus, all three seasons would have lead to different conclusions and only one, the summer season, would have supported the "junk food" hypothesis.

The younger seals that were still growing in some cases, though the ASLC did not let them breed. Therefore, while reproductive costs were not an issue, growth costs were. During the summer periods (Season 3, Figure 11), the young seals gained mass on both diets and body fat on pollock. There was no change in body fat, in the face of tremendous changes in mass, when fed herring during the summer. Thus, these younger animals produce the opposite conclusion to the older animal summer results: for the young animals, diet made no difference during the summer. Therefore, had we conducted a feeding study only during the summer, we would have concluded that the junk food hypothesis was relevant for adults, but not for young animals. During the winter (Season 2, Figure 11), we found that the younger seals did not gain mass on herring and lost mass on pollock, showed not change in body fat on pollock and lost fat on herring. Thus, a winter study would have given equivocal results for the "junk food" hypothesis: it would have held for mass changes, but not for body condition. Finally, during the fall (Season 1, Figure 11), we found the animals lost mass and body fat on pollock and gained mass but not body fat on herring. Therefore, we would have concluded that the "junk food" hypothesis was not supported for younger animals in the fall in terms of body condition, though perhaps it applied for body mass.

The mixed diet data were critical in that they demonstrated that there could be changes in mass and body condition regardless of diet (Figures 8,9). In the wild, harbor seals eat a complex diet Iverson et al. 1997) and it has been documented that there are seasonal changes in body mass and condition in wild Alaskan harbor seals (Fadely et al. 1998). The results of this study indicate that field observations of changes in body mass or body fat of harbor seals are not sufficient to imply changes in food quality or quantity.

Clearly, patterns of mass change and body fat variations are dependent on season and seal age. A number of factors may contribute to these differences, including different energetic demands during biological cycles, seasonal variables such as temperature and light, differences in activity level either during different seasons or at different ages, etc. Analysis as to which of these factors were significant was beyond the design scope of this study. The repeated crossover nature of the experimental design allowed us to compensate for internal variability, since we were able to compare each animal with itself on different diets and at different seasons.

These studies were designed to allow seals to adjust intake as naturally as possible to match their energy needs. The essential question was not whether seals need *more* of a given prey item to meet their energy requirements, but simply whether the composition (ie. low fat vs. high fat) of the diet alone would prove detrimental to seal health or body condition. The diets therefore, were not designed to be isocaloric and the seals did adjust intake as diet changed.

Taken together, we believe these data strongly argue against the broad statement that pollock is not an appropriate food item for seals. There was no consistent pattern that demonstrated any change in body condition that could be linked to diet.

Blood patterns.

Our data demonstrate that at least 10 different blood chemistry/hematology variables commonly measured in field studies of harbor seals are influenced by diet or season. The remaining variables we tested may not be reflective of diet or season but could be useful in

determining medical or other issues. For example, we found in the our field work that LDH values responded significantly to capture handling (Fadely et al. 1998) and thus may be useful to characterize responses to handling, but would not be useful for determining diet or seasonal changes.

This study was not designed to assess the biochemical or metabolic rationale for why certain blood parameters would change with diet or season. Such a study would need to involve controlled biochemical studies of the fish, the breakdown products from normal metabolic processes in the seals, turnover rates of the compounds, etc. Having discerned which metabolites fall into different categories, future studies could focus on these groupings for this type of analysis and detailed mechanisms.

There are several blood variables that should be discussed. As noted above, this study verifies that, regardless of diet, hematocrit and Hb change with season such that low values occur during the molting period. During this time, seals tend to haul out on dry land to facilitate skin re-growth. Regardless of the physiological mechanism for Hct alteration, it is clear that any study of seals on different diets would need to correct for this seasonal change in Hct. For example, it would be incorrect for a study to examine a seal on diet 1 during the spring and on diet 2 during the fall and conclude that Hct declined on the fall diet. This is why we conducted this experiment over 2 years with the crossover design. Recent studies (Rosen and Trites 2000a,b) on feeding trials in Steller sea lions were for very short time periods (less than 20 days) and in only one season. This EVOS project demonstrates that such short-term studies can lead to conclusions that would not be borne out in longer experiments.

The enzymes ALT, AST and GGT responded to dietary changes in the seals. These enzymes are included in standard blood chemistry panels because of their usefulness in diagnosing cell/tissue damage, particularly, but not exclusively in the liver. In this study, AST was elevated whenever the seals consumed herring, regardless of season. Classically, elevated AST levels in marine mammals are associated with liver or tissue damage (Bossart and Dierauf, 1990). Certainly the seals in this study did not go through periods of tissue damage, but rather responded to different diets. What would be different about the diets of pollock and herring that would induce different enzyme release rates? As noted above, a completely different set of experiments would need to be designed to find out why AST, for example, would be elevated in harbor seals eating herring.

In summary, this study was able to classify at least 5 blood chemistry/hematology values that responded to diet and 5 that responded to season. These distinctions can be used to not only interpret patterns seen in wild seals, but also to help refine future studies so that blood metabolites of interest can be identified

Digestion trials

During this study we observed a greater mean retention time (MRT) and apparent dry matter digestibility (DMD) of herring when compared with pollock. In other words, herring tended to stay in the gut longer and be more fully digested. Feeding frequency also affected these measurements, such that increased feeding frequency (4 meals/day vs 1 meal/day) resulted in lower MRT and digestibility. The MRT was calculated for both the solid and liquid phase markers and in herring ranged from 18.4 h to 29.4 h for the single meal/day regimen and 15.4 h to 27.1 h for the 4 meal/day regimen. These values were lower for pollock at both feeding frequencies, ranging from 12 h to 24.9 h (1meal/day) and 9.2 h to 22.7 h (4 meals/day).

We noted a positive correlation between body mass of the seals and MRT for liquid and solid phases during all seasons (Figure 16). To our knowledge, this is the first study to report the MRT for any pinniped over various size ranges. It has been reported that MRT increases with body mass in mammals; however, these results are usually determined from data collected across species (Robbins 1993). Our MRT data are consistent with other studies using inert markers in that fluids and very small particles moved through the gastrointestinal tract faster than larger solid particles. In ruminants and macropods, fluids move through the GI tract twice as fast as solids (Robbins 1993), however, in species such as carnivores, which have tubular, non-complex digestive systems, the gap between liquid and solid passage is bridged. The excretion curves generated from our data are consistent with batch-reactor digestion (Penry and Jumars 1987), which assumes the consumption of discreet meals. Greater mixing occurred during the one feeding per day (Figure 14), which is apparent from the separation in excretion curves and longer retention times. In marine mammals, foraging occurs while diving, thus increasing the importance of meeting an optimal retention time of digesta, which is correlated with the length of time between meals. This is in turn correlated with the time and energy invested in food acquisition and processing.

Our results are consistent with previously reported dry matter digestibility values. During the feeding trials for this study, we calculated DMD values averaging 92% for herring and 88.5% for pollock. In studies of California sea lions, Fadely et al. (1994) reported DMD values ranging from 87.7 - 91% for herring and 83.2 - 90.9% for pollock and in similar studies of captive Steller sea lions, Rosen and Trites (2000) reported DMD values of 86.5% for pollock and 90.1% for herring. The lower values of both sea lion studies may be a result of using manganese (Mn^{2+}) as a marker where we used total balance methods. Barboza and Jorde (2001) stated that Mn^{2+} concentrations underestimated DMD calculations in black ducks during long-term fasting studies. Our reports of lower DMD for pollock (relative to herring) are similar to reports form Rosen and Trites (2000), although in our study we noticed an increase in the digestibility during the one feeding per day regimen. This may provide a clue to the plasticity of the pinniped gastrointestinal tract. We believe our study gave adequate time for changes to occur in the digestive system of the captive harbor seals (approximately 3 months on each diet prior to the experimental treatment). Most studies to date have not employed such a feeding schedule, which may account for the discrepancies in results. Robbins (1993) points out that, when making comparisons, several weeks should separate feeding trails to reduce the influence of gut fullness and changes in the size of the body water pool or the effect of hormones.

