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# A Comparison of the Imapct of Environmental Stressors on Blood Parameters of Captive Harbor Seals (Phoca Vitulina)

Maura Allard
*University of Rhode Island*, maura.allard@gmail.com

# A COMPARISON OF THE IMPACT OF ENVIRONMENTAL STRESSORS ON BLOOD PARAMETERS OF CAPTIVE HARBOR SEALS *(PHOCA VITULINA)*

MAURA K. ALLARD, University of Rhode Island, Kingston
JAMES R. CARPENTER, Human Nutrition, Food and Animal Sciences, CTAHR
University of Hawaii at Manoa, Honolulu
SHANNON ATKINSON and LORI POLASEK, Alaska SeaLife Center, Seward

**Introduction:** Since the mid-1970s, the population of harbor seals (*Phoca vitulina)* in the Gulf of Alaska and Prince William Sound has been significantly declining (*Exxon Valdez* Oil Spill Trustee Council, 2001). Because of potential decreases in the quality, distribution and abundance of prey, harbor seals may be feeling the repercussions of nutritional stress. This stress can thus have an effect on the blood chemistry values of the animal. Little research has been established in determining blood chemistry values for harbor seals in Alaska. It has been suggested that various environmental factors can influence the blood chemistry values due to temperature, day length (photoperiod), restraint and diet amongst other variables. It has been shown that photoperiod has a direct correlation with the secretion of hormones from the pineal and pituitary glands (Oki and Atkinson, 2004). These hormones, such as cortisol and thyroid, help the body maintain homeostasis and may also play a role in the effect on blood parameters. Prey sources and levels of nutrient intake, particularly fat and protein, might also alter metabolites as well. The air temperature can affect a seal's resting metabolic rate (Rosen and Renouf, 1998). These shifts in temperature, throughout seasons or even on a day-to-day basis, may affect an animal's circulatory system. Currently there are no guidelines for veterinarians and rehabilitators to refer to when analyzing blood chemistry values for sick animals. The hope for this research is that it will aide in establishing guidelines for specific circumstances due to environmental stressors. It is hoped that in the future, this study will be used to determine normal blood levels along with extent of variation so that veterinarians can simply refer to in a manual.

**Hypothesis:** Because captivity poses several impending stressors on animals, it is important to understand their effects upon an animal's circulating metabolites and physiological regulating systems. Our hypothesis is that age, diet, photoperiod and degree of stress (due to handling and/or confinement) may all alter harbor seals' blood parameters.

**Objectives:**

1. Determine the affects of an animal's environment (diet, photoperiod, and physical confinement) on various blood parameters of harbor seals held in captivity.
2. Identify which blood characteristics are most susceptible to changing due to various environmental stressors and to subsequently determine exactly how these stressors impact or are related to nutrient intake, body weight changes, and blood parameters.
3. Travel to Alaska to observe and participate in actual research data collection, observe the various procedures and learn about careers with the Alaska SeaLife Center and marine mammals in general.

**Review of Literature:** An important parameter to keep in mind when conducting and evaluating the data from this study is that animals that are studied should be within a given population, as there may be differences within populations coming from differing geographical areas (Trumble and Castellini, 2002). It has been suggested that in newborns, HCT and Hb values are higher, taper off during the nursing period and then increase throughout the weaning

and adult period (Burns, 2005). During molting periods which are generally in the autumn, HCT values decrease (Castellini *et al*., 1996). It is important to note that erythrocyte counts and Hb concentrations are generally lower in captive versus wild marine mammals (McConnell & Vaughn, 1983). Pinnipeds have large spleens which can contract from stress due to fear, excitement or apprehension. This contraction can thus cause an increase in circulating RBC and then causes a shift in HCT values when an animal is handled or restrained (Castellini *et al*., 1996). Studies have also suggested that an animal can adjust to captivity and habituate, whereby the physiological responses to stress may not occur (Lander *et al*., 2003). Captivity and molt status do have an effect on HCT values (Castellini et al., 1996). Individual pinnipeds have extremely dynamic HCT regulation (Castellini, 1996).

