Exxon Valdez Oil Spill
Restoration Project Annual Report

Recovery of Harbor Seals from EVOS: Condition and Health Status

Restoration Project 95001
Annual Report

This annual report has been prepared for peer review as part of the *Exxon Valdez* Oil Spill Trustee Council restoration program for the purpose of assessing project progress. Peer review comments have not been addressed in this annual report.

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Study History: This project began in FY93 as a Research Service Agreement with the Alaska Department of Fish and Game. In FY95 it was initiated as Restoration Project 95001 and this is the first annual report. A journal article covering portions of this project is in press (Castellini, J.M., H.J. Meiselman and M.A. Castellini. Understanding and interpreting hematocrit measurements in pinnipeds. *Marine Mammal Science*.1996). This project was funded to continue in FY 96 and it is anticipated that it will continue through FY97 and will be closed out with a Final Report prepared in FY98.

Abstract: The objectives of this project were to establish the criteria to evaluate the health and body condition of harbor seals (*Phoca vitulina)* inside and outside of Prince William Sound in special reference to potential problems induced by the *Exxon Valdez* Oil Spill. The four primary objectives were to begin the collection of blood chemistry data, initiate the modeling of body condition via morphometric indices, correct body condition and blood indices for age, sex, season and location and finally, to compare morphological criteria to animals both pre and post-spill and inside and outside of the Sound. Major progress was made in all four areas and work continues in each. Thus far, we have constructed plasma chemistry and hematological reference ranges based on up to 245 blood samples collected between 1989-95 from free-ranging seals in the Gulf of Alaska, and conducted preliminary analyses to determine which blood parameters are sensitive to non-health effects. While body condition indices may be poor indicators of health, we have found evidence of interannual changes in some blood values from seals, particularly females, sampled in the southwestern area of Prince William Sound since 1992 that may reflect shifting dietary habits.

Key Words: Blood chemistry, body condition, *Exxon Valdez* oil spill, harbor seals, health, Kodiak Island, *Phoca vitulina*, Prince William Sound, Southeast Alaska, traditional knowledge.

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

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INTRODUCTION

Harbor seal (*Phoca vitulina*) populations in Alaska show evidence of decline over portions of their range. Prince William Sound (PWS) harbor seal populations, further impacted by the spill (Frost and Lowry 1994*a*, Frost et al. 1995), have essentially stabilized at decreased levels, but have shown no signs of population recovery (Frost et al. 1995). Assessment and interpretation of harbor seal body condition and nutritional status data can 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 into other areas that may better explain their current recovery status.

OBJECTIVES

There were four major objective to this project as originally defined and they remained of primary importance throughout the first year.

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, sex, 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, sex, season and location.
4. Compare blood and morphological indices of health and condition in light of the above to examine interannual changes, potential EVOS-related impacts, and to help interpret changes in population status.

METHODS

Field Techniques

Harbor seals were live-captured by net entanglement, in conjunction with EVOS Project Number 95064, using methods previously described by Frost et al. (1995). Once captured, the seals were transported to shore or ship, anesthetized if required, weighed with an electronic hanging scale, and morphometric measurements gathered. Blood samples were drawn for laboratory analysis.

Blood chemistry

Blood samples were prepared in the field for shipment and ultimately transferred to the University of Alaska for further analysis. Plasma samples were sent to a veterinary laboratory for assessment of "standard" health indices and analyzed at our laboratory for indicators of dehydration, malnutrition and hormonal imbalance.

Body Condition

Linear and curvilinear length, a series of girths at 7 locations, and mass were collected from each animal. Blubber depths at 2-3 sites at each girth ring were measured using a portable ultrasound unit. In the laboratory, the data were placed into models of how length, girth and mass are related for harbor seals and used to evaluate body condition. Condition indices were compared using a database of morphometric measurements and corresponding body and sculp masses collected during 1973-1978 by Alaska Department of Fish and Game (ADFG) (Pitcher and Calkins 1979, Pitcher 1986). In an effort to incorporate local Native knowledge about harbor seal condition and health, we utilized information from the WHISKERS! (ADFG) database into our models. This data-base is built on interviews with subsistence users and their knowledge of local resources.

RESULTS

Blood chemistry

Two Prince William Sound Cruises in 1995 resulted in captures of 22 (May) and 20 (September) seals. An additional 15 samples from PWS were collected by National Marine Fisheries Service (NMFS) personnel during August. Kodiak Island cruises resulted in captures of 8 seals during March and 9 in October. Southeast Alaska samples provided by ADFG were 19 (April) and 9 (September). Morphometric data were available from 546 seals collected in the northern Gulf of Alaska, including PWS (n = 146) and Kodiak Island (n = 194) regions during 1973-1978 (Pitcher and Calkins 1979, Pitcher 1986).

Blood chemistry and hematological values measured from harbor seals from three geographic sampling regions within the Gulf of Alaska were combined to calculate reference ranges. While two-way analyses of variance indicated there were no significant affects ($P >$ 0.05) of gender or drugging on many blood chemistry parameters, significant effects ($P <$ 0.05) of gender were found on several others including alkaline phosphatase, creatinine, glucose, and haptoglobin. Albumin, globulin, and total protein are examples of some variables affected differently depending on season.

Liver associated plasma enzyme activities were found to not vary significantly with season or location within PWS. However, in May 1995 the mean red blood cell volume (MCV) of seals from the northern Sound was significantly lower than that of the western-southwestern zone or of the eastern zone. Likewise, the MCV of the northern and western PWS zones were significantly higher than MCV of seals from Kodiak.

Body Condition

Seasonal variability in body mass and composition were evident from calculations using data collected by Pitcher and Calkins (1979), and show considerable differences between males and females. Male body mass did not vary greatly during the year, but relative amounts of core and blubber changed by 10-15%. The core component was greatest in August, corresponding to the period when seal blubber was found to be thinnest (Pitcher and Calkins 1979, Pitcher 1986). Conversely, females exhibited large seasonal fluctuations in body and core mass. Sculp masses of both males and females were lowest in the summer months and greatest during winter months. This seasonal blubber change was also evident from analysis of local knowledge contained within the Whiskers database. Of the 65 household responses found by the database search, 49% stated the seasonality pertained to changes in blubber content or quality, 29% suggested shooting technique was important, 29% of the responses did not state causes, 18% noted sex-related differences, 9% mentioned freshwater glacial bays or inlets as being significant contributors to sinking, 5% note age-related fat content differences, 5% mentioned seasonal diet changes, and 1% did not observe any seasonal pattern to floating.

Using data from Pitcher and Calkins (1979), sculp and core masses were found to scale directly with body mass. Condition indices ranged widely in predictive power for sculp mass, and were all poorly related to blubber content. Model performance improved if gender, age class and season were included in a multiple regression against sculp mass.

Interannual changes in seal condition based on local knowledge chronicled in the Whiskers database indicates it is of most concern in the western Prince William Sound and Cook Inlet region. Relative to the past, 5 households reported more thinner or skinnier seals, or increased incidences of sinking, 2 households reported indications of poorer quality fat, 0 households reported fatter seals or greater floatability, and 1 reported observing no condition differences. There were no reports (0/3) of sick, diseased, or starving seal observations.

DISCUSSION

Blood Chemistry and Hematology

This study sampled greater numbers of harbor seals with broad geographic and seasonal distributions than any previous examination. Preliminary screening of panels based on these reference ranges did not present indications of population-level chronic diseases, consistent with findings from serological survey data for common phocid diseases (Frost et al. 1995). Development of reference ranges appropriate for free-ranging Gulf of Alaska harbor seals permits examination of veterinary blood panels with more confidence than would have been possible utilizing ranges published from small sample sizes, or from captive or free-ranging seals of other geographic regions.

In contrast to sea otters (Ballachey et al. 1994), we found no significant differences for liver-associated enzymes among regions of PWS. However, there were differences in mean corpuscular volume (MCV) within the Sound, and between the western Sound and Kodiak Island seals sampled during the same season. Seals sampled within the western-southwestern Sound showed a significant increase in MCV during 1991-95. Increases in MCV of the magnitude found in these comparisons is consistent with those observed in response to dietary shifts from clupeid to gadoid diets for free-ranging harbor seals in Moray Firth, Scotland (Thompson et al. in review).

We have also found gender-specific interannual differences in two liver-associated enzyme activities for seals within the western-southwestern region of the Sound. It is immediately important to note that none of these differences indicate diseased seals, as these activities were all within the normal reference ranges we established.

Morphometrics and Condition

Indices of body condition ranged from poor to moderate predictors of sculp mass, which improved somewhat when gender, age class, and season were included in the regression models. They were all very poorly related to blubber content. We found a strong relationship between mass and volume index in harbor seals. Little additional variability was explained by changes in gender, age or season. Indices based on mass at length may prove useful, given the moderate correlation with sculp mass. Ultimately, when the appropriateness and behavior of condition indices are established by this study, comparisons of condition will be interpreted by integrating findings from other PWS projects, such as fish abundance estimates and trophic relationships derived from fatty acid or stable isotope analyses.

Seasonal changes in body composition described here are not new information. Rather, they are presented to illustrate the magnitude of this variation, and to show that interannual comparisons cannot be performed without consideration of the season of measurement. More uniquely, however, the comparison to seasonal patterns generated from traditional knowledge serves as an example of how the gap between quantitative western science and traditional knowledge of seal biology may be bridged. It also underscores the importance of this type of subsistence data gathering. With more comments regarding condition, patterns of changes can be detected that would otherwise elude current sampling regimes.

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INTRODUCTION

Harbor seal (*Phoca vitulina*) populations in Alaska show evidence of decline over portions of their range. Prior to the *Exxon Valdez* Oil Spill 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 (Hoover-Miller 1994). Prince William Sound (PWS) harbor seal populations, further impacted by the spill (Frost and Lowry 1994*a*, Frost et al. 1995), have essentially stabilized at decreased levels, but have shown no signs of population recovery (Frost and Lowry 1994*b*, Frost et al. 1995). Assessment and interpretation of harbor seal body condition and nutritional status data can 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 into other areas that may better explain their current recovery status.

Changes in ecosystems or in prey availability due to natural or anthropogenic causes can be reflected in the body condition or nutritional status of top trophic-level consumers, such as harbor seals. However, indices used to assess body condition may also vary with season, age, or gender (Pitcher 1986, Trites and Bigg 1992, Beck et al. 1993, Renouf et al. 1993) independent of foraging ability or prey availability. Therefore, normal ranges of body size, shape and blubber distribution must be quantified before useful interannual comparisons can be performed. Furthermore, few studies have critically examined the assumptions used in assessing body condition from morphometric measurements in harbor seals. Likewise, blood chemical and hematological parameters also change significantly in response to environmental or nutritional effects (Seal et al. 1975, Geraci et al. 1979, McConnell and Vaughan 1983, Kuiken 1985, Roletto 1993). Chemical profiles and complete blood counts can identify potential homeostatic imbalances in organ systems or metabolic pathways if the effects of non-health related variation can be quantified (Payne and Payne 1987, Kerr 1989, Castellini et al. 1993).

Interpretation of blood chemistry and body condition data collected from harbor seals during the spill and subsequent studies can be improved by quantifying non-health related effects and by establishing normal values for free-ranging animals. At the time of the spill, comparative hematological values for harbor seals mostly derived from a few studies with small sample sizes from captive animals, sufficient for examining general health, but insufficient for more detailed interpretations of health status (McConnell and Vaughan 1983, Bossart and Dierauf 1990). The ultimate goal of this project is 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, will enable interannual and interregional comparisons of nutritional and health status. Thus far, we have constructed plasma chemistry and hematological reference ranges based on up to 245 blood samples collected between 1989-95 from free-ranging seals in the Gulf of Alaska, and conducted preliminary analyses to determine which blood parameters are sensitive to non-health effects. We have found evidence of interannual changes in some blood values from seals, particularly females, sampled in the southwestern area of Prince William Sound since 1992 that may reflect shifting dietary habits. We have also begun to quantify patterns in blubber mobilization within a seal, and examine how this affects our ability to utilize morphometric based condition indices for comparisons with historic data.

OBJECTIVES

The objectives set forth for this multi year project were:

1. Collect additional 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.

METHODS

Seal Capture Locations

Within Prince William Sound, 1995 field work was conducted during May and September using the chartered vessels *Provider* and *Pacific Star*, respectively, in conjunction with Project 95064. Additional samples were collected from the eastern Sound by National Marine Fisheries Service (NMFS) personnel during August, operating out of Cordova. We also collected samples from seals captured at Kodiak Island during March and October in association with Alaska Department of Fish and Game (ADFG) utilizing the ADFG vessel *Pandalus*. Samples were provided by ADFG personnel from southeast Alaska during April and September on board the National Oceanic and Atmospheric Administration vessel *John Cobb*. Similar data were collected within PWS in association with Project /064 between 1992-1994, and data collected by Frost and Lowry (1994*a*) from 1989-92 were included in analyses. Seals captured during 1992-1995 in spring and fall months in PWS were also utilized by Frost and Lowry (1994*a*) and Frost et al. (1995) for satellite-tagging and trophic interaction studies. Seals were also captured during 1993-94 around Kodiak Island and southeast Alaska during spring and/or fall seasons in association with ADFG (Lewis 1995). A historical database of seals collected during 1973-78 (Pitcher and Calkins 1979) was provided by ADFG for morphometric analyses and interannual comparisons.