Digestion is often seen as a tradeoff between the rate and thoroughness of processing (Robbins 1993). Some animals process quickly and incompletely and others slowly and thoroughly (Robbins 1993). Harbor seals, like most pinnipeds, are opportunistic feeders and thus may need to compensate aspects of digestive processing when faced with large changes in food availability or quality. The question is, can these animals adjust their digestive processes to maximize intake and digestibility of specific prey? In this study, there were significant differences between MRT and DMD based on both diet and feeding frequency. The shorter retention time coupled with decreased digestibility for pollock may be an indispensable adaptation of mongastrics. By processing pollock more quickly, nutrient uptake is maintained at a relatively high level because these seals can keep their guts full, even in the face of one feeding per day, by continually providing new ingesta. This assumes that there is plentiful pollock available for seals during foraging.

A few studies have reported that poor quality prey (low lipid) fed to pinnipeds may result in reduced body condition (Martensson et al. 1994, Rosen and Trites 2000a,b). Martensson et al. (1994) reported that harp seal pups fed crustaceans lost mass and would have ultimately died, while Rosen and Trites (2000) maintain that Steller sea lion juveniles would continue to lose mass while eating pollock. An EVOS report concerning kittiwakes in the Gulf of Alaska found that seabirds grew at faster while feeding on high-lipid fish as opposed to low lipid fish (Romano and Roby 1996). However, they also reported that there was no difference in the growth and development of puffins whether they ate a high lipid or low lipid/high protein diet. Rosen and Trites (2000) report that because of the energetic differences in prey items (herring, 6.4-7.6 kJ/g; pollock, 4.5-4.7 kJ/g) that Steller sea lion juveniles would have to eat 35-65% more pollock to acquire the same energy reserves. During this study, four meals/day appeared to buffer the differences between herring and pollock. We would suggest that the four meal/day feeding scenario is more consistent to what is found in nature for pinnipeds.

Rosen and Trites (2000a,b) reported that the possible implications of their findings are more critical to younger sea lions because they have higher mass specific energy needs than adults (Kleiber 1975), and their smaller stomachs (Calder 1984) might restrict intake and storage capacity for any given meal. Our data show that compared with adults, younger sub-adult harbor seals have longer retention times for both herring and pollock. At this time we can only speculate that these longer retention times in sub-adult harbor seals may be a response to some developmental, behavioral (eg. less efficient foraging) or physiological mechanism. In any case, if the main prey available to harbor seals or sea lions is pollock, then a slightly longer retention time might be an advantage in younger animals if they are less efficient at foraging, or have a smaller intake capacity.

Summary. The feeding trials involving captive harbor seals demonstrated greater mean retention times and apparent dry matter digestibility for herring diets when compared with pollock and also during the one feeding/day regimen when compared with four feedings/day. We documented a difference in the digestibility of herring in the sub-adult age class of harbor seals as well as increased retention time of prey for these younger animals. We believe that many of these observations reflect adaptations to a fluctuating food source and give clues to the plasticity of the gastrointestinal tract of the harbor seal.

Rehabilitation studies

Mass and Morphometric Measurements. Pups from Groups A and B grew more slowly than wild seals and did not reach 25 kg until they were at least 10 weeks old (Figure 19). By contrast, the mean mass from 2 – 6 week old pups from the Gulf of Alaska is about 27-30 kg (Trumble and Castellini 2001). Similarly, it took pups from Groups A and B 9 – 13 weeks to achieve standard lengths, axillary girths and blubber depths typical of 2 – 6 week old wild harbor seals (Trumble and Castellini 2001). Although most of the pups were alone in the field for only 2 – 3 days, this suggests that the nursing phase is extremely critical to future growth, and/or that some aspect of rehabilitation itself restricts growth rate.

Hematology.

White blood cell counts fell within expected ranges (Bossart and Dierauf 1990, Hall 1998) and decreased with age in Group A seals (Figure 22). This suggests a developmental or dietary effect on WBC counts, since the greatest drop occurred between 2 – 6 weeks old, when

the seals' diets changed from formula to fish. White blood cell counts of seals in Group C were variable but counts measured just prior to death were elevated (> 2 S.D. from Group A, similar age) in at least 3 of the 5 animals. Elevated WBC count appears to be a good indicator of health status, although consideration should be given to age and nutritional status. All seals with WBC values above 15 million cells/ml belonged to either Group B or C. At least 2 of the 5 individuals with high values had no obvious external symptoms at the time that WBC values were high. This is pertinent to studies of wild populations, where animals are often assumed to be healthy on the basis of external appearance (Small 2001), or where outlier statistics are starting to be used to assess overall population health (Fadely 1998, Trumble and Castellini 2002).

Hematocrit decreased over time in all seals and was not significantly affected by year of rehabilitation or health status (Figure 24). Decreases in Hct occurred in two phases – an early abrupt decrease that spanned the first 3 weeks of rehabilitation and a later, more gradual decrease. It is common for neonatal mammals to have elevated Hct that drops sometime shortly after birth (Altman 1961, Spensley et al. 1987). While this was found not to be the case in neonatal northern elephant seals (Castellini et al. 1990), it has been observed in gray seals (Hall 1998) and Weddell seals (pers. obs.). Hct levels in many of the rehabilitation seals decreased to very low levels (34 – 40%). It is difficult to compare these values to those of similarly aged wild harbor seals, since most field studies sample pups either before or right after weaning (typically 2 – 6 weeks old). However, most reports of harbor seal Hct at various ages fall in the range of 40 – 65% (Bossart and Dierauf 1990, Fadely et al. 1998).

While chronic blood loss is a potential cause of anemia (eg. weekly blood draws), the small blood sample size in this study was insufficient to cause changes of the magnitude observed in this study. The weekly sample size ranged from 0.5 – 2.5% of total blood volume and was calculated to cause a Hct drop of no more than 0.5 %units (ie. 56% - 55.5%) between weekly samplings. Studies of chronic blood sampling support these conclusions. In a study of repeated blood sampling from rats, Cardy and Warner (1979) reported that monthly blood samples ranging from 7 – 25% of total blood volume (depending on the size of the rat) resulted in no change in any hematological parameters (McGuill and Rowan 1989).

Mean cell hemoglobin content (MCHC) remained constant except at the most extreme values of low Hct (34%) (Figure 25b). While MCHC is traditionally used as an indicator of iron deficiency, it is not very sensitive (Tvedten and Weiss 1999). Therefore, while there is no indication of iron deficiency in these seals on the basis of MCHC, it is possible that insufficient iron was available to maintain Hct.

The secondary decrease in Hct was associated with increases in growth rate as measured both by a volume index (S.L x Axillary Girth^2 *as per* Castellini and Kooyman, 1990) and by girth (Figure 27). The 3 seals that had the highest growth rates in the later phase of rehabilitation also experienced some of the lowest Hct values (Figures 19, 24). Absolute plasma volume (l) increased with age, while relative plasma volume (% mass) declined (Figure 28). This suggests that the late phase Hct decline was not a result of a sudden disproportionate increase of plasma volume associated with increased growth (ie. dilution of RBC by increased relative plasma volume.

