Exxon Valdez Oil Spill
Restoration Project Annual Report

Recovery of Harbor Seals from EVOS: Condition and Health Status

Restoration Project 96001
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. An annual report was issued in 1996 by Fadely and Castellini under the title Recovery of Harbor Seals from EVOS: Condition and Health Status. Two journal articles covering portions of this project have been published (Castellini, J. M., H. J. Meiselman and M. A. Castellini. 1996. Understanding and interpreting hematocrit measurements in pinnipeds. *Marine Mammal Science* 12(2);251-264; Zenteno-Savin, T., M. A. Castellini, L. D. Rea and B. S. Fadely. 1997. Plasma haptoglobin levels in threatened Alaskan pinniped populations. *Journal of Wildlife Diseases* 33(1);64-71). An additional journal article covering portions of this project has been submitted for publication (Zenteno-Savin, T. and M. A. Castellini. Plasma angiotensin II, arginine vasopressin and atrial natriuretic peptide in free ranging and captive seals and sea lions. *Comparative Biochemistry and Physiology.* Submitted). The project effort was continued under Restoration Project 96001, the subject of this annual report. Blubber analyses were initiated in FY95 as a Broad Agency Announcement award (95117-BAA), and was rolled over in FY96 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. The program is currently active under Restoration Project 97001 and is expected to continue into FY98. The project will be closed out with a Final Report to be 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*) within Prince William Sound and the Gulf of Alaska in special reference to potential problems induced by the *Exxon Valdez* Oil Spill. We constructed plasma chemistry and hematology reference ranges based on nearly 300 samples collected during 1991 - 1996, that can be used to screen seals for clinically significant conditions. Analyses of variance found up to half of the total variation in blood parameters could be attributed to handling, individual, regional, seasonal, or interannual effects. Power analysis modeling of interannual and interregional comparisons showed that small differences in blood variables could be detected with high statistical power, and that these differences were similar in magnitude to effects produced by handling or individual factors. We have determined that some of the seals sampled during this project exhibited indications of clinical conditions. Seal blubber from Prince William Sound was found to be more energy dense than blubber from southeast Alaska seals, and energy density also varied with gender, season and body mass. Condition indices of blubber content and body size did not show conclusive differences among the two regions.

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

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

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EXECUTIVE SUMMARY

Harbor seal (*Phoca vitulina*) populations in Alaska have declined significantly over portions of their range, particularly in the Gulf of Alaska. Prince William Sound (PWS) populations, further impacted by the spill, have essentially stabilized at decreased levels, but show no signs of population recovery. Assessment and interpretation of harbor seal health status data will help resolve multiple hypotheses proposed to explain these declines, and help focus future studies. This study program 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, but such interpretations require establishment of a set of reference or 'normal' ranges for these blood values. 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. However 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. 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 Prince William Sound and the Gulf of Alaska, and quantify sources of variation from handling, age, gender, seasonal, regional, and interannual sources. We are also testing and utilizing various indices of body condition related to total blubber content and energy status, including assessments of blubber quality from samples provided by subsistence harvesters.

This report describes work accomplished under Restoration Project 96001, subsequent to our previous Annual Report for 95001 completed in April 1996. It also describes analyses of blubber quality which were initiated as Restoration Project 95117-BAA (reported on in an Annual Report completed in Sep 1996), and combined into project 96001. In our previous report preliminary reference ranges for plasma chemistries, hemograms and leukograms were generated and initial examinations of interannual changes within PWS conducted. However, interpretations were limited by an incomplete understanding of individual (seal age and gender), handling, sample processing, seasonal, and regional effects on blood parameters. We also reported on a possible difference in blubber quality between PWS and southeast Alaska (SE) seals in our Project 95117-BAA Annual Report, but the effects of region and season were inseparable. Emphasis was placed on resolving these issues during 1996, and we describe in this report completed analyses of plasma chemistry and hematology reference ranges, with detailed analyses of sources of variance due to individual and environmental factors. We also focused on analyzing blubber samples collected from the harbor seal biomonitoring program (Restoration Project 96244) to resolve the regional comparison between PWS and SE.

In 1996, 89 seals were captured and sampled from haul-outs at Kodiak Island (n = 13), Prince William Sound (n = 62), and southeast Alaska (n = 14). These data were combined into a database of hematological samples comprised of nearly 300 samples collected since 1991. Reference or 'normal' ranges for plasma chemistry and hematological variables were calculated to include ranges within ±2 sd of the mean, thereby including 95% of the data. Of the 23 plasma chemistry analytes measured, 19 were non-normally distributed. Most distributions were

transformable, and reference ranges for the remainder were calculated utilizing non-parametric techniques. Estimates of 95% confidence intervals around range limits were likewise calculated. Of 307 seals sampled between 1993 - 1996 that were screened for outliers based on these reference ranges, 274 had at least one statistically outlying variable. A maximum of 16 outlying variables occurred in two orphaned pups that were captured for rehabilitation. Statistical outliers will not necessarily be indications of clinical disease. However, as numbers of outliers increase within one seal, particularly if these were systematically-related, then the likelihood of clinical significance must increase. Outlier frequency was found to follow a Poisson distribution, with clinically unhealthy animals representing a small fraction of the animals sampled. We propose that statistical models based on this distribution might help separate clinically unhealthy seals from a background frequency expected to occur randomly. Combined with interannual and interregional analyses of the type presented in our 1996 Annual Report, we will be able to describe clinical and subclinical health trends.

Handling, individual, regional, and temporal factors accounted for up to 55% of the variation in plasma chemistries, up to 54% in hemograms, and up to 40% in leukograms. The delay between seal capture and blood sampling significantly affected plasma potassium, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine phosphokinase (CPK), and lactate dehydrogenase (LDH) values, but only affected absolute lymphocyte and eosinophil counts among hematological variables. Age, gender, seasonal, and regional effects were apparent in most parameters, and magnitudes of each effect were 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.

Comparisons of blubber quality, measured as energy density (cal/g), were performed on 28 samples from PWS and 27 SE samples collected by subsistence harvesters and provided to us by the biomonitoring program (Project 96244). We found that blubber energy density apparently declined with increasing mass in males, but not females. Gender-specific regional and seasonal differences in blubber energy density were found. Blubber from PWS females was significantly more energy dense than SE females (spring 1.5%, autumn 4.2% greater; $F_{(1,22)} = 16.099$, $P = 0.001$), and varied significantly with season ($F_{(1,22)} = 7.277$, $P = 0.013$). Energy density of male blubber was 1.6% (spring) to 2.3% (autumn) greater in PWS seals ($F_{(1,25)} = 4.492$, $P = 0.044$), though neither difference was independently significant. There was no significant seasonal component to male blubber energy density ($F_{(1,25)} = 0.141$, $P = 0.711$). These differences in blubber energy density can arise by two mechanisms; either lipids were being selectively removed and stored, or blubber hydration states were changing. These two mechanisms can be differentiated by comparing blubber water and dry-mass basis lipid contents, and we are currently conducting these analyses. There was no clear evidence of differences in whole-seal blubber content or body size between the two regions, based on indices of body condition derived from mass, length, and blubber thickness.

Future field work on blood indices must focus on pup and yearling age classes, which were largely unrepresented in our database. We are continuing analyses of blubber to determine whether these differences were due to changes in hydration state or lipid content. Additionally,

increased regional and seasonal distributions of subsistence harvest-derived blubber samples will expand comparisons with archived (pre-decline) blubber samples (performed in our 95117-BAA Annual Report) to Lower Cook Inlet and Yakutat.

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 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 health status data may 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 program 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 (Engelhardt 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 still comprised 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 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 296 blood samples collected between 1991-96 from free-ranging seals in the Gulf of Alaska, and conducted analyses to determine which blood parameters are sensitive to non-health effects. Preliminary analyses produced evidence of interannual changes in some blood values from seals (Fadely and Castellini 1996), but full interpretations of interannual effects were inappropriate because analyses of handling, age, gender and season factors was incomplete. In this report we have estimated reference ranges for plasma chemistries, hemograms and leukograms, and estimated confidence intervals for these ranges. We also found that handling, individual, temporal

and regional factor effects were sufficiently large to influence results of statistical population comparisons. These effects must therefore be considered when performing interannual or interregional comparisons. We have also begun to develop models that will statistically identify seals with clinically significant blood variable profiles. Some of the seals we have captured may have been clinically unhealthy at the time of sampling.

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. Fadely et al. (1996) found a small (about 2%) difference in blubber energy density between PWS and Southeast Alaska, but could not separate the effects of season from geographic location. With additional blubber samples received from the subsistence harvest biosampling program (Project 96244), work completed since 95117-BAA report (Fadely et al. 1996) has focused on calorimetric determinations to perform appropriate regional comparisons. We have found gender-specific effects of body mass on blubber energy quality , and have found seasonal and regional differences in blubber energy content. Blubber from PWS seals was more energy dense than SE seal blubber, though there were no regional differences in body composition or size.

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.
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

Seal capture locations

Harbor seals were captured from three general geographic regions; Kodiak and Sitkinak Islands (grouped as Kodiak Island, KI), Prince William Sound (PWS) and southeast Alaska (SE). Captures were conducted during Spring (March-May) or Autumn (September-October) months throughout 1993-96, during April 1991 and 1992 in PWS (Frost and Lowry 1994*b*), and during

August 1994 in SE and PWS in July-August 1995 and 1996. Within PWS, 1995 and 1996 field work was conducted during May and September using the chartered vessels *Provider* and *Pacific Star*, in conjunction with Project /064. We also collected samples from seals captured at Kodiak and Sitkinak Islands during March and October of 1995, and October 1996 in association with Alaska Department of Fish and Game (ADFG) utilizing the vessels *Pandalus* and *Big Valley*. Samples were provided by ADFG personnel from southeast Alaska during April and September 1995. October 1996 SE seals were collected operating from the *Quest*. Similar data were collected within PWS in association with Project /064 between 1992-1994, and data collected by Frost and Lowry (1994*a,b*) from 1989-92 were included in some analyses. Seals captured during 1992-1996 in spring and fall months in PWS were also utilized by Frost and Lowry (1994*b*) and Frost et al. (1995) for satellite-tagging and trophic interaction studies. Seals were also captured during 1993-1994 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 1972-1978 (Pitcher and Calkins 1979) was provided by ADFG for morphometric analyses and comparisons.

Animal handling and sample collection

Seals were live-captured from 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). 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, and gender was recorded. Seals were held for variable periods to recover from drugging effects before being allowed to return to water.

Plasma chemistry and hematology

Blood was sampled from the intervertebral extradural vein (Geraci and Smith 1975) using 3.5 inch 18 or 20 G 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 pups and some yearlings were taken by flipper venipuncture, using 1.5 inch 18 or 20 G 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 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 aliquoted into 1.5-2.0 mL cryogenic storage vials (Nalgene® Brand, Nalge Co., Rochester, NY) and frozen in liquid nitrogen until return 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. 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. Recorded handling factors which might effect blood parameter variability 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.

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 (Ektachem Analyzer) by technicians at the Fairbanks Memorial Hospital (FMH) on plasma collected in sodium or lithium-heparin collection tubes. 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 collection tubes 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 by Dr. Terry Spraker. 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.

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 (Kopec and Harvey 1995). 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 1984). Reference ranges were calculated as being within two standard deviations of the mean (Kerr 1989), 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 1987). Reference range 95% confidence limits for normally distributed data were calculated as:

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

where q is the quantile, and as:

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

for other data (Bland 1987). Based on calculated reference ranges, the blood chemistry and hematology database was filtered for statistical outliers among seals captured during 1993-96 for which plasma was collected. Regional and temporal comparisons were conducted by calculating weighted outlier proportions, where the proportion of outliers for an analyte (p_o) was calculated as:

$$p_o = n_o \cdot (n_s/N)$$

where n_o was the number of seals exhibiting an outlying value for the blood variable of interest, n_s was the number of seals sampled at the same time and location as n_o, and N was the total number of seals with outlying variables.

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. 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). Models were recalculated with few or no handling effects (if appropriate) to expand the comparisons to years prior to 1995 which did not have all handling effects recorded. 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 1984) 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 1973). Tests were conducted using two examples from the data, a regional comparison among Prince William Sound, Kodiak Island and southeast Alaska based on sample sizes 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© version 4.1 (Analytical Software) or Systat© version 6.1 (SPSS Inc.).

Blubber analyses

Harbor seal blubber samples were collected through the subsistence harvest biosampling program (Project No. 96224). Seal hunters and trained assistants removed blubber samples (approximately 100 - 200 g) from the ventral 'hip' region (about 60 - 70 % of standard length from the nose) of seals collected during subsistence harvests. Measurements of body mass, standard length, curvilinear length, axillary girth, hip girth, xiphosternal and ventral hip blubber thickness were also collected when possible. Blubber collected from the seal was placed in a plastic storage bag and frozen at -5 C. Blubber samples were kept frozen for transport to the

University of Alaska Fairbanks (UAF) Museum, where they were recorded into a chain of custody database and redistributed to our laboratory. Blubber samples were double or triple bagged in freezer storage bags (Ziploc Brand, DowBrands L.P., Indianapolis, IN) and frozen at -80 C.