Animal Handling and Sample Collection

Harbor seals were live-captured by net-entanglement using techniques described in Frost et al. (1995). After removal from the net, seals were transported to ship or shore, and were restrained manually or chemically by intramuscular injection with a ketamine/diazepam mixture. Weights were measured (± 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 categorized into age classes of pup, yearling, subadult or adult on the basis of size and time of year. Seals were held for variable periods to recover from drugging effects before being allowed to return to water.

Blood Collection, Processing and Analyses

Blood was sampled from the intervertebral extradural vein using 3.5 inch 18 or 20 G spinal needles (Monoject) into various blood collection tubes (Vacutainer). Typically up to 40 mL of blood was collected for serum, 25 mL for plasma, and 12 mL in EDTA tubes for complete blood counts (CBC) and hormone analyses. Blood samples from pups and some yearlings were taken by flipper venipuncture, using 1.5 inch 18 or 20 G needles drawing into blood collection tubes. In the field, blood hematocrit (% red blood cells by volume) was measured using a portable centrifuge (Compur M1100). Samples of whole blood were pipetted into Drabkin's reagent for hemoglobin analysis. Blood was then centrifuged and plasma, serum, and whole blood samples were frozen in liquid nitrogen for later laboratory analyses. Blood smear slides were made for determination of differential leukocyte counts.

Plasma samples were assessed for "standard" health indices (such as cholesterol level, salts, and enzymes characteristic of tissue damage) and also analyzed for indicators of dehydration (water content), malnutrition (ketones), stress (haptoglobin), and hormone imbalance (angiotensin, atrial natriuretic peptide). 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), gammaglobulin transferase (GGT), creatinine phosphokinase (CPK), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were performed by automated machine analysis at the Fairbanks Memorial Hospital (FMH) using an Ektachem Analyzer. Additionally, concentrations of ketone bodies (β-HBA), iron, and hemoglobin were or will be determined using standard kits from Sigma Chemical Co. and performed in our laboratory. Complete blood counts of white and red blood cells, platelet and differential white blood cell counts were performed by technicians at FMH from blood collected in EDTA collection tubes 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).

Hormone imbalance and ketone body determinations are in progress and will not be discussed in this report. Reference ranges for blood chemistries and hematologies were calculated as being within two standard deviations of the mean (Kerr 1989). Non-normally distributed data were first arcsin or square-root transformed (Zar 1984). Initial tests of non-health related effects on blood parameter values were conducted utilizing data collected in September 1994 and May 1995 from Prince William Sound. Two-way analyses of variance (ANOVA) tested for gender and drug application affects and interactions.

A selection of liver enzyme activities and other blood parameters were compared both within PWS and interannually for seals within the western-southwestern Sound region as an example of how we will perform population level comparisons. ALT, AST, GGT and absolute eosinophil counts were the focus of this comparison since these were recently found to differ between western and eastern Sound populations of sea otters (*Enhydra lutris*; Ballachey et al. 1994, 1995). Values presented in the text are means with standard deviations. Seal capture locations within the Sound were grouped into an eastern region (Gravina Island, Hawkins Island Cut-off, Olsen Bay, Port Fidalgo), a northern region (Dutch Group, Fairmount Point, Herring Bay, Lone Island, Long Bay) and a western-southwestern region (Applegate Rocks, Bay of Isles, Channel Island, Little Green Island, Green Island, Port Chalmers, Stockdale Harbor, Seal Island). These roughly correspond to the PWS divisions created by the herring research group associated with the *Exxon Valdez* Oil Spill Trustee Council funded Sound Ecosystem Assessment (SEA) project.

Morphometric Measurements and Analyses

In addition to mass, a series of lengths and girths were measured (Figure 1). Standard length (SL; straight line distance between tip of nose and tip of tail), total curvilinear length (CL; distance between tip of nose and tip of tail with measuring tape laying on animal) and CL to each girth location were measured ($\pm$ 1 cm) with the seal positioned on its belly, dorsal side up. Blubber thickness was measured at up to three locations (dorsal, lateral, ventral) at each girth measurement location (except at ear girth ring) using a portable ultrasonic unit (Scanoprobe II, Model 7310, Scanco, Inc.), similar to Gales and Burton (1987). Blubber thickness relative to body thickness was calculated by dividing the mean blubber thickness by the body radius at that girth site. Total body fat can be estimated using whole-animal bioimpedance (BIA) if BIA data can be regressed with other estimates of body fat. Bioelectrical impedance was measured using a portable BIA unit (RJL Systems BIA-101A) with 21G 1.5" electrodes attached at the head and base of flippers, in anticipation of 96001 studies that will estimate total body fat by injection of doubly-labeled water.

A database of morphometric measurements and corresponding body and sculp masses collected during 1973-1978 by Pitcher and Calkins (1979) and Pitcher (1986) was utilized to examine seasonal trends in body composition and to test the utility of condition indices derived from morphometric measurements. Sculp mass (mass of blubber and skin) and blubber content (ratio of sculp mass to body mass) were compared to three condition indices; the ratio of axillary girth (G) to standard length (CI; McLaren 1958, Pitcher 1986), the ratio of body mass to a volume index, calculated as $SL*G^2$ (DI; modified from Castellini and Calkins 1993), and the ratio of body mass to standard length after log transformation (MI; Usher and Church 1969). Indices were tested for predictability of sculp mass or blubber content alone and in combination with other variables such as gender, age class and time of year in linear or stepwise regression models using Statistix® software.

A preliminary interannual comparison of condition indices was performed for seals collected or captured in Prince William Sound during spring months, combining data from this study, Frost and Lowry (1994*a*), and Pitcher and Calkins (1979) to illustrate the direction this type of analysis could proceed. Standard length data from 1989-90 measured with the seal in a belly-up position were converted to 'belly-down' standard lengths using the regression $Y_e = 2.35 + 1.0x$ (where Y_e is belly-up, x is belly-down length) from Pitcher and Calkins (1979).

Incorporation of Local Traditional Knowledge and Wisdom

Local knowledge of harbor seal body condition was compiled from the 'Whiskers!' database (Version 1.0) produced by ADFG Division of Subsistence. This database is comprised of interviews with Alaska Natives between 1992 and 1995 from communities around the Gulf of Alaska. Three of the regions represented in the database (Kodiak, North Pacific Rim (Cook Inlet and Prince William Sound), and southeast Alaska) were searched using the keywords fat, blubber, nutrition, condition, float, floating, sink and sinking. Responses found by this search were quantified by encoding comments regarding floating, sinking, fat quantity and quality, seasonality, factors affecting these parameters of seal condition, and comparisons to past seal condition. Results were then calculated as the proportion of responses within the category to the total number of households satisfying the search conditions.

RESULTS

Data Collection

Two Prince William Sound cruises in 1995 resulted in captures of 22 (May) and 20 (September) seals. An additional 15 samples from PWS were collected by NMFS personnel during August. Kodiak Island cruises resulted in captures of 8 seals during March and 9 in October. Southeast Alaska samples provided by ADFG were 19 (April) and 9 (September). Comparative hematological and morphometric data available from 1989-1994 are summarized with 1995 captures in Table 1. Morphometric data were available from 546 seals collected in the northern Gulf of Alaska, including PWS (n = 146) and Kodiak Island (n = 194) regions during 1973-1978 (Pitcher and Calkins 1979, Pitcher 1986).

Capture of seals by net-entanglement resulted in a larger proportion of smaller animals (Figure 2) compared to collections made by Pitcher and Calkins (1979) in the mid-1970's. Based on growth data from Pitcher and Calkins (1979), these smaller animals were 1-3 years old. Though these juveniles were predominantly male (60%), there was no significant difference (Pearson's χ^2 = 0.22, P = 0.640) with the sex ratio of juveniles collected in 1973-75 (56% male, 44% female, n = 77). There was also no significant difference in the overall sex ratio of collected seals (58% male, 42% female, n = 146) in 1973-75 and of net-captured seals (55% male, 46% female, n = 119) during 1992-95 (Pearson's χ^2 = 0.11, P = 0.735).

Blood Chemistry and Hematology

Blood chemistry and hematological values measured from harbor seals from three geographic sampling regions within the Gulf of Alaska were combined to calculate reference ranges (Tables 2 and 3). Data derived from seals sampled posthumously, or from lipemic or hemolytic plasma samples were excluded from this analysis. Gravimetric units, rather than SI units, were utilized since these are still commonly used by veterinary science in this country. A table of conversion factors are listed in Appendix A. Because of the large sample sizes, most parameters were normally distributed (Figure 3). Blood variables such as enzyme activities or count data (CBC) that often are not normally distributed (Figure 4a), were normalized by appropriate transformations (Figure 4b). Two examples of seals that exhibited clinically significant outliers when compared to these ranges are shown in Table 4.

From Prince William Sound, fall 1994 and spring 1995 data provided sufficient contrasts to examine the effects of gender or drugging. However, some variables which were unaffected in September were affected in May, thus more samples are required to fully resolve these interactions. Two-way analyses of variance indicated there were no significant affects ($P > 0.05$) of gender or drugging on AG ratio (ratio of albumin to globulin), albumin, ALT, CPK, direct or total bilirubin, GGT, globulin, LDH, MCH, MCV, potassium, phosphorus, platelets, red blood cell count, sodium, or white blood cell count. Significant effects ($P < 0.05$) of gender were found on alkaline phosphatase, AST, BUN, creatinine, glucose, and haptoglobin. Injection of chemical anesthetics had significant effects on creatinine, glucose, hemoglobin, calcium, BUN, hematocrit and MCHC. Albumin, globulin, and total protein are examples of some variables affected differently depending on season.

Liver associated plasma enzyme activities such as AST, ALT and GGT were found to not vary significantly with season ($P > 0.05$; df = 29, 55 and 55 respectively). Within PWS, there were no significant differences in absolute eosinophil counts (ANOVA, $F_{[2,34]} = 0.06$, $P = 0.942$), ALT ($F_{[2,34]} = 0.15$, $P = 0.8642$) or GGT ($F_{[2,32]} = 0.29$, $P = 0.754$), nor was there a significant difference in AST for males ($F_{[2,18]} = 0.11$, $P = 0.901$) or females ($F_{[1,13]} = 1.49$, $P = 0.244$) among the three zones within the Sound during fall of 1994. Likewise, there were no differences among regions for these variables during spring 1995 (eosinophils $F_{[2,39]} = 1.68$, $P = 0.2001$; ALT $F_{[2,39]} = 0.29$, $P = 0.750$; GGT $F_{[2,39]} = 0.44$, $P = 0.649$; male AST $F_{[2,18]} = 3.01$, $P = 0.075$; female AST $F_{[1,19]} = 1.92$, $P = 0.182$). However, in May 1995 the mean MCV of seals from the northern Sound was significantly lower ($\bar{x} = 103$ fL) than that of the western-southwestern zone ($\bar{x} = 114$ fL) or of the eastern zone ($\bar{x} = 112$ fL; $F_{[2,43]} = 10.97$, $P = 0.0001$), though there was no difference in MCHC ($F_{[2,51]} = 2.51$, $P = 0.091$). Likewise, the MCV of the northern and western PWS zones were significantly higher than MCV of seals from Kodiak ($\bar{x} = 110 \pm 6$ fL; $F_{[1,56]} = 6.67$, $P = 0.0124$), and MCHC was significantly lower in these Sound seals ($\bar{x} = 39.8 \pm 4.3$ %) than in seals from Kodiak ($\bar{x} = 42.6 \pm 4.3$ %; $F_{[1,64]} = 5.38$; $P = 0.0286$).

In interannual comparisons within the western Sound, there was no clear pattern in ALT activity in subadult or adult males since 1989, but ALT increased in each year since 1992 among females (Figure 5). These patterns were also evident for males and females in AST activity (Figure 6). Both sexes showed indistinguishable interannual trends in GGT activity, which increased between 1992-1994 and decreased in 1995, and absolute eosinophil counts

(Figure 7). Other combined blood parameters showed no interannual trend in AG ratio, a significant increase in MCV between 1991-1995, but alternating levels of MCHC (Figure 8). There were no significant yearly differences in body mass among these adult females ($F_{[3,14]}$ = 0.558, P = 0.558) or males ($F_{[2,18]}$ = 2.58, P = 0.103).

Body Composition and Morphometrics

Volume index was an excellent predictor of body mass ($M_b = 2.86+4.64*SL*G^2$; n = 790, $r^2 = 0.933$, $P < 0.0001$), explaining 93% of the variation (Figure 9). A stepwise regression model showed significant effects of gender, fall season and adult age class (all $P <$ 0.0001), but this only accounted for an additional 0.9 % of the variation ($r^2 = 0.942$, n = 542). An analysis of regression residuals from the volume index model shows that females tend to fall above the regression line ($\bar{x} = 1.9 \pm 6.5$, n = 392) while males were significantly lower ($\bar{x} = -1.2 \pm 5.7$, n = 397; $F_{[1,787]} = 29.31$, $P < 0.0001$). Standard length alone was not a better predictor of body mass (linear regression after log transformation; $r^2 = 0.824$, $P <$ 0.0001, n = 800), and an additional 3.7% of variation was explained by season, location, and age class ($r^2 = 0.861$, n = 547).