While there were many changes in Hct and associated blood variables in pups undergoing rehabilitation at the ASLC, none of these changes appeared to be related to differences in health status (ie. no statistical difference between Group A and B). There are many ways that health status can influence Hct (Tvedten and Weiss 1999) and any potential effect of health status on

Hct in this study may have been overshadowed by the dramatic decreases that occurred even in relatively healthy pups

Blood chemistry. Upon admission, Group A pups had several blood variables for which the median values fell outside published ranges for wild harbor seal pups in the Gulf of Alaska (Trumble and Castellini 2002). Even though the pups in this group were healthy compared with Groups B and C, they were significantly younger (3 – 10 days old) than pups for which reference ranges have been constructed (2 – 6 weeks old, Trumble and Castellini 2002). For example, by 2 weeks of age, bilirubin and GGT values were within normal ranges. Variables associated with lack of food (ie. BUN, glucose) and dehydration (sodium and total protein) all appeared normal in Group A pups upon admission. This suggests that while they may have lacked maternal care they were in no immediate danger of dying from starvation or dehydration.

One pup was admitted to the ASLC in such critical condition that it was euthanized that day (PV98009, 2 months old). Admission values from this pup are included (Table 4) as an example of values from a severely compromised individual. In this individual, 14 of 22 blood chemistry variables were outside the min/max values from Group A pups at admission, 16 of 22 variables were outside the min/max values of Group A pups at a similar age (9 weeks old) and 8 of 20 variables were outside normal published ranges for 2 – 6 week old wild harbor seal pups from the Gulf of Alaska.

Before addressing the possible effects of health status on blood chemistry parameters, it is necessary to have a sense of how these values might change over time. Changes that occur over time might be a result of normal development, nutritional changes (ie. weaning), recovery and acclimation to captivity. In some cases, blood chemistry values appeared to change gradually over time (eg. GGT, glucose), or after weaning (eg. creatinine, BUN/creatinine). Elevated GGT has been observed in sea otters injured by the oil spill (Rebar et al. 1995). In seals admitted to the ASLC, GGT values were generally high and extremely variable (Table 4), however both mean levels and variability decreased throughout rehabilitation. Creatinine values were low relative to normal wild values (Trumble and Castellini 2002).

In some cases, such as AST, cholesterol, triglyceride, and potassium, the greatest age-related differences in values occurred between pre- (2 weeks old) and post-weaning (>6 weeks old). Cholesterol values from seals in Group A fell sharply after being admitted to the ASLC and remained low until weaning occurred, at which time they increased (Figure 31). The lowest cholesterol values corresponded to when the seals were being fed formula. The subsequent increase in cholesterol appeared to be more closely linked to weaning than to age.

By 6 – 7 weeks old all seals in Groups A and B were weaned and ostensibly healthy. Even so, many blood chemistry values from Groups A and B varied significantly as a function of health status (Figure 32, Table 5). Mean blood chemistry values for seals from Group C just prior to death are presented for comparison. Statistical comparison is not possible because of the variability in age of the seals when they died (5 – 60 days old). However, many variables appear different with respect to mean value or variability in seals just prior to death than in the relatively healthy seals in Group A.

While many blood chemistry median values were not significantly different between Groups A and B, many displayed differences in distribution between the two groups. Upon admission and at 2 weeks old (pre-weaning) there was significant variability in many of the blood chemistry values of both groups (Table 4, Figures 33-36). By 6 – 7 weeks old, most Group A blood chemistry values had stabilized and were within published reference values (Trumble

and Castellini 2001,2002). This resulted in wider distribution of blood chemistry values wherein many values from Group B seals fell well outside the min/max values from Group A and often outside published ranges for 2 – 6 week old wild harbor seals in the Gulf of Alaska (Trumble and Castellini 2001,2002).

Haptoglobin is an acute phase protein associated with a wide range of physiological stresses and is used as a sensitive, although non-specific indicator of disease in human and veterinary medicine (Henry et al. 1974). Application of acute phase protein analyses has been extended to wild populations to assess potential stressors (Duffy et al. 1993, Zenteno-Savin et al. 1997). Plasma haptoglobin concentrations were highly variable in harbor seal pups from Group A compared with wild pups in Prince William Sound (PWS) in 1997 (Figure 38a,b). There was no obvious effect of age on these values. While Group A seals exhibited variable haptoglobin concentrations, the scale of this variability was much smaller that observed in Groups B and C, confirming the usefulnes of this protein as an indicator of physiological stress.

Since each individual in Group B was admitted with, or developed, different conditions for which they were treated, it is not surprising that there was no single blood chemistry profile that describes them all. Many, such as alkaline phosphatase, creatinine and BUN/creatinine may be influenced by delayed growth. Others such as total protein, globulin and haptoglobin, may reflect exposure to serious infection. Some, such as unusual values at various times for glucose (pre-weaning), BUN, creatinine, BUN/creatinine, GGT and AST in Kali (PV00002) may be indicative of some undiagnosed systemic problem that resulted in a failure to thrive starting at about 10 weeks old. Regardless of the cause, seals in Groups B and C developed blood chemistry profiles that were different from both those of Group A and published reference ranges, even long after (or long before) their symptoms were obvious.

Many of the symptoms exhibited by pups in Group B could have been considered mild, or gone unnoticed in wild pups, particularly once they had begun to resolve. The possibility of subclinical conditions in healthy looking animals is well accepted in veterinary or wildlife medicine. This study confirms that analysis of outlier frequency in wild populations could reveal differences in population health status, even at a fairly moderate level. It also highlights the need for caution in assuming that animals sampled from wild populations are healthy based on a brief external assessment (eg. no obvious lacerations, infections or lethargy). In fact the statistical probability that, in spite of appearance, some animals in any random field sample are compromised to some degree is exactly why analysis of statistical outliers is valuable.

CONCLUSIONS

In its simplest form, this study was designed to test how differing diets, seasons and health status impact the blood chemistry and body condition of harbor seals. It was carried out in order to better understand the variability seen in those parameters from wild caught animals. Never before has such an intense effort been conducted on a marine mammal of any species using tightly controlled diets, digestion studies, continual monitoring and body condition assessment.

We believe that the results from this study, while complex, have a common theme. That theme is that the harbor seal exhibits a plastic response to season and diet such that it can adapt its physiology as necessary to best cope with the metabolic demands placed upon it by those conditions. Thus, we conclude that it is not possible to predict harbor seal health, body condition

or energy balance based solely on the diet that the animal is consuming. This is a critical finding in that it challenges the "junk food" hypothesis and those of similar format that claim, on an *a priori* basis, that because a certain prey item is of different caloric content, that it is therefore not an acceptable food item. Within the limits of comparing herring, which is considered a "high quality" food item and pollock, which is considered a "low quality" item, we believe that harbor seals are capable of adapting their biology and behavior to utilize these two fish species as a food source. Of course, in nature, a seal would not likely eat a diet of 100% of any species. However, even at those extremes, the animals responded appropriately to these different food items. The fact that other variables such as activity level, age, captivity, social structure, etc. may also influence these responses was factored into the study design and in no way changes our conclusions.

The seals in this study altered their food intake and their digestive physiology to match the seasonal and dietary changes that they were experiencing. They remained healthy by all standards regardless of diet. This was made clear by the straightforward comparison to the rehabilitation pups which showed clear metabolic problems. The information from the rehabilitation pups demonstrated that using outlier theory to characterize blood chemistry patterns and assess populations is a viable method of analysis. Furthermore, these data re-emphasize that seals can appear healthy, but still exhibit blood chemistry values that suggest they are compromised. Thus, a field comment that seals "appear healthy" may not be a complete or adequate description of an animal.