Using data provided by the biosampling program, an index of harbor seal body condition was calculated using standard length, body mass and xiphosternal blubber depth (mLMD) in an equation modified from Ryg et al. (1990) that is related to the total blubber content (Ryg et al. 1990, Gales and Renouf 1994, Fadely et al. 1995). Comparisons of blubber thickness were performed by calculating relative blubber thickness as the ratio of xiphosternal blubber depth to axillary radius, calculated as girth/2π (Ryg et al. 1988, Beck and Smith 1995).

Samples for bomb calorimetry were prepared by cutting cubes of frozen blubber. All edge, muscle or visibly oxidized surfaces were trimmed away. Duplicate 0.9 - 1.0 g samples (measured to ±0.0001 g) were kept frozen in calorimeter crucibles until analyzed in an automated non-adiabatic bomb calorimeter (Parr Co.). Energy densities were expressed as a per wet-mass basis. Duplicate 0.3 - 0.5 g blubber samples were cut and freeze-dried to constant mass at -70 C under vacuum (Labconco Freeze Dryer Model 5) and the water content calculated based on the mass difference. Lipid content was determined as the mass difference after extraction of wet or dried blubber samples of initial wet masses between 0.5 - 0.7 g (measured to ±0.0001 g) for >24 h in a 2:1 chloroform-methanol mixture in a Soxhlet extraction apparatus.

RESULTS

Plasma chemistry and hematology samples

Data from up to 296 seals sampled during 1992 - 1996 were utilized in plasma chemistry reference range calculations (Table 1), because not all analytes were necessarily measured for each seal. 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 were also 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, $P < 0.089$; Table 2). Likewise, 249 samples utilized for hematology ranges (Table 3) 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 (Table 4; overall $\chi^2 = 5.17$, df = 3, $P = 0.160$).

Reference ranges

Of the 23 plasma chemistry analytes measured, 19 exhibited non-normal distributions (Table 5; Figures 1, 2). Distributions of enzyme activities were successfully normalized utilizing logarithmic transformations (Figure 2), though distributions of potassium, creatinine and bilirubin could not be normalized by any transformation and non-parametric statistics were calculated. The remainder of analytes deviated only slightly from normality and parametric statistics were used. Mean corpuscular hemoglobin content, MCH (field-derived only) and platelet count were not normally distributed (Table 6; Figures 3 and 4), but MCH and platelet count were successfully transformed. White blood cell counts (WBC) and absolute counts of banded neutrophils and basophil were not normally distributed, nor were differential counts of banded neutrophils,

monocytes, eosinophils or basophil (Table 7; Figure 5). Of these, only WBC and differential eosinophil counts were transformable.

Plasma chemistry variability

Individual, handling and environmental factors explained up to 55% of the variation in plasma chemistries collected during 1995 - 96 for which handling factors were included (Table 8). Handling factors only affected 6 of the 23 analytes, and the elapsed time between blood sampling and processing (up to 8 h) did not account for variation in any analyte. Application of ketamine/diazepam for chemical restraint produced effects on phosphorus and total bilirubin. Elapsed time between seal capture and blood sampling time (0.2 - 8.3 h) produced effects in potassium, AST, CPK and LDH (Table 8). Partial F test statistics were calculated to test the effects of removing this variable from each model (Neter et al. 1990). With the exception of AST which was marginally significant ($F^*_{(1,71)} = 4.107$; $P < 0.05$) and explained only 5.5% of the variation, the elapsed time between capture and sampling was a significant component of the regression models, and accounted for large portions of variability (potassium $F^*_{(1,71)} = 16.7$, $P < 0.001$, $R^2 = 0.226$; CPK $F^*_{(1,71)} = 14.5$, $P < 0.001$, $R^2 = 0.196$; LDH $F^*_{(1,71)} = 19.5$, $P < 0.001$, $R^2 = 0.325$). To increase sample sizes for comparison, multiple regression models were run excluding handling effects for the other analytes, and were limited to seals which had been drugged prior to sampling. In these models, individual, regional and temporal effects explained 2.1 - 39.2% of the variation in plasma chemistries (Table 9). Potassium levels were not significantly affected by the elapsed time between blood sampling and sample processing in this comparison. Partitioning total variation among significant parameters showed that only sodium and alkaline phosphatase were effected by all age class, gender, region, season and interannual effects, while gender only affected 5 of the 19 variables (Table 10). 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 11).

Hematological variability

Handling, individual, regional and temporal factors accounted for up to 54% of the variation in hematological measurements in the 1995 - 96 data subset (Table 12). 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 (Table 12). However, the elapsed time between sample collection and CBC analysis (range 0 - 19 days, median = 4 days) produced effects in field-derived MCV, hospital-derived MCHC and RBC (Table 12). The elapsed time effect on RBC was small ($F^*_{(1,82)} = 19.5$, $P = 0.05$, $R^2 = 0.055$) and only accounted for 5.5% of the variability. When adjusted for gender, region and season, RBC counts did not exhibit noticeable effects of elapsed processing time for up to 6 days (Figure 6). Elapsed time between sample collection and CBC analysis accounted for 27% of the variation in hospital-derived MCHC ($F^*_{(1,85)} = 23.6$, $P < 0.001$, $R^2 = 0.269$). As above, regression models were reconstructed excluding handling effects for appropriate analytes to increase sample sizes for comparison. Elapsed time was limited to <6 days for field-derived MCV and the clinical laboratory RBC count. This factor proved not to be significant in the expanded data set for hospital-derived MCHC ($F^*_{(1,218)} = 0.13$, $P > 0.5$), and was dropped. Combinations of

individual, regional and temporal factors accounted for 4 - 56% of hematological variability (Table 13), which are partitioned among individual and environmental factors in Table 14.

Leukogram variability

Administration of anesthesia and gender did not account for significant amounts of variation in leukograms when modeled with environmental factors (Table 15). Elapsed capture to sampling time caused decreases in absolute lymphocyte and eosinophil counts, while the elapsed time between sampling and creation of blood smears tended to decrease absolute and differential basophil counts (Table 15). Elapsed time between sampling and CBC processing caused a 1% per day increase in differential eosinophil counts (Table 15). Recalculated models with reduced handling parameters are presented in Table 16, and relative contributions of individual and environmental factors on leukograms are summarized in Table 17.

Blubber analyses

Gender specific differential effects of body mass on blubber energy content were found (Figure 7). Blubber energy densities of male seals tended to decline with increasing body mass, though this regression was significant for PWS (Energy density (cal/g) = 8943 - 3.12 · Mass; $F_{(1,4)} = 12.622$, $P = 0.024$, $r^2 = 0.759$), but not in SE (Energy density (cal/g) = 8786 - 3.04 · Mass; $F_{(1,15)} = 3.346$, $P = 0.087$, $r^2 = 0.182$). There were no significant trends for females from PWS ($F_{(1,8)} = 2.353$, $P = 0.164$, $r^2 = 0.227$) or SE ($F_{(1,15)} = 3.346$, $P = 0.087$, $r^2 = 0.182$).

Regional and seasonal differences in blubber energy density were found in samples from PWS and SE (Figure 8). Blubber from PWS females was significantly more energy dense than SE females (spring 1.5%, autumn 4.2% greater; $F_{(1,22)} = 16.099$, $P = 0.001$), and varied significantly with season ($F_{(1,22)} = 7.277$, $P = 0.013$). This seasonal difference was only evident in southeast Alaska (3.3% decline from spring to autumn; Bonferroni-adjusted post-hoc comparison $P = 0.039$). Energy density of male blubber was 1.6% (spring) to 2.3% (autumn) greater between PWS and SE seals ($F_{(1,25)} = 4.492$, $P = 0.044$), though neither difference was independently significant (Bonferroni-adjusted post-hoc comparison $P > 0.05$). There was no significant seasonal component to male blubber energy density ($F_{(1,25)} = 0.141$, $P = 0.711$).

Relative blubber thickness (Table 18) was slightly greater in SE seals for both sexes in spring (5% difference) and autumn (3% difference; $F_{(1,51)} = 0.007$, $P = 0.007$), but there were no significant seasonal or sex differences ($P > 0.05$). Sufficient morphometric data were collected for two mLMD comparisons, and only females in autumn ($t_s = -2.739$, $P = 0.019$, df = 11) were significantly different (Table 18). There was no significant difference between males in spring ($t_s = -0.440$, $P = 0.668$, df = 12). There was also no detectable difference in scaling of mass and length between the two regions ($F_{(1,32)} = 0.077$, $P = 0.783$, regression on log-transformed variables, adjusted for sex and season; Figure 9).

DISCUSSION

Plasma chemistry and hematology reference ranges

Plasma chemistry and hematology measurements from free-ranging animals can provide critical data for determining health status of individuals, 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). Determination of possible clinical conditions using this technique requires the establishment of reference ranges, preferably from known healthy or unhealthy subjects (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). Thus, construction of reference ranges requires large sample sizes distributed throughout the age and sex structure of a population to be representative. Sample sizes used in constructing reference ranges for Gulf of Alaska harbor seals in this study (n = 296 for plasma chemistries) are much larger than have been previously available (Roletto (1993) n = 101; Kopec and Harvey (1995) n = 53; de Swart et al. (1995) n = 22). Estimation of 95% confidence limits around lower or upper reference ranges is important especially for blood parameters for which elevated values are clinically significant (Kopec and Harvey 1995), yet in practice these are rarely calculated (Bland 1987) presumably due to sample size limitations. Reference ranges presented here can ultimately 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 would not be appropriate. However, collection of additional pup and yearling data could be critical, since these age classes may be particularly sensitive to food limitations and disease (Roletto 1993, Rea 1995).

Of 307 seals screened for outliers that were sampled between 1993 - 1996, 274 had at least one statistically outlying variable (Figure 10). A maximum of 16 outlying variables occurred among two orphaned pups that were captured for rehabilitation. As stated above, statistical outliers will not necessarily be indicative of clinical disease. However as numbers of outliers increase within one seal, particularly if these were systematically-related blood variables, then the likelihood of clinical significance must increase. According to the distribution in Figure 10, these follow a Poisson distribution, with clinically unhealthy animals representing a small fraction of the animals sampled. In a Poisson distribution, probabilities of occurrence must be independent (Sokal and Rohlf 1973). 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' will broaden the shoulder compared to that of a randomly-generated Poisson distribution. We are currently deriving the appropriate statistical models for this type of analysis. With this type of modeling, the distribution of outliers within the sample 'space' of time and region might be separable from a background frequency expected to occur randomly. A relative distribution of plasma sodium concentration, haptoglobin and WBC outliers were plotted with respect to year and region to detect whether non-random clusters might occur (Figure 11). Though we have yet to derive appropriate statistical tests to formalize this approach, there were regionally-specific trends in distributions (Figure 11). Combined with interannual and interregional analyses of the type presented in Fadely and Castellini (1996), we will track patterns of clinical and subclinical health parameters.

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), diet or contaminants (Duffy et al. 1993, Kopec and Harvey 1995, Schumacher et al. 1995, de Swart et al. 1995). However, few studies have had the scope to address sources of variability such as handling, age, gender or season, though recently Kopec and Harvey (1995) and de Swart et al. (1995) tested for age, gender and season effects. In comparisons of free-ranging populations, isolation and quantification of these sources of variation could be critical to interpreting observed differences in blood chemistries or hematologies. Handling effects, defined as administration of anesthesia, elapsed time before blood collection, and elapsed time between collection and processing of blood samples had relatively few significant effects on blood chemistries relative to total variability. The amount of time a seal was held prior to sampling accounted for significant variability in AST, CPK and LDH (Tables 8, 10) as would be expected from acute stress-related enzymes (Bossart and Dierauf 1990), but the magnitude of the effects relative to mean values was small (Table 8). Larger effects were produced by differences in blood collection tubes (Table 11), and many were greater in magnitude than individual or environmental effects (Table 8, 9). The type of blood collection tube is therefore a critical consideration when comparing regional or temporal data. For example, it is important to note when comparing samples from Prince William Sound, that samples collected immediately following the oil spill were collected in serum tubes (Frost and Lowry 1994*a*).

Individual and environmental factors explained up to 39% of the variation in plasma chemistries, but no single factor was consistently the largest contributor (Table 10). Single factors explained up to 18% of total variation (except gender which accounted for 3% at most), but the median R^2 were 0.0 for gender, region and season, and only 0.033 and 0.029 for age and year, respectively. This suggests that a great deal of variation remains to be attributed to other sources such as seal health or condition, unquantified handling techniques, or analytical laboratory variability. Variation explained by environmental factors does not preclude the possibility of health concerns. For example, the regional differences in haptoglobin levels found by the model (Table 9) have been considered to be indicative that the Prince William Sound population is affected by stressors differentially from seals in other regions (Zenteno-Savin et al. 1997). Likewise there are undoubtedly many of other sources of variability that have not been quantified by our approach, such as blood collection tube drawing order, which can affect some chemical and hematological analytes (McClatchey 1994). Power modeling of minimum detectable differences in regional or interannual comparisons indicate that we have the ability to detect relatively small differences in plasma chemistries with high statistical power (Table 19). These differences are sufficiently small that comparisons could easily be biased by age and gender effects, and temporal or regional comparisons must be carefully constructed.