Seasonal variability in body mass and composition were evident from calculations using data collected by Pitcher and Calkins (1979), and show considerable differences between males and females (Figure 10). Male body mass did not vary greatly during the year, but relative amounts of core and blubber changed by 10-15%. The core component was greatest in August, corresponding to the period when seal blubber was found to be thinnest (Pitcher and Calkins 1979, Pitcher 1986). Conversely, females exhibited large seasonal fluctuations in body and core mass. Sculp masses of both males and females were lowest in the summer months and greatest during winter months. This seasonal blubber change was also evident from analysis of local knowledge contained within the Whiskers database. Responses from 68 households satisfied the search conditions, and 65 responses discussed seasonality of blubber thickness or quality, or variability in floating and sinking of shot seals. The proportion of households reporting when seals sink or are thinner, versus times when seals floated or were fat generated two normal distributions with modal sinking occurring during summer, and modal floating occurring during winter months (Figure 11). Of the 65 household responses found by the database search, 49% (32) stated the seasonality pertained to changes in blubber content or quality, 29% (19) suggested shooting technique was important, 29% of the responses did not state causes, 18% (12) noted sex-related differences, 9% (6) mentioned freshwater glacial bays or inlets as being significant contributors to sinking, 5% (3) note age-related fat content differences, 5% mentioned seasonal diet changes, and 1% (1) did not observe any seasonal pattern to floating.

Using data from Pitcher and Calkins (1979), sculp and core masses were found to scale directly with body mass (sculp mass = $0.342M_b^{1.0}$, $r^2 = 0.900$, n = 343; core mass = $0.648M_b^{1.0}$, $r^2 = 0.970$, n = 343). Condition indices ranged widely in predictive power for sculp mass (CI $r^2 = 0.138$, $P < 0.0001$; DI $r^2 = 0.021$, $P = 0.0035$; MI $r^2 = 0.796$, $P <$ 0.0001; n = 406 for all comparisons), and were all poorly related to blubber content (CI r^2 = 0.084, $P < 0.0001$; DI $r^2 = 0.025$, $P = 0.0007$; MI $r^2 = 0.0015$, $P = 0.208$; n = 406). Model performance improved if gender, age class and season was included in a multiple regression against sculp mass (CI R^2 = 0.706, P < 0.0001; DI R^2 = 0.572, P < 0.0001; MI R^2 = 0.831, P < 0.0001). Residuals from the regression models generating the indices were poor indicators of sculp mass (rCI r^2 = 0.134, P < 0.0001; rDI r^2 = 0.0184, P = 0.0035; rMI r^2 = 0.206, P < 0.0001) and of relative blubber content (rCI r^2 = 0.076, P < 0.0001; rDI r^2 = 0.026, P = 0.0011; MI r^2 = 0.020, P = 0.0025). Residual index model fit with sculp mass improved when gender, age class, and season were included (rCI R^2 = 0.675; rDI R^2 = 0.547; rMI R^2 = 0.638).

Underlying blubber thicknesses were significantly but poorly correlated with girth (Table 5) since the core component also changes (Figure 10). Blubber depths among sites on a seal were only moderately correlated (Table 6). This was also evident in comparisons of the variability in girths relative to position on the seal (Figure 12). Coefficients of variation in girth measurements were highest at the neck and hip regions, and posterior to the neck increased in variability rearwards. Relative to seal thickness, the neck and hip regions had about 40% thicker blubber depths than the other body locations (Figure 13).

Measurement errors are partly responsible for some of the poor condition index performance. Replicate standard length measurements performed on 38 seals in 1994 resulted in an absolute measurement error of 2.9 cm (±2.6 cm), independent of standard length. This would create a 3% error in both CI and DI. Repeated independent measures of axillary girth in 6 seals also resulted in a 2.9 cm absolute error, which would generate a 10% error in DI, but only a 3% error in CI.

Interannual changes in seal condition based on local knowledge chronicled in the Whiskers database indicates it is of most concern in the western Prince William Sound and Cook Inlet region. Of 68 households found in the database search, there were 10 (15%) mentions of long-term seal condition comparisons of any kind. One response was from the lower Cook Inlet area, 3 from southeast (of 34 households), and 6 were from the Prince William Sound region (of 18 households). Relative to the past, 5 households reported more thinner or skinnier seals, or increased incidences of sinking, 2 households reported indications of poorer quality fat, 0 households reported fatter seals or greater floatability, and 1 reported observing no condition differences. There were also no reports (0/3) of sick, diseased, or starving seal observations. Estimates of blubber thickness were about 19-25 cm during winter 1991 at Port Graham, and 25 mm during October-December 1993 and September 1994 at Chenega Bay (Table 7). Blubber thicknesses measured over the xiphisternum during 1973-78 (Pitcher and Calkins 1979) and from the mid-trunk (1993-95) are presented for comparison. No other measurements were available for lower Cook Inlet during winter months.

Interannual changes in body condition based on the three morphometric models seem to indicate changes since the mid-1970's (Figures 14, 15). Spring males show changes in girth-length and mass-length relationships, but while adults show differences in mass-volume, the subadults did not (Figure 14). Conversely, spring adult females show little changes in these relationships between the two decades (Figure 15).

DISCUSSION

Blood Chemistry and Hematology

Of the studies presenting plasma chemical and hematological reference ranges for harbor seals, this study sampled greater numbers of seals with broad geographic and seasonal distributions (McConnell and Vaughan (1983) $n = 15$, Roletto (1993) $n = 26$, de Swart et al. (1995) $n = 22$, Kopec and Harvey (1995) $n = 53$). Preliminary screening of panels based on these reference ranges did not present indications of population-level chronic diseases, consistent with findings from serological survey data for common phocid diseases (Frost et al. 1995, Lewis 1995). We have established ranges not available at the time of the spill, but this does not appear to require altering conclusions of Frost and Lowry (1994*a*). However, we have yet to closely examine each panel for every seal. This analysis is continuing and will be refined once non-health sources of variability can be more adequately considered. Without histological determinations of disease state, diseased seals may have been included in our reference ranges. The assumption in setting a normal reference range within two standard deviations is that outliers will be mostly comprised of disease-state animals, though this is not necessarily true in reality (Kerr 1989).

Development of reference ranges appropriate for free-ranging Gulf of Alaska harbor seals permits examination of veterinary blood panels with more confidence than would have been possible utilizing ranges published from small sample sizes, or from captive or free-ranging seals of other geographic regions. In the examples presented in Table 4, PV94PW13 was a male yearling captured at Channel Island on 18 September, 1994. Elevated AST, CPK, LDH, decreased total protein, elevated white blood cell count and neutrophilia were consistent with symptoms of acute stress (Bossart and Dierauf 1990). Though this seal was held 3 hours 40 minutes before a blood sample was taken, it showed no obvious signs to differentiate it from other seals. Seal PV95RH02 was an orphaned pup transported to Alpine Animal Clinic in Anchorage on 12 May, 1995. While the leukogram did not indicate problems, the large number of significantly out of range plasma chemistries suggests gross homeostatic imbalances. Most intriguing is the elevated BUN. At 104 mg/dL (37 mM), this may indicate prolonged fasting (Rea 1995) though we have yet to confirm this with a laboratory determination of ketone bodies.

Because many of the interannual or interregional population level differences in chemistries would be subtle, it is important to quantify other sources of variability. Other recent studies have found age, gender and season effects on many blood parameters (Kopec and Harvey 1995, de Swart et al. 1995), and we will also be examining factors not often quantified or considered in field studies, such as handling technique, time between capture and sampling, and time between blood collection and processing. As shown in the results, we have initiated tests of gender and drugging effects. However, complex interactions may exist between several of these factors. For example, we performed a stepwise regression analysis of hematocrit to determine the effects of body mass, age class, gender, drugging, season, year, location, and elapsed handling time before blood sampling. Of these variables, there were highly significant ($P < 0.001$) effects of body mass, season and location, accounting for 43% of the variability ($n = 131$). Surprisingly, drugging was excluded from this model as being insignificant, yet the within-season comparisons showed a high degree of significance (P = 0.0022). Further analyses will continue this type of modeling to quantify interactions of environmental variables. Other recent studies of harbor seals in California (Kopec and Harvey 1995) and the Netherlands (de Swart et al. 1995) also examined interactions of many of these factors, and will be extremely useful sources of comparison for this study.

In contrast to sea otters (Ballachey et al. 1994, 1995), we found no significant differences for liver-associated enzymes among regions of PWS. However, there were differences in mean corpuscular volume (MCV) within the Sound, and between the western Sound and Kodiak Island seals sampled during the same season. Seals sampled within the western-southwestern Sound showed a significant increase in MCV during 1991-95. Increases in MCV of the magnitude found in these comparisons is consistent with those observed in response to dietary shifts from clupeid to gadoid diets for free-ranging harbor seals in Moray Firth, Scotland (Thompson et al. in review). Declining herring stocks in the western-southwestern region of PWS have been documented (Brown et al. 1996). Reports of starving seals were not found in the Whiskers database compilation of local knowledge for this area, but whether these diet shifts represent subtle levels of food limitation is not clear since the condition indices infer relatively good condition during this period. Notably, Thompson et al. (in review) found that MCV did not differ between seals in good or poor condition, only between seals sampled during 'good' and 'poor' clupeid abundance years. Since seals in this area tend to be very localized in their foraging patterns (Frost et al. 1995), further analyses will refocus on differences among capture locations within this western zone.

We have also found gender-specific interannual differences in liver-associated enzyme AST and ALT activities for seals within the western-southwestern region of the Sound. It is immediately important to note that none of these differences indicate diseased seals, as these activities were all within the normal reference ranges we established. Because of the non-specificity of enzymes, it is clinically more significant when suites of enzyme levels change, as has been shown here. Medway (1980) cites domestic animal studies that show a relationship between AST and ALT content with body size, but whether this relationship exists for marine mammals is unclear (Bossart and Dierauf 1990). There was no significant year effect on body mass of females or males so this effect, if it exists, did not account for the trend shown by these enzymes. It is unclear why GGT would show the same increasing trend during 1992-94, but then decline again in 1995. The lack of a yearly trend of AG is consistent with the absence of a severe or widespread disease impacting the population (Duncan and Prasse 1986), which thus far has been confirmed by serological surveys (Frost et al. 1995) and the other standard panel chemistries. However, Zenteno-Savin et al. (in review) have suggested that based on elevated levels of the acute-phase stress protein haptoglobin relative to harbor seals from southeast Alaska, there may indeed be some chronic stressor affecting this population. This interpretation, however, is currently being tested as we continue to analyze more samples.

Morphometrics and Condition

The best method to determine body composition of harbor seals is to directly measure the component core and sculp masses for an animal of known age, as was performed by

Pitcher and Calkins (1979) and Pitcher (1986). This provides data not only on body composition, but also on mass and length at age data which provide direct measures of environmental conditions being experienced by seals of different ages. This type of comparison found evidence of decreased body condition in Steller sea lions in the Gulf of Alaska (*Eumetopias jubatus*; Calkins and Goodwin 1988), but these comparative data do not exist for harbor seals. In the absence of terminal collections, we are applying ultrasonic and bioimpedance techniques to determine the proportion of blubber in live seals, the techniques and results of which will be addressed in subsequent reports. Some useful comparative data are also becoming available through a cooperative tissue and data collection program with local villagers. However, if body composition could be meaningfully related to an index derived from commonly collected morphometric measurements, such as length, axillary girth and body mass, then the sample sizes for comparison between years and locations increases greatly. For this analysis, improved condition is assumed to be related to relatively greater blubber content.

The three direct-ratio indices of body condition ranged from poor (DI) to moderate (MI) predictors of sculp mass, which improved somewhat when gender, age class, and season were included in the regression models. They were all very poorly related to blubber content. We found a strong relationship between mass and volume index in harbor seals, as was also shown for Steller sea lions (Castellini and Calkins 1993) and Weddell seals (*Leptonychotes weddellii*; Castellini and Kooyman 1990). The large coefficient of determination for harbor seals suggests that little variability remains to be explained by changes in gender, age or season, and this was shown to be true. Therefore, a condition index based on density changes (DI) also may be rather insensitive to changes in body composition. Pitcher (1986) reported a blubber content range of 20-60% among harbor seals. Assuming densities of 0.9 g/mL for adipose tissue and 1.1 g/mL for lean tissue (Nordøy and Blix 1985), the total body density would vary between 0.98-1.06 g/mL, a change of only 7%. Based on our estimates, changes within this range would be difficult to discriminate from measurement error.

Pitcher (1986) previously reported a weak correlation of CI with blubber thickness, and others have noted its equivocal performance as a condition index (Gales and Renouf 1994, Kopec and Harvey 1995). Examination of seasonal changes of body composition (Figure 10) and of correlations among girth measurements and underlying blubber thicknesses (Table 5) give indications why the above condition indices are relatively poor predictors of blubber content or sculp mass. Most of condition-related changes occur rearward of the typically measured axillary girth, and these changes were not well correlated with changes in axillary girth. We found that girth measurements were poorly correlated with underlying blubber thickness, and that blubber thickness was only moderately correlated around the seal body. The greatest variability in girth occurred at the neck and hip (approximately 70-80% SL from the nose) regions. Neck girth variability may be a sampling artifact resulting from the extensibility of phocid necks. Large variability in girth and blubber thickness, as well as relatively greater blubber thicknesses in the posterior regions (60-80% of SL) have also been shown in other seal species (Ryg et al.1990, Gales and Renouf 1994, Beck and Smith 1995).