This study verified that there are biochemical blood markers in seals that respond to diet and season and that there are some markers that respond to neither. It also verified that the seals show clear seasonal patterns in their metabolic profiles such that a seal fed a certain diet in June has a different metabolic response to the same diet in December. The seals could adjust their digestive physiology to match the diet and they adjusted their food intake according to diet and season. The animals demonstrated strong plasticity in regards to diet and season.

Therefore, on the basis of this study, we conclude that harbor seals can adapt their physiology as necessary to match the type of food item available. It is not possible to predict seal health, body condition or energy balance based solely on the type of food consumed.

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Table 1. Admission information for harbor seal pups brought to the ASLC for rehabilitation during 1998 and 1999. Condition was assessed by the ASLC veterinarian (P. Tuomi, DVM).

	I.D.	*Sex*	*Admission Date*	*Approximate Age at Admission*	*Condition at Admission*	*Date of Release/ Death*
Group A	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
	Kenai (99004)	M	5-20-99	7-10 days	Thin	9-23-99
	Mackenzie (99007)	F	6-14-99	3 days	Thin, dehydrated,	9-23-99
	Iggy (99009)	M	6-30-99	10 days	Dehydrated	9-23-99
Group B	Iliamna (98009)	F	7-10-98	3 weeks	Injured, laceration, pus, thin	10-3-98
	S.K. (99008)	F	6-22-99	7-10 days	Dehydrated, infection within 1 week	9-23-99
	Kali (00002)	F	4-23-00	3 days	Dehydrated latent failure to thrive	8-24-00
	Rainbow (00003)	F	5-17-00	5 days	Anal inflammation	8-24-00
	Bristol (00006)	F	6-24-00	4 weeks	Emaciated, diarrhea, lethargic	8-24-00
Group C	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]
	00001	M	4-16-00	2 days	Cesarean, premature drowned	5-28-00
	00004	F	5-26-00	5 days	Lethargic, yellowish gums, Puncture/scratch wounds	6-1-00
	00005	F	6-8-00	7 – 10 days	Lethargic - unresponsive Greenish stools	6-26-00

[a]picked up by tourist
[b]euthanized

Table 2. Serum chemistry values for ALT and AST for all six harbor seals fed either pollock or herring. ANOVA, mean (S.E.).

SEAL NAME		Pollock	Herring	P
Cecil	ALT AST	19.0 (2.8) 65.4 (4.7)	29.0(2.9) 42.7 (4.6)	0.013 0.001
Poco	ALT AST	41.2 (5.4) 58.1 (4.5)	55.0 (4.0) 42.2 (6.0)	0.003 0.041
Skeezix	ALT AST	38.2 (3.7) 70.5 (4.6)	52.3 (4.9) 57.3 (6.0)	0.050 0.031
Pender	ALT AST	70.8 (7.9) 183.0 (50.4)	206.4 (34.3) 63.1 (15.6)	0.024 0.039
Sydney	ALT AST	45.4 (5.4) 91.3 (7.5)	65.9 (5.4) 79.8 (5.5)	0.026 0.012
Travis	ALT AST	70.5 (5.2) 98.5 (12.2)	96.8 (7.1) 60.7 (4.9)	0.005 0.006

Table 3. Serum chemistry and blood hematological variables that changed with diet or season during feeding trials with harbor seals. "No response" variables did not show diet or seasonal patterns.

DIET	**SEASON**	**NO RESPONSE**	
ALT	Albumin	AP	Potassium
AST	Globulin	BUN	Protein
BUN/CR	A:G Ratio	Calcium	CK
GGT	Hemoglobin	Cholesterol	Sodium
Creatinine	Hematocrit	Phosphorus	Chloride
		LDH	Bilirubin

Table 4a. Serum chemistry values for harbor seal pups upon admission to the ASLC during 1998 - 2000.Values are expressed as median (italics), minimum, and maximum. Individual serum chemistry values for PV98009 (60 days old) at the time of admission/just prior to death. Bold font and asterisks represent values beyond the min/max values observed in Group A seals upon admission and at similar age, respectively.

GROUP		***ALB g/dL***	***ALKP U/L***	***ALT U/L***	***AMYL U/L***	***AST U/L***	***BUN mg/dL***	***CREA mg/dL***	***BUN/ CREA***	***Ca mg/dL***	***CHOL mg/dL***	***CK U/L***	***GGT U/L***
A	*median*	*3.64*	*230*	*236*	*312*	*89*	*36.4*	*0.36*	*90.6*	*9.5*	*316.6*	*141*	*51*
	min	3.03	150	86	139	57	21.3	0.31	59.2	9.3	243.0	95	32
	max	4.27	384	703	404	135	63.3	0.47	204.2	10.1	446.6	298	109
	n	5	5	5	5	5	5	5	5	5	5	5	5
B <2wks	*median*	*3.7*	*241*	*58*	*206*	*92*	*28.0*	*0.42*	*63.6*	*9.7*	*191.0*	*480*	*15*
	min	3.2	204	46	140	62	19.5	0.35	55.7	9.0	145.4	57	11
	max	4.1	282	66	235	102	73.0	0.44	173.8	10.1	308.0	1109	31
	n	3	3	3	3	3	3	3	3	3	5	3	3
C < 2wks	*median*	*3.3*	*166*	*60*	*197*	*60*	*27.0*	*0.35*	*80.9*	*9.2*	*301.5*	*605*	*65*
	min	3.0	161	57	167	35	25.3	0.33	72.3	8.9	294.7	131	52
	max	3.3	234	191	330	107	35.6	0.44	81.8	9.4	448.3	798	88
	n	3	3	3	3	3	3	3	3	3	3	3	3
B	*median*	*3.23*	*222*	*46*	*235*	*62*	*28.0*	*0.35*	*63.6*	*9.7*	*279.6*	*209*	*31*
	min	2.63	193	13	140	27	8.0	0.29	27.6	9.0	145.4	57	11
	max	4.11	287	66	622	102	73.0	0.44	173.8	10.1	415.2	1109	275
	n	5	5	5	5	5	5	5	5	5	5	5	5
C	*median*	*3.11*	*164*	*59*	*263*	*60*	*31.3*	*0.40*	*81.4*	*9.0*	*374.9*	*381.5*	*66*
	min	1.79	41	10	167	35	25.3	0.33	72.3	8.7	294.7	131	52
	max	3.34	234	191	438	107	99.2	0.66	150.3	9.4	452.6	798	88
	n	4	4	4	4	4	4	4	4	4	4	4	4
98009		***1.79****	***41****	***10****	***438***	*59*	***99.2****	***0.66****	*150.3**	***8.65****	*452.6**	*158*	*67**

ALB – albumin, ***ALKP*** – alkaline phosphatase, ***ALT*** – alanine transferase, ***AMYL*** – amylase, ***AST*** – aspartate aminotransferase, ***BUN*** – blood urea nitrogen, ***CREA*** – creatinine, ***Ca*** – calcium, ***CHOL*** – cholestrerol, ***CK*** – creatine phosphokinase, ***GGT*** – gamma-glutamyl transaminase

Table 4b. Serum chemistry values for harbor seal pups upon admission to the ASLC during 1998 – 2000 (cont'd).

