

*Exxon Valdez* Oil Spill  
Restoration Project Final Report

Recovery of Harbor Seals from EVOS: Condition and Health Status

Restoration Project 97001  
Final Report

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April 1998

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### Restoration Project 97001

### Final Report

**Study History:** This project began in FY 93 as a Research Service Agreement with the Alaska Department of Fish and Game. In FY 95 it was initiated as Restoration Project 95001. An annual report was issued in 1996 by Fadely and Castellini under the title Recovery of Harbor Seals from EVOS: Condition and Health Status. The project effort was continued under Restoration Project 96001. Blubber analyses were initiated in FY 95 as a Broad Agency Announcement award (95117-BAA), and rolled over in FY 96 as part of project 96001. An annual report was issued in 1996 by Fadely, Castellini and Castellini under the title Harbor Seals and EVOS: Blubber and Lipids as Indices of Food Limitation. A further annual report was issued in 1997 by Fadely, Castellini and Castellini under the title Recovery of Harbor Seals from EVOS: Condition and Health Status. Three journal articles have been published covering portions of this project (Castellini, J.M., H.J. Meiselman and M.A. Castellini. 1996. Understanding and interpreting hematocrit measurements in pinnipeds. *Marine Mammal Science* 12(2):251-264; Zenteno-Savin, T., M.A. Castellini, L.D. Rea and B.S. Fadely. Plasma haptoglobin levels in threatened Alaskan pinniped populations. *Journal of Wildlife Disease* 33(1):64-71. 1997; Zenteno-Savin, T. and M.A. Castellini. 1998. Plasma angiotensin II, arginine vasopressin and atrial natriuretic peptide in free ranging and captive seals and sea lions. *Comparative Biochemistry and Physiology* 119C(1):1-6. The project effort was continued under Restoration Project 97001, closed out in FY 98 as Restoration Project 98001 and is summarized in this Final Report.

**Abstract:** The objectives of this project were to establish criteria to evaluate health and body condition of harbor seals (*Phoca vitulina*) within Prince William Sound and the Gulf of Alaska in reference to potential problems induced by the *Exxon Valdez* Oil Spill. We constructed plasma chemistry and hematology reference ranges from 296 seals collected during 1991-1996. Significant handling, individual and seasonal effects were found. Small differences in blood variables could be detected with high statistical power. While some seals sampled showed more clinically aberrant values than expected by chance, the majority appeared healthy. Blubber from Prince William Sound seals was less hydrated than blubber from seals sampled from non-declining areas. Blubber samples from 1977 were slightly less hydrated and slightly more energy-rich than matched contemporary samples. Condition indices of blubber content and body size did not show differences among Prince William Sound, Kodiak Island and southeast Alaska seals or pre- and post-decline in the Gulf of Alaska. Temporal and regional differences in carrying capacity are consistent with these data, although other factors such as predation, human-caused mortality and contaminant exposure should continue to be explored.

**Key Words:** Blood chemistry, blubber, body condition, harbor seals, health, hematology, Kodiak Island, *Phoca vitulina*, Prince William Sound, southeast Alaska, subsistence harvest, physiology.

**Project Data:** *Description of data* - The data collected during this project include blood chemistry and hematology measurements (standard veterinary panel) from 296 harbor seals collected during 1991–1996 in Prince William Sound and the Gulf of Alaska. Other measurements include morphometric data (mass, standard length, girths and blubber depths). Proximate analyses of blubber samples obtained from the biosampling program (Restoration Project /244) cover samples from the Prince William Sound and the Gulf of Alaska and include lipid content, energy density and water content. Proximate analyses of archived blubber samples cover samples from Kodiak Island and southeast Alaska collected by the Alaska Department of Fish and Game from 1976–1978. *Format* - All the aforementioned data are recorded in Microsoft Excel and Microsoft Access 97. Histological data (measurement of adipocytes) from blubber samples are recorded in Microsoft Excel97. *Custodian* - Dr. Michael A. Castellini, Institute of Marine Science, University of Alaska Fairbanks, Fairbanks, AK 99775-7220, Phone: (907) 474-6825, -7204, e-mail: mikec@ims.alaska.edu

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Appendix 2. Errata from Fadely, B. S. 1997. Investigations of harbor seal (*Phoca vitulina*) health status and body condition in the Gulf of Alaska. Ph.D. Thesis. Univ. of Alaska, Fairbanks, Alaska. 183pp.

## 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. **Assessment and interpretation of harbor seal health status data may help resolve multiple hypotheses proposed to explain these declines and focus future studies.** This study describes harbor seal health and condition based on hematological, morphometric and body fat indices.

Blood chemistry and hematology values can be indicative of health status, disease, nutritional status, or environmental conditions. Such interpretations require establishment of a set of reference or 'normal' ranges for these blood values and indications of clinical or sub-clinical disease can be detected if the effects of non-health related variation can be distinguished for free-ranging animals. At the time of the *Exxon Valdez* oil spill, most comparative hematological values for harbor seals derived from a few captive animal studies with small sample sizes, sufficient for examining general health, but insufficient for more detailed interpretations of health status. Body condition can be used as another indicator of population health. If declining populations are nutritionally limited, decreases in body condition may be evident as foraging expenditures increase. Likewise, because harbor seals rely on subcutaneous blubber for insulation and energy storage, changes in blubber quantity or quality may indicate environmental differences.

This study was designed to develop standardized blood profiles of seals from PWS and the Gulf of Alaska and to quantify sources of variation from handling, age, gender, seasonal, regional and interannual sources. Various indices of body condition related to total blubber content and energy status have also been tested, including assessments of blubber quality from samples provided by subsistence harvesters.

This final report summarizes all work accomplished under Restoration Project /001 and Restoration Project 95117-BAA (which was combined with project 96001). It also describes work accomplished under Restoration Project 97001, subsequent to our previous Annual Report for 96001 completed in April 1997. In previous reports we established reference ranges for plasma chemistries, hemograms and leukograms, including analyses of sources of variance due to individual and environmental factors such as age, gender, seasonal and regional effects. Estimates of 95% confidence intervals around range limits were calculated. We proposed using statistical models based on outlier frequency to help separate clinically unhealthy animals from a background frequency expected to occur randomly. We had also reported on regional variations in blubber quality from blubber samples provided by Restoration Project /244.

### **Objectives**

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

1. Collect hematological data to establish reference ranges of blood chemistries and hematologies of PWS harbor seals and determine variation attributable to sampling technique, age, gender, or season and location of capture.
2. Estimate our ability to detect changes in body condition using morphometric measurements.



3. Assess body condition using morphometric measures of body shape, density and fat content, and determine the effects of age, gender, season and location.
4. Compare blood and morphological indices of health and condition in light of the above to examine interannual changes, potential spill-related impacts and to help interpret changes in population status.
5. Assess blubber quantity and quality with respect to fat and water content and energy density and determine variation attributable to age, gender, or season and location of capture.

### ***Plasma Chemistry and Hematology***

Using up to 296 samples collected during 1991–1996, we constructed reference ranges for 36 blood and plasma variables. Handling, individual, regional and temporal factors accounted for up to 55% of the variation in plasma chemistries in the 1995–1996 data subset. For example, the delay between seal capture and blood sampling significantly affected plasma potassium concentrations and creatine phosphokinase, lactate dehydrogenase and aspartate aminotransferase activities. In a second run of regression models that removed handling factors, individual, regional and temporal effects explained 2.1–39.2% of the variation in plasma chemistries. White blood cell properties were relatively insensitive to effects of drugging and sample handling and were unaffected by gender. However, individual, regional and temporal factors explained up to 19% of variation in leukogram measurements. Handling, individual, regional and temporal factors accounted for up to 56% of the variation in hematological measurements in the 1995–1996 data subset. Hematocrit, hemoglobin and red blood cell count varied directly with each other and had large seasonal components to their variability.

Age, gender, seasonal and regional effects were apparent in most blood parameters, and the magnitude of each effect was calculated. By conducting an analysis of statistical power for interannual and interregional comparisons, we found that small differences were detectable with high statistical power, and that the variability introduced by individual or environmental effects were of similar magnitude. Therefore, temporal and regional comparisons must be carefully constructed to avoid biasing by non-health effects.

### ***Outlier Theory***

One hundred and fifty eight seals sampled between 1993–1996 were screened for outliers among the 36 chemical and hematological variables, relative to reference ranges. Of these seals, 84% had at least one statistically outlying variable and the outlier frequency distribution was similar to that predicted by a binomial distribution. For field applications where reference ranges were determined without other knowledge of the animals' health status, these distributions suggest that only subjects with at least four outliers could be considered to have clinical concerns beyond that expected by chance. This number will depend on the number of chemical and hematological variables being considered. Approximately 21% of seals which were screened for outliers had at least four outlying blood analyte values. These seals were not clustered by region, season, or year of sampling. This suggests that no sampled group was disproportionately compromised with respect to health status.

### *Juvenile vs. Adult Comparisons*

Comparisons of blood chemistry values from juvenile and adult PWS harbor seals show trends that are consistent with dietary differences. Juveniles had elevated white blood cell and neutrophil counts, higher red blood cell counts, higher hemoglobin concentrations, elevated mean corpuscular hemoglobin content and decreased mean corpuscular volume. These values may also be influenced by developmental changes. Further study of these indices with captive seals fed controlled diets and with pups and juveniles should help clarify the interpretation of these data.

### *Condition Indices*

In general, morphometric condition indices did not provide adequate precision in reflecting changes in true condition for either sex, although measurements of length and girth provided excellent mass prediction. There were no detectable differences in body condition of seals (body mass adjusted for size) from the Gulf of Alaska sampled during 1963–1964 (pre-decline), 1976–1978 (during major decline) and 1989–1996 (after major decline). However, sample sizes were small with a patchy distribution throughout locations and years, reducing the likelihood of detecting body condition changes in response to environmental conditions. There was no evidence of long term blubber thickness changes for males or females between 1973 and 1996. As with long-term comparisons, there was little evidence of condition differences among Kodiak Island (KI), PWS and southeast Alaska (SE) seals during 1993–1996. Kodiak Island seals tended to be more massive at length than in SE or PWS, but only during autumn. There was no apparent difference in sculp content based on blubber depth measurements among the three regions, or among years within regions. Combining the lack of condition differences with a population decline in PWS suggests that differences in carrying capacity could exist. This is also consistent with the lack of population recovery following the *Exxon Valdez* oil spill.

### *Blubber Quality*

Comparisons of blubber quality, measured as energy density (kJ/g), were performed on samples ( $n=74$ ) collected from subsistence harvesters in PWS, Yakutat and SE and provided to us by the biosampling program (Restoration Project /244). There were no geographical differences of blubber energy densities (dry-mass basis) among seals sampled during spring 1995–1996 from PWS ( $39.1 \pm 0.2$  kJ/g,  $n = 14$ ), Yakutat ( $38.8 \pm 0.2$  kJ/g,  $n = 10$ ) or SE ( $38.8 \pm 0.3$  kJ/g,  $n = 5$ ,  $P = 0.371$ ). Energy densities were also not significantly different between sexes ( $P = 0.169$ ), but male blubber was more hydrated ( $6.5 \pm 0.3\%$ ,  $n = 19$ ) than female blubber ( $5.3 \pm 0.3\%$ ,  $n = 14$ ,  $P = 0.011$ ). There were no geographical differences in blubber energy densities among seals sampled during autumn 1995–1996 from PWS ( $39.1 \pm 0.2$  kJ/g,  $n = 14$ ) and SE ( $39.0 \pm 0.2$  kJ/g,  $n = 12$ ,  $P = 0.697$ ) and there were no gender differences of energy density ( $P = 0.768$ ) or water content ( $P = 0.227$ ). However, blubber from PWS seals was significantly less hydrated ( $5.6 \pm 1\%$ ,  $n = 14$ ) than blubber from SE seals ( $8.4 \pm 1\%$ ,  $n = 12$ ,  $P = 0.001$ ). Blubber energy density (dry-mass basis) did not vary seasonally within PWS or SE, although water content of blubber from SE males was significantly different between autumn ( $8.8 \pm 0.8\%$ ,  $n = 8$ ) and winter ( $6.1 \pm 0.6\%$ ,  $n = 8$ ,  $P = 0.040$ ), but not spring ( $6.9 \pm 1.1\%$ ,  $n = 3$ ).

### *Interannual Comparisons using Archived Samples*

Using all available archived blubber, samples collected from SE and KI during 1976–1977 had significantly lower water content and greater lipid content (dry-mass basis) than the 1995–1996 samples. However, energy densities (dry-mass basis) were not significantly different between archived and recently collected blubber. There were no significant differences in how energy density (wet-mass basis) varied with water content or with lipid content, which may have been expected if lipids had been degraded or oxidized over time. Using a more conservative approach in which archived and contemporary samples were matched by season and region (winter, SE), it was determined that blubber samples from 1977 were less hydrated and slightly more energy-rich than contemporary samples. The 1977 samples had a slightly higher lipid content (dry-mass basis) (1977,  $98.6 \pm 0.2\%$ ; 1996,  $97.6 \pm 0.2\%$ ;  $t_s = 3.35$ ,  $df = 12$ ,  $P = 0.006$ ) and energy density (dry-mass basis) (1977,  $39.1 \pm 0.1$  kJ/g; 1996,  $38.7 \pm 0.1$  kJ/g,  $t_s = 2.076$ ,  $df = 19$ ,  $P = 0.006$ ). A further comparison of matched samples collected from Yakutat in 1976 and 1996 produced similar results. While the water content of samples collected in 1976 was not significantly different than samples collected in 1996 (1976,  $5.2 \pm 0.6\%$ ; 1996,  $5.9 \pm 0.6\%$ ,  $P = 0.151$ ), as in the previous analysis of matched samples the 1976 values were clustered at the low end of the range. The 1976 and 1996 samples from Yakutat had similar energy densities (dry-mass basis) (1976,  $39.3 \pm 0.1$  kJ/g; 1996,  $39.16 \pm 0.4$  kJ/g,  $P = 0.498$ ). Based on these comparisons, blubber quality did not show obvious changes during this 20 year period.

### *Histology*

Histological analysis of blubber samples proved to be a poor indicator of blubber quality. Adipocyte size was not correlated to blubber depth, blubber hydration state or energy density. Histological examination revealed groups of large adipocytes ranging from 85–130  $\mu\text{m}$  in diameter ( $104 \pm 9$   $\mu\text{m}$ ,  $n = 36$ ) separated by bands of collagen.

### *Conclusion*

In conclusion, blood chemistries were found to vary with handling technique, gender and regional and temporal effects, and reference ranges were constructed for detection of outliers with respect to the overall population. Blood profiles were sensitive to environmental factors and aspects of seal behavior. Analyses of contemporary blood profile data found regional and interannual trends that differed among juvenile and adult age classes. While these differences are consistent with different diets, developmental influences have not been assessed. A significant number of seals had greater than 3 blood analyte values classified as outliers, however there was no trend with respect to region or time period in which these occurred and most seals appeared healthy. Morphometric indices of condition were generally poor predictors of condition. Though sample sizes for comparison were limited, no evidence was found for changes in body condition of harbor seals sampled during 1963–1964, 1972–1978 and 1989–1996. There was also no evidence of condition differences among current KI, PWS and SE harbor seals. Seal blubber from PWS was slightly more hydrated, and thus had a slightly higher energy content (about 1% for a 50 kg seal) than seals from SE. A plausible conclusion based on population declines and no apparent change in body condition is that environmental carrying capacity decreased near the oceanic ‘regime shift’ in 1976. A similar conclusion is postulated for current differences in population trajectories among PWS, KI and SE. Other factors may include predation, human-

caused mortality, contaminants accumulation, or lack of sample coverage. Many of the blood profile results were intriguing and more detailed examination of the interrelationship between body condition, blood chemistry, hematology, endocrinology and diet in captive seals would be useful.

This final report has been prepared according to the EVOS Trustee Council guidelines. In agreement with the Trustee Council, this report consists primarily of the Ph.D. thesis of Brian Fadely (Fadely, B. S. 1997. Investigations of harbor seal (*Phoca vitulina*) health status and body condition in the Gulf of Alaska. Ph.D. Thesis, Univ. of Alaska, Fairbanks, Alaska. 183pp.). Included are the sections as outlined in *Procedures for Preparation and Distribution of Reports*, provided by the Trustee Council, with summaries of Methods, Results and Discussion, referring as appropriate to the thesis for details. A final, original copy of the thesis is attached as Appendix 1).

## INTRODUCTION

Harbor seal (*Phoca vitulina*) populations in Alaska have declined significantly over portions of their range, particularly in the Gulf of Alaska (Small and DeMaster 1995). Prior to the *Exxon Valdez* oil spill in 1989, population declines of 85% had been reported from Tugidak Island (Pitcher 1990), and declines may also have occurred in the eastern Bering Sea and Aleutian Islands, while populations in southeast Alaska (SE) have been stable or increasing (Hoover-Miller 1994, Small 1996, Lewis 1996). Prince William Sound (PWS) harbor seal populations, further impacted by the spill (Frost and Lowry 1994a, Frost et al. 1995) have continued to decline at about 6% per year (Frost et al. 1997). Assessment and interpretation of harbor seal health status data will help resolve multiple hypotheses proposed to explain these declines and to help focus future studies. If the PWS harbor seals are compromised, then we will know some of the directions that should be followed towards potential restoration. If they are not compromised, then we can focus our attention on other areas that may better explain their current recovery status. This study attempts to describe harbor seal health and condition based on hematological, morphometric and body fat indices.

Blood chemistry and hematology values can be indicative of health status, disease, nutritional status or environmental conditions (Seal et al. 1975, Geraci et al. 1979, McConnell and Vaughan 1983, Kuiken 1985, Roletto 1993, Schumacher et al. 1995). Such interpretations require establishment of a set of reference or 'normal' ranges for these blood values, and potential homeostatic imbalances in organ systems or metabolic pathways can be detected if the effects of non-health related variation can be distinguished for free-ranging animals (Seal et al. 1975, Payne and Payne 1987, Kerr 1989, Castellini et al. 1993, Schumacher et al. 1995). For example, at the time of the *Exxon Valdez* oil spill, most comparative hematological values for harbor seals derived from a few captive animal studies with small sample sizes, sufficient for examining general health, but insufficient for more detailed interpretations of health status (Englehardt 1979, McConnell and Vaughan 1983, Bossart and Dierauf 1990). McConnell and Vaughan (1983) and Schumacher et al. (1995) have also demonstrated that blood chemistries differ between captive and free-ranging seals. Subsequent field and captive studies have expanded on this knowledge and included analyses of variation due to animal and environmental factors, but were still comprised of relatively small sample sizes (Kopec and Harvey 1995, de Swart et al. 1995, Schumacher et al. 1995), or were biased towards pups (Roletto 1993). The ultimate goal of this project was to derive useful indices of condition and hematology, that when controlled for other sources of variation such as gender, age, season of capture, and animal and sample handling techniques, would enable interannual and interregional comparisons of

nutritional and health status. We constructed plasma chemistry and hematological reference ranges based on up to 296 blood samples collected between 1991–1996 from free-ranging seals in the Gulf of Alaska and estimated confidence intervals for these ranges. We found that handling, individual, temporal and regional effects were sufficiently large to influence results of statistical population comparisons. These effects must therefore be considered when performing interannual or interregional comparisons. We analyzed statistical outlier distributions in an attempt to identify seals with clinically significant blood variable profiles. Approximately 21% of seals screened had more than three outlier variables, and may have been clinically unhealthy at the time of sampling. No trends were evident in terms of location and time of capture of these seals or which variables were outside reference range confidence limits. Differences were observed between some hematological variables of juvenile and adults seals in PWS. These differences may be related to diet or other environmental factors but may also be influenced by developmental processes.

Harbor seals rely on subcutaneous blubber for insulation and energy storage (Ryg et al. 1988), and the quantity and thickness of blubber have been found to vary with season and energy intake (Nordøy and Blix 1985, Pitcher 1986, Ryg et al. 1988, Beck et al. 1993). Because one proposed hypothesis for population declines in PWS has been food limitation and nutritional stress (Alaska Sea Grant 1993), we examined whether compositional or quantitative differences existed in blubber from seals among regions exhibiting different population dynamics around the Gulf of Alaska. There were no geographical differences in blubber energy densities among seals sampled in the autumn (PWS, SE) or spring (PWS, Yakutat and SE) of 1995–1996. Blubber from PWS was slightly less hydrated than SE seal blubber, resulting in slightly higher energy density (wet-mass basis). However, there was no difference in energy density between PWS and SE blubber samples on a dry-mass basis. Using matched contemporary and archived samples (winter, SE), it was determined that blubber samples from 1977 were less hydrated and slightly more energy rich (dry-mass basis) than contemporary samples.

If declining populations are nutritionally limited, decreases in body condition or composition may be evident as foraging expenditures increase. One goal of this study was to determine whether size or compositional changes have occurred among harbor seals from the Gulf of Alaska between the 1970's and 1990's. There were no detectable differences in body condition of seals from the Gulf of Alaska sampled during 1963–1964 (pre-decline), 1976–1978 (during major decline) and 1989–1996 (after major decline) and there was no evidence of long term blubber thickness changes for males or females between 1973 and 1996. There was also little evidence of condition differences among Kodiak Island, PWS and SE seals during 1993–1996. These results suggest potential temporal and regional differences in carrying capacity but do not preclude the involvement of other factors such as predation, human-caused mortality and contaminants.

## **OBJECTIVES**

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

1. Collect hematological data to establish reference ranges of blood chemistries and hematologies of PWS harbor seals and determine variation attributable to sampling technique, age, gender, or season and location of capture.

2. Estimate our ability to detect changes in body condition using morphometric measurements.
3. Assess body condition using morphometric measures of body shape, density and fat content, and determine the effects of age, gender, season and location.
4. Compare blood and morphological indices of health and condition in light of the above to examine interannual changes, potential spill-related impacts and to help interpret changes in population status.
5. Assess blubber quantity and quality with respect to fat and water content and energy density and determine variation attributable to age, gender, or season and location of capture.

## **METHODS**

Harbor seals were captured from three general geographic regions within the Gulf of Alaska; Kodiak and Sitkinak Islands (grouped as Kodiak Island, KI), Prince William Sound (PWS) and southeast Alaska (SE). Captures were conducted during spring (March–May) and/or autumn (September–October) throughout 1993–1996 in all or some of the three regions. In PWS, captures also occurred during April 1991–1992 (Frost and Lowry 1994*b*), and July–August 1995–1996. July–August SE captures also occurred in 1994. Seals captured during 1992–1996 in spring and autumn within PWS were also utilized by Frost and Lowry (1994*b*) and Frost et al. (1995) for satellite-tagging and trophic interaction studies. Seals captured during 1993–1994 at Kodiak Island and southeast Alaska were also studied by Swain (1996) and Lewis (1995, 1996) for movement and dive behavior. Some of the plasma chemistry data from these seals have been included in studies by Castellini et al. (1996) and Zenteno-Savin et al. (1997, 1998). Sampling distributions and biases are presented in Appendix 1 (Chapter 2, pages 21 - 22 and Table 1).

Additional historical data were derived from several sources including morphometric measurements of harbor seals during 1963 (April - July) from Aialik and Harris Bays, Kenai Peninsula and during 1964 (April - July) from Tugidak Island (Bishop 1967), during 1972–1978 from the Gulf of Alaska (Pitcher 1977, 1986; Pitcher and Calkins, 1983), during 1979 and 1985 from the Bering Sea (Frost and Lowry, unpublished data) and during 1989–1990 from PWS and the Barren and Kodiak Islands (Frost and Lowry 1994*a*). These data were collected at various times of the year and have been grouped seasonally for analysis.

Morphometric measurements and blubber samples were provided by the biosampling program (Restoration Project /244), conducted in cooperation with Native subsistence hunters, the Alaska Native Harbor Seal Commission, and the National Marine Fisheries Service. Samples were lumped categorically according to time of year, region, year and sex and were probably representative of what was available to the hunter. Age has not yet been determined.

### ***Animal handling and sample collection***

Seals were live-captured near haul-outs by net-entanglement as described in Frost and Lowry (1994*b*) and Lewis (1995, 1996). After removal from the net, seals were bundled individually in hoop-net bags made of fine-mesh nylon webbing attached to rubber hoops. Seals were transported to ship or shore, and placed in relatively quiet locations until further restrained either manually, or chemically by intramuscular injection with a ketamine/diazepam mixture

(Frost et al. 1995). Body mass was measured ( $\pm 0.1$  kg) with a hanging electronic load cell balance (Ohaus Model I-20W), and blood samples were collected prior to any other invasive procedures. Morphometric measurements were then completed and other procedures performed as detailed in Frost et al. (1995) and Lewis (1995). Seals were subjectively categorized into age classes of pup, yearling, subadult or adult on the basis of size and time of year, and gender was recorded. Seals were held for variable periods to recover from anesthetic effects before being allowed to return to water.

### ***Plasma chemistry and hematology***

Blood was usually sampled from the intervertebral extradural vein (Geraci and Smith 1975), using 3.5 inch 18 or 20 gauge spinal needles (Monoject<sup>®</sup>, Sherwood Medical Co., St. Louis, MO) into various blood collection tubes (Vacutainer<sup>®</sup>, Becton-Dickinson Vacutainer Systems, Rutherford, NJ). Blood samples from some pups were taken by flipper venipuncture (Geraci 1971), using 1.5 inch 18 or 20 gauge needles drawing into blood collection tubes. Samples were processed in the field or prepared for storage at  $-80^{\circ}\text{C}$  for later laboratory analysis. Field processing included pipetting aliquots of whole blood into Drabkin's reagent (for hemoglobin analysis), measuring hematocrit and preparing smear slides (for differential leukocyte counts). Tubes containing 5 mL of whole blood in EDTA were kept refrigerated until hematological analysis by a hospital laboratory.

Plasma chemistry measurements included sodium, potassium, chloride, phosphorous, blood urea nitrogen (BUN), creatinine, direct and total bilirubin, total protein, albumin, globulin, alkaline phosphatase, glucose, lactate dehydrogenase (LDH), gammaglutamyl transferase (GGT), creatine phosphokinase (CPK), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and whole blood hemoglobin. Hematology measurements included hematocrit, complete blood counts of white and red blood cells, platelet counts, differential white blood cell counts, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin content (MCHC).

Several factors related to blood and animal handling techniques were monitored or tested to determine their effect on data variability. These included the elapsed time between seal capture and blood sampling, administration of chemical anesthesia, elapsed time between blood sampling and sample processing, and the elapsed time between sample collection and CBC analyses by hospital laboratories.

For more detailed methods associated with chemical and hematological analyses as well as details of statistical analyses see Appendix 1, Chapter 2, pages 17–21 and Chapter 5, pages 140–141.

### ***Condition indices***

Condition index comparisons used morphometric and age data collected by Pitcher (1977, 1986) and Pitcher and Calkins (1983) and included 405 harbor seals collected from the Gulf of Alaska. Measurements included whole body mass ( $M_b$ ), axillary girth ( $G$ ), standard length ( $L$ ; dorsal side up), xiphosternal blubber depth (XBT), sculp (blubber plus pelage) mass ( $M_s$ ), core mass ( $M_c$ ) and age. Similar data were collected during 1979 and 1985 from the Bering Sea (Frost and Lowry, unpublished data), although standard length was measured ventral-side up



(Scheffer 1967). Contemporary data included measurements of standard length (L; dorsal-side up), curvilinear length ( $L_c$ ), a series of girth rings taken at the ears, neck, shoulder, axilla, maximum diameter, mid-trunk, hips and ankles (Appendix 1, Chapter 3, Figure 1), curvilinear distance to each girth ring, and blubber depth at lateral and dorsal sites along each girth ring except ears and ankles. Additional morphometric data were provided by the biosampling program (EVOS Restoration Project /244) and included dorsal standard length, axillary girth and xiphosternal blubber thickness. Morphometric data similar to the historic data set were also collected during 1989–1990 from Prince William Sound and the Barren and Kodiak Islands (Frost and Lowry 1994a).

See Appendix 1, Chapter 3, pages 63–66 and Chapter 5, pages, 139–141 for a more detailed description of morphometric measurements and calculations, including analyses of condition indices and interannual and interregional comparisons of body condition.

### ***Blubber quality***

Harbor seal blubber samples were obtained from two sources; through a subsistence harvest program (EVOS Restoration Project /244), and from archived samples that had been collected in 1976–1977 and subsequently archived by the Alaska Department of Fish and Game. Samples obtained from the subsistence harvest program were collected during October 1995 to May 1996 from southeast Alaska, March through November 1996 from PWS and during May 1996 from the Yakutat area. Measurements of body mass, standard length, curvilinear length, axillary girth, hip girth, and xiphosternal and ventral hip blubber thickness were also collected when possible. Analyses of blubber quality included energy density, lipid content, water content and ash content. See Appendix 1, Chapter 4, pages 117–120 for a detailed description of blubber collection and analyses.

A group of blubber samples obtained through the program (February to May, 1996; Ketchikan, Klawock and Yakutat) was examined histologically to determine if adipocyte size would be a useful indicator of blubber quality. Samples were embedded in paraffin, sectioned (7  $\mu\text{m}$ ) and stained with hematoxylin and eosin. Two sections were mounted from each sample, each cut at right angles to the other. Both sections were analyzed. Adipocyte measurements were made at 400 $\times$  magnification. Measurements excluded cells or fields in close proximity to other blubber structures (e.g., collagen, blood vessels) or the edge of the section. Cell count measurements included five complete field counts from each section. Counts included partial cells along the top and right side of the field and excluded partial cells along the bottom and left side of the field. Mean adipocyte size was determined by measuring 5 individual cells from each of 4 fields. Cells were chosen as being of fairly regular shape and occurring in the middle of the field and adjacent to each other. Each cell was measured across two diameters at right angles to each other.

## **RESULTS**

### ***Plasma chemistry and hematology***

Data from 296 seals sampled during 1992–1996 were utilized in reference range calculations (Appendix 1, Chapter 2, Table 1). Reference ranges for plasma chemistries,

hematologies and leukograms are presented in Appendix 1, Chapter 2, Tables 2, 3 and 4, respectively.

Individual (age and gender), handling, regional and temporal (season and year) factors explained up to 55% of the variation in plasma chemistries collected during 1995–1996. For example, elapsed time between seal capture and blood sampling accounted for a large portion of the variability in potassium ( $P < 0.001$ ,  $R^2 = 0.226$ ), CPK ( $P < 0.001$ ,  $R^2 = 0.196$ ), LDH ( $P < 0.001$ ,  $R^2 = 0.325$ ) and, to a lesser degree, AST ( $P < 0.05$ ,  $R^2 = 0.055$ ). When handling factors were removed from all but these four analytes (potassium, CPK, LDH and AST), individual, regional and temporal effects explained up to 39% of variation (Appendix 1, Chapter 2, Table 5), although no single factor was consistently the largest contributor (Appendix 1, Chapter 2, Table 6).

Individual, handling, regional and temporal factors accounted for up to 56% of the variation in hematological measurements in the 1995–1996 data subset. Hematocrit, hemoglobin and RBC counts varied directly with each other (Appendix 1, Chapter 2, Table 8), and had large seasonal components to their variability (Appendix 1, Chapter 2, Table 9).

Leukograms were relatively insensitive to handling effects and were unaffected by gender. The elapsed time between capture and sampling caused decreases in absolute lymphocyte ( $P < 0.05$ ,  $R^2 = 0.041$ ) and eosinophil counts ( $P < 0.05$ ,  $R^2 = 0.046$ ). The elapsed time between sampling and creation of blood smears tended to decrease absolute ( $P < 0.05$ ,  $R^2 = 0.065$ ) and differential ( $P < 0.05$ ,  $R^2 = 0.064$ ) basophil counts. In recalculated models which excluded elapsed time from capture to sampling (for all but absolute lymphocyte and eosinophil counts), individual, regional and temporal factors explained up to 19% of leukogram variability.

Modeling of statistical power and minimum detectable hematological differences in regional or interannual comparisons shows detectable differences similar in magnitude to changes caused by handling, individual, regional or temporal factors. Of the 158 seals (sampled between 1993–1996) screened for outliers among 36 chemical and hematological variables, 84% had at least one statistically outlying variable and the outlier frequency distribution was similar to that predicted by a binomial distribution (Appendix 1, Chapter 2, Figure 1).

Appendix 1, Chapter 2, pages 21–25 provides more detailed results, including analysis of sampling bias, a more complete description of factors affecting variability and more complete statistical analyses.

Interannual and interregional comparisons of blood chemistry and hematology are presented in Appendix 1, Chapter 5, pages 143–144. Mean corpuscular volume (MCV) increased in seals within PWS during 1992–1996 (Appendix 1, Chapter 5, Figure 4). Other interannual and interregional relationships are detailed in Appendix 1, Chapter 5, Figures 5–12. While 21% of seals screened for outliers had  $\geq 4$  statistically significant outlying blood analyte values, there was no definable trend in year or location of occurrence (Appendix 1, Chapter 5, Figure 13).

### ***Condition indices***

Appendix 1, Chapter 3, pages 66–77 provides a detailed analysis of condition indices based on body mass, sculp mass, length, girths and blubber depths. Absolute and indexed measures of condition were compared among locations and seasons for males and females. In

general, morphometric condition indices did not provide adequate precision in reflecting changes in true condition.

Analysis of blubber distribution patterns and girth measurements revealed that the relationship was unpredictable (Appendix 1, Chapter 3, Figure 13) and that blubber distribution was somewhat different between males and females. Blubber thickness was most variable at the dorsal maximum girth site for males and at the dorsal neck site for females (Appendix 1, Chapter 3, Table 7). When dorsal and lateral blubber depths were combined, the most variable sites for females were found anterior to the axillary girth and in males in the mid 30–60% of the body (Appendix 1, Chapter 3, Table 8). Seasonal declines in blubber thickness (spring to autumn) were generally greater for females than males and varied depending on girth site (Appendix 1, Chapter 3, Figure 16).

Calculations of the LMD (length, mass, blubber depth) index using the most variable blubber depth location, as recommended by Ryg et al. (1990), resulted in only moderate prediction of sculp content for males and was a poor predictor for females. Mean blubber thickness at the maximum girth site alone was a better predictor of sculp content for females than for males. Taking the mean of 5 blubber measurements from any combination of locations produced slightly better predictions of blubber content for males and females. More detailed results and statistics are presented in Appendix 1, Chapter 3, pages 66–77.

Interannual and interregional comparisons of body condition are presented in Appendix 1, Chapter 5, pages 141–142. While there were some interannual, seasonal and interregional differences, there was no evidence of changed body condition among seals before (1963–1964), during (1973–1978) or after (1989–1996) the major population decline or among seals from KI, PWS or SE during 1993–1996.

### ***Blubber quality***

Mean water content of blubber samples collected during 1995–1996 was  $6.5 \pm 0.3\%$  ( $n = 74$ ). Lipid content was  $91.8 \pm 0.5\%$  (wet-mass basis) or  $97.8 \pm 0.2\%$  ( $n = 29$ ; dry-mass basis). Ash content was negligible, so this determination was discontinued. Energy densities from bomb calorimetry were  $36.4 \pm 0.1$  kJ/g (wet-mass basis) or  $39.0 \pm 0.1$  kJ/g ( $n = 69$ ; dry-mass basis). Blubber lipid and energy density were inversely related to water content (Appendix 1, Chapter 4, Figure 1).

There were no geographical differences in blubber energy densities (dry-mass basis) among seals sampled during spring 1995–1996 from PWS ( $39.1 \pm 0.2$  kJ/g,  $n = 14$ ), Yakutat ( $38.8 \pm 0.2$  kJ/g,  $n = 10$ ) or SE ( $38.8 \pm 0.3$  kJ/g,  $n = 5$ ;  $P = 0.371$ ). Energy densities were also not significantly different between sexes, but male blubber was more hydrated ( $6.5 \pm 0.3\%$ ,  $n = 19$ ) than female blubber ( $5.3 \pm 0.3\%$ ,  $n = 14$ ;  $P = 0.011$ ). There were no geographical or gender differences of blubber energy densities (dry-mass basis) among seals sampled during autumn 1995–1996 from PWS and SE. However, blubber collected from PWS seals was significantly less hydrated ( $5.6 \pm 1\%$ ) than blubber from SE seals ( $8.4 \pm 1\%$ ,  $P = 0.001$ ).

Blubber energy density (dry-mass basis) did not vary seasonally within PWS or SE. Blubber water content also did not vary seasonally except for males from SE between autumn and winter ( $8.8 \pm 0.8\%$ ,  $n = 8$ ;  $6.1 \pm 0.6\%$ ,  $n = 8$ , respectively;  $P = 0.040$ ).

Archived blubber samples collected from SE and KI during 1976–1977 had significantly lower water content and greater lipid content (dry-mass basis) than the 1995–1996 samples when all available samples were considered. However, energy densities (dry-mass basis) were not significantly different between archived and recently collected blubber. There were no significant differences in how energy density (wet-mass basis) varied with water content or with lipid content, which may have been expected if lipids had been degraded or oxidized over time. Using a more conservative approach in which archived and contemporary samples were matched by season and region (winter, SE), it was determined that blubber samples from 1977 were less hydrated and more energy rich than contemporary samples. The 1977 samples had a slightly higher lipid content (dry-mass basis) (1977,  $98.6 \pm 0.2\%$ ; 1996,  $97.6 \pm 0.2\%$ ;  $t_s = 3.35$ ,  $df = 12$ ,  $P = 0.006$ ) and energy-density (dry-mass basis) (1977,  $39.1 \pm 0.1$  kJ/g; 1996,  $38.7 \pm 0.1$  kJ/g,  $t_s = 2.076$ ,  $df = 19$ ,  $P = 0.006$ ). A further comparison of matched samples collected from Yakutat in 1976 and 1996 produced similar results. While the water content of samples collected in 1976 was not significantly different than samples collected in 1996 (1976,  $5.2 \pm 0.6\%$ ; 1996,  $5.9 \pm 0.6\%$ ,  $P = 0.151$ ), as in the previous analysis of matched samples the 1976 values were clustered at the low end of the range. The 1976 and 1996 samples from Yakutat had similar energy densities (dry-mass basis) (1976,  $39.3 \pm 0.1$  kJ/g; 1996,  $39.16 \pm 0.4$  kJ/g,  $P = 0.498$ ).

Appendix 1, Chapter 4, pages 120–124, provides more detailed results from blubber quality analyses including relationships of blubber quality with seal morphometrics and with sample handling procedures.

The utility of histology as a measure of blubber quality was examined with a subset of blubber samples collected in spring 1996 from Ketchikan, Klawock and Yakutat. Mean adipocyte diameter was  $104 \pm 9 \mu\text{m}$  ( $n = 25$ ), with a range of 85–130  $\mu\text{m}$ . Analysis of blubber histology revealed that cell counts were strongly correlated with direct adipocyte measurements. For each blubber sample, 2 sections were cut at right angles to each other. The strongest correlation occurred when counts and measurements from both sections were averaged (Figure 1). Since direct adipocyte measurement is time-consuming and somewhat subjective (cells are not always regularly shaped), cell counts were used in further analyses.

There was no relationship between mean combined adipocyte count and blubber depth ( $Corr = -0.396$ ), an index of blubber depth at a given body size (blubber depth/seal radius;  $Corr = -0.233$ ) or any other measure of blubber quality (water content,  $Corr = -0.137$ ; energy density,  $Corr = 0.2475$ ).

## DISCUSSION

Declines of marine mammals have been documented over large portions of the Bering Sea and Gulf of Alaska (Alaska Sea Grant 1993, Small and DeMaster 1995). Harbor seal population declines were first noted by Pitcher (1990) in 1976 at Tugidak Island, Alaska. Declines had already been noted in Prince William Sound (Frost and Lowry 1994a, Frost et al. 1994) before the *Exxon Valdez* oil spill in March 1989. This spill caused the mortality of approximately 36% of the seal population in oiled areas (Frost et al. 1994) and declines continue at about 6% per year (Frost et al. 1997). Conversely, harbor seal populations have remained stable or have slightly increased in southeast Alaska (Small and DeMaster 1995, Small 1996, Lewis et al. 1996).

Many hypotheses have been proposed for these recent harbor seal declines (Sease 1992, Hoover-Miller 1994), including reduced environmental carrying capacity (nutritional limitation), habitat reduction, infectious disease, predation, past commercial harvests, subsistence harvests, direct and indirect fisheries take, contaminants and emigration. Many of these factors have been partially addressed in other studies (Frost and Lowry 1994*b*, Frost et al. 1995, Lowry et al. 1996, Swain et al. 1996, Frost et al. 1997, Zarnke et al. 1997). This report provides additional data for interpretation of these population changes.

### ***Plasma chemistry and hematology***

Plasma chemistry and hematology measurements from free-ranging animals can provide critical data for determining individual health status, and for interpretation of population trends (Seal et al. 1975, Geraci et al. 1979, McConnell and Vaughan 1983, Kuiken 1985, Roletto 1993, Schumacher et al. 1995, de Swart et al. 1995). Determination of possible clinical conditions using this technique requires the establishment of reference ranges, preferably from subjects of known health status (Kerr 1989). Clinical determinations of health are usually not available in free-ranging conditions, and reference ranges constructed from wild animals often utilize statistical exclusion to determine outliers as possible health concerns (Kopec and Harvey 1995, de Swart et al. 1995). In addition, isolation and quantification of sources of variability due to handling, age, gender or season are critical for interpretation of observed differences in chemistry and hematology (Geraci et al. 1979).

For a full discussion of sources of variability in blood values as well as interannual and interregional comparisons, see Appendix 1, Chapter 2, pages 25–35. Handling factors, particularly the elapsed time between seal capture and blood sampling, strongly influenced plasma potassium, CPK and LDH values and had moderate effects on AST, and absolute lymphocyte and eosinophil counts. Individual, regional and temporal factors accounted for up to 39% of the variation in plasma chemistries, 19% in leukograms and up to 56% in hematologies. Seasonal patterns detected in sodium, glucose, calcium, total protein, alkaline phosphatase, AST, hematocrit, hemoglobin, RBC count, neutrophils and lymphocytes may be related to endogenous endocrinological changes, which may obscure other effects due to causes such as dietary changes. Magnitudes of most handling, individual and seasonal effects were within the minimum detectable differences predicted from power models of sample interannual and interregional comparisons. Thus, interannual and interregional comparisons of population blood parameters should be constructed to compare within season, and interpreted carefully in light of potential differences in sample processing.

Comparisons among harbor seal populations from KI, PWS and SE performed since 1989 have included blood profile analyses, a methodology that may provide more sensitive indicators of seal health than gained from condition indices alone. For a full discussion of the relationships between blood profiles and the health status of harbor seals in the Gulf of Alaska see Appendix 1, Chapter 5, pages 148–155. While a significant number of seals had  $\geq 4$  statistically outlying blood analyte values, the distribution in year or location of these seals did not indicate that particular periods or locations experienced manifestations of poorer clinical health. Blood values showed direct or indirect fluctuations with many environmental and behavioral trends, indicating that blood profiles were sensitive to environmental change. Trends of many blood factors

differed between juveniles and adults. While these trends are consistent with dietary differences they may be affected by other factors such as development or exposure to contaminants. Developmental differences will be addressed in future studies of pups and juveniles (Trumble and Castellini 1997).

### ***Condition Indices***

Two important goals of this study were to determine whether significant changes in body condition could be detected using morphometric measurements and to determine whether size or compositional changes have occurred among harbor seals from the Gulf of Alaska between the 1970's and 1990's.

For a complete discussion of the effects of harbor seal body shape and blubber distribution on the performance of morphometric indices of condition, see Appendix 1, Chapter 3, pages 77–87. Condition indices based on length and girth were generally poorly correlated with any condition measure for either sex. Girth measurements were poorly correlated with underlying blubber thickness and correlations among girths or blubber thickness were only moderate, thus the underlying assumptions of the G/L condition index were not supported. Indices based on length and girth provided excellent mass prediction. No single measure or combination of measures, including ultrasound blubber depths, girths, lengths or mass was found to predict sculp content with the precision or consistency desired in a condition index. The 'best' predictor of blubber content was obtained by taking the mean of any 5 blubber depths, however, about 40% of the variability in blubber content remains to be explained.

Comparisons of body condition of harbor seals in the Gulf of Alaska during 1963–1996 and the implications to health status are discussed in Appendix 1, Chapter 5, pages 144–155. No evidence was found of changed condition among harbor seals sampled before (1963–1964), during (1973–1978) or after (1989–1996) the major population decline. There was no evidence of long-term blubber thickness changes for males or females between 1973–1996. As with long-term comparisons, there was little evidence of condition differences, including sculp content (based on ultrasonic blubber depth measurements), among PWS, KI and SE between 1993–1996. Combining the lack of condition differences with a long-term population decline in Prince William Sound suggests that temporal differences in carrying capacity could exist. This would be consistent with the lack of population recovery after the *Exxon Valdez* oil spill. Similarly, carrying capacity differences could explain current differences in population trajectories in PWS, KI and SE. There are, however, other factors which are still being investigated which may impact the population status and recovery of harbor seals in the Gulf of Alaska, including exposure to contaminants, predation and human-caused mortality.

### ***Blubber quality***

Since harbor seals rely on subcutaneous blubber for insulation and energy storage (Ryg et al. 1988) and the quantity and thickness of blubber is variable according to season and energy intake (Nordøy and Blix 1985, Pitcher 1986, Ryg et al. 1988, Beck et al. 1993), we examined whether compositional or quantitative differences existed in seal blubber which might be associated with nutritional or population status.

A discussion of proximate analyses of blubber and comparisons to other studies is presented in Appendix 1, Chapter 4, pages 124–126. There was no evidence of changes in blubber energy density (dry-mass basis) among seals from PWS and SE when compared within seasons. However, seal blubber from PWS was slightly less hydrated than blubber collected from SE (spring and autumn) and Yakutat (spring). There are likely energetic differences associated with the differing hydration states, but they were statistically undetectable. It is possible that during spring, seals from PWS had lower overall energy stores than seals from the other two regions, given body size and blubber thickness differences. These morphometric differences were not apparent during autumn, however.

Interannual comparisons of blubber quality including blubber collected in 1977, suggested that, while water content measurements fell within the range of values from recently collected samples, there is evidence of dehydration in the archived samples. Based on energy density data, we have concluded that no lipid degradation has occurred. We have added more archived samples to the database, and the results and conclusions remain unchanged. We are continuing to receive blubber samples collected by the biosampling program and they will continue to be analysed and added to our current database and statistical analyses. For a complete discussion on seasonal, regional and interannual comparisons of blubber quality see Appendix 1, Chapter 4, pages 126–128.

Histological examination of harbor seal blubber revealed no relationship between adipocyte size and blubber depth or an index of blubber depth at a given body size (blubber depth/body radius). We observed differences in hydration state of blubber from different regions, but adipocyte measurements were not correlated to water content or any other measure of blubber quality and so were considered a poor indicator of quality.

## CONCLUSIONS

Blood parameters were found to vary with handling technique, gender, and regional and temporal effects, and reference ranges were constructed for detection of outliers with respect to the overall population. However, blood profiles were also sensitive to environmental factors and aspects of seal behavior. While a significant number of seals had greater than 3 blood analytes which were classified as outliers, there was no trend with respect to season or location of occurrence. Based on the measured blood values, the majority of seals sampled showed no indication of poor health. Analyses of blood profile data showed regional and interannual trends that differed among juvenile and adult age classes. More study of the influence of seal development on these trends is required. More detailed examination of the interrelationships between body condition, blood chemistry and hematology, endocrinology and diet need to be performed in captive studies. Since dehydration and water balance are critical components to many diseases, details of electrolyte balance and water regulatory hormones with respect to geographic location would be useful.

Morphometric indices of condition were generally poor predictors of condition, and sampling techniques limited the direct comparison of measurements taken before, during and after population declines. Though samples sizes for comparison were limited, no evidence was found for changes in body condition of harbor seals sampled during 1963–1964, 1972–1978 and



1989–1996. There was also no evidence of condition differences among Prince William Sound, Kodiak Island and southeast Alaska seals between 1993–1996.

Harbor seal blubber quality was similar to that of other phocids. Blubber from Prince William Sound seals was slightly more hydrated, and thus had slightly higher energy content than seals from southeast Alaska. This difference was small, only about a 1% difference in blubber energy stores for a 50 kg seal. Archived blubber samples from the 1970's showed evidence of dehydration but no apparent lipid degradation. On the basis of energy content, there has been no appreciable change in blubber quality between the 1970's and 1990's.

A complicating factor in the detection of health problems with harbor seals during 1989–1996 was a lack of sampling during the winter, and of limited sampling of young age classes. Thus, the conclusions of this study are limited to subadult and adults captured during spring, summer and autumn. The techniques used for seal capture may have biased our samples toward more healthy individuals. Further study of rehabilitated seals should help to address this potential limitation.

One plausible conclusion, based on population declines and no apparent change in body condition was that environmental carrying capacity decreased near the period of an oceanic 'regime shift' in 1976. A similar conclusion is proposed for current differences in population trajectories among Prince William Sound, Kodiak Island and southeast Alaska. However, other potential causes or contributors to the decline, such as increased predation, human-caused mortality or exposure to contaminants are not discounted by these findings.

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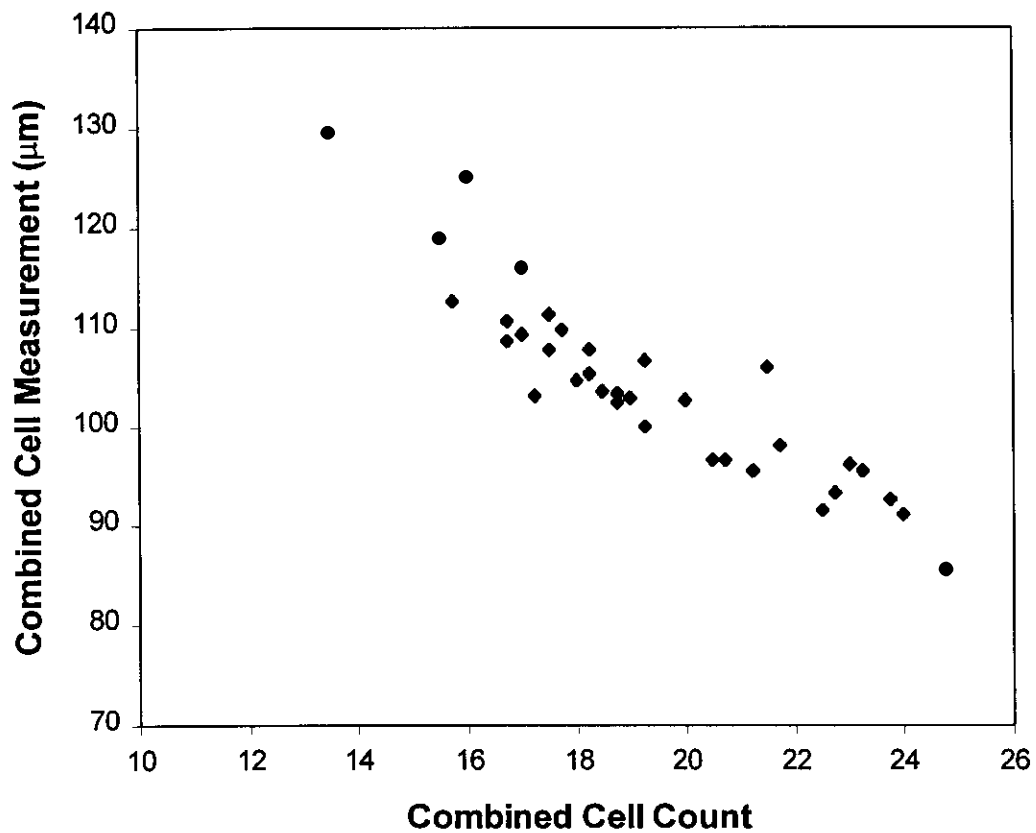


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## **APPENDIX 1**

Fadely, B. S. 1997. Investigations of harbor seal (*Phoca vitulina*) health status and body condition in the Gulf of Alaska. Ph.D. Thesis. Univ. of Alaska, Fairbanks, Alaska. 183pp.