Hematological variability

Most handling effects did not account for significant variability in hematologies (Table 12) or leukograms (Table 15). Elapsed time between sample collection and CBC processing affected RBC count and some associated variables (Table 12), though this effect seemed to occur mostly after 7 days of refrigeration (Figure 6). 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 1 day which were not evident in these 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 (Castellini et al. 1996). These effects were evident by comparing field and clinical laboratory derived hematologies (Table 12, 13), and will therefore need to be considered in comparisons 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 is not to say the handling effect does not produce changes in an analyte. For example, it is well known that handling stresses can elevate hematocrit, while drugging causes decreases (Castellini et al. 1996). What has been shown by modeling with these data is that given handling, individual, temporal and regional factors, the handling effects may not have contributed significantly to explaining additional variability when the other factors are considered.

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, 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 (Kopec and Harvey 1995, de Swart et al. 1995). Hematocrit, hemoglobin and RBC had large seasonal components to their variability (Table 14), and varied directly with each other (Table 13). This seasonal effect has previously been noted for harp seals (Ronald et al. 1969) during summer molt periods, for harbor seals captured in San Francisco Bay (Kopec and Harvey 1995), and for captive harbor seals (de Swart et al. 1995). Power modeling of minimum detectable hematological differences in regional or interannual comparisons shows detectable differences (Table 20) similar in magnitude to changes caused by handling, individual or environmental factors (Tables 12, 13). Platelet counts were an exception, being highly variable (Table 6).

Leukogram variability

As with plasma chemistries and hemograms, assessment of non-health related sources of variability is 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). Leukograms were relatively insensitive to handling effects of drugging and sample handling (Tables 15, 16), and were completely unaffected by gender. As with hemograms, other studies have found varied effects. Kopec and Harvey (1995), found that neutrophils, monocytes and lymphocytes were effected by gender, though de Swart et al. (1995) did not find significant effects of gender on leukograms. All three studies detected significant age-related effects on several leukocyte values (Tables 15, 16). In contrast to plasma chemistries and hemograms, however, combinations of handling, individual, temporal or regional factors did not account for much of the overall variability in leukocytes (Tables 15, 16, 17). It is also evident that because of very small detectable differences based on power analyses (Table 21), these factors must be considered when performing interannual or interregional comparisons.

Blubber analyses

Fadely et al. (1996) found no detectable effect of handling, measured as the elapsed time from seal collection to blubber sample freezing, on blubber energy content. If effects of this type were operating, then they occur within 1 hour of collecting a seal. Otherwise, handling effects can be ignored for other comparisons. Previously, regional differences in blubber energy density and lipid content were inseparable from seasonal effects (Fadely et al. 1996), but we have now found that seal blubber from Prince William Sound seals was more energy dense than from seals collected in southeast Alaska in both spring and autumn periods (Figure 8). We also found that blubber energy density apparently declined with increasing mass for males, but not females (Figure 7). This mass difference did not alter the results of regional comparisons for males. These differences in blubber energy density can arise by two mechanisms; either lipids were being selectively removed and stored, or blubber hydration states were changing. These two mechanisms can be differentiated by comparing blubber water and dry-mass basis lipid contents (Fadely et al. 1996), and we are currently conducting these analyses on the samples. However, regional and seasonal differences in blubber quality arising from changes in lipid content have also been shown in other pinnipeds including harbor seals (Bowen et al. 1992).

Differences in blubber quality can be compensated by changes in quantity (Beck et al. 1993, Gales et al. 1994). Though blubber mass is not directly measured as part of the biosampling program, several morphometric measurements were collected that were used as indices of blubber content. There was marginal evidence for increased blubber content of southeast Alaska seals based on xiphosternal blubber thickness measurements (Table 18). Xiphosternal blubber depth, however, is poorly correlated with the total blubber content of a harbor seal (Pitcher 1986; Fadely and Castellini 1994, 1995). Better estimates of blubber stores were achieved by Ryg et al. (1990) using an index based on mass, length and a dorsal blubber depth taken at about 60% of body length. This index was modified to incorporate xiphosternal blubber depth measurements, which has modest correlation with total blubber content (Fadely and Castellini 1995). Comparison of this index suggests that regional differences in blubber content were minimal or absent, and that lower blubber energy densities of SE seals were not compensated for by increased blubber mass. Likewise, there was no detectable difference in the mass-length relationship of seals between the two regions (Figure 9), and though this index has no relationship with changes in blubber content (Fadely and Castellini 1995), the lack of differences suggest SE seals were not more or less massive than PWS seals. The utility of this index increases if mass and length can be measured against animal age. Teeth were extracted from harvested seals for aging as part of the biosampling program, but have yet to be analyzed by ADFG. Size ranges at age were taken from aged-seals during 1972 - 1978 (Pitcher and Calkins 1979) and delineated on Figure 9. Based on this, 1 - 4 year-old seals were well represented in the sampling, an age group that could be susceptible to nutritional compromise.

Seasonal decreases in blubber quality and quantity have been shown in lactating harbor seals (Bowen et al. 1992). Several studies have now shown a positive relationship between blubber energy density and total body fat stores (Worthy and Lavigne 1983, Bowen et al. 1992, Gales et al. 1994). Therefore these regional differences in quality, but not quantity, can also be interpreted as reflecting total energy stores. Differences in condition could result from either differences in prey quality, effecting either energy intake or assimilation, or from different energy

expenditures in prey acquisition. We are currently testing methods to analyze blubber biopsy samples taken from live-captured seals, that when combined with estimates of blubber volume, will help add resolution to the problem. We are also currently analyzing recently acquired subsistence harvest samples from elsewhere in Alaska (Bering Sea, Yakutat, lower Cook Inlet), and have subsampled appropriately season-region matched specimens from the ADFG archived blubber collected during 1973 - 1978, continuing analyses initiated in 1995 (Fadely et al. 1996).

CONCLUSIONS

1. Reference ranges were constructed from a core database of up to 296 seals for plasma chemistries, 349 for hematologies, and 275 for leukograms. However, samples were somewhat biased towards adult males from Prince William Sound. Pups and yearlings were nearly absent from the database, accounting for only 3% and 8%, respectively. Future sampling efforts should attempt to increase numbers of these age classes.
2. Seal handling, blood sample processing, individual (age and gender), seasonal, regional, and interannual factors accounted for up to 55% of variation in plasma chemistries, 54% in hemograms, and 40% in leukograms. Seal handling and blood processing factors in general did not affect many variables with respect to the total variation observed from other sources.
3. Power analysis of interregional and interannual comparisons showed that small differences in blood variables can be detected with high statistical power. These minimum detectable differences were within the magnitude of effects resulting from handling or individual factors. Therefore, they must be incorporated in statistical comparisons of temporal or spatial differences of blood analytes.
4. Based on a frequency of occurrence distribution of clinically outlying blood variables, some seals sampled during this project were clinically unhealthy. A preliminary comparison indicated that there were probably temporal and regional patterns to outlying blood values. These analyses will be compared and contrasted with examinations of sub-clinical interannual or regional patterns in blood indices.
5. Gender-specific differences were found in blubber quality (measured as energy density). Within seasons and regions, female blubber was always more energy dense than male blubber, and male blubber decreased in energy density as body mass increased.
6. Prince William Sound seal blubber was up to 4% more energy dense than blubber from southeast Alaska seals in both spring and autumn. There was no clear evidence of differences in whole-seal blubber content or body size between the two regions. We are continuing analyses to determine whether these differences were due to changes in hydration state or lipid content. Additionally, increased regional and seasonal distributions of subsistence harvest-derived blubber samples will expand comparisons with archived (pre-decline) blubber samples to Lower Cook Inlet and Yakutat.

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Table 1. Seasonal and regional sampling distribution for calculation of plasma chemistry reference ranges for harbor seals (*Phoca vitulina*) captured during Spring (Mar - May), Summer (Jul - Aug) or Autumn (Sep - Oct).

Year	Season	Kodiak Island	Prince William Sound	Southeast	Seasonal Totals
1992	Spring		8		8
1993	Spring	4	12	8	24
	Autumn	1		8	9
1994	Spring		10		10
	Summer			31	31
	Autumn	9	23	7	39
1995	Spring	8	22	19	49
	Summer		6		6
	Autumn	8	14	9	31
1996	Spring		21		21
	Summer		26		26
	Autumn	13	15	14	42
Regional Totals		43	157	96	296

Table 2. Breakdown by age and sex of harbor seals (*Phoca vitulina*) used to calculate plasma chemistry reference ranges, captured during 1992 - 96 from Prince William Sound, Kodiak Island or southeast Alaska.

Age Class	Male	Female	Undetermined	Age Total
Pup	4	5		9
Yearling	11	12		23
Sub Adult	46	39		85
Adult	113	57		170
Undetermined Age	5	2	2	9
Gender Total	179	115	2	296

Table 3. Seasonal and regional sampling distribution for calculations of hematological and leukocyte reference ranges for harbor seals (*Phoca vitulina*) captured during Spring (Mar - May), Summer (Jul - Aug) or Autumn (Sep - Oct) of 1991 - 96.

Year	Season	Kodiak Island	Prince William Sound	Southeast	Seasonal Totals
1991	Spring		4		4
1992	Spring		4		4
1993	Spring		12		12
1994	Spring		9		9
	Summer			30	30
	Autumn		23		23
1995	Spring	8	22		30
	Summer		4		4
	Autumn	9	20		29
1996	Spring		22		22
	Summer		34		34
	Autumn	15	17	16	48
Regional Totals		32	171	46	249

Table 4. Breakdown by age and sex of harbor seals (*Phoca vitulina*) used to calculate hematological and leukogram reference ranges for seals captured during 1991 - 96 in Prince William Sound, Kodiak Island, or southeast Alaska.

Age Class	Male	Female	Undetermined	Age Total
Pup	3	6		9
Yearling	9	13		22
Sub Adult	39	31		70
Adult	85	55		140
Undetermined Age	4		4	8
Gender Total	140	105	4	249

Table 5. Harbor seal (*Phoca vitulina*) plasma chemistry reference ranges calculated from samples collected during 1992 - 1996 in southeast Alaska, Prince William Sound and Kodiak Island regions.

Variable	$\bar{x}$	sd	*n*	Reference Range	95%CI[i]	Total Range (min - max)
Sodium[a,b]	148	5	291	138 - 157	1	136 - 167
Chloride[a,b]	108	4	296	99 - 117	1	84 - 122
Potassium[a,b,c]	3.9	0.7	291	3.1 - 4.6	ns[j]	2.7 - 12.2
Calcium[d]	2.4	0.2	291	2.1 - 2.7	0.0	2.0 - 3.0
Phosphorus[d]	1.6	0.4	291	0.7 - 2.5	0.1	0.5 - 3.6
Glucose[d]	9.0	1.4	291	6.2 - 11.9	0.3	3.6 - 15.2
Blood Urea Nitrogen (BUN)[b,d]	15.7	4.6	296	6.8 - 24.6	0.7	6.1 - 28.6
Creatinine[b,c,d]	72	27	296	44 - 133	ns[k]	35 - 159
BUN:Creatinine Ratio[b]	52	20	296	12 - 92	4	12 - 155
Cholesterol[b,d]	5.78	1.24	296	3.29 - 8.26	0.23	3.44 - 10.64
Total Bilirubin[b,c,d]	6.8	3.4	286	1.7 - 13.7	ns[l]	1.7 - 18.8
Direct Bilirubin[b,c,d]	5.1	3.4	284	0.0 - 10.3	ns[m]	0.0 - 17.1
Total Protein[e]	78.7	7.1	293	64.5 - 92.9	1.4	53.0 - 96.0
Albumin[b,e]	31.1	2.5	293	26.1 - 36.1	0.5	23.0 - 49.0
Globulin[b,e]	47.6	6.4	293	34.8 - 60.4	1.3	25.0 - 66.0
Albumin:Globulin[b]	0.7	0.1	293	0.5 - 0.9	0.0	0.4 - 1.2
Alkaline Phosphatase[f,g]	57	26	290	24 - 135	5	20 - 179
Aspartate Aminotransferase[f,g]	155	81	293	56 - 425	16	53 - 1164
Alanine Aminotransferase[f,g]	57	37	293	17 - 195	7	9 - 930
Creatine Phosphokinase[f,g]	1038	1286	296	129 - 8318	254	146 - 20000
Gammaglutamyl Transferase[f,g]	19	8	269	9 - 41	2	5 - 197
Lactate Dehydrogenase[f,g]	3837	1880	268	1493 - 9863	390	626 - 21500
Haptoglobin[b,h]	1131	411	223	311 - 1951	93	259 - 2447

Table 5. Continued.

[a]mmol/L

[b]Non-normal distribution ($P < 0.05$; Kolmogorov-Smirnoff Probability Test).

[c]Reference range and 95% CI calculated from 2.5% and 97.5% quantiles of ranked data.

[d]µmol/L

[e]g/L

[f]iu/L

[g]Statistics derived from log-transformed data, therefore listed sd are not symmetrical about the mean.

[h]mg/L

[i]95% confidence intervals around lower and upper reference range values.

[j]Non-symmetrical intervals: lower limits 2.9 - 3.2; upper limits 4.6 - 5.4

[k]Non-symmetrical intervals: lower limits 35 - 44; upper limits 124 - 144

[l]Non-symmetrical intervals: lower limits 1.7 - 1.7; upper limits 10.3 - 17.1

[m]Non-symmetrical intervals: lower limits 0.0 - 0.0; upper limits 8.6 - 13.7

Table 6. Harbor seal (*Phoca vitulina*) hematological reference ranges calculated from samples collected during 1991 - 1996 in southeast Alaska, Prince William Sound, and Kodiak Island regions.