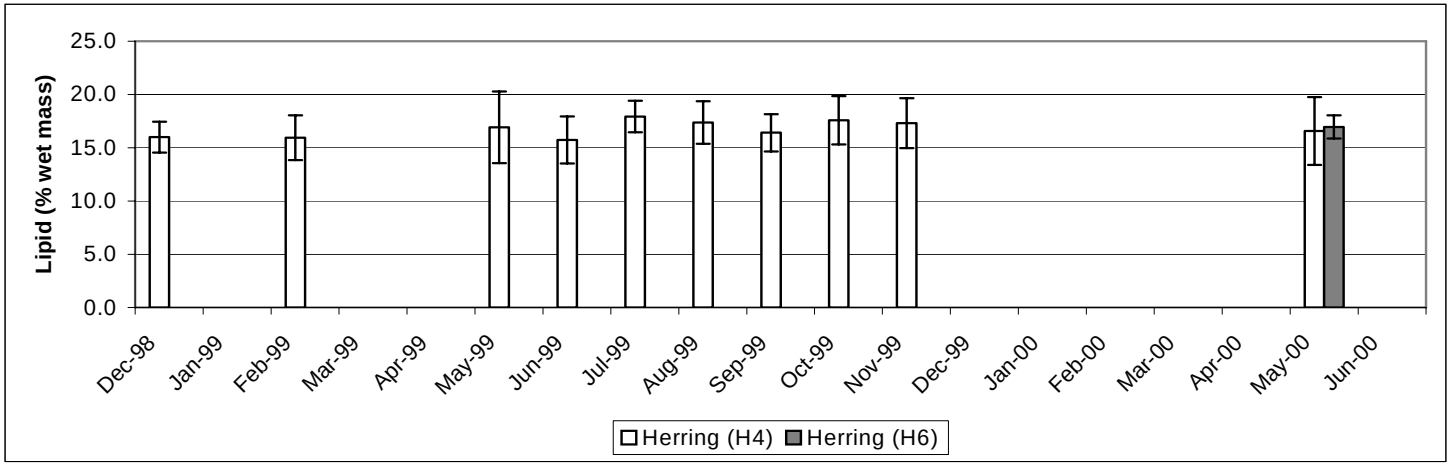
GROUP		*GLU mg/dL*	*PHOS mg/dL*	*TBIL mg/dL*	*TP g/dL*	*GLOB g/dL*	*ALB/ GLOB*	*TRIG mg/dL*	*Na mM*	*K mM*	*Cl mM*
A	***median***	***186.0***	***7.34***	***2.59***	***6.54***	***2.53***	***1.40***	***112.8***	***153.6***	***4.61***	***104.8***
	min	156.0	5.90	1.20	5.94	2.27	0.83	78.7	148.0	3.70	102
	max	190.2	8.98	6.06	6.69	3.66	1.88	149.5	160.0	5.15	113.1
	n	5	5	5	5	5	5	4	5	5	5
B <2wks	***median***	***152.6***	***7.4***	***12.5***	***6.2***	***2.6***	***1.46***	***80.8***	***154.1***	***4.9***	***107.4***
	min	137.6	6.9	2.5	5.8	2.5	1.24	69.0	153.8	4.9	102.5
	max	172.3	8.4	14.8	6.7	2.6	1.59	121.3	155.8	5.2	107.9
	n	3	3	3	3	3	3	3	3	3	3
C < 2wks	***median***	***180.8***	***7.4***	***3.1***	***6.0***	***2.8***	***1.2***	***94.7***	***151.6***	***5.2***	***104.8***
	min	150.4	6.3	1	6.0	2.7	1.0	82.4	151.6	4.8	103.5
	max	227.1	8.2	7.4	6.2	3.0	1.2	134.5	153.8	5.5	107.3
	n	3	3	3	3	3	3	3	3	3	3
B	***median***	***152.6***	***7.41***	***2.47***	***6.16***	***2.61***	***1.24***	***81.0***	***155.3***	***4.91***	***107.4***
	min	137.6	6.91	0.61	5.32	2.50	0.61	69.0	153.8	4.41	102.5
	max	201.5	8.42	14.78	6.98	4.34	1.59	121.3	157.6	5.26	115.8
	n	5	5	5	5	5	5	5	5	5	5
C	***median***	***165.6***	***7.79***	***3.87***	***5.98***	***2.91***	***1.09***	***114.6***	***151.5***	***5.35***	***105.7***
	min	109.2	6.33	0.99	5.90	2.73	0.44	82.4	145.1	4.80	103.5
	max	227.1	10.46	7.39	6.15	4.11	1.19	334.4	153.8	6.25	107.3
	n	4	4	4	4	4	4	4	4	4	4
98009		***109.2****	***10.46****	*4.63**	*5.90*	***4.11***	***0.44****	***334.4****	***145.1****	***6.25****	*106.6*

GLU – glucose, ***PHOS*** – phosphorous, ***TBIL*** – total bilirubin, ***TP*** – total protein, ***GLOB*** – globulin, ***ALB/GLOB*** – albumin/globulin, ***TRIG*** – triglyceride, ***Na*** – sodium, ***K*** – potassium, ***Cl*** – chloride

Table 5. Blood chemistry variables that were affected by pup age or health status. *P* values were determined using Kruskal-Wallis, non-parametric analysis. Effect of age (2 – 13 weeks old) was determined in pups from Group A (n = 5) as well as Groups A and B, combined (n = 8 at 2 weeks old, n = 10 at all other ages). Values in bold were significantly affected both in Group A alone and in the two groups combined. Values in italics were not significant, but still had a relatively low probability and were included only when there was a significant effect of age in the other category (Group A or Combined). Effect of health status (Group A *vs.* Group B) was determined for each age group. Pups from Group C were not included in any of these analyses.

	Kruskal-Wallis (P =)		
	Age		**Health Status**
	Group A	**Combined**	**Group A *vs.* Group B**
Albumin			
Alkaline Phosphatase			0.028[4]
Alanine Aminotransferase			0.025[1], 0.024[2]
Aspartate Aminotransferase	**0.022**	**0.005**	
Amylase			
Blood Urea Nitrogen	*0.112*	*0.075*	
Calcium			
Cholesterol	**0.019**	**0.002**	
Creatinine	**0.010**	**0.001**	0.024[2], 0.047[4]
BUN/Creatinine	**0.018**	**0.001**	0.025[2]
Glucose	0.021	*0.075*	
Phosphorous			0.027[5]
Total Bilirubin	*0.189*	0.049	
Total Protein			
Globulin	*0.222*	0.021	
Albumin/Globulin			
Sodium			
Potassium	0.007	*0.139*	0.009[3]
Chloride			
Triglyceride	**0.019**	**0.001**	
GGT	**0.002**	**0.001**	0.025[1]

[1] At admission (< 2 weeks old), [2] Approximately 2 weeks old, [3] 6-7 weeks old, [4] 9-10 weeks old, [5] 12-13 weeks old