INVESTIGATIONS OF HEALTH STATUS AND BODY CONDITION OF HARBOR  
SEALS (*PHOCA VITULINA*) IN THE GULF OF ALASKA

A  
THESIS

Presented to the Faculty  
of the University of Alaska Fairbanks  
in Partial Fulfillment of the Requirements  
for the Degree of  
DOCTOR OF PHILOSOPHY

By  
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## ABSTRACT

Harbor seal (*Phoca vitulina*) declines during the past 20 years in the Kodiak Island and Prince William Sound regions contrast with stable or slightly increasing populations in southeastern areas of Alaska. Aspects of health status and body condition were investigated to test the hypothesis that these declines were driven by nutritional limitation, and to determine whether recent differential population trajectories among Kodiak Island, Prince William Sound, and southeast Alaska could have health-related components. For comparisons between 1992-96, three aspects of health status were examined; blood chemistry, blubber distribution and quantity; and blubber quality. Clinical ranges of plasma chemistries and hematologies were established for free-ranging seals in the Gulf of Alaska. Significant handling, individual, and seasonal effects were found on many blood parameters that could bias interannual and interregional comparisons if not incorporated in models. Based on statistical modeling, some seals showed more clinically aberrant values than expected by chance, but these were not clumped among regions or years. Differences existed in interannual blood chemistry and hematology patterns between juveniles and adults. Likewise, there were regional differences in blood chemistries of unknown significance. Morphometric indices were poor indicators of condition defined as size-at-age or blubber content. This was related to patterns of blubber distribution and variability, which differed between males and females. Blubber quality, measured as lipid content, did not substantially vary seasonally or between geographic regions, but blubber from Prince William Sound was less hydrated than blubber from non-declining areas. There were no detectable differences in body condition of seals from the Gulf of Alaska sampled during 1963/64 (pre-decline), 1976-78 (during decline) and 1995-96. However, sample sizes were small and patchily distributed throughout locations and years. Thus, the likelihood of detecting body condition changes in response to environmental conditions was poor. Body condition was not substantially different among seals from Prince William Sound,

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# 1 Introduction

Declines of marine mammals and seabirds have been documented over large portions of the Bering Sea and Gulf of Alaska (Alaska Sea Grant 1993; Small and DeMaster 1995). In the case of Steller sea lions (*Eumetopias jubatus*), these declines evidently began during the 1960's in the Aleutian Islands, and spread eastward such that in 1989 the remaining population was only 54-87% of the early counts (Loughlin et al. 1992). This stock declined a further 37% during 1989-1994 (Small and DeMaster 1995). Harbor seal (*Phoca vitulina*) population declines were first noted by Pitcher (1990) in 1976 at Tugidak Island, Alaska. By 1992, this population had declined by about 92%, and only since 1992 has an increasing trend been observed (Figure 1). Likewise, declines were already noted for Prince William Sound (Frost and Lowry 1994a; Frost et al. 1994) before the *Exxon Valdez* oil spill in March 1989 (Figure 1). This spill directly caused the mortality of approximately 36% of the seal population in oiled areas (Frost et al. 1994), and declines continue at about 6% per year (Frost et al. 1997). Conversely, harbor seal (Figure 1) and Steller sea lion populations have remained stable or have slightly increased in southeast Alaska (Small and DeMaster 1995; Small 1996; Lewis et al. 1996).

Harbor seals (*P. v. richardsi*) in the North Pacific range from the eastern Aleutian Islands to the Baja Peninsula in Mexico (Hoover-Miller 1994), typically inhabiting coastal and estuarine habitats (Small and DeMaster 1995). There is a gradation into *P. v. stejnegeri* along the Aleutian Islands, and this subspecies inhabits the Aleutian, Commander and Kuril Islands (Burns et al. 1984). As a species, harbor seals are found around the North Pacific rim, Bering Sea, and North Atlantic Ocean coastal waters (Riedman 1990). Three management stocks had been identified within Alaska: Bering Sea, Gulf of Alaska, and southeast Alaska, based primarily on the different population trends (Small and DeMaster 1995). However, genetic studies utilizing mitochondrial DNA indicated that though differentiation throughout Alaska appeared to occur as a clinal

continuum from southeast Alaska through Bristol Bay, on a large scale there appeared to be a substructure split at the lower Cook Inlet (Westlake and O'Corry-Crowe 1997).

Many hypotheses have been proposed for these recent harbor seal declines (Sease 1992; Hoover-Miller 1994), including reduced environmental carrying capacity (nutritional limitation), habitat reduction, infectious disease, predation, past commercial harvests, subsistence harvests, direct and indirect fisheries take, contaminants, and emigration. Population declines may have resulted from combinations of these variables, and different factors may have been operating at different times and locations. Also, these factors are not necessarily independent. In contrast to the massive harbor seal population die-off in the North Atlantic during 1988-89 (Heide-Jørgensen et al. 1992), there has been no evidence of massive die-off events in Alaska, and determination of cause must be made by inference from capture or collection of seals, rather than from necropsies of beach-cast carcasses. This makes it difficult to determine specific causative factors initiating the decline. Recent studies have therefore focused on factors currently affecting harbor seal populations in the Gulf of Alaska (Frost et al. 1995), particularly with respect to the different trends between Prince William Sound and southeast Alaska populations (Figure 1). To provide additional data for interpretation of these population changes, my dissertation research focused on assessing health status and body condition of harbor seals in the Gulf of Alaska.

Many of the above factors have been partially addressed in other studies. Based on satellite transmitter tagging data, harbor seal movements were mostly local to the tagging location, or the seals returned to the general tagging area after longer distance movements with few exceptions (Frost and Lowry 1994b; Frost et al. 1995; Swain et al. 1996; Frost et al. 1997). Thus, emigration from areas of decline seems unlikely. Zarnke et al. (1997) and Lowry et al. (1996) tested harbor seal sera collected during 1978-1994 for exposure to infectious diseases, and found evidence of exposure to phocid herpesvirus, phocine distemper virus, *Brucella* spp., *Toxoplasma gondii*, and *Chlamydia psittaci*. However, there have been no known outbreaks of these diseases in Alaskan harbor seals,

and there were no detectable differences in exposure between southeast Alaska and regions of decline (Lowry et al. 1996; Zarnke et al. 1997). Both studies therefore suggested that the available evidence did not support these infectious diseases as being important in the decline of these surveyed populations.

Changes in the carrying capacity of the ecosystem may have resulted from warming trends present since a 'regime-shift' occurrence in 1976-77, associated with changes in position and intensity of the Aleutian Low (Niebauer 1997), and linked to a decrease in the mean anomaly of the southern oscillation index (Niebauer 1988, 1997). This regime-shift was correlated with the onset of major changes in commercial and forage fish stock distribution, abundance, and composition in the Gulf of Alaska and Bering Sea (Alverson 1992). Though some fishery stocks in the North Pacific Ocean exhibited strong recruitment during the transition years (Niebauer and Hollowed 1993), in general there have been declines in forage fish abundance (Alverson 1992). This could result in nutritional limitation in two ways; through increased foraging costs due to changes in prey availability or abundance, or to changes in the nutritional quality of the diet.

#### *Use of blood parameters to determine individual or population health status*

Population level comparisons of blood chemistry have been utilized to determine nutritional insufficiencies (Payne and Payne 1987), and to examine differences in population status or environmental quality of terrestrial herbivores and carnivores (Seal et al. 1975, 1978; Franzmann 1985; Messier 1987; Hellgren et al. 1989; Knick et al. 1993). Fewer studies of this type have been performed on marine mammals, and until the recent studies of Castellini et al. (1993), Roletto (1993), Kopec and Harvey (1995), Rea (1995), Schumacher et al. (1995), Thompson et al. (1997), and Zenteno-Savin et al. (1997), few pinniped studies had sufficient sample sizes for these types of contrasts. These types of studies assume that environmental or disease factors apply to an entire local population, and sub-clinical conditions can be determined by statistical examination of blood profile data. Determination of health status requires quantification of natural variability in

hematological parameters, which has not been described for Alaskan harbor seal populations in particular, or for many free-ranging pinniped populations in general.

#### *Determination of body condition*

Considerable literature exists on the determination of human and animal body condition (see Hanks 1981, Franzmann 1985, Lukaski 1987, and Harder and Kirkpatrick 1994 for review). Use of body condition measurements assumes that changes in condition result in changes in fitness, such that survival or reproductive output are changed (Harder and Kirkpatrick 1984). Examination of relative adipose tissue stores or comparisons of size may therefore help interpret changes in population status. If declining populations are nutritionally limited, decreases in body condition or composition may be evident as foraging expenditures increase. Determinations of body condition utilize size (mass or length) relative to age, or mass relative to a size or shape assessment, typically incorporating a body length or girth measurement, such as the body mass index in humans (Smalley et al. 1990). Compositional indices of condition in pinnipeds determine or index percent body fat relative to body mass either directly (Pitcher 1986; Beck et al. 1993) or indirectly using morphometric indices (Smirnov 1924; Ryg et al. 1990), bioelectrical impedance analysis (Gales et al. 1994), isotopic water injection (Gales et al. 1994; Arnould 1995), or by ultrasonic determinations of blubber volume (Gales and Burton 1987; Slip et al. 1992; Worthy et al. 1992; Rosen and Renouf 1997). However, most commonly used morphometric indices of condition had not been validated until recently, and generally these were found to be poor predictors of body composition (Pitcher 1986; Ryg et al. 1990; Gales and Renouf 1994). Furthermore, recent phocid studies have found that changes in distribution of blubber along the body of a seal may not be reflected by many commonly collected condition indices (Beck et al. 1993; Gales and Renouf 1994), and that certain body sections selectively retain a relatively thick blubber layer (Ryg et al. 1990; Beck and Smith 1995; Rosen and Renouf 1997). Finally, it has been recently shown that an assumption of constant blubber composition (measured by lipid content or energy density) is incorrect; blubber quality can change seasonally and with seal condition

(Bowen et al. 1992; Beck et al. 1993; Gales et al. 1994). Though Rosen and Renouf (1997) examined seasonal blubber distribution in captive harbor seals, similar measurements of body condition have not been performed on free-ranging populations of harbor seals.

### *Scope of study*

This research project was designed to determine health and condition through blood chemistries and measurements of size and blubber content of free-ranging harbor seals. For these comparisons to be performed, however, reference ranges for hematological variables needed to be established, and effects of handling, individual, temporal and regional factors measured. Likewise, the appropriateness of condition indices needs to be established. Thus, in Chapters 2-4 I explore fundamental concepts required to assess harbor seal health and condition, and these concepts are utilized in Chapter 5 to draw conclusions about harbor seal health in the Gulf of Alaska. Chapter 2, *Plasma chemistry and hematology reference ranges of free-ranging Gulf of Alaska harbor seals, and the effects of handling, individual, regional, and temporal factors*, presents reference ranges generated for plasma chemistries, hematologies and leukograms from free-ranging seals, and quantification of the effects of sample processing, seal handling, gender, age, time of year, and region on these ranges. I also determined the statistical power of performing these temporal and regional comparisons, and related that to the magnitude of handling and individual factors. Finally, I addressed the problem of spontaneous outlier creation that arises from the statistical generation of reference ranges.

In Chapter 3, *Effects of body shape and blubber distribution on the performance of morphometric indices of condition in harbor seals*, I examined the utility of commonly collected morphometric condition indices to reflect true measures of body condition. This treatment relied on morphometric data collected by Ken Pitcher and other Alaska Department of Fish and Game researchers during 1972-78. Other analyses of these data were presented in Pitcher (1977, 1986) and Pitcher and Calkins (1979). If appropriate indices can be found, then interannual and interregional comparisons of seal body



condition would be greatly facilitated, and issues of nutritional compromise could be addressed directly. This chapter also describes topographical patterns of blubber distribution among harbor seals, and examines hydrodynamic, thermoregulatory, and energetic considerations underlying blubber distribution.

Chapter 4, *Seasonal and regional differences in proximate composition of blubber from harbor seals*, examines the idea that condition is determined not only by blubber quantity, but also quality. Proximate composition of blubber samples collected through a subsistence harvest by Alaskan Natives was examined for sex and age differences, and compared among regions and seasons. This chapter was compiled from two previously presented reports (Fadely et al. 1996, 1997), and is included with permission of the co-authors.

Chapter 5, *Health status and condition of harbor seals in the Gulf of Alaska, 1972-1996*, combines the appropriate condition indices determined in Chapters 2-4 to examine interannual changes during population declines, and to compare regions with different population trajectories during 1993-96. The previous chapters dealt with the establishment of relationships and examined criteria with which to base population-level comparisons of health status and condition in these harbor seal populations. With these established, in Chapter 5 these principles were combined into conclusions regarding harbor seal health in the Gulf of Alaska. Particular attention was given to detection of chronic effects following the *Exxon Valdez* Oil Spill that may be limiting harbor seal population recovery in Prince William Sound, and to factors related to food or energy intake.

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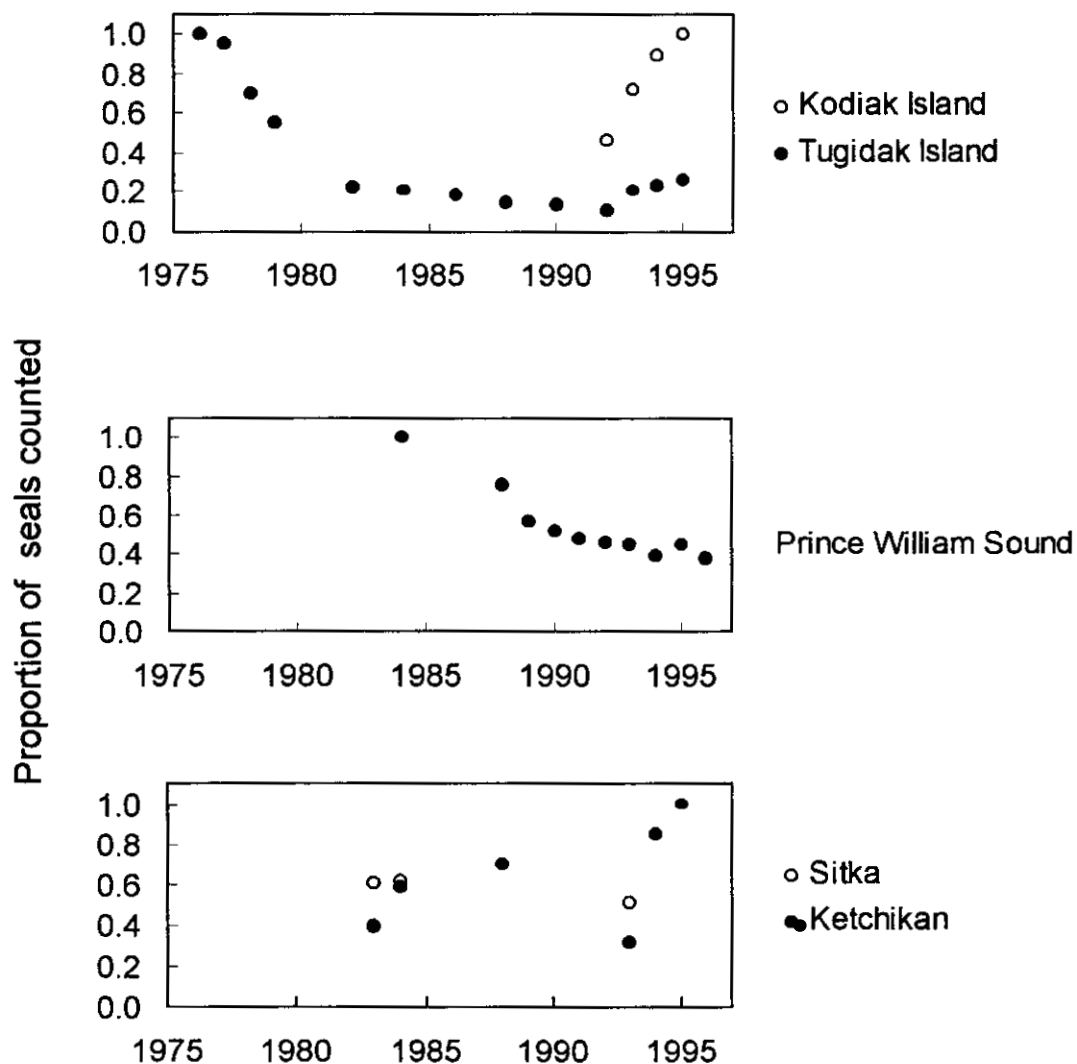


Figure 1. Temporal change in harbor seal populations (relative to largest count) of Kodiak and Tugidak Islands, Prince William Sound, and southeast Alaska. Counts expressed as proportion of maximal seal count for each location. Data taken from Pitcher (1989, 1990), Frost et al. (1997), Calkins and Pitcher (1984), and Small (1996). Maximum population counts were Tugidak (6619), Kodiak (3286), Prince William Sound (2583), Ketchikan (2604), and Sitka (1945).

## **2 Plasma Chemistry and Hematology Reference Ranges of Free-ranging Gulf of Alaska Harbor Seals, and the Effects of Handling, Individual, Regional, and Temporal Factors**

### **INTRODUCTION**

Harbor seal (*Phoca vitulina*) populations in Alaska have declined significantly over portions of their range, particularly in the Gulf of Alaska (Small and DeMaster 1995). Prior to the *Exxon Valdez* oil spill in 1989, population declines of 85% had been reported from Tugidak Island (Pitcher 1990), and declines may have occurred in the eastern Bering Sea and Aleutian Islands, while populations in southeast Alaska (SE) were stable or increasing (Small and DeMaster 1995; Small 1996). Prince William Sound (PWS) harbor seal populations, further impacted by the *Exxon Valdez* oil spill (Frost and Lowry 1994a; Frost et al. 1995), continue to decrease at about 6% per year (Frost and Lowry 1994b; Frost et al. 1995, 1997). Assessment and interpretation of harbor seal health status data may help resolve multiple hypotheses proposed to explain these declines.

Blood chemistry and hematology values can be indicative of health status, disease, nutritional status, or environmental conditions (Seal et al. 1975; Geraci et al. 1979; McConnell and Vaughan 1983; Kuiken 1985; Franzmann 1985, 1986; Roletto 1993; Schumacher et al. 1995). However, such interpretations require establishment of a set of reference or 'normal' ranges for these blood values. Potential homeostatic imbalances in organ systems or metabolic pathways can be detected if the effects of non-health related variation in blood reference values can be quantified (Seal et al. 1975; Medway 1980; Franzmann 1985; Payne and Payne 1987; Kerr 1989; Castellini et al. 1993; Harder and Kirkpatrick 1994; Schumacher et al. 1995). There is extensive literature regarding derivation of reference ranges based on population sampling (Murphy 1982; Kerr 1989; Duncan et al. 1994; McClatchey 1994), and subsequent application to free-ranging populations of both terrestrial and marine mammals (Seal et al. 1975; Franzmann 1985,

1986; Kopec and Harvey 1995; de Swart et al. 1995). However, the differentiation of true clinical outliers from those occurring by chance (Rebar and Boon 1983) is rarely discussed, and the effectiveness of population comparisons based on statistical power considerations has not been calculated.

At the time of the *Exxon Valdez* oil spill in 1989, most hematological values for harbor seals were derived from captive animal studies with generally small sample sizes, sufficient for examining general health, but insufficient for more detailed interpretations of population health status (Hubbard 1968; Ridgeway 1972; Sweeney 1974; Engelhardt 1979; McConnell and Vaughan 1983). This problem was also noted by Schumacher et al. (1995) in regards to the north Atlantic phocine distemper virus episode among harbor seals (Heide-Jørgensen et al. 1992). Furthermore, McConnell and Vaughan (1983) and Schumacher et al. (1995) demonstrated that blood chemistries differed between captive and free-ranging seals. Several studies of pinniped population medicine have had sufficient scope to address boundaries for blood chemistry interpretation (Ronald et al. 1969; Geraci 1971; Geraci et al. 1979; Worthy and Lavigne 1982; Horning and Trillmich 1997), but not until recently have field and captive studies included analyses of variation due to animal, regional, or temporal factors for harbor seals. However these are still relatively small sample sizes (Kopec and Harvey 1995; de Swart et al. 1995; Schumacher et al. 1995), or are biased towards pups (Roletto 1993). The purpose of this study was to generate plasma chemistry and hematology reference ranges from a large sample of free-ranging harbor seals, and to statistically quantify affects of handling and sampling techniques, age, gender, season and region. Once these methodological and statistical factors were described, then interannual and interregional comparisons of the blood chemistry of free-ranging harbor seals could be performed. This chapter seeks to describe the methods and limitations involved in population level determinations of blood chemistry. The data from this chapter are then used in Chapter 5 to describe the overall apparent health of harbor seals in the Gulf of Alaska.

## METHODS

### *Seal capture locations*

Harbor seals were captured from three general geographic regions within the Gulf of Alaska; Kodiak and Sitkinak Islands (grouped as Kodiak Island, KI), Prince William Sound (PWS) and southeast Alaska (SE). Captures were conducted during spring (March-May) and/or autumn (September-October) throughout 1993-96 in all or some of the three regions, detailed in Table 1. In PWS, captures also occurred during April 1991/92 (Frost and Lowry 1994b), and July-August 1995/96. July-August SE captures also occurred in 1994. Seals captured during 1992-1996 in spring and autumn within PWS were also utilized by Frost and Lowry (1994b) and Frost et al. (1995) for satellite-tagging and trophic interaction studies. Seals captured during 1993-1994 at Kodiak Island and southeast Alaska were also studied by Swain (1996) and Lewis (1995, 1996) for movement and dive behavior. Some of the plasma chemistry data from these seals have been included in studies by Castellini et al. (1996) and Zenteno-Savin et al. (1997a,b).

### *Animal handling and sample collection*

Seals were live-captured near haul-outs by net-entanglement as described in Frost and Lowry (1994b) and Lewis (1995, 1996). After removal from the net, seals were bundled individually in hoop-net bags made of fine-mesh nylon webbing attached to rubber hoops. Seals were transported to ship or shore, and placed in relatively quiet locations until further restrained either manually, or chemically by intramuscular injection with a ketamine/diazepam mixture (Frost et al. 1995). Weights were measured ( $\pm 0.1$  kg) with a hanging electronic load cell balance (Ohaus Model I-20W), and blood samples were collected prior to any other invasive procedures. Morphometric measurements were then completed and other procedures performed as detailed in Frost et al. (1995) and Lewis (1995). Seals were subjectively categorized into age classes of pup, yearling, subadult or adult on the basis of size and time of year, and gender was recorded. Seals were held for variable periods to recover from anesthetic effects before being allowed to return to water.

### *Plasma chemistry and hematology*

Blood was usually sampled from the intervertebral extradural vein (Geraci and Smith 1975), using 3.5 inch 18 or 20 gauge spinal needles (Monoject®, Sherwood Medical Co., St. Louis, MO), into various blood collection tubes (Vacutainers®, Becton-Dickinson Vacutainer Systems, Rutherford, NJ). Typically up to 40 mL of blood was collected for serum, 25 mL for plasma, and 12 mL in ethylenediaminetetraacetic acid (EDTA) tubes for complete blood counts (CBC). Blood samples from some pups were taken by flipper venipuncture (Geraci 1971), using 1.5 inch 18 or 20 gauge needles drawing into blood collection tubes. Collection tubes were kept cool on ice or refrigerated until processed. In the field, blood hematocrit (% red blood cells by volume) was measured using a portable centrifuge (Compur MI 100). Samples of whole blood were pipetted into Drabkin's reagent (for conversion into cyanomethemoglobin) for hemoglobin analysis. Blood was then centrifuged and plasma, serum, and whole blood samples were aliquoted into 1.5-2.0 mL cryogenic storage vials (Nalgene® Brand, Nalge Co., Rochester, NY) and frozen in liquid nitrogen until returned to the laboratory, where they were kept frozen at -80 °C for later laboratory analyses. Blood smear slides were made in the field for determination of differential leukocyte counts. Tubes containing 5 mL whole blood in EDTA were kept refrigerated until hematological analysis by a hospital laboratory.

Several factors related to blood-handling techniques were monitored or tested to determine their effect on data variability. These included the elapsed time between seal capture and blood sampling, administration of chemical anesthesia, elapsed time between blood sampling and sample processing (centrifugation and storage for plasma/serum, creation of blood smear for differential leukocyte counts), and the elapsed time between sample collection and CBC analyses by hospital laboratories. In addition, blood from two seals was collected into sodium-heparin, lithium-heparin and serum Vacutainer collection tubes. Replicate 1 mL aliquots of fresh plasma from each heparinized tube and replicate 1

mL aliquots of serum were compared to determine how much variability of measured parameters occurred as a result of the type of collection tube.

Standard panels that assay plasma sodium, potassium, chloride, phosphorus, blood urea nitrogen (BUN), creatinine, cholesterol, direct and total bilirubin, total protein, albumin, globulin, alkaline phosphatase, glucose, lactate dehydrogenase (LDH), gammaglutamyl transferase (GGT), creatine phosphokinase (CPK), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were performed using automated machine analysis (Kodak Ektachem 550 Analyzer) by technicians at the Fairbanks Memorial Hospital (FMH) on plasma or serum. Ratios of albumin to globulin (AG) and blood urea nitrogen to creatinine were calculated from measured values. Hemoglobin was determined using standard kits from Sigma Chemical Co. and performed in our laboratory. Complete blood counts of white and red blood cells, platelet counts and differential white blood cell counts were performed by technicians at FMH from blood collected in EDTA using a Coulter Model S-Plus-4 Counter, and from blood smears produced in the field. Some white blood cell counts were performed directly in the field using light microscopy (Unopette). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin content (MCHC) were calculated from combinations of measured hematocrit, hemoglobin and red blood cell count (RBC) following Kerr (1989). Separate MCV, MCH, and MCHC were calculated using our own measurements of hematocrit and hemoglobin (rather than those of FMH) but using the FMH RBC determination. These values were compared to those determined using the Coulter counter at FMH. Not all plasma or hematology variables were measured for each seal due to limited sample volumes or other factors.

#### *Statistical analysis of plasma and hematological data*

Plasma chemistry reference range calculations excluded samples that were lipemic, hemolytic, or collected posthumously. Hematology reference ranges excluded samples collected posthumously. Otherwise, all data samples were included in the absence of other clinical indicators to categorize the seals as unhealthy. Sample distributions were tested

for goodness of fit with expected normal distributions using Kolmogorov-Smirnoff Probability Tests (Zar 1996). Some values (particularly proportional or count data) were transformed using logarithmic, angular or square-root transformations to improve the normality of their distributions prior to statistical calculations (Murphy 1982; Zar 1996). Reference ranges were calculated as being within two standard deviations of the mean (Kerr 1989; Duncan et al. 1994), unless transformations did not correct large deviations from normality. In these cases, reference ranges were calculated as the 2.5% and 97.5% quantiles of ranked data (Bland 1995). Reference range 95% confidence limits (CI) for normally distributed data were calculated as:

$$CI = \pm 1.96 (3s^2/n)^{0.5}$$

where  $s$  is the variance and  $n$  is sample size, and for other data as:

$$CI = nq \pm 1.96 (nq(1-q))^{0.5}$$

where  $q$  is the quantile (Bland 1995).

Plasma chemistry and hematology panel data from all seals sampled during 1993-96 were screened for outliers based on calculated reference range criteria. A subset of these seals was created by including only panels for which all 36 chemical and hematological variables were measured. Expected frequencies of numbers of outliers per seal were calculated from a binomial expansion of  $(p + q)^k$ , where  $p$  was the probability of an outlier (set at 0.05 from reference range calculations),  $q$  was the probability of no outliers (0.95), and  $k$  was the number of variables considered (36).

Effects of individual, handling, temporal and spatial factors on variability were modeled in a forward stepwise multiple regression analysis model for each blood parameter (transformed if necessary). All categorical factors were treated as dummy variables, and were entered into the model in the order of handling effects first (drug application, elapsed time between capture and sampling, elapsed time between sampling and blood specimen processing, and elapsed time between sampling and CBC processing), individual effects second (gender and age), then season, region and year. To increase sample sizes for comparison, partial  $F$  test statistics (Neter et al. 1990) were calculated to

test the effects of removing handling effects from each model. Multiple regression models were then repeated with handling effects removed (if  $P > 0.05$  for  $F$  of handling), and were limited to seals which had been drugged prior to sampling. This expanded comparisons for seals sampled prior to 1995, which did not have all handling effects recorded. Relative contributions of each parameter to the overall explained variation were calculated by taking the ratio of the individual sum of squares (when all other variables were included in the model) to the total sum of squares for all parameters (Neter et al. 1990). Based on the variation found in plasma chemistries and hematologies, it was possible to calculate the minimum differences that would be detectable in regional or interannual comparisons. Power calculations for analyses of variance (Zar 1996) were utilized assuming a conservative model of  $\alpha = 0.05$  and a power of  $(1 - \beta) = 0.95$ , or a 95% probability of rejecting a false null hypothesis (Sokal and Rohlf 1987). Tests were conducted using two examples from the data, a regional comparison among Prince William Sound, Kodiak Island and southeast Alaska based on samples collected during Autumn 1996, and an interannual comparison based on seals captured during 1992-96 within Prince William Sound. All statistics were calculated using Statistix<sup>®</sup> version 4.1 (Analytical Software) or Systat<sup>®</sup> version 6.1 (SPSS Inc.).

## RESULTS

### *Plasma chemistry and hematology samples*

Data from 296 seals sampled during 1992-1996 were utilized in plasma chemistry reference range calculations (Table 1). There were significant regional and seasonal sampling biases weighted towards PWS (53% of samples, Kodiak Island 15%, and SE 32%) and autumn months (41%, spring 38%, and summer 21%; overall  $\chi^2 = 33.9$ ,  $df = 4$ ,  $P < 0.0001$ ). These samples also were not homogeneously distributed among males (61%) and females (39%), nor among age classes (3% pup, 8% yearling, 29% subadults and 57% adults; overall  $\chi^2 = 6.51$ ,  $df = 3$ ,  $P < 0.089$ ). Likewise, 249 samples utilized for hematology ranges (Table 1) derived primarily from PWS (69%; KI 13%, SE 18%) and autumn months (40%; spring 33%, summer 27%; overall  $\chi^2 = 63.6$ ,  $df = 4$ ,  $P < 0.0001$ ).



There were also similar gender (56% male, 42% female) and age class (4% pup, 9% yearling, 28% subadult, 56% adult) trends (overall  $\chi^2 = 5.17$ ,  $df = 3$ ,  $P = 0.160$ ).

#### *Reference ranges*

Reference ranges for plasma chemistries, hematologies and leukograms are presented in Tables 2, 3, and 4, respectively. Of the 23 plasma chemistry analytes measured, 19 exhibited non-normal distributions (Table 2), as did two of seven hematological variables (Table 3), and six of 13 leukograms (Table 4). Mean values and ranges of hematological variables were different between field and clinical laboratory determinations (Table 3), particularly among measures of erythrocyte morphology. Clinical laboratory Coulter-counter determined values were less variable (CV 6-10%) than field determinations (CV 8-14%).

#### *Plasma chemistry variability*

Individual (age and gender), handling, regional and temporal (season and year) factors explained up to 55% of the variation in plasma chemistries collected during 1995-96, for which handling factors were included. Application of ketamine/diazepam for chemical restraint impacted inorganic phosphorus (regression coefficient  $\beta = 0.4$  mmol/L;  $F^*_{(1,71)} = 10.62$ ;  $P = 0.003$ ;  $R^2 = 0.144$ ) and total bilirubin ( $\beta = 1.7$   $\mu$ mol/L;  $F^*_{(1,71)} = 5.74$ ;  $P < 0.05$ ;  $R^2 = 0.042$ ). Sample sizes for this comparison were small (2 of 73 not drugged during spring, 3 of 69 not drugged during autumn) and may be inseparable from seasonal effects since all 32 summer-sampled seals were not drugged. By contrast, elapsed time between seal capture and blood sampling (0.2-8.3 h) was a significant component of the regression models, and accounted for large portions of variability in potassium ( $\beta = -0.3$  mmol/L;  $F^*_{(1,71)} = 26.6$ ,  $P < 0.001$ ,  $R^2 = 0.226$ ), CPK ( $\beta = 2$  iu/L;  $F^*_{(1,71)} = 23.9$ ,  $P < 0.001$ ,  $R^2 = 0.196$ ), and LDH ( $\beta = 2$  iu/L;  $F^*_{(1,71)} = 29.8$ ,  $P < 0.001$ ,  $R^2 = 0.325$ ), but less of the variation in AST ( $\beta = 1$  iu/L;  $F^*_{(1,71)} = 4.91$ ,  $P < 0.05$ ,  $R^2 = 0.055$ ). Elapsed time between blood sample collection and processing (up to 8 h) did not account for variation in any analyte. In a second run of regression models that removed handling factors from all but the four analytes (potassium, AST, CPK, and LDH), individual, regional and temporal

effects explained 2.1-39.2% of the variation in plasma chemistries (Table 5). Potassium levels were not significantly affected by the elapsed time between seal capture and blood sampling in this comparison. Partitioning total variation among significant parameters showed that only sodium and alkaline phosphatase were affected by all five tested factors, and gender differences only affected 5 of the 19 variables (Table 6). Individual, regional, and temporal factors explained up to 39% of the variation in plasma chemistries, but no single factor was consistently the largest contributor (Table 6). Single factors explained up to 18% of total variation, but most  $R^2$  values for gender, region and season were zero, and median  $R^2$  were only 0.033 for age and 0.035 for year. The type of blood collection tube used produced significant effects (2-way ANOVA) in 8 of 22 chemistry analytes, relative to sodium-heparin collection tubes (Table 7).

#### *Hematological variability*

Handling, individual, regional and temporal factors accounted for up to 56% of the variation in hematological measurements in the 1995-96 data subset. Drug application, elapsed time between capture and blood sampling, and elapsed time between blood sampling and time of processing did not explain significant amounts of variation in any hematological analyte (all  $P > 0.05$ ). However, only 3 of 88 (spring) and 3 of 71 (autumn) seals were not drugged, while all 48 seals handled during summer were not drugged. Thus, some effects attributed to season may be inseparable from drugging effects. Elapsed time between sample collection and CBC analysis (range 0-19 days, median = 4 days) affected field-derived MCV ( $\beta = -1.8$  fL,  $F^*_{(1,85)} = 6.87$ ,  $P = 0.010$ ,  $R^2 = 0.063$ ), hospital-derived MCHC ( $\beta = -0.9$  g/L,  $F^*_{(1,85)} = 43.1$ ,  $P < 0.001$ ,  $R^2 = 0.269$ ), and RBC ( $\beta = 0.02$   $10^{12}/L$ ,  $F^*_{(1,82)} = 9.55$ ,  $P < 0.01$ ,  $R^2 = 0.055$ ). When adjusted for gender, region and season, RBC counts did not exhibit noticeable effects of elapsed processing time for up to 6 days for males ( $<6$  days  $F_{(6,95)} = 1.139$ ,  $P = 0.346$ ;  $>6$  days  $F_{(2,14)} = 7.601$ ,  $P = 0.006$ ), and was unaffected in female samples. Regression models were repeated without handling effects except for limiting elapsed time between sample collection and CBC processing to  $<6$  days for field-derived MCV and laboratory RBC counts. This factor

proved to be not significant in the expanded data set for hospital-derived MCHC ( $F^*_{(1,218)} = 0.13, P > 0.5$ ), and was dropped. Hematocrit, hemoglobin and RBC varied directly with each other (Table 8), and had large seasonal components to their variability (Table 9). Gender influenced hematological parameters less than other factors (Table 9).

#### *Leukogram variability*

Leukograms were relatively insensitive to handling effects of drugging and sample handling, and were unaffected by gender. Neither seal gender nor administration of anesthesia accounted for significant amounts of variation in leukograms when modeled with regional and temporal factors ( $P > 0.05$ ). Elapsed capture to sampling time caused decreases in absolute lymphocyte ( $\beta = -0.7 \text{ } 10^9/\text{L}; F^*_{(1,85)} = 5.31; P < 0.05; R^2 = 0.041$ ) and eosinophil counts ( $\beta = -0.3 \text{ } 10^9/\text{L}; F^*_{(1,85)} = 5.97; P < 0.05; R^2 = 0.046$ ), while the elapsed time between sampling and creation of blood smears tended to decrease absolute ( $\beta = -0.05 \text{ } 10^9/\text{L}; F^*_{(1,85)} = 7.30; P < 0.05; R^2 = 0.065$ ) and differential ( $\beta = -0.4 \text{ } \%; F^*_{(1,85)} = 7.13; P < 0.05; R^2 = 0.064$ ) basophil counts. Recalculated models included capture to blood sampling elapsed time only for absolute lymphocyte and eosinophil counts. Individual, regional and temporal factors explained up to 19% of variation in leukogram parameters in this model (Table 10). Most  $R^2$  values of age were zero for differential counts (Table 11), and median  $R^2$  values were 0.030 (region), 0.020 (season), and 0.047 (year).

#### *Statistical power and probability distributions*

Minimum detectable differences of plasma chemistries (Table 12), hematologies (Table 13), and leukograms (Table 14) for interregional and interannual population comparisons were relatively small, particularly with respect to the magnitude of handling, individual, or seasonal effects. Power modeling of minimum detectable hematological differences in regional or interannual comparisons shows detectable differences similar in magnitude to changes caused by handling, individual, regional, or temporal factors. Platelet counts were an exception, being highly variable (Table 3). One hundred and fifty eight seals sampled between 1993-1996 were screened for outliers among the 36 chemical

and hematological variables, relative to the reference ranges of Tables 2-4. Of these seals, 84% had at least one statistically outlying variable (Figure 1a), and the outlier frequency distribution was similar to that predicted by a binomial expansion for 36 factors with a 5% probability of occurrence. Deviations between observed and expected outliers per individual seal were clumped, indicating a lack of independence among events (Figure 1b).

## DISCUSSION

### *Plasma chemistry and hematology reference ranges*

Plasma chemistry and hematology measurements from free-ranging animals can provide critical data for determining individual health status, and for interpretation of population trends over time or between localities (Seal et al. 1975; Geraci et al. 1979; McConnell and Vaughan 1983; Kuiken 1985; Roletto 1993; Schumacher et al. 1995; de Swart et al. 1995). Determination of possible clinical conditions using this technique requires the establishment of reference ranges, preferably from subjects of known health status (Kerr 1989). Clinical determinations of health are usually not available in free-ranging conditions, and reference ranges constructed from wild animals often utilize statistical exclusion to determine outliers as possible health concerns (Kopec and Harvey 1995; de Swart et al. 1995). To be representative, reference ranges must be constructed with large sample sizes distributed throughout the age and sex structure of a population. Estimation of 95% confidence limits around lower or upper reference ranges are important especially for blood parameters for which elevated values are clinically significant (Kopec and Harvey 1995), yet in practice these are rarely calculated (Bland 1995) presumably due to sample size limitations. Clinical determinations of health status were not performed for seals in this study, though (with very few exceptions) there were no externally or behaviorally detectable indications of disease. Sample sizes used in constructing reference ranges for Gulf of Alaska harbor seals in this study were much larger than have been previously available. Reference ranges presented here can be further broken down based on seasonal and regional differences, but because of the limited samples from pups and yearlings (3% and 8% of the data, respectively), decomposition by age group was not

appropriate. However, collection of additional pup and yearling data from the Gulf of Alaska could be critical, since these age classes may be particularly sensitive to food limitations and disease (Roletto 1993; Rea 1995).

In the absence of other indicators of health status, or of an ability to recapture and resample animals suspected of clinical disease, there is great reliance on utilizing statistical methods to identify potentially unhealthy subjects. However, statistical outliers will not necessarily be indicative of clinical disease (Rebar and Boon 1983). As numbers of outliers increase within an individual, particularly if these are system-related blood variables, then the likelihood of clinical significance must increase. Because of the defining criteria for reference range construction, frequencies of outlier occurrence within individuals follow a binomial distribution. In a binomial distribution, probabilities of occurrence must be independent (Sokal and Rohlf 1987). However, groups of clinically significant outliers will most likely be interdependent. For example, water balance problems can affect sodium, chloride, hematocrit, and a number of enzymes and other analytes. This resultant statistical 'clumping' was evident by examining the differences between expected and observed outlier per seal frequencies (Figure 1b). For field applications where reference ranges were determined without other knowledge of the animals health status, these distributions suggest that only subjects with at least 4 outliers could be considered to have clinical concerns beyond that expected by chance. This number will change depending on the number of chemical and hematological variables being considered.

#### *Plasma chemistry variability*

Blood chemistry and hematology data comparisons of populations, between regions or over time, seek to determine differences related to disease (Kopec and Harvey 1995), food limitation (Seal et al. 1975; Geraci et al. 1979; Worthy and Lavigne 1982), diet (Thompson et al. 1997) or contaminants (Duffy et al. 1993; Kopec and Harvey 1995; Schumacher et al. 1995; de Swart et al. 1995). In these comparisons, isolation and quantification of sources of variability due to handling, age, gender or season is critical for

interpretation of observed differences in blood chemistries or hematologies (Geraci et al. 1979). However, besides Geraci et al. (1979), few pinniped studies have had sufficient scope to address these sources of variation within the same study, though recently Kopec and Harvey (1995) and de Swart et al. (1995) tested for age, gender and season effects.

There is extensive literature regarding biochemical profile interpretation relative to disease, and some of the underlying physiological mechanisms for both terrestrial and marine mammals (Rebar and Boon 1983; Bossart and Dierauf 1990; Duncan et al. 1994). Thus, this discussion focuses primarily on effects attributed to individual and seasonal factors that will influence interregional and interannual comparisons. Additional discussion of the biological and ecological significance of some findings are presented in Chapter 5.

Patterns of individual, regional, or temporal effects on plasma chemistries were similar in some respects to those found in other harbor seal studies (Table 15). The major difference was that I found age effects to be more common among plasma chemistries than Kopec and Harvey (1995) or de Swart et al. (1995). In the present study, age-related effects were detected in 14 of 23 variables, while only creatinine was significantly different in the Kopec and Harvey (1995) study, and de Swart et al. (1995) found no age effects. Kopec and Harvey (1995) split age into two categories of sub-adult or adult based on size, while this study had four categories which added pups and yearlings. This difference may account for the finer resolution. De Swart et al. (1995) however, compared serial samples from same-age seals for slightly more than a two-year period. Their study found no age effect on plasma chemistries for seals during growth periods from 15 months to about 2.4 years old. These seals had been acclimated to captivity for 1 year prior to their study, thus their study was structured to show age effects if present. In comparisons of free-ranging ringed seal (*Phoca hispida*) pups and adults, Geraci et al. (1979) found adults had decreased potassium, decreased calcium, increased cholesterol, increased glucose, and decreased alkaline phosphatase activity relative to pups. Similar findings were found in this study with two notable exceptions; glucose was unaffected by age, and cholesterol

declined with increasing age class (Table 5). The pup ages were similar in both studies (<5 months), so were nutritionally independent. Since cholesterol is sensitive to dietary fat (Bossart and Dierauf 1990), the difference may be related to dietary differences between the two pup species. In relation to other harbor seal studies, therefore, age effects found here either arose from inclusion of pups, or from other environmental factors, such as dietary differences or foraging behaviors, acting differentially between the age groups. These interactions are discussed further in Chapter 5. Based on blood chemistry comparisons with similar factors among free-ranging herbivores, disparity between studies is not uncommon, and ultimately age affected most plasma variables (Franzmann 1985).

More gender effects were found by the de Swart et al. (1995) study that compared gender groups matched in age and size. Thus, gender effects were more likely to be isolated from other factors, in contrast to my study and that of Kopec and Harvey (1995), which both utilized free-ranging seals. As with age effects, gender differences found in the field may arise not only from endogenous differences in physiology between the two groups, but also from different foraging or dietary habits.

In contrast to age and gender, seasonal effects on plasma chemistries were detected in this study and that of Kopec and Harvey (1995), but not among captive seals (de Swart et al. 1995) (Table 15). Thus, either the captive seal group was isolated from environmental cues necessary to induce seasonal changes in metabolism, or other environmental factors such as diet were responsible for seasonal differences among the free-ranging seals. However, harbor seals in captivity reduced metabolic rates by 17-35% following molt, and metabolism was typically elevated in summer relative to winter months (Ashwell-Erickson and Elsner 1981; Rosen and Renouf 1995). These changes in metabolic rate were linked to increases of plasma cortisol and decreases of plasma thyroxine during the molt (Riviere et al. 1977; Ashwell-Erickson and Elsner 1981; Renouf and Noseworthy 1991), and reflected by large seasonal changes in body mass and composition (Pitcher and Calkins 1979; Pitcher 1986). Thus, many observed seasonal

changes in plasma chemistry may be linked to this endocrinological variation or to associated changes in food intake. Varying thyroxine levels affect plasma sodium, cholesterol, glucose, calcium, phosphorus, alkaline phosphatase, CPK, AST, ALT, GGT, and LDH (Wallach 1996). Increased cortisol promotes gluconeogenesis and stimulates protein metabolism, and thus is associated with increased plasma glucose and decreased total protein (Green 1981), both patterns shown by seals in this study. Seasonal changes in plasma chemistry among harbor seals in this system may be adequately attributed to endogenous changes in metabolism, and seasonal comparisons of chemistries in an attempt to examine environmental changes (such as foraging patterns or seasonal dietary shifts) may be obscured. For this reason it is critical to not compare across seasons for many plasma chemistries when performing interregional or interannual comparisons.

Seasonal changes in the acute-phase stress protein haptoglobin were also found in brown bears (Mominoki et al. 1996), but bears showed declines in spring and increased levels during autumn and winter. This seasonality was presumed to be associated with metabolic changes due to hibernation. Elevated plasma haptoglobin can be indicative of chronic stress, infection, or inflammation (Gordon and Koj 1985). Haptoglobin release is stimulated by increased levels of plasma cortisol, and corticosteroid elevation may be necessary for haptoglobin production (Gordon and Limaos 1979; Silveira and Limaos 1990; Limaos et al. 1985). High cortisol associated with late pregnancy and parturition (Raeside and Ronald 1981) should increase haptoglobin seasonality in females. However, no gender differences were found in this study of haptoglobins, and the seasonal effect was only within Prince William Sound (Chapter 5). Thus corticosteroids alone may not be the reason for these patterns.

In this study, handling had relatively few significant effects on blood chemistries. The amount of time a seal was held prior to sampling accounted for most of the variability in potassium, AST, CPK and LDH, rather than by individual, regional or temporal factors. These results were consistent with expected responses to acute stress (Bossart and Dierauf 1990), but the magnitudes of these effects relative to mean values were small.



Chemical anesthesia produces numerous physiological changes that are reflected in plasma chemistries (Wallach 1996), though these effects were mostly absent from this study. This was probably due to a sampling regime that resulted in an unbalanced design to adequately show this effect, since though relatively few seals were not drugged during captures in spring and autumn, seals captured during summer months were always manually restrained. This complicated separation of handling and seasonal effects.

The utilization of different blood collection tubes produced variable effects on analyte values, and some effects were greater in magnitude than could be attributed to individual, regional or temporal effects. For most analytes, however, the type of blood collection tube would not be a critical factor when comparing regional or temporal population data. Based on this study and other comparisons of serum versus anticoagulant tube type (Ladenson et al. 1974; Smith, Jr. et al. 1987; Doumas et al. 1989), values of potassium, glucose, total bilirubin, total protein, and AST should not be considered interchangeable between the two types of tubes. For determination of clinical health based on reference ranges, however, the magnitude of most of these differences is minor enough that the effect can be ignored. For example, although there were significant effects of tube type on ALT (Table 7), the magnitude of the effect was only 1% of the reference range (Table 2). This was also true for chloride (1%), glucose (1.7%), BUN (2.8%), and BUN:creatinine ratio (0.2%). Larger effects that could influence comparisons were found for potassium (13%), total (14%) and direct (25%) bilirubin, and GGT (6%).

Decomposition of variances attributable to handling, individual, regional, or temporal effects suggests that at least 60% of variation remains to be explained by other sources such as seal health or condition, unquantified handling techniques, or analytical laboratory variability. However, variation explained by regional or temporal factors does not preclude the possibility of health concerns. For example, regional differences in haptoglobin levels were considered indicative that the Prince William Sound population was affected by stressors differentially from seals in other regions (Zenteno-Savin et al. 1997b). I found in this study that there were elevated haptoglobin levels during spring.

However, this seasonal effect only occurred during 1994 and 1996, but not 1995, within Prince William Sound (Chapter 5). Considering only adult seals, in 1994 three of five seals had haptoglobin values at or exceeded the upper limit of the reference range, and in 1996 five of nine seals met or exceeding the upper limit, while none of the nine seals in 1995 did so. Thus, even though a seasonal pattern was suggested, it did not occur every year. It was intermittently of sufficient magnitude to be clinically significant. Further discussions of interannual and interregional affects are presented in Chapter 5.

There were undoubtedly other sources of variability that were not quantified by my approach, such as blood collection tube drawing order or time of day, which can affect some chemical and hematological analytes (McClatchey 1994). Power modeling of minimum detectable differences in regional or interannual comparisons indicates an ability to detect relatively small differences in plasma chemistries with high statistical power. These differences were sufficiently small that comparisons could easily be biased by age and gender effects, and therefore temporal or regional comparisons must be carefully constructed.

#### *Hematological variability*

In this study most handling effects did not account for significant variability in hemograms. Geraci and Engelhardt (1974) found no changes in RBC or WBC for harp seal (*Phoca groenlandica*) blood held for up to 14 days at 4 °C when in EDTA, but did find significant increases in hematocrit within one day which were not evident in my data. It has also been shown that optical counting methods utilized by Coulter-type laboratory machines overestimate phocid MCV, and therefore report elevated hematocrits and lower MCHC (Bossart and Dierauf 1990; Castellini et al. 1996). These effects were evident by comparing field and clinical laboratory derived hematologies in this study, and must be considered in studies using multiple methodologies, such as within Prince William Sound. It is important to note that even though a particular handling effect did not account for significant portions of variation, this does not imply that the handling method does not produce changes in an analyte. For example, it is well known that handling stress can

elevate hematocrit, while drugging causes decreases (Castellini et al. 1996). As pointed out above, due to handling methodologies this study probably did not maximize differences in hematologies due to drugging. However, modeling these data showed that, given handling, individual, temporal and regional effects, the handling effects probably did not contribute significantly to explaining variability.

As with the plasma chemistries, gender differences did not explain much of the hematological variability. However, males had significantly lower hematocrits, hemoglobin and red blood cell counts, a pattern also detected in San Francisco Bay harbor seals (Kopec and Harvey 1995), but not among captive harbor seals (de Swart et al. 1995). Hematocrit, hemoglobin and RBC counts decreased with age (Table 8), a pattern which has also been reported for Galapagos fur seals (*Arctocephalus galapagoensis*; Horning and Trillmich 1997), but has not consistently been found among harbor seals. Thompson et al. (1997) detected lower RBC and larger MCV of adults relative to juveniles, similar to this study. Serial samples from captive seals did not show an age effect on RBC count (de Swart et al. 1995), but did detect age-related increases in hemoglobin, MCH, MCV and MCHC. Though an increase in MCV was similar to this study and Thompson et al. (1997), the captive seals used by de Swart et al. (1995) were <2.5 years old at the end of their study, and thus are not directly comparable to the other studies comparing adult age classes. Conversely, no age effects were detected on hematological parameters between subadult and adult groups by Kopec and Harvey (1995). McConnell and Vaughan (1983) found a declining trend of RBC count with age between 3 month old pups and adults, but interpretation is difficult due to a small adult sample size ( $n=2$ ). Geraci (1971) compared neonatal harp seal (*Phoca groenlandica*) pups to adults and found no age effect on RBC count, but an increase of MCV, MCH and MCV with age class. Inconsistent findings between studies of age effects may thus mostly be related to comparisons of different age classes.