Based on the greater variability in posterior blubber thickness, Ryg et al. (1990) described a condition index that incorporated blubber thickness from the most variable point with mass and standard length. This model also outperformed CI in a study by Gales and Renouf (1994). We are currently trying to utilize this model, but since our blubber thicknesses do not correspond directly with the sampling method of Pitcher and Calkins (1979) we are proceeding cautiously. Continued analyses will utilize comparisons of indices against the seasonal composition change data (Figure 10) from Pitcher and Calkins (1979). Since changes in body and component masses are known, this will help interpret the behavior of the many indices.

These indices may also be ineffective for representing relative condition in young seals. In a similar study, neither CI nor DI discriminated among young, old or orphaned Steller seal lion pups (Rea 1995). Even in pups girth changes of posterior regions may be better indicators of condition. After a 10 day rehabilitation period following arrival at the Alpine Veterinary Clinic, axillary girth in the orphaned harbor seal pup PV95RH02 increased only 2 cm, while hip girth increased by 8 cm.

Indices based on mass at length (MI) may prove useful, given the moderate correlation with sculp mass. However, even after log transformation, this relationship was still curvilinear and size dependent. Further analyses with this relationship will utilize exponential curve-fitting to remove this effect, following (Trites and Bigg 1992). However, size variation bias is inherent in the use of each of these condition index ratios (Packard and Boardman 1988), since sculp mass scales proportionately with body mass. Using regression residuals from the relationships that generated these indices may be an appropriate method to reduce this variation (Reist 1985). Regression residuals were therefore utilized for testing hypotheses about condition and seasonal or interannual effects, though they were only moderately associated with sculp mass when gender, age class, and season were included.

Seasonal changes in body composition described here are not new information, having been presented as seasonal changes in blubber thickness by Pitcher and Calkins (1979) and Pitcher (1986). Rather, they are presented to illustrate the magnitude of this variation, and to show that interannual comparisons cannot be performed without consideration of the season of measurement. More uniquely, however, the comparison to seasonal patterns generated from traditional knowledge serves as an example of how the gap between quantitative western science and traditional knowledge of seal biology may be bridged. It also underscores the importance of this type of subsistence data gathering. With more comments regarding condition, patterns of changes can be detected that would otherwise elude current sampling regimes.

Residual variation in condition indices for spring adult females (Figure 15) were consistent with their improved spring condition shown in Figure 10. Adult males, however, seemed to be in better condition during 1993 and 1994 than in other years. Ultimately, when the appropriateness and behavior of condition indices are established by this study, comparisons of condition will be interpreted by integrating findings from other PWS projects, such as fish abundance estimates and trophic relationships derived from fatty acid or stable isotope analyses.

Reports of seasonally thinner than normal seals from fall/winter 1994 in PWS and 1991 winter in lower Cook Inlet (Table 7) were difficult to evaluate given the large number of potential biases generated in this comparison. Blubber thickness was not well correlated around the body, so the site of measurement can influence the comparison, as well as whether the assessment was made in fall or winter, or on males or females (Pitcher 1986). Methodologies of Pitcher and Calkins (1979) and this study were not directly comparable, as they directly measured blubber thickness over the xiphisternal process, while our values were generated from the average of dorsal, lateral and ventral ultrasonic blubber thicknesses from the mid-trunk region. Since there was no mention of gender in the comments from the Whiskers database, sexes were combined to generate the values in Table 7. There were no comparable lower Cook Inlet or Kenai Peninsula data appropriate for these seasons (Pitcher and Calkins 1979). However, relative to the other estimates seals during fall-winter of 1993/94 tended to be thinner, while this was less clear for lower Cook Inlet seals. This comparison also serves as a prototype to integrate traditional ecological knowledge and western science. If enough of these types of observations can be compiled, trends might become evident that could improve the understanding of factors affecting the fluctuating or declining populations of harbor seals.

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Table 1. Harbor seals collected or captured in the Gulf of Alaska during 1989-1995 used for hematological or morphological analyses.

	Kodiak Archipelago	Prince William Sound	Southeast Alaska
1989			
Spring[a]		1	
Summer[b]	2	10	
Fall[c]	1		
Winter[d]		1	
1990			
Spring		7	
1991			
Spring		4	
Fall		5	
1992			
Spring		8	
1993			
Spring	4	13	9
Fall	1	15	12
1994			
Spring		10	
Summer			38
Fall	10	31	9
1995			
Spring	8	22	19
Summer		15	
Fall	9	20	9

[a]March-May
[b]June-August
[c]September-October
[d]November-December

Table 2. Plasma chemistry reference ranges generated from harbor seals sampled during 1989-1995 in southeast Alaska, Prince William Sound and the Kodiak archipelago.

Variable	*n*	$\bar{x}$	se	Reference range ±2sd	Total range min-max
Electrolytes					
Sodium[a]	205	146	0.4	135-157	132-167
Potassium[a]	205	3.9	0.1	2.4-5.3	2.9-12.2
Calcium[b]	205	9.6	0.04	8.3-10.9	8.2-11.8
Chloride[a]	205	106	0.6	98-114	6-120
Phosphorus[b]	204	5.0	0.1	2.0-7.9	1.6-14.5
Proteins					
Albumin[c]	205	3.1	0.02	2.5-3.6	0.7-3.7
Globulin[c]	205	4.7	0.05	3.2-6.2	2.2-6.7
A:G Ratio	205	0.7	0.01	0.4-1.0	0.4-1.5
Total Protein[c]	205	7.8	0.05	6.2-9.3	5.0-9.4
Haptoglobin[d]	66	105.0	6.4	1.6-208	20.7-244.7
Total Bilirubin[b]	183	0.5	0.02	0.1-0.7	0.1-2.4
Direct Bilirubin[b]	185	0.3	0.01	0.0-0.6	0.0-0.8
Enzymes					
ALT[e,f]	204	63	5	4-187	12-798
AST[e,f]	199	159	9	25-400	44-1164
Alkaline Phosphatase[e,f]	204	66	3	28-152	20-440
CPK[e,f]	202	1270	161	32-5928	130-16000
GGT[e,f]	176	19	1	5-44	7-197
LDH[e,f]	176	4005	215	777-9739	422-21500
Metabolism Status					
BUN[b]	205	44	1	19-69	17-104
Creatinine[b]	205	0.9	0.01	0.4-1.4	0.3-1.8
Glucose[b]	205	159	2	107-211	65-239

[a]meq/L
[b]mg/dL
[c]g/dL
[d]mg Hb bound/dL
[e]IU/L
[f]Statistics derived from square-root transformations

Table 3. Hematological reference ranges generated from harbor seals sampled during 1989-1995 in southeast Alaska, Prince William Sound and the Kodiak archipelago.

Variable	*n*	$\bar{x}$	se	Reference range ±2sd	Total range min-max
Red blood cell count[a]	128	5.23	0.05	4.02-6.43	3.62-7.87
Hematocrit[b]	245	56	0.4	42-69	32-74
Hemoglobin[c]	192	23.3	0.3	16.7-29.9	14.7-35.8
MCH[d]	120	47	0.6	34-59	29-83
MCV[e]	122	110	0.7	95-125	85-132
MCHC[b]	192	42	0.4	32-52	25-72
Platelet count[f,g]	125	316	18	47-826	5.6-1278
White blood cell count[f,g]	173	11.3	0.2	6.0-18.2	4.9-25.3
Neutrophils (%)[b]	147	55.0	1.0	30.3-78.4	25.0-88.0
Lymphocytes (%)[b]	147	30.1	0.1	11.7-52.5	10.0-61.0
Eosinophils (%)[b]	147	8.3	0.1	0.7-23.0	0.0-28.0
Monocytes (%)[b]	147	2.4	0.1	0.2-12.2	0.0-15.0
Basophils (%)[b]	147	1.1	0.1	0.5-7.8	0.0-12.0

[a] $10^6/cm^2$
[b] %
[c] g/dL
[d] pg
[e] fL
[f] $10^3/cm^2$
[g] arcsin transformed for normalization

Table 4. Examples of harbor seal blood chemistry profiles illustrating clinically unhealthy animals. Seal PV94PW13 was a male yearling captured at Channel Island on 18 Sep, 1994. Seal PV95RH02 was an orphaned pup transported from Ketchikan to Alpine Veterinary Clinic in Anchorage on 18 May, 1995.

Variable	PV94PW13	PV95RH02
Na[a]	144	157
K[a]	3.2	6.4 *
Cl[a]	107	
Ca[b]	10.4	10.0
Phosphorus[b]	4.6	14.5 *
Albumin[c]	3.3	3.5
Globulin[c]	4.1	2.3 *
AG Ratio	0.8	1.5 *
Protein[c]	7.4	5.8 *
Total Bilirubin[b]	0.8 *	2.4 *
Direct Bilirubin[b]	0.5	
ALT[d]	82	76
AST[d]	403 *	
AP[d]	105	440 *
CPK[d]	8250 *	
GGT[d]	15	
LDH[d]	9959 *	
BUN[b]	30	104 *
Creatinine[b]	0.7	0.9
Glucose[b]	138	236 *
Hematocrit[e]	62	
WBC[f]	14.8	7.3
Neutrophils[e]	76	56

*Value outside of normal reference range.
[a]meq/L
[b]mg/dL
[c]g/dL
[d]IU/L
[e]%
[f]$10^3/cm^2$

Table 5. Correlations of girth measurements with underlying mean blubber depth for harbor seals from Kodiak, Prince William Sound and southeast Alaska 1993-1995.

Girth Location	*r*	*P*	*n*
Neck	0.452	0.0345	22
Shoulder	0.299	0.0387	48
Axillary	0.407	0.0006	68
Maximum	0.410	0.0015	57
Mid-trunk	0.422	0.0001	85
Hip	0.418	0.0001	86

Table 6. Correlations of mean blubber depth among measurement sites on harbor seals captured during 1993-1995 at Kodiak Island, Prince William Sound and southeast Alaska (n = 42).

	Shoulder	Axillary	Mid-trunk
Axillary	0.506**		
Mid-trunk	0.595**	0.595**	
Hip	0.507**	0.340*	0.514**

*$P < 0.05$
**$P < 0.001$

Table 7. Comparisons of blubber thicknesses (mm) for combined sexes of harbor seals.

	Blubber thickness	*n*	Source
Prince William Sound			
1973/75 (Oct-Nov)	28±8[a]	146	Pitcher and Calkins (1979)
1993/94 (fall-winter)	6-25[b]	>4	Whiskers database Code 082-13-092994
1993-95 (Sep)	22±6[c]	26	This study
Lower Cook Inlet			
1991(winter)	19-25[b]	na	Whiskers database Code 282-119-042893
Kodiak			
1976/78 (winter)	28±12[a]	4	Pitcher and Calkins (1979)
1994/95 (Sep)	25±5[c]	9	This study

[a] Direct-measure xiphisternal blubber thickness.
[b] Estimated general blubber thickness.
[c] Mean mid-trunk ultrasonic blubber thickness.

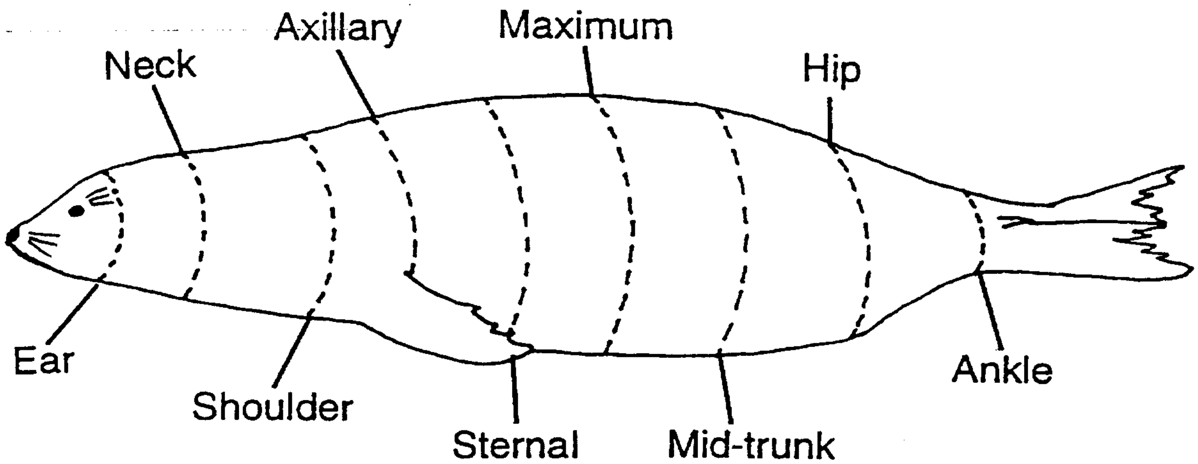

Figure 1. Locations of girth measurements gathered from harbor seals.