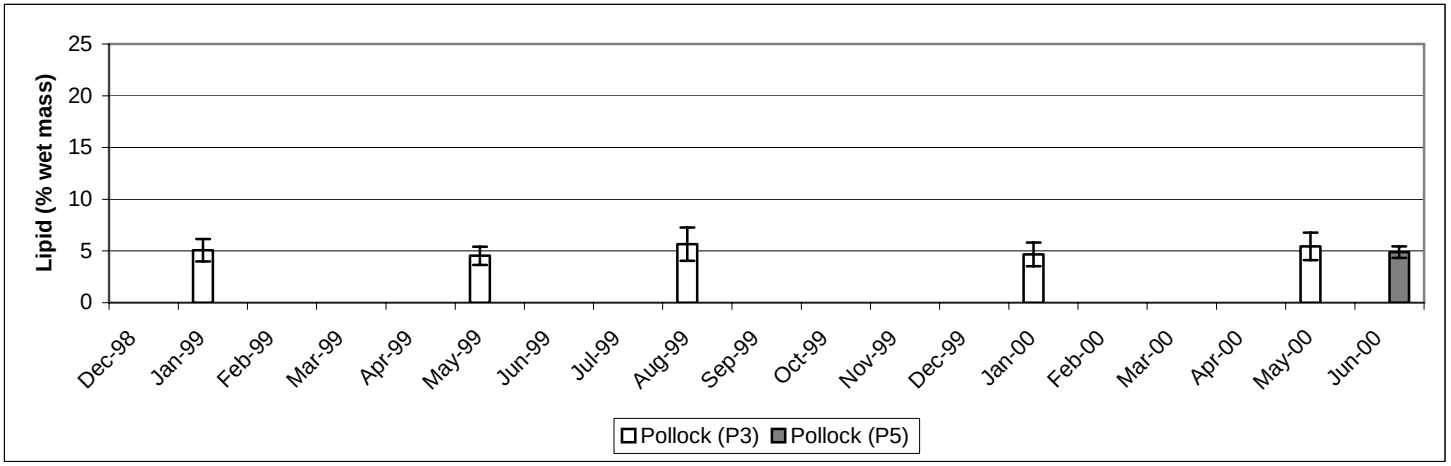
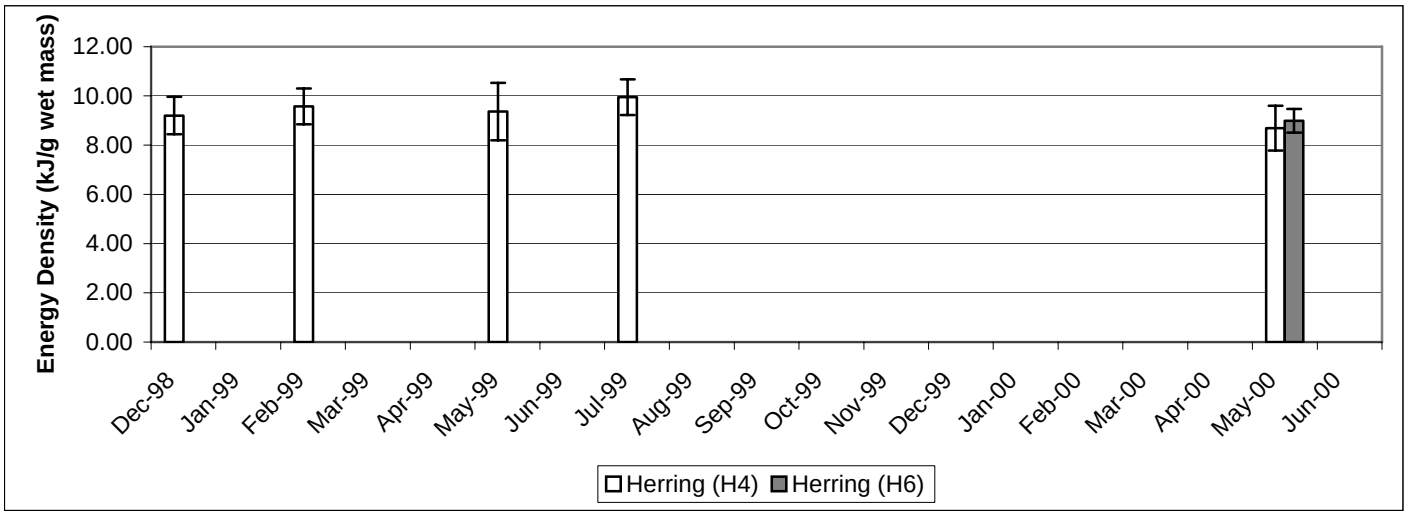
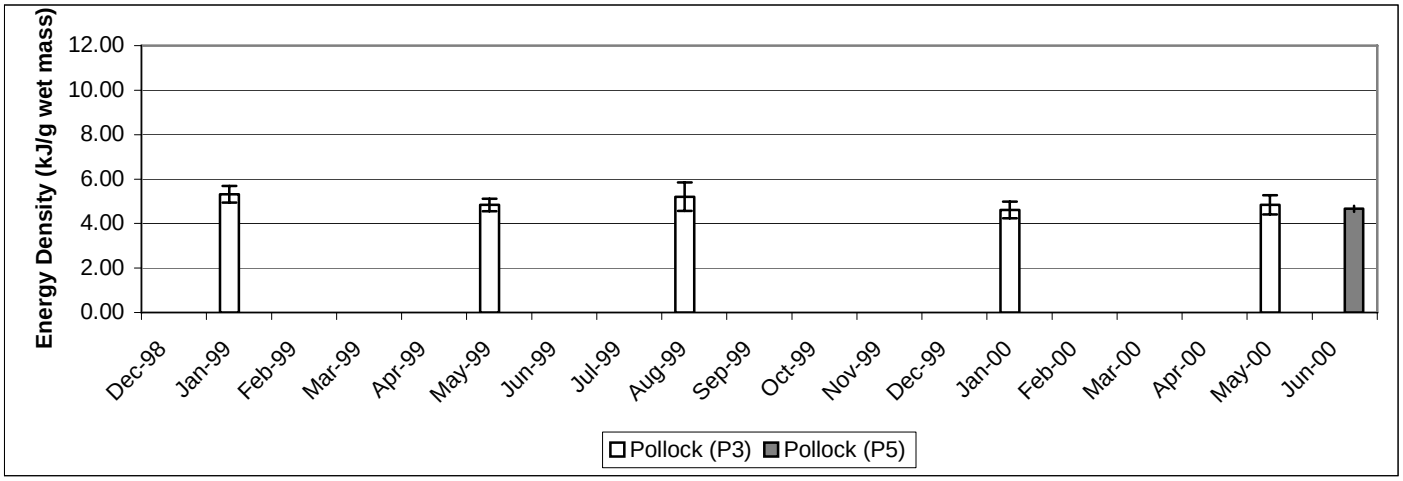


Figure 1. Lipid content (% of wet mass) of stored, frozen herring (***a***) and pollock (***b***) fed to harbor seals during feeding trial at the ASLC, expressed as mean ± S.D. H# and P# represent the batch numbers of herring and pollock used as food at the ASLC (see Methods).

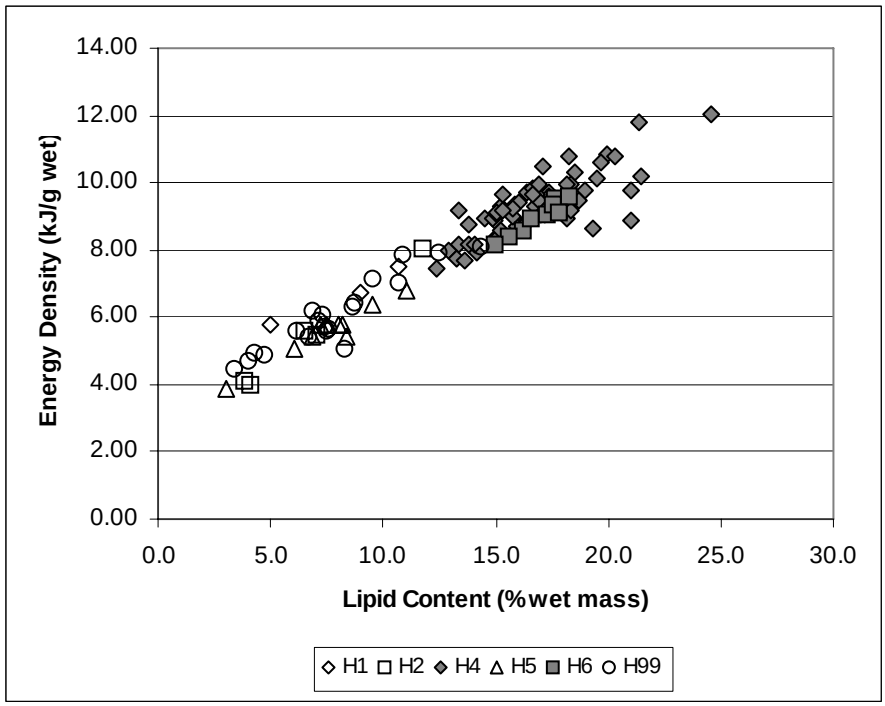


a



b

Figure 2. Energy density (kJ/g wet mass) of stored, frozen herring (***a***) and pollock (***b***) fed to harbor seals during feeding trials at the ASLC, expressed as mean ± S.D. H# and P# represent the batch numbers of herring and pollock used as food at the ASLC (see Methods).