Because of lower variability in clinical laboratory assessments of MCV relative to field derived measurements, clinical laboratory determined MCV detected a small increase

among adults relative to other age classes. Though both methods detected higher MCV in pups, field-derived MCV did not show a difference among adults (Table 8). This translated into a slightly higher age contribution to explaining overall variance when compared to all handling, individual, temporal, and spatial factors (Table 9).

Seasonal variability of hematocrit and RBC was found by this and other studies (Table 15). This seasonal effect was also noted for harp seals (Ronald et al. 1969) and was related to molt status. de Swart et al. (1995) suggested that seasonal decreases in RBC may result from decreased oxygen needs during this period when seals spend an increased proportion of time hauled-out. However, summer declines and autumn increases in RBC count, hematocrit and hemoglobin have been reported for terrestrial carnivores such as bobcat (*Felis rufus*; Knick et al. 1993) and black bear (*Ursus americanus*; Hellgren et al. 1989). Decreased triiodothyronine ( $T_4$ ) levels and decreased food intake have been related to higher hemoglobin concentrations, higher hematocrits, and higher red blood cell counts (Harlow and Seal 1981; Eales 1988). Likewise, increased thyroxine ( $T_3$ ) levels have been associated with decreases in hemoglobin concentration (Wallach 1996). Based on plasma thyroxine levels measured by Ashwell-Erickson and Elsner (1981), these patterns do not specifically match, and there may be other considerations of the molt involved. Simultaneous measurements of serum  $T_3/T_4$  and hemograms are required to test this association. However, because similar seasonal changes in erythrocytes occur in marine and terrestrial mammals, these patterns may be the result of endogenous cycles, as suggested by Knick et al (1993), rather than as a response to decreased diving needs as suggested by de Swart et al. (1995).

#### *Leukogram variability*

As with plasma chemistries and hemograms, assessment of external sources of variability is also essential for leukograms. Not only do leukocytes respond to infection and inflammation (Bossart and Dierauf 1990), but they may be more sensitive indicators of contaminant intake than plasma chemistries (de Swart et al. 1996). As with plasma chemistries and hemograms, other studies have found varied effects. Kopec and Harvey

(1995) found that neutrophils, monocytes and lymphocytes were affected by gender, though de Swart et al. (1995) did not find significant effects of gender on leukograms (Table 15). I found an age-related decline of WBC count between pups and adults, but no differences among yearlings and subadults. Similarly, no trend was detected by de Swart et al. (1995) for seals between 1.3-2.4 years old. Though pups are born with low WBC counts, these increase within 20 days of parturition (Ross et al. 1993). There were, however, seasonal and regional differences in relative neutrophil and lymphocyte counts common to the three studies. I found summer declines in neutrophil and increases in lymphocyte differential counts, as did Kopec and Harvey (1995). Captive seals from the de Swart et al. (1995) study did not show these seasonal patterns. Plasma cortisol levels are highest during summer molting periods (Ashwell-Erickson and Elsner 1981), and increased corticosteroids induce elevated neutrophil counts (Wallach 1996). However, longer exposure to corticosteroids results in relative lymphocytopenia (Wallach 1996). Thus, the harbor seal pattern is not entirely consistent with seasonally elevated cortisol levels, but there may be an association. In contrast to plasma chemistries and hemograms, however, combinations of handling, individual, temporal or regional factors accounted for less than 20% of the overall variability in leukocytes. It was also evident that because of very small detectable differences based on power analyses, these factors must be considered when performing interannual or interregional comparisons.

### *Summary*

Plasma chemistry and hematological reference ranges were established for free-ranging populations of harbor seals from the Gulf of Alaska. These ranges can be used to examine regional and temporal health trends for individuals or populations. However, a certain number of blood parameters will be classified as statistical outliers by chance alone, as modeled by the binomial distribution. Handling factors, particularly the elapsed time between seal capture and blood sampling, strongly influenced plasma potassium, CPK, and LDH values, and had moderate effects on absolute lymphocyte and eosinophil counts. The elapsed time between blood sample collection and Coulter-counter analysis

significantly affected MCV, MCHC and RBC counts, particularly after 6 days. The amount of variation attributable to handling exceeded that attributable to individual, regional, and temporal factors for AST, CPK, and LDH. Handling, individual, regional and temporal factors accounted for  $\leq 39\%$  of the variation in plasma chemistries, only 19% for leukograms, and  $\leq 56\%$  in hematologies. Seasonal patterns detected in sodium, glucose, calcium, total protein, alkaline phosphatase, AST, hematocrit, hemoglobin, RBC count, neutrophils and lymphocytes may be related to endogenous endocrinological changes, which may obscure other effects due to causes such as dietary changes. Magnitudes of most handling, individual and seasonal effects were within the minimum detectable differences predicted from power models of sample interannual and interregional comparisons. Thus, interannual and interregional comparisons of population blood chemistries should be constructed to compare within seasons, and interpreted carefully in light of potential differences in sample processing.

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Table 1. Seasonal and regional sampling distribution for calculation of plasma chemistry and hematological (in parentheses) reference ranges for harbor seals captured during spring (Mar-May), summer (Jul-Aug) or autumn (Sep-Oct).

Year	Season	Kodiak Island	Prince William Sound	Southeast	Seasonal Totals
1991	Spring		(4)		(4)
1992	Spring		8 (4)		8 (4)
1993	Spring	4	12 (12)	8	24 (12)
	Autumn	1		8	9
1994	Spring		10 (9)		10 (9)
	Summer			31 (30)	31 (30)
	Autumn	9	23 (23)	7	39 (23)
1995	Spring	8 (8)	22 (22)	19	49 (30)
	Summer		6 (4)		6 (4)
	Autumn	8 (9)	14 (20)	9	31 (29)
1996	Spring		21 (22)		21 (22)
	Summer		26 (34)		26 (34)
	Autumn	13 (15)	15 (17)	14 (16)	42 (48)
<b>Regional Totals</b>		<b>43 (32)</b>	<b>157 (171)</b>	<b>96 (46)</b>	<b>296 (249)</b>

Table 2. Harbor seal plasma chemistry reference ranges calculated from samples collected during 1992-1996 in southeast Alaska, Prince William Sound and Kodiak Island regions.

Variable	Avg	sd	n	Reference Range	CI <sup>d</sup>	Total Range (min-max)
Sodium (mmol/L) <sup>a</sup>	148	5	291	138 - 157	1	136 - 167
Chloride (mmol/L) <sup>a</sup>	108	4	296	99 - 117	1	84 - 122
Potassium (mmol/L) <sup>a,b</sup>	3.9	0.7	291	3.1 - 4.6	ns <sup>e</sup>	2.7 - 12.2
Calcium (mmol/L)	2.4	0.2	291	2.1 - 2.7	0.0	2.0 - 3.0
Phosphorus (mmol/L)	1.6	0.4	291	0.7 - 2.5	0.1	0.5 - 3.6
Glucose (mmol/L)	9.0	1.4	291	6.2 - 11.9	0.3	3.6 - 15.2
Blood Urea Nitrogen (mmol/L) <sup>a</sup>	15.7	4.6	296	6.8 - 24.6	0.7	6.1 - 28.6
Creatinine ( $\mu$ mol/L) <sup>a,b</sup>	72	27	296	44 - 133	ns <sup>f</sup>	35 - 159
BUN:Creatinine Ratio <sup>a</sup>	52	20	296	12 - 92	4	12 - 155
Cholesterol (mmol/L) <sup>a</sup>	5.78	1.24	296	3.29 - 8.26	0.23	3.44 - 10.64
Total Bilirubin ( $\mu$ mol/L) <sup>a,b</sup>	6.8	3.4	286	1.7 - 13.7	ns <sup>g</sup>	1.7 - 18.8
Direct Bilirubin ( $\mu$ mol/L) <sup>a,b</sup>	5.1	3.4	284	0.0 - 10.3	ns <sup>h</sup>	0.0 - 17.1
Total Protein (g/L)	78.7	7.1	293	64.5 - 92.9	1.4	53.0 - 96.0
Albumin (g/L) <sup>a</sup>	31.1	2.5	293	26.1 - 36.1	0.5	23.0 - 49.0
Globulin (g/L) <sup>a</sup>	47.6	6.4	293	34.8 - 60.4	1.3	25.0 - 66.0
Albumin:Globulin <sup>a</sup>	0.7	0.1	293	0.5 - 0.9	0.0	0.4 - 1.2
Alkaline Phosphatase (iu/L) <sup>c</sup>	57	26	290	24 - 135	5	20 - 179
Aspartate Aminotransferase (iu/L) <sup>c</sup>	155	81	293	56 - 425	16	53 - 1164
Alanine Aminotransferase (iu/L) <sup>c</sup>	57	37	293	17 - 195	7	9 - 930
Creatine Phosphokinase (iu/L) <sup>c</sup>	1038	1286	296	129 - 8318	254	146 - 20000
Gammaglutamyl Transferase (iu/L) <sup>c</sup>	19	8	269	9 - 41	2	5 - 197
Lactate Dehydrogenase (iu/L) <sup>c</sup>	3837	1880	268	1493 - 9863	390	626 - 21500
Haptoglobin (mg/L) <sup>a</sup>	1131	411	223	311 - 1951	93	259 - 2447

<sup>a</sup>Non-normal distribution ( $P < 0.05$ ; Kolmogorov-Smirnoff Probability Test).

<sup>b</sup>Reference range and 95% CI calculated from 2.5% and 97.5% quantiles of ranked data.

<sup>c</sup>Statistics derived from log-transformed data, therefore listed sd are not symmetrical about the mean.

<sup>d</sup>95% confidence intervals around lower and upper reference range values.

<sup>e</sup>Non-symmetrical intervals: lower limits 2.9-3.2; upper limits 4.6-5.4

<sup>f</sup>Non-symmetrical intervals: lower limits 35-44; upper limits 124-144

<sup>g</sup>Non-symmetrical intervals: lower limits 1.7-1.7; upper limits 10.3-17.1

<sup>h</sup>Non-symmetrical intervals: lower limits 0.0-0.0; upper limits 8.6-13.7



Table 3. Harbor seal hematological reference ranges calculated from samples collected during 1991-1996 in southeast Alaska, Prince William Sound, and Kodiak Island regions.

Variable	Avg	sd	n	Reference range	CI <sup>d</sup>	Total range (min-max)
<b>Field</b>						
Hematocrit	0.55	0.07	349	0.41 - 0.68	1	0.32 - 0.74
Hemoglobin (g/dL)	23.2	3.2	302	16.8 - 29.7	0.6	14.8 - 35.5
MCHC (g/L) <sup>a</sup>	43	5	301	33 - 53	1	25 - 72
MCV (fL)	110.9	8.8	228	93.2 - 128.5	2.0	75.2 - 143.2
MCH (pg) <sup>b</sup>	47.2	6.0	216	36.6 - 60.8	1.4	29.1 - 82.6
<b>Clinical Laboratory</b>						
Hematocrit	0.60	0.06	232	0.47 - 0.72	1	0.44 - 0.76
Hemoglobin (g/dL)	21.9	1.9	232	17.4 - 26.4	0.6	15.5 - 28.4
MCHC (g/L) <sup>a</sup>	36	2	232	33 - 40	0	29 - 47
MCV (fL)	119.0	5.7	232	107.6 - 130.5	1.3	82.6 - 131.0
MCH (pg) <sup>a</sup>	43.4	2.6	232	38.2 - 48.6	0.6	31.5 - 52.5
Red Blood Cell Count (10 <sup>12</sup> /L)	5.03	0.58	232	3.86 - 6.20	0.13	3.62 - 7.87
Platelet Count (10 <sup>9</sup> /L) <sup>c</sup>	340	251	224	111 - 694	57	33 - 1278

<sup>a</sup>Non-normal distribution ( $P < 0.05$ ; Kolmogorov-Smirnoff Probability Test).

<sup>b</sup>Statistics calculated from log-transformed data. Standard deviation is not symmetrical around mean.

<sup>c</sup>Statistics calculated from square-root transformed data. Standard deviation is not symmetrical around mean.

<sup>d</sup>95% confidence intervals around lower and upper reference range values.

Table 4. Harbor seal white blood cell count and differential leukocyte count reference ranges calculated from samples collected during 1991-1996 in southeast Alaska, Prince William Sound and Kodiak Island regions.

Variable	Avg	sd	n	Reference range	CI <sup>d</sup>	Total range (min-max)
<b>Absolute counts (10<sup>9</sup>/L)</b>						
White Blood Cell Count <sup>a</sup>	11.4	3.0	275	6.7 - 19.3	0.3	5.2 - 25.3
Neutrophils	6.8	2.5	219	1.8 - 11.8	0.3	2.2 - 18.2
Banded Neutrophils <sup>b</sup>	0.0	0.02	219	0.0 - 0.0	ns <sup>e</sup>	0.00 - 0.14
Lymphocytes	3.45	1.42	219	0.61 - 6.29	0.30	0.96 - 9.00
Monocytes <sup>b</sup>	0.47	0.4	219	0.0 - 1.4	ns <sup>f</sup>	0.00 - 2.40
Eosinophils <sup>d</sup>	0.84	0.04	219	0.04 - 2.65	0.01	0.0 - 3.2
Basophils <sup>b</sup>	2.2	2.8	82	0.0 - 11.5	ns <sup>g</sup>	0.0 - 11.8
<b>Differential Counts (%)</b>						
Neutrophils	56	12	249	31 - 81	3	25 - 88
Banded Neutrophils <sup>b</sup>	0	0	249	0 - 1	ns <sup>h</sup>	0 - 1
Lymphocytes	30	11	249	8 - 51	2	8 - 61
Monocytes <sup>b</sup>	3	1-7	249	0 - 11	ns <sup>i</sup>	0 - 15
Eosinophils <sup>c</sup>	8	6	249	1 - 22	1	0 - 29
Basophils <sup>b</sup>	1	0-4	249	0 - 7	ns <sup>j</sup>	0 - 12

<sup>a</sup>Statistics calculated from log-transformed data; sd is non-symmetrical about mean.

<sup>b</sup>Non-normal distribution (Kolmogorov-Smirnoff Probability Test;  $P > 0.05$ ). Statistics calculated from quantiles of ranked data.

<sup>c</sup>Statistics calculated from angular-transformed data; sd is asymmetrical about mean.

<sup>d</sup>95% confidence intervals around lower and upper reference range values.

<sup>e</sup>Asymmetrical intervals: upper limits 0.0-0.12.

<sup>f</sup>Asymmetrical intervals: lower limits 0.0-0.0; upper limits 1.3-1.8.

<sup>g</sup>Asymmetrical intervals: lower limits 0.0-0.0; upper limits 8.4-11.8.

<sup>h</sup>Asymmetrical intervals: upper limits 0-1.

<sup>i</sup>Asymmetrical intervals: lower limits 0.0-0.0; upper limits 10-15.

<sup>j</sup>Asymmetrical intervals: lower limits 0-0; upper limits 6-12.

Table 5. Forward stepwise multiple regression matrix showing statistically significant ( $P < 0.05$ ) regression coefficients of individual, regional, or temporal factors on plasma chemistries of chemically anaesthetized harbor seals sampled in Prince William Sound, southeast Alaska, and the Kodiak archipelago during 1992-1996 ( $n = 201$  unless noted otherwise). Regression coefficients represent magnitude of effect (positive or negative) that adjusts variable for that factor. For example, when all individual, regional and temporal factors are considered, adult sodium concentration is 1.7 mmol/L greater relative to other age classes.

Variable	Age Class <sup>l</sup>				Gender <sup>m</sup>		Region <sup>n</sup>			Season <sup>o</sup>		Year					R <sup>2</sup>
	P	Y	S	A	M	F	PWS	KI	SE	Sp	Au	92	93	94	95	96	
Sodium <sup>a</sup>				1.7	-1.7				2.8		-3.7		-4.0				0.232
Chloride <sup>a</sup>					-1.3			-1.9			2.5			1.9			0.140
Potassium <sup>a,b</sup>		0.6															0.048
Calcium <sup>c</sup>				-0.1							0.1				0.1		0.183
Phosphorus <sup>a</sup>									0.1								0.023
Glucose <sup>a</sup>										-0.7				-0.6		0.6	0.167
Blood Urea Nitrogen (BUN) <sup>a</sup>				2.2										2.1			0.098
Creatinine <sup>c</sup>				17							-6						0.185
BUN:Creatinine Ratio				-6										8			0.034
Cholesterol <sup>a</sup>	2.83			-0.51				0.40					-0.51	-0.75			0.237
Total Bilirubin <sup>c,d</sup>		2.9													1.3	-1.5	0.113
Direct Bilirubin <sup>c,d</sup>		2.1						-1.1								-1.1	0.120
Total Protein <sup>o</sup>				5.3							-3.1				3.0	2.2	0.217
Albumin <sup>o</sup>						0.8											0.021
Globulin <sup>o</sup>				4.7							-2.5			-2.6			0.224
Albumin:Globulin Ratio			-0.07	-0.11		0.03								0.05			0.185
Alkaline Phosphatase <sup>f</sup>				-1	1			-1	-2		1						0.352
Aspartate Aminotransferase <sup>f,g</sup>		1											-1				0.064
Alanine Aminotransferase <sup>f</sup>								1									0.153
Creatine Phosphokinase <sup>f,h</sup>																	0.357
Gamma-glutamyl Transferase <sup>f,i</sup>	1							1			1						0.098
Lactate Dehydrogenase <sup>f,j</sup>				-1													0.345
Haptoglobin <sup>k</sup>								355			263				-176		0.392

Table 5. Continued.

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<sup>a</sup>mmol/L

<sup>b</sup>Included elapsed capture to blood sampling time in model, but its effect was non-significant ( $n = 147$ ).

<sup>c</sup>μmol/L

<sup>d</sup> $n = 194$ .

<sup>e</sup>g/L.

<sup>f</sup>iu/L.

<sup>g</sup>Includes elapsed capture to blood sampling time in model ( $\beta = 1$ ;  $R^2 = 0.182$ ;  $n = 147$ ).

<sup>h</sup>Includes elapsed capture to blood sampling time in model ( $\beta = 3$ ;  $R^2 = 0.357$ ;  $n = 147$ ).

<sup>i</sup> $n = 180$ .

<sup>j</sup>Includes elapsed capture to blood sampling time in model ( $\beta = 2$ ;  $R^2 = 0.308$ ,  $n = 127$ ).

<sup>k</sup>mg/L,  $n = 154$ .

<sup>l</sup>P = pup; Y = yearling; S = subadult; A = adult.

<sup>m</sup>M = male; F = female.

<sup>n</sup>PWS = Prince William Sound; KI = Kodiak Island; SE = southeast Alaska.

<sup>o</sup>Sp = spring; Au = autumn.

Table 6. Relative contribution ( $R^2$ ) of individual, regional, and temporal factors to total variance in harbor seal plasma chemistries, based on decomposed multiple regression sums of squares with all other factors included in model (Table 5;  $n=201$  unless noted).  $R^2$  values of zero are blank.

Variable	Age Class	Gender	Region	Season	Year
Sodium	0.025	0.028	0.063	0.135	0.074
Chloride		0.024	0.057	0.098	0.035
Potassium <sup>a</sup>	0.048				
Calcium	0.082			0.076	0.063
Phosphorus			0.023		
Glucose				0.072	0.096
Blood Urea Nitrogen (BUN)	0.068				0.038
Creatinine	0.178			0.022	
BUN:Creatinine Ratio	0.020				0.024
Cholesterol	0.128		0.025		0.060
Total Bilirubin	0.040				0.093
Direct Bilirubin	0.033		0.032		0.069
Total Protein	0.154			0.052	0.037
Albumin		0.021			
Globulin	0.156			0.044	0.029
Albumin:Globulin Ratio	0.115	0.017			0.042
Alkaline Phosphatase	0.061	0.018	0.173	0.147	0.016
Alanine Aminotransferase			0.184	0.052	0.184
Aspartate Aminotransferase <sup>b</sup>			0.035		
Creatine Phosphokinase <sup>c</sup>					
Gammaglutamyl Transferase	0.029		0.046	0.031	
Lactate Dehydrogenase <sup>d</sup>	0.041		0.022		
Haptoglobin			0.170	0.095	0.041

<sup>a</sup> $n = 147$ .

<sup>b</sup>Elapsed time between capture and blood sampling  $R^2 = 0.153$  ( $n = 147$ ).

<sup>c</sup>Elapsed time between capture and blood sampling  $R^2 = 0.357$  ( $n = 147$ ).

<sup>d</sup>Elapsed time between capture and blood sampling  $R^2 = 0.308$  ( $n = 127$ ).

Table 7. Effects of blood collection tube-type (relative to values derived from sodium-heparin collection tube samples) on plasma chemistries of two harbor seals (ANOVA,  $df = 2,6$ ).

Variable	Lithium-heparin	Serum	Significance
Sodium (mmol/L)	-3	-2	.
Chloride (mmol/L)	-3	-1	*
Potassium (mmol/L)	-0.3	0.2	***
Calcium (mmol/L)	-0.1	-0.0	.
Phosphorus (mmol/L)	0.1	0.2	.
Glucose (mmol/L)	-0.4	-0.4	*
Blood Urea Nitrogen (mmol/L)	-0.5	-0.5	*
Creatinine ( $\mu\text{mol/L}$ )	0	-2	.
BUN:Creatinine Ratio	-2	-1	.
Cholesterol (mmol/L)	-0.14	0.01	*
Total Bilirubin ( $\mu\text{mol/L}$ )	0.0	1.7	***
Direct Bilirubin ( $\mu\text{mol/L}$ )	0.0	2.6	***
Total Protein (g/L)	-1.3	-1.3	.
Albumin (g/L)	0.3	0.0	.
Globulin (g/L)	-1.0	-1.3	.
Albumin:Globulin	0.0	0.0	.
Alkaline Phosphatase (iu/L)	-1	1	.
Aspartate Aminotransferase (iu/L)	5	-3	.
Alanine Aminotransferase (iu/L)	-4	6	***
Creatine Phosphokinase (iu/L)	17	-10	.
Gammaglutamyl Transferase (iu/L)	0	2	*
Lactate Dehydrogenase (iu/L)	201	59	.

$P > 0.05$ ; \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

Table 8. Forward stepwise multiple regression matrix of statistically significant ( $P < 0.05$ ) coefficients of individual, regional, or temporal factors on expanded data set of harbor seal hematological variables collected during 1991-1996. Regression coefficients represent magnitude of effect (positive or negative) on variable by factor. For example, when all individual, regional and temporal factors are considered, male hematocrits are 2% points lower than females.

Variable	Gender <sup>j</sup>		Age Class <sup>k</sup>			Region <sup>l</sup>			Season <sup>m</sup>			Year <sup>n</sup>				n	R <sup>2</sup>		
	M	F	P	Y	S	A	PWS	KI	SE	Sp	Su	Au	93	94	95			96	
<b>Field</b>																			
Hematocrit	-0.02					-0.04			-0.04									344	0.484
Hemoglobin <sup>a</sup>		0.6			-1.3	-2.6	1.3			0.6	-2.6		-1.3		-1.3			298	0.333
MCHC <sup>b</sup>													-3		-3			296	0.097
MCV <sup>c,d</sup>			-9															201	0.031
MCH <sup>e</sup>														1.1	1.1			211	0.072
<b>Clinical Laboratory</b>																			
Hematocrit	-0.02				-0.03	-0.05			-0.03	0.03	-0.06							228	0.439
Hemoglobin <sup>a</sup>	-0.6				-0.6	-1.9			-1.3	1.3	-1.9						0.6	228	0.041
MCHC <sup>b,f</sup>										1	1		-1				1	214	0.393
MCV <sup>c</sup>			-6			3					-2							228	0.133
MCH <sup>e</sup>			-3.8	-1.4											1.0	2.5		228	0.281
Red Blood Cell Count <sup>d,g</sup>		0.15			-0.45	-0.72			-0.49	0.72		0.41	0.22					203	0.564
Platelet Count <sup>i</sup>							-4			-2				-15				216	0.248

<sup>a</sup>g/dL.

<sup>b</sup>Mean corpuscular hemoglobin concentration; g/L.

<sup>c</sup>Mean corpuscular volume; fL.

<sup>d</sup>Model limited to elapsed sample to CBC process time <6 days.

<sup>e</sup>Mean corpuscular hemoglobin;  $\mu$ g.

<sup>f</sup>Elapsed time between sampling and CBC processing excluded from model ( $F^*_{(1,218)} = 0.13$ ;  $P > 0.50$ ).

<sup>g</sup> $10^{12}/L$ .

<sup>i</sup> $10^9/L$ .

<sup>j</sup>M = male; F = female.

<sup>k</sup>P = pup; Y = yearling; S = subadult; A = adult.

<sup>l</sup>PWS = Prince William Sound; KI = Kodiak Island; SE = southeast Alaska.

<sup>m</sup>Sp = spring; Su = summer; Au = autumn.

<sup>n</sup>93 = 1993; 94 = 1994; 95 = 1995; 96 = 1996.

Table 9. Relative contribution ( $R^2$ ) of individual, regional, and temporal factors to total variance in harbor seal hematologies, based on decomposed multiple regression sums of squares with all other model factors included (Table 8).  $R^2$  values of zero blank.

Variable	Gender	Age Class	Region	Season	Year	<i>n</i>
<b>Field</b>						
Hematocrit	0.013	0.081	0.063	0.170	0.015	344
Hemoglobin	0.016	0.070	0.032	0.172	0.027	298
MCHC					0.097	296
MCV		0.031				201
MCH					0.072	211
<b>Clinical Laboratory</b>						
Hematocrit	0.021	0.071	0.023	0.226		228
Hemoglobin	0.025	0.077	0.033	0.187	0.020	228
MCHC				0.080	0.121	214
MCV		0.139		0.026		228
MCH		0.096			0.191	228
Red Blood Cell Count	0.016	0.154	0.053	0.167	0.014	203
Platelet Count			0.032	0.008	0.116	216



Table 10. Forward stepwise multiple regression matrix of statistically significant ( $P < 0.05$ ) coefficients of individual, regional, and temporal factors on leukograms of harbor seals, sampled during 1991-1996. Regression coefficients represent magnitude of effect (positive or negative) on variable by factor. For example, when all individual, regional and temporal factors are considered, pup WBC counts are  $1.3 \times 10^9/L$  higher, and adults  $-1.1 \times 10^9/L$  lower, relative to other age classes.

Variable	Age Class <sup>e</sup>				Region <sup>f</sup>			Season <sup>g</sup>			Year <sup>h</sup>						n	R <sup>2</sup>
	P	Y	S	A	PWS	KI	SE	Sp	Su	Au	91	92	93	94	95	96		
<b>Absolute Counts</b>																		
White Blood Cell Count <sup>a</sup>	1.3			-1.1												1.1	271	0.085
Neutrophils <sup>a</sup>	2.3			-0.9	-1.3								-1.6				217	0.131
Banded Neutrophils <sup>a</sup>																	217	0.000
Lymphocytes <sup>a,b</sup>			0.6						0.6					0.7			212	0.188
Monocytes <sup>a</sup>													-0.5		-0.2		217	0.096
Eosinophils <sup>a,c</sup>																	212	0.066
<b>Differential Counts</b>																		
Neutrophils <sup>d</sup>					-5				-8				-7	-6			247	0.141
Banded Neutrophils <sup>d</sup>			0.05		-0.05				0.04								247	0.065
Lymphocytes <sup>d</sup>					4				4				10	4			247	0.090
Monocytes <sup>d</sup>													-4		-2		214	0.108
Eosinophils <sup>d</sup>				0.2					-0.1					0.3			247	0.108

<sup>a</sup> $10^9/L$ .

<sup>b</sup>Elapsed capture to blood sample time included in model (coefficient =  $-0.8 \times 10^9/L$ ).

<sup>c</sup>Elapsed capture to blood sample time included in model (coefficient =  $-0.3 \times 10^9/L$ ).

<sup>d</sup>%.

<sup>e</sup>P = pup; Y = yearling; S = subadult; A = adult.

<sup>f</sup>PWS = Prince William Sound; KI = Kodiak Island; SE = southeast Alaska.

<sup>g</sup>Sp = spring; Su = summer; Au = autumn.

<sup>h</sup>91 = 1991; 92 = 1992; 93 = 1993; 94 = 1994; 95 = 1995; 96 = 1996.

Table 11. Relative contribution ( $R^2$ ) of individual, regional, or temporal factors to total variance in harbor seal leukograms, based on decomposed multiple regression sums of squares with all other model factors considered (Table 10).  $R^2$  values of zero are blank.

Variable	Age Class	Region	Season	Year	<i>n</i>
<b>Absolute Counts</b>					
White Blood Cell Count	0.082			0.019	271
Neutrophils	0.072	0.047		0.020	217
Banded Neutrophils					217
Lymphocytes <sup>a</sup>	0.034		0.017	0.034	212
Monocytes				0.096	217
Basophils <sup>b</sup>		0.205			88
Eosinophils <sup>c</sup>					212
<b>Differential Counts</b>					
Neutrophils		0.037	0.062	0.047	247
Banded Neutrophils	0.030	0.032	0.020		247
Lymphocytes		0.030	0.023	0.051	247
Monocytes				0.108	214
Eosinophils	0.038		0.027	0.050	247
Basophils <sup>d</sup>		0.202			88

<sup>a</sup>Elapsed time between capture and blood sampling time included in model ( $R^2 = 0.082$ ).

<sup>b</sup>Elapsed time between blood sampling and blood smear processing time included in model ( $R^2 = 0.065$ ).

<sup>c</sup>Elapsed capture to blood sample time included in model ( $R^2 = 0.066$ ).

<sup>d</sup>Elapsed time between blood sampling and blood smear processing time included in model ( $R^2 = 0.064$ ).

Table 12. Minimum detectable differences ( $\delta$ ) in harbor seal plasma chemistries for sample regional and interannual comparisons. Regional comparison was based on detecting differences among Prince William Sound, Kodiak Island and southeast Alaska using seals sampled during autumn 1996. Interannual comparison test based on Prince William Sound seals sampled between 1992-96. Both models tested at  $\alpha = 0.05$  and  $(1-\beta) = 0.95$ , following power calculations in Zar (1996).

Variable	Regional $\delta^a$	Interannual $\delta^b$
Sodium (mmol/L)	4	3
Chloride (mmol/L)	3	3
Potassium (mmol/L)	0.6	0.5
Calcium (mmol/L)	0.2	0.1
Phosphorus (mmol/L)	0.3	0.3
Glucose (mmol/L)	1.2	0.9
Blood Urea Nitrogen (BUN; $\mu\text{mol/L}$ )	3.9	3.1
Creatinine ( $\mu\text{mol/L}$ )	23	18
BUN:Creatinine Ratio	17	13
Cholesterol (mmol/L)	1.05	0.83
Total Bilirubin ( $\mu\text{mol/L}$ )	2.9	2.3
Direct Bilirubin ( $\mu\text{mol/L}$ )	2.9	2.3
Total Protein (g/L)	6.0	4.8
Albumin (g/L)	2.1	1.7
Globulin (g/L)	5.4	4.3
Albumin:Globulin Ratio	0.1	0.1
Alkaline Phosphatase (iu/L)	22	17
Aspartate Aminotransferase (iu/L)	69	54
Alanine Aminotransferase (iu/L)	31	25
Creatine Phosphokinase (iu/L)	1094	860
Gammaglutamyl Transferase (iu/L)	7	5
Lactate Dehydrogenase (iu/L)	1599	1257
Haptoglobin (mg/L)	350	275

<sup>a</sup> $v_1 = 2, v_2 = 123, \phi = 2.25.$

<sup>b</sup> $v_1 = 4, v_2 = 360, \phi = 1.95.$

Table 13. Minimum detectable differences ( $\delta$ ) in harbor seal hematologies for sample regional and interannual comparisons. Regional comparison based on detecting differences among Prince William Sound, Kodiak Island and southeast Alaska seals using seals sampled during autumn 1996. Interannual comparison test based on Prince William Sound seals sampled between 1992-96. Both models tested at  $\alpha = 0.05$  and  $(1-\beta) = 0.95$ , following ANOVA power calculations in Zar (1996).

Variable	Regional $\delta^a$	Interannual $\delta^b$
<b>Field</b>		
Hematocrit	0.06	0.04
Hemoglobin (g/dL)	2.6	1.9
MCHC (g/L)	4	3
MCV (fL)	7.0	5.1
MCH ( $\mu$ g)	4.8	3.5
<b>Clinical Laboratory</b>		
Hematocrit	0.05	0.03
Hemoglobin (g/dL)	1.6	1.6
MCHC (g/L)	2	1
MCV (fL)	4.5	3.3
MCH ( $\mu$ g)	2.1	1.5
Red Blood Cell Count ( $10^{12}/L$ )	0.46	0.33
Platelet Count ( $10^9/L$ )	200	144

<sup>a</sup> $v_1 = 2$ ,  $v_2 = 141$ ,  $\phi = 2.25$ .

<sup>b</sup> $v_1 = 4$ ,  $v_2 = 340$ ,  $\phi = 1.95$ .

Table 14. Minimum detectable differences ( $\delta$ ) in harbor seal leukograms for sample regional and interannual comparisons. Regional comparison based on detecting differences among Prince William Sound, Kodiak Island and southeast Alaska seals using seals sampled during autumn 1996. Interannual comparison test based on Prince William Sound seals sampled between 1992-96. Both models tested at  $\alpha = 0.05$  and  $(1-\beta) = 0.95$ , following ANOVA power calculations in Zar (1996).

Variable	Regional $\delta^a$	Interannual $\delta^b$
<b>Absolute Counts (<math>10^9/L</math>)</b>		
White Blood Cell Count	2.4	1.7
Neutrophils	2.0	1.4
Banded Neutrophils	0.02	0.01
Lymphocytes	1.13	0.82
Monocytes	0.32	0.23
Eosinophils	0.03	0.02
Basophils	2.2	1.6
<b>Differential Counts (%)</b>		
Neutrophils	10	7
Banded Neutrophils	0	0
Lymphocytes	9	6
Monocytes	5	3
Eosinophils	5	3
Basophils	3	2

<sup>a</sup> $v_1 = 2, v_2 = 141, \phi = 2.25.$

<sup>b</sup> $v_1 = 4, v_2 = 340, \phi = 1.95.$

Table 15. Comparison of individual, regional and temporal effects on blood chemistries and hematologies of harbor seals from this study and the literature. Studies compared were 1) this study; 2) Kopec and Harvey (1995); 3) de Swart et al. (1995); 4) Schumacher et al. (1995); and 5) Thompson et al. (1997). Significant effects denoted by ●, no effect denoted by ○, and · denotes an unmeasured parameter.

Variable	Age					Gender					Season					Region			
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4
Sodium	●	○	○	·	·	●	○	●	·	·	●	○	○	·	·	●	○	○	·
Chloride	○	○	○	·	·	●	○	○	·	·	●	●	○	·	·	●	●	○	·
Potassium	●	○	○	·	·	○	○	●	·	·	○	○	○	·	·	○	○	○	·
Calcium	○	○	○	·	·	○	○	○	·	·	●	○	○	·	·	○	○	○	·
Phosphorus	○	○	○	·	·	○	○	●	·	·	○	●	○	·	·	●	○	○	·
Glucose	○	○	○	·	·	○	○	○	·	·	●	○	○	·	·	○	○	○	·
Blood Urea Nitrogen	●	○	○	·	·	○	●	○	·	·	○	○	○	·	·	○	○	●	●
Creatinine	●	●	○	·	·	○	○	○	·	·	●	○	○	·	·	○	○	●	●
BUN:Creatinine Ratio	●	○	·	·	·	○	○	·	·	·	○	○	·	·	·	○	○	·	·
Cholesterol	●	○	○	·	·	○	○	○	·	·	○	○	○	·	·	●	○	●	●
Total Bilirubin	●	○	○	·	·	○	○	○	·	·	○	○	○	·	·	○	○	○	○
Direct Bilirubin	●	·	·	·	·	○	·	·	·	·	○	·	·	·	·	●	·	·	·
Protein	●	○	○	·	·	○	○	○	·	·	●	●	○	·	·	○	○	○	●
Albumin	○	○	·	·	·	●	○	·	·	·	○	○	·	·	·	○	○	·	·
Globulin	●	○	·	·	·	○	○	·	·	·	●	●	·	·	·	○	○	·	·
AG	●	○	·	·	·	●	○	·	·	·	○	○	·	·	·	○	○	·	·
Alkaline Phosphatase	●	○	○	·	·	●	○	○	·	·	●	●	○	·	·	●	○	○	○
Aspartate Aminotransferase	○	○	○	·	·	○	○	●	·	·	○	○	○	·	·	●	○	○	●
Alanine Aminotransferase	○	○	○	·	·	○	○	●	·	·	●	○	○	·	·	●	○	○	●
Creatine Phosphokinase	○	○	○	·	·	○	○	○	·	·	○	○	○	·	·	○	○	○	·
Gamma-glutamyl Transferase	●	○	○	·	·	○	○	○	·	·	●	○	○	·	·	●	○	○	●
Lactate Dehydrogenase	●	○	○	·	·	○	○	●	·	·	○	○	○	·	·	●	○	○	·
Red Blood Cell Count	●	○	○	·	●	●	●	○	·	○	●	●	●	·	○	●	○	●	·
Hematocrit	●	○	○	·	○	●	●	○	·	○	●	●	●	·	●	●	○	●	·
Hemoglobin	●	○	●	·	○	●	●	○	·	○	●	○	○	·	●	●	●	○	·
MCHC	○	○	●	·	○	○	○	○	·	○	○	○	○	·	○	○	○	○	·
MCV	●	○	●	·	●	○	○	○	·	○	○	○	○	·	○	○	○	○	·
MCH	○	○	●	·	·	○	○	○	·	·	○	○	○	·	·	○	○	○	·
Platelets	○	·	·	·	·	○	·	·	·	·	●	·	·	·	·	●	·	·	·
White Blood Cell Count	●	○	○	·	○	○	○	○	·	○	○	○	○	·	○	○	○	●	·
Neutrophils	○	○	○	·	○	○	●	○	·	●	●	●	○	·	○	●	○	●	·
Lymphocytes	○	○	●	·	○	○	●	○	·	●	●	●	○	·	○	●	○	○	·
Monocytes	○	○	○	·	○	○	●	○	·	○	○	○	○	·	○	○	○	○	·
Eosinophils	●	○	●	·	○	○	○	○	·	○	●	○	○	·	○	○	○	○	·
Basophils	○	○	·	·	●	○	○	·	·	●	○	○	·	·	○	○	·	·	·

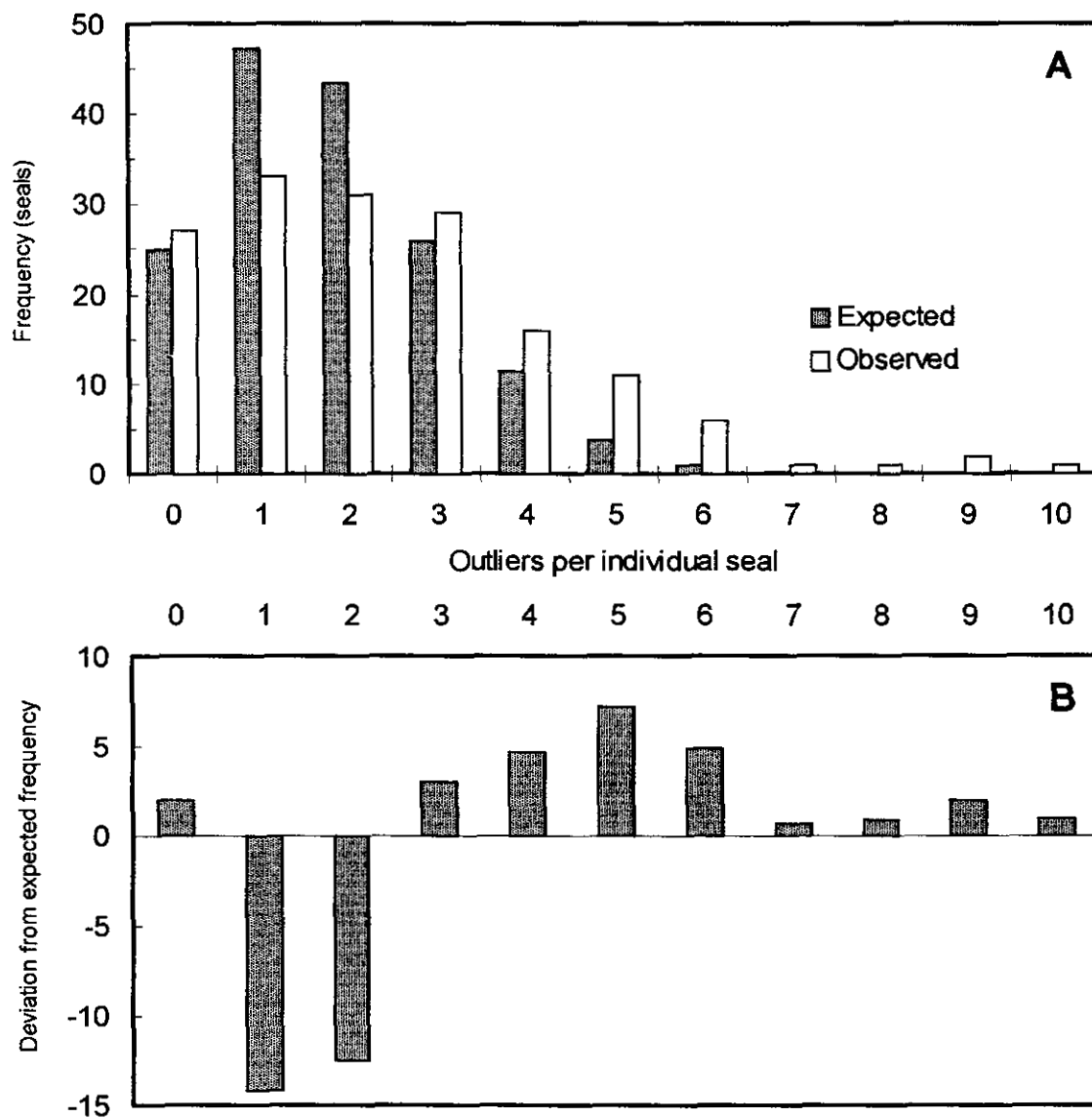


Figure 1. Distributions of expected and observed plasma chemistry and hematological outliers (A), and deviations between frequencies (B) of 36 variables for 158 harbor seals sampled in Alaska during 1993-1996.

# **3 Effects of Body Shape and Blubber Distribution on the Performance of Morphometric Indices of Condition in Harbor Seals**

## **INTRODUCTION**

Nutritional limitation as a result of changes in prey distribution or abundance has been proposed as one of multiple hypotheses to explain harbor seal (*Phoca vitulina*) population declines throughout parts of Alaska (Sease 1992; Alaska Sea Grant 1993; Hoover-Miller 1994). Nutritional limitations in turn affect the nutritional status of an animal, and changes in body size or composition reflect responses to energy expenditure and intake relative to maintenance requirements (Harder and Kirkpatrick 1994). Body condition affects fecundity and survival rates (Harder and Kirkpatrick 1994), and was shown to be related to timing of implantation in grey seals (*Halichoerus gypus*; Boyd 1984). Relative to an overall population of animals, body condition can be measured as relative size (body mass or length) at age (Walker and Bowen 1993), as rate of growth relative to age (Trites and Bigg 1992; Harder and Kirkpatrick 1994), or by determination of body composition as fat and fat-free components, also measured as adipose tissue or lipid stores relative to body mass (Bowen et al. 1992; Worthy et al. 1992; Beck et al. 1993; Gales et al. 1994; Arnould 1995). Direct morphological measurements of body composition in phocids are facilitated by the storage of 90% of total body lipids within a subcutaneous blubber layer (Beck et al. 1993; Gales et al. 1994), which has multiple functions as energy storage, insulation, and shaping for streamlining (Ryg et al. 1988; Beck and Smith 1995; Rosen and Renouf 1997).

An ultimate goal of this study was to determine whether size or compositional changes have occurred among harbor seals from the Gulf of Alaska between the 1970's and 1990's. Seals collected during 1972-78, near the beginning of population declines



(Pitcher 1990), were sampled with different techniques than seals captured during 1991-1996 (Chapter 5, this dissertation). Current non-destructive techniques to assess phocid body composition involve the use of ultrasonic determinations of blubber depth (Gales and Burton 1987; Slip et al. 1992; Rosen and Renouf 1997), isotope dilution estimation of fat and fat-free mass (Lifson and McClintock 1966; Worthy et al. 1992; Gales et al. 1994; Arnould 1995), or the use of bioelectrical impedance analysis (Gales et al. 1994; Arnould et al. 1995), and are not directly comparable to measurements of blubber or sculp (blubber with attached skin) mass collected for harbor seals during 1972-78. Also, measures of condition related to size (mass or length at age) require determination of age, available only by counting tooth enamel layers (Scheffer 1950), a method not typically utilized on live marine mammal subjects. Thus, body composition has often been indexed by combinations of morphometric measurements involving body mass, length, or girth (Smirnov 1924; Sivertsen 1941; McLaren 1958; Sergeant 1973; Boulva and McLaren 1979; Castellini and Calkins 1993; Sweitzer and Berger 1993; Kopec and Harvey 1995), or by a measure of blubber thickness taken over the xiphosternal process (Sergeant 1973; Pitcher 1986). Until recently these indices had not been validated by rigorous quantitative studies, and there have been indications that these measures may be relatively poor indicators of individual condition (Pitcher 1986; Gales and Burton 1987; Ryg et al. 1990; Beck et al. 1993; Gales and Renouf 1994; Arnould 1995). It is possible that these indices may perform better for tracking general condition of populations of animals, for example if statistical means of groups of animals are compared. Population-level index performance or tracking utility for seasonal and interannual changes in condition among groups of animals have not previously been tested.

The first goal of this study was to evaluate the utility of morphometric-based condition indices to predict size or compositional condition at individual or population levels. Secondly, this study examined the effect of body scaling with respect to mass, length and girth, and the limitations this places on the use of morphometric indices of

condition. Finally, topographical blubber distribution was examined in cross-sectional studies of free-ranging animals to examine age, gender, seasonal, regional and interannual patterns of blubber distribution, with respect to effects on condition index interpretation. This technique was recently applied to a longitudinal study of captive harbor seals by Rosen and Renouf (1997). More involved discussions relevant to long-term and recent body condition among Gulf of Alaska harbor seals are found in Chapter 5.

## METHODS

### *Historical data*

This study of condition index comparisons used morphometric and age data collected by Pitcher (1977, 1986) and Pitcher and Calkins (1983) in 1972-78. During these studies, 405 harbor seals from the Gulf of Alaska were collected and sampled. Seals were collected by shooting, then weighed ( $M_b$ ), and measured for axillary girth ( $G$ ), standard length ( $L$ ; dorsal-side up), and xiphosternal blubber depth (XBT). Sculp (blubber plus pelage) was separated and weighed ( $M_s$ ). Core mass ( $M_c$ ) was determined by subtracting sculp from body mass. Canines were extracted, sectioned, stained and age determined by counting cementum rings. Details of these procedures can be found in Pitcher (1977, 1986) and Pitcher and Calkins (1983). Similar data were collected during 1979 and 1985 from the Bering Sea (Frost and Lowry, unpublished data). Frost and Lowry measured standard length with the seal ventral-side up (Scheffer 1967), and these were converted to dorsal-side up standard lengths using a regression equation from Pitcher and Calkins (1983).

### *Contemporary data*

Harbor seals were captured as part of several studies designed to investigate long-term population declines in Alaska harbor seals (Frost and Lowry 1994*a,b*; Lewis 1995, 1996; Frost et al. 1995, 1996, 1997). Seals were captured from three general geographic regions within the Gulf of Alaska; Kodiak and Sitkinak Islands (grouped as Kodiak Island,

KI), Prince William Sound (PWS), and southeast Alaska (SE). Captures were conducted during spring (March-May), summer (July-August) and autumn (September-October) in 1991-96. Seal captures during summer from PWS (1995, 1996) and SE (1994) were performed by personnel from the National Marine Mammal Laboratory as part of a radio-tagging study.

Seals were captured by entangling them in large-mesh nets near haul-outs. Seals were removed from nets and placed individually in hoop-net bags constructed of small-mesh nylon webbing sewn to rubber rings, and kept in quiet locations on ship or shore until processed. During spring and autumn collections, seals were typically anaesthetized with an intramuscular injection of a ketamine hydrochloride (Ketaset) and valium (Diazepam) mixture (Frost et al. 1995, 1996, 1997). Seals were weighed to  $\pm 0.1$  kg with a hanging electronic load cell balance (Ohaus Model I-20W), and standard length ( $L$ ; dorsal side up), curvilinear length ( $L_c$ ), and a series of girth rings taken at the ears, neck, shoulder, axilla, maximum diameter, mid-trunk, hips, and ankles (Figure 1) were measured to  $\pm 1.0$  cm. Curvilinear distances to each girth ring were also measured. Blubber depths were determined using a portable ultrasonic unit (Scanoprobe II, Model 7310, Scanco, Inc.) at lateral and dorsal sites along each girth ring except ears and ankles. Blubber depth relative to body thickness ( $d/r$ ) was calculated as the blubber depth divided by ( $\text{girth}/2\pi$ ), and blubber volume was determined by summation of a series of truncated cones (Gales and Burton 1987; Slip et al. 1992; Rosen and Renouf 1997). Blubber volume was converted to blubber mass by assuming a blubber density of 0.95 g/mL (Chapter 4, this dissertation). Fineness ratio, and index of slenderness and streamlining, was calculated as the standard length divided by maximum girth.

Additional morphometric data were provided by a harbor seal biosampling program conducted in cooperation with Native subsistence hunters, the Alaska Native Harbor Seal Commission, and the National Marine Fisheries Service. After seals were shot by hunters, trained sampling personnel weighed and measured dorsal standard length,

axillary girth and xiphosternal blubber thickness. Morphometric data similar to the historic data set were collected during 1989-90 from Prince William Sound, and the Barren and Kodiak Islands (Frost and Lowry 1994a). As above, ventral-side up standard lengths were converted to dorsal-side up lengths.

There was no direct way to assess the effect of measuring standard lengths on live versus shot seals. Therefore, comparisons of post-mortem versus live-measured length to mass scaling were performed using data from seals captured by net-entanglement (measured while alive), and seals collected by shooting during subsistence harvests from the same location and time of year. Seals were captured or shot in Prince William Sound in autumn 1995 and 1996. Standard lengths were compared between groups with mass as a covariate.

#### *Condition index calculations*

Utilizing data collected from shot seals, sculp mass (blubber with attached skin) and sculp content (proportion of body mass that was sculp) were assumed to be absolute measures of body composition condition. Observed body mass, length or axillary girth at age relative to that expected from regressions of these were assumed to be absolute measures of condition related to size or growth. Measured length relative to expected length at age was based on regressions calculated by McLaren (1993) from these data. Regressions of mass and girth at age were assumed to be the simplest non-linear form resulting in the highest regression coefficient. I tested the following morphometric indices of condition: xiphosternal blubber thickness (XBT; Sivertsen 1941; McLaren 1958; Pitcher 1986),  $G/L$  (Smirnov 1924; Sivertsen 1941; Sergeant 1973; Pitcher and Calkins 1983; Pitcher 1986),  $M_b/L$  (Boyd 1984; Arnould 1995; Kopec and Harvey 1995),  $M_b/L^2$  (Smalley et al. 1990),  $M_b/L^3$  (Bailey 1968; Virgl and Messier 1993; Arnould 1995), residual  $M_b$  at length from the least-squares best-fit sex-specific regression of  $M_b$  and  $L^a$ ; Smalley et al. 1990; Trites and Bigg 1992),  $M/LG^2$  and  $M/L^aG^b$  (volume or density indices; Castellini and Calkins 1993; Sweitzer and Berger 1993), and a length, mass and

blubber depth index (LMD; Ryg et al. 1990) modified to use the available XBT measure rather than the Ryg et al. (1990) recommended dorsal blubber thickness at 60% of standard body length.