Variables such as changes in diet, disease, experimental regimes and artificial environments can also create stress on an animal (McConnell and Vaughn, 1983). Nutritional stress has been one of the many reasons posed as to why seals' population may be declining. The "junk food" hypothesis is one of the current theories of a variable that may be inducing physiological stress in seals (Trumble and Castellini, 2001). Studies have shown that seals that consumed herring harvested after a 'bad' winter had a significant reduction in erythrocytes and MCHC and an increase in MCV (Thompson *et al*., 1997). Plasma BUN, creatinine and protein values are also influenced by dietary quality and hydration state of the animals (Schweigert, 1993). Certain prey may also have nutrients or antimetabolites that may interact with the absorption of the essential nutrients that the seals need (Mazzaro *et al*., 1995). If a seal is not able to absorb its necessary nutrients it may have an adverse effect on its blood parameters. However, it has also been concluded that there are physiological responses to prey switching that are unrelated to differences in the energetic content of the prey (Thompson *et al*., 1997).

Because seals are diving animals by nature, they often face varying temperature extremes and also a great deal of hydrostatic pressure. Harbor seals have a relatively high level of blood cholesterol. The erythrocyte membranes are fairly insensitive in comparison to terrestrial animals because of this (Williams *et al*., 2001). Cholesterol keeps the membranes stiff.

A harbor seal's fur is principally used as a streamlining and protective function which is why a drastic change in temperature would not stimulate molting (Rosen and Renouf, 1997). For animals that use their fur as a source of thermal regulation, their molt period can often be triggered by temperature change. As with a harbor seal, it has been established that the photoperiod plays a key role in determining the molt period (Mo and Gili, 2000)

**Materials and Methods:** Animals and Facilities: Four weaned Harbor Seal pups were housed at the Alaska SeaLife Center (ASLC) and were part of a long-term dietary study (high-fat vs low-fat). At weaning the seals averaged 26.0 kg body weight (Atuun - 26.4 kg, Miki - 21.5 kg, Qilak - 22.5 kg, and Susitna - 33.6 kg). All four pups were fed a mixed diet comprised of herring (30%), pollock (30%), capelin (20%), and squid (20%). Feeding either high-fat, or low-fat herring in the mixed diet altered the energy density of the diet. Animals were fed twice daily (8:30-9:00 am and 4:00-4:30 pm). The high-fat seals (Group A- Atuun and Qilak) received high fat herring for the herring component of the diet and were fed to a level of satiation that allowed training methods to continue using routine animal handling protocols by the husbandry staff at the ASLC. Group B seals (Miki and Susitna) received the same mass of each species of foods (as

a proportion of the seal's individual weight), except that low fat herring was substituted for high fat herring. The weight of each prey item consumed daily was recorded for each seal. Seals were weighed periodically on a platform scale so that dry matter and nutrient intakes as a proportion of the individual seal's weight could be determined for each seal and averaged across treatments. The diets were not designed to be iso-caloric and the mass of food being offered was adjusted throughout the experiment based on growth rate and body condition. Group B was expected to be more nutritionally stressed than seals in Group A due to lower lipid and caloric intake.

This "tracking diet" design allows the seals to adjust their intake naturally by season as determined by the appetite of Group A seals. With the exception of short pulse switches in the diet, each group will remain on their respective diet through reproductive maturity. Comprehensive condition assessments will be made throughout the study to monitor growth and condition of each seal. In addition, the seals digestive efficiency will be contrasted to ascertain how effectively seals are able to respond to short-term ecological changes in forage fish availability.

**Diet Sampling, Tracking and Proximate Analysis for Nutrient Composition:**

Diet prey items were weighed and recorded daily before each feeding so that both fresh food and dry matter intake could be determined for each harbor seal. Representative samples of each of the dietary prey items were sampled periodically, particularly during the rate of passage and assimilation efficiency studies. Samples of the prey items were kept frozen (-80$^{o}$C) until being homogenized for proximate analysis. Proximate analysis was performed using standard methods of the Association of Official Analytical Chemists (1990) to determine the dry matter, moisture, ash, crude protein, and crude fat content of prey and fecal samples.