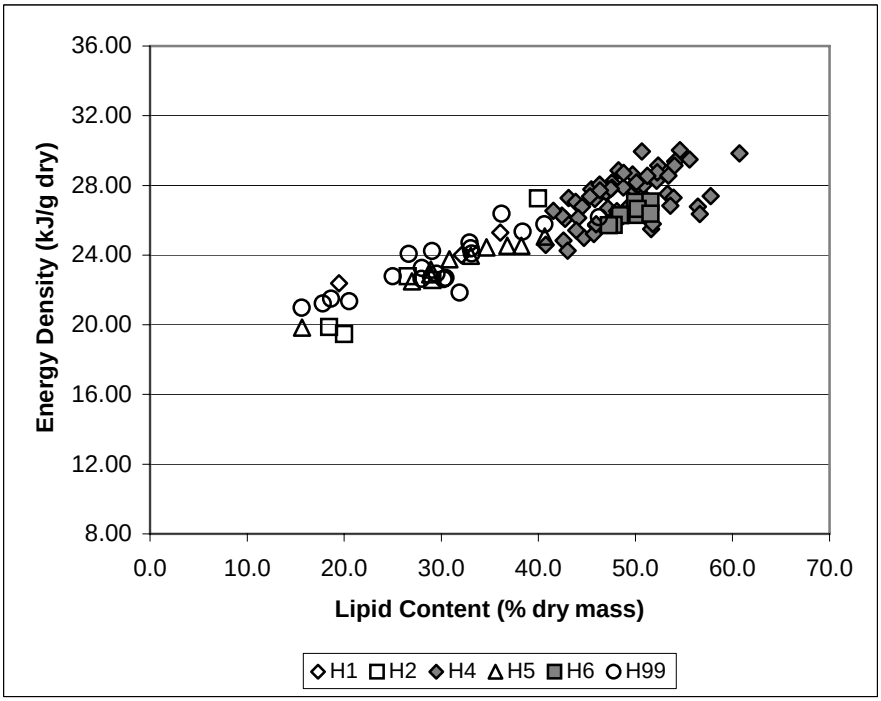


Figure 3. Relationship between lipid content and energy density of herring, including herring fed to seals during feeding trials at the ASLC (shaded symbols, H4 and H6). ***a.*** lipid content and energy density expressed as a function of wet mass (*Corr* = 0.963) ***b.*** lipid content and energy density as a function of dry mass (*Corr* = 0.902). H# represents the batch numbers of herring used as food (except H99) at the ASLC (see Methods).

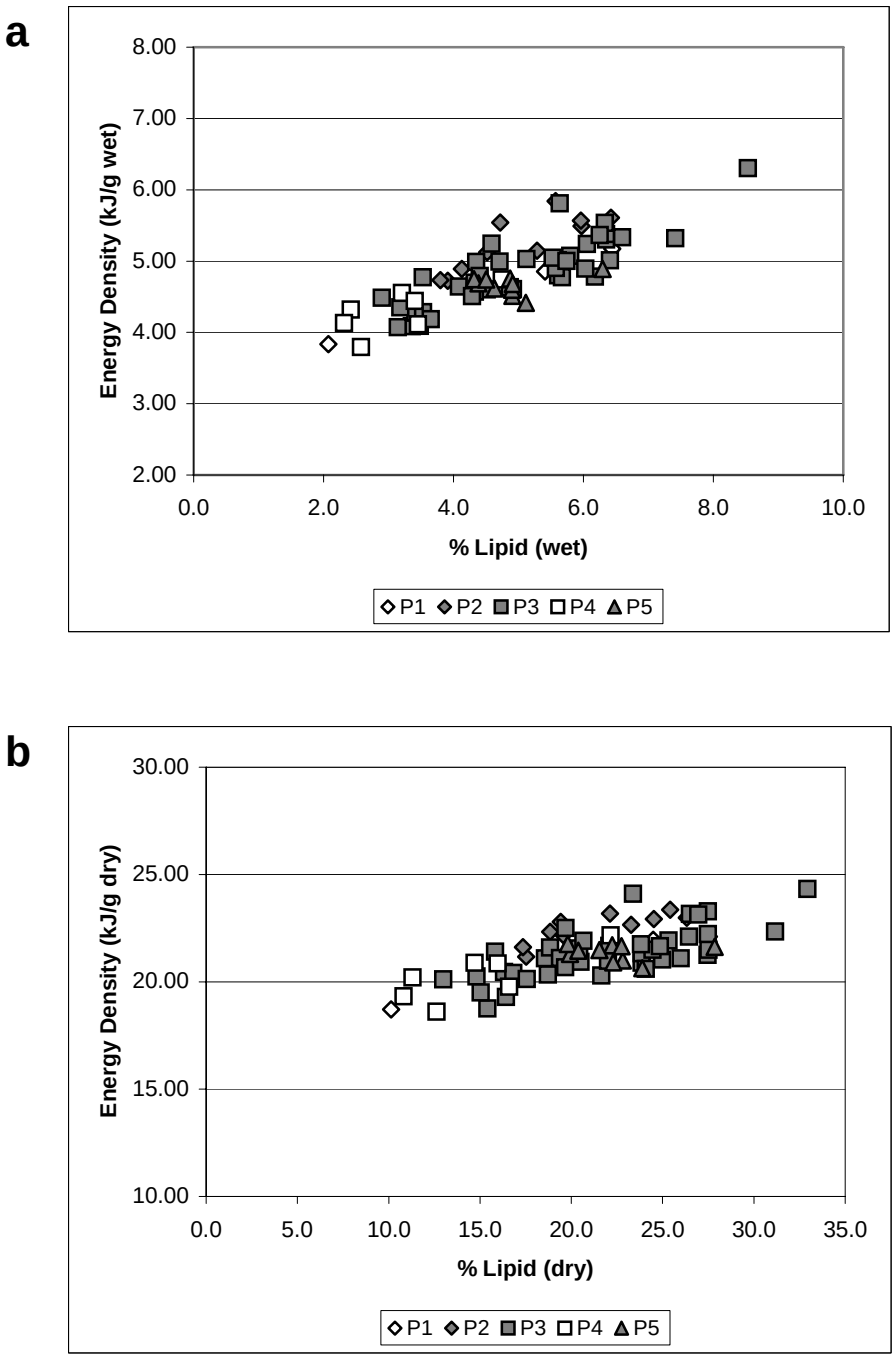
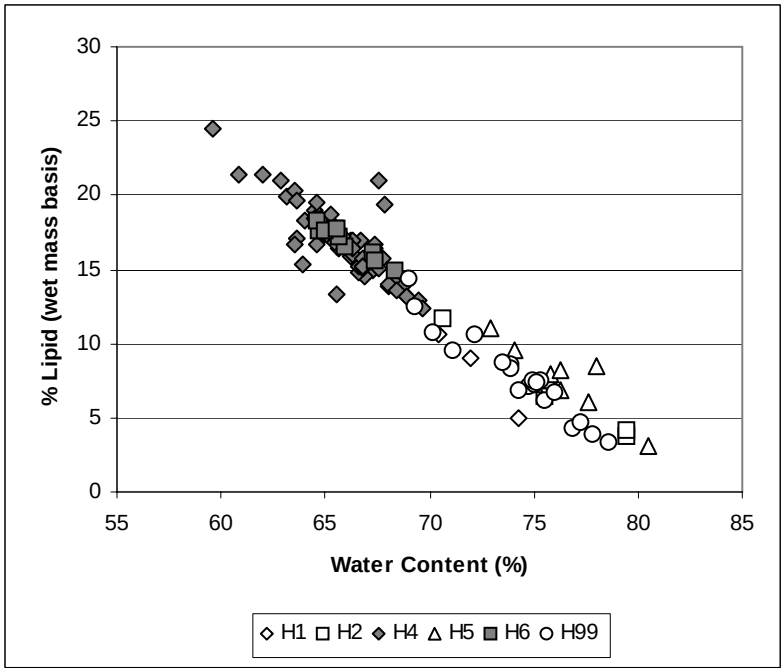