Condition indices were compared to absolute measures of condition (defined above) for the entire data set, and to two subsets of the data grouped by season and location to maximize sample sizes within each group. Seasonal comparisons were performed using seal data collected during 1975 from Prince William Sound, the only year and locality which had four-season coverage. Locational comparisons were performed using data collected during spring 1976, a season and year which had maximum variety of sampling locations and the largest available sample sizes. Sampling locations were Kodiak Island, Kenai Peninsula, and various locations around the Gulf of Alaska, grouped to increase sample size: Icy Bay, Middleton Island, Kayak Island, and Yakutat.

Statistics were calculated using Systat<sup>®</sup> version 7.0 (SPSS Inc.), Statistix<sup>®</sup> version 4.1 (Analytical Software), or Excel<sup>®</sup> (Microsoft Corp.), and some regressions were determined using Table Curve v.3.0.5 (Jandel Scientific). All regression and correlation *P*-values presented were adjusted for degrees of freedom or numbers of contrasts using Bonferonni adjustments (Systat 1996). Residuals used in condition index comparisons were the deviation from the regression line calculated from two variables (for example mass and length) (Sokal and Rohlf 1987). Thus, if a standardized residual value is 1.0, that parameter was on the regression line. Values >1.0 are greater than expected, and values <1.0 are less than expected from the overall regression between two variables.

## RESULTS

### *Body size and scaling*

Relationships between mass, length, and axillary girth were examined to generate some of the condition indices tested. There was a significant difference in length scaling depending upon whether seals were measured alive or dead (Figure 2). For seals collected

during the same season from the same general location, least square mean lengths of seals (adjusted for body mass) measured postmortem were  $1.32 \pm 0.02$  m ( $n = 14$ ), 10.8 cm longer than live-measured seals ( $1.21 \pm 0.01$  m,  $n = 37$ ;  $F_{(1,48)} = 31.471$ ,  $P < 0.001$ ;  $M_b^{0.31}$  as covariate). Therefore, scaling relationships were determined on groups of data separated by capture technique.

Postmortem standard length ( $L$ , in m) scaled with body mass ( $M_b$ , in kg) as:

$$L = 0.396M_b^{0.31} \quad (1)$$

( $r^2 = 0.894$ ,  $F_{(1,674)} = 5702.5$ ,  $P < 0.001$ ), and live-measured length scaled as:

$$L = 0.368M_b^{0.31} \quad (2)$$

( $r^2 = 0.864$ ,  $F_{(1,344)} = 2193.1$ ,  $P < 0.001$ ), both shown in Figure 2. Likewise, there was a significant difference of the scaling of axillary girth with length depending on whether measurements were gathered from live or postmortem seals ( $F_{(1,48)} = 51.364$ ,  $P < 0.001$ , with  $L$  as covariate). Thus,  $G$  scaled with  $L$  for postmortem measurements as:

$$G = 0.69L^{0.96} \quad (3)$$

( $r^2 = 0.765$ ,  $F_{(1,710)} = 2316.4$ ,  $P < 0.001$ ), and for live captures as:

$$G = 0.77L^{0.94} \quad (4)$$

( $r^2 = 0.730$ ,  $F_{(1,341)} = 925.1$ ,  $P < 0.001$ ), though the exponents were not significantly different. Because of the length difference, seals measured live were apparently thicker compared to similarly sized seals measured postmortem. The condition index of  $G/L$  was calculated assuming a linear relationship.

Core mass ( $M_c$ , kg) and sculp mass ( $M_s$ , kg) were found to scale with total body mass as:

$$M_c = 0.604M_b^{1.02} \quad (5)$$

( $r^2 = 0.963$ ,  $P < 0.001$ ,  $n = 450$ ), and as:

$$M_s = 0.385M_b^{0.97} \quad (6)$$

( $r^2 = 0.872$ ,  $P < 0.001$ ,  $n = 450$ ), as shown in Figure 3. Neither exponent was significantly different than  $M_b^{1.00}$ , thus sculp and core components varied isometrically with body mass. Inclusion of sex, season, location and year as factors did not substantially change these relationships ( $M_c$   $R^2 = 0.967$ ,  $P < 0.001$ ;  $M_b$   $R^2 = 0.883$ ,  $P < 0.001$ ). That is, relative to the variability in the data (the 95% confidence intervals around 0.385 'sculp content' were 0.333-0.446), inclusion of these other factors only explained about 10% additional variability in the overall regression. There were significant effects on sculp mass relative to body mass attributable to gender (females had greater sculp mass than males when adjusted for body mass,  $F_{(1,440)} = 27.066$ ,  $P < 0.001$ , corresponding to a mean female sculp content of  $38.3 \pm 0.5$  %, males  $34.1 \pm 0.4$  %), season ( $F_{(3,440)} = 11.444$ ,  $P < 0.001$ ), and the interaction of the two factors ( $F_{(3,440)} = 4.573$ ,  $P = 0.004$ ). There was no residual relationship of sculp mass with size ( $r^2 = 0.003$ ,  $P = 0.138$ ) or age ( $r^2 = 0.0$ ,  $P = 0.476$ ), thus condition index performance tests were not restricted to adult seals. Sculp mass was proportional to core mass as  $M_s \propto M_c^{0.823}$  ( $r^2 = 0.994$ ,  $F_{(1,449)} = 0.994$ ,  $P < 0.001$ ).

#### *Condition index performance*

Performance tests of condition indices were limited to seals of known age, hence the 1972-78 Gulf of Alaska group, using data previously presented in Pitcher and Calkins (1983) and Pitcher (1986). Absolute measures of condition were calculated from this group from the following relationships. For these data, mass was found to be related to length as:

$$M_b = 22.9L^{3.08} \quad (7)$$

for females ( $r^2 = 0.892$ ,  $F_{(1,283)} = 2348$ ,  $P < 0.001$ ), and:

$$M_b = 22.3L^{2.90} \quad (8)$$

for males ( $r^2 = 0.932$ ,  $F_{(1,262)} = 3587$ ,  $P < 0.001$ ). Females tended to be slightly more massive for a given length than males, particularly for longer (or older) seals. These regressions were used to calculate residual mass at length for index comparisons. Mass was found to be related to age by:

$$M_b = 81.4 - 65.5e^{(-0.196T)} \quad (9)$$

for females, where T is age in years ( $r^2 = 0.745$ ,  $F_{(2,291)} = 428$ ,  $P < 0.001$ ). This relationship for males was:

$$M_b = 91.3 - 74.4e^{(-0.195T)} \quad (10)$$

( $r^2 = 0.843$ ,  $F_{(2,269)} = 428$ ,  $P < 0.001$ ). Males were heavier than females at similar ages, particularly after ages of 2-3 years. Equations 9 and 10 were used to calculate mass at age residuals. Girth was related to age by:

$$G = 1.03 - 0.390e^{(-0.265T)} \quad (11)$$

for females ( $r^2 = 0.652$ ,  $F_{(2,293)} = 277.4$ ,  $P < 0.001$ ), and:

$$G = 1.10 - 0.437e^{(-0.240T)} \quad (12)$$

for males ( $r^2 = 0.775$ ,  $F_{(2,273)} = 472.4$ ,  $P < 0.001$ ). Males were slightly larger in girth at age than females, particularly after about 8 years of age. Residual girth at age was calculated from equations 11 and 12 for condition index comparisons.

Body mass was found to be significantly related to a volume index such that:

$$M_b = 42.4L^{1.44}G^{1.63} \quad (13)$$

for females ( $R^2 = 0.956$ ,  $F_{(2,276)} = 3048$ ,  $P < 0.001$ ), and:

$$M_b = 38.5L^{1.51}G^{1.42} \quad (14)$$

for males ( $R^2 = 0.967$ ,  $F_{(2,260)} = 3817$ ,  $P < 0.001$ ). Females thus tended to be more massive for their body volume than males. These regressions were used to calculate residual mass at body volume. Likewise, mass was strongly related to  $LG^2$  for females:



$$M_b = 49.6LG^2 \quad (15)$$

( $r^2 = 0.986$ ,  $F_{(1,487)} = 35053$ ,  $P < 0.001$ ), and for males:

$$M_b = 47.5LG^2 \quad (16)$$

( $r^2 = 0.989$ ,  $F_{(1,521)} = 48006$ ,  $P < 0.001$ ). As with the volume index, regressions were significantly different between sexes ( $F_{(1,1005)} = 13.08$ ,  $P < 0.001$ ), but were unaffected by whether seals were measured alive or dead ( $F_{(1,1005)} = 0.133$ ,  $P = 0.716$ ). Sculpt mass was also moderately predictable from regressions on length and girth:

$$M_s = 20.0L^{0.63}G^{2.38} \quad (17)$$

for females ( $R^2 = 0.897$ ,  $F_{(2,196)} = 865$ ,  $P < 0.001$ ), and:

$$M_s = 16.3L^{0.81}G^{2.11} \quad (18)$$

for males ( $R^2 = 0.861$ ,  $F_{(2,204)} = 640$ ,  $P < 0.001$ ).

Correlations of absolute and indexed measures of condition were highly variable for males (Table 1) and females (Table 2). The range of sculp contents available for comparison were 20.6-48.9% for males, and 23.4-55.4% for females. Sculpt content was poorly correlated with all indices except LMD index for both sexes. Sculpt mass was highly correlated with 5 condition indices for both males (Table 1) and females (Table 2), more than any other condition measure. However, this was probably because of the high degree of correlation between sculp mass and body mass (Figure 3) producing an autocorrelation affect. Residual mass at age was highly and positively correlated with residual length at age ( $r = 0.608$ ,  $P < 0.001$ ,  $n = 549$ ), suggesting that lighter or heavier than normal seals also tended to be shorter or longer, respectively, than expected.

Based on Tables 1 and 2, several condition indices were tested for their sensitivity to changes in absolute condition among seals grouped by season for males (Figures 4, 5) and females (Figures 6, 7), and between locations within a season (Figures 8-11). Within

Prince William Sound during 1975, males did not exhibit significant seasonal changes in body mass ( $L^3$  as covariate to remove size effect;  $F_{(3,68)} = 0.541, P = 0.656$ ), but had significant reductions in sculp ( $F_{(3,68)} = 25.01, P < 0.001$ ) with increased core ( $F_{(3,68)} = 14.384, P < 0.001$ ) content relative to body mass during summer (Figure 4a). Male seals were fattest during winter. There was no statistically significant seasonal trend in residual mass at age ( $F_{(3,68)} = 0.50, P = 0.683$ ) or residual mass at length ( $F_{(3,68)} = 0.525, P = 0.666$ ; Figure 4b), though heavier than average for length (standardized residual  $>1$ ) tended to be lighter than average for age (standardized residual  $<1$ ). Apparent seasonal oscillations in residual girth and length at age (Figure 4c) were not significant (girth  $F_{(3,68)} = 2.281, P = 0.087$ ; length  $F_{(3,69)} = 0.408, P = 0.748$ ). Both of these condition measures followed the seasonal pattern shown by sculp content (Figure 4a) during spring-autumn, but not from autumn-winter or winter-spring. There was no significant seasonal difference in age composition (Kruskal-Wallis test statistic = 0.508,  $P = 0.917$ ) between spring (median age = 4 yr old), summer (median age = 5 yr), autumn (median age = 4 yr), and winter (median age = 4.5 yr).

Changes in the sculp component were detected by significant changes in the LMD index ( $F_{(3,68)} = 11.00, P < 0.001$ ) between spring and summer ( $P < 0.001$ , Bonferonni adjusted pairwise comparison), summer and autumn ( $P = 0.009$ ), and summer and winter ( $P = 0.017$ ), but not between autumn and winter, or winter and spring (Figure 5a). Seasonal changes in the LMD index were similar to deviations from typical length at age (Figure 4c). Though there was a significant seasonal effect on G/L (Figure 5b;  $F_{(3,69)} = 6.51, P = 0.001$ ), and significant differences detected between spring and winter ( $P = 0.002$ , Bonferonni adjusted pairwise comparison) and autumn and winter ( $P = 0.001$ ), this index did not correspond to seasonal patterns in any condition measure (Figure 5). The winter decline in this ratio was similar to that of residual girth at age (Figure 4c), but there was not a similar correspondence in summer. Mass relative to length (M/L) did not vary significantly with season ( $F_{(3,69)} = 0.61, P = 0.980$ ; Figure 5c), and did not track any

condition measure (Figure 4a,c), though it did reflect the residual mass at length derived from Equation 8 (Figure 4b). Residual body mass relative to body volume (Figure 5d) varied seasonally ( $F_{(3,69)} = 6.752, P < 0.001$ ), and male seals were significantly lighter relative to body volume in spring and autumn (winter to spring difference  $P = 0.004$ ; summer to autumn difference  $P = 0.061$ ; autumn to winter difference  $P = 0.001$ , Bonferonni adjusted pairwise comparisons). With the exception of winter, this tracked changes in the core component (Figure 4a), and inversely tracked residual girth at age (Figure 4c). That is, male seals that had larger axillary girths relative to others at that age were below the typical relationship for their body mass at volume.

In contrast to males, female body mass varied significantly throughout the year ( $F_{(3,36)} = 5.569, P = 0.003, L^3$  as a covariate to remove size variation). Scaled for size differences, females were heaviest in spring ( $60.4 \pm 1.5$  kg,  $n = 22$ ) and lightest in autumn ( $49.2 \pm 2.4$  kg,  $n = 9$ ). Summer ( $56.3 \pm 4.1$  kg,  $n = 3$ ) and winter ( $53.4 \pm 2.7$  kg,  $n = 7$ ) mass were intermediate. Similar to males, female sculp mass relative to body mass (Figure 6a) varied through the year ( $F_{(3,37)} = 6.440, P = 0.001$ ), and was greatest in winter relative to spring (Bonferonni adjusted pairwise comparison  $P = 0.002$ ) and summer ( $P = 0.020$ ). Like males, females exhibited no significant seasonal deviations from normal mass at length ( $F_{(3,37)} = 1.683, P = 0.187$ ) or mass at age relationships ( $F_{(3,37)} = 2.368, P = 0.086$ ; Figure 6b). However, unlike males, deviations from both relationships tracked directly. That is, females that tended to be heavy for their age were also generally heavy for their length. There were no significant seasonal trends to deviations from girth at age ( $F_{(3,37)} = 1.137, P = 0.347$ ) or length at age ( $F_{(3,37)} = 0.162, P = 0.921$ ) relationships (Figure 6c). Age composition of the sampled seals did not vary seasonally (Kruskall-Wallis test statistic = 2.012,  $P = 0.570$ ) between spring (median age = 4.0 years), summer (median age = 3.0 years), autumn (median age = 5.0 years), and winter (median age = 4.0 years).

There was no seasonal trend to the LMD index (Figure 7a;  $F_{(3,37)} = 0.585, P = 0.628$ ) for females, in contrast to males (Figure 5a). As with males, this index seemed

more sensitive to the length at age (Figure 6c) than to sculp content (Figure 6a) changes. Girth/Length did not significantly vary seasonally ( $F_{(3,37)} = 1.977$ ,  $P = 0.134$ ), though spring and summer ratios were higher than autumn and winter (Figure 7b). As with males, this ratio was most related to deviations of girth at age (Figure 6c). That is, spring and summer female seals that had greater girth at age than normal had slightly larger G/L ratios. There was also no seasonal difference in the M/L index (Figure 7c;  $F_{(3,37)} = 0.201$ ,  $P = 0.895$ ), nor in the residual mass at volume index (Figure 7d;  $F_{(3,37)} = 2.236$ ,  $P = 0.100$ ). Unlike males, there was no apparent agreement between this index and deviations from the girth at age regression (Figure 6c).

Body masses of male seals were significantly greater at Kodiak Island ( $80.3 \pm 2.5$  kg,  $n = 13$ ) than at either Kenai Peninsula ( $62.3 \pm 2.2$  kg,  $n = 16$ ) or Gulf of Alaska ( $67.2 \pm 3.5$  kg,  $n = 6$ ) locations during spring 1976 ( $F_{(2,31)} = 13.102$ ,  $P < 0.001$ ;  $L^3$  as a covariate to remove size variation). This was also reflected by significant differences in residual mass at length ( $F_{(2,32)} = 6.695$ ,  $P = 0.004$ ) and at age ( $F_{(2,32)} = 6.453$ ,  $P = 0.004$ ; Figure 8b). Sculp and core content (Figure 8a), however, were only significantly different between Kodiak Island and the Gulf of Alaska locations (overall location effect:  $F_{(2,32)} = 3.758$ ,  $P = 0.034$ ; Kodiak Island and Gulf of Alaska difference  $P = 0.038$ , Bonferonni adjusted pairwise comparison). There was no significant residual variation in length at age (Figure 8c) among the three location groups ( $F_{(2,32)} = 0.567$ ,  $P = 0.572$ ). There was, however, a location effect on residual girth at age (Figure 8c;  $F_{(2,32)} = 3.687$ ,  $P = 0.036$ ), that was only significant between Kodiak Island and the Gulf of Alaska (Bonferonni adjusted pairwise comparison  $P = 0.052$ ). The age composition of Kodiak Island male seals was significantly older (median age = 16 years) than that of Kenai Peninsula (median age = 6 years) and Gulf of Alaska (median age = 9 years; Kruskal-Wallis test statistic = 17.512,  $P < 0.001$ ) seals.

Unlike the male seasonal contrasts, LMD index did not accurately reflect location differences in sculp content (Figure 9a), and there was no significant location effect

( $F_{(2,32)} = 1.750, P = 0.190$ ). However, as with the seasonal contrasts LMD index seemed related to the direction of the length at age residual (Figure 8c). The G/L index varied significantly with location ( $F_{(2,32)} = 3.401, P = 0.046$ ; Figure 9b), with Kodiak Island males being slightly larger in girth at length than Kenai Peninsula seals (Bonferonni adjusted pairwise comparison  $P = 0.074$ ). This trend was apparently unrelated to any measure of condition (Figure 8). Kodiak Island male seals were also categorized as significantly heavier per body length by the M/L index ( $F_{(2,32)} = 17.743, P < 0.001$ ) relative to Kenai Peninsula ( $P < 0.001$ ) and Gulf of Alaska ( $P = 0.009$ ) seals (Figure 9c). In relation to mass at age (Figure 8b), however, Kodiak Island seals essentially fell on the overall regression line, while Kenai Peninsula and Gulf of Alaska seals were slightly underweight for their age. Kenai Peninsula males were also significantly less massive at body volume (Figure 9d) than Kodiak Island males ( $F_{(2,32)} = 6.272, P = 0.004$ ), but there were no other significant pairwise comparisons. This index did not reflect differences in body composition among the three locations (Figure 8a).

In contrast to males, females did not differ significantly in body mass ( $F_{(2,22)} = 0.217, P = 0.806$ ;  $L^3$  as covariate to remove size variation), or sculp and core mass ( $F_{(2,23)} = 0.130, P = 0.878$ ) among the three locations (Figure 10a). There were also no differences among mass at age ( $F_{(2,23)} = 2.352, P = 0.118$ ) or at length ( $F_{(2,23)} = 0.028, P = 0.973$ ; Figure 10b). There was a weak trend of Kodiak Island females to be longer at age than Gulf of Alaska females ( $F_{(2,23)} = 3.032, P = 0.065$ ; Figure 10c), but there was no girth at age difference ( $F_{(2,23)} = 1.957, P = 0.164$ ). As with males, however, the age composition of females from Kodiak Island (median = 14 years) was significantly older (Kruskal-Wallis test statistic = 6.976,  $P = 0.031$ ) than that of females from the Kenai Peninsula (median age = 6.5 years) and Gulf of Alaska (median age = 8 years).

Reflecting the lack of condition differences among the three regions, condition indices (Figure 11a-d) did not show significant differences either (LMD Index  $F_{(2,23)} = 1.025, P = 0.375$ ; G/L  $F_{(2,23)} = 0.257, P = 0.775$ ; M/L  $F_{(2,23)} = 2.815, P = 0.081$ ;

mass at volume residual  $F_{(2,23)} = 0.694, P = 0.510$ ). Mass at length index (Figure 11c) for Kodiak Island showed a slightly heavier at length trend than the other two sites.

#### *Blubber distribution and body thickness*

Mean fineness ratios calculated for live-captured seals were  $4.0 \pm 0.02$  ( $n = 202$ ). Fineness ratio did not differ significantly among sex ( $F_{(1,191)} = 0.265, P = 0.607$ ), age class ( $F_{(3,191)} = 2.238, P = 0.085$ ), or year ( $F_{(2,191)} = 0.441, P = 0.644$ ), but differed significantly among seasons ( $F_{(2,191)} = 15.89, P < 0.001$ ) and regions ( $F_{(2,191)} = 8.16, P < 0.001$ ). Maximum diameter among these seals scaled with length as  $L^{0.88}$  ( $r^2 = 0.703, P < 0.001$ ), and was at 50% of the curvilinear length (Figure 1). However, if lengths adjusted to postmortem measurements were used, overall scaling changed to  $L^{0.95}$  ( $r^2 = 0.704, P < 0.001$ ) which was not significantly different than  $L^{1.0}$ . Fineness ratio with adjusted lengths was significantly greater at  $4.3 \pm 0.023$  ( $t_s = -100.0, P < 0.001$ ).

Girths were not uniformly variable along the body (Figure 12), but were significantly and highly correlated for both sexes (Tables 3, 4). Female girths were more variable than male girths posterior to the maximum diameter, at 50% of length (Figure 12). Anterior to the maximum body diameter, neck girths were most variable in both sexes. Within the range of this data, head extension did not account for this variability. Neck girths standardized for size by the axillary to total curvilinear length were weakly and positively correlated with similarly standardized ear to shoulder distance ( $r = 0.202, P = 0.016, n = 143$ ). If neck girth variability had been related to changes in head extension, there would have been a negative relationship between these two variables, as compressed distances between ear and shoulder, relative to overall body size, should have produced larger neck girths. Degree of girth correlation generally declined as distances between girth rings increased (Tables 3, 4). Girth measurements, however, were weakly correlated with underlying dorsal or lateral blubber thickness (Tables 5, 6). No dorsal blubber thickness measurements were significantly correlated with girth among males (Table 5), though there were moderate, significant correlations for females (Table 6). An

example of one of these relationships is presented in Figure 13. Two distinct patterns between dorsal blubber thickness and girth were evident, though these were unrelated to gender ( $F_{(1,90)} = 0.076$ ,  $P = 0.784$ ), age class ( $F_{(3,90)} = 0.475$ ,  $P = 0.700$ ), season ( $F_{(1,90)} = 0.054$ ,  $P = 0.816$ ), region ( $F_{(2,90)} = 0.028$ ,  $P = 0.972$ ), or year of capture ( $F_{(2,90)} = 1.632$ ,  $P = 0.201$ ). Thus, there is occasionally excellent correspondence between these two parameters, but it is apparently unpredictable in occurrence.

Mean absolute blubber thickness for females was thinnest at the lateral shoulder girth site ( $1.9 \pm 0.1$  cm,  $n = 25$ ) and thickest at the lateral axillary girth site ( $2.8 \pm 0.2$  cm,  $n = 24$ ). Mean absolute blubber thickness in males was thinnest at the lateral shoulder girth site ( $2.0 \pm 0.1$  cm,  $n = 43$ ), and thickest at the lateral maximum girth site ( $3.1 \pm 0.1$  cm,  $n = 63$ ). Neither the mean absolute minimum or maximum blubber thickness was significantly different between sexes (minimum  $t_s = -0.776$ ,  $df = 66$ ,  $P = 0.440$ ; maximum  $t_s = -1.194$ ,  $df = 84$ ,  $P = 0.236$ ). Blubber depths relative to body thickness were greatest in neck and hip regions of both sexes, and lateral blubber was thicker than dorsal blubber near the body midpoint (30-60% of  $L_c$ ) (Figures 14, 15). Blubber thickness variability was fairly uniform among all dorsal and lateral sites, but most variable for males at the dorsal maximal girth site, and at the dorsal neck for females (Table 7). When dorsal and lateral blubber depths were combined, the most variable sites for females were found anterior to the axillary girth for both absolute and relative blubber thicknesses (Table 8). Conversely, males were most variable in mean absolute and relative blubber thickness in the mid 30-60% of the body (Table 8). Seasonal declines (spring to autumn) in blubber thickness (absolute and relative to body radius) were, overall, greater for females than males, and varied depending upon girth site (Figure 16). The least amount of seasonal change occurred in the maximum or midtrunk girth region, 50-62% of curvilinear length.

Sculp content calculated by the truncated cone method was best correlated with mean absolute and relative blubber thickness measured at the maximum body girth site in both sexes (Tables 9, 10). The level of correlation was higher than between XBT and

sculp content (Tables 1, 2). Calculations of the LMD index using the most variable blubber depth location, as recommended by Ryg et al. (1990), resulted in only moderate prediction of sculp content for males (with dorsal blubber thickness at maximum girth site;  $r^2 = 0.525$ ,  $F_{(1,38)} = 44.11$ ,  $P < 0.001$ ). This relationship was slightly improved if the mean of dorsal and lateral blubber thickness from this site was used ( $r^2 = 0.568$ ,  $F_{(1,36)} = 49.61$ ,  $P < 0.001$ ). For females, the LMD index calculated using dorsal neck blubber thickness was a poor predictor of sculp content ( $r^2 = 0.160$ ,  $F_{(1,14)} = 3.85$ ,  $P = 0.070$ ), and using the maximum girth site blubber depths generated weaker relationships (for mean blubber depth;  $r^2 = 0.398$ ,  $F_{(1,23)} = 16.87$ ,  $P < 0.001$ ) than found in males. Mean blubber thickness at the maximum girth site alone was a better predictor of sculp content for females ( $r^2 = 0.558$ ,  $F_{(1,23)} = 31.31$ ,  $P < 0.001$ ) than for males ( $r^2 = 0.505$ ,  $F_{(1,38)} = 40.72$ ,  $P < 0.001$ ). By taking the mean of 5 blubber measurements from any combination of locations, slightly better predictions of blubber content were obtained for males ( $r^2 = 0.645$ ,  $F_{(1,41)} = 77.26$ ,  $P < 0.001$ ) and females ( $r^2 = 0.610$ ,  $F_{(1,24)} = 40.16$ ,  $P < 0.001$ ). The sensitivity of this index was such that a 1 mm change in mean blubber thickness was equivalent to a 0.8% change in blubber content.

## DISCUSSION

### *Scaling*

Phocid body shape and blubber distribution reflect energetic, thermal and hydrodynamic demands, as well as geometric limitations. Mammals living in a buoyant aquatic medium do not have support constraints to body shape scaling that are found in terrestrial mammals (Economos 1983), yet geometric scaling (mass  $\propto$  length<sup>3</sup>, maximum diameter (D)  $\propto$  L<sup>1.0</sup>) seems to be prevalent among terrestrial and aquatic animal species, with empirically determined scalings of  $M \propto L^{2.95}$  (Economos 1983; Niklas 1994). Absolute harbor seal mass at length was affected by whether measurements were taken from living or postmortem seals, but this did not affect the proportionality, which was



consistent with geometric scaling. Mass to length scaling of harbor seals in this study ( $L^{2.9-3.1}$ ) was similar to that found for harp, grey, bearded and harbor seals ( $L^{3.1}$ ; Innes et al. 1990), ringed seals ( $L^{2.86-2.97}$ ; Usher and Church 1969), and northern elephant seals ( $L^{3.0}$ ; *Mirounga angustirostris*; Haley et al. 1991). One assumption in comparing mass and size of geometrically similar objects is that body densities are nearly  $1 \text{ g/mL}^3$ , and uniform throughout the body (Niklas 1994). Male and female harbor seal masses scaled differently with length, but in a manner consistent with an overall 4% greater sculp content of females, which would therefore be less dense. Variability in body composition may also explain slight differences among or within species.

Size and scaling relationships affect interpretations of morphometric condition measures. Because of the geometric scaling of mass with length, and the relationship of  $M_b$  and  $L$  to age, indices based on  $M_b/L$  will always suggest that longer or older seals will be in better condition than younger seals. Thus, although this index is highly correlated with sculp mass (this study) and moderately correlated with total body lipids (Arnould 1995), it is not age-independent and condition differences could be inferred if comparisons were performed between seals or groups of seals with different ages or age structures. This was evident in the regional comparisons (Figures 8-11), where the Kodiak Island seal group had a much older age structure than the other two locations. Mass/length index suggested relatively 'better' condition of Kodiak Island seals, yet while this seemed to be an accurate assessment for males, it was not for females. Because of an isometric relationship, similar problems do not exist for  $G/L$  indices, nor indices utilizing  $L^3$  which normalize the  $M_b/L$  relationship.

Sculp and core masses scaled isometrically with body mass, typical for most animals (Schmidt-Nielsen 1984). Likewise, scaling of sculp with core, or lean body mass, was similar to that found in 28 orders of terrestrial Carnivora by Pond and Ramsay (1992). Though Pond and Ramsay (1992) did not directly present scaling of whole-body adipose stores, the combination of superficial adipose tissue ( $M^{0.743}$ ) and intra-abdominal adipose

tissue ( $M^{1.014}$ ) mass scalings ( $M_c^{0.879}$ ) was similar to the  $M_c^{0.823}$  found for harbor seals. Thus, though harbor seal blubber (subcutaneous adipose tissue) stores increased with core mass at a greater rate than those of terrestrial carnivores, if it is assumed that these are essentially equivalent to whole-body adipose tissue (Beck et al. 1993; Gales and Renouf 1994), there was otherwise no difference in adipose tissue scaling between harbor seals and terrestrial carnivorous mammals.

Harbor seal maximum diameter scaling was similar to the  $D \propto L^{0.98}$  found by Niklas (1994) for 67 animal species. Fineness ratios were at the optimum value for minimizing surface drag (4.5) and volume drag (4.2; Hertel 1966), similar to FR of 3.8-4.6 measured for three captive harbor seals (Williams and Kooyman 1985) and equivalent to Weddell seal (*Leptonychotes weddelli*) FR of  $4.0 \pm 0.5$  (Castellini and Kooyman 1990). Location of maximum diameter at 50% of the body length was consistent with other pinniped studies (Aleyev 1977; Innes 1984), and similar to streamlined laminar profiles of technical bodies of rotation (Hertel 1966). Maintenance of a streamlined profile may influence how blubber is distributed (Ryg et al. 1990; Beck and Smith 1995). Maximum girth in harbor seals was the least variable girth site in males, and among the least in females (Figure 12), suggesting that maximum cross-sectional areas were preferentially maintained near the optimal FR. This is even more striking considering that this site was one of the most variable in blubber thickness relative to body radius for males (Table 7,8). Adjustments to FR could be made by neck extensions or retraction (Fish 1993). However, the neck (males) or shoulder (females) were the most seasonally variable blubber depth sites, indicating that profile maintenance did not limit blubber plasticity.

An 11 cm decrease in length of live-measured seals relative to postmortem measurements greatly complicates condition comparisons between studies that use different collection methods. This postmortem extension probably arises from overall muscular relaxation contributing to spinal extension, and also to extension of the thoracic region, which during life is often held contracted with the vertebral column in a sigmoidal

shape (King 1964). Though it is compelling to apply a 'correction factor', there may be a considerable difference in measurement precision between the two techniques. Length measurements of dead seals are performed with the seal lying ventral or dorsal side up, in a 'relaxed' state, not overly extended or compressed (Scheffer 1967). Consequently, length measurements should have a high degree of precision, though some variability may occur depending upon the state of rigor (Pitcher 1986). Measurements of live seals are taken with the seal anesthetized, manually restrained, or confined by a restraint device. Not only does this generate variability through differences in neck extension or other movements, but also through observer reading of measuring tapes. Fadely and Castellini (1996) found an absolute measurement error of  $2.9 \pm 2.6$  cm ( $n = 34$ ) over a harbor seal size range of 88-158 cm, from which observers recorded 2-3 independent standard length measures. This error was found to be independent of seal length over that range (Fadely and Castellini 1996). Thus, any correction factor derived from the methodology followed in this study to detect a difference between the two groups would likely also be biased. A carefully constructed study is required to determine an appropriate correction factor.

#### *Morphometric measures and condition prediction*

Condition indices based on length and girth were generally poorly correlated with any condition measure for either sex. Some of this may be attributed to variability within regressions used for condition determination relative to age. However, this poor performance reiterates the findings of Pitcher (1986), and was consistent with findings for harp (Ryg et al. 1990; Beck et al. 1993; Gales and Renouf 1994), ringed (Ryg et al. 1990), and grey seals (Ryg et al. 1990).

Poor performance of G/L has been suggested to result from errors in measurement of sculp mass or the commencement of rigor (Pitcher 1986). However, I have shown that girth measurements were poorly correlated with underlying blubber thickness, and that correlations among girths or blubber thickness were only moderate. Therefore, the assumptions underlying G/L, that girth changes from a single location scaled for size

reflect blubber thickness and mass changes over the body, are incorrect. Length and girth can be eliminated mathematically from models predicting blubber or sculp content. For example, male body mass related to LG (Equation 14) can be rewritten as  $38.5(LG)^{1.47}$ , and sculp mass (Equation 18) as  $16.3(LG)^{1.46}$ . Dividing to find sculp as a proportion of body mass gives  $0.42(LG)^{-0.01}$ , agreeing with direct regressions of sculp and body mass (Figure 3) that sculp mass is isometrically related to body mass. Similar manipulations for females resulted in  $0.47(LG)^{-0.08}$ .

Burns and Gol'tsev (1984) presented G/L and sculp content data for harbor seals collected from the Aleutian, Pribilof and Commander Islands during 1968 and 1973/74. Statistical comparison of sculp percentage and G/L from their Table 4 shows that, while there was an overall correlation of G/L with sculp percentage ( $r = 0.812$ ,  $P = 0.008$ ,  $n = 9$ ) among means grouped by age, there was no relationship for seals  $\geq 2$  years old ( $r = 0.029$ ,  $P = 0.971$ ,  $n = 4$ ), but a strong relationship for seals  $< 2$  years old ( $r = 0.940$ ,  $P = 0.017$ ,  $n = 5$ ). When data from 1972-78 were broken into similar groups, relationships were significant but weaker ( $\geq 2$  years old  $r = 0.267$ ,  $P < 0.001$ ,  $n = 349$ ;  $< 2$  years old  $r = 0.435$ ,  $P = 0.001$ ,  $n = 57$ ). Thus it seems that in harbor seals G/L may occasionally reflect proportions of sculp and blubber accurately, but there was seemingly no consistent relationship with age, season or location for when this might occur. Resolution of appropriate times to utilize G/L will require tests with whole-body blubber distribution data.

Though length and girth were poor condition predictors, they provided excellent mass prediction relationships (Equations 13-16). Predictive relationships for mass from a  $LG^2$  volume index (Hofman 1975) have also been determined for Weddell seals (Hofman 1975, Castellini and Kooyman 1990), Steller sea lions (Castellini and Calkins 1993), Australian fur seals (Pemberton et al. 1993), and northern elephant seals (Haley et al. 1991). In each study, volume index mass prediction regressions were highly significant, accounting for up to 98% of the variation in mass (this study). This result would not

occur if body densities varied greatly. Harbor seal blubber density was found to be 0.95 g/mL (Chapter 4, this dissertation), and core tissue density is approximately 1.1 g/mL (Nordøy and Blix 1985). Since changes in sculp and core mass tend to occur simultaneously in response to energy demands in harbor seals (Bowen et al. 1992; Markussen et al. 1992; Muelbert and Bowen 1993), whole body density would not change greatly, and mass predictor equations would therefore be relatively insensitive to changes in body composition. For example, the range of sculp contents used for condition index testing in this study from the Pitcher and Calkins (1983) and Pitcher (1986) data was essentially 20-50%. Subtracting 6% skin mass (Pitcher and Calkins 1983) results in a blubber content of 14-44%, with core content of 80-50%, respectively. Ignoring bone density and lung inflation, calculated body densities based on these compositions were 1.09 g/mL<sup>3</sup> (at 50% blubber) to 1.14 g/mL<sup>3</sup> (at 20% blubber). Because of the model assumptions these should not be considered estimates of true seal densities, but they do illustrate that over the maximum range of field-recorded composition of 20-50% blubber content, body density only changes by 4%. Seasonal changes of male seals collected from Prince William Sound during 1975 (Figure 4) of 24% (summer) to 40% (winter) sculp content results in a body density difference of 2.6%. This was similar to the conclusions of Haley et al. (1991), who calculated that compositional changes accounted for 3% of the error in morphometric mass prediction in northern elephant seals. This is within the error expected from the precision of standard length and axillary girth measurements determined for live-measured harbor seals (Fadely and Castellini 1996). Fadely and Castellini (1996) found that based only on length or girth measurement precision, a 3-10% error could be expected in a density index. Thus, when using a density index, any true change in condition could not confidently be differentiated from measurement error.

Summer decreases and winter increases of sculp content, as well as increased sculp content, mass and age of Kodiak Island seals relative to other sites were previously noted by Pitcher and Calkins (1983) and Pitcher (1986). These seasonal and location population

contrasts performed here illustrate how using condition indices can go wrong and right in these types of comparisons. The main point is that for harbor seals, morphometric indices may or may not accurately reflect true condition, but which occurs is not yet predictable. The differences in mean sculp content between these groups were much less than the range used in the overall correlation contrasts of Tables 1 and 2. Thus, as stated by McLaren and Smith (1985), classic morphometric indices may be relatively poor indicators of condition unless a seal is extremely nutritionally compromised, in which case statistical analyses become unnecessary for discrimination.

#### *Blubber distribution and condition estimation*

Patterns of blubber distribution and variation among free-ranging harbor seals were similar to those found within individual captive seals (Rosen and Renouf 1997), and these both differed from patterns determined for harp (Gales and Renouf 1994; Beck and Smith 1995), ringed (Ryg et al. 1988) and southern elephant (Slip et al. 1992) seals. The most notable difference was that harbor seal blubber was relatively thicker and more variable in the anterior 20-29% of the body, consistent with greater girth variability at the neck site. Though female harbor seals were most variable in girth within the rear 50% of the body, presumably due to changes associated with gestation, seasonal depletion of blubber occurred near the axillary area. Male harbor seals were more similar to patterns of blubber distribution found in ringed and harp seals, where blubber is thickest and most variable near 50-60% of the body length (Ryg et al. 1988; Beck and Smith 1995). The differential patterns of blubber distribution and seasonal change between sexes preclude the use of a single morphometric body composition index.

It is curious that at times there was seemingly excellent correspondence between girth measurements and underlying blubber thickness, and at other times no correlation at all. Further longitudinal studies of captive seals may provide a perspective on this phenomenon, which may be related to the energy status of the seal. In both sexes, changes in sculp mass were associated with changes in core mass. This is consistent with

maintenance of relative blubber thickness for insulation (Ryg et al. 1990), and patterns of blubber and core usage for energy requirements during lactation (Bowen et al. 1992) and fasting (Markussen et al. 1992) in harbor seals.

Rosen and Renouf (1997) hypothesized these differences in topographical blubber distribution may be physiological/anatomical adaptations related to the degree of seasonal food availability associated with the latitudinal differences in habitat of the different seal species measured. As they point out, too few studies of this type have been performed to fully test this hypothesis. However, another possible contributing factor to these differences may be in the different measurement techniques utilized. Both the harp (Beck et al. 1993; Beck and Smith 1995) and ringed (Ryg et al. 1988) seal studies measured blubber thickness on blubber removed completely from the carcass and laid flat for blubber depth measurements. Conversely, both harbor seal studies (Rosen and Renouf 1997; this study) utilized ultrasonic blubber depth determinations performed upon live subjects. This adds at least two additional sources of error. First, extensions of the thoracic region may contribute to the variability of girth and blubber thickness measurements. Over the range of sizes measured in this study, no compelling link between neck contraction and increased girth was found. However, since this was compared within a set of live animals, there may not have been sufficient variability to fully evaluate this effect. Conversely, while the neck girths were among the most variable site for both males and females (Figure 12), blubber thickness was most variable in the neck and shoulder regions for females, but males were most variable in the axillary to maximum girth region (Tables 7, 8). Also, blubber thickness in the neck and shoulders were the sites of greatest seasonal change in males, and the shoulder site showed the greatest seasonal change for females (Figure 16). Thus, unless there was a seasonal bias to a neck extension effect, it does not seem to account for the greater variability of this region.

A second source of technique differences was found in evidence of fat slumping. This effect was first detected by Slip et al. (1992) measuring blubber thickness with

ultrasound in southern elephant seals. They found a significant slumping of the blubber layer when sites were measured perpendicular to the ground, as the lateral sites were in this study. Thus, in spring when harbor seals were fatter relative to autumn, lateral sites at 37-50% of body length (axilla to maximum girth) had greater blubber thickness than dorsal sites (Figures 14, 15). Gales and Renouf (1994) measured blubber thickness of harp seals collected during March-June by direct measure through slits in the skin taken while the seals was positioned with dorsal side up. Aside from a greater blubber thickness of lateral versus dorsal blubber depth at the axilla, there was no evidence of blubber slumping. This was in agreement with Beck and Smith (1995), who found larger blubber depths at dorsal sites posterior to 40% of the body length, but greater lateral depths anterior to this site. Thus, while blubber slumping does occur, there is also evidence for regionally variable blubber depths between dorsal and lateral sites at the same girth rings. Thus, to resolve the apparent differences in blubber distribution between harbor seals and other phocids would require a study directly comparing ultrasonic determinations of blubber depth to determinations made by laying the flensed sculp flat, or performing this latter technique on a group of harbor seals.

Gales and Renouf (1994) found that truncated cone approximations overestimated blubber mass by about 17%. This could be improved with increased numbers of girth and blubber thickness measurements, but with 21 input parameters, this technique is already time-consuming for convenient field use. Another factor influencing the relative insensitivity of morphometric indices may have been the relatively limited scope of body conditions observed in the ultrasonic blubber determination portion of the study. Similar measurements performed on orphaned pups, otherwise noticeably compromised seals, or increased summer sampling would show whether these indices could be used to discriminate seals in diminished nutritional states.

No single measure or combination of measures, including ultrasound blubber depth, girths, lengths, or mass was found to predict sculp content with the precision or



consistency desired in a condition index. This was attributable to a poor correlation between body girth and underlying blubber thickness, to spatially (within the body) variable seasonal blubber utilization patterns, and to spatially heterogeneous variability in blubber depths. This variability was such that a 'best' predictor of blubber content was obtainable by taking the mean of any 5 blubber depths. Unfortunately, about 40% of the variability in blubber content remains to be explained. Also, at a sensitivity of 0.8% sculp content per millimeter of blubber depth, small measurement errors in ultrasound readings could result in condition 'changes' similar in magnitude to some observed seasonal and regional differences. Though sculp content was moderately related to the mean blubber depth at the maximum body thickness alone, or to an LMD index of Ryg et al. (1990), body condition estimates based on sculp mass percentage for harbor seals may be inherently too variable for precise comparisons. This is in contrast to other phocid species such as ringed or harp seals, which have excellent agreement between these factors. Also, in harbor seals less than 2 years old (Burns and Gol'tsev 1984; Bowen et al. 1992) there were high degrees of correlation among morphometric measurements or sculp mass and total body fat.

A final complicating factor in using morphometric estimates of sculp and fat mass is the lipid content of adipose tissue. Bowen et al. (1992) found excellent agreement of total body fat (from deuterated water dilution) and sculp mass (postmortem direct weighing) in pups, but this relationship was much weaker among lactating females. Blubber tissue lipid content has been found to change in response to energy demands in harp (Beck et al. 1993; Gales et al. 1994) and harbor (Bowen et al. 1992; Chapter 4, this dissertation) seals. Thus, accurate assessment of harbor seal condition will require the use of indirect measures of total body fat and body composition, such as isotope dilution (Bowen et al. 1992; Worthy et al. 1992) or bioelectrical impedance analysis (Gales et al. 1994).

Satisfactory assessments of live-seal body condition would be obtainable by collecting mass and age measurements, providing size comparisons standardized for somatic growth. Because mass and length may vary simultaneously with condition at age, and because the precision and accuracy of length measured on live seals is questionable, length measurements may be a poor standard on which to base mass or girth comparisons. On seals measured postmortem, mass, length, age, and blubber thickness measured at the xiphosternal process and between 50-78% of the body length should provide sufficient condition assessment. However, as stated above, a future study should measure blubber thickness on flensed sculps, to verify patterns of variability determined from ultrasound measurements.

#### LITERATURE CITED

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Table 1. Pearson correlation coefficient matrix of absolute and index measures of body condition in male harbor seals. Seals collected during 1972-78 from the Gulf of Alaska ( $n = 207$ ), by Pitcher and Calkins (1983) and Pitcher (1986). Overall Bartlett  $\chi^2 = 14905$  ( $df = 77, P < 0.001$ ). Comparisons of xiphosternal blubber thickness (XBT) and girth/length (G/L) with sculp content and sculp mass were also presented in Pitcher (1986), though not grouped by gender.

Condition index	Sculp Content (%)	Sculp Mass (kg)	$M_b$ at Length Residual	$M_b$ at Age Residual	Length at Age Residual	Girth at Age Residual
Body mass ( $M_b$ )	-0.042	0.916***	0.345***	0.342***	-0.188	0.260*
Standard length (L)	-0.093	0.834***	0.026	0.213	-0.104	0.144
Axillary girth (G)	0.008	0.897***	0.347***	0.342***	-0.194	0.419***
G/L	0.240*	0.261**	0.770***	0.343***	-0.215	0.669***
$M_b/L$	-0.013	0.918***	0.472***	0.376***	-0.253*	0.297***
$\log M_b / \log L$	-0.009	-0.057	-0.034	0.108	0.210	0.097
$M_b$ at L residual	0.184	0.388***		0.420***	-0.386***	0.383***
$M_b/L^2$	0.055	0.850***	0.728***	0.439***	-0.350***	0.367***
$M_b/L^3$	0.196	0.295***	0.994***	0.397***	-0.370***	0.366***
$M_b/LG^2$	-0.058	0.055	0.344***	0.087	-0.237*	-0.439***
$M_b$ at volume index	0.041	0.311***	0.757***	0.298***	-0.373***	-0.090
XBT	0.528***	0.587***	0.459***	0.433***	-0.045	0.474***
LMD index	0.596***	0.052	0.236*	0.260**	0.132	0.355***

\* =  $P < 0.05$ ; \*\* =  $P \leq 0.01$ ; \*\*\* =  $P \leq 0.001$

Table 2. Pearson correlation coefficient matrix of absolute and index measures of body condition in female harbor seals. Seals collected during 1972-78 from the Gulf of Alaska ( $n = 198$ ), by Pitcher and Calkins (1983) and Pitcher (1986). Overall Bartlett  $\chi^2 = 15364$  ( $df = 77, P < 0.001$ ). Comparisons of xiphosternal blubber thickness (XBT) and girth/length (G/L) with sculp content and sculp mass were also presented in Pitcher (1986), though not grouped by gender.

Condition index	Sculp Content (%)	Sculp Mass (kg)	Mass at Length Residual	Mass at Age Residual	Length at Age Residual	Girth at Age Residual
Body mass ( $M_b$ )	-0.144	0.903 <sup>***</sup>	0.534 <sup>***</sup>	0.546 <sup>***</sup>	-0.011	0.426 <sup>***</sup>
Standard length (L)	-0.136	0.816 <sup>***</sup>	0.115	0.293 <sup>**</sup>	0.058	0.205
Axillary girth (G)	-0.014	0.923 <sup>***</sup>	0.503 <sup>***</sup>	0.549 <sup>***</sup>	0.007	0.583 <sup>***</sup>
G/L	0.263 <sup>*</sup>	0.464 <sup>***</sup>	0.805 <sup>***</sup>	0.603 <sup>***</sup>	-0.076	0.808 <sup>***</sup>
$M_b/L$	-0.140	0.891 <sup>***</sup>	0.637 <sup>***</sup>	0.583 <sup>***</sup>	-0.062	0.461 <sup>***</sup>
$\log M_b/\log L$	-0.206	-0.127	-0.093	-0.197	-0.148	-0.168
$M_b$ at length residual	-0.026	0.463 <sup>***</sup>		0.633 <sup>***</sup>	-0.224	0.550 <sup>***</sup>
$M_b/L^2$	-0.118	0.825 <sup>***</sup>	0.809 <sup>***</sup>	0.641 <sup>***</sup>	-0.135	0.522 <sup>***</sup>
$M_b/L^3$	-0.309	0.519 <sup>***</sup>	0.997 <sup>***</sup>	0.647 <sup>***</sup>	-0.219	0.557 <sup>***</sup>
$M_b/LG^2$	-0.436 <sup>***</sup>	0.199	0.478 <sup>***</sup>	0.205	-0.242 <sup>*</sup>	-0.239
$M_b$ at volume index	-0.360 <sup>***</sup>	0.250 <sup>*</sup>	0.698 <sup>***</sup>	0.343 <sup>***</sup>	-0.277 <sup>**</sup>	-0.044
XBT	0.537 <sup>***</sup>	0.641 <sup>***</sup>	0.354 <sup>***</sup>	0.430 <sup>***</sup>	0.068	0.561 <sup>***</sup>
LMD index	0.672 <sup>***</sup>	0.194	0.081	0.174	0.108	0.375 <sup>***</sup>

\* =  $P < 0.05$ ; \*\* =  $P \leq 0.01$ ; \*\*\* =  $P \leq 0.001$

Table 3. Pearson correlation coefficient matrix for girth measurements of male harbor seals collected during 1993-96 from Kodiak Island, Prince William Sound, and southeast Alaska. Overall Bartlett  $\chi^2 = 753.2$  (df = 28,  $P < 0.001$ ). All coefficients were significant at  $P < 0.001$ , and  $n = 60$  for each case.

	Ear	Neck	Shoulder	Axillary	Maximum	Midtrunk	Hip
Neck	0.859						
Shoulder	0.829	0.865					
Axillary	0.872	0.886	0.880				
Maximum	0.878	0.892	0.893	0.979			
Midtrunk	0.827	0.868	0.843	0.945	0.954		
Hip	0.727	0.800	0.830	0.862	0.871	0.887	
Ankle	0.653	0.703	0.785	0.776	0.762	0.729	0.797

Table 4. Pearson correlation coefficient matrix for girth measurements of female harbor seals collected during 1993-96 from Kodiak Island, Prince William Sound, and southeast Alaska. Overall Bartlett  $\chi^2 = 630.3$  (df = 28,  $P < 0.001$ ). All coefficients were significant at  $P < 0.001$ , and  $n = 44$  for each case.

	Ear	Neck	Shoulder	Axillary	Maximum	Midtrunk	Hip
Neck	0.801						
Shoulder	0.807	0.905					
Axillary	0.795	0.902	0.918				
Maximum	0.779	0.886	0.900	0.973			
Midtrunk	0.761	0.862	0.888	0.958	0.990		
Hip	0.724	0.852	0.834	0.913	0.941	0.953	
Ankle	0.743	0.786	0.785	0.832	0.831	0.835	0.878

Table 5. Pearson correlation coefficients ( $r$ ) of girth measurement with underlying dorsal and lateral blubber thickness for male harbor seals. Seals captured from Kodiak Island, Prince William Sound, and southeast Alaska during 1993-96. Sample sizes in parantheses.