Spreadsheets were developed that enabled the tracking of daily food consumption, body weight changes, rate of growth, nutrient intake, and diet consumption as percent of body mass (weight). Weekly averages were also calculated to monitor and track changes in the diet over the course of the sampling.

**Blood Sampling and Analysis:**

Blood samples (for hormone and metabolite determination) were collected either voluntarily while the seals are under mild sedation and while physically restrained by trained husbandry staff. Blood was collected from the intervertebral sinus at a site ~60% of their body length. Injection sites were cleaned with 70% isopropyl alcohol and a dilute solution of povidone iodine in saline (30ml in 500ml NaCl) prior to injection. During blood collections, animals were either confined in a cage prior to blood draws or were unconfined, in which they just received bloodwork without prior holding. Blood collection volumes did not exceed 5% total blood volume (based on weight at the time of sampling) or 5 ml/kg. For this project, ~40ml of blood was collected every four to six weeks from each seal. For methodology, see ASLC blood sampling protocol (p28) either at the protocol here from the IACUC or remove this statement. The levels of the blood metabolites BUN, triglycerides, and glucose were determined directly following blood collection by standard IDEXX vet chemistries using an IDEXX VetTest Blood Chemistry Analyzer 8008 (IDEXX Laboratories, Inc., Westbrook, Maine). Serum was also harvested from each blood sample through centrifugation and stored at -80ºC until analysis.

Air temperature was collected from the National Climatic Data Center website and used to formulate graphs by which to compare the effect of temperature against consumption and the

effect on blood characteristics. The photoperiods were calculated through the Online Photoperiod calculator and the website for the US Naval Observatory. Photoperiods were observed to reference the potential effects of day length on consumption which could ultimately have an effect on blood parameters.

**Results:**

The julian dates and photoperiods are presented in Figure 1 and the photoperiod ranged from a high of about 18 hours and 53 minutes during June to a low of about 5 hours and 5 minutes during February. As can be seen from Figure 1, the photoperiods are very consistent for both years 1 (2004) and 2 (2005) and overlap enough that you can not tell them apart. Figure 2 presents the maximum, minimum and average temperatures during the trail period. Average temperatures ranged from a low of 5°F during the winter to a high of 85°F during the summer. The daily mean temperatures were similar for both years. Figure 3 presents the weekly averages for dietary food mass consumed per seal each day. You will note that Susitna, a low-fat fed animal, consumed the most and Quilak, a high-fat fed animal, consumed the least each day. One can also see in this figure that intake also varies with age and time of year. As one would expect, the intakes generally increased as the animals got larger (older) and during the winter/early spring time period. Figure 4 depicts the changes in body weight and you'll notice that body weight directly parallel the average consumptions shown in Figure 3. Tables 5 and 6 present the daily protein and lipid consumption by the seals and the seals fed the high-fat diet consumed more fat and less protein than the low-fat fed seals. Figure 7 presents the average energy consumption for each animal and those animals consuming the higher fat level were also consuming more total energy (Mcal.day).

Tables 1-6 present the blood protein and enzyme concentrations, and the blood concentrations of Ca, Phos, electrolytes, fibrinogen and various blood cell types of Harbor seals from weaning through 18 months of post-weaning growth. Values in the tables are the means $\pm$ standard deviations and where significance occurs the level of probability is also presented. The NS in all tables indicates that the means are not significantly different from one another. Tables 1 and 2 present the average means for each of the four seals, Tables 3 and 4 compare the means for high-fat and low-fat fed animals and the differences in blood values for confined and unconfined seals, and Tables 5 and 6 provide the means for all animals during the long (>12hr/d), intermediate (>8 and <12hr/d) and short (<8h/d) photoperiods. Most of the blood parameters were not significantly different from one another for each of the analyzed variables.

Figure 1.