Variable	$\bar{x}$	sd	*n*	Reference range	95% CI[j]	Total range (min - max)
Field						
Hematocrit	0.55	0.07	349	0.41 - 0.68	1	0.32 - 0.74
Hemoglobin[a]	3.6	0.5	302	2.6 - 4.6	0.1	2.3 - 5.5
MCHC[b,c]	43	5	301	33 - 53	1	25 - 72
MCV[d]	110.9	8.8	228	93.2 - 128.5	2.0	75.2 - 143.2
MCH[e,f]	47.2	6.0	216	36.6 - 60.8	1.4	29.1 - 82.6
Clinical Laboratory						
Hematocrit	0.60	0.06	232	0.47 - 0.72	1	0.44 - 0.76
Hemoglobin[a]	3.4	0.3	232	2.7 - 4.1	0.1	2.4 - 4.4
MCHC[b,c]	36	2	232	33 - 40	0	29 - 47
MCV[d]	119.0	5.7	232	107.6 - 130.5	1.3	82.6 - 131.0
MCH[b,e]	43.4	2.6	232	38.2 - 48.6	0.6	31.5 - 52.5
Red Blood Cell Count[g]	5.03	0.58	232	3.86 - 6.20	0.13	3.62 - 7.87
Platelet Count[h,i]	340	251	224	111 - 694	57	33 - 1278

[a]mmol/L.

[b]Non-normal distribution ($P < 0.05$; Kolmogorov-Smirnoff Probability Test).

[c]Mean corpuscular hemoglobin concentration; g/L.

[d]Mean corpuscular volume; fL.

[e]Mean corpuscular hemoglobin; ρg.

[f]Statistics calculated from log-transformed data. Standard deviation is not symmetrical around mean.

[g]10^{12}/L.

[h]10^{9}/L.

[i]Statistics calculated from square-root transformed data. Standard deviation is not symmetrical around mean.

[j]95% confidence intervals around lower and upper reference range values.

Table 7. Harbor seal (*Phoca vitulina*) 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	$\bar{x}$	sd	*n*	Reference range	95% CI[f]	Total range (min - max)
Absolute counts						
White Blood Cell Count[a,b]	11.4	3.0	275	6.7 - 19.3	0.3	5.2 - 25.3
Neutrophils[a]	6.8	2.5	219	1.8 - 11.8	0.3	2.2 - 18.2
Banded Neutrophils[a,c]	0.0	0.02	219	0.0 - 0.0	ns[g]	0.00 - 0.14
Lymphocytes[a]	3.45	1.42	219	0.61 - 6.29	0.30	0.96 - 9.00
Monocytes[a,c]	0.47	0.4	219	0.0 - 1.4	ns[h]	0.00 - 2.40
Eosinophils[a,d]	0.84	0.04	219	0.04 - 2.65	0.01	0.0 - 3.2
Basophils[a,c]	2.2	2.8	82	0.0 - 11.5	ns[i]	0.0 - 11.8
Differential Counts						
Neutrophils[d]	56	12	249	31 - 81	3	25 - 88
Banded Neutrophils[c,d]	0	0	249	0 - 1	ns[j]	0 - 1
Lymphocytes[d]	30	11	249	8 - 51	2	8 - 61
Monocytes[c,d]	3	1-7	249	0 - 11	ns[k]	0 - 15
Eosinophils[d,e]	8	6	249	1 - 22	1	0 - 29
Basophils[c,d]	1	0-4	249	0 - 7	ns[l]	0 - 12

[a] 10^9/L.

[b] Statistics calculated from log-transformed data; sd is non-symmetrical about mean.

[c] Non-normal distribution (Kolmogorov-Smirnoff Probability Test; $P > 0.05$). Statistics calculated from quantiles of ranked data.

[d] %.

[e] Statistics calculated from angular-transformed data; sd is asymmetrical about mean.

[f] 95% confidence intervals around lower and upper reference range values.

[g] Asymmetrical intervals: upper limits 0.0 - 0.12.

[h] Asymmetrical intervals: lower limits 0.0 - 0.0; upper limits 1.3 - 1.8.

[i] Asymmetrical intervals: lower limits 0.0 - 0.0; upper limits 8.4 - 11.8.

[j] Asymmetrical intervals: upper limits 0 - 1.

[k] Asymmetrical intervals: lower limits 0.0 - 0.0; upper limits 10 - 15.

[l] Asymmetrical intervals: lower limits 0 - 0; upper limits 6 - 12.

Table 8. Forward stepwise multiple regression matrix showing statistically significant ($P < 0.05$) regression coefficients of individual or environmental variables on plasma chemistries of harbor seals (*Phoca vitulina*) sampled in Prince William Sound, southeast Alaska, and the Kodiak archipelago during 1995 - 1996 ($n = 75$).

	Age Class[f]				Gender[g]		Region[h]				Season[i]			Year		Elapsed Capture to Sampling Time[j]	Drugs[k]		
Variable	P	Y	S	A	M	F	PWS	KO	SE	Sp	Su	Au	95	96			Y	N	R^2
Sodium[a]				2.1								-2.7							0.261
Chloride[a]												-2.5							0.149
Potassium[a]	-0.4				-0.2											-0.3			0.371
Calcium[b]				-0.1					-0.1		0.2								0.399
Phosphorus[b]													-0.4				0.4		0.330
Glucose[b]																			0.000
Blood Urea Nitrogen (BUN)[b]				4.0							2.0								0.285
Creatinine[b]			18	44		9			-18	27									0.553
BUN:Creatinine Ratio										-20									0.107
Cholesterol[b]																			0.000
Total Bilirubin[b]									-1.7	3.4							1.7		0.458
Direct Bilirubin[b]								1.7		3.4									0.490
Total Protein[c]				4.3															0.104
Albumin[c]												-1.9							0.250
Globulin[c]				4.1															0.107
Albumin:Globulin Ratio				-0.1			0.1												0.184
Alkaline Phosphatase[d]																			0.000
Alanine Aminotransferase[d]																			0.000
Aspartate Aminotransferase[d]							2							-1		1			0.163
Creatine Phosphokinase[d]	-3									2						2			0.393
Gammaglutamyl Transferase[d]		1									1								0.227
Lactate Dehydrogenase[d]							2							-1		2			0.345
Haptoglobin[e]		229																	0.069

Table 8. Continued.

[a]mmol/L
[b]μmol/L
[c]g/L
[d]iu/L
[e]mg/L
[f]P = pup; Y = yearling; S = subadult; A = adult.
[g]M=male; F=female.
[h]PWS = Prince William Sound; KO = Kodiak Island; SE = southeast Alaska.
[i]Sp = spring; Su = summer; Au = autumn.
[j]Hours.
[k]Y = yes; N = no.

Table 9. Forward stepwise multiple regression matrix showing statistically significant ($P < 0.05$) regression coefficients of individual or environmental variables on plasma chemistries of chemically anaesthetized harbor seals (*Phoca vitulina*) sampled in Prince William Sound, southeast Alaska, and the Kodiak archipelago during 1992 - 1996 ($n = 201$ unless noted otherwise).

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

Table 9. Continued.

[a]mmol/L
[b]Included elapsed capture to blood sampling time in model, but in was non-significant (n = 147).
[c]µmol/L
[d]n = 194.
[e]g/L.
[f]iu/L.
[g]Includes elapsed capture to blood sampling time in model (β = 1; R^2 = 0.182; n = 147).
[h]Includes elapsed capture to blood sampling time in model (β = 3; R^2 = 0.357; n = 147).
[i]n = 180.
[j]Includes elapsed capture to blood sampling time in model (β = 2; R^2 = 0.308, n = 127).
[k]mg/L, n = 154.
[l]P = pup; Y = yearling; S = subadult; A = adult.
[m]M = male; F = female.
[n]PWS = Prince William Sound; KO = Kodiak Island; SE = southeast Alaska.
[o]Sp = spring; Au = autumn.

Table 10. Relative contribution (R^2) of individual or environmental factors to total variance in harbor seal (*Phoca vitulina*) plasma chemistries, based on decomposed multiple regression sums of squares with all other factors included in model (Table 9; $n = 201$). R^2 values of zero left 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[a]	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[b]			0.035		
Creatine Phosphokinase[c]					
Gammaglutamyl Transferase	0.029		0.046	0.031	
Lactate Dehydrogenase[d]	0.041		0.022		
Haptoglobin			0.170	0.095	0.041

[a] $n = 147$.
[b] Elapsed time between capture and blood sampling $R^2 = 0.153$ ($n = 147$).
[c] Elapsed time between capture and blood sampling $R^2 = 0.357$ ($n = 147$).
[d] Elapsed time between capture and blood sampling $R^2 = 0.308$ ($n = 127$).

Table 11. Effects of blood collection tube-type (relative to values derived from sodium-heparin collection tube samples) on plasma chemistries of two harbor seals (*Phoca vitulina*).

Variable	Lithium-heparin	Serum
Sodium[a]	-3	-2
Chloride[a*]	-3	-1
Potassium[a***]	-0.3	0.2
Calcium[b]	-0.1	-0.0
Phosphorus[b]	0.1	0.2
Glucose[b*]	-0.4	-0.4
Blood Urea Nitrogen (BUN) [b*]	-0.5	-0.5
Creatinine[b]	0	-2
BUN:Creatinine Ratio	-2	-1
Cholesterol[b*]	-0.14	0.01
Total Bilirubin[b***]	0.0	1.7
Direct Bilirubin[b***]	0.0	2.6
Total Protein[c]	-1.3	-1.3
Albumin[c]	0.3	0.0
Globulin[c]	-1.0	-1.3
Albumin:Globulin	0.0	0.0
Alkaline Phosphatase[d]	-1	1
Aspartate Aminotransferase[d]	5	-3
Alanine Aminotransferase[d***]	-4	6
Creatine Phosphokinase[d]	17	-10
Gammaglutamyl Transferase[d*]	0	2
Lactate Dehydrogenase[d]	201	59

[a]mmol/L.

[b]µmol/L.

[c]g/L.

[d]iu/L.

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; 2-way ANOVA df = 2,6.

Table 12. Forward stepwise multiple regression matrix of statistically significant ($P < 0.05$) coefficients of individual or environmental factors on hematological variables ($n = 88$) of harbor seals (*Phoca vitulina*).

Variable	Age Class[g]				Gender[h]		Region[i]			Season[j]			Year[k]		Elapsed Sampling to CBC[l] Process Time[m]	R^2
	P	Y	S	A	M	F	PWS	KO	SE	Sp	Su	Au	95	96		
Field																
Hematocrit				-0.04					-0.05	0.05	-0.03					0.509
Hemoglobin[a]				-0.3				0.2								0.216
MCHC[b]										-4						0.076
MCV[c]															-1.8	0.063
MCH[d]										-1.0						0.067
Clinical Lab					3											
Hematocrit				-0.02	-0.02						-0.11	-0.05				0.491
Hemoglobin[a]				-0.2					-0.2		-0.3					0.352
MCHC[b]													-1		-0.9	0.451
MCV[c]	-11	-4						2	5							0.237
MCH[d]	-4.3	-2.3											-1.9			0.218
Red Blood Cell Count[e]			-0.40	-0.65		0.19		0.27		0.44					0.02	0.540
Platelet Count[f]																0.000

[a]mmol/L.
[b]Mean corpuscular hemoglobin concentration; g/L.
[c]Mean corpuscular volume; fL.
[d]Mean corpuscular hemoglobin; ρg.
[e]10^{12}/L.
[f]10^{9}/L.
[g]P = pup; Y = yearling; S= subadult; A = adult.
[h]M = male; F = female.
[i]PWS = Prince William Sound; KO = Kodiak Island; SE = southeast Alaska.
[j]Sp = Spring; Su = Summer; Au = Autumn.
[k]95 = 1995; 96 = 1996.
[l]Complete blood count.
[m]Hours.

Table 13. Forward stepwise multiple regression matrix of statistically significant ($P < 0.05$) coefficients of individual or environmental factors on expanded data set of harbor seal (*Phoca vitulina*) hematological variables collected during 1991 - 1996.

	Gender[j]		Age Class[k]				Region[l]			Season[m]			Year[n]					
Variable	M	F	P	Y	S	A	PWS	KO	SE	Sp	Su	Au	93	94	95	96	*n*	R^2
Field																		
Hematocrit	-0.02					-0.04			-0.04		-0.08	-0.04	0.02				344	0.484
Hemoglobin[a]		0.1			-0.2	-0.4	0.2			0.1	-0.4		-0.2		-0.2		298	0.333
MCHC[b]													-3		-3		296	0.097
MCV[c,d]			-9														201	0.031
MCH[e]														1.1	1.1		211	0.072
Clinical Lab																		
Hematocrit	-0.02				-0.03	-0.05			-0.03	0.03	-0.06						228	0.439
Hemoglobin[a]	-0.1				-0.1	-0.3			-0.2	0.2	-0.3					0.1	228	0.041
MCHC[b,f]										1	1		-1			1	214	0.393
MCV[c]			-6			3					-2						228	0.133
MCH[e]			-3.8	-1.4											1.0	2.5	228	0.281
Red Blood Cell Count[d,g]		0.15			-0.45	-0.72			-0.49	0.72		0.41	0.22				203	0.564
Platelet Count[i]							-4			-2				-15			216	0.248

[a]mmol/L.
[b]Mean corpuscular hemoglobin concentration; g/L.
[c]Mean corpuscular volume; fL.
[d] Model limited to elapsed sample to CBC process time <6 days.
[e]Mean corpuscular hemoglobin; pg.
[f]Elapsed time between sampling and CBC processing excluded from model ($F^*_{(1,218)} = 0.13$; $P > 0.50$).
[g]10^{12}/L.
[i]10^9/L.
[j]M = male; F = female.
[k]P = pup; Y = yearling; S= subadult; A = adult.
[l]PWS = Prince William Sound; KO = Kodiak Island; SE = southeast Alaska.
[m]Sp = Spring; Su = Summer; Au = Autumn.
[n]93 =1993; 94 = 1994; 95 = 1995; 96 = 1996.