Girth Location	Dorsal Blubber Thickness		Lateral Blubber Thickness	
Neck	0.225	(40)	0.218	(33)
Shoulder	0.188	(61)	0.128	(42)
Axillary	0.097	(75)	0.115	(44)
Maximum	0.154	(65)	0.116	(62)
Mid-trunk	0.180	(87)	0.336**	(85)
Hip	0.164	(93)	0.368***	(88)

\*\*  $P = 0.003$ , \*\*\*  $P = 0.001$

Table 6. Pearson correlation coefficients ( $r$ ) of girth measurement with underlying dorsal and lateral blubber thickness for female harbor seals. Seals captured from Kodiak Island, Prince William Sound, and southeast Alaska during 1993-96. Sample sizes in parantheses.

Girth Location	Dorsal Blubber Thickness		Lateral Blubber Thickness	
Neck	0.323	(27)	0.245	(19)
Shoulder	0.563***	(39)	0.539*	(25)
Axillary	0.547***	(40)	0.650***	(24)
Maximum	0.581***	(36)	0.574***	(47)
Mid-trunk	0.649***	(62)	0.609***	(61)
Hip	0.520***	(63)	0.583***	(62)

\*  $P = 0.011$ , \*\*\*  $P \leq 0.001$

Table 7. Variability (coefficients of variation) of relative blubber depths ( $d/r$ ) at harbor seal girth sites. Seals collected from Kodiak Island, Prince William Sound, and southeast Alaska during 1993-96. Sample sizes in parantheses.

Girth Site	Males		Females	
	Dorsal	Lateral	Dorsal	Lateral
Neck	0.280 (40)	0.290 (33)	0.369 (27)	0.313 (19)
Shoulder	0.338 (61)	0.358 (42)	0.337 (39)	0.310 (25)
Axillary	0.439 (75)	0.330 (44)	0.312 (40)	0.211 (24)
Maximum	0.443 (65)	0.341 (62)	0.229 (36)	0.298 (47)
Midtrunk	0.348 (87)	0.267 (85)	0.262 (62)	0.208 (61)
Hip	0.368 (93)	0.274 (88)	0.280 (63)	0.336 (62)

Table 8. Mean absolute (cm) and relative (blubber depth/body radius) blubber depths for harbor seals captured during 1993-96 from Kodiak Island, Prince William Sound, and southeast Alaska.

Girth Site	Males							Females						
	Absolute			Relative				<i>n</i>	Absolute			Relative		
	$\bar{x}$	<i>se</i>	CV	$\bar{x}$	<i>se</i>	CV			$\bar{x}$	<i>se</i>	CV	$\bar{x}$	<i>se</i>	CV
Neck	2.4	0.1	0.25	0.22	0.01	0.26	29	2.5	0.2	0.33	0.25	0.02	0.33	17
Shoulder	2.1	0.1	0.27	0.14	0.01	0.27	37	2.1	0.2	0.39	0.16	0.01	0.35	22
Axillary	2.6	0.2	0.36	0.17	0.01	0.36	37	2.4	0.2	0.29	0.16	0.01	0.20	17
Maximum	2.7	0.1	0.34	0.17	0.01	0.34	60	2.4	0.1	0.25	0.16	0.01	0.17	35
Midtrunk	2.2	0.1	0.23	0.15	0.00	0.23	82	2.3	0.1	0.27	0.16	0.00	0.19	60
Hip	2.4	0.1	0.26	0.20	0.01	0.25	88	2.3	0.1	0.30	0.20	0.01	0.24	62

Table 9. Pearson correlation coefficients of mean absolute and relative (d/r) blubber thickness measurements with truncated-cone estimates of blubber content for male harbor seals. Seals captured during 1994-96 from Kodiak Island, Prince William Sound, or southeast Alaska.

Girth Site	Absolute		Relative		<i>n</i>
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	
Neck	0.640	<0.001	0.722	<0.001	32
Shoulder	0.348	0.134	0.527	<0.001	43
Axillary	0.640	<0.001	0.695	<0.001	43
Maximum	0.719	<0.001	0.755	<0.001	40
Midtrunk	0.423	0.035	0.678	<0.001	41
Hip	0.433	0.022	0.511	<0.001	43

Table 10. Pearson correlations of mean absolute and relative (blubber depth/body radius) blubber thickness measurements with truncated-cone estimates of blubber content for female harbor seals. Seals captured during 1994 -96 from Kodiak Island, Prince William Sound, or southeast Alaska.

Girth Site	Absolute		Relative		<i>n</i>
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	
Neck	0.618	0.008	0.483	0.049	17
Shoulder	0.726	<0.001	0.567	0.003	26
Axillary	0.713	<0.001	0.536	0.006	25
Maximum	0.759	<0.001	0.627	<0.001	25
Midtrunk	0.714	<0.001	0.536	0.005	26
Hip	0.702	<0.001	0.393	0.047	26

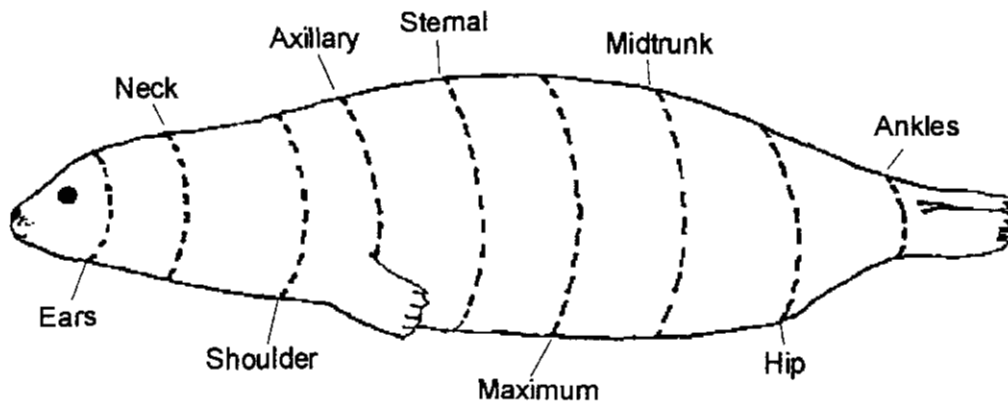


Figure 1. Locations of girth rings measured on harbor seals captured from the Gulf of Alaska during 1993-96. Sternal girth measurements were replaced with maximum girth in 1994. Expressed as a percentage of curvilinear length from the nose, girth rings measured along the body at: ear  $\bar{x} = 0.098 \pm 0.001$ , ( $n = 153$ ); neck  $\bar{x} = 0.203 \pm 0.002$  (180); shoulder  $\bar{x} = 0.285 \pm 0.002$  (170); axillary  $\bar{x} = 0.366 \pm 0.002$  (186); maximum  $\bar{x} = 0.500 \pm 0.004$  (140); midtrunk  $\bar{x} = 0.615 \pm 0.004$  (184); hip  $\bar{x} = 0.777 \pm 0.002$  (186); and ankle  $\bar{x} = 0.907 \pm 0.001$  (172).

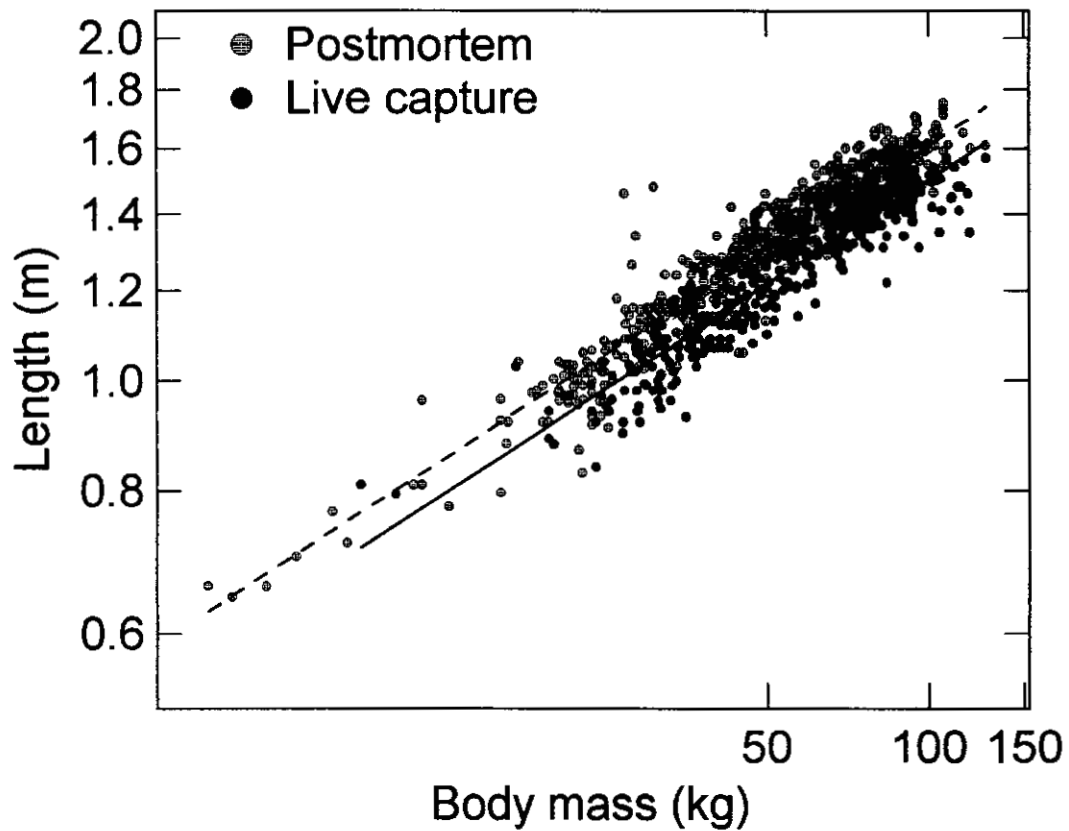


Figure 2. Allometric scaling of dorsal standard length with body mass for harbor seals. Seals were collected from the Gulf of Alaska and Bering Sea during 1972-79, 1985, 1989-90, and 1995-96, or captured from the Gulf of Alaska during 1993-96. Postmortem measurement scaling (dashed line) was  $L = 0.396M_t^{0.31}$  ( $r^2 = 0.894$ ,  $F_{(1,674)} = 5702.5$ ,  $P < 0.001$ ), live capture scaling (solid line)  $L = 0.368M_t^{0.31}$  ( $r^2 = 0.864$ ,  $F_{(1,344)} = 2193.1$ ,  $P < 0.001$ ).



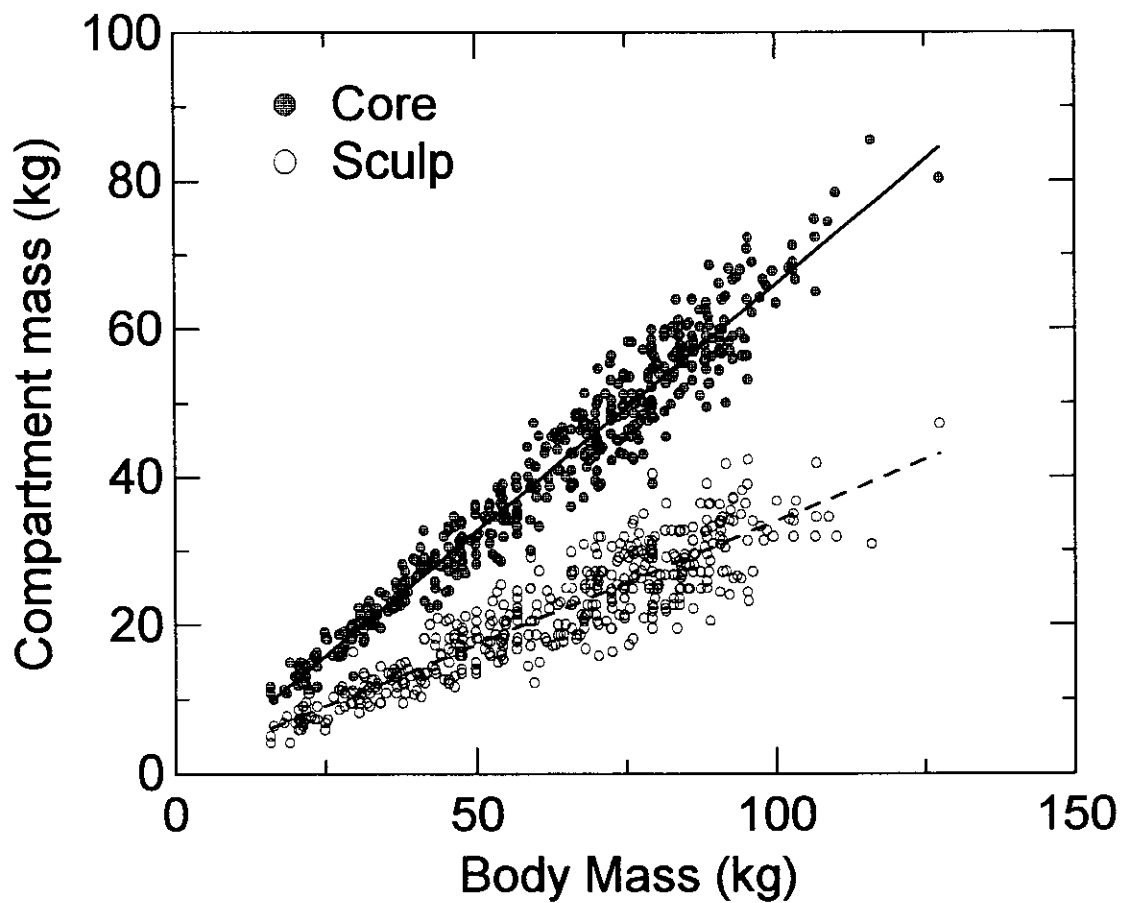


Figure 3. Sculp and core compartment mass scaling with total body mass for harbor seals collected from the Gulf of Alaska, 1972-78, and Bering Sea 1979 and 1985. Regression equations are sculp mass =  $0.385M_t^{0.97}$  ( $r^2 = 0.871$ ,  $P < 0.001$ ,  $n = 450$ ), core mass =  $0.604M_t^{1.02}$  ( $r^2 = 0.963$ ,  $P < 0.001$ ,  $n = 450$ ).

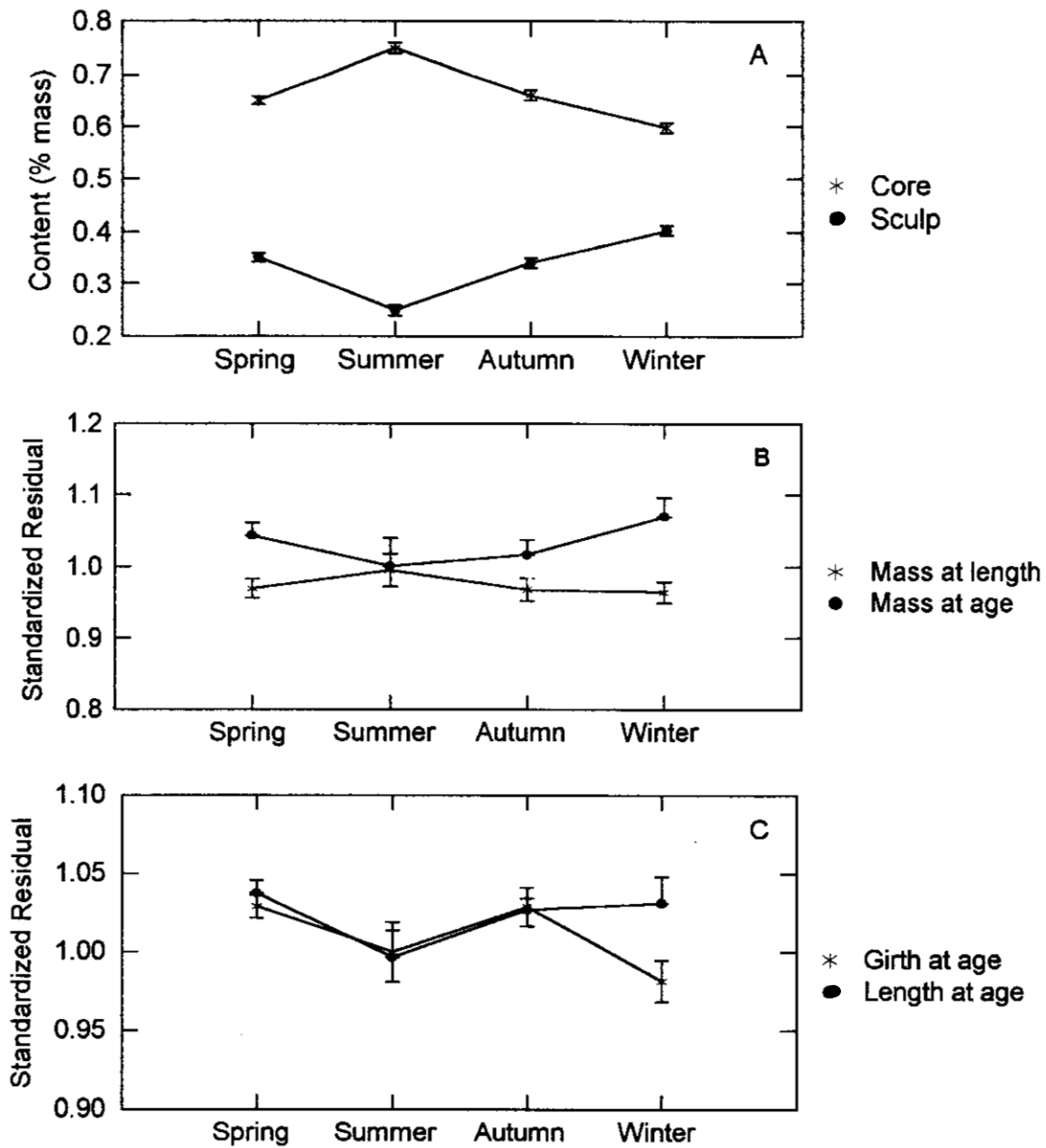


Figure 4. Seasonal condition changes (mean  $\pm$ se) of male harbor seals collected during 1975 from Prince William Sound: a) sculp and core content (relative to body mass); b) observed masses relative to those expected from age or length regressions; and c) observed length and girth relative to at age regressions. Sample sizes: spring 34, summer 12, autumn 13, and winter 14.

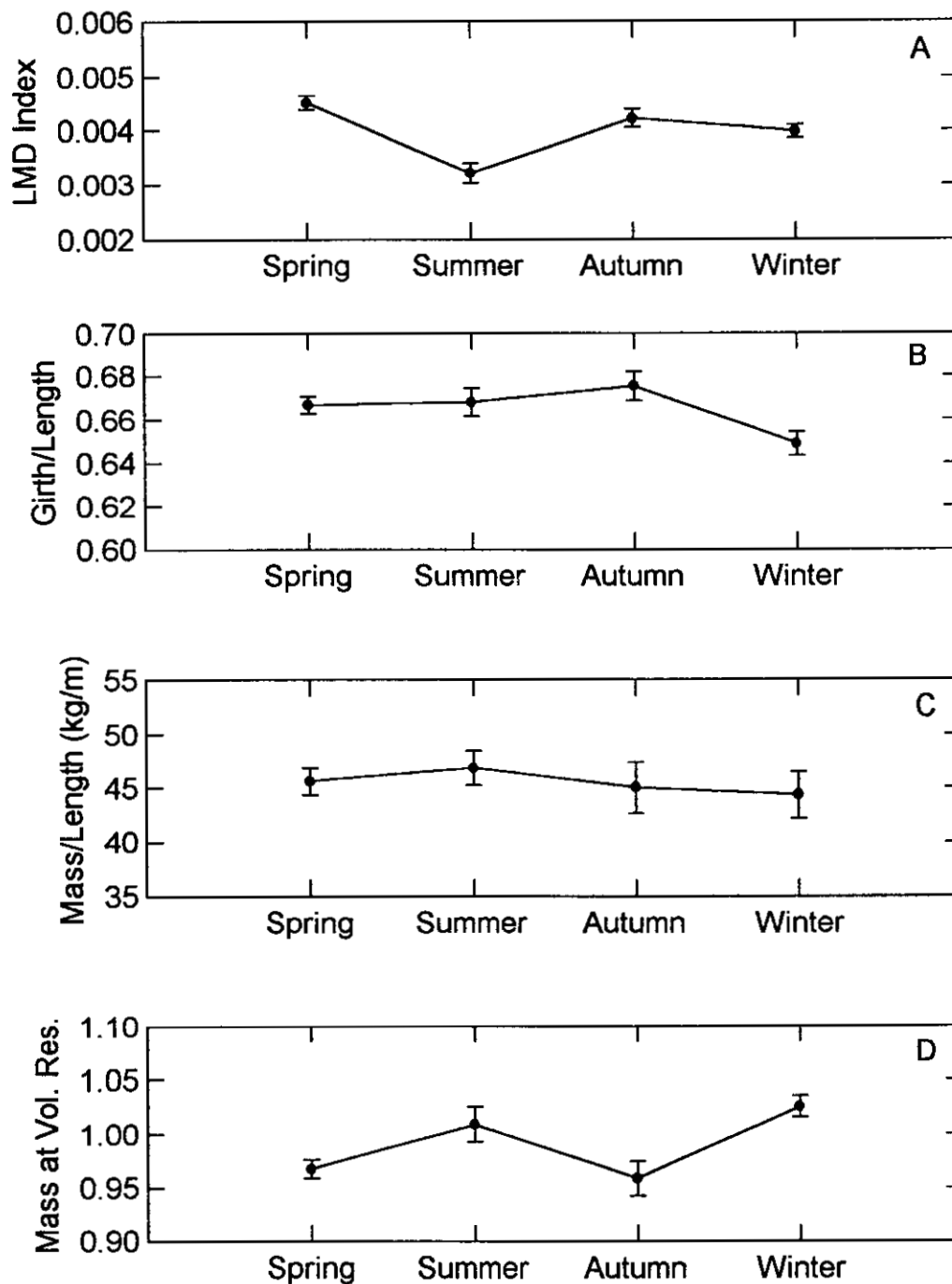


Figure 5. Seasonal condition index changes (mean  $\pm$  se) for male harbor seal collected from Prince William Sound during 1975; a) modified LMD index; b) G/L; c) M/L; d) observed mass relative to mass expected from sex-specific regression (Equation 8). Sample sizes were: spring 34, summer 12, autumn 13, and winter 14.

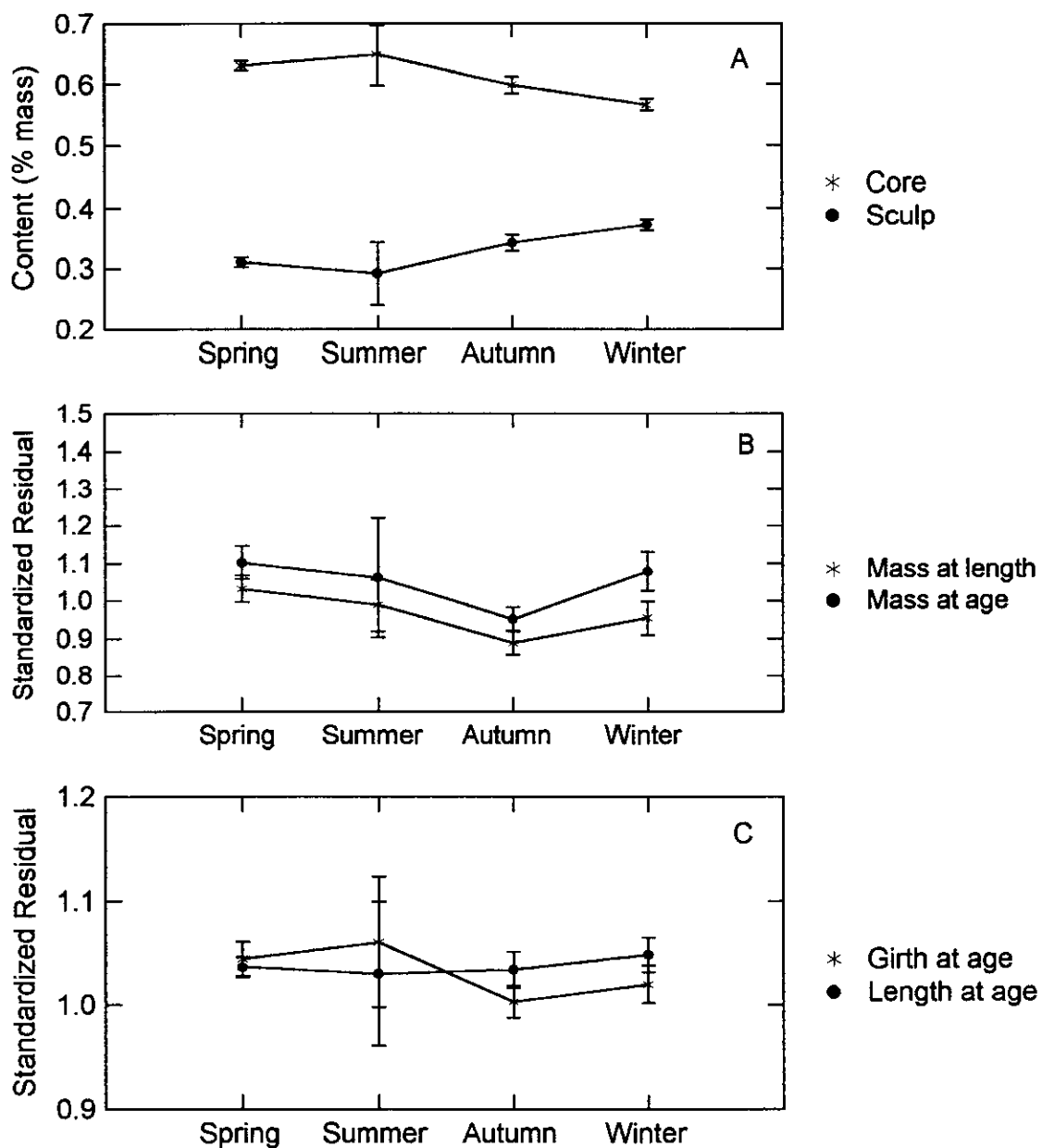


Figure 6. Seasonal condition changes (mean  $\pm$  se) of female harbor seals collected during 1975 from Prince William Sound: a) sculpin and core content (relative to body mass); b) observed mass relative to that expected from age or length regressions; and c) observed length and girth relative to expected from age regressions. Sample sizes: spring 22, summer 3, autumn 9, and winter 7.

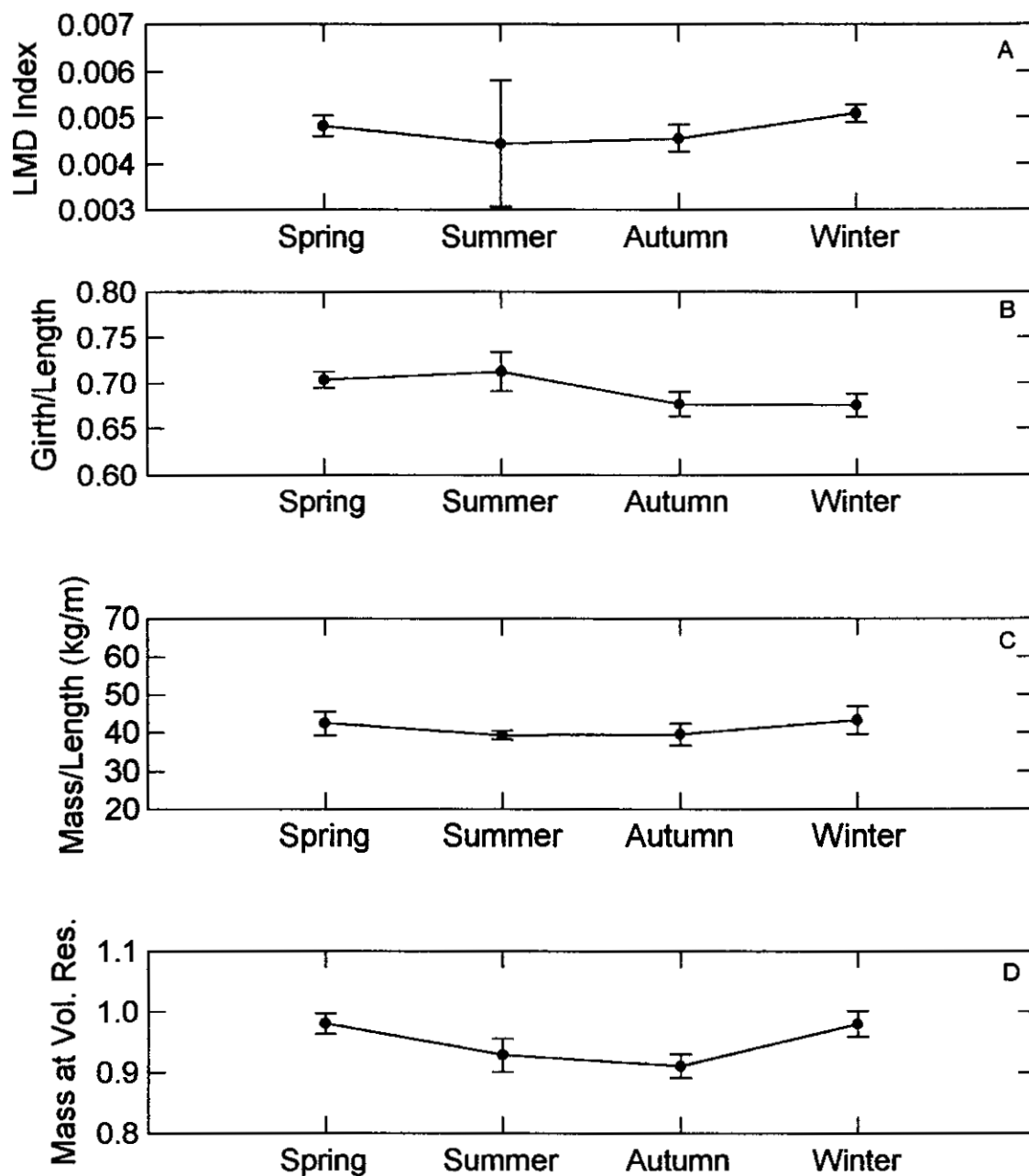


Figure 7. Seasonal condition index changes (mean  $\pm$  se) of female harbor seals collected from Prince William Sound during 1975: a) modified LMD index; b) G/L; c) M/L; d) observed mass relative to mass expected at volume index (Equation 14). Sample sizes were spring 22, summer 6, autumn 10, and winter 9.

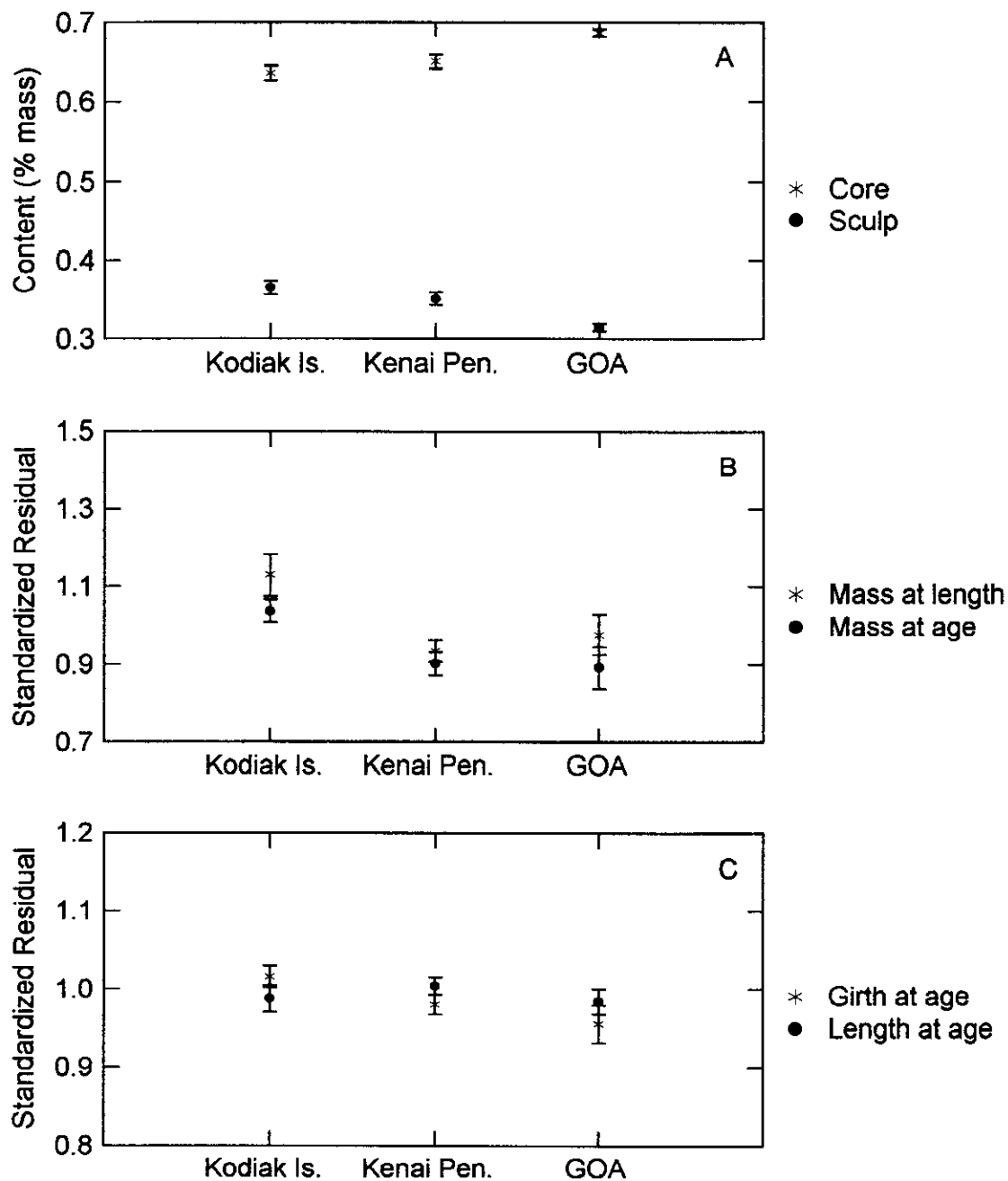


Figure 8. Locational condition differences (mean  $\pm$ se) of male harbor seals collected during spring 1976 from Kodiak Island ( $n = 13$ ), Kenai Peninsula ( $n = 16$ ), and the Gulf of Alaska (GOA;  $n = 6$ ): a) sculp and core content (relative to body mass); b) observed masses relative to those expected from age or length regressions, and; c) observed length and girth relative to at age regressions.

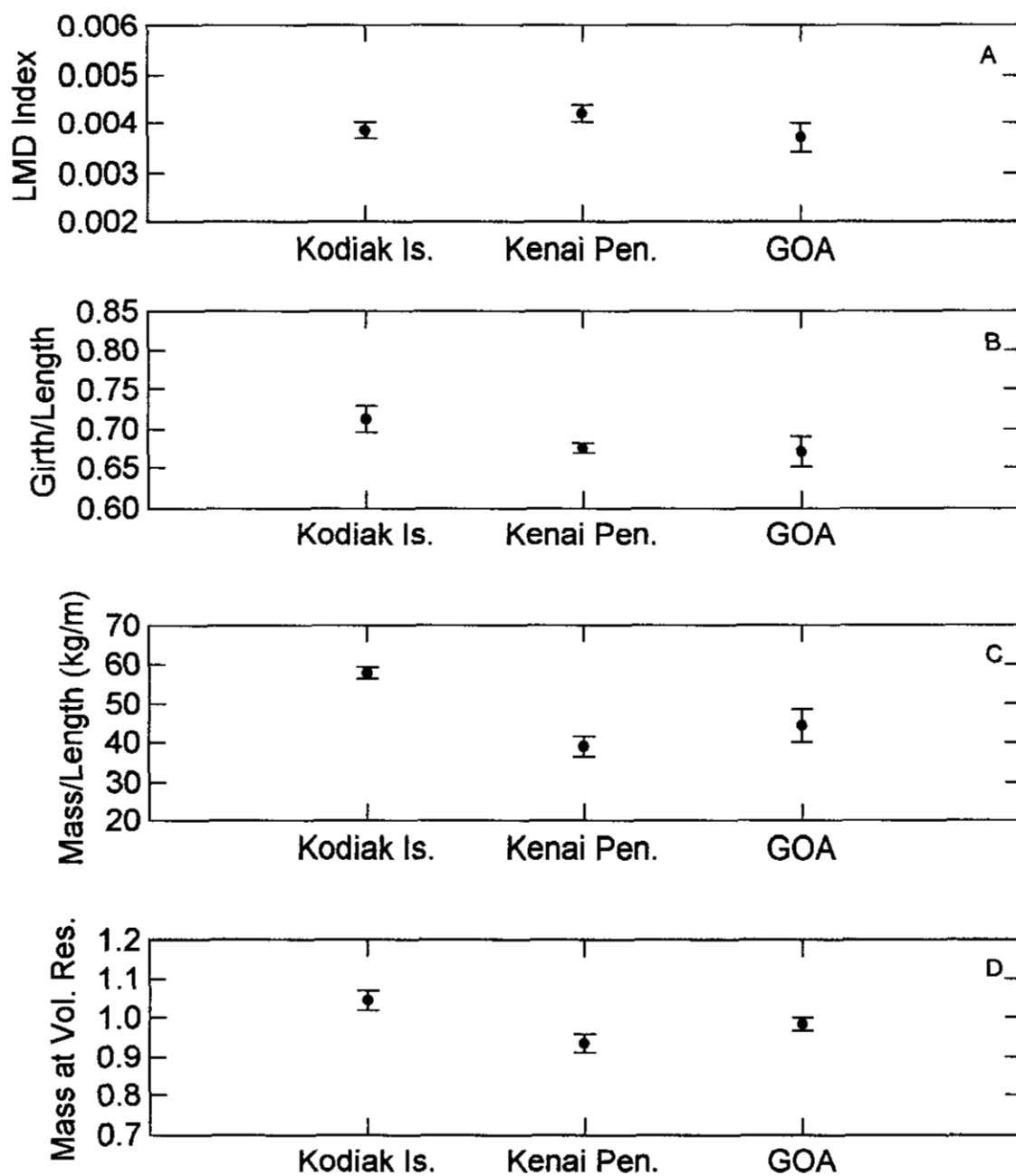


Figure 9. Locational condition index differences (mean  $\pm$  se) for male harbor seals collected during spring 1976 from Kodiak Island ( $n = 13$ ), Kenai Peninsula ( $n = 16$ ), and the Gulf of Alaska (GOA;  $n = 6$ ): a) modified LMD index; b) G/L; c) M/L; d) observed mass relative to mass expected from sex-specific regression (Equation 8).

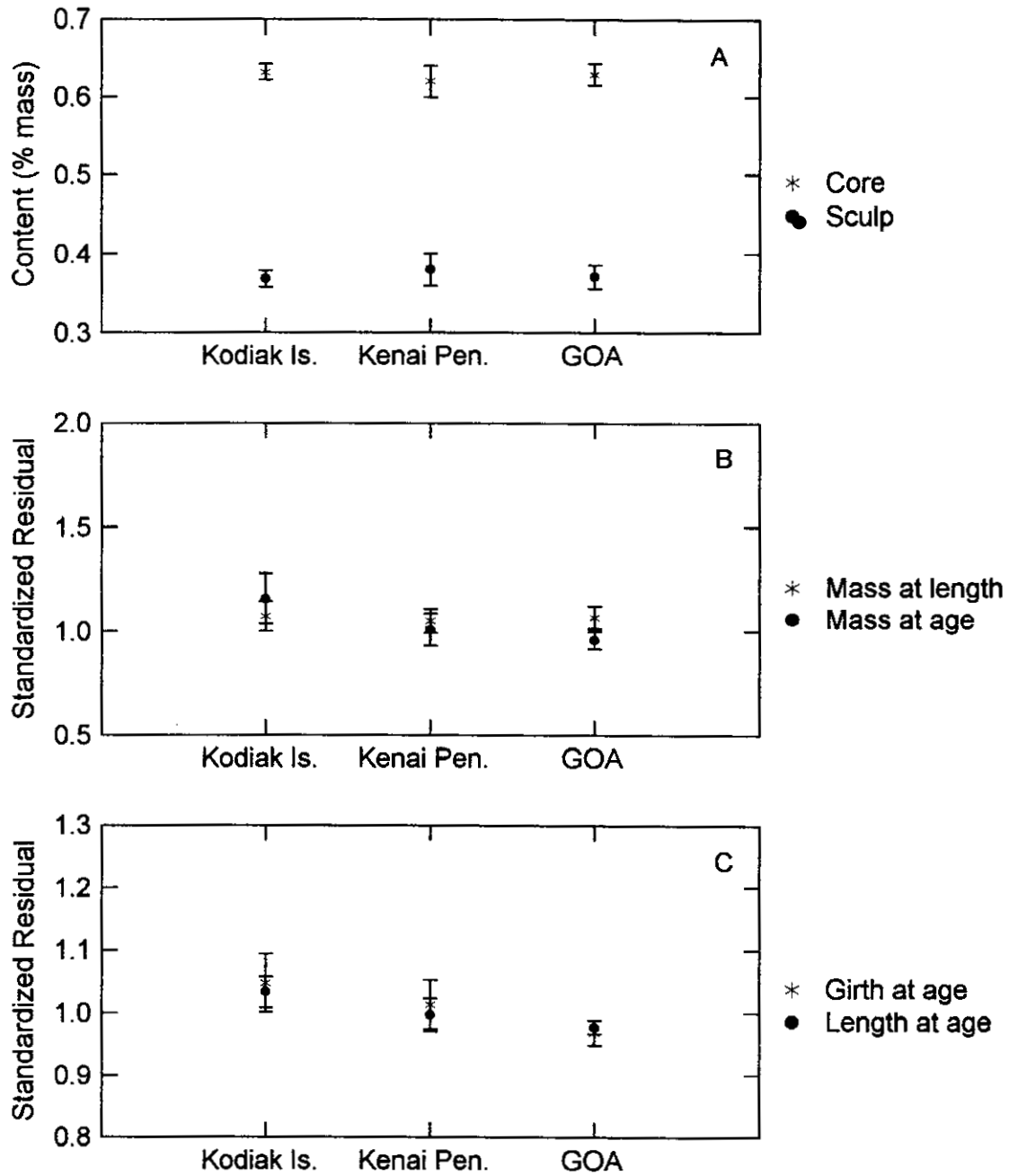


Figure 10. Locational condition differences (mean  $\pm$ se) of female harbor seals collected during spring 1976 from Kodiak Island ( $n = 7$ ), Kenai Peninsula ( $n = 6$ ), and the Gulf of Alaska (GOA;  $n = 13$ ): a) sculp and core content (relative to body mass); b) observed masses relative to those expected from age or length regressions, and; c) observed length and girth relative to at-age regressions.



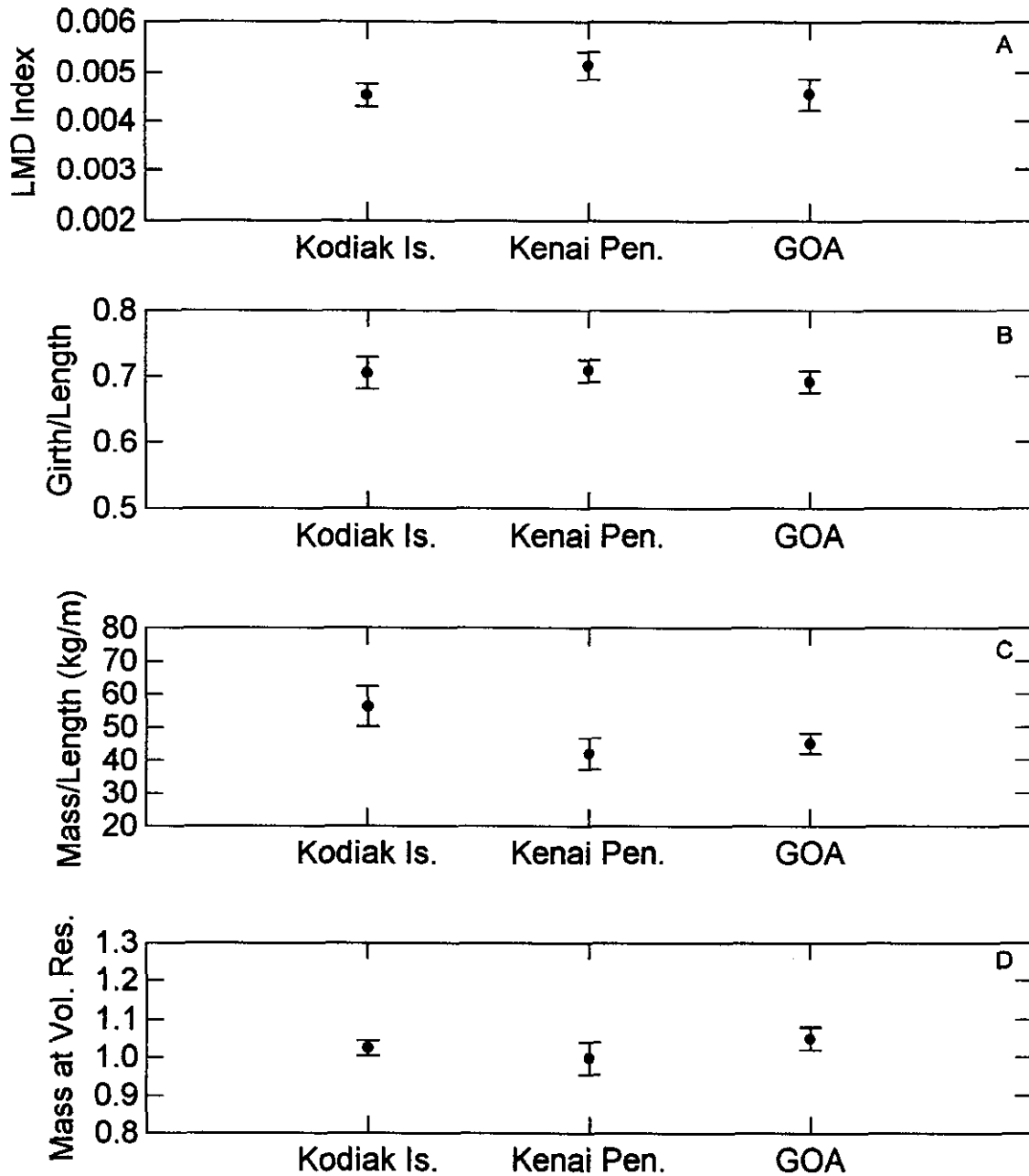


Figure 11. Locational condition index differences (mean  $\pm$  se) for female harbor seals collected during spring 1976 from Kodiak Island ( $n = 7$ ), Kenai Peninsula ( $n = 6$ ), and the Gulf of Alaska (GOA;  $n = 13$ ): a) modified LMD index; b) G/L; c) M/L; d) observed mass relative to mass expected from sex-specific regression (Equation 8).

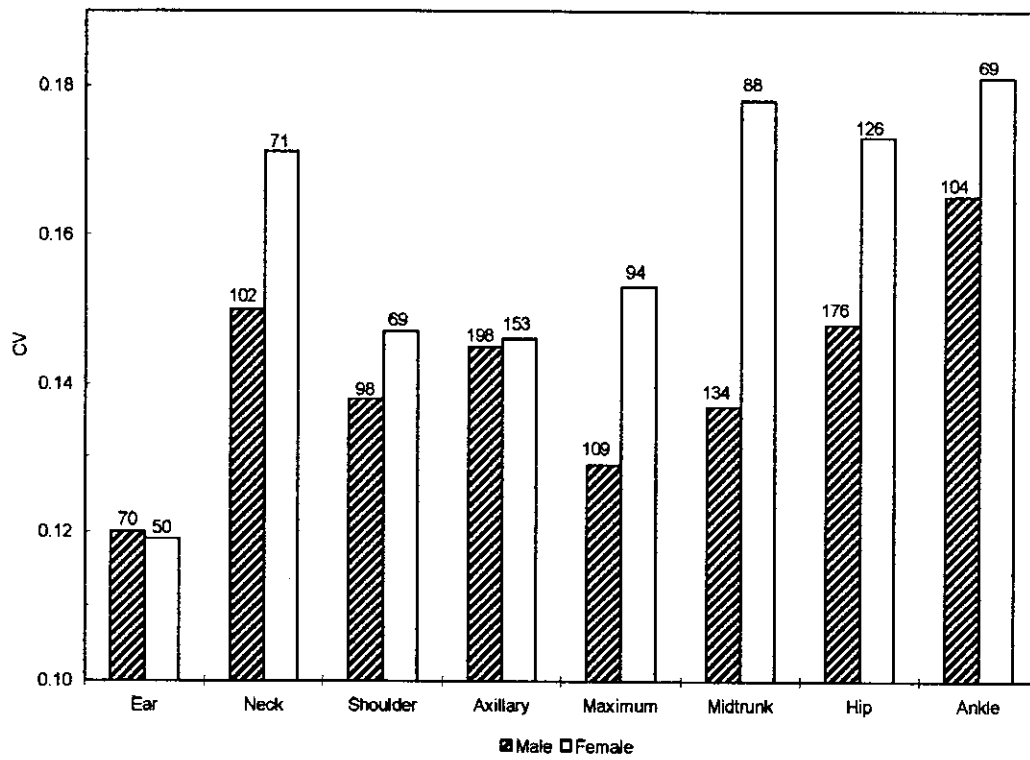


Figure 12. Girth coefficients of variation (CV) for harbor seals collected during 1993-96, from Kodiak Island, Prince William Sound, and southeast Alaska. Sample sizes listed above bars.

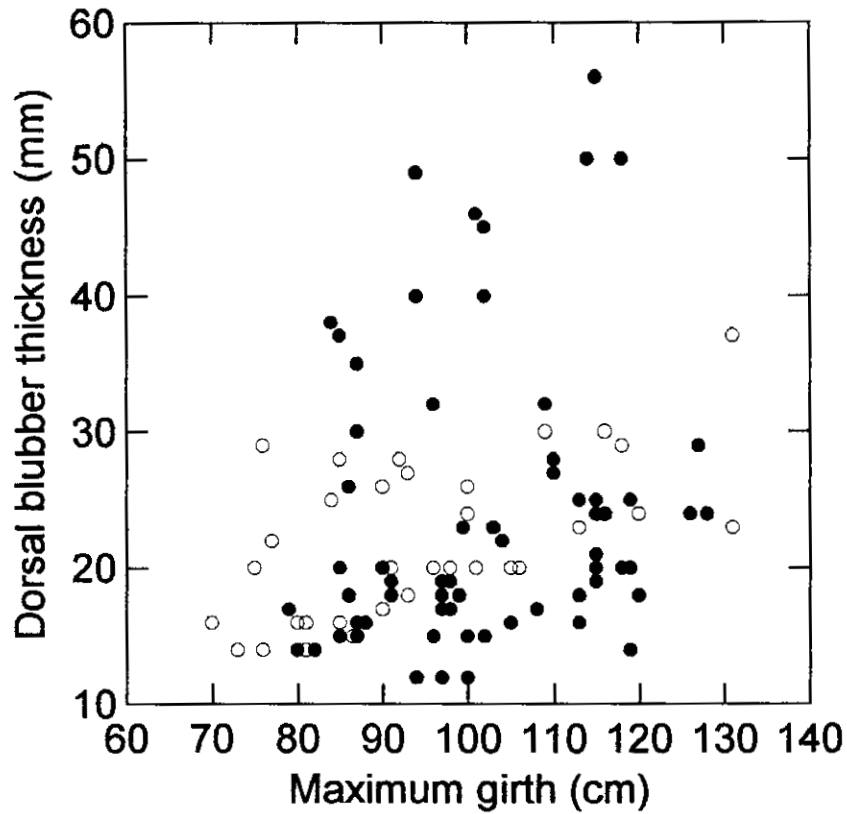


Figure 13. Relationship between maximum girth and dorsal blubber thickness for male (●) and female (○) harbor seals collected from the Gulf of Alaska during 1993-96. The two groupings in the data were not explained by age, gender, season, region, or year of capture.