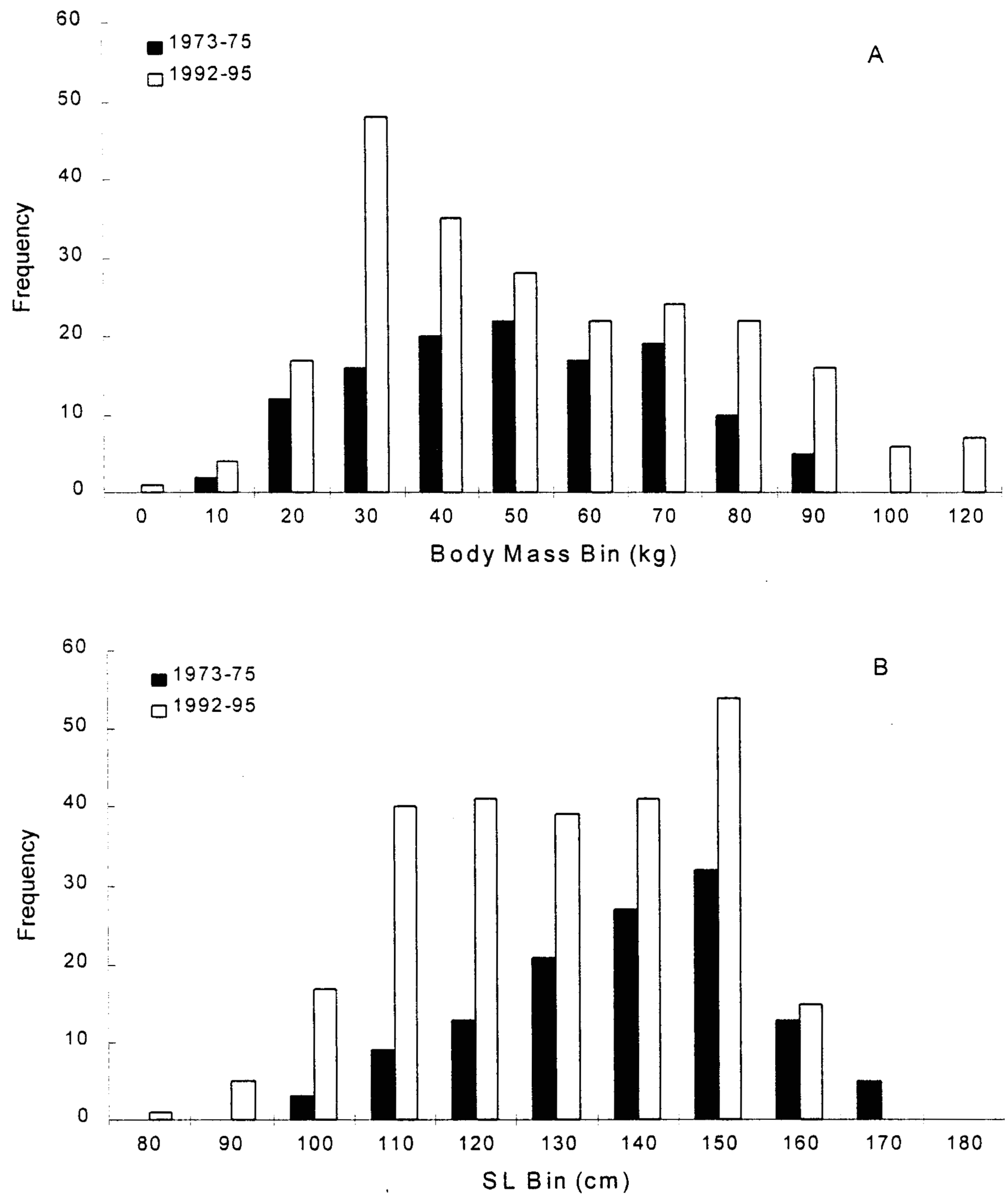

Figure 2. Frequency distributions of body mass (A) and standard length (B) for harbor seals collected (1973-75) or captured by net-entanglement (1992-95) in Prince William Sound. Bin axes denote upper bin limits.

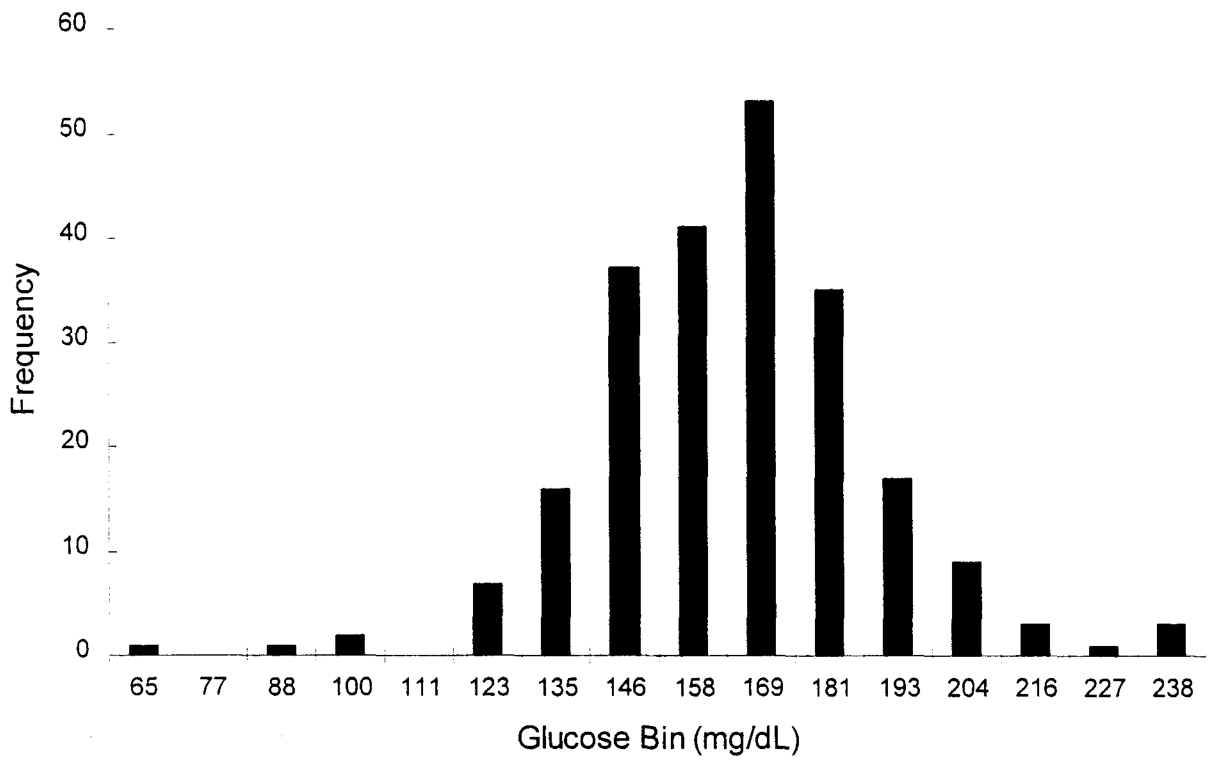

Figure 3. Frequency distribution of glucose for harbor seals sampled during 1989-1995 from Kodiak Island, Prince William Sound, and southeast Alaska. Bin axis denotes upper bin limits.

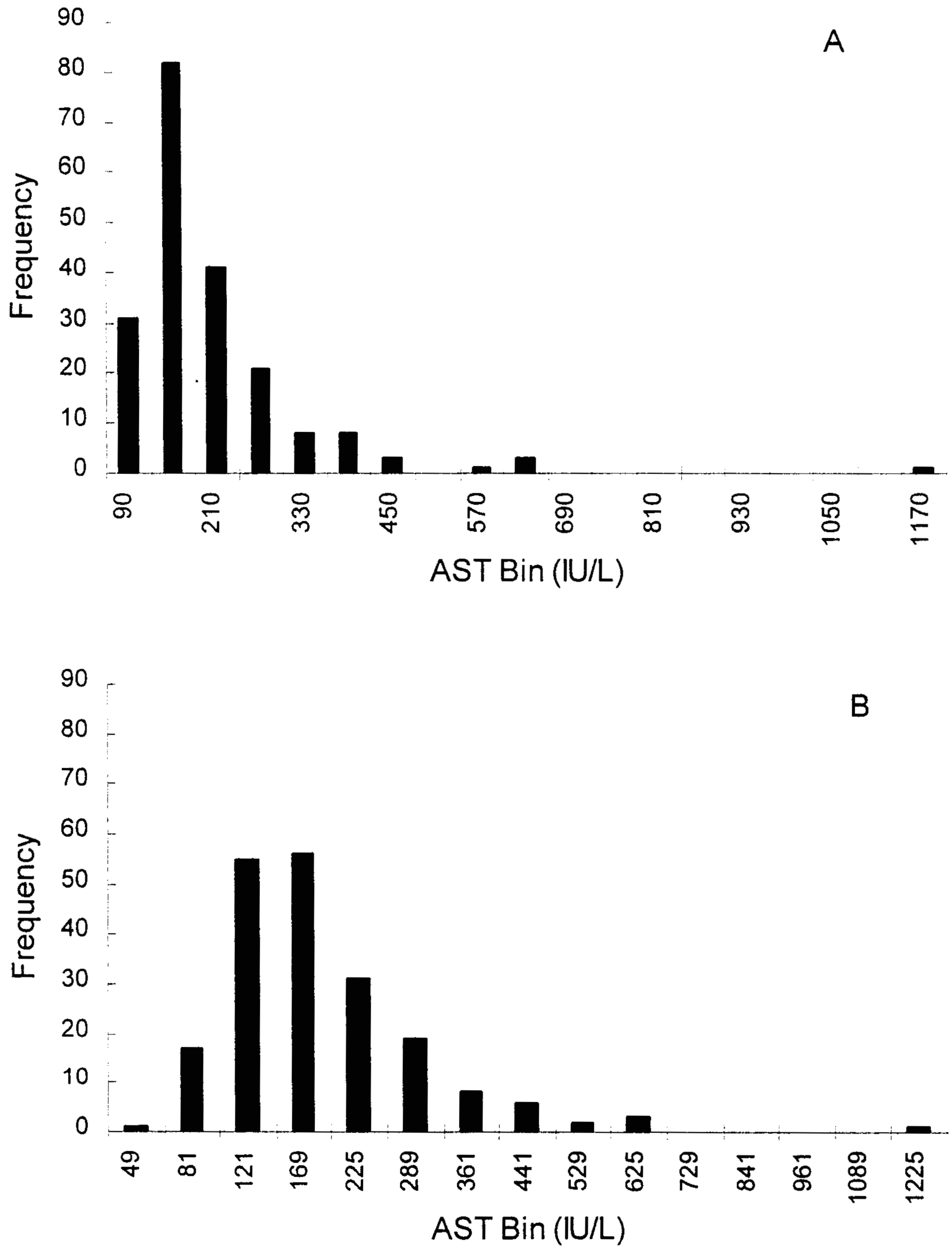

Figure 4. Frequency distributions of plasma aspartate aminotransferase (AST) (A) and square-root transformed AST (B) for harbor seals sampled during 1989-1995 from Kodiak Island, Prince William Sound and southeast Alaska. Bin axes denote upper bin limits.

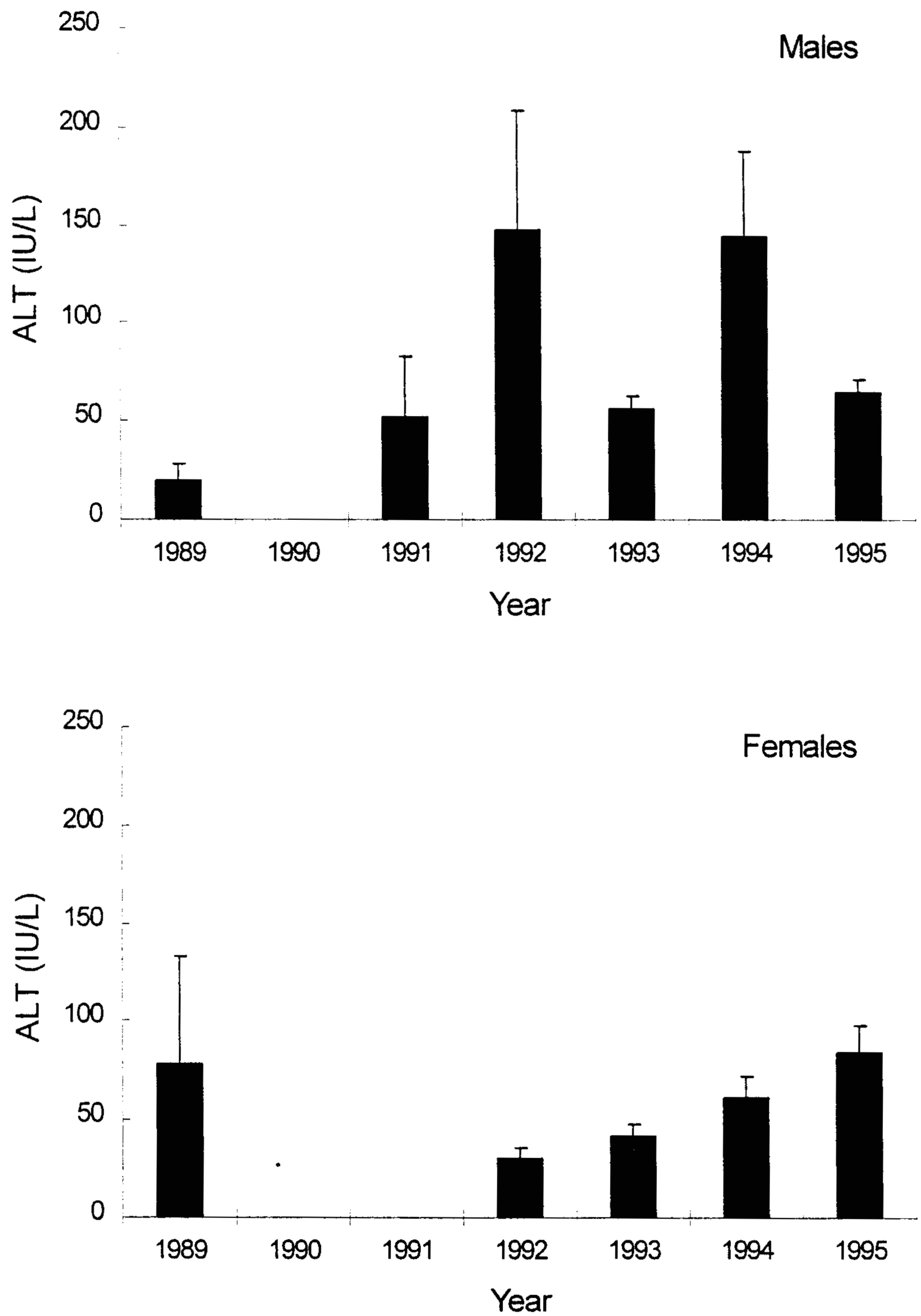

Figure 5. Mean plasma alanine aminotransferase (ALT) activities (International Units per Liter) for male and female harbor seals sampled within western-southwestern Prince William Sound. Error bars denote 1 SE. Sample sizes for males/females: 1989 n = 2/5, 1991 n = 3/0, 1992 n = 6/2, 1993 n = 9/4, 1994 n = 18/13, 1995 n = 15/18.