Table 14. Relative contribution (R^2) of individual or environmental factors to total variance in harbor seal (*Phoca vitulina*) hematologies, based on decomposed multiple regression sums of squares with all other model factors included (Table 13). R^2 values of zero left blank.

Variable	Gender	Age Class	Region	Season	Year	*n*
Field						
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
Clinical Lab						
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 15. Forward stepwise multiple regression matrix of statistically significant ($P < 0.05$) coefficients of individual or environmental factors on leukograms ($n = 88$) of harbor seals (*Phoca vitulina*).

Variable	Age Class[c]				Region[d]			Season[e]			Year[f]		Elapsed Capture to Blood Sample Time[g]	Elapsed Blood Sample to Processing Time[g]	Elapsed Sampling to CBC[h] Process Time[g]	R^2
	P	Y	S	A	PWS	KO	SE	Sp	Su	Au	95	96				
Absolute Counts																
White Blood Cell Count[a]				-1.2												0.104
Neutrophils[a]				-1.5					-2.3		-1.6					0.238
Banded Neutrophils[a]			0.01					-0.02			0.01					0.114
Lymphocytes[a]			1.0					-1.1	0.8				-0.7			0.323
Monocytes[a]			0.2													0.043
Eosinophils[a]				0.3									-0.3			0.089
Basophils[a]					-0.2	-0.4								-0.05		0.213
Differential Counts																
Neutrophils[b]							-7		-34	-17	-12					0.397
Banded Neutrophils[b]			0.01					-0.1				-0.1				0.114
Lymphocytes[b]									25	15	8					0.329
Monocytes[b]										-3					1	0.072
Eosinophils[b]				1												0.097
Basophils[b]					-2	-3								-0.4		0.208

[a] 10^9/L.

[b] %.

[c] P = pup; Y = yearling; S = subadult; A = adult.

[d] PWS = Prince Wiliam Sound; KO = Kodiak Island; SE = southeast Alaska.

[e] Sp = Spring; Su = Summer; Au = Autumn.

[f] 95 = 1995; 96 = 1996.

[g] Hours.

[h] Complete blood count.

Table 16. Forward stepwise multiple regression matrix of statistically significant ($P < 0.05$) coefficients of individual or environmental factors on leukograms of harbor seals (*Phoca vitulina*), sampled during 1991 - 1996.

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

[a] 10^9/L.
[b] Elapsed capture to blood sample time included in model (coefficient = -0.8).
[c] Elapsed capture to blood sample time included in model (coefficient = -0.3).
[d] %.
[e] P = pup; Y = yearling; S = subadult; A = adult.
[f] PWS = Prince William Sound; KO = Kodiak Island; SE = southeast Alaska.
[g] Sp = Spring; Su = Summer; Au = Autumn.
[h] 91= 1991; 92 = 1992; 93 = 1993; 94 = 1994; 95 = 1995; 96 = 1996.

Table 17. Relative contribution (R^2) of individual or environmental factors to total variance in harbor seal (*Phoca vitulina*) leukograms, based on decomposed multiple regression sums of squares with all other model factors considered (Table 16). R^2 values of zero left blank.

Variable	Age Class	Region	Season	Year	*n*
Absolute Counts					
White Blood Cell Count	0.082			0.019	271
Neutrophils	0.072	0.047		0.020	217
Banded Neutrophils					217
Lymphocytes[a]	0.034		0.017	0.034	212
Monocytes				0.096	217
Basophils[b]		0.205			88
Eosinophils[c]					212
Differential Counts					
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[d]		0.202			88

[a]Elapsed time between capture and blood sampling time included in model ($R^2 = 0.082$).
[b]Elapsed time between blood sampling and blood smear processing time included in model ($R^2 = 0.065$).
[c]Elapsed capture to blood sample time included in model ($R^2 = 0.066$).
[d]Elapsed time between blood sampling and blood smear processing time included in model ($R^2 = 0.064$).

Table 18. Condition indices for harbor seals (*Phoca vitulina*) collected during subsistence harvests from Prince William Sound and southeast Alaska, during spring and autumn months of 1995 - 1996.

	Relative Blubber Thickness						mLMD					
	Prince William Sound			Southeast			Prince William Sound			Southeast		
	$\bar{x}$	se	*n*	$\bar{x}$	se	*n*	$\bar{x}$	se	*n*	$\bar{x}$	se	*n*
Spring												
Males	0.193	0.018	11	0.250	0.021	12	0.005	0.001	4	0.005	0.000	10
Females	0.200	0.021	7	0.249	0.016	8						
Autumn												
Males	0.189	0.017	5	0.219	0.029	3						
Females	0.191	0.007	9	0.225	0.024	4	0.004	0.000	9	0.005	0.001	4

Table 19. Minimum detectable differences (δ) in harbor seal (*Phoca vitulina*) plasma chemistries for sample regional and interannual comparisons. Regional comparison was based on detecting differences among Prince William Sound, Kodiak Island, and southeast Alaska seals using seals sampled during autumn 1996. Interannual comparison test was 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 (1984).

Variable	Regional δ[f]	Interannual δ[g]
Sodium[a]	4	3
Chloride[a]	3	3
Potassium[a]	0.6	0.5
Calcium[b]	0.2	0.1
Phosphorus[b]	0.3	0.3
Glucose[b]	1.2	0.9
Blood Urea Nitrogen (BUN)[b]	3.9	3.1
Creatinine[b]	23	18
BUN:Creatinine Ratio	17	13
Cholesterol[b]	1.05	0.83
Total Bilirubin[b]	2.9	2.3
Direct Bilirubin[b]	2.9	2.3
Total Protein[c]	6.0	4.8
Albumin[c]	2.1	1.7
Globulin[c]	5.4	4.3
Albumin:Globulin Ratio	0.1	0.1
Alkaline Phosphatase[d]	22	17
Aspartate Aminotransferase[d]	69	54
Alanine Aminotransferase[d]	31	25
Creatine Phosphokinase[d]	1094	860
Gammaglutamyl Transferase[d]	7	5
Lactate Dehydrogenase[d]	1599	1257
Haptoglobin[e]	350	275

[a]mmol/L.
[b]μmol/L.
[c]g/L.
[d]iu/L.
[e]mg/L.
[f]$\nu_1 = 2$, $\nu_2 = 123$, $\phi = 2.25$.
[g]$\nu_1 = 4$, $\nu_2 = 360$, $\phi = 1.95$.

Table 20 Minimum detectable differences (δ) in harbor seal (*Phoca vitulina*) hematologies for sample regional and interannual comparisons. Regional comparison was based on detecting differences among Prince William Sound, Kodiak Island, and southeast Alaska seals using seals sampled during autumn 1996. Interannual comparison test was based on Prince William Sound seals sampled between 1992 - 1996. Both models tested at $\alpha = 0.05$ and $1-\beta = 0.95$, following ANOVA power calculations in Zar (1984).

Variable	Regional δ[g]	Interannual δ[h]
Field		
Hematocrit	0.06	0.04
Hemoglobin[a]	0.4	0.3
MCHC[b]	4	3
MCV[c]	7.0	5.1
MCH[d]	4.8	3.5
Clinical Laboratory		
Hematocrit	0.05	0.03
Hemoglobin[a]	0.2	0.2
MCHC[b]	2	1
MCV[c]	4.5	3.3
MCH[d]	2.1	1.5
Red Blood Cell Count[e]	0.46	0.33
Platelet Count[f]	200	144

[a]mmol/L.
[b]Mean corpuscular hemoglobin concentration; g/L.
[c]Mean corpuscular volume; fL.
[d]Mean corpuscular hemoglobin; ρg.
[e]10^{12}/L.
[f]10^{9}/L.
[g]$\nu_1 = 2$, $\nu_2 = 141$, $\phi = 2.25$.
[h]$\nu_1 = 4$, $\nu_2 = 340$, $\phi = 1.95$.

Table 21. Minimum detectable differences (δ) in harbor seal (*Phoca vitulina*) leukograms for sample regional and interannual comparisons. Regional comparison was based on detecting differences among Prince William Sound, Kodiak Island, and southeast Alaska seals using seals sampled during autumn 1996. Interannual comparison test was based on Prince William Sound seals sampled between 1992 - 1996. Both models tested at $\alpha = 0.05$ and $1 - \beta = 0.95$, following ANOVA power calculations in Zar (1984).

Variable	Regional δ[c]	Interannual δ[d]
Absolute Counts[a]		
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
Differential Counts[b]		
Neutrophils	10	7
Banded Neutrophils	0	0
Lymphocytes	9	6
Monocytes	5	3
Eosinophils	5	3
Basophils	3	2

[a] 1^{09}/L.

[b] %.

[c] $\nu_1 = 2$, $\nu_2 = 141$, $\phi = 2.25$.

[d] $\nu_1 = 4$, $\nu_2 = 340$, $\phi = 1.95$.

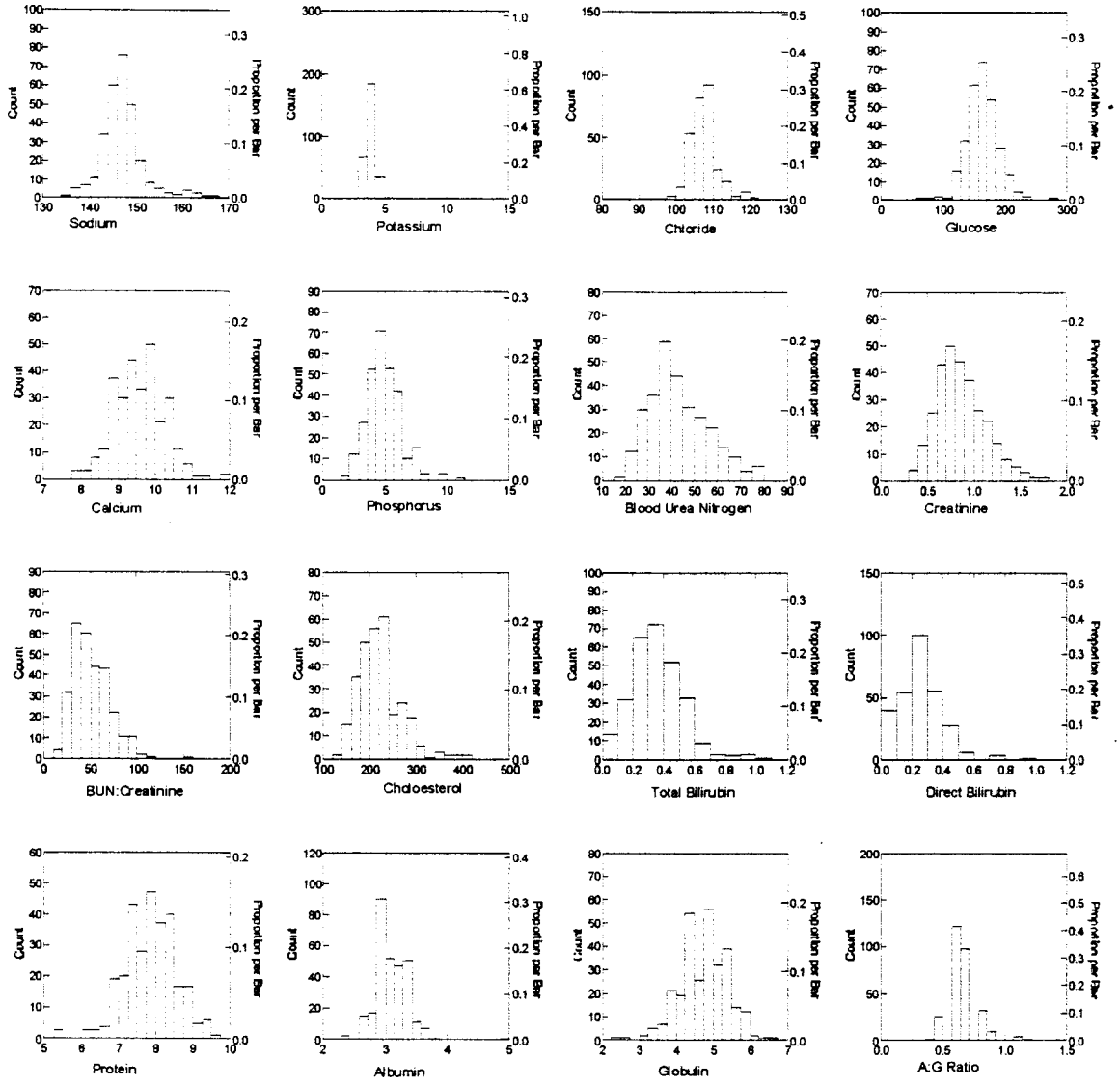

Figure 1. Plasma chemistry frequency histograms for harbor seals (*Phoca vitulina*) captured during 1992 - 1996 from Kodiak Island, Prince William Sound, and southeast Alaska.