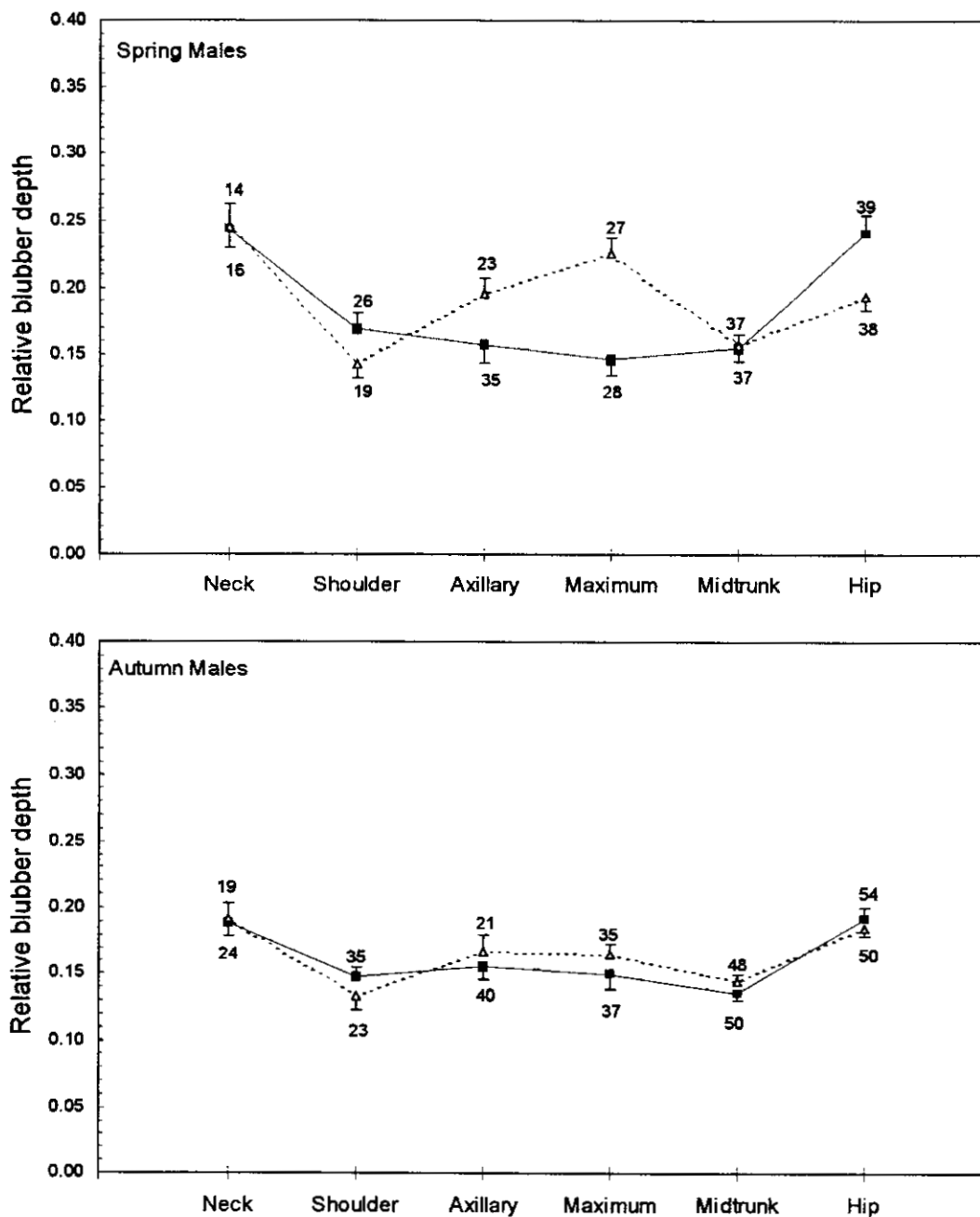


Figure 14. Distribution of dorsal (■) and lateral (△) blubber thickness relative to body radius for male harbor seals captured during spring (top) and autumn (bottom) of 1993-96 from Kodiak Island, Prince William Sound, and southeast Alaska. Error bars denote  $\pm 1$  se (sample sizes listed).

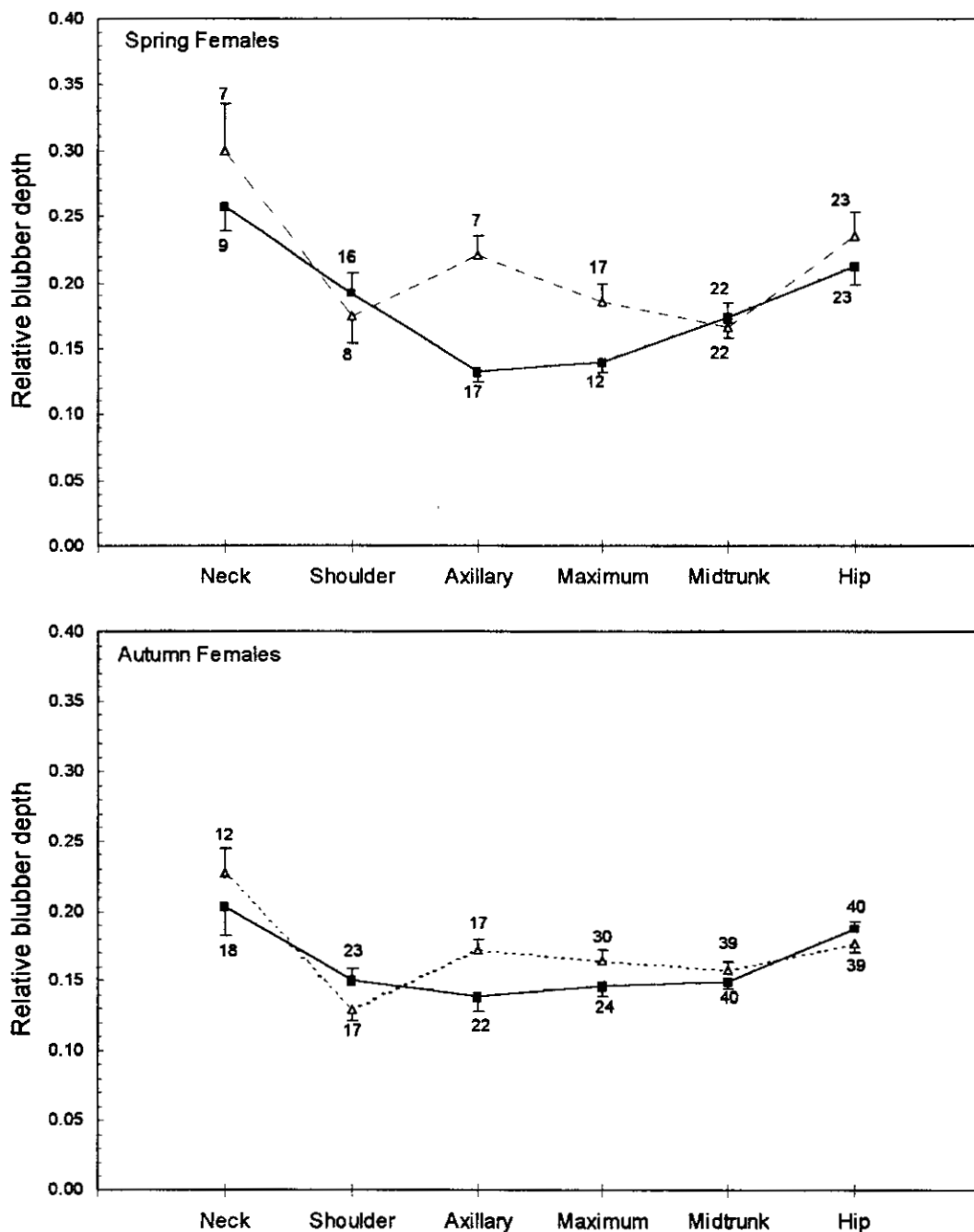


Figure 15. Distribution of dorsal (■) and lateral (Δ) blubber thickness relative to body radius for female harbor seals captured during spring (top) and autumn (bottom) of 1993-96 from Kodiak Island, Prince William Sound, and southeast Alaska. Error bars denote  $\pm 1 se$  (sample sizes listed).

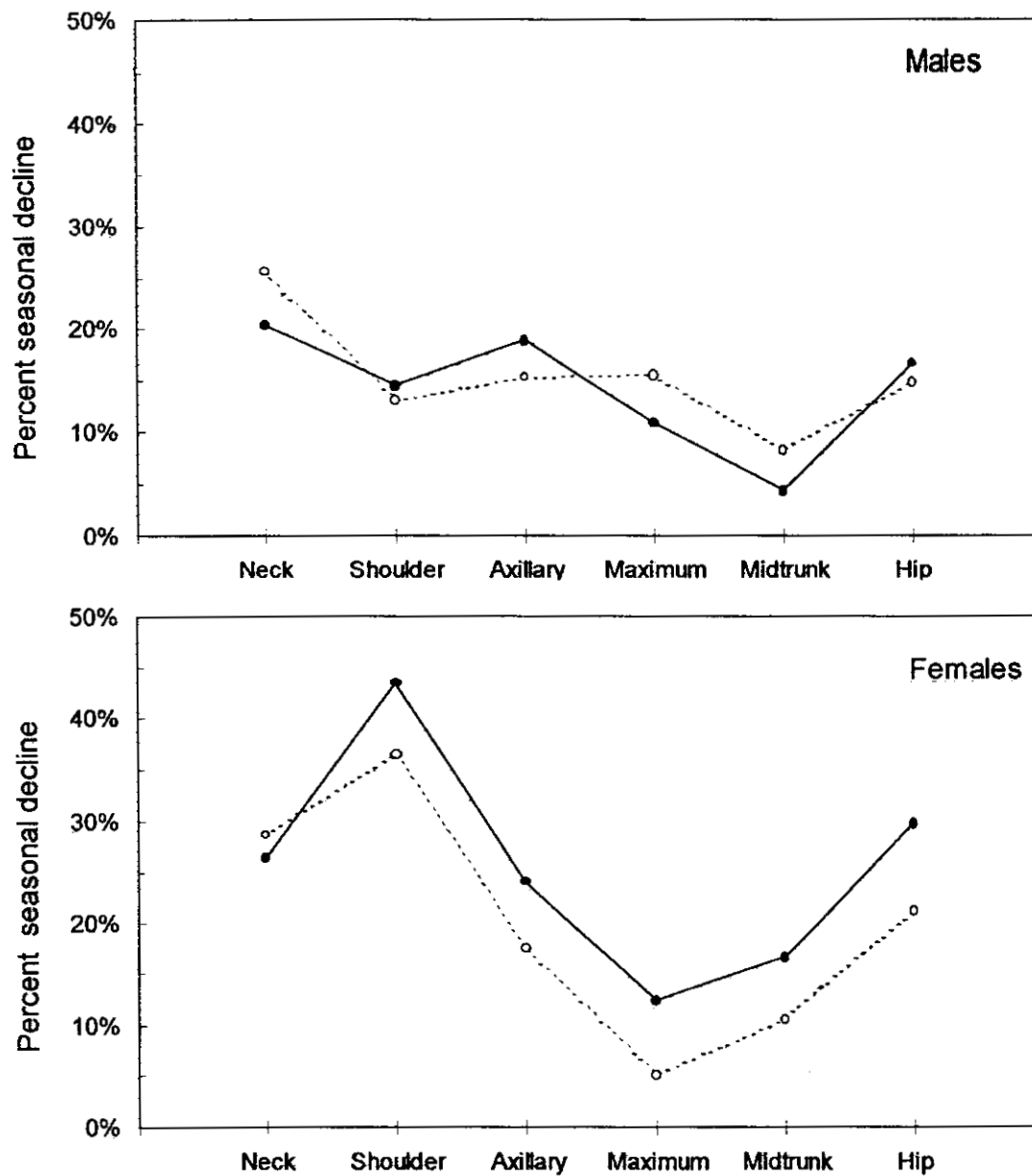


Figure 16. Percent seasonal (spring to autumn) decrease in mean blubber thickness (●) and blubber depth relative to body radius (○), for harbor seals captured during 1993-96 from Kodiak Island, Prince William Sound, and southeast Alaska. Blubber thickness was mean of dorsal and lateral measurements. Sample sizes similar to Figures 14 and 15.

## 4 Seasonal and Regional Differences in Proximate Composition of Blubber from Harbor Seals<sup>1</sup>

### INTRODUCTION

Harbor seals (*Phoca vitulina*) rely on subcutaneous blubber for insulation and energy storage (Ryg et al. 1988), and the quantity and thickness of blubber have been found to vary with age, sex, season, and energy intake (Nordøy and Blix 1985; Pitcher 1986; Ryg et al. 1988; Bowen et al. 1992; Beck et al. 1993). A key issue surrounding the decline of harbor seal populations in Alaska has been the role of food limitation and nutritional stress (Alaska Sea Grant 1993). If this has been an important component of the decline, then differences in body condition may be evident (Chapter 3). Seal body composition, measured as relative amounts of adipose tissue, varies with levels of energy intake (Nordøy and Blix 1985; Markussen 1995), and is reflected by changes in the subcutaneous blubber layer, which comprises up to 90% of the lipid stores of a seal (Beck et al. 1993). In phocids, lipid mobilization from blubber accounts for 75-94 % of energy requirements during fasting (Nordøy and Blix 1985; Bowen et al. 1992; Beck et al. 1993; Markussen 1995). While the volume of blubber changes in relation to energy intake, it has been only recently that blubber quality has also been shown to vary. On Sable Island, Nova Scotia, the lipid content of blubber from lactating harbor seals declined from about 93% to 77% during lactation (Bowen et al. 1992), and a seasonal decline of 3% in blubber lipid content was found between winter and spring for harp seals (*Phoca groenlandica*)

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<sup>1</sup>Portions of this chapter were previously presented as Fadely, B. S., M. A. Castellini, and J. M. Castellini. 1996. Harbor seals and EVOS: Blubber and lipids as indices as food limitation. *Exxon Valdez Oil Spill Restor. Study 95117-BAA Annu. Rep.*, Univ. of Alaska, Fairbanks, Alaska. 34 pp.; and as Fadely, B. S., J. M. Castellini, and M. A. Castellini. 1997. Recovery of harbor seals from EVOS: Condition and health status. *Exxon Valdez Oil Spill Restor. Study 96001 Annu. Rep.*, Univ. of Alaska, Fairbanks, Alaska. 53 pp.

from Les Escoumins, Quebec (Beck et al. 1993). Gales et al. (1994) found that blubber lipid increased with increasing body condition (defined as total body fat) in harp seals. As suggested by Gales et al. (1994), comparisons of seal condition which measure or index blubber stores implicitly assume constant lipid content of blubber. However, blubber quality has been shown to vary in response to energy status, and lipid content can vary seasonally, reflecting reproductive demands or prey availability (Bowen et al. 1992; Beck et al. 1993; Gales et al. 1994). By describing both the quantity and quality (i.e. lipid content) of blubber, a more complete understanding of seal condition can be obtained.

This study was designed to determine whether quantitative or compositional differences existed in blubber of seals from regions exhibiting different population trends around the Gulf of Alaska. We also sought to determine whether the quality of archived blubber samples collected from harbor seals during the late 1970's, before or near the beginning of detected population declines (Pitcher 1990; Small 1996), was suitable for comparison to recently collected samples from similar geographic locations.

## METHODS

Harbor seal blubber samples were obtained from two sources; through a subsistence harvest biosampling program, and from archived samples that had been collected in 1976-77 and subsequently archived by Alaska Department of Fish and Game. Blubber samples obtained from the subsistence harvest tissue program (a cooperative effort of the Alaska Native Harbor Seal Commission, Alaska Department of Fish and Game, National Marine Fisheries Service, and the University of Alaska Fairbanks) were collected during October 1995 to May 1996 from southeast Alaska, March through November 1996 from Prince William Sound, and during May 1996 from the Yakutat area. Seal hunters and trained assistants removed blubber samples (approximately 100-200 g) from the ventral 'hip' region (about 70-80 % of curvilinear length from the nose) of seals collected during subsistence harvests. Measurements of body mass, standard length (L), curvilinear length, axillary girth, hip girth, xiphosternal and ventral hip blubber thickness were also collected when possible. Blubber collected from a seal was placed in a plastic



storage bag and frozen at  $-5^{\circ}\text{C}$ . Blubber samples were kept frozen for transport. Blubber samples were double or triple bagged in freezer storage bags (Ziploc Brand, DowBrands L.P., Indianapolis, IN) and frozen at  $-80^{\circ}\text{C}$ .

Using measurements provided by the biosampling program, an index of harbor seal body condition was calculated as body mass relative to  $L^3$  (Chapter 3). Blubber thickness among seal populations was compared by calculating relative blubber thickness as the ratio of blubber depth to body radius, calculated as  $\text{girth}/2\pi$  (Chapter 3, Ryg et al. 1988, Beck and Smith 1995).

Seal blubber collected during 1976-77 was wrapped in aluminum foil or bagged in plastic bags and frozen at  $-30^{\circ}\text{C}$  by Alaska Department of Fish and Game. Subsamples were taken from archived frozen blubber of seals collected at Kodiak Island during April 1976 ( $n = 2$ ) and southeast Alaska during January-February 1977 ( $n = 7$ ). Specimens for analysis were chosen based on time and location of collection, size of specimen, and external appearance of the sample. Selected specimens were removed from the freezer, subsampled and double-bagged in plastic freezer storage bags (Ziploc). Original blubber and samples were returned to  $-30^{\circ}\text{C}$  before thawing occurred. Subsampled blubber specimens were transported to our laboratory packed in dry ice, and stored at  $-80^{\circ}\text{C}$  upon arrival.

Samples for bomb calorimetry were prepared by cutting cubes of frozen blubber, from which all edge, muscle, or visibly oxidized surfaces were trimmed away. Duplicate samples (measured to  $\pm 0.1$  mg) were kept frozen in calorimeter crucibles until analyzed in an automated non-adiabatic bomb calorimeter (Parr). Blubber cubes of less than 0.6 g did not completely combust, and the optimum cube size range was determined to be 0.9-1.0 g.

We tested oven-drying and freeze-drying as methods for determining water content. Oven-dried blubber samples did not achieve stable dry masses within 7 days at  $80 - 110^{\circ}\text{C}$ , so this technique was abandoned. Duplicate blubber samples were cut and freeze-dried to constant mass at  $-70^{\circ}\text{C}$  under vacuum (Labconco Freeze Dryer Model 5). The effect of sample mass on drying time and final dry mass was tested using 16 replicates of blubber from one seal ranging in size from 0.05-1.0 g (measured to  $\pm 0.1$  mg) and

dried up to 190 h. There was a significant effect of sample mass on measured water content below 0.1000 g. All samples changed in mass by less than 0.01% after 55 h in the freeze-drier. Thus the optimum sample mass range was determined to be 0.3-0.5 g as a compromise between variability and drying time.

Lipid content was determined as the mass difference after extraction of wet or dried blubber samples. Blubber samples of 0.5-0.7 g (wet mass, measured to  $\pm 0.1$  mg) were rinsed for >24 h in a 2:1 chloroform-methanol mixture in a Soxhlet apparatus (Bligh and Dyer 1959; Lockyer 1987; Beck et al. 1993). Sample mass had no effect on lipid content over a range of 0.3-0.9 g. If wet blubber samples were used, dry-masses were calculated from water content determined in separate samples of the same blubber section. Lipid content was expressed as a percent of wet or dry blubber mass.

Ash content was determined for blubber samples that had been dried and lipid extracted. Samples were placed in crucibles and ashed in a muffle furnace at 600°C for 6 hours (Sybron Thermolyne 1500). Residue mass was determined directly, and ash content expressed as a percentage of the initial wet or dry blubber mass.

As a check on bomb calorimetry and proximate composition analyses, combusted energy densities were compared to energy densities expected based on composition analysis. Assuming lipid, ash and protein comprised 100% of the dry mass, protein content was determined by subtraction of empirically determined lipid and ash content. Lipid and protein content were converted to energy densities by assuming an energy content of 39.3 kJ/g for lipid, and 17.99 kJ/g for protein (Schmidt-Nielsen 1994).

Blubber density was determined by measuring the buoyant force of 1-2 g blubber samples immersed in 250 mL chilled, deionized water into which 0.1 mL wetting agent (Foto-Flo 200, Kodak Co.) had been mixed (Mettler ME-33360 Density Determination Kit, attached to a Mettler Model AE100 balance). The addition of this volume of wetting agent produced a negligible ( $<0.001$  g/mL) density change to the water, but facilitated the prevention of air bubble formation on blubber samples during submersion. Water temperature was recorded to the nearest 0.1°C with a thermocouple (Physitemp BAT-10), and corresponding densities were determined from standard tables (Weast 1984).

Percentages were transformed using the arcsine angular transformation (Sokal and Rohlf 1987) prior to statistical testing. All statistical procedures were conducted using Statistix<sup>®</sup> version 4.1 (Analytical Software) or Systat<sup>®</sup> version 6.1 (SPSS Inc.) software packages. Results are presented as mean  $\pm$  1 standard error.

## RESULTS

### *Proximate blubber composition*

Blubber samples collected during 1995-96 combining southeast Alaska, Yakutat, and Prince William Sound had a mean water content of  $6.5 \pm 0.3$  % ( $n = 74$ ), and a lipid content of  $97.8 \pm 0.2$  % ( $n = 29$ ; dry-mass basis) or  $91.8 \pm 0.5$  % (wet-mass basis). Ash content was a negligible proportion of blubber ( $0.037 \pm 0.006$  %, dry-mass basis;  $0.036 \pm 0.006$  %, wet-mass basis, both  $n = 4$ ), so this determination was discontinued. Energy densities from bomb calorimetry were  $39.0 \pm 0.1$  kJ/g ( $n = 69$ ; dry-mass basis) or  $36.4 \pm 0.1$  kJ/g (wet-mass basis). Protein content was estimated at  $2.0 \pm 0.2$  % ( $n = 29$ ; dry-mass basis) or  $1.7 \pm 0.3$  % (wet-mass basis). This value also included any accumulated measurement errors. Blubber density was  $0.954 \pm 0.003$  g/mL ( $n = 8$ ). Water expressed on a fat-free basis was  $78.7 \pm 0.02$  % ( $n = 29$ ). No significant differences were found in energy density ( $t_s = 0.48$ ;  $P = 0.650$ ;  $df = 6$ ) or water content ( $t_s = 1.95$ ;  $P = 0.191$ ;  $df = 2$ ) of blubber samples derived from the head or hip areas. Thus, head blubber values were utilized for comparison if hip samples were unavailable. There was no significant difference between dry-mass basis energy content estimated from proximate composition analysis ( $38.9 \pm 0.03$  kJ/g) and bomb calorimetry ( $39.0 \pm 0.1$  kJ/g;  $t_s = -1.375$ ,  $P = 0.177$ ,  $df = 37$ ), indicating that lipid content was measured accurately by the Soxhlet extraction technique.

Blubber lipid content was directly and inversely related to water content (Figure 1). Because blubber is predominantly lipid, protein only contributed 1.0% of the total energy content. Thus, energy density decreased with increasing water content, and increased with increasing lipid content. Comparison of fat-free basis water content and lipid content (wet-mass basis) did not reveal a direct association (Figure 2). However,

visually the data clustered into two groups of seals split at 91% lipid content (Figure 2). The lower group (A) showed increased fat-free basis water with decreasing lipid content, while the upper group (B) exhibited a slight increasing trend between fat-free basis water and lipid content. Group A was comprised of seven males and one female, two collected during spring of 1996 from Prince William Sound, the others from autumn/winter of 1995/96 from southeast Alaska. There were no significant differences among the groups in blubber energy density ( $t_s = -1.145$ ,  $P = 0.886$ ,  $df = 36$ ) or mass to length scaling ( $F_{(1,9)} = 0.064$ ,  $P = 0.805$ ,  $L^3$  as a covariate). However, Group A males had a significantly lower blubber thickness to body radius ratio at the hips than Group B (Group A mean  $0.235 \pm 0.02$  (5); Group B mean  $0.322 \pm 0.03$  (10);  $t_s = -2.595$ ,  $P = 0.022$ ,  $df = 12.9$ ). The blubber thickness relative to body radius for the single Group A female (0.215) was also much less than the mean of Group B females ( $0.375 \pm 0.002$ ,  $n = 9$ ).

There was no effect of the elapsed time between seal kill and sample freezing on energy density (dry-mass basis;  $F_{(1,31)} = 0.198$ ,  $P = 0.659$ ), water content ( $F_{(1,32)} = 1.242$ ,  $P = 0.273$ ), or lipid content (dry-mass basis;  $F_{(1,8)} = 0.066$ ,  $P = 0.803$ ), for periods of 1-18 hours. None of the parameters measured varied with standard length (energy density  $F_{(1,68)} = 2.136$ ,  $P = 0.148$ ; water content  $F_{(1,72)} = 0.083$ ,  $P = 0.774$ ; lipid content  $F_{(1,31)} = 0.275$ ,  $P = 0.604$ ; fat-free basis water content  $F_{(1,30)} = 2.196$ ,  $P = 0.149$ ) over the size range (0.92-1.85 m) represented. Likewise, there were no significant relationships between body mass and energy density (dry-mass basis  $F_{(1,19)} = 2.994$ ,  $P = 0.100$ ), or water content ( $F_{(1,19)} = 1.611$ ,  $P = 0.220$ ). Blubber thickness measurements, both absolute and relative to body radius were not significantly correlated with blubber energy density ( $n = 55$ ,  $P > 0.10$ ), or water content ( $n = 27-31$ ; all  $P > 0.10$ ). There was no correlation between relative blubber thickness and lipid content, except when separated by sampling location (Figure 3). Blubber thickness relative to body radius at the hips, the site from which blubber samples were removed, was correlated with wet-mass basis lipid content ( $r = 0.638$ ,  $P = 0.035$ ,  $n = 11$ ) for samples collected during autumn and winter of 1995/96 from southeast Alaska. There was no relationship between these factors ( $r = 0.364$ ,  $P = 0.201$ ,  $n = 14$ ) for samples collected during spring 1996 from Prince William Sound

(Figure 3). A condition index of axillary girth divided by standard length was likewise not correlated with any measure of blubber composition (all  $P > 0.10$ ,  $n = 25$  comparisons).

#### *Geographic and seasonal effects on blubber quality*

There were no geographical differences of blubber energy densities (dry-mass basis) among seals sampled during spring 1995-96 from Prince William Sound ( $39.1 \pm 0.2$  kJ/g,  $n = 14$ ), Yakutat ( $38.8 \pm 0.2$  kJ/g,  $n = 10$ ), or southeast Alaska ( $38.8 \pm 0.3$  kJ/g,  $n = 5$ ;  $F_{(2,23)} = 1.017$ ,  $P = 0.371$ ). Energy densities were also not significantly different between sexes ( $F_{(2,23)} = 2.014$ ,  $P = 0.169$ ), but male blubber was more hydrated ( $6.5 \pm 0.3$  %,  $n = 19$ ) than female blubber ( $5.3 \pm 0.3$  %,  $n = 14$ ;  $F_{(1,27)} = 7.389$ ,  $P = 0.011$ ). There was also a trend for Prince William Sound blubber to be less hydrated ( $5.3 \pm 0.3$  %,  $n = 18$ ) relative to Yakutat ( $6.3 \pm 0.3$  %,  $n = 10$ ) and southeast ( $6.1 \pm 0.5$  %,  $n = 5$ ), but this was not statistically significant ( $F_{(2,27)} = 2.610$ ,  $P = 0.092$ ). These Prince William Sound seals, however, were less massive at length ( $42.5 \pm 4.8$  kg,  $n = 5$ ) than seals from Yakutat ( $56.0 \pm 2.8$  kg,  $n = 10$ ;  $F_{(1,10)} = 5.758$ ,  $P = 0.037$ ;  $L^3$  as a covariate, no southeast measurements available for comparison; Figure 4), and had thinner blubber relative to body girth at the sternum (Prince William Sound mean ratio  $0.196 \pm 0.16$  (18); Yakutat  $0.258 \pm 0.02$  (10); southeast  $0.251 \pm 0.03$  (5);  $F_{(2,27)} = 3.513$ ,  $P = 0.044$ ).

Likewise, there were no geographical differences of blubber energy densities (dry-mass basis) among seals sampled during autumn 1995-96 from Prince William Sound ( $39.1 \pm 0.2$  kJ/g,  $n = 14$ ) and southeast ( $39.0 \pm 0.2$  kJ/g,  $n = 12$ ;  $F_{(1,22)} = 0.156$ ,  $P = 0.697$ ). There were no gender differences of energy density ( $F_{(1,22)} = 0.089$ ,  $P = 0.768$ ) or water content ( $F_{(1,22)} = 1.544$ ,  $P = 0.227$ ). However, Prince William Sound blubber was significantly less hydrated ( $5.6 \pm 1$  %,  $n = 14$ ) than blubber from southeast Alaska seals ( $8.4 \pm 1$  %,  $n = 12$ ;  $F_{(1,22)} = 14.395$ ,  $P = 0.001$ ). In contrast to spring, though, there were no significant differences in morphometric condition measures between Prince William Sound and southeast (mass relative to  $L^3$   $F_{(1,15)} = 0.516$ ,  $P = 0.484$ ; blubber thickness relative to girth  $F_{(1,18)} = 0.035$ ,  $P = 0.853$ ).

Blubber energy density (dry-mass basis) did not vary significantly for either sex among spring and autumn within Prince William Sound (males  $F_{(1,10)} = 0.048$ ,  $P = 0.831$ ; females  $F_{(1,13)} = 0.05$ ,  $P = 0.828$ ), or among spring, autumn, and winter within southeast Alaska (males  $F_{(2,16)} = 2.345$ ,  $P = 0.128$ ; females  $F_{(2,9)} = 0.034$ ,  $P = 0.967$ ). Blubber water content of males from southeast Alaska was significantly different between autumn ( $8.8 \pm 0.8$  %,  $n = 8$ ) and winter ( $6.1 \pm 0.6$  %,  $n = 8$ ;  $F_{(2,16)} = 3.955$ ,  $P = 0.040$ ), but not spring ( $6.9 \pm 1.1$  %,  $n = 3$ ). There were no significant seasonal effects on water content for females from southeast Alaska ( $F_{(2,10)} = 0.472$ ,  $P = 0.637$ ) or Prince William Sound ( $F_{(1,13)} = 0.050$ ,  $P = 0.827$ ).

#### *Interannual comparisons with archived blubber*

Using all available samples, archived blubber collected from southeast Alaska and Kodiak Island during 1976-77 were significantly lower in water content ( $4.6 \pm 0.3$  %,  $n = 9$ ) than 1995-96 samples ( $6.5 \pm 0.3$  %;  $t_s = -4.483$ ,  $P < 0.001$ ,  $df = 36.9$ ). Lipid content (dry-mass basis) of the 1976/77 samples ( $98.4 \pm 0.2$  %,  $n = 9$ ) was greater than the 1995/96 samples ( $91.8 \pm 0.5$  %;  $t_s = 2.422$ ,  $P = 0.021$ ,  $df = 32.6$ ). However, energy densities (dry-mass basis) of archived blubber ( $39.1 \pm 0.1$  kJ/g,  $n = 9$ ) were not significantly different than energy densities of recently collected blubber ( $39.0 \pm 0.1$  kJ/g;  $t_s = 0.488$ ,  $P = 0.627$ ,  $df = 76$ ). There were no significant differences in how energy density (wet-mass basis) varied with water content ( $F_{(1,18)} = 1.79$ ,  $P = 0.197$ ) or with lipid content ( $F_{(1,16)} = 0.90$ ,  $P = 0.356$ ), which may have been expected if lipids had been degraded or oxidized over time. There were also no significant differences between periods in fat-free basis water content ( $t_s = -0.468$ ,  $P = 0.643$ ,  $df = 29.9$ ).

A more conservative comparison utilized the archived samples collected during winter from southeast Alaska ( $n = 7$ ) with samples collected from southeast Alaska during winter of 1995/96. Thus matched in season and region, the archived samples were likewise less hydrated ( $4.5 \pm 0.1$  %) than blubber from 1995/96 ( $7.2 \pm 1.1$  %;  $t_s = -2.773$ ,  $df = 17.3$ ,  $P = 0.013$ ), and had slightly greater dry-mass basis lipid content (1977,  $98.6 \pm 0.2$  %; 1996,  $97.6 \pm 0.2$ ;  $t_s = 3.365$ ,  $df = 12$ ,  $P = 0.006$ ). Thus, energy densities

(dry-mass basis) were higher in 1977 samples ( $39.1 \pm 0.1$ ) than in 1995/96 samples ( $38.7 \pm 0.1$ ,  $n = 14$ ;  $t_s = 2.076$ ,  $df = 19$ ,  $P = 0.052$ ). Lipid content on a wet-mass basis was likewise 2.3% greater in 1977 ( $94.3 \pm 0.4$  %) than 1995/96 ( $92.0 \pm 0.6$  %;  $t_s = 3.313$ ,  $df = 12$ ,  $P = 0.006$ ). Fat-free basis water content from 1977 was  $78.9 \pm 1.6$  %, significantly higher than a  $73.8 \pm 1.1$  % from 1995/96 ( $t_s = 2.581$ ,  $df = 12$ ,  $P = 0.024$ ).

## DISCUSSION

### *Proximate blubber composition*

Because of the negligible ash content and low protein component, changes in adult phocid blubber composition can be regarded as a two-component system consisting of lipid and water (Figure 1). Essentially, fat is mobilized to and from an otherwise relatively constant fat-free body (Pace and Rathbun 1945; Spray and Widdowson 1950). Although seal blubber is vertically heterogenous in fatty acid composition (Fredheim et al. 1995), lipid content (% mass basis) does not vary with blubber depth or body sampling location (Beck et al. 1993). Thus, analysis of single body site blubber samples should reflect whole-body blubber composition.

The chemical composition of blubber from harbor seals in the Gulf of Alaska region was similar to that measured for adult harbor seals from eastern Canada (Bowen et al. 1992), and to other phocid species (Table 1). The range of lipid content found among Gulf of Alaska seals (86.5-95.6 %, a 9.1% difference; wet-mass basis) was less than the range found for lactating adult females (15%, Bowen et al. 1992), and shifted upward. These differences may arise through two mechanisms. First, though pregnant females were collected, none of the seal blubber analyzed in this study came from lactating females. Thus, seals that may be expected to have minimal energy stores were missing from this sample set. Second, Bowen et al. (1992) utilized petroleum ether lipid extraction, while we used a 2:1 chloroform-methanol extraction. These different techniques may account for some of the difference, but Lockyer (1987) did not find a significant difference between the two solvent systems on the amount of lipid extracted from cetacean blubber. However, Beck et al. (1993) also report high lipid content for

harp seal blubber collected during spring, autumn and winter from Quebec using the chloroform-methanol solvent system. Much lower harp seal blubber lipid content was found during late March to mid-June from Newfoundland using centrifugation techniques. Because the season and locations were different between these two studies, the effect of different lipid determination techniques cannot be assessed. Future studies should carefully consider the potential effect of solvent system or lipid extraction technique if comparisons among studies are desired.

We did not find detectable handling effects, measured as the elapsed time from seal collection to blubber sample freezing, on blubber energy content or proximate composition. This was a concern because lipids separate from harbor seal blubber when left standing at room temperatures, a rendering technique utilized by local Natives<sup>2</sup>. Because of the practicalities of seal hunting in remote areas, considerable time could elapse before blubber samples were collected and frozen. However, if effects of this type were operating, they occurred within 1 hour of seal collection, less than the minimum elapsed time measured between seal kill and sample freezing in this study.

In general we found no association between condition indices and blubber composition. However, there may have been a relationship between the relative thickness of blubber and lipid content at the sampling site (Figure 3). Evidence of a relationship was found only for seals sampled during autumn and winter, when harbor seals in this region are generally in positive energy balance, and gain weight and blubber mass (Pitcher and Calkins 1983; Pitcher 1986; Chapter 3). Above about 91% lipid content, however, an increase of nearly 15% in blubber thickness relative to body radius was associated with a minor (<1%) increase in lipid content. The seals below 91% lipid content were also separated from other seals as Group A in a plot of fat-free basis water content with lipid content (Figure 2). As lipid content increased from 86-91%, more water was lost from

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<sup>2</sup> Personal communications found in Whiskers! (1996, version 1.1), an electronic database of local indigenous knowledge. Available from the Division of Subsistence, Alaska Department of Fish and Game, 333 Raspberry Rd., Anchorage, AK 99518.



adipocytes than from blubber above 91% lipid content. These relationships suggest that the dynamics of lipid and water exchange in harbor seals require further study in relation to energy status. Because of this disparity, reliance on only quantitative measures of blubber content may present inadequate measures of condition, as suggested by Gales et al. (1994).

Seal blubber serves a dual role as insulator and energy store, and for insulation it is the thickness of the blubber relative to the thickness of the seal which is significant (Ryg et al. 1988). The regions of a seal posterior to the maximum girth have been shown to be over-insulated relative to the thickness of the animal (Ryg et al. 1988, Beck and Smith 1995), and this region was also the most variable in blubber thickness (Beck et al. 1993; Gales and Renouf 1994; Beck and Smith 1995) indicating that blubber stores in this region were linked to energy status. However, Fadely (Chapter 3) and Rosen and Renouf (1997) found that the neck and shoulder regions of harbor seals were more variable in blubber thickness than the posterior regions, and Fadely (Chapter 3) found differential variability of sites between sexes. Blubber thickness and lipid content were not measured at these sites in this study, which may partly account for the lack of correlation between lipid content and blubber thickness.

#### *Regional and seasonal blubber composition*

No evidence was found of changes in blubber energy density (dry-mass basis) among seals from areas of population decline (Prince William Sound) and stability (southeast), when compared within seasons. However, seal blubber from Prince William Sound was less hydrated than blubber collected from southeast Alaska (spring and autumn) and Yakutat (spring). Decreases in blubber water content have been associated with increased body fat in grey seals (*Halichoerus grypus*; Reilly 1989), and harp seals (Worthy and Lavigne 1983; Gales et al. 1994) since most phocid lipid stores are contained within the blubber (Beck et al. 1993). Within spring, the hydration difference of blubber between Prince William Sound and Yakutat was 1%, which would produce a 0.4 kJ/g difference in energy content based only on lipid change. The dry-mass energy basis

difference between these two locations was 0.3 kJ/g, consistent with that expectation. Thus, there were likely energetic differences associated with the differing hydration states, but they were statistically undetectable. However, Prince William Sound seals during spring were less massive (relative to scaled body length; Figure 4) and had thinner relative blubber thickness than seals from Yakutat and southeast Alaska. Thus, though there were minor differences in blubber composition, there was evidence that Prince William Sound had lower overall energy stores than seals from the other two regions. These morphometric differences were not apparent, however, among seals collected during autumn from Prince William Sound and southeast Alaska. If condition changes as a result of differences in prey quality, affecting either energy intake or assimilation, or from different energy expenditures in prey acquisition, then these factors may only be critical seasonally among these locations. Also, the age distribution represented among these comparisons is composed largely of 1–4 year olds, an age at which food limitations may have a significant impact (Alaska Sea Grant 1993).

Within regions, the only seasonal effect on blubber composition was found to be increased blubber hydration in autumn relative to winter for southeast Alaska males. This is consistent with the general improvement in body mass and condition observed from this time of year (Pitcher and Calkins 1983; Pitcher 1986; Chapter 3). Seasonal declines in blubber quality and quantity have been shown in lactating harbor seals (Bowen et al. 1992). This period of time was not represented in among samples used in this study and this may have contributed to the lack of seasonal variation in blubber composition.

#### *Interannual comparisons with archived blubber*

Because of the duration the archived samples were frozen, the quality of this 20-year-old blubber was a foremost concern. Archived samples were carefully trimmed to avoid rancid, oxidized or desiccated tissue, yet it is apparent they were excessively dehydrated relative to recently collected blubber samples. Comparing the southeast Alaska samples collected during winter of 1977 and 199/96, water content variance was significantly reduced for the archived samples. Unfortunately, not knowing the original hydration status of these samples precludes a definitive conclusion, particularly since

archived samples still fall within a range of lipid and water content observed from recently collected blubber (Figure 1). However, consistent with overall dehydration, archived samples were clustered at one end of the relationship. A second concern was of fatty acid degradation or overall lipid loss during storage. If this did not occur, then regardless of the hydration state, comparisons of dry-mass basis lipid or energy content would address the issue of overall seal condition between the two decades. Within the southeast Alaska winter samples, the slightly higher lipid content of 1977 samples should produce a 0.39 kJ/g higher energy content relative to the 1995/96 samples. The energy density difference between the two groups based on bomb calorimetry was 0.4 kJ/g, suggesting that lipid degradation did not occur. Seal populations in southeast Alaska have remained stable or have slightly increased during the past 20 years (Small and DeMaster 1995; Small 1996), and we conclude that the quality of harbor seal blubber based on dry-mass basis energy density does not appear to show obvious changes over that time (1977-1996). However, it must be considered that this was based on small sample sizes from only one year in each decade.

Increased lipid content of blubber results in decreased thermal conductance (Worthy and Edwards 1990). Specific relationships between lipid content of harbor seal blubber and thermal conductance have yet to be determined. However, based on comparisons from 4 cetacean species (Worthy and Edwards 1990), differences in lipid content on the order of 10% shown in this study for harbor seals did not substantially change thermal conductance within a species. Thermal conductance over the range of blubber lipid content shown for lactating harbor seals and nursing pups (Bowen et al. 1992) may change significantly, and determination of this relationship would enhance models of harbor seal thermoregulation created by Watts (1992), Watts et al. (1993), and Hansen et al. (1995).

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Table 1. Proximate blubber composition of phocid species from this study and the literature. Mean values ( $\pm 1se$ ) are presented, or ranges if mean values were unavailable from publications.

Species	Energy Density (kJ/g)	Water (%)	Lipid (%)	Protein (%)	Ash (%)	Density (g/mL <sup>3</sup> )	Reference
Harbor seal	36.4 (0.1)	6.5 (0.3)	91.8 (0.5)		0.04 (0.01)	0.954 (0.003)	This study
Adult		5 - 15	77.0 - 92.1	2 - 5			Bowen et al. 1992 <sup>a</sup>
Pup		7 - 17	75.8 - 90.0	2 - 6			Bowen et al. 1992
Grey seal	38.8 (0.1)	7.4 (1.1)				0.90	Nordøy and Blix 1985 <sup>b</sup>
Harp seal		9.5 (0.6)	87.7 (0.8)	2.0 (0.2)	0.01 (0.01)		Gales et al. 1994 <sup>c</sup>
			93.8 - 98.9				Beck et al. 1993 <sup>d</sup>
						0.937 (0.008)	Gales and Renouf 1994 <sup>e</sup>
						0.921 (0.008)	Beck and Smith 1995 <sup>f</sup>
Southern elephant seal						0.95	Gales and Burton 1987 <sup>g</sup>

<sup>a</sup>Adult  $n = 12$ , pup  $n = 13$ .

<sup>b</sup> $n = 4$ .

<sup>c</sup> $n = 26$ .

<sup>d</sup> $n = 33$ .

<sup>e</sup> $n = 12$ .

<sup>f</sup> $n = 30$ .

<sup>g</sup> $n = 5$ , no  $se$  reported.

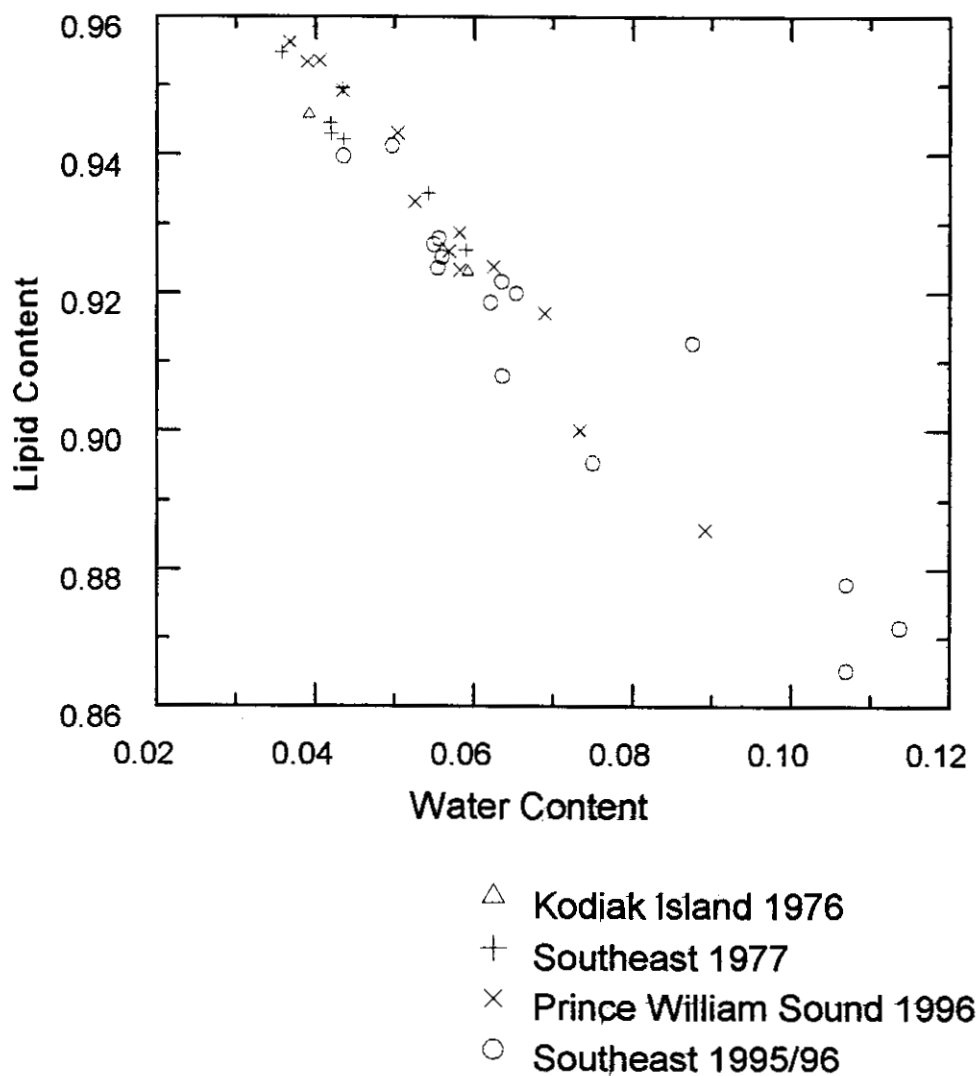


Figure 1. Relationship between lipid content (wet-mass basis) and water content for harbor seal blubber from seals collected in southeast Alaska, Prince William Sound, and Kodiak Island.



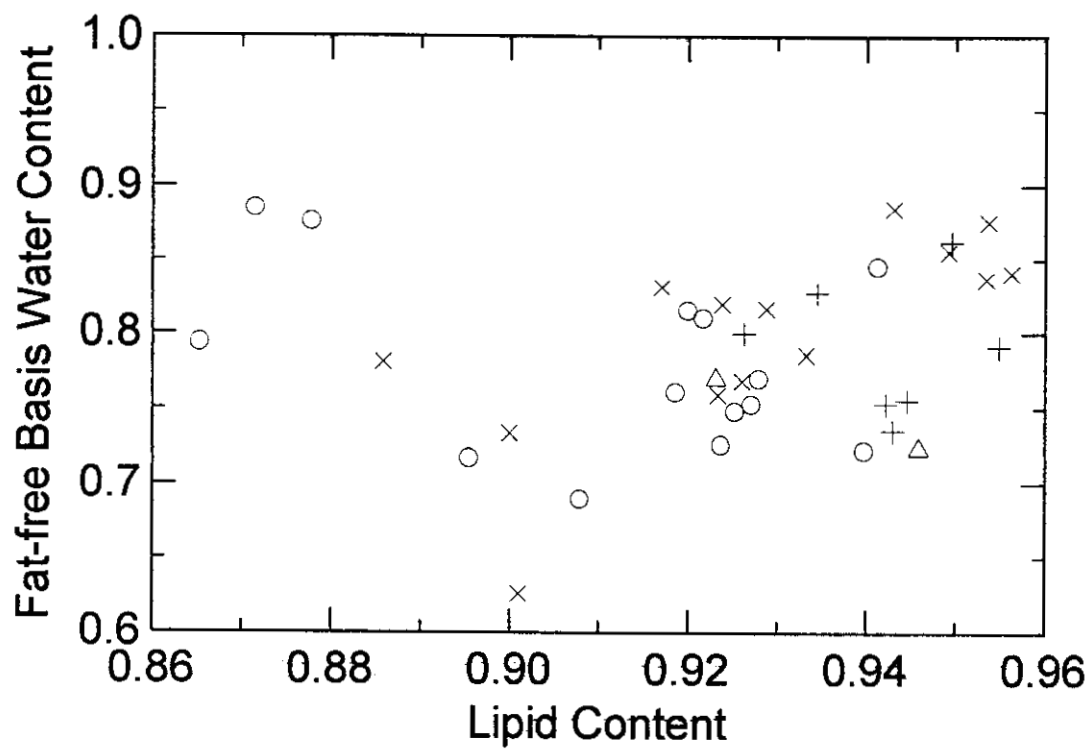


Figure 2. Relationship between fat-free basis water content and lipid content (wet-mass basis) for harbor seal blubber. Samples collected from southeast Alaska during 1977 (+) and 1995/96 (o), Prince William Sound 1996 (x), and Kodiak Island 1976 (Δ).

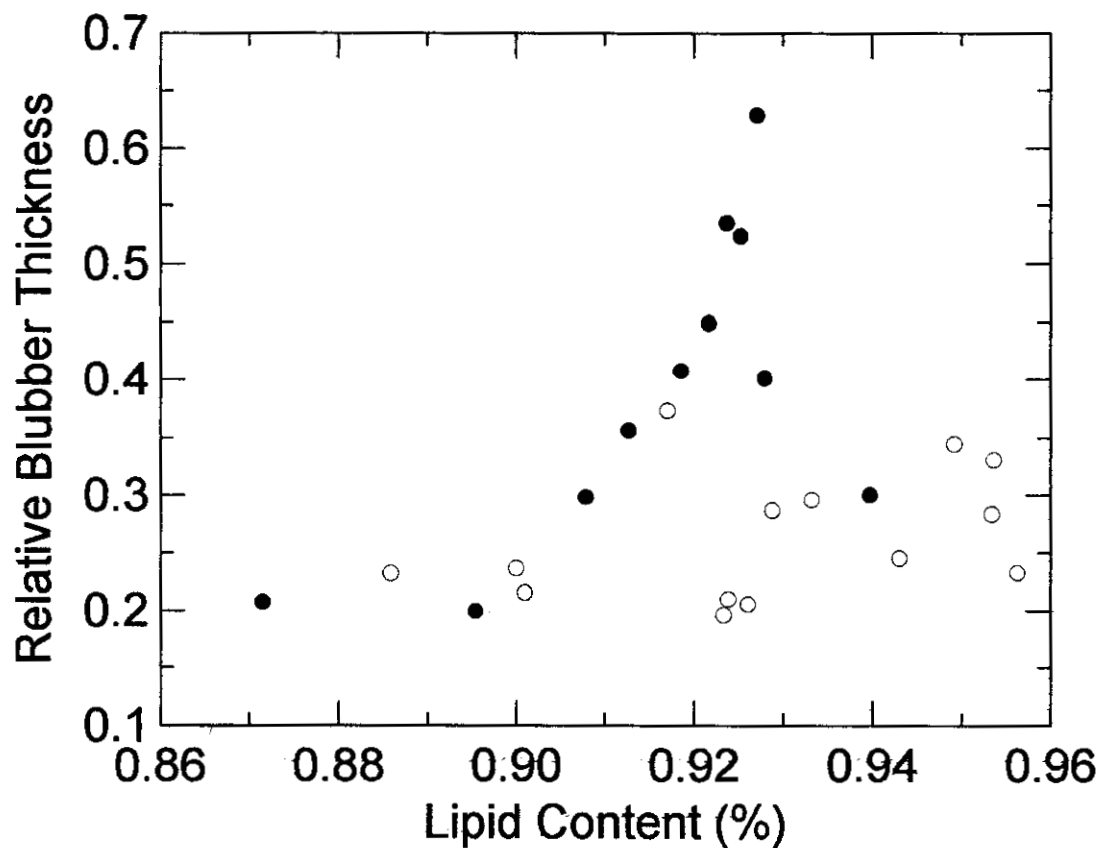


Figure 3. Relationship between relative blubber thickness (to body radius) at the hips (about 78% of curvilinear length) and lipid content (wet-mass basis). For harbor seals collected from Prince William Sound (○) and southeast Alaska (●) during 1995-96. Samples from Prince William Sound were all collected during spring, samples from southeast were collected during autumn and winter.