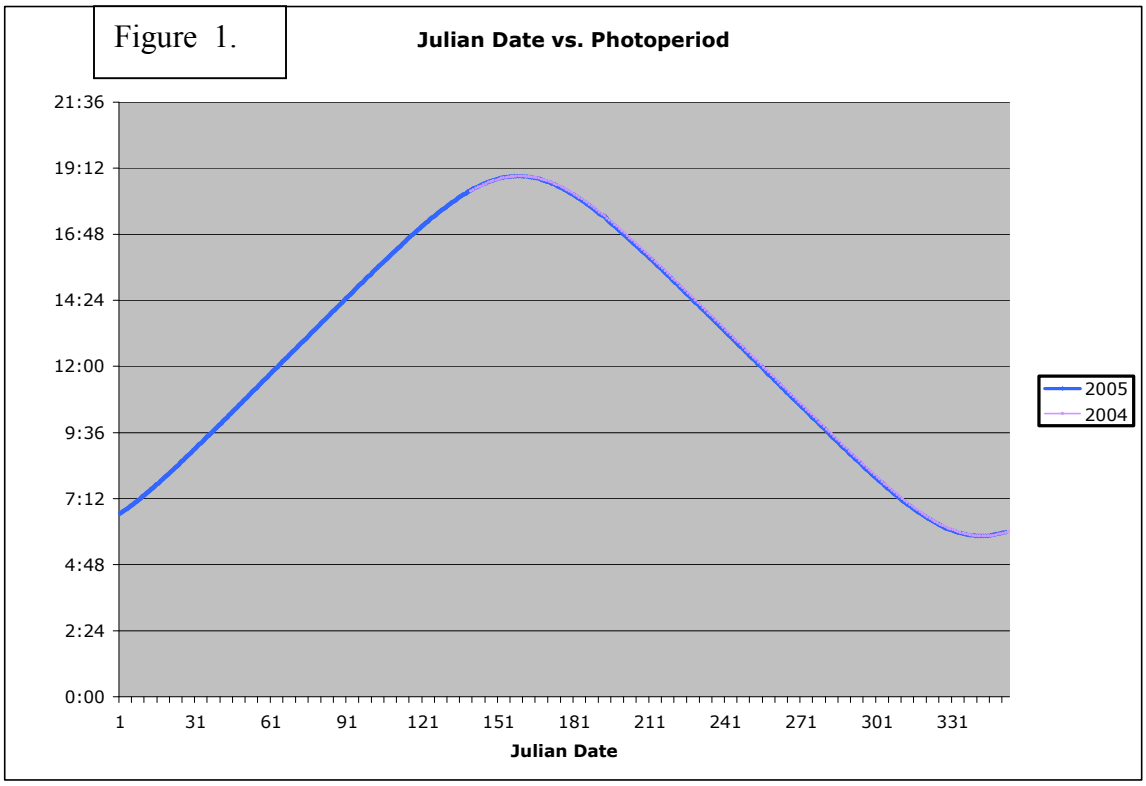


Figure 2.