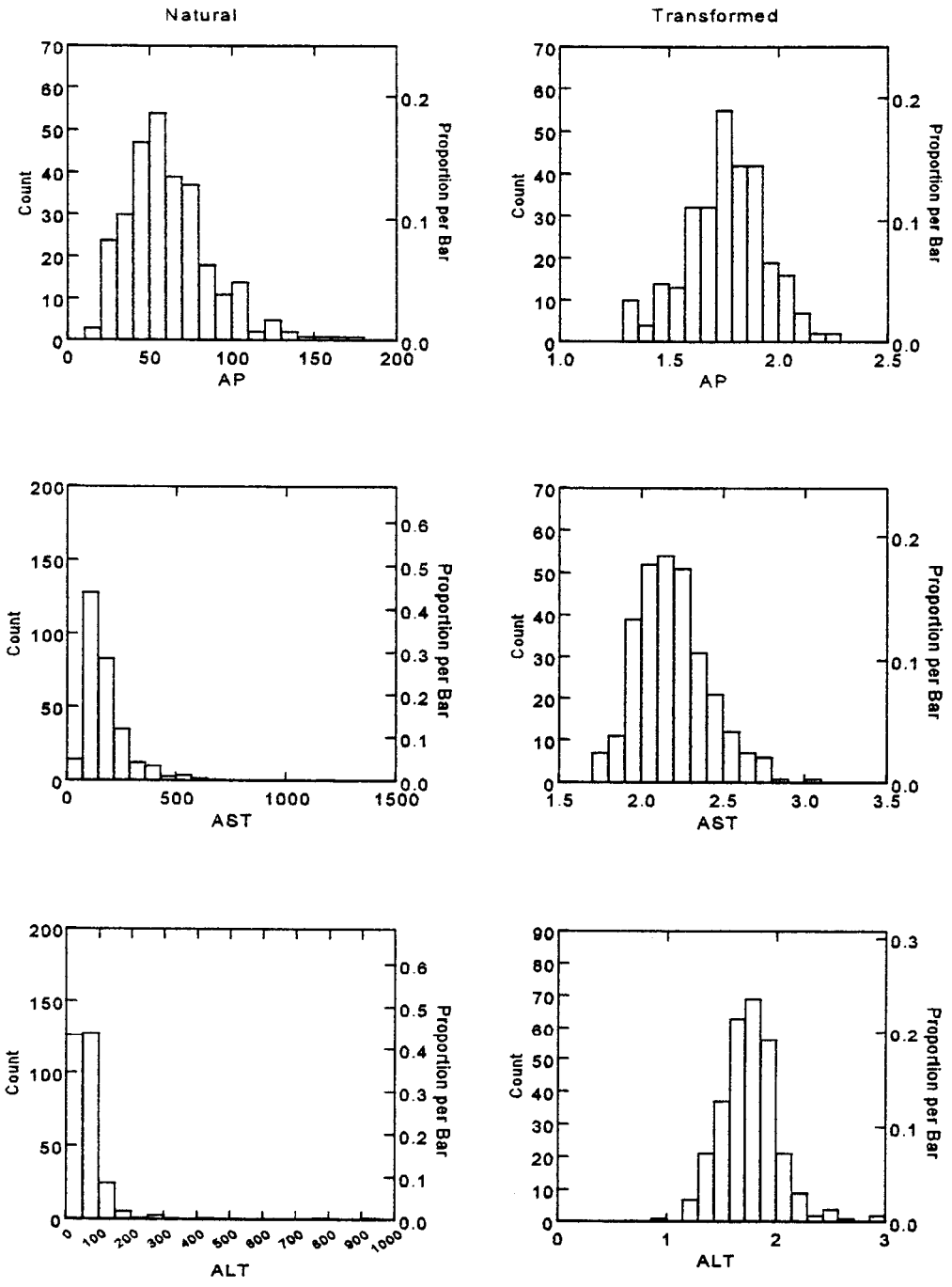

Figure 2. Plasma enzyme activity frequency histograms (natural and log-transformed) for harbor seals (*Phoca vitulina*) captured from Kodiak Island, Prince William Sound, and southeast Alaska during 1992 - 1996.

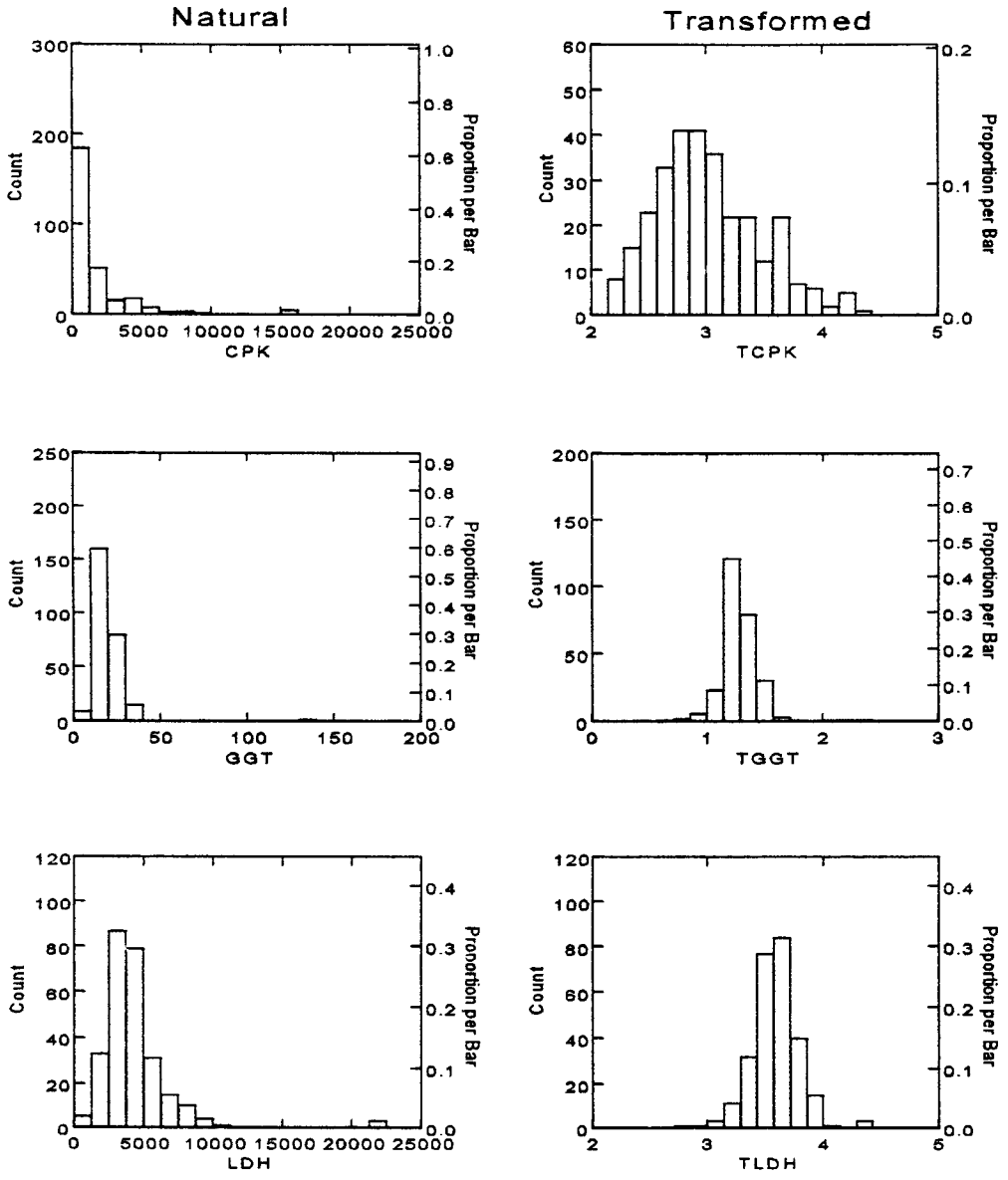

Figure 2. Continued.

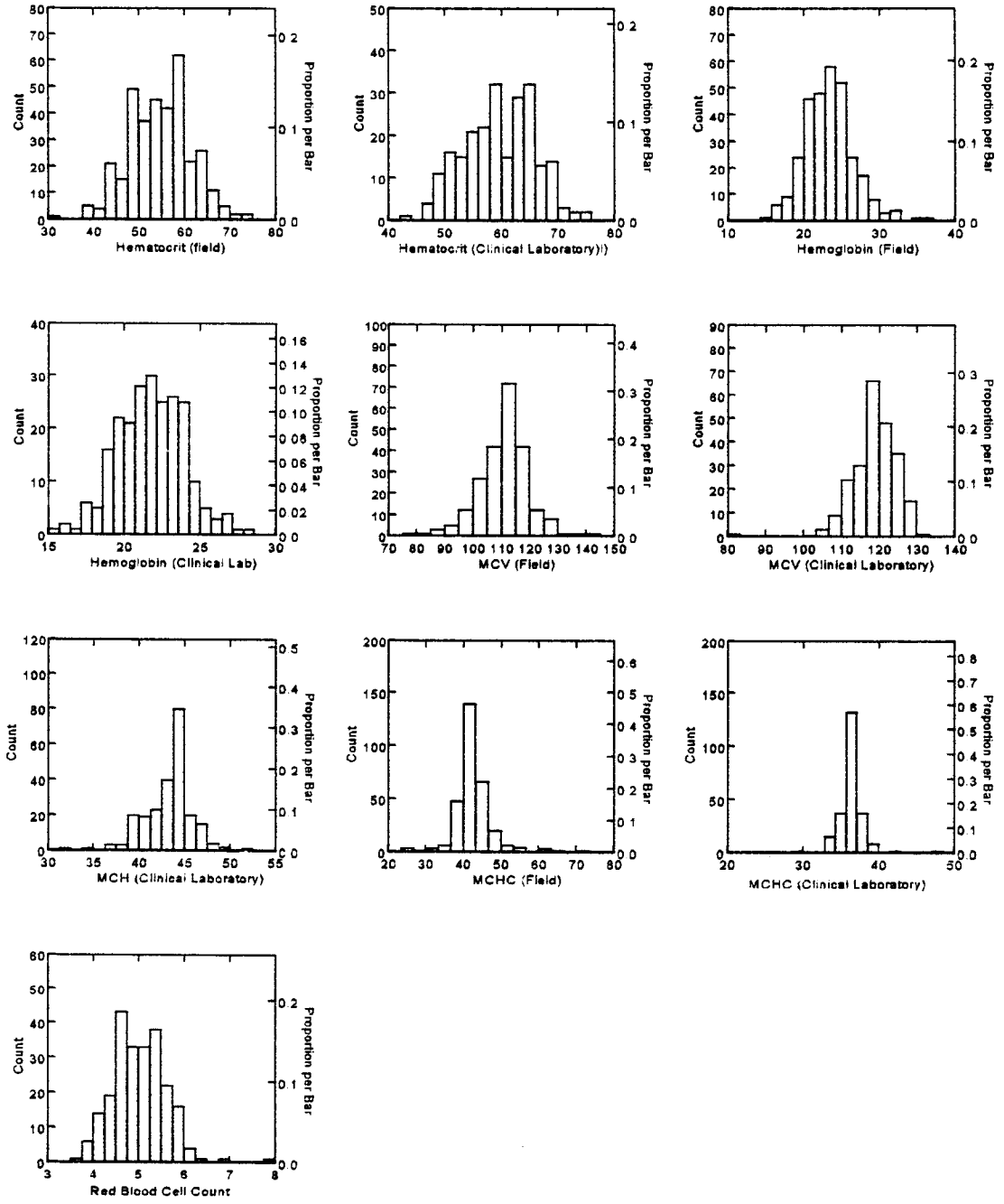

Figure 3. Hematological frequency histograms for harbor seals (*Phoca vitulina*) captured during 1991 - 1996 from Kodiak Island, Prince William Sound, and southeast Alaska.