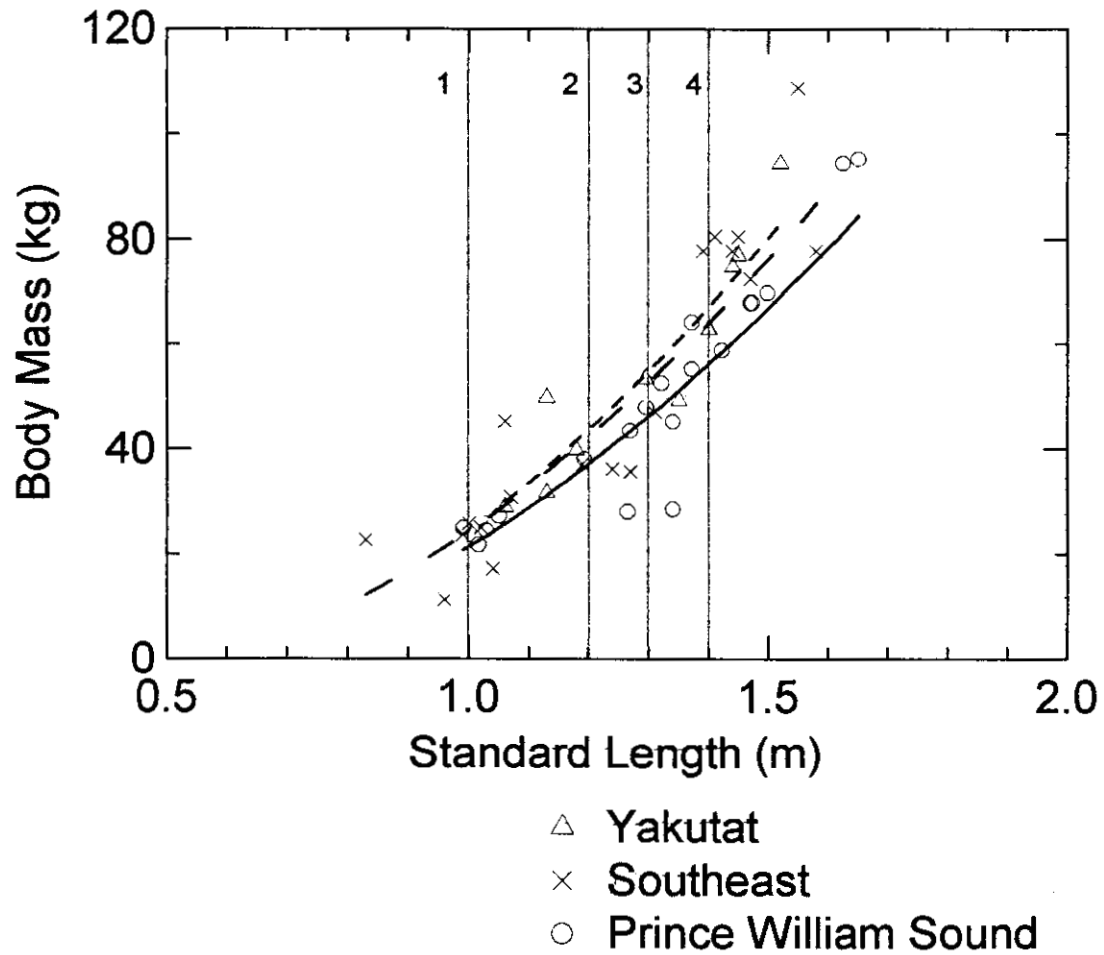


Figure 4. Scaling of body mass with length for harbor seals collected during subsistence harvests 1995-1996. Approximate divisions in age class (in years) length from Pitcher and Calkins (1983). Regression lines presented for each region; Yakutat (---), southeast (-.-); Prince William Sound (—).

## **5 Health Status and Body Condition of Harbor Seals in the Gulf of Alaska during 1963-1996**

### **INTRODUCTION**

Harbor seals (*Phoca vitulina*) range throughout coastal Alaska along the north Pacific rim from the Aleutian Islands to the southeast panhandle, and extend into the Bering Sea (Hoover-Miller 1994). Population declines of up to 87% were recorded at Tugidak Island, near the center of this range, beginning at least in 1976 (Pitcher 1990). Declines were also noted in Prince William Sound prior to the March 29, 1989 *Exxon Valdez* Oil Spill, which directly claimed an estimated 36% of the local population (Frost et al. 1994). Since then, seals throughout Prince William Sound continued to decline at about 6% per year (Frost et al. 1995, 1997). In contrast, populations in southeast Alaska remained stable, or increased slightly throughout the same period (Small and DeMaster 1995; Small 1996).

Many pinniped and seabird populations in the northern Gulf of Alaska and Bering Sea have displayed similar patterns of population decline since the late 1960's (Alaska Sea Grant 1993), and multiple hypotheses involving nutritional limitation, disease, predation, environmental contamination, emigration and direct or indirect fisheries harvests have been examined in attempts to explain these patterns (Sease 1992; Alaska Sea Grant 1993; Hoover-Miller 1994; Frost et al. 1994; National Research Council 1996). Nutritional limitation is proposed to have resulted from changes in prey distribution, abundance, or species composition through independent or synergistic mechanisms of natural climate change and fisheries harvests (Alverson 1992; Alaska Sea Grant 1993; National Research Council 1996).

If nutritional limitations contributed to harbor seal declines, or to the continuing non-recovery following the oil spill in Prince William Sound, then it may be possible to detect changes in seal body condition or composition, which reflect changes in energy

intake status (see Costa 1987 for review). Many pinniped indices of condition which can be used for comparison to historic data have been shown to be poorly to moderately correlated with absolute condition or body composition (Ryg et al. 1990; Beck et al. 1993; Gales and Renouf 1994; Chapter 3, this dissertation). Additionally, differences were found in the scaling of mass with length dependent upon whether measurements were collected on live seals or postmortem, precluding direct comparisons of morphometric condition indices (Chapter 3, this dissertation). McLaren and Smith (1985) suggested combining morphometric condition indices with other sensitive indicators of health derived from blood chemistry profiles, a methodology commonly applied in terrestrial ecosystems (Seal et al. 1975; Hanks 1981; Franzmann 1985; Messier 1987; Payne and Payne 1987; Hellgren et al. 1989; Knick et al. 1993; Harder and Kirkpatrick 1994) and recently used in comparisons of Steller sea lion (*Eumetopias jubatus*) pup condition (Castellini et al. 1993; Rea 1995), harbor seal health status in San Francisco Bay (Kopeck and Harvey 1995), and harbor seal dietary patterns in Scotland (Thompson et al. 1997).

Blood chemistries and hematologies provide diagnostic data for determination of acute and chronic conditions, and by examining groups of analytes the organ or metabolic systems being affected can be detected (Rebar and Boon 1983; Duncan et al. 1994; Roletto 1993). Hematological values which may indicate chronic oil effects or environmental contamination include increased activities (levels) of alanine (ALT) and aspartate aminotransferase (AST), and lactate dehydrogenase (LDH), enzymes associated with the liver (St. Aubin 1990; Hartung 1995; Rebar et al. 1995). Changes to the immune system may also result from chronic exposure to environmental contaminants, manifested as neutrophilia and increased white blood cell counts (de Swart et al. 1995; Schumacher et al. 1995). Changes in nutritional status, energy balance, or even diet composition can also be reflected in blood chemistries, providing an avenue to test degrees of nutritional limitation (Castellini and Rea 1992; Rea 1995; Knick et al. 1993; Thompson et al. 1997). Infection, inflammation, or trauma cause increased levels of haptoglobin, one of the acute-

phase proteins (Gordon and Koj 1985). Increases in haptoglobin have also been associated with chronic oil exposure (Duffy et al. 1993).

This study examined harbor seal health and condition in the Gulf of Alaska from two perspectives. First, by the comparison of body size or composition indices from seals collected within and outside of decline areas, periodically between 1963 and 1996; and secondly by contrasting blood chemistries and condition indices of seals between 1992-96 among three major regional groupings; Kodiak Island, Prince William Sound and southeast Alaska, each with different population trajectories (Chapter 1, this dissertation).

## METHODS

### *Body condition*

Comparative long-term condition data were derived from several sources. Bishop (1967) collected measurements of body mass, standard length, xiphosternal blubber thickness, and age during 1963 from Aialik and Harris Bays on the Kenai Peninsula, and from Tugidak Island during April-July, 1964. Pitcher (1977), Pitcher and Calkins (1983), and Pitcher (1986) presented condition data collected from harbor seals during 1972-1978 from the Gulf of Alaska, also used in Chapter 3 of this dissertation. Similar data were collected during 1989-90 from Prince William Sound and the Barren and Kodiak Islands (Frost and Lowry 1994). Bering Sea data were collected in 1979 and 1985 by Frost and Lowry (unpublished). Morphometric data collected during 1995-96 were provided by the harbor seal biosampling program. All of these data were collected from postmortem seals. Condition data were also collected from live seals captured by net-entanglement during 1992-1996 (details of the methodologies for these studies can be found in Chapters 3 and 4, this dissertation).

Comparisons of body mass ( $M$ ) were performed by controlling for body size using standard length cubed ( $L^3$ ) as a covariate, which provides an age-independent length reference (Chapter 3, this dissertation). Indices of blubber content for 1993-96 were

calculated by taking the mean of at least 5 blubber thickness measurements (Chapter 3, this dissertation).

#### *Blood chemistry and hematology*

Seals live-captured during 1992-96 also had blood withdrawn for profile determination, as detailed in Chapter 2 of this dissertation. Briefly, standard plasma chemistry panels were generated using automated machine analysis (Ektachem Analyzer) by technicians at the Fairbanks Memorial Hospital (FMH) on plasma which had been collected in sodium or lithium-heparin collection tubes. Analytes used for comparisons in this study were aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH). Hemoglobin was determined using standard kits from Sigma Chemical Co. in our laboratory. Complete blood counts of white and red blood cells, platelet counts and differential white blood cell counts were performed by technicians at FMH from blood collected in EDTA using a Coulter Model S-Plus-4 Counter, and from blood smears produced in the field. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin content (MCHC) were calculated from combinations of measured hematocrit, hemoglobin and red blood cell count (RBC) following Kerr (1989). These were calculated using our own hematocrit and hemoglobin determinations in combination with the FMH RBC determination. Haptoglobin was determined as the hemoglobin-binding capacity of haptoglobin, using methods detailed in Zenteno-Savin et al. (1997).

#### *Statistical comparisons*

Interannual and interregional comparisons of body condition and blood chemistry were performed within boundaries of sex, age, and season determined to have significant effects (Chapters 2 and 3, this dissertation). Months were categorized into seasons as winter (Dec-Feb), spring (Mar-May), summer (Jun-Aug), and autumn (Sep-Nov). For hematological comparisons, seasons were limited to spring and autumn, and seals were split into age categories of juveniles (yearlings through subadults) and adults based on mass, length, and time of year. Juveniles were generally less than 4 years old based on

size-at-age relationships from Pitcher and Calkins (1983). Enzyme activities were log-transformed before statistical testing (Chapter 2, this dissertation).

To examine distribution patterns of statistically significant plasma chemistry and hematological outliers among years and regions, seal blood chemistry profiles were screened based on reference ranges established in Chapter 2. The total number of outliers (values above or below 95% reference range) were summed for each seal, and seals with less than 4 outlying variables were excluded. Exclusion was based on a binomial probability model indicating fewer than 3 outlying variables were more likely to occur by chance (Chapter 2).

Statistics were calculated using Systat<sup>®</sup> version 7.0 (SPSS Inc.). All *P*-values presented were adjusted for degrees of freedom or numbers of contrasts using Bonferroni adjustments (Systat 1996). Error ranges in text and figures represent  $\pm 1 se$ .

## RESULTS

### *Body condition*

In general there were no detectable differences in body mass adjusted for size among male (Table 1) or female (Table 2) harbor seals collected from three Gulf of Alaska regions. The only statistically significant comparison was a decline of body mass among males from Kodiak Island between springs of 1976 and 1977 (Table 1). Interannual differences within decades were similar to differences between decades (Tables 1, 2), and no consistent interannual trend was detectable. Bering Sea males did not differ in body mass between 1979 and 1985 (Table 1), though females tended to be lighter in 1985 (Table 2).

Among seals collected from the Kodiak region, there were no discernible differences in mass-at-age relationships between 1963/64 and 1976-78 (Figures 1a, 2a), nor in standard length-at-age comparisons (Figures 1b, 2b). Male blubber thickness during 1963/64 tended to be greater than in 1976-78 among males (Figure 1c), but were similar between periods among females (Figure 2c). Blubber thickness did not show



interannual trends among males in Prince William Sound (Table 3) and southeast Alaska (Table 4), or among females of southeast Alaska (Table 5) between the 1970's and mid-1990's.

Body mass (adjusted for size) varied with year and region for both sexes captured during 1993-96 from the Kodiak, Prince William Sound, or southeast Alaska regions ( $F_{(6,218)} = 1.581, P = 0.154$ ; Figure 3). Prince William Sound male body mass varied with year in spring ( $F_{(3,37)} = 3.54, P = 0.024$ ), but not during autumn (Figure 3). In contrast, Prince William Sound female masses did not vary interannually during spring ( $F_{(3,36)} = 2.161, P = 0.110$ ), but were higher in 1993 compared to 1994-96 during autumn ( $F_{(3,33)} = 2.849, P = 0.052$ ). Southeast Alaska males were heavier than Prince William Sound or Kodiak Island seals during spring of 1995 ( $F_{(2,26)} = 8.13, P = 0.002$ ), but there was no difference between southeast and Prince William Sound males in spring of 1993 (Figure 3). Kodiak Island males were heaviest among the three regions during autumn 1995 ( $F_{(1,13)} = 91.00, P < 0.001$ ) and 1996 ( $F_{(2,29)} = 3.74, P = 0.036$ ), but not in 1994. Prince William Sound and southeast Alaska males did not differ in their body masses during autumn throughout 1993-96. Likewise, there were no regional differences among years for females during autumn ( $F_{(7,49)} = 0.767, P = 0.617$ ), though Kodiak Island seals tended to be more massive.

There was significant interannual, seasonal, and regional variability in mean blubber thickness based on ultrasonic measurements of males captured during 1993-96 from the Gulf of Alaska ( $F_{(9,60)} = 2.241, P = 0.031$ ), but there were no significant regional differences within years ( $F_{(1,60)} = 0.001, P = 0.973$ ), or among years within regions (Table 6). Females also exhibited variation in mean blubber thickness among years, regions and seasons ( $F_{(11,47)} = 2.178, P = 0.032$ ), and again there was no significant year and region interaction ( $F_{(1,60)} = 2.040, P = 0.104$ ), or any significant trends among years within regions (Table 7).

### *Blood chemistry and hematology*

Mean corpuscular volume (MCV) increased in seals within Prince William Sound during 1992-1996 (Figure 4) for both adult and juvenile age groups ( $F_{(3,153)} = 2.963$ ,  $P = 0.034$ ). There were no significant interannual differences detected among seals from the Kodiak Island ( $F_{(1,27)} = 0.155$ ,  $P = 0.697$ ) or southeast Alaska ( $F_{(1,20)} = 0.001$ ,  $P = 0.985$ ) regions (Figure 4). Mean corpuscular hemoglobin concentration (MCHC; Figure 5) varied with year among seals from Prince William Sound ( $F_{(3,277)} = 12.834$ ,  $P < 0.001$ ), and southeast ( $F_{(3,61)} = 6.506$ ,  $P = 0.001$ ), but not Kodiak ( $F_{(5,35)} = 0.390$ ,  $P = 0.852$ ). Within age group and season, there were no detectable differences in red blood cell count (Figures 6, 7) between years within Prince William Sound ( $F_{(4,131)} = 0.450$ ,  $P = 0.772$ ), the Kodiak archipelago ( $F_{(1,20)} = 0.013$ ,  $P = 0.912$ ), or southeast ( $F_{(1,17)} = 1.192$ ,  $P = 0.290$ ). Within age group and seasons, there was a significant interannual effect on hemoglobin concentration within Prince William Sound ( $F_{(2,135)} = 4.105$ ,  $P = 0.019$ ), but not within southeast Alaska ( $F_{(2,56)} = 0.781$ ,  $P = 0.463$ ) or Kodiak ( $F_{(13,27)} = 1.860$ ,  $P = 0.084$ ) regions (Figures 8, 9).

White blood cell counts (WBC) varied significantly with year in Prince William Sound ( $F_{(3,158)} = 3.125$ ,  $P = 0.028$ ) and southeast ( $F_{(2,46)} = 3.477$ ,  $P = 0.039$ ), but not among seals from Kodiak ( $F_{(3,38)} = 2.115$ ,  $P = 0.114$ ; Figure 10). Absolute neutrophil counts mirrored the WBC patterns (Figure 10), but interannual differences were not significant (Prince William Sound  $F_{(3,156)} = 2.212$ ,  $P = 0.089$ ; Kodiak Island  $F_{(1,27)} = 3.469$ ,  $P = 0.073$ ).

Plasma haptoglobin concentration (Figure 11) varied with year, season and age group in Prince William Sound ( $F_{(5,129)} = 4.460$ ,  $P = 0.001$ ), and with year and season in Kodiak regions ( $F_{(2,24)} = 7.109$ ,  $P = 0.004$ ), but there was no detected trend in southeast ( $F_{(2,65)} = 0.033$ ,  $P = 0.968$ ). There were significant year effects on ALT among all three regions (Figure 12; Prince William Sound  $F_{(4,185)} = 3.178$ ,  $P = 0.015$ ; Kodiak  $F_{(3,38)} = 4.221$ ,  $P = 0.011$ ; southeast  $F_{(3,89)} = 6.465$ ,  $P = 0.001$ ), but no differences among age groups (Prince William Sound  $F_{(1,185)} = 0.336$ ,  $P = 0.563$ ; Kodiak  $F_{(1,38)} = 0.668$ ,

$P = 0.419$ ; southeast  $F_{(1,89)} = 3.794$ ,  $P = 0.055$ ). Prince William Sound AST activities varied with year ( $F_{(5,179)} = 4.424$ ,  $P = 0.001$ ), and differed among age groups ( $F_{(3,179)} = 2.618$ ,  $P = 0.052$ ; Figure 12). Year and age group effects were not significant among seals from southeast or Kodiak regions (all  $P > 0.1$ ). Lactate dehydrogenase activities varied significantly with year (Figure 12) in Prince William Sound ( $F_{(5,163)} = 9.757$ ,  $P < 0.001$ ) and southeast ( $F_{(2,67)} = 3.040$ ,  $P = 0.054$ ), but not in Kodiak ( $F_{(3,38)} = 0.342$ ,  $P = 0.795$ ).

The number of seals that had greater than 3 statistically outlying plasma chemical or hematological values was about 21% of the numbers of seals sampled (Figure 13;  $r^2 = 0.366$ ,  $F_{(1,37)} = 0.404$ ,  $P = 0.004$ ). Seals sampled from Prince William Sound during spring/autumn of 1995, and autumn 1996 had fewer seals than expected from the overall relationship with  $\geq 4$  statistical outliers (Figure 13, data points k, l, q).

## DISCUSSION

### *Long-term comparisons of body condition*

There was no apparent long-term change in harbor seal body condition evident among the comparisons performed here. At least three interpretations exist for this finding; that changes in body condition occurred but were not detectable with the given sampling regime, methods, or indices; that weak evidence was found to support differential body condition differences between sexes; or that changes in condition truly did not occur among seals during this period. Comparisons of harbor seal body condition within the Gulf of Alaska during the past 30 years was complicated by the use of different sampling techniques, and sampling periods that varied with respect to season, year, and location. Collectively, these placed limitations on the comparisons that reduced sample sizes available for comparison. Seals collected during 1963-64 (Bishop 1967) and 1972-78 (Pitcher and Calkins 1983; Pitcher 1986) were measured postmortem. Though there were some collections in 1989 associated with the *Exxon Valdez* Oil Spill (Frost and Lowry 1994), the majority of body condition data collected during 1991-96 has been measured from live seals. Live-sampled seals not only tend to be about 10 cm shorter than

seals measured postmortem, but these measurements may also be more variable (Chapter 3). Thus, a raw comparison would have resulted in recently collected seals being apparently more massive at length than in prior decades. Though it may have erred on the conservative side, correction factors were not applied to live-captured seals for condition comparisons to avoid any other undetected bias introduced by live-measurements. Data contributed by the subsistence harvest and associated biosampling program during 1995-96 has produced the only directly comparable measurements. Thus, sample sizes available for comparison were low.

During 1972-78, an intensive study focusing on harbor seal biology throughout the Gulf of Alaska, with particular respect to issues surrounding potential offshore oil or mineral development, was designed and implemented by Pitcher and Calkins (1983). To maximize geographic coverage during the course of the research program, their sampling protocol covered different regions in each of the years of the study. Thus, not all locations were sampled in all years of the study, complicating any potential interannual and interregional effects. Harbor seal condition varies with gender, season, and region (Pitcher and Calkins 1983; Pitcher 1986; Chapter 3, this dissertation), and to avoid bias comparisons must be performed within these categories. Low statistical power was therefore associated with these long-term comparisons because of sample size alone. However, it is also possible that, because of the limited sampling, periods of relatively low condition among seals may not have been detected. For example, the population decline was so rapid that transitory periods of decreased body condition, if present, might have been undetected. If winter was a nutritionally stressful period, changes in condition resulting from this may have been undetected, and seals surviving into spring that were sampled could have represented the only portion of the population in adequate condition to survive.

It is also important to consider the generally poor utility of condition indices to reflect body condition (Chapter 3). Mass at length may be a useful indicator of relative condition (Trites and Bigg 1990), but it does not tend to reflect mass or length at age

(Chapter 3), a more sensitive indicator of body condition (Pitcher 1977, McLaren 1993). That is, seals may have been shorter and less massive, but still remain proportionally similar to seals in better condition. Thus, the lack of length-at-age data from recently collected seals precludes a definitive comparison to historic data. However, there did not appear to be differences in growth rates between 1964 and 1978, years prior to and during the population decline. Likewise, changes in sculp content are in general only moderately predicted by changes in xiphosternal blubber thickness (Pitcher 1986; Gales et al. 1994; Chapter 3). Thus, in addition to already low sample sizes for comparison, this relationship contributed to a lack of statistical power. Conversely, the body condition of free-ranging animals may only improve with environmental conditions to a certain level, beyond which improvements in food availability would not be evident in improved body condition, but rather in life-history traits such as improved survivorship or reproductive success.

Within these sampling limitations, no evidence was found of changed condition among harbor seals sampled before (1963/64), during (1973-78), and after (1989-96). Only one interannual comparison, 1977-78 for males collected during spring at Kodiak Island, showed a decline in mass at length between subsequent years (Table 1). Given the timing and proximity to large population declines, this difference is notable. However, females sampled during the same period did not show a similar pattern (Table 2). A trend for thicker xiphosternal blubber thickness among 1963/64 male seals relative to 1976-78 from Tugidak Island is also noteworthy (Figure 1). However, there may have been a seasonal bias to this sampling that could produce this difference. Bishop (1967) stated that seals were collected from April-July, but it was not possible to determine the exact sampling periods. Collection timing is an important consideration in blubber thickness comparisons, since differences of only 1-2 months could account for this magnitude of thickness change (Pitcher and Calkins 1983; Pitcher 1986). Pitcher and Calkins (1983) also noted the decreased blubber thickness of these seals relative to other areas, but were unable to determine its significance. Again, however, females did not show a similar

pattern (Figure 2). There was also no evidence of longer-term blubber thickness changes for males or females between 1973 and 1996.

If it is accepted that harbor seal body condition did not change throughout the periods of population decline, then this must be interpreted with respect to hypotheses regarding the decline. It is equally important to note that no evidence was found of increased harbor seal body condition throughout this time period. An increase in condition, coupled with a declining population, would have suggested that carrying capacity had not changed, and that other factors were affecting the population (Gerrodette and DeMaster 1990).

Assuming a constant environment, if predation, harvest pressure, infectious disease or contaminants accumulation were individually primarily responsible for the decline, body condition would not be expected to change. Thus, the findings of this study do not dismiss these factors as potential mechanisms for the decline. Though data are insufficient to evaluate the role of increased predation (Hoover-Miller 1994), fisheries take and subsistence harvest levels were determined to be too low to have been the likely primary factors initiating the declines (Small and DeMaster 1995). Likewise, no detectable differences in exposure to infectious diseases such as phocid herpesvirus, phocine distemper virus, *Brucella* spp., *Toxoplasma gondii*, and *Chlamydia psittaci* were detected between seals from southeast Alaska versus regions of decline (Lowry et al. 1996; Zarnke et al. 1997). Thus, the available evidence did not support these infectious diseases as being important in the decline of the surveyed populations. The role of contaminants in the decline is unknown, and studies examining this have just begun (Becker and Papa 1997).

No change in harbor seal body condition is also consistent with reductions in environmental carrying capacity (Gerrodette and DeMaster 1990). Reduced environmental carrying capacity in the Gulf of Alaska has been suspected as a result of an oceanic condition regime shift. In 1976/77 the sea surface temperature increased by about 2 °C (Niebauer 1997). This regime shift has been associated with major changes in

commercial and forage fish stock distribution, abundance, and composition (Alverson 1992; Mantua et al. 1997). Evidence for reductions in environmental carrying capacity relative to this regime shift is decreases in carbon stable isotope ( $\delta^{13}\text{C}$ ) abundance in comparisons of harbor seal tissues sampled during 1950-1996 (Hirons and Schell 1997). Though there were no differences in  $\delta^{15}\text{N}$  to indicate shifts in trophic level, decreases in  $\delta^{13}\text{C}$  during 1975-1996 were indicative of lower primary productivity and less energy available to the ecosystem after the regime shift. However, a direct link between primary productivity and harbor seal carrying capacity has not been established, and decreases in harbor seal  $\delta^{13}\text{C}$  may also have occurred if primary productivity remained stable, but was shifted to other consumers in the food web.

Steller sea lions (*Eumetopias jubatus*) populations have also undergone large declines in the Aleutians and western Gulf of Alaska (Loughlin et al. 1992). In contrast to harbor seals, sea lions sampled in the 1980's were less massive, shorter, and thinner at age than sea lions sampled during the 1970's (Calkins et al. 1997). It is possible that when age information becomes available from the 1995-96 subsistence harvest animals, a similar condition difference may be found. Conversely, reductions in the environmental carrying capacity for sea lions may have been greater than for harbor seals, if undernutrition was indeed a cause of the decline.

#### *Recent regional comparisons*

Comparisons among harbor seal populations from Kodiak Island, Prince William Sound, and southeast Alaska performed since 1989 have included blood profile analyses in addition to collection of condition index data, a methodology that may provide more sensitive indicators of seal health than gained from condition indices (McLaren and Smith 1985). This was evident from the interannual and interregional variability found among blood analytes relative to fewer indications of condition differences. It is important to note that while some seals did exhibit  $\geq 4$  statistically significant outlying blood analyte values, the distribution in year or location of these seals did not indicate that particular periods or locations experienced manifestations of poorer clinical health. Likewise, the

magnitude of population-level interannual and interregional variability among blood analytes were within the reference ranges established in Chapter 2.

As with long-term comparisons, there was little evidence of condition differences among the three regions during 1993-96. Kodiak Island seals tended to be more massive at length than at southeast Alaska or Prince William Sound, but this was evident only during autumn. Likewise, there was no apparent difference in sculp content based on ultrasonic blubber depth measurements among the three regions, or among years within regions. Similar problems of detection and statistical power that were noted for long-term comparisons also affect interpretation of these data. Most notable is the lack of sampling during winter periods. If seals were compromised in health or condition and failed to survive winter, then sampling surviving seals in spring would show a bias towards healthy and fit animals. However, this would require some acute mechanism by which compromised seals had a decreased likelihood of winter survival. If there were a wide-spread incidence of winter undernutrition, this should have been reflected in spring condition indices or blood chemistries, but this was not observed.

A second sampling error consideration was whether seals that were compromised in health or condition had the same likelihood of being captured as 'healthy' seals. For example, if compromised seals hauled-out in different locations, or did not haul-out at all, they would be underrepresented in the sampling. Based on the occurrence of seals with  $\geq 4$  statistically outlying blood analytes, a number that is unlikely based on probability to occur by chance (Chapter 2), sampling of this category did occur. However, since these seals were detected based on statistically generated reference ranges, rather than from reference ranges derived from comparisons of seals with known health status, inferences of relative clinical health must be made cautiously. For example, no evidence of chronic effects that influence liver function were found specific to Prince William Sound. Indeed, most interannual trends among liver enzymes were similar among regions, suggesting that either similar environmental effects were operating over all regions, or that some change was occurring in laboratory assay techniques over time.



If there were no differences in body condition among harbor seals from the three regions, then as for long-term comparisons, this must be interpreted with respect to alternate hypotheses regarding regional differences in population trends. There seem to be no differential exposures to a suite of infectious diseases that could account for population declines among the three regions (Zarnke et al. 1997), though the role of contaminants are still being evaluated (Becker and Papa 1997). The effect of commercial fisheries incidental mortality, however, is currently unknown due to a lack of fisheries observers in nearshore fisheries (Small and DeMaster 1995). Likewise, though subsistence harvest rates have been relatively constant during 1992-95 (Wolf and Mishler 1996), the impact could increase as local populations decrease (Hoover-Miller 1994). Currently there is no consensus on whether human-caused mortality is contributing to regional declines (Small and DeMaster 1995). As with the long-term population declines, the finding of no change in body condition does not disprove these mechanisms.

However, combining the lack of condition differences with a population decline in Prince William Sound suggests that differences in carrying capacity could exist. Prince William Sound seal populations have been declining while southeast Alaska populations have remained stable, and Kodiak Island seal populations may have been increasing throughout the duration of this study (Small 1996). Thus, this suggests the possibility that Prince William Sound harbor seal carrying capacity continued to decline, while remaining stable in southeast Alaska but increasing, or greatly increasing (based on slightly higher mass at length for Kodiak seals) for Kodiak Island (Gerrodette and DeMaster 1990). This is also consistent with the lack of population recovery following the *Exxon Valdez* Oil Spill.

Multitudes of factors can influence blood parameters, and regional and temporal effects were detected on harbor seal blood parameters (Chapter 2). Considerable other data regarding oceanography, fish abundance, and harbor seal biology has been collected within Prince William Sound following the *Exxon Valdez* Oil Spill in March of 1989.

Thus, blood chemistry interpretations presented here will primarily focus on seals sampled within Prince William Sound, which also had the best sampling coverage for this study.

Throughout the 1993-96 sampling period, Prince William Sound has experienced two different types of spring blooms in response to small changes in air temperature and wind speed (Eslinger 1997). In 1993 and 1996, spring phytoplankton blooms were earlier and rapid, causing most of the production to fall to the benthic system. In contrast, spring blooms during 1994 and 1995 were later and of longer duration, hence primary productivity was better coupled with zooplankton abundance, and the productivity remained in the pelagic system (Eslinger 1997). These differences in spring productivity should ultimately affect the abundance of forage fish species for harbor seals. Herring biomass in the Sound increased throughout the 1980's, but crashed after 1992, and declined through 1995 (Bailey et al. 1995; Thornton and Eslinger 1997). In contrast, pollock (*Theragra chalcogramma*) biomass has steadily increased in Prince William Sound throughout 1993-97 (Thomas 1997).

Harbor seal abundance has also fluctuated throughout 1993-96, based on aerial surveys conducted during August and September (Frost et al. 1997). Abundance on Prince William Sound haulouts was relatively higher in 1993 and 1995 compared to 1994 and 1996 (Frost et al. 1997). There were also changes in traveling, and presumably foraging, habits of harbor seals. Prior to autumn 1995, only two of 30 satellite-tagged harbor seals swam on extended trips out of Prince William Sound (Frost et al. 1997). However, in 1996 seven of eight tracked seals left Prince William Sound to apparently forage in the Gulf of Alaska (Frost et al. 1997). Presumably associated with this change in foraging patterns was a different diet composition in 1996 relative to 1994-95, based on fatty-acids analyses (Frost et al. 1997). Diet also varied with age group, with fatty acid profiles from juvenile harbor seals suggesting diets of capelin, sandlance, and herring (Frost et al. 1997). In contrast, adults fed on capelin, but also pollock, herring, and yellowfin sole.

Blood values and condition indices showed direct or indirect fluctuations with many of the above environmental and behavioral trends (Table 8), indicating that blood profiles were sensitive to environmental changes. Additionally, trends of most blood factors differed among juveniles and adults. In particular, interannual trends of hemoglobin concentration showed opposite directional changes among the two age groups (Figure 8), and liver enzyme activities varied more interannually among juveniles than among adults (Figure 12). Increases in summer-autumn seal haul-out abundance were associated with decreased mass relative to length (males), decreased MCHC, and decreased plasma haptoglobin (adults) during spring. Conversely, decreased seal abundance was associated with increased mass relative to length (males), increased MCHC, and increased plasma haptoglobin during spring (Table 8).

Recent studies of harbor seals have found changes in certain blood parameters relative to diet or contaminant intake (Schumacher et al. 1995, de Swart et al. 1995, Thompson et al. 1997). Thompson et al. (1997) described changes in harbor seal hematologies relative to 'good' (>50% of diet) and 'bad' (<11% of diet) clupeid years. In good clupeid years, they recorded increased WBC (2% absolute increase) and neutrophilia ( $\sim 2 \times 10^{12}/L$  increase), and in bad clupeid years they measured decreased RBC ( $\sim 0.4 \times 10^9/L$ ), hemoglobin (1-2 g/dL), MCHC ( $\sim 3$  g/L), and increased MCV (6-16 fL). An apparent macrocytic anemia was hypothesized to result from anti-metabolites contained within gadids that restrict iron absorption (Thompson et al. 1997). De Swart et al. (1995) found increases in WBC, absolute neutrophil count, and RBC in response to a diet of herring from heavily polluted waters, while other blood parameters did not change significantly. As with Thompson et al. (1997), all values remained within established normal ranges, and as such would not typically be detected as clinically significant in a single-sample assessment.

When Prince William Sound harbor seal blood chemistries are compared among juvenile and adult age classes, trends become evident that were consistent with dietary differences. As noted above, Frost et al. (1997) suggested dietary differences between

juvenile and adult harbor seals; only the adult diets included pollock and sole. Comparing blood profiles of adults and juveniles from within Prince William Sound, juveniles had elevated white blood cell and neutrophil counts, higher red blood cell counts, higher hemoglobin concentrations, elevated MCHC, and decreased MCV. These findings were consistent with those expected from seals with greater gadid dietary components, based on Thompson et al. (1997). However, they may also have been developmental differences, and this possibility will be addressed in future studies involving pups and juveniles (Trumble and Castellini 1997). The range of increase observed in MCV and the magnitude of fluctuations of MCHC, WBC and neutrophil counts within Prince William Sound were comparable or larger than the differences found due to diet (Thompson et al. 1997) or contaminants intake (de Swart et al. 1995). Further interpretation of blood profiles of these factors can be attained by reducing the geographic level of comparison to haul-out locations, rather than broad geographic regions. Harbor seals have been shown to generally forage local to haul-out sites (Frost et al. 1995), and dietary patterns were found to vary between haul-outs as close together as 15 km (Iverson et al. 1997).

Zenteno-Savin et al. (1997) found elevated haptoglobin levels among seals from Prince William Sound relative to southeast Alaska, and concluded that some stressor was differentially affecting the populations. With broader interannual and seasonal comparisons, it is evident that stressor effects, if any, were occurring differentially among age groups, seasons and regions (Figure 11). The only consistent elevation of haptoglobin level of seals from Prince William Sound over all other sites was among juveniles sampled during spring. However, haptoglobin levels were apparently inversely related to harbor seal haul-out abundance, lower when greater numbers of seals were hauled-out (Table 8). This was consistent with a larger-scale trend for increased haptoglobin levels in areas of Steller sea lion decline relative to areas of population stability (Zenteno-Savin et al. 1997). However, because haptoglobin scavenges hemoglobin from the blood, these patterns were also consistent with levels of hemoglobin among harbor seals.

In long-term studies of blood chemistry and hematology, changes in clinical laboratory techniques or methods could generate seemingly significant changes in values. An ideal control would be a perpetually-maintained plasma solution of known analyte concentrations. In the absence of this, however, it seems unlikely that laboratory precision changes occurred since juvenile and adult seals, and often different regions sampled and analyzed during the same periods showed different patterns. It is premature to speculate about possible causative mechanisms underlying the observed patterns, and it may never be possible to produce anything but a list of differential possibilities. However, clear differences were evident among juveniles and adults, and among regions. Continued monitoring of blood profiles with respect to population trends could provide useful information regarding health status.

#### *Conclusions and future directions*

Blood chemistries were found to vary with handling technique, gender, and regional and temporal effects, and reference ranges were constructed for detection of outliers with respect to the overall population. However, blood profiles were also sensitive to environmental factors and aspects of seal behavior. More detailed examination of the interrelationship between body condition, blood chemistry and hematology, and endocrinology and diet need to be performed in captive studies. Since dehydration and water balance are a critical components of many diseases, details of electrolyte balance and water regulatory hormones with respect to geographic location should also be examined.

Morphometric indices of condition were generally poor predictors of condition, and sampling techniques limited the direct comparison of measurements taken before, during, and after population declines. Though sample sizes for comparison were limited, no evidence was found for changes in body condition of harbor seals sampled during 1963/64, 1972-78, and 1989-1996. There may have been evidence of a decline in body mass relative to length among male seals sampled during spring between 1976 and 1977, and for thinner blubber in 1976-78 relative to 1963-64. These differences were not

apparent, however, among females sampled during the same periods. Thus, one plausible conclusion based on population declines and no apparent change in body condition was that environmental carrying capacity had in fact decreased near the period of an oceanic “regime-shift” in 1976. However, other potential causes or contributors to the decline, such as increased predation, fisheries mortality, or subsistence harvest were not discounted by this finding of no body condition change.

A similar conclusion was proposed for current differences in population trajectories among Prince William Sound, Kodiak Island, and southeast Alaska. There was no evidence of condition differences among these regions, though Kodiak Island seals had a tendency to be slightly larger at length. Seal blubber from Prince William Sound, however, was slightly more hydrated, and thus had slightly more energy content than seals from southeast Alaska. This difference was small, only about a 1% difference in blubber energy stores for a 50 kg seal. Thus, population declines in Prince William Sound with the absence of condition change is also suggestive of a decreased environmental carrying capacity for harbor seals, or of factors other than nutritional limitation, such as predation or human-caused mortality, being important. Analyses of blood profile data found regional and interannual trends that differed among juvenile and adult age classes, yet there was no indication that any region or time period showed greater than expected numbers of seals with blood values exceeding reference range limits. There were changes in blood profiles that were consistent with differences in chemistries between seals thought to be feeding primarily on gadids versus seals with no gadid component. A complicating factor in the detection of health problems with harbor seals during 1989-1996 was a lack of sampling during winter, and of limited sampling of young age classes. Thus, the conclusions of this study were limited to subadult and adults captured during spring, summer, and autumn.

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Table 1. Interannual comparisons of male harbor seal body mass (kg) adjusted for size ( $L^3$ ). Seals measured postmortem during 1963-96. Least squares means results of analysis of covariance within regions. Thus, mean values can be compared interannually and interseasonally within, but not between, regions.

		1963	1973	1975	1976	1977	1978	1979	1985	1989	1990	1996	F	P
<b>Prince William Sound</b>														
Spring	$\bar{x}$			114.3 <sup>a</sup>							116.8 <sup>c</sup>	109.4	1.670	0.196
	se			2.3							2.9	3.6		
	n			35							5	4		
Summer	$\bar{x}$		113.0	117.4						122.6			1.373	0.285
	se		5.6	2.5						4.5				
	n		1	15						2				
Autumn	$\bar{x}$		116.1 <sup>a</sup>	113.7 <sup>a</sup>						117.1		115.4	1.121	0.345
	se		3.6	2.3						4.4		3.1		
	n		4	15						1		5		
-----														
<b>Kodiak Archipelago</b>														
Spring	$\bar{x}$				144.9 <sup>a</sup>	135.8 <sup>a</sup>							5.963	0.004
	se				3.7	3.0								
	n				13	34								
Summer	$\bar{x}$	134.2 <sup>d</sup>					137.2 <sup>a</sup>			132.0 <sup>c</sup>			0.553	0.585
	se	3.5					4.5							
	n	15					5			1				
Autumn	$\bar{x}$				131.9 <sup>a</sup>		133.8 <sup>a</sup>						0.232	0.634
	se				3.3		5.0							
	n				23		7							
-----														
<b>Bering Sea</b>														
Spring	$\bar{x}$							55.0 <sup>b</sup>	46.8 <sup>b</sup>				2.429	0.136
	se							2.2	4.6					
	n							10	2					

<sup>a</sup>Pitcher (1977), Pitcher and Calkins (1983).

<sup>b</sup>Frost and Lowry, unpublished data.

<sup>c</sup>Frost and Lowry (1994).

<sup>d</sup>Bishop (1967).

Table 2. Interannual comparisons of female harbor seal body mass (kg) adjusted for size ( $L^3$ ). Seals measured postmortem during 1963-96. Least squares means results of analysis of covariance within regions. Thus, mean values can be compared interannually and interseasonally within, but not between, regions.

		1963	1972	1973	1975	1976	1977	1978	1979	1985	1989	1990	1995	1996	F	P
<b>Prince William Sound</b>																
Spring	$\bar{x}$				108.1 <sup>a</sup>							107.7 <sup>a</sup>			0.395	0.536
	se				3.3							6.0				
	n				22							2				
Summer	$\bar{x}$			105.2 <sup>a</sup>	103.1 <sup>a</sup>						98.4 <sup>c</sup>				2.286	0.144
	se			4.6	4.1						4.6					
	n			4	6						6					
Autumn	$\bar{x}$			101.2 <sup>a</sup>	98.3 <sup>a</sup>									99.9	0.220	0.804
	se			3.8	3.7									4.3		
	n			11	10									9		
-----																
<b>Southeast Alaska</b>																
Autumn	$\bar{x}$		43.2 <sup>a</sup>										46.0		0.238	0.635
	se		2.6										4.6			
	n		10										4			
-----																
<b>Kodiak Archipelago</b>																
Spring	$\bar{x}$					127.6 <sup>a</sup>	125.3 <sup>a</sup>								0.002	0.966
	se					4.5	4.2									
	n					7	44									
Summer	$\bar{x}$	115.5 <sup>d</sup>						112.4 <sup>a</sup>				117.2 <sup>c</sup>			0.418	0.661
	se	4.5						4.8				9.1				
	n	24						12				2				
Autumn	$\bar{x}$					116.3 <sup>a</sup>		117.0 <sup>a</sup>							0.176	0.840
	se					3.9		5.8								
	n					28		9								

<sup>a</sup>Pitcher (1977), Pitcher and Calkins (1983).

<sup>b</sup>Frost and Lowry, unpublished data.

<sup>c</sup>Frost and Lowry (1994).

<sup>d</sup>Bishop (1967).

Table 2, continued.

		1963	1972	1973	1975	1976	1977	1978	1979	1985	1989	1990	1995	1996	<i>F</i>	<i>P</i>
<b>Bering Sea</b>																
Spring	$\bar{x}$								67.8 <sup>b</sup>	58.8 <sup>b</sup>					4.860	0.055
	se								2.3	3.3						
	<i>n</i>								8	4						
Summer	$\bar{x}$									60.2				52.2	0.674	0.458
	se									5.1				8.2		
	<i>n</i>									5				2		

<sup>a</sup>Pitcher (1977), Pitcher and Calkins (1983).

<sup>b</sup>Frost and Lowry, unpublished data.

<sup>c</sup>Frost and Lowry (1994).

<sup>d</sup>Bishop (1967).



Table 3. Least squares means xiphosternal blubber thickness (cm) of male harbor seals collected within Prince William Sound. Results of analysis of covariance adjusted for body size ( $L^3$ ). Data from 1973/75 from Pitcher and Calkins (1983) and Pitcher (1986).

		1973	1975	1996	<i>F</i>	<i>P</i>
Spring	$\bar{x}$	2.9	2.6	2.5	1.186	0.326
	se	0.2	0.1	0.2		
	<i>n</i>	4	15	5		
Autumn	$\bar{x}$		2.9	2.7	1.344	0.253
	se		0.1	0.2		
	<i>n</i>		35	11		

Table 4. Least squares means xiphosternal blubber thickness (cm) of male harbor seals collected within southeast Alaska. Results of analysis of covariance adjusted for body size ( $L^3$ ). Data from 1972 from Pitcher and Calkins (1983) and Pitcher (1986).

		1972	1975	1995	1996	<i>F</i>	<i>P</i>
Spring	$\bar{x}$		2.8		3.4	3.217	0.100
	se		0.3		0.2		
	<i>n</i>		3		11		
Autumn	$\bar{x}$	3.4		3.1		0.251	0.651
	se	0.4		0.4			
	<i>n</i>	3		3			

Table 5. Least squares means xiphosternal blubber thickness (cm) of female harbor seals collected within southeast Alaska. Results of analysis of covariance adjusted for body size ( $L^3$ ). Data from 1972 from Pitcher and Calkins (1983) and Pitcher (1986).

		1972	1975	1995	1996	<i>F</i>	<i>P</i>
Spring	$\bar{x}$		3.6		3.5	0.014	0.909
	se		0.5		0.3		
	<i>n</i>		3		8		
Autumn	$\bar{x}$	3.2		3.0		0.232	0.647
	se	0.2		0.2			
	<i>n</i>	5		5			

Table 6. Mean blubber depth (cm) of at least 5 body sites for male harbor seals captured during 1993-96, based on ultrasound measurements.

		1993	1994	1995	1996	<i>F</i>	<i>P</i>
<b>Prince William Sound</b>							
Spring	$\bar{x}$	2.7		2.5	2.6	0.659	0.526
	se	0.3		0.1	0.1		
	<i>n</i>	4		12	14		
Autumn	$\bar{x}$		2.6	2.3	2.0	0.752	0.490
	se		0.4	0.3	0.1		
	<i>n</i>		2	8	7		
<b>Southeast</b>							
Spring	$\bar{x}$						
	se						
	<i>n</i>						
Autumn	$\bar{x}$	2.3			2.1		
	se	0.1			0.1		
	<i>n</i>	8			11		
<b>Kodiak Island</b>							
Spring	$\bar{x}$			2.1			
	se			0.1			
	<i>n</i>			4			
Autumn	$\bar{x}$			2.3	2.5	0.488	0.496
	se			0.2	0.2		
	<i>n</i>			4	12		

Table 7. Mean blubber depth (cm) of at least 5 body sites for female harbor seals captured during 1993-96, based on ultrasound measurements.

		1993	1994	1995	1996	<i>F</i>	<i>P</i>
<b>Prince William Sound</b>							
Spring	$\bar{x}$	2.4		2.3	2.8	1.953	0.174
	se	0.2		0.1	0.2		
	<i>n</i>	2		9	8		
Autumn	$\bar{x}$		2.4	1.9	2.1	2.130	0.141
	se		0.1	0.2	0.2		
	<i>n</i>		6	11	10		
<b>Southeast</b>							
Autumn	$\bar{x}$				2.2		
	se				0.3		
	<i>n</i>				4		
<b>Kodiak Island</b>							
Spring	$\bar{x}$			2.7			
	se			0.3			
	<i>n</i>			3			
Autumn	$\bar{x}$			2.5	2.2	1.031	0.349
	se			0.2	0.4		
	<i>n</i>			5	3		

Table 8. Interannual changes in environmental factors and selected morphometric and hematological values for Prince William Sound, during 1993-96.

	1993	1994	1995	1996
Phytoplankton bloom <sup>a</sup>	↓	↑	↑	↓
Herring biomass <sup>b</sup>	↓	↓	↓	↑
Pollock biomass	↑	↑	↑	↑
Seal abundance <sup>c</sup>	↑	↓	↑	↓
Seal diet <sup>d</sup>		=	=	△
Seal mass (males)	↓	↑	↓	↑
Seal mass (females)	↔	↔	↔	↔
MCV	↓	↑	↓	↑
MCHC	↓	↑	↓	↑
Hemoglobin (juv. males)	↓	↑	↓	↑
Hemoglobin (ad. males)	↑	↓	↑	
Haptoglobin (juv.)		↔	↔	↑
Haptoglobin (ad.)		↑	↓	↑
WBC (juv.)	↓	↑	↓	↔
WBC (ad.)	↓	↑	↔	↔
Rel. neutrophils (juv.)	↑	↓	↑	↔
Rel. neutrophils (ad.)	↑		↑	↑

<sup>a</sup>↓ denotes spring productivity fell to benthos, ↑ spring productivity remained in pelagic system (Eslinger 1997).

<sup>b</sup>For other categories, ↓ denotes relatively lower, ↑ relatively higher, and ↔ no change relative to other values.

<sup>c</sup>From Table 6 of Frost et al. (1997).

<sup>d</sup>= equivalent diet composition, △ dietary change (Frost et al. 1997).