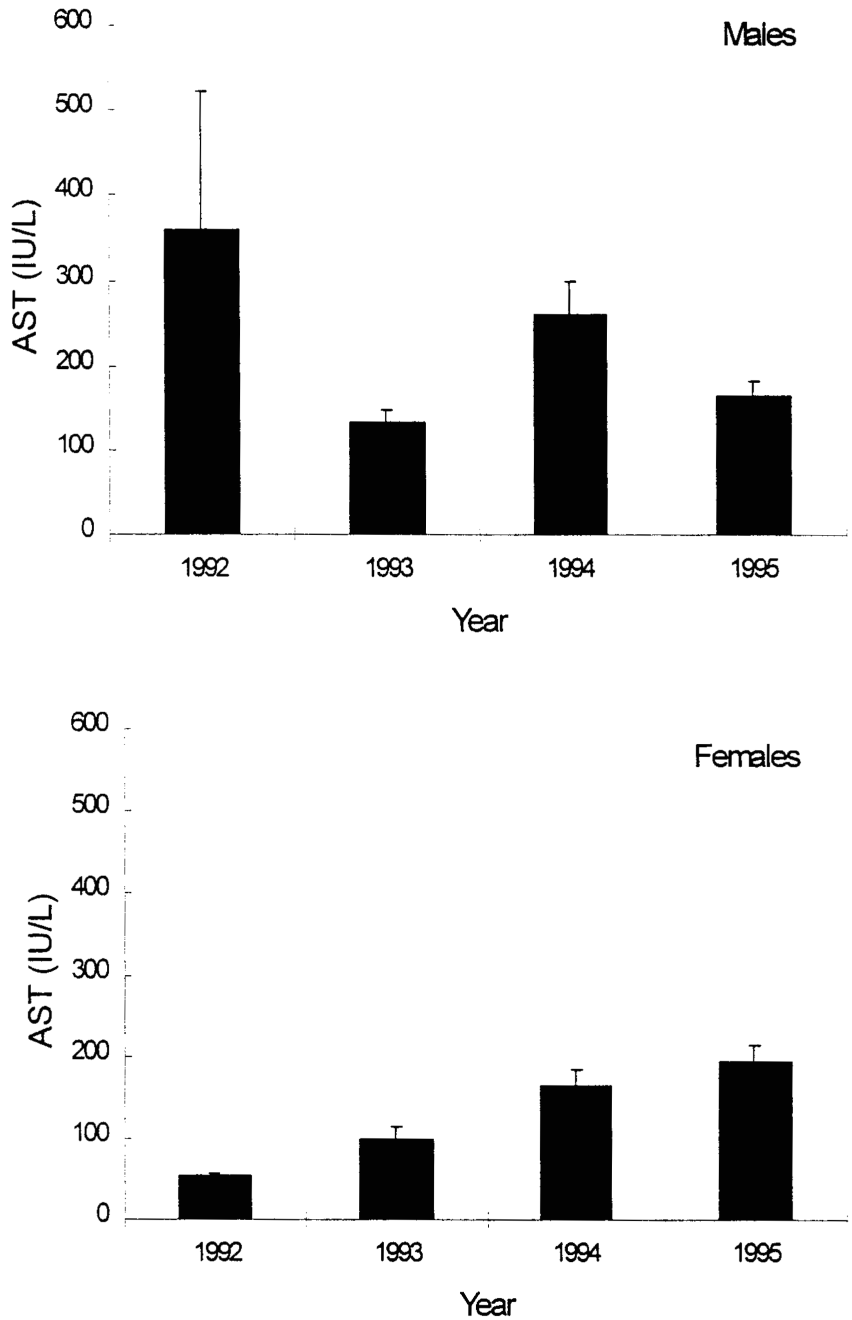

Figure 6. Mean plasma aspartate aminotransferase (AST) activities (error bar is 1 SE) for male and female harbor seals sampled from within western-southwestern Prince William Sound. Sample sizes for males/females: 1992 n = 6/2, 1993 n = 9/4, 1994 n = 17/12, 1995 n = 15/18. Year effect is significant (P = 0.0290) for females.

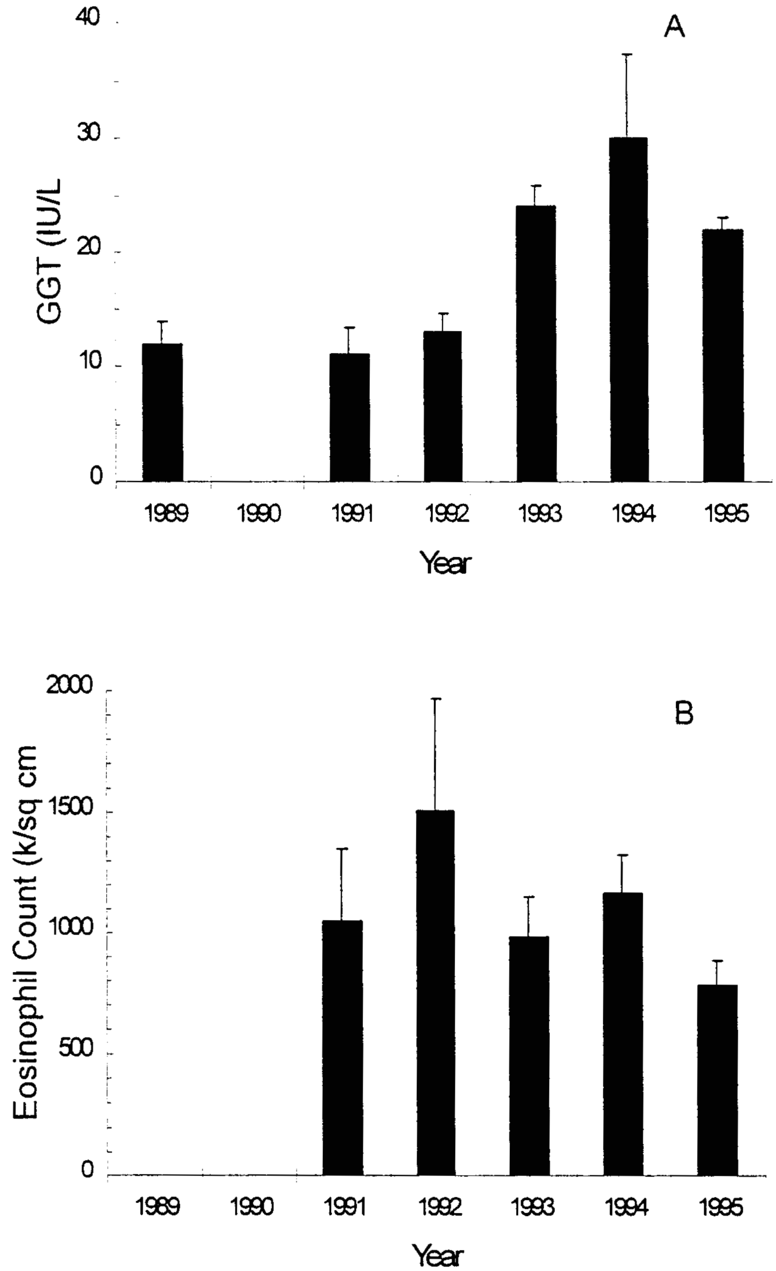

Figure 7. Mean plasma gamma globulin transferase (GGT) activity (A) and absolute eosinophil counts (B) for harbor seals captured within western-southwestern Prince William Sound, both sexes combined (error bars are 1 SE). Both males and females showed the same patterns, but 1993-95 GGT values were significantly higher (P = 0.0001) than 1989-92 for females only. Sample sizes for GGT: 1989 n =7, 1991 n = 3, 1992 n = 8, 1993 n = 12, 1994 n = 29, 1995 n = 33; for eosinophils: 1991 n = 4, 1992 n = 4, 1993 n = 13, 1994 n = 29, 1995 n = 33.

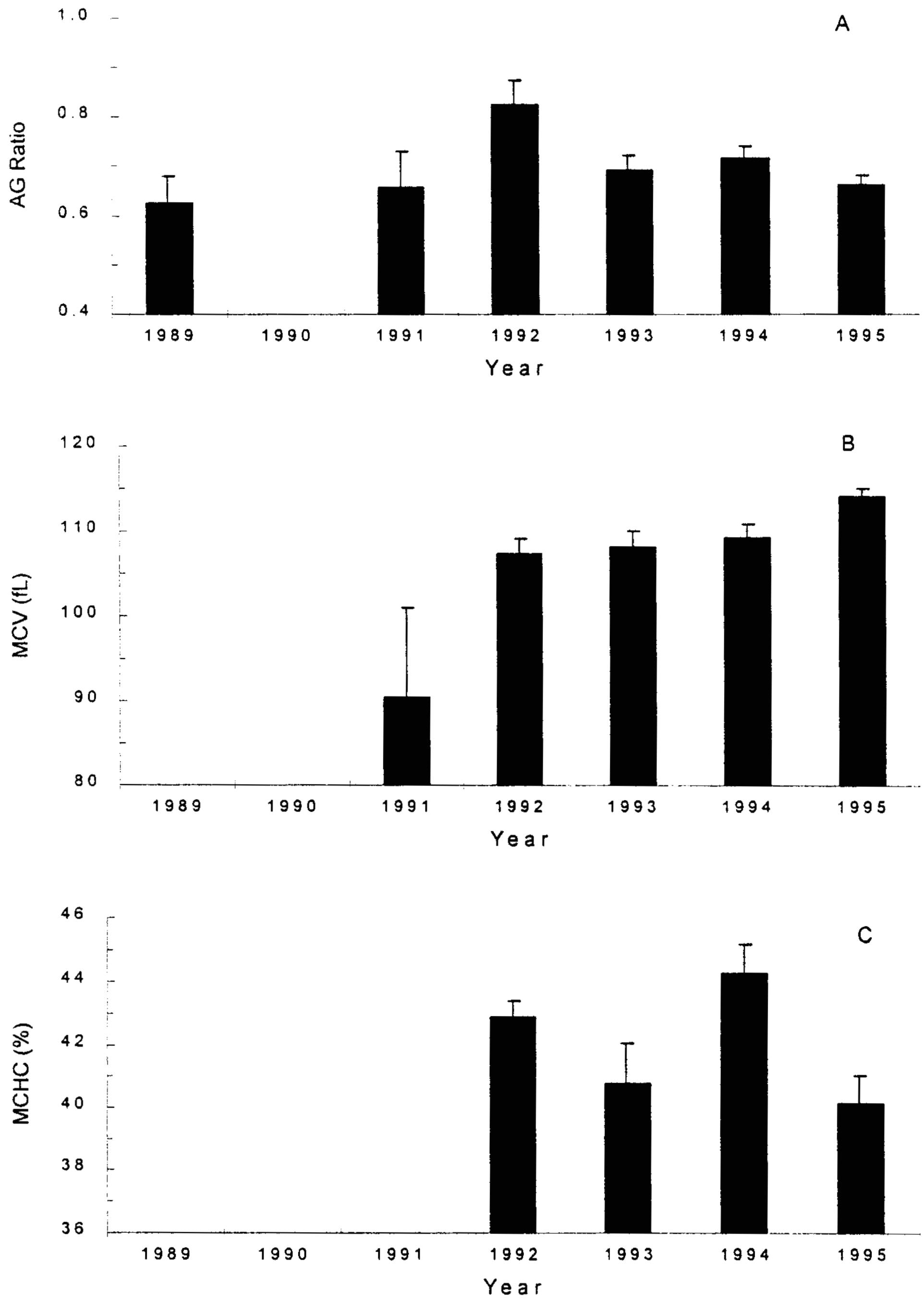

Figure 8. Mean AG ratio (A), MCV (B), and MCHC (C) for harbor seals captured within western-southwestern Prince William Sound, both sexes combined (error bars are 1 SE). MCV values for 1991 and 1995 are significantly different ($P = 0.0005$). AG sample sizes: 1989 $n = 7$, 1991 $n = 7$, 1992 $n = 8$, 1993 $n = 26$, 1994 $n = 28$, 1995 $n = 32$. MCV sample sizes: 1991 $n = 4$, 1992 $n = 6$, 1993 $n = 15$, 1994 $n = 29$, 1995 $n = 33$. MCHC sample sizes: 1991 $n = 4$, 1992 $n = 6$, 1993 $n = 28$, 1994 $n = 31$, 1995 $n = 33$.