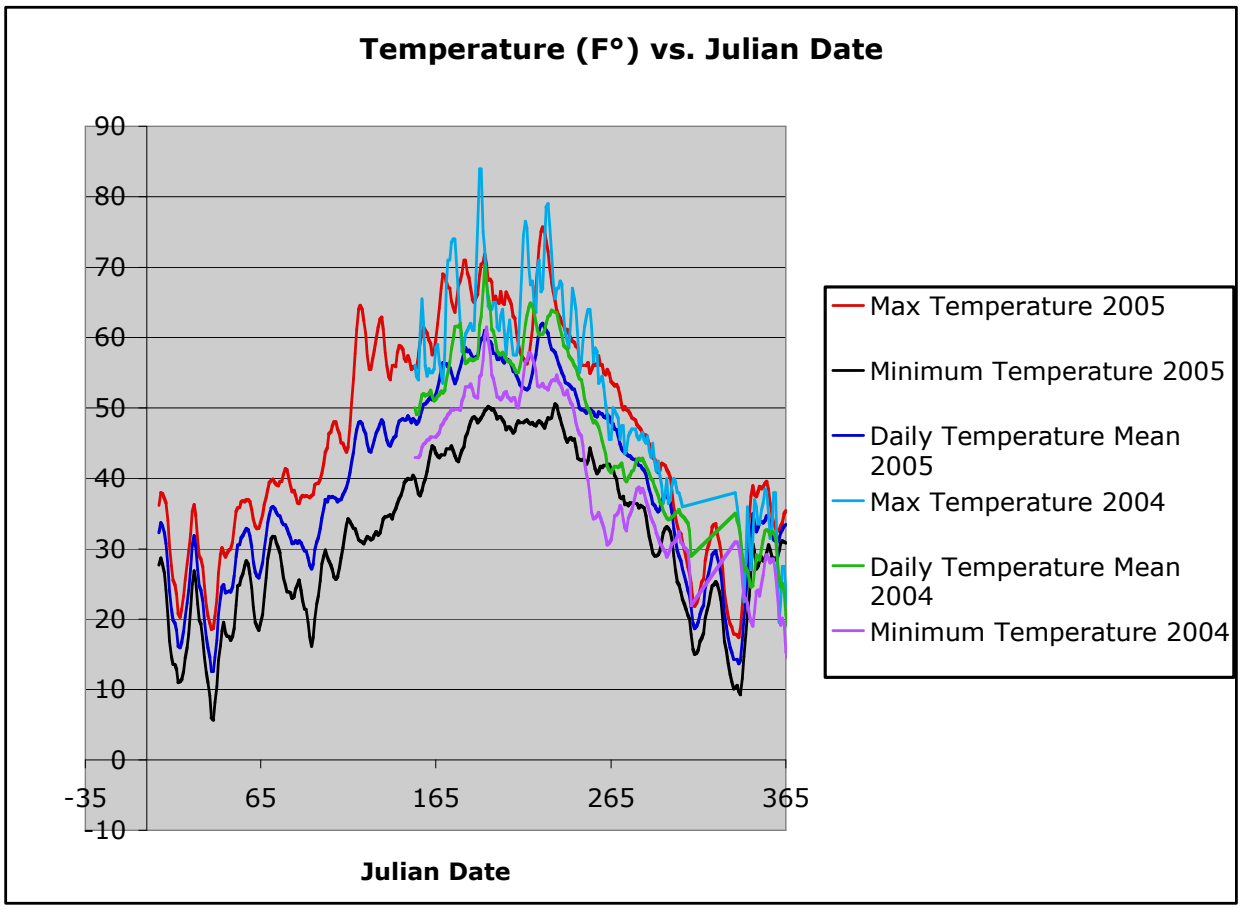

Figure 3.