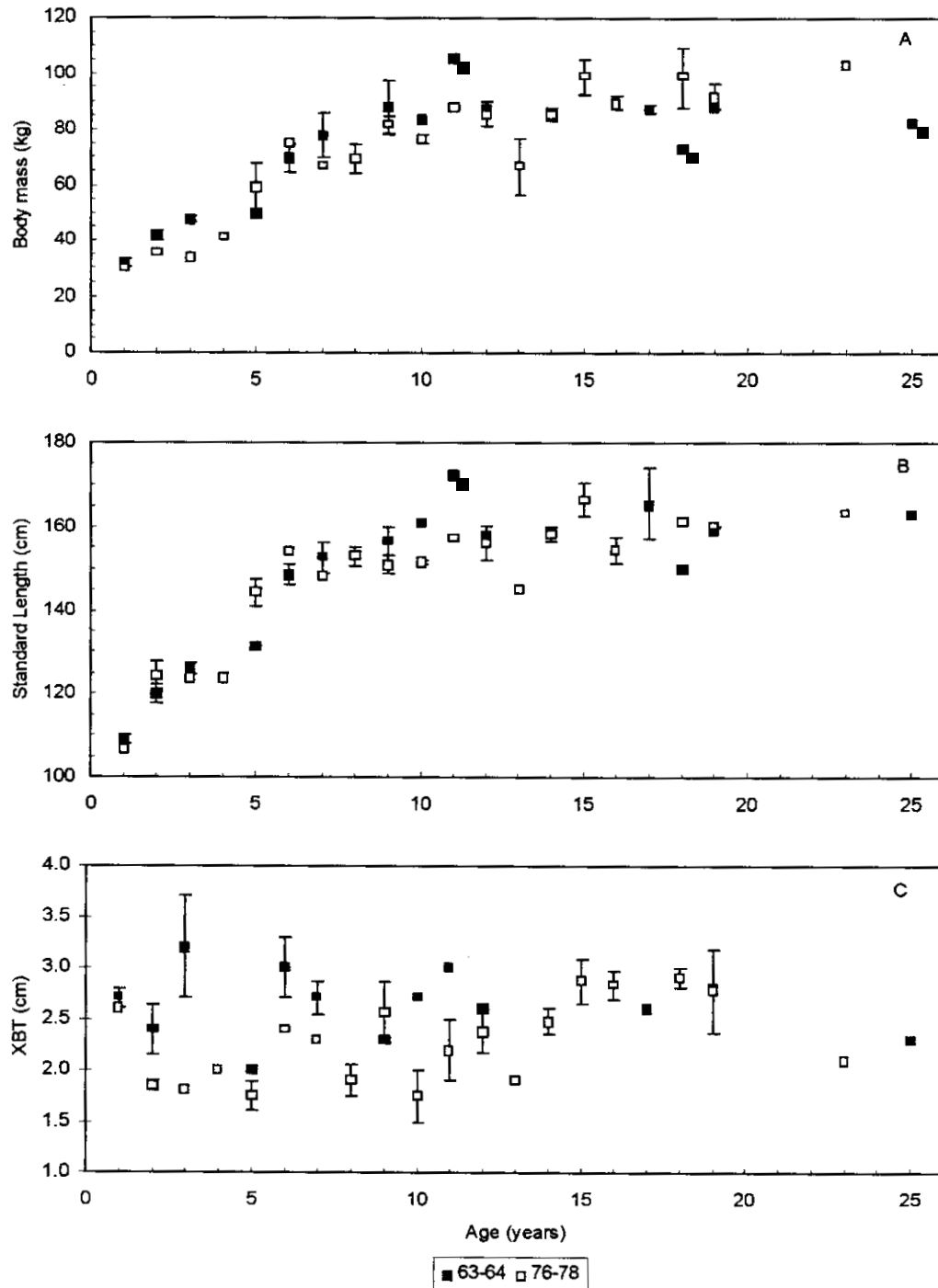


Figure 1. Body mass (a), length (b), and xiphosternal blubber thickness (XBT) (c) of male harbor seals collected from the Kodiak Island region in 1963/64 (Bishop 1967) and 1976-78 (Pitcher and Calkins 1983). Error bars equal  $\pm 1$  SE, if no error bar then  $n = 1$ .

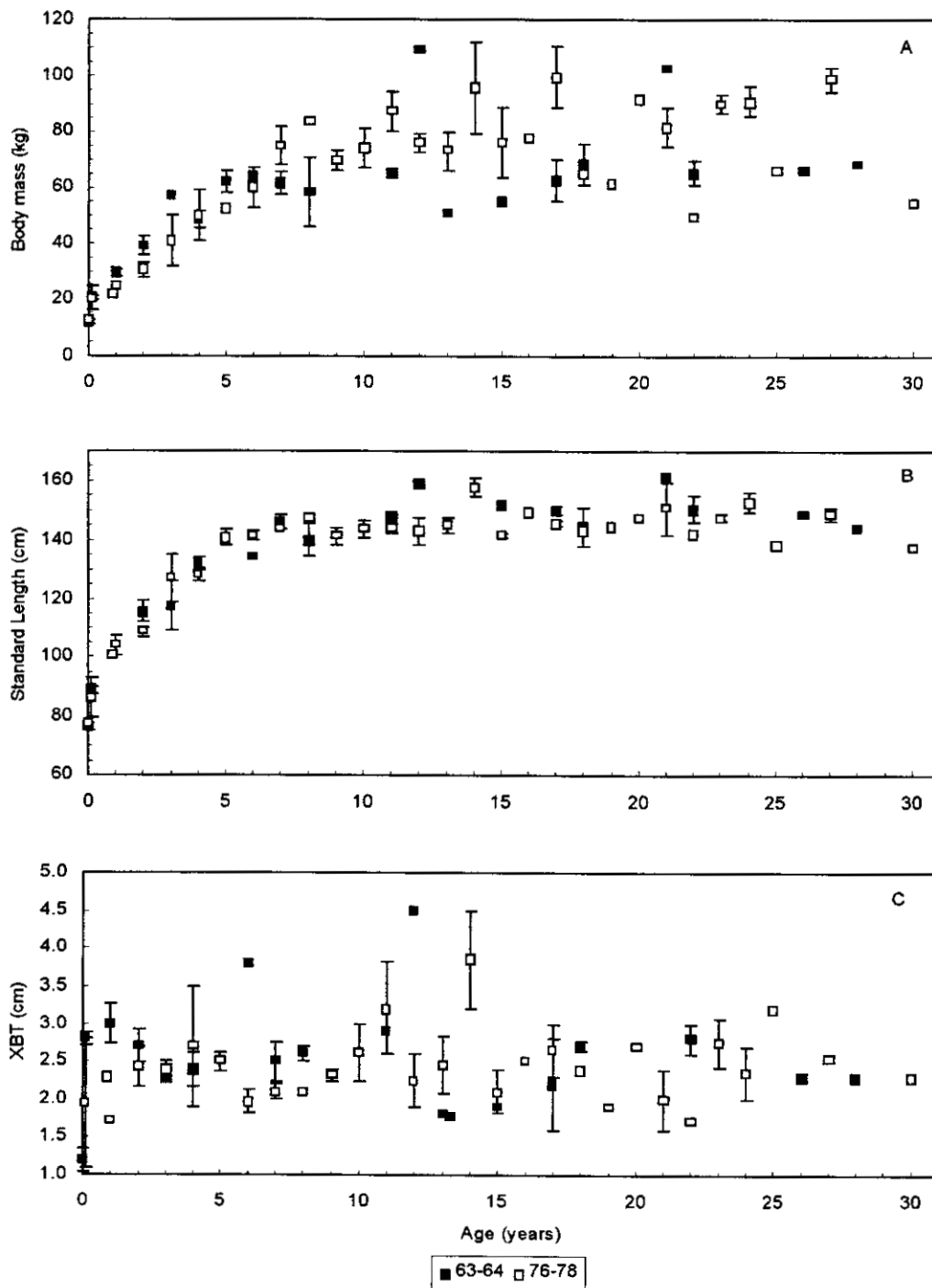


Figure 2. Body mass (a), length (b), and xiphosternal blubber thickness (XBT) (c) of female harbor seals collected from the Kodiak Island region in 1963/64 (Bishop 1967) and 1976-78 (Pitcher and Calkins 1983). Error bars equal  $\pm 1$  SE, if no error bar then  $n = 1$ .

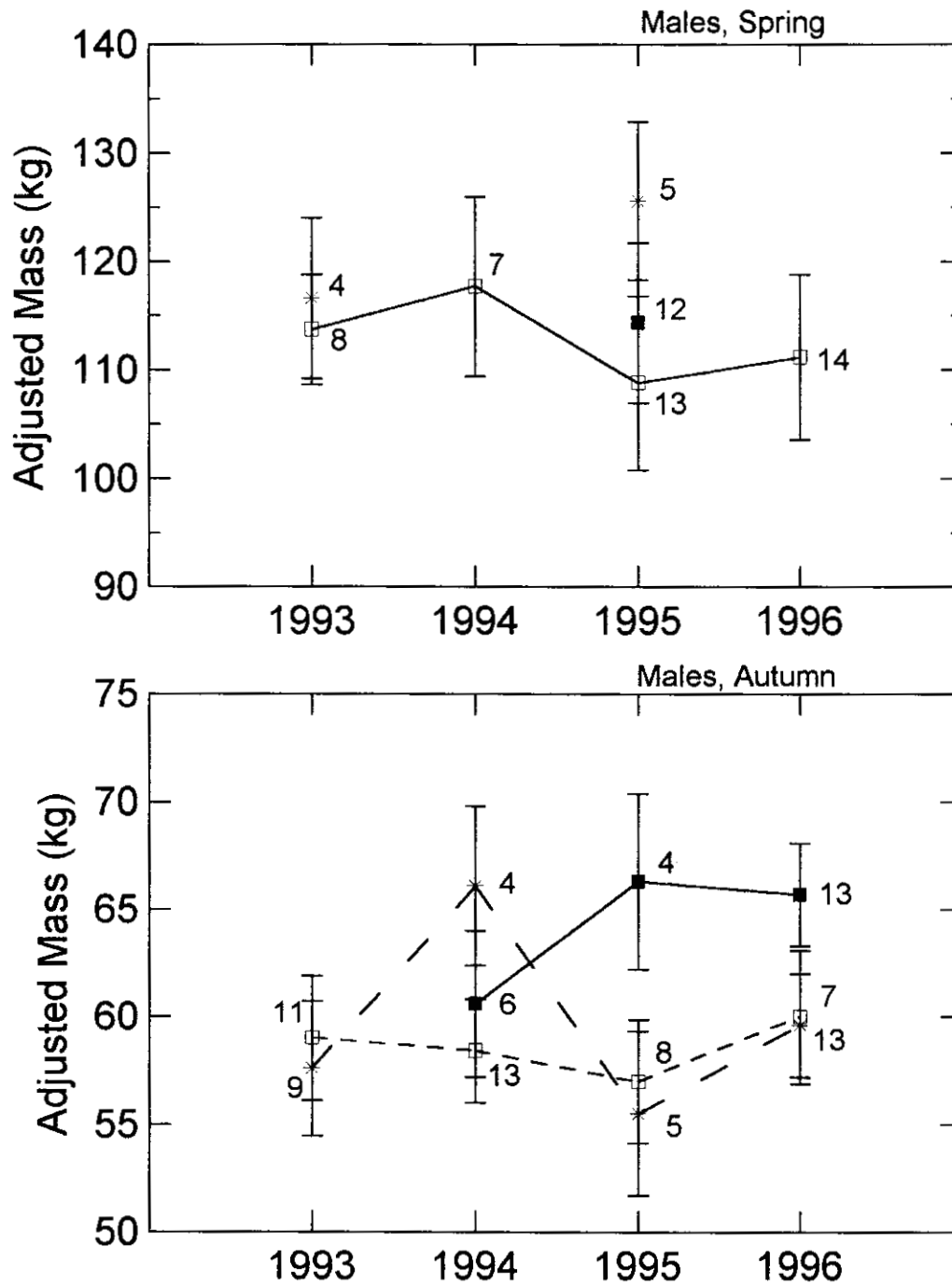


Figure 3. Mass index (adjusted with  $L^3$  as a covariate) of male and female harbor seals captured in spring and autumn. Seals captured from Kodiak Island (■), Prince William Sound (□), and southeast Alaska (\*), regions. Sample sizes noted.

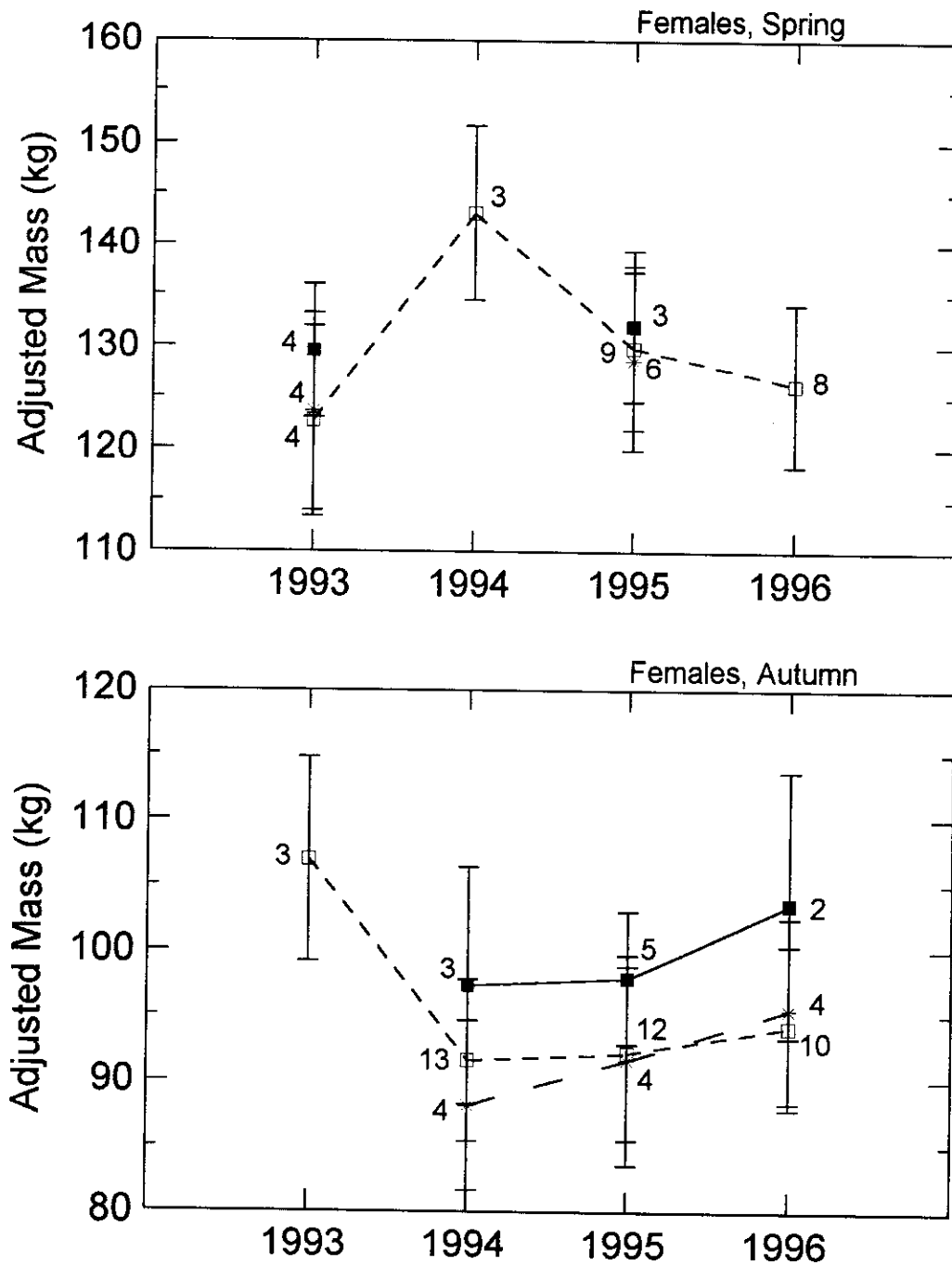


Figure 3, continued. Mass index (adjusted with  $L^3$  as a covariate) of male and female harbor seals captured in spring and autumn. Seals captured from Kodiak Island (■), Prince William Sound (□), and southeast Alaska (\*) regions. Sample sizes noted.



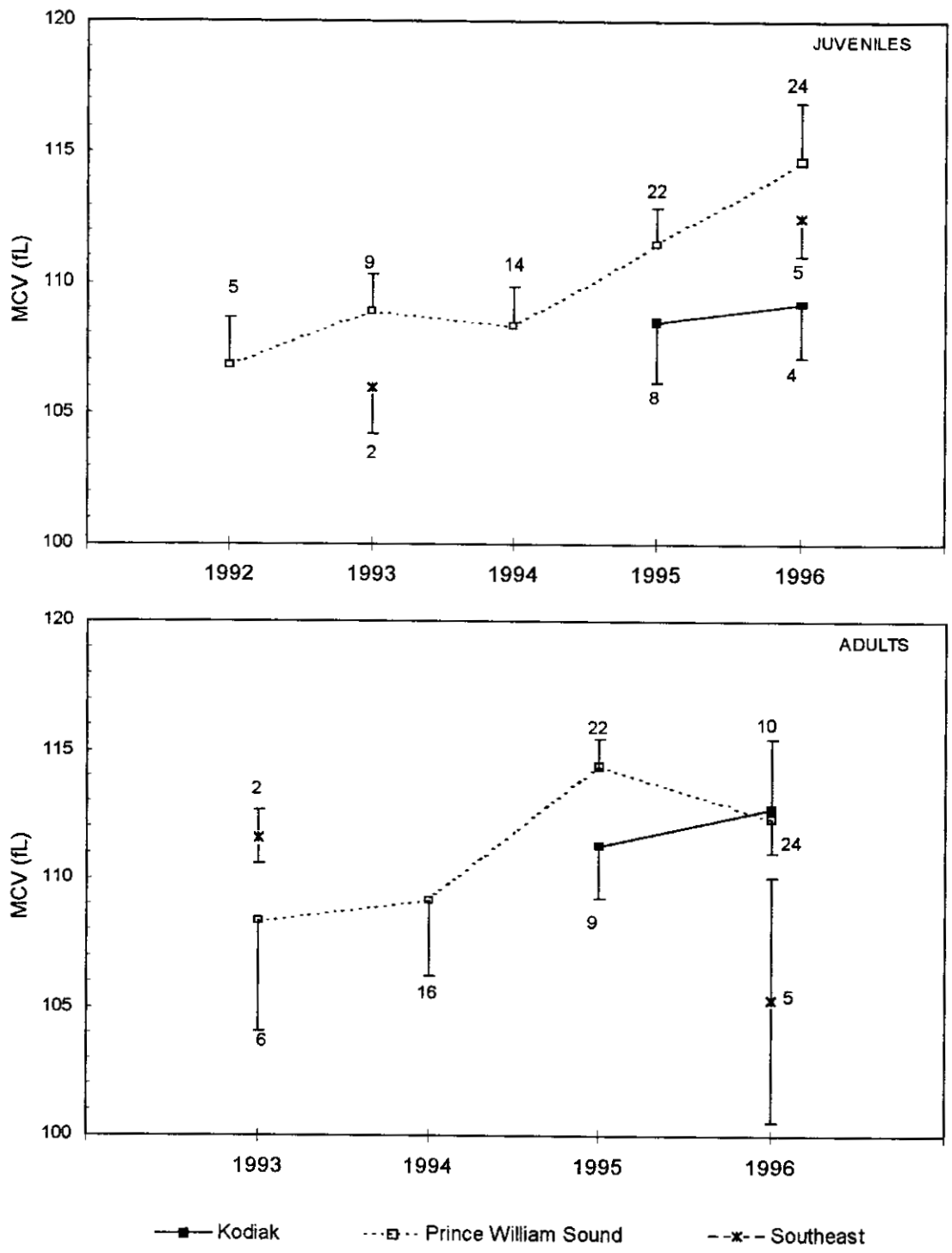


Figure 4. Interannual and regional comparisons of juvenile and adult harbor seal mean corpuscular volume (MCV).

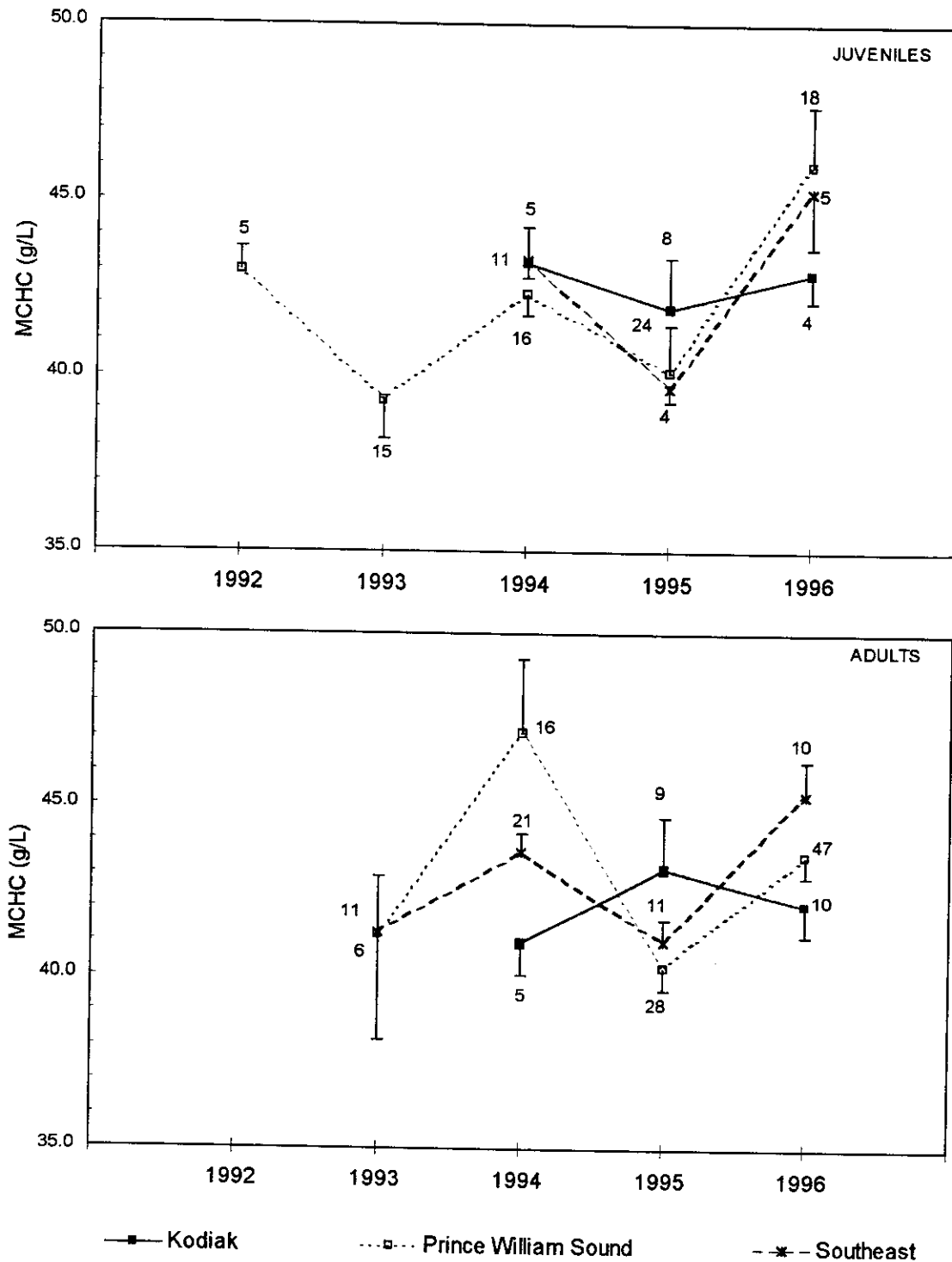


Figure 5. Interannual and regional comparisons of juvenile and adult harbor seal mean corpuscular hemoglobin concentration (MCHC).

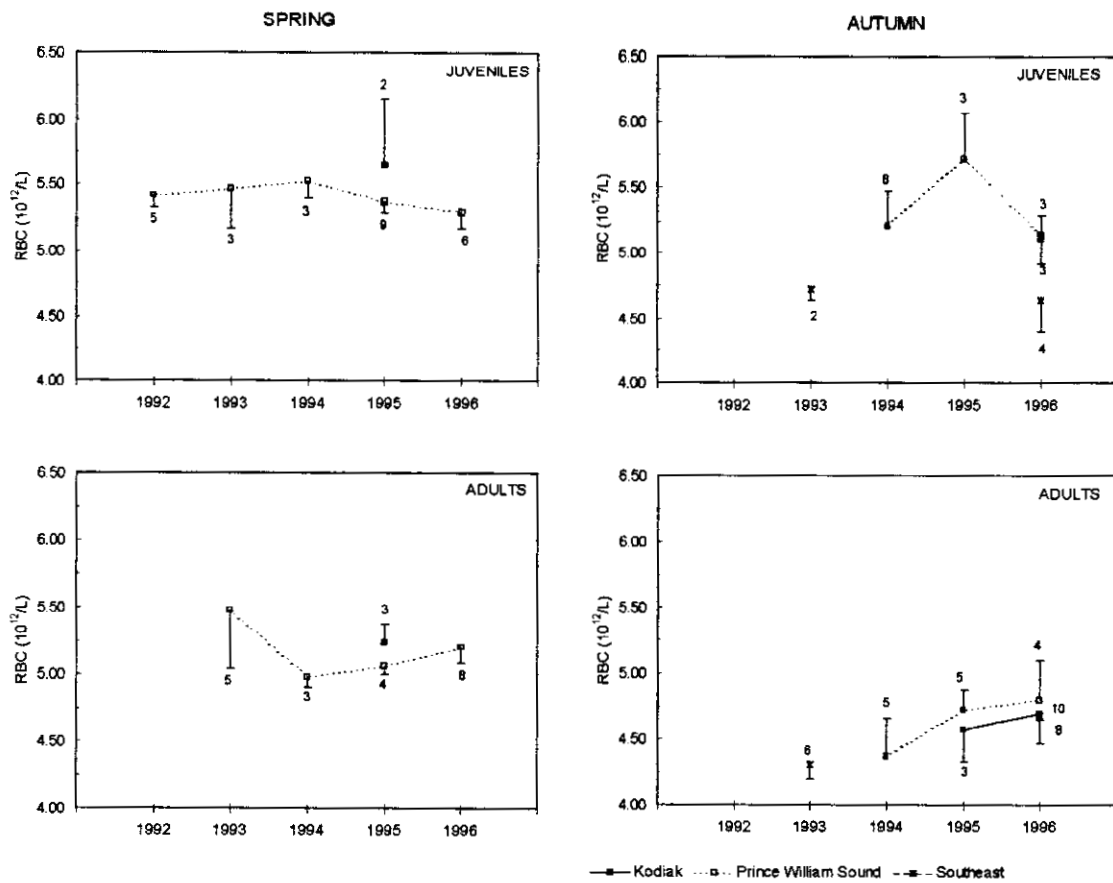


Figure 6. Interannual and regional comparisons of male harbor seal red blood cell count (RBC), during spring and autumn.

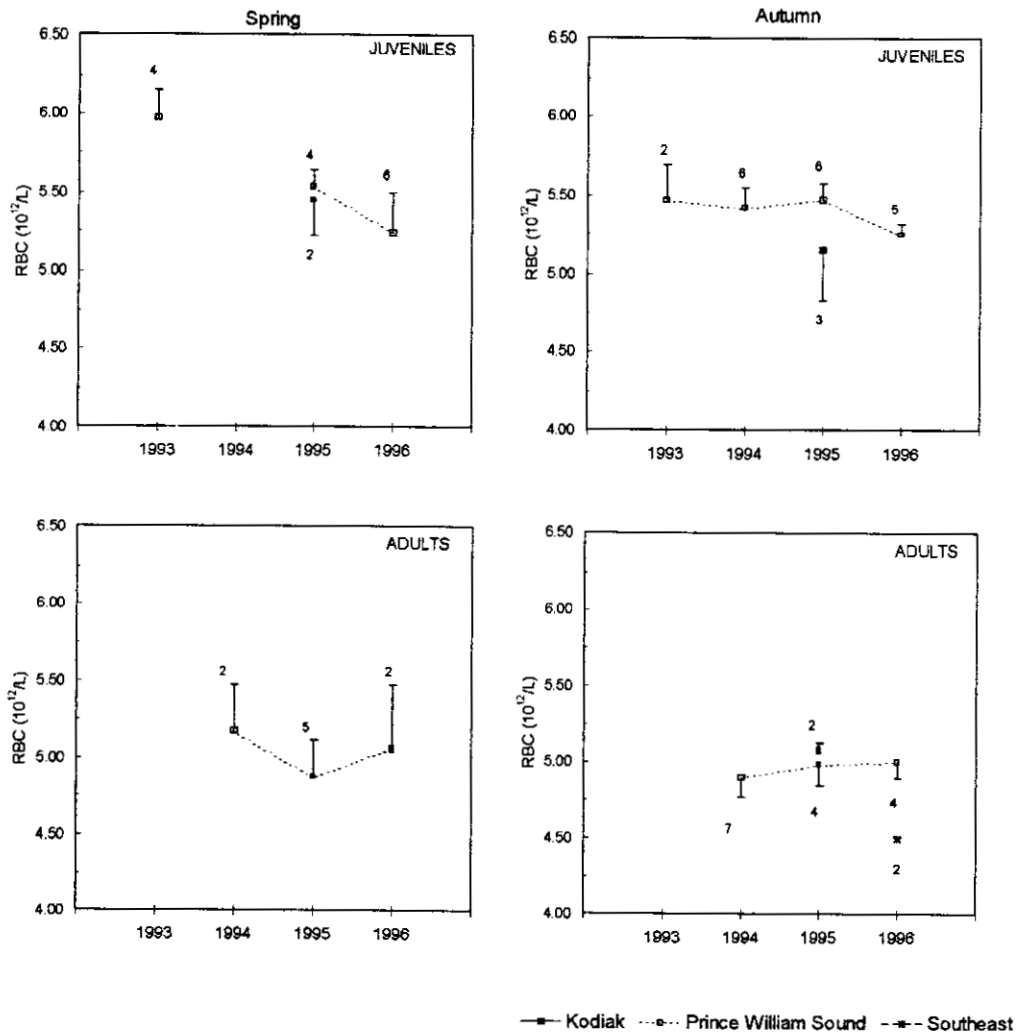


Figure 7. Interannual and regional comparisons of female harbor seal red blood cell count (RBC), during spring and autumn.

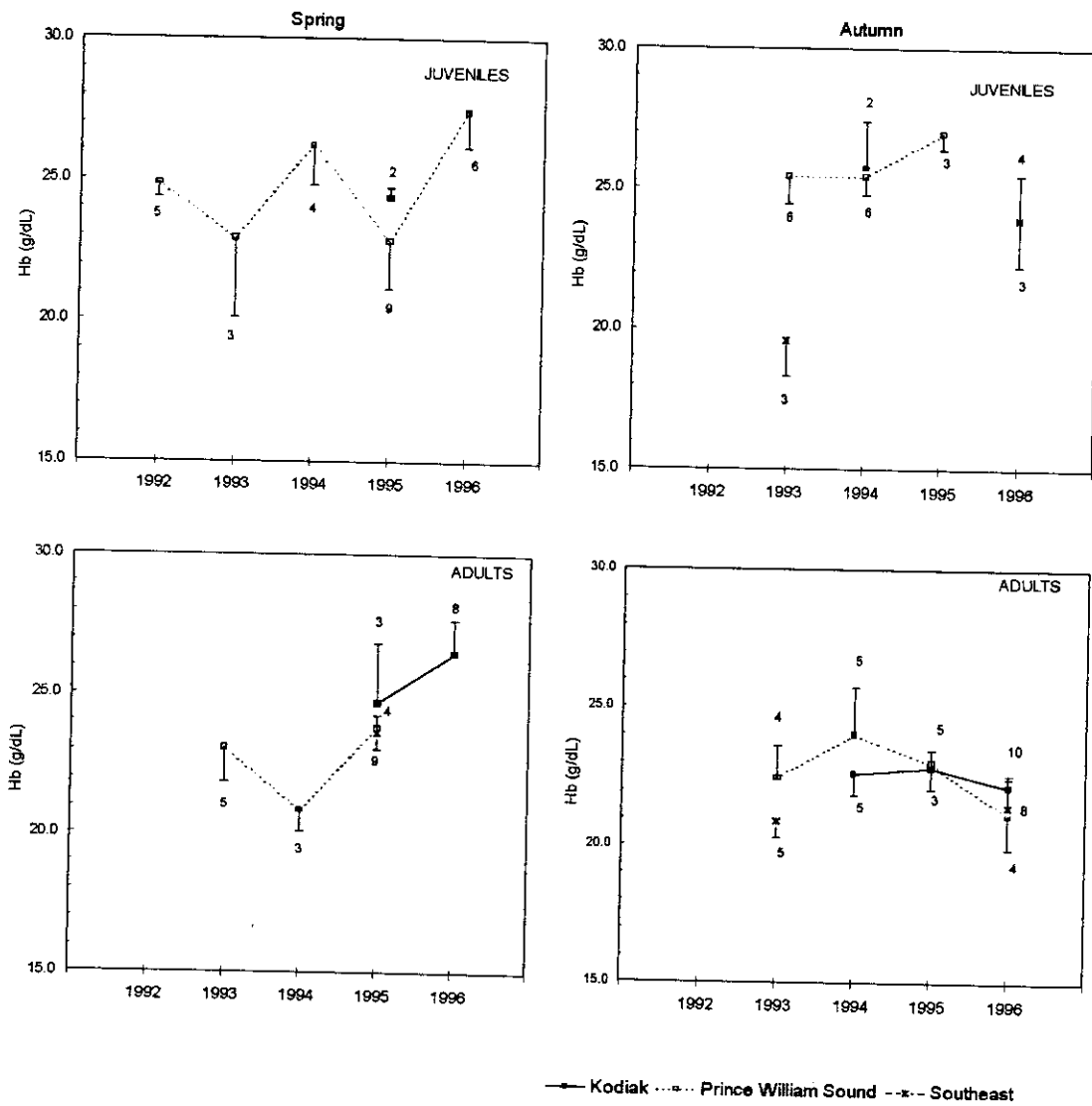


Figure 8. Interannual and regional comparisons of male harbor seal hemoglobin (Hb) concentration during spring and autumn.

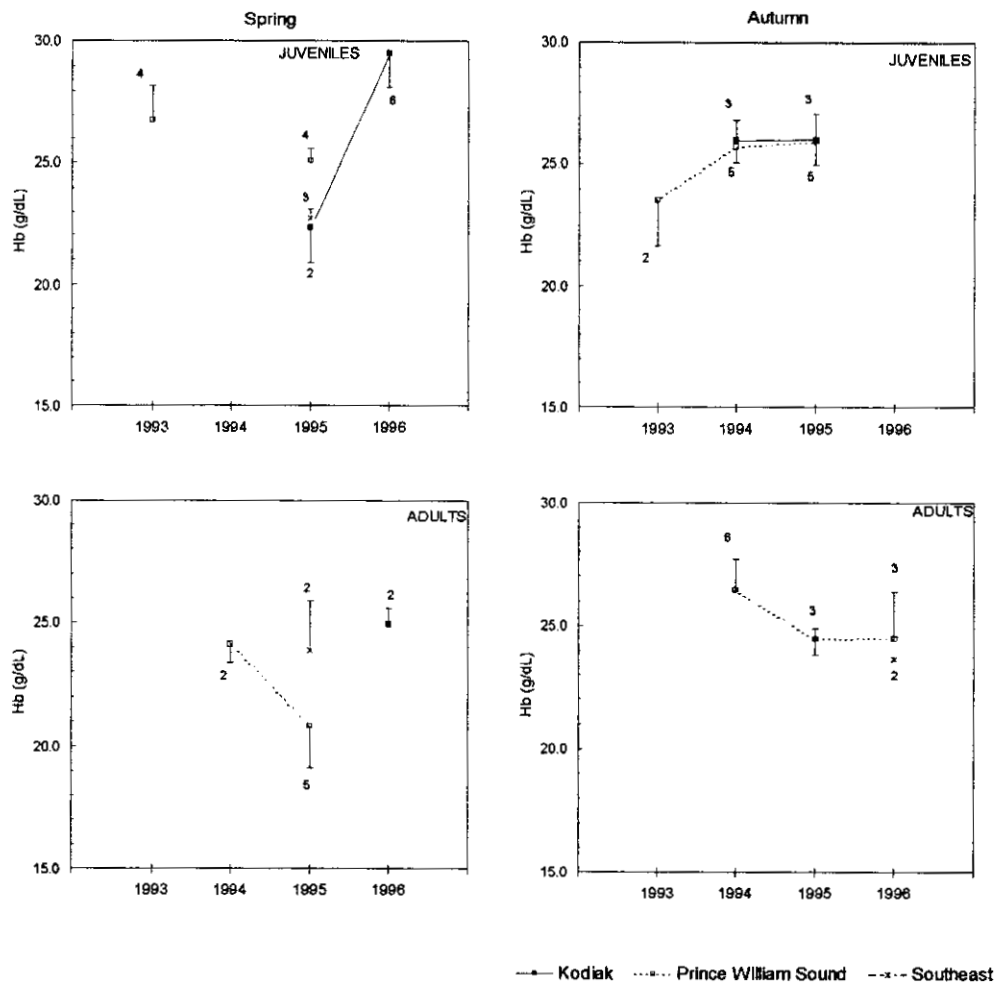


Figure 9. Interannual and regional comparisons of female harbor seal whole-blood hemoglobin (Hb) concentration during spring and autumn.

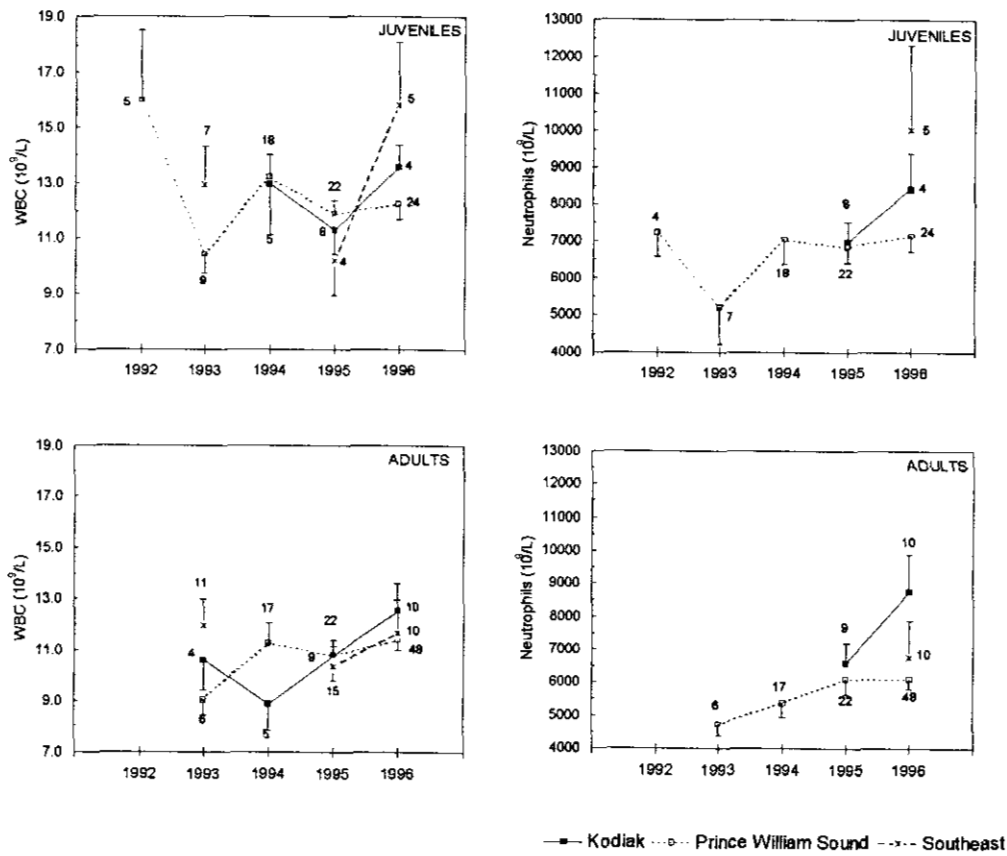


Figure 10. Interannual contrasts of juvenile and adult harbor seal white blood cell (WBC) and absolute neutrophil counts.

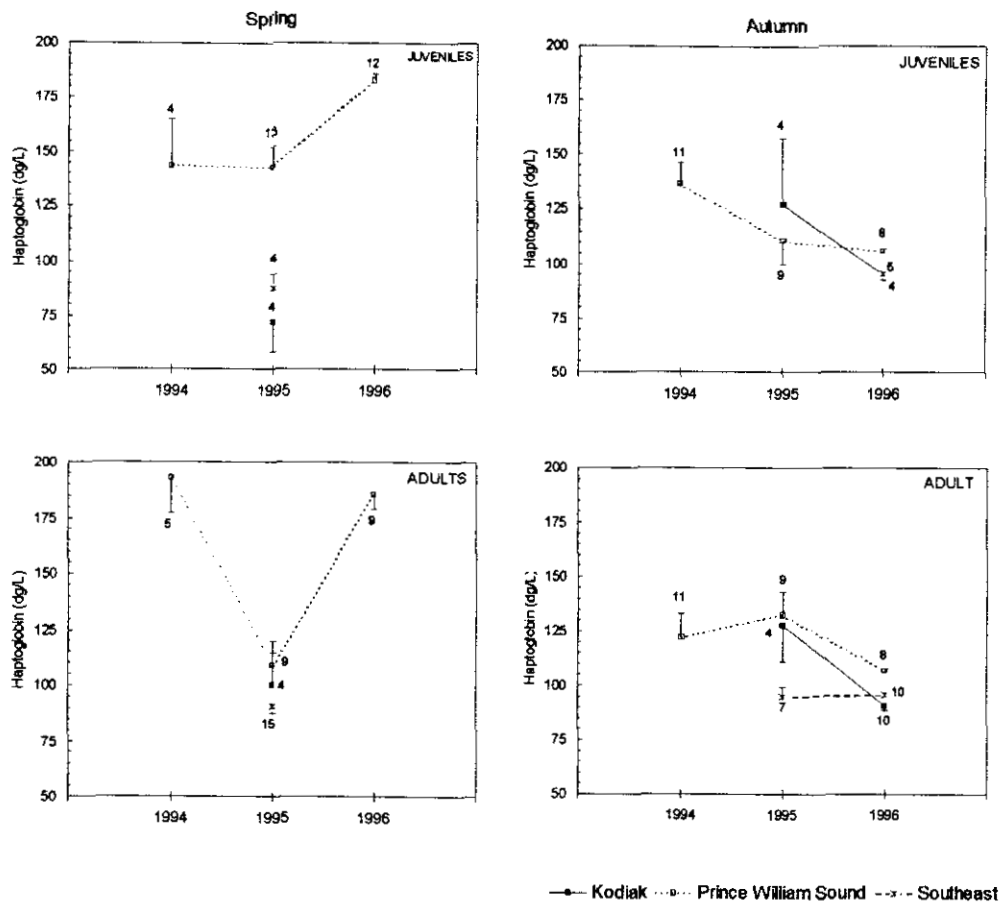


Figure 11. Interannual, regional, seasonal and age comparisons of harbor seal haptoglobin concentration.



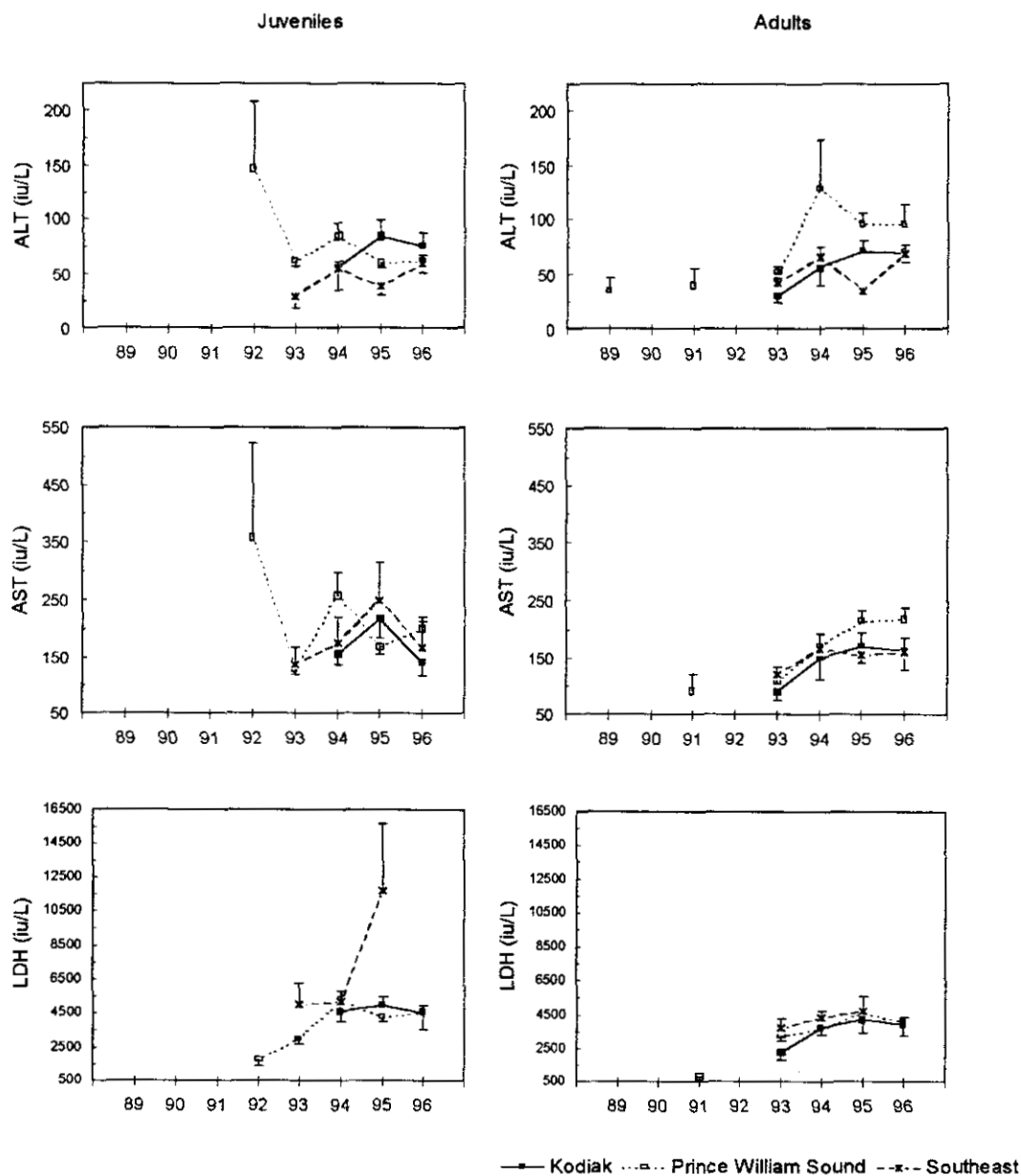
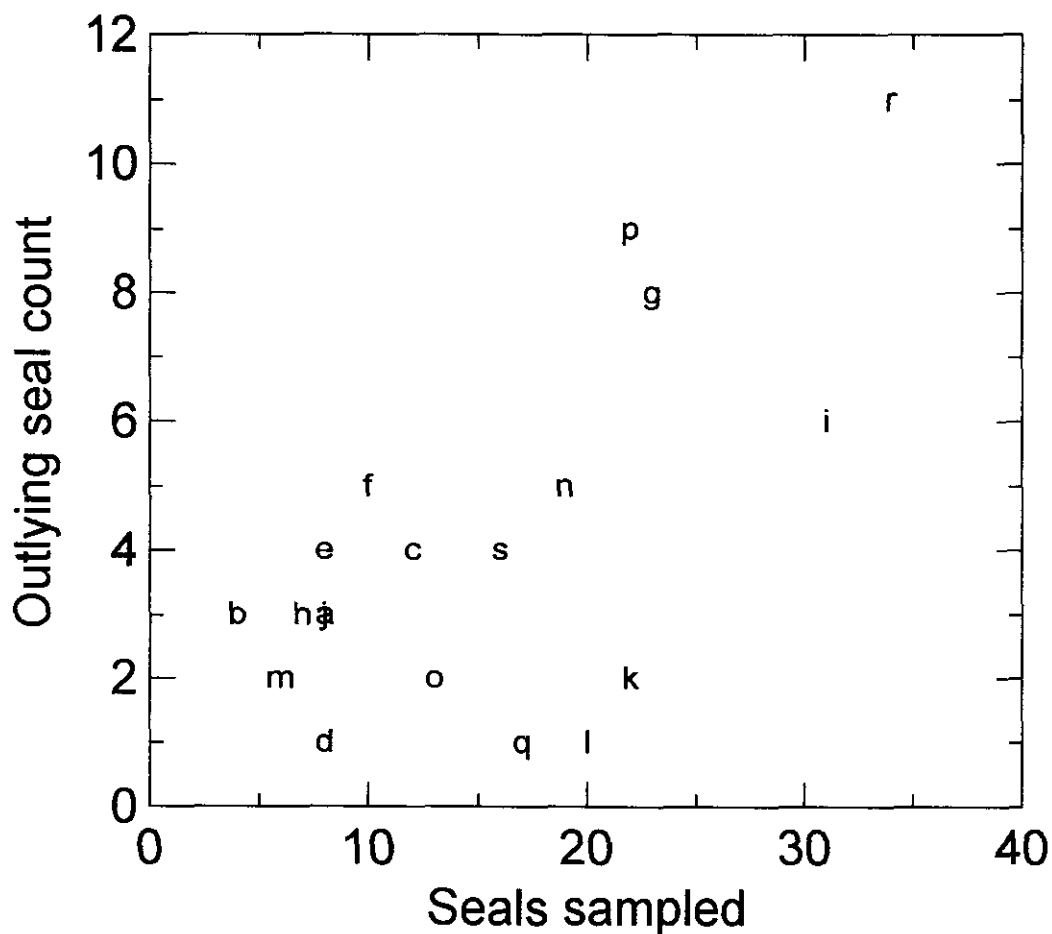


Figure 12. Interannual, regional, seasonal and age comparisons of harbor seal alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) activities. Sample sizes as follows: juveniles from Kodiak 1994 (5), 1995 (8), 1996 (4); Prince William Sound 1992 (6), 1993 (14), 1994 (18), 1995 (25), 1996 (24); southeast 1993 (7), 1994 (12), 1995 (5), 1996 (5). Adults from Kodiak 1993 (4), 1994 (5), 1995 (9), 1996 (10); Prince William Sound 1989 (2), 1991 (2), 1993 (11), 1994 (17), 1995 (29), 1996 (48); southeast 1993 (11), 1994 (25), 1995 (22), 1996 (10).



- |                      |                      |                      |
|----------------------|----------------------|----------------------|
| a) PWS, spring 1992. | h) SE, autumn 1994.  | o) KI, autumn 1996.  |
| b) KI, spring 1993.  | i) SE, summer 1994.  | p) PWS, spring 1996. |
| c) PWS, spring 1993. | j) KI, spring 1995.  | q) PWS, autumn 1996. |
| d) SE, autumn 1993.  | k) PWS, spring 1995. | r) PWS, summer 1996. |
| e) SE, spring 1993.  | l) PWS, autumn 1995. | s) SE, autumn 1996.  |
| f) PWS, spring 1994. | m) PWS, summer 1995. |                      |
| g) PWS, autumn 1994. | n) SE, spring 1995.  |                      |

Figure 13. Number of seals with  $\geq 4$  plasma chemistry or hematology statistically outlying variables relative to number of seals sampled. Abbreviations for regions: PWS, Prince William Sound; KI, Kodiak Island; SE, southeast Alaska.

## APPENDIX 2

**Errata for Fadely, B. S. 1997.** Investigations of harbor seal (*Phoca vitulina*) health status and body condition in the Gulf of Alaska. Ph.D. Thesis. Univ. of Alaska, Fairbanks. Alaska. 183pp.

### Chapter 2,

Table 3, Page 46; Table 8, Page 52; Table 13, Page 57:

Units for Red Blood Cell Counts ( $10^{12}/L$ ), while accurate, are more often expressed as  $10^6/\mu L$ .

Units for Platelet Counts ( $10^9/L$ ), while accurate, for the sake of consistency should be expressed as  $10^3/\mu L$ .

Table 4, Page 47; Table 10, Page 54; Table 1, Page 58:

Units for White Blood Cell Absolute Counts ( $10^9/L$ ), while accurate, are more often expressed as  $10^3/\mu L$ .

Table 12, Page 56:

Blood Urea Nitrogen (BUN;  $\mu mol/L$ ) should read Blood Urea Nitrogen (BUN;  $mmol/L$ ).

### Chapter 3,

Page 72:

2<sup>nd</sup> Paragraph: P = 921 should read P = 0.921.

### Chapter 5,

Figure 6, Page 176; Figure 7, Page 177:

Units for Red Blood Cell Counts ( $10^{12}/L$ ), while accurate, are more often expressed as  $10^6/\mu L$ .

Figure 10, Page 180:

Units for White Blood Cell and Neutrophil Counts ( $10^9/L$ ), while accurate, are more often expressed as  $10^3/\mu L$ .