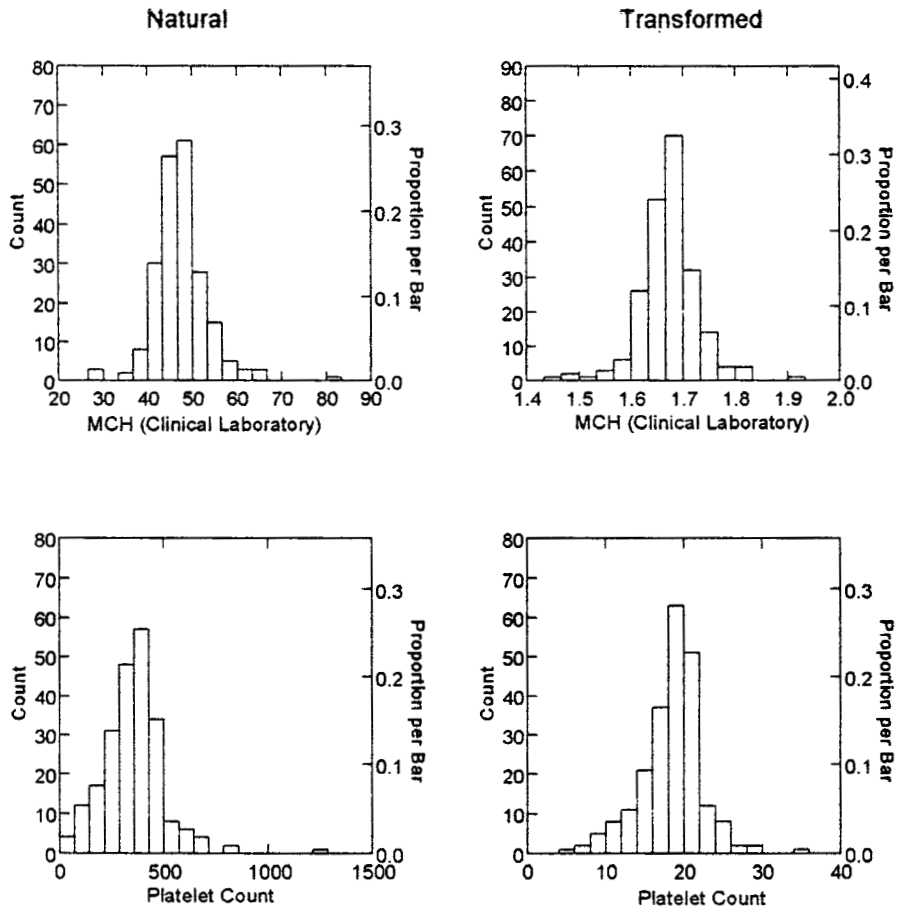

Figure 4. Frequency histograms (natural and transformed) of mean corpuscular hemoglobin (MCH) and platelet counts for harbor seals (*Phoca vitulina*) captured during 1991 - 1996 from Kodiak Island, Prince William Sound, and southeast Alaska.

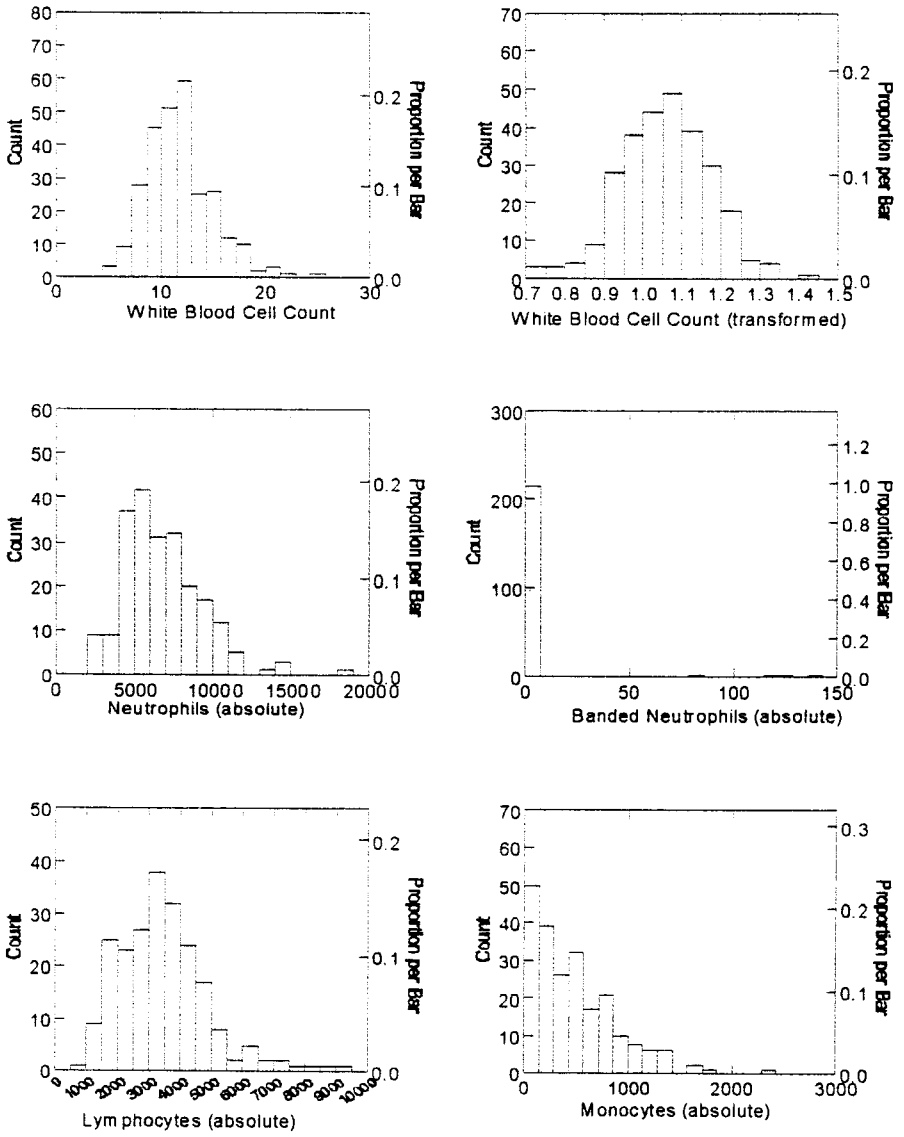

Figure 5. Frequency distribution of white blood cell counts (natural and log-transformed) and absolute leukocyte counts for harbor seals (*Phoca vitulina*) captured during 1991- 1996 from Kodiak Island, Prince William Sound, and southeast Alaska.

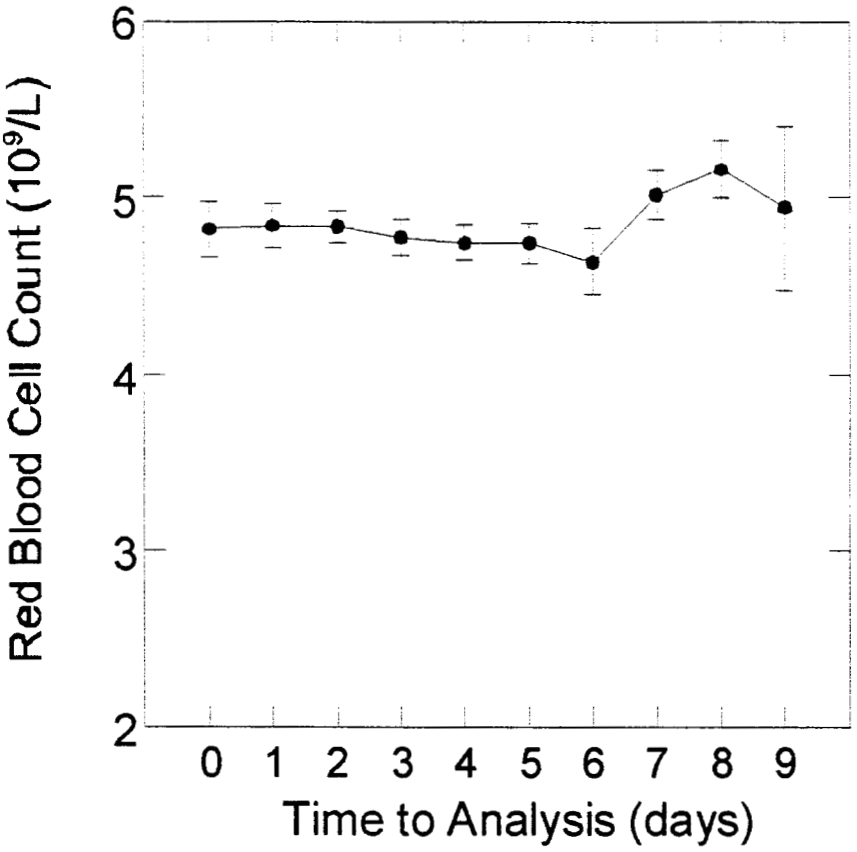

Figure 6. Effect of elapsed time between blood sampling and sample processing on red blood cell counts of harbor seals (*Phoca vitulina*). Least squares means adjusted for gender, season, and region (n = 220).

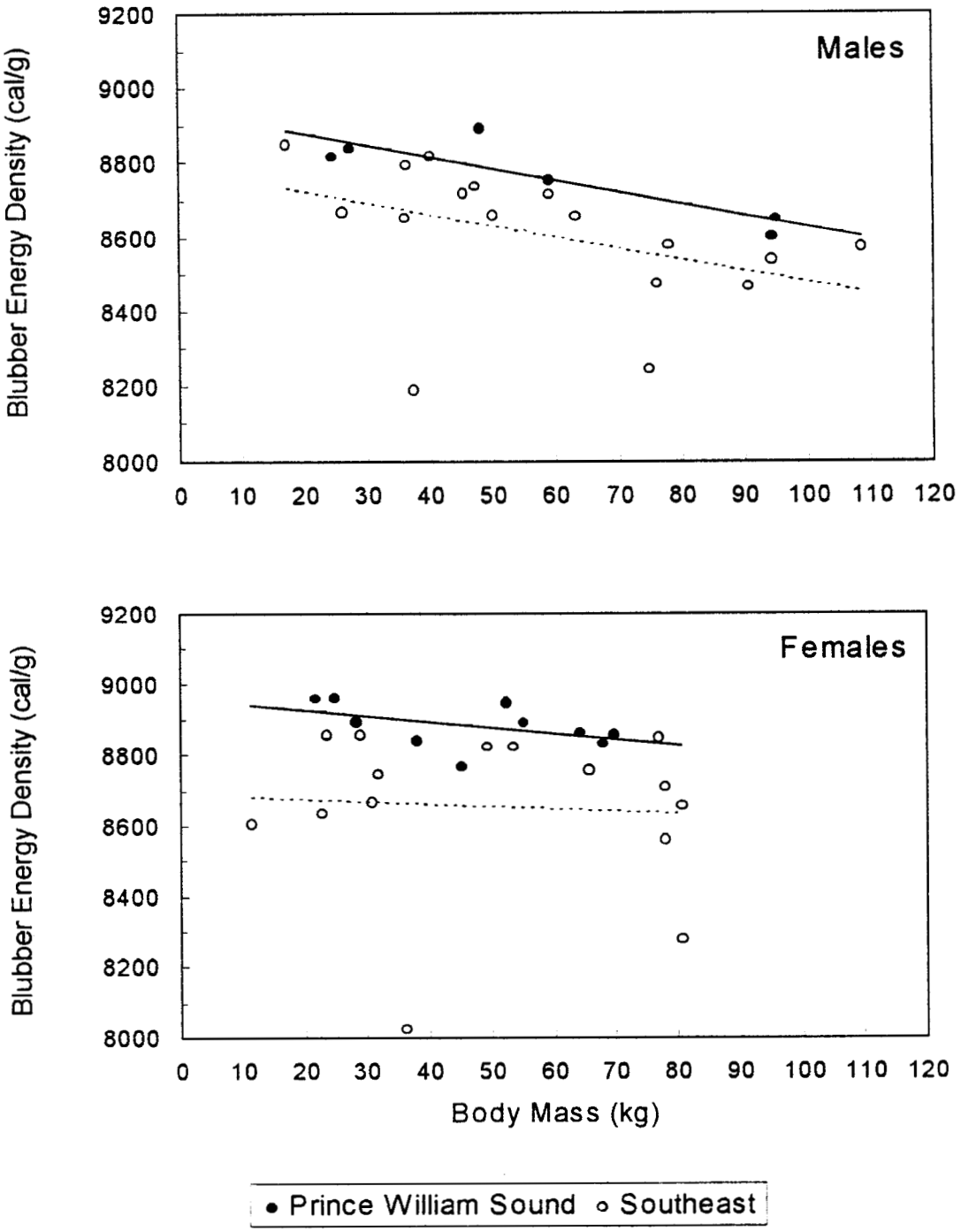

Figure 7. Relationships between blubber energy densities and male or female harbor seal (*Phoca vitulina*) body mass, for seals collected during subsistence harvests during 1995 - 1996 in Prince William Sound and Southeast Alaska.