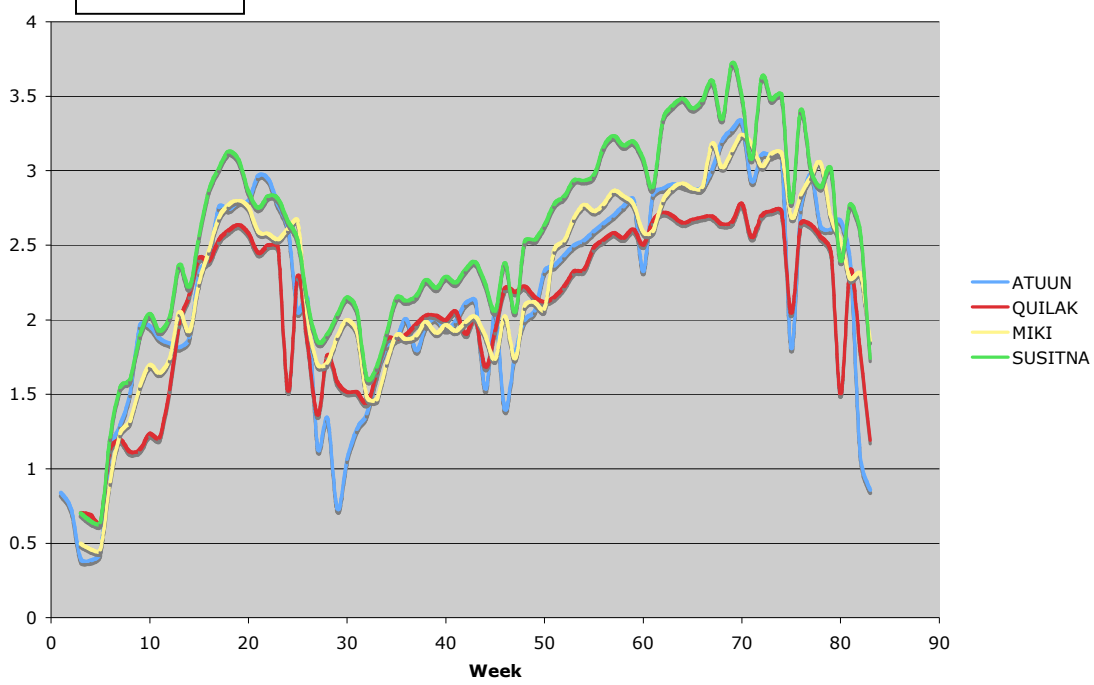


Figure 4.

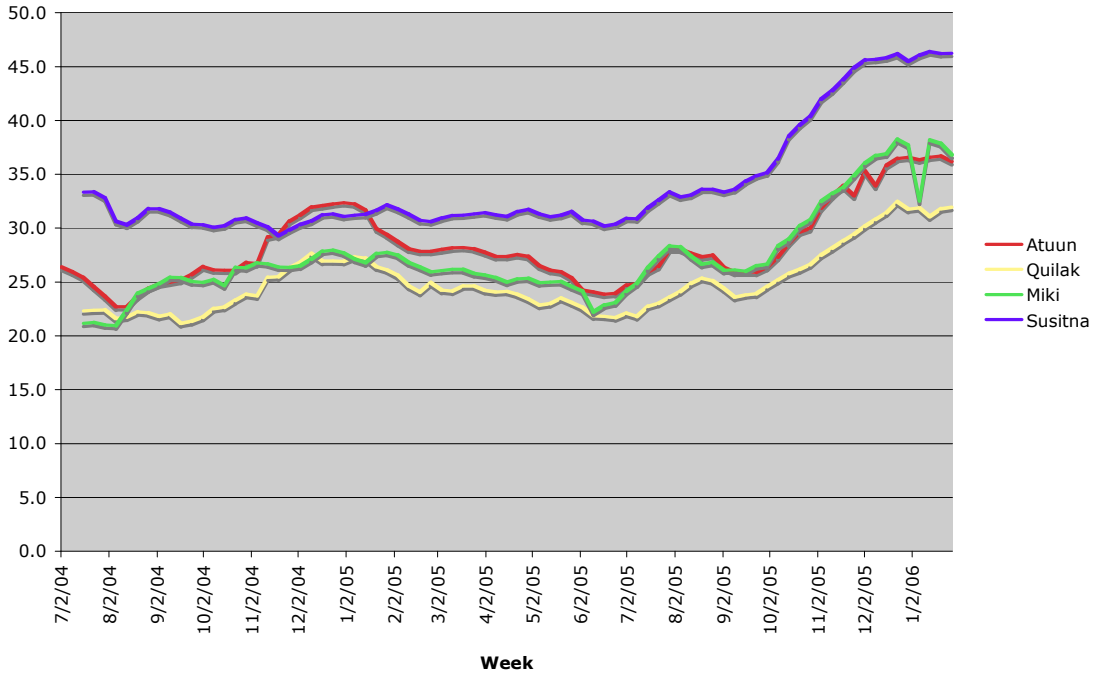

Figure 5.