Figure 4. Relationship between lipid content and energy density of pollock, including pollock fed to seals during feeding trials at the ASLC (shaded symbols, P2, P3 and P5). ***a***. lipid content and energy density expressed as a function of wet mass ($r = 0.819$, $r^2 = 0.666$). ***b***. lipid content and energy density as a function of dry mass ($r = 0.708$, $r^2 = 0.493$). P# represents the batch numbers of pollock used as food at the ASLC.



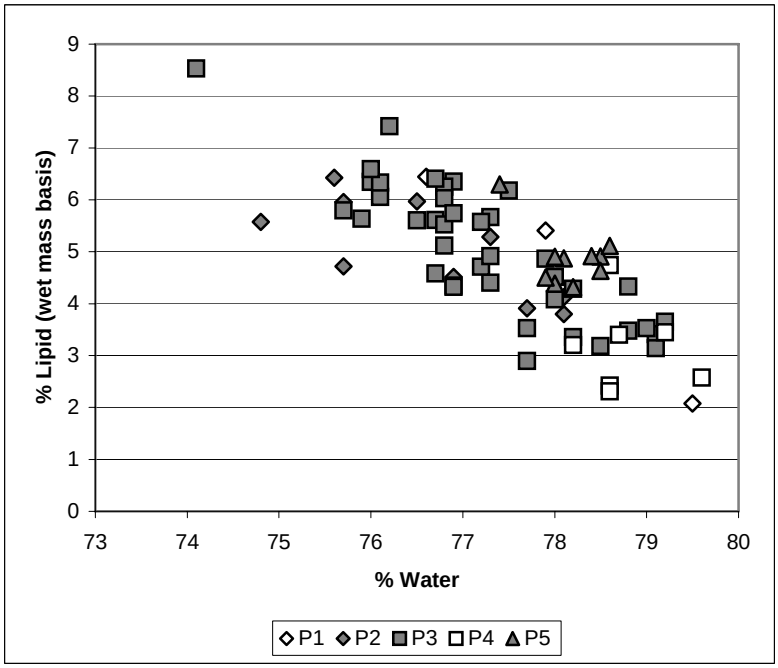


Figure 5. Relationship between water content and lipid content (wet mass basis) of herring and pollock, including herring (H4 and H6) and pollock (P2, P4 and P5) fed to seals during feeding trials at the ASLC (shaded symbols). ***a***. herring, (*Corr* = -0.966) ***b.*** pollock (*Corr* = -0.793). H# and P# represent the batch numbers of herring and pollock used as food at the ASLC (see Methods).

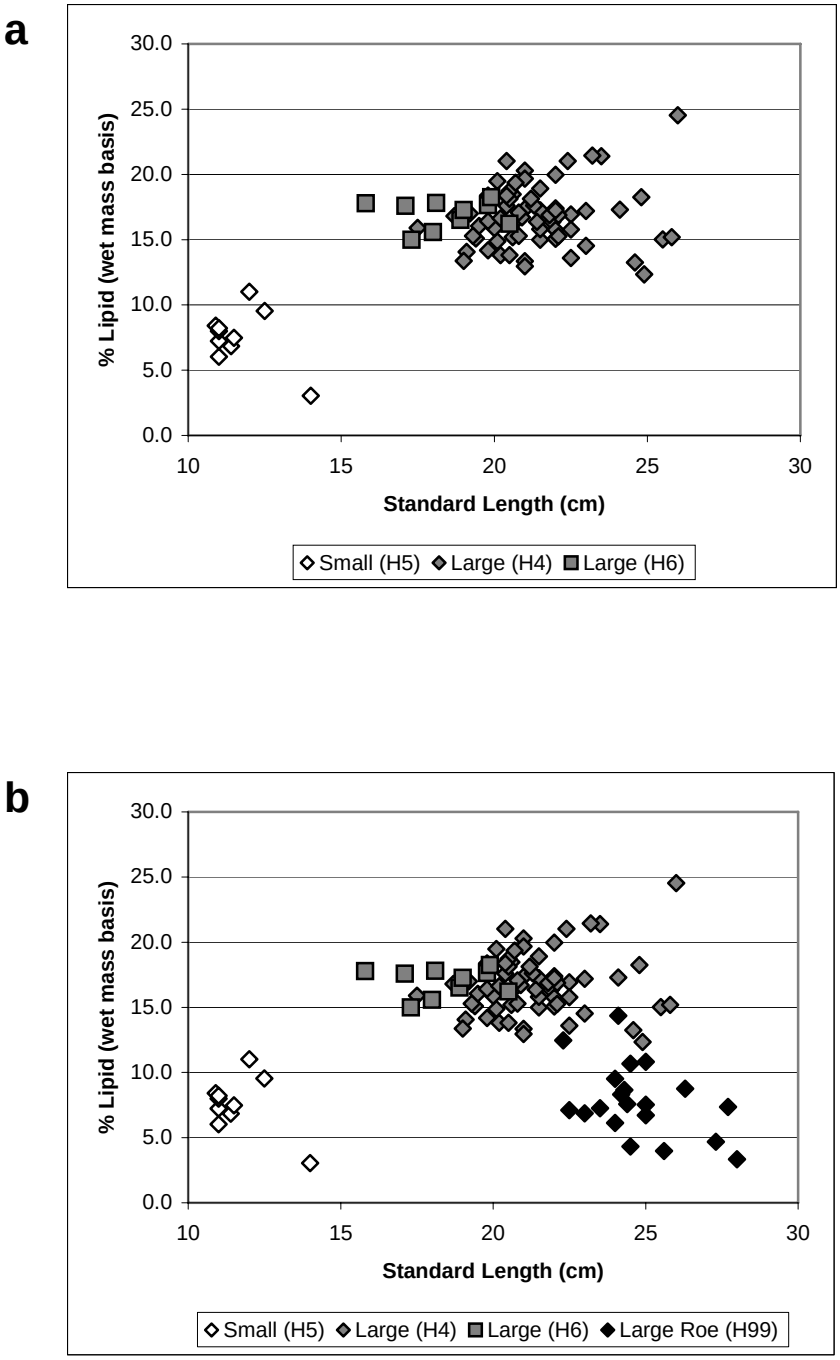


Figure 6. Relationship between standard length and lipid content (wet mass basis) of herring. ***a.*** small (H5) and large (H4 and H6) herring (*Corr* = 0.707) ***b.*** small (H5), large (H4 and H6) and large roe (H99) herring (*Corr* = -0.070). H# represent the batch numbers of herring used as food (except H99) at the ASLC (see Methods).