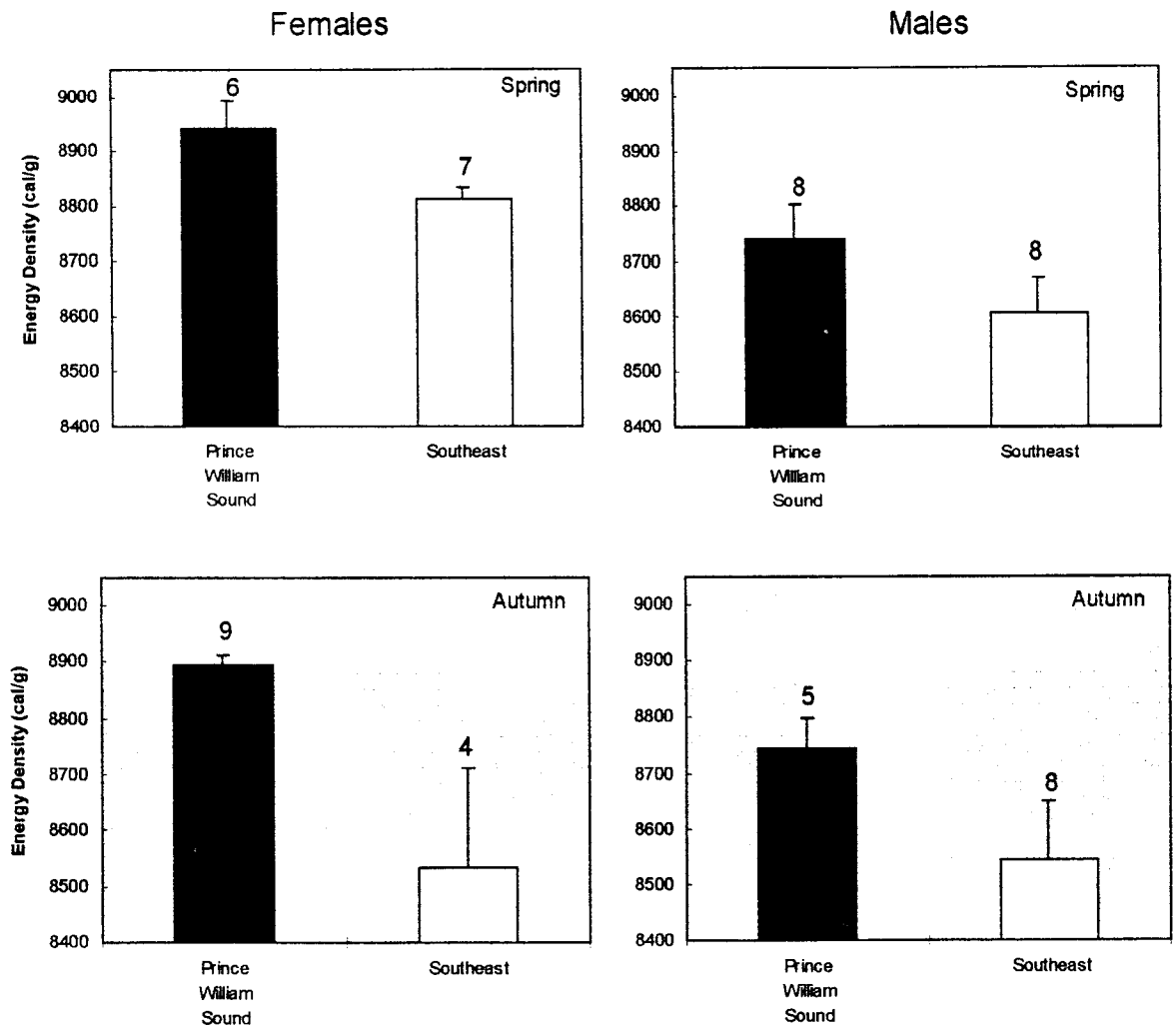

Figure 8. Comparisons of blubber energy density among gender, season and region for harbor seals (*Phoca vitulina*) collected during subsistence harvests in Prince William Sound and southeast Alaska, 1995 - 1996.

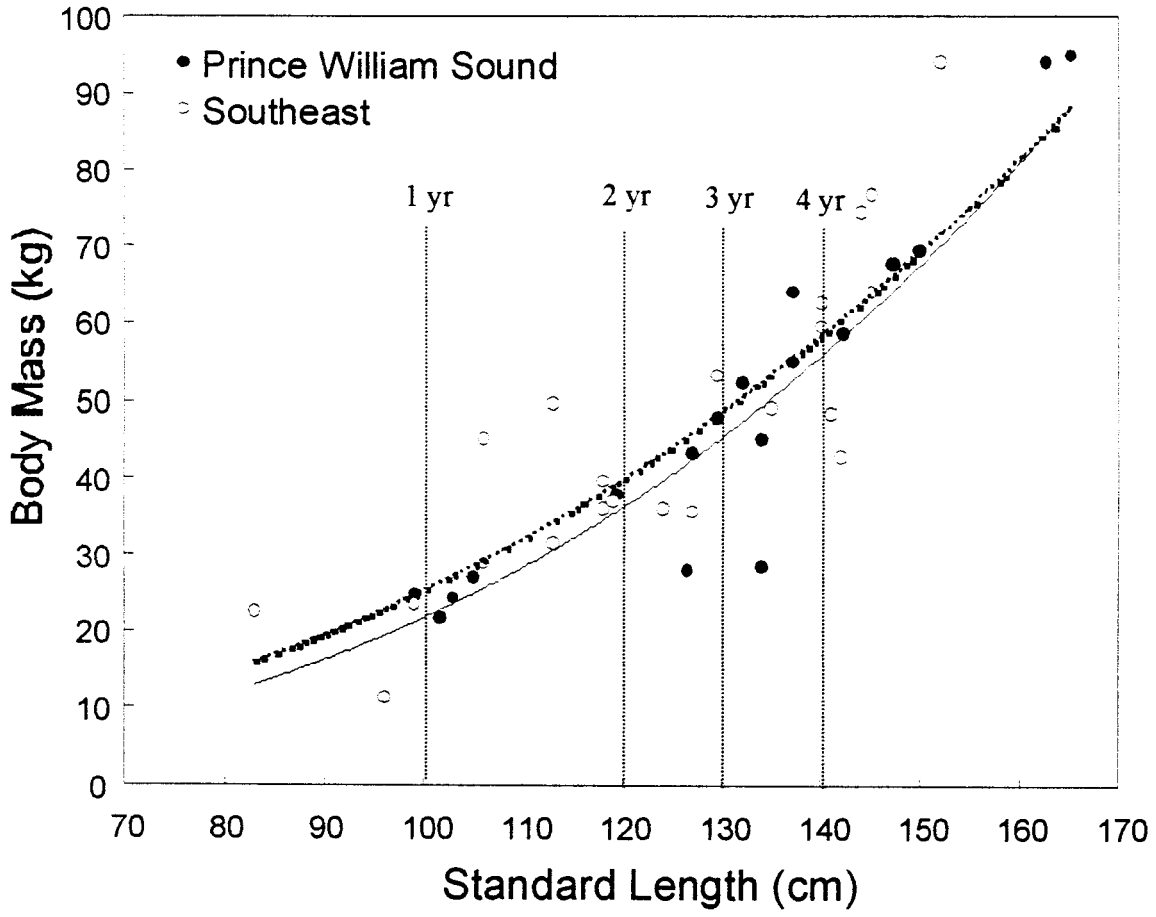

Figure 9. Scaling of body mass with length for harbor seals (*Phoca vitulina*) collected during subsistence harvests 1995 - 1996 in Prince William Sound and southeast Alaska. No significant difference between regions ($F_{(1,32)} = 0.077$, $P = 0.783$), overall $R^2 = 0.808$. Approximate divisions in age class length from Pitcher and Calkins (1979).

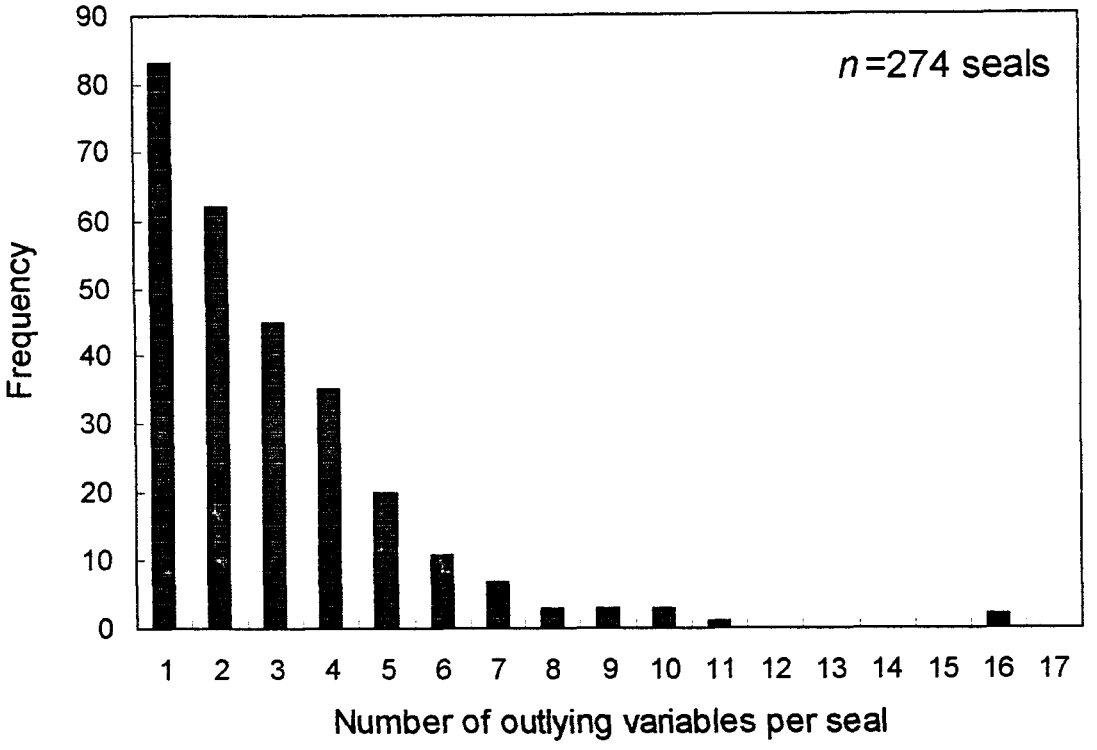

Figure 10. Frequency histogram of total numbers of plasma chemical or hematological outlying variables per harbor seal (*Phoca vitulina*) sampled during 1993 - 1996 from Prince William Sound, Kodiak Island or southeast Alaska. Total of 48 blood analytes surveyed among 307 seals.

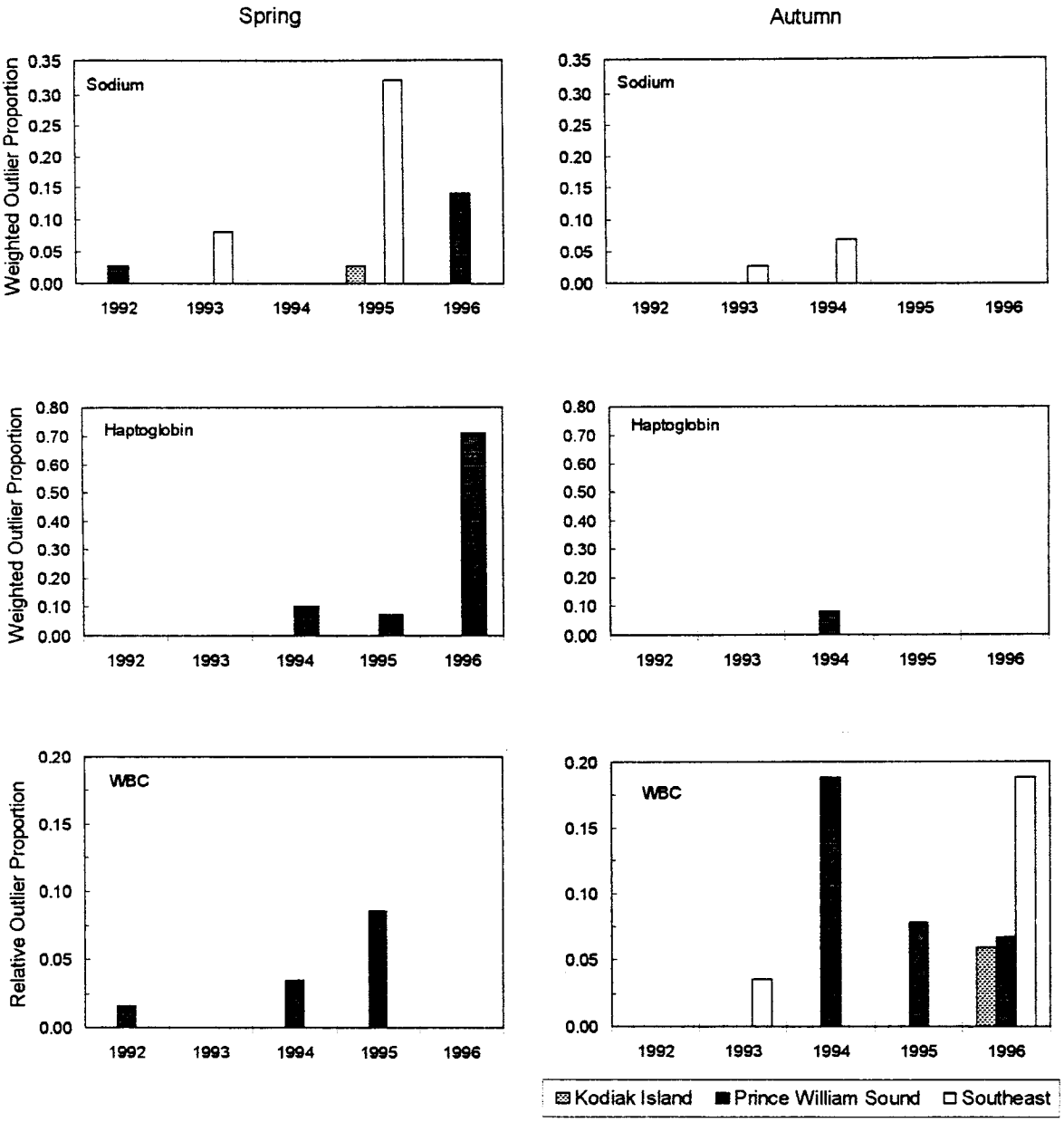

Figure 11. Regional, seasonal and interannual distributions of plasma sodium, haptoglobin and white blood cell count (WBC) outliers (expressed as relative outlier proportion, based on weighted samples size) for harbor seals (*Phoca vitulina*).