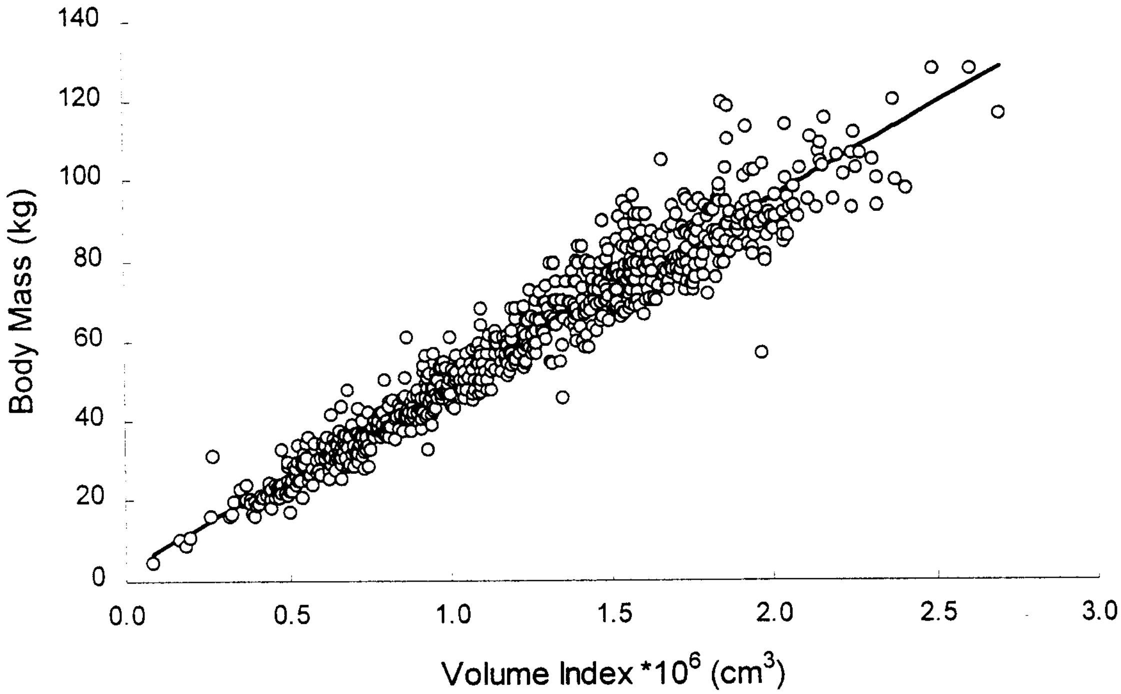

Figure 9. Relationship between body mass and volume index ($SL*G^2$) for harbor seals collected or captured in the Gulf of Alaska, 1973-1995. Regression equation is: $Mass = 2.86 + 4.64*10^{-5}$; $r^2 = 0.933$; $P < 0.0001$; $n = 790$.

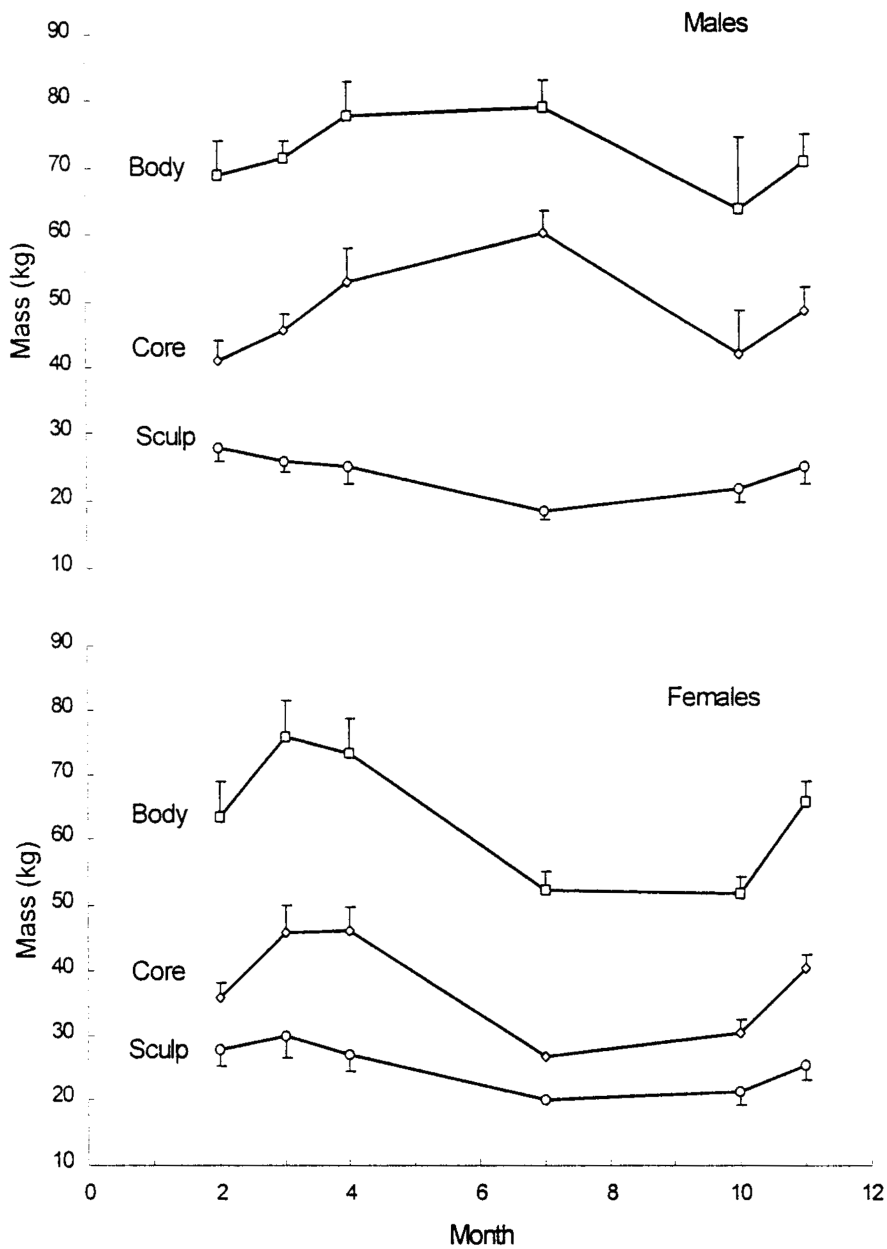

Figure 10. Seasonal body mass and compositional changes for Gulf of Alaska harbor seals (>3 yr old) collected during 1975, calculated with data from Pitcher and Calkins (1979). Error bars are 1 SD. Sculp is mass of skin and subcutaneous blubber. Sample sizes for males/females: Feb n = 9/6, Mar n = 15/4, Apr n = 5/9, Jul n = 8/4, Oct n = 2/3, Nov n = 8/5.

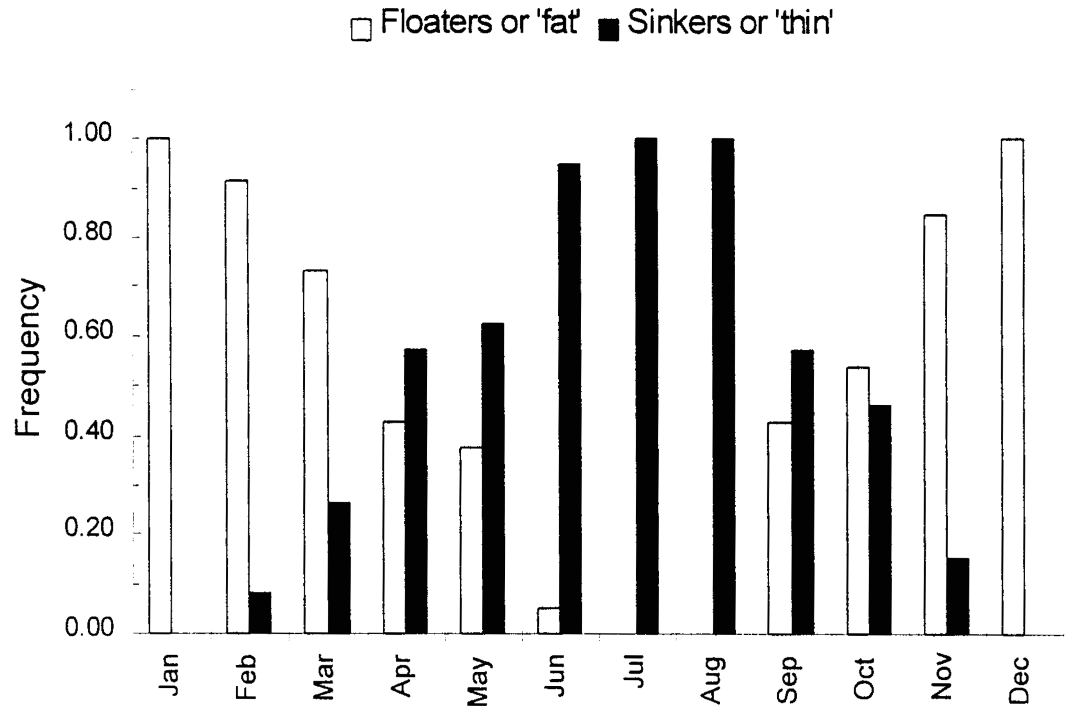

Figure 11. Relative harbor seal body condition quantified from local knowledge as chronicled in the 'Whiskers!' database (Ver. 1.0) of ADFG Division of Subsistence during 1992-95. Proportions are of households within each month categorizing seals as floaters/fat or sinkers/thin. Sample sizes are: Jan $n = 24$, Feb $n = 12$, Mar $n = 15$, Apr $n = 14$, May $n = 16$, Jun $n = 20$, Jul $n = 18$, Aug $n = 17$, Sep $n = 14$, Oct $n = 13$, Nov $n = 13$, Dec $n = 26$.

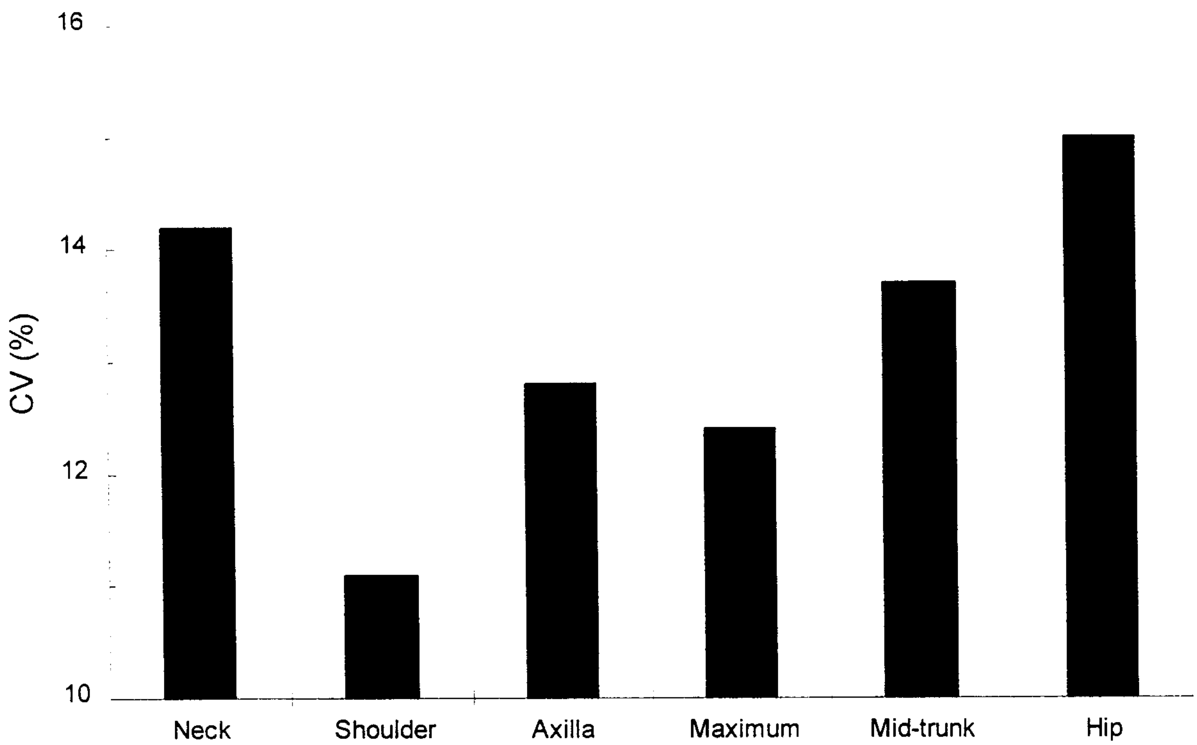

Figure 12. Coefficients of variation in girth measurements of harbor seals captured from Kodiak Island, Prince William Sound and southeast Alaska during 1993-1995. Sample sizes: neck $n = 58$, shoulder $n = 51$, axillary $n = 162$, maximum $n = 65$, mid-trunk $n = 93$, hip $n = 130$.

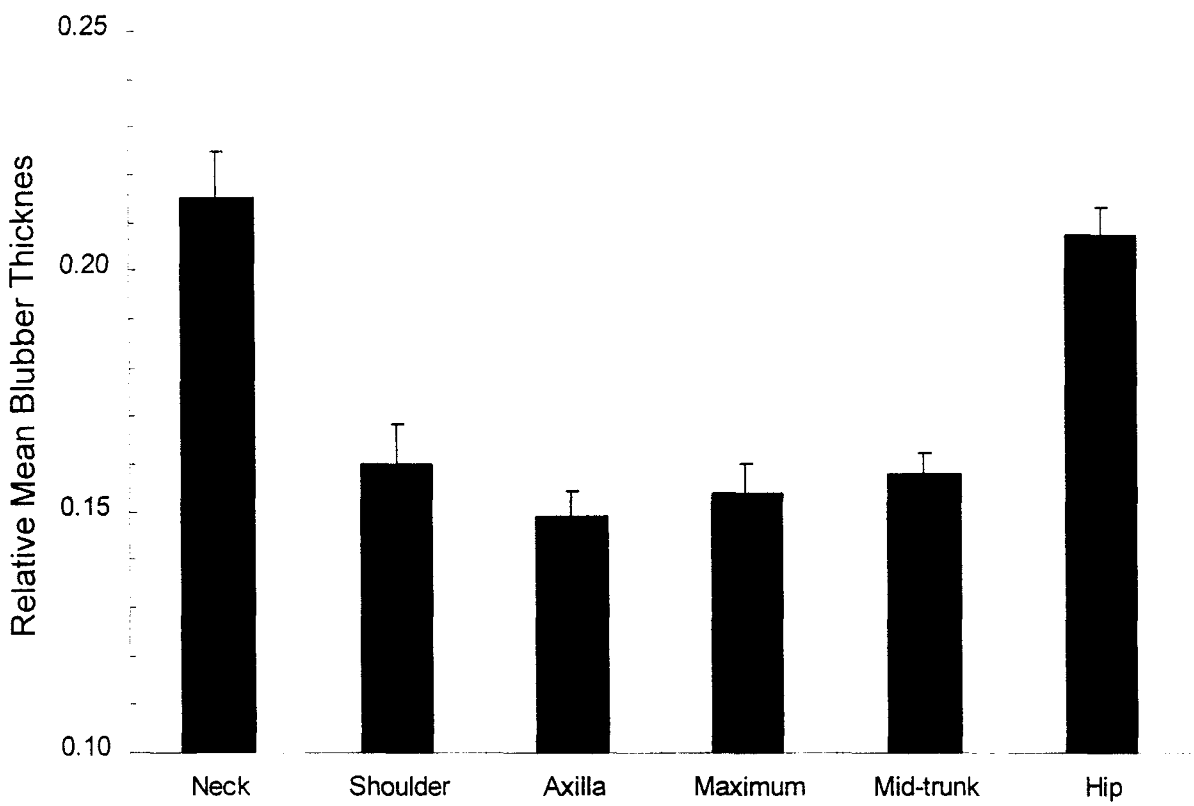

Figure 13. Relative mean blubber thickness (ratio of mean blubber thickness to body radius) for harbor seals captured from Kodiak Island, Prince William Sound and southeast Alaska during 1993-1995. Neck and hip relative blubber thickness are significantly different than others ($P < 0.0001$; Bonferroni comparison after ANOVA). Samples sizes: neck $n = 22$, shoulder $n = 22$, axilla $n = 68$, maximum $n = 57$, mid-trunk $n = 85$, hips $n = 86$.

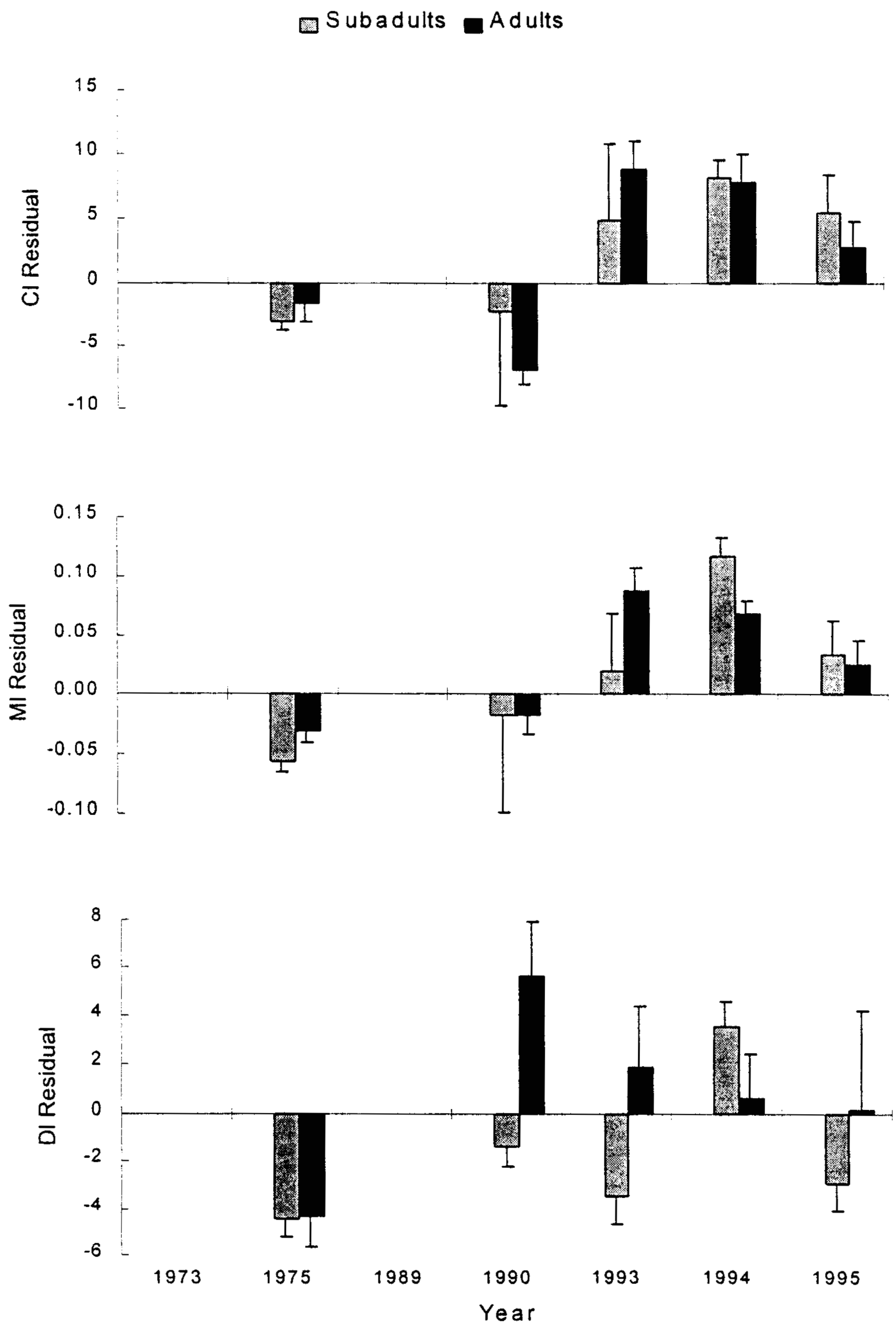

Figure 14. Residual variation from condition index regressions for male harbor seals collected or captured in Prince William Sound during Mar-May. Subadult/adult sample sizes: 1975 n = 13/15, 1990 n = 2/3, 1993 n = 3/5, 1994 n = 4/2, 1995 n = 8/4.

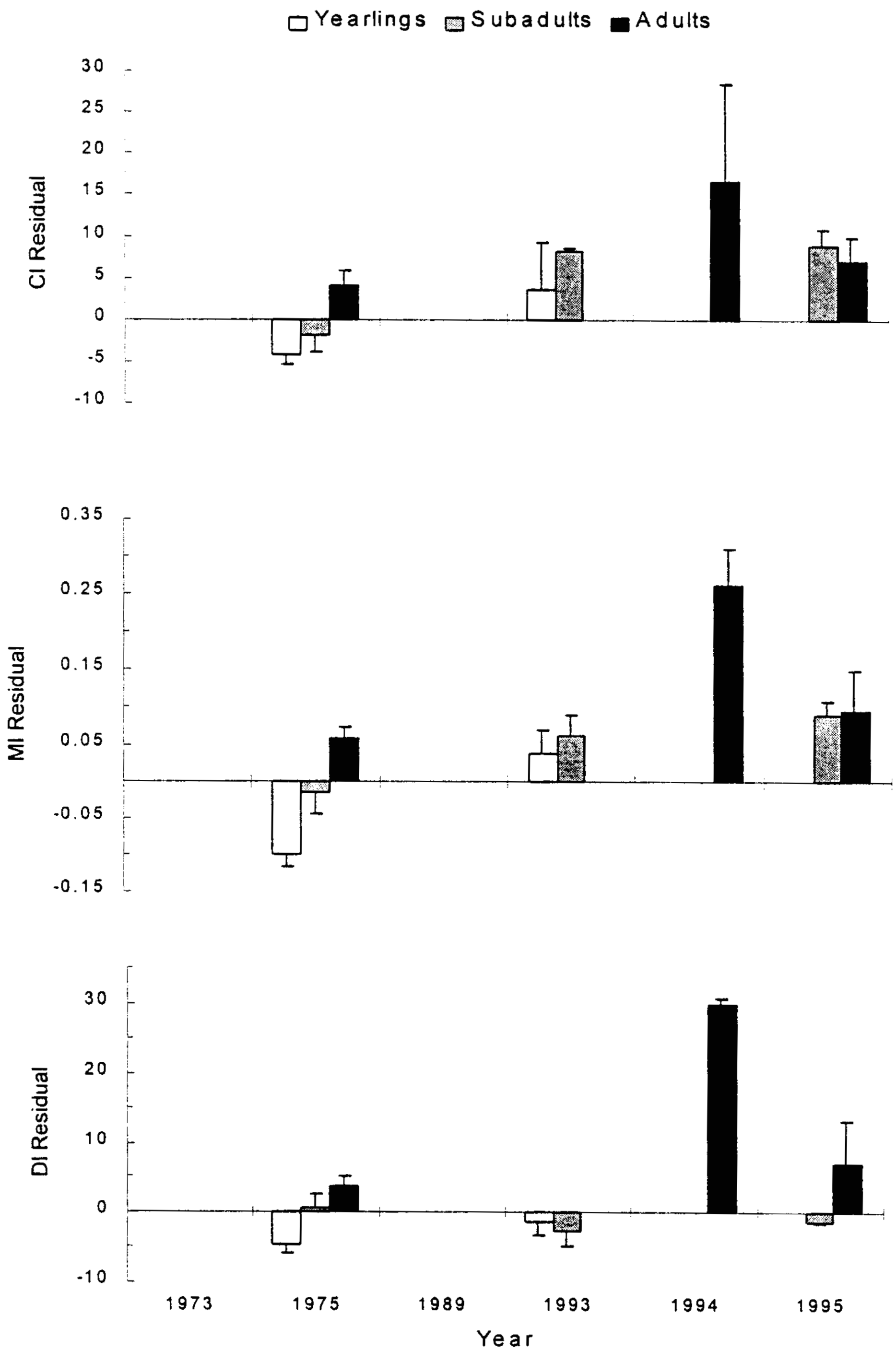

Figure 15. Mean residual variation (error bars are 1 SE) from condition index regressions for female harbor seals collected or captured in Prince William Sound during Mar-May. Sample sizes for yearling/subadult/adult: 1975 n = 5/8/9, 1993 n = 2/2/0, 1994 n = 0/0/2, 1995 n = 0/3/5.

Appendix A. Conversion factors for changing blood chemistry gravimetric units to SI units (from Table A.1 in Kerr 1989).

Variable	Gravimetric unit	SI unit	Conversion factor
Sodium	meq/L	mmol/L	none
Potassium	meq/L	mmol/L	none
Calcium	mg/dL	mmol/L	0.25
Chloride	meq/L	mmol/L	none
Phosphorus	mg/dL	mmol/L	0.32
Protein, albumin, globulin	g/dL	g/L	10
Bilirubin	mg/dL	μmol/L	17.1
BUN	mg/dL	mmol/L	0.36
Creatinine	mg/dL	μmol/L	88.4
Glucose	mg/dL	mmol/L	0.056