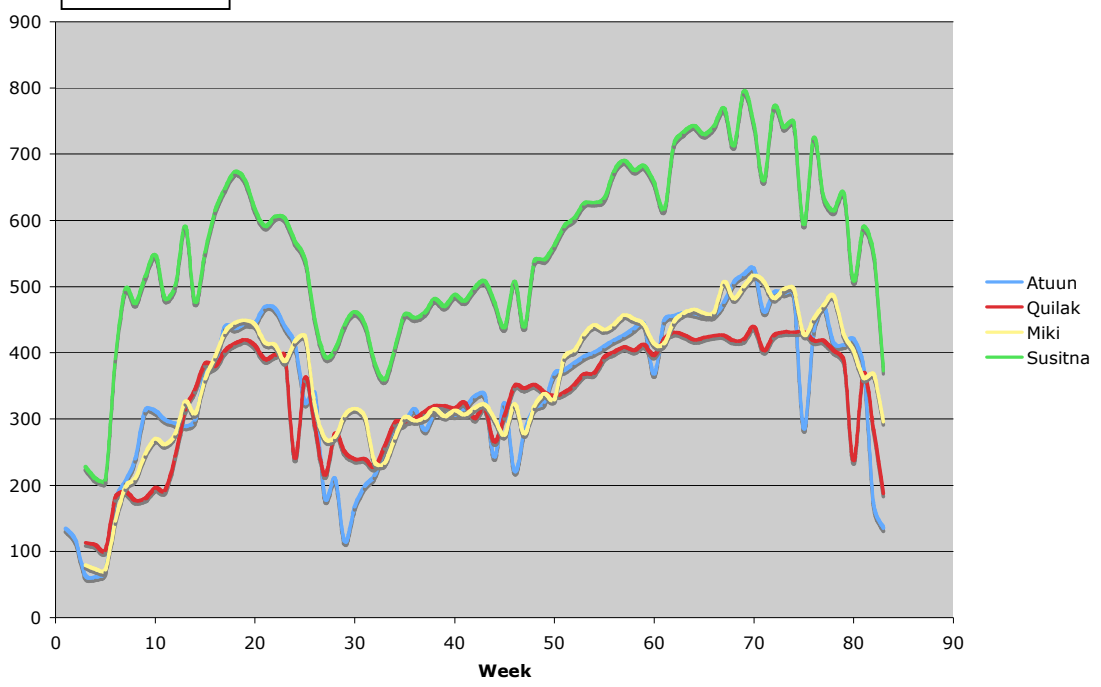


Figure 6.

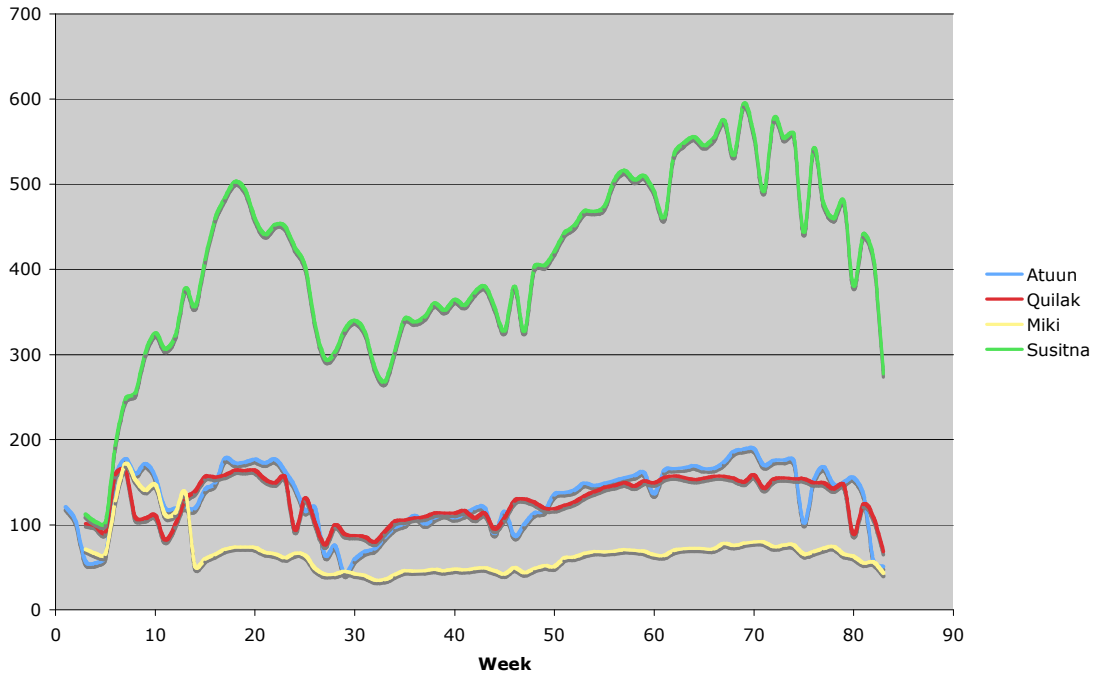

Figure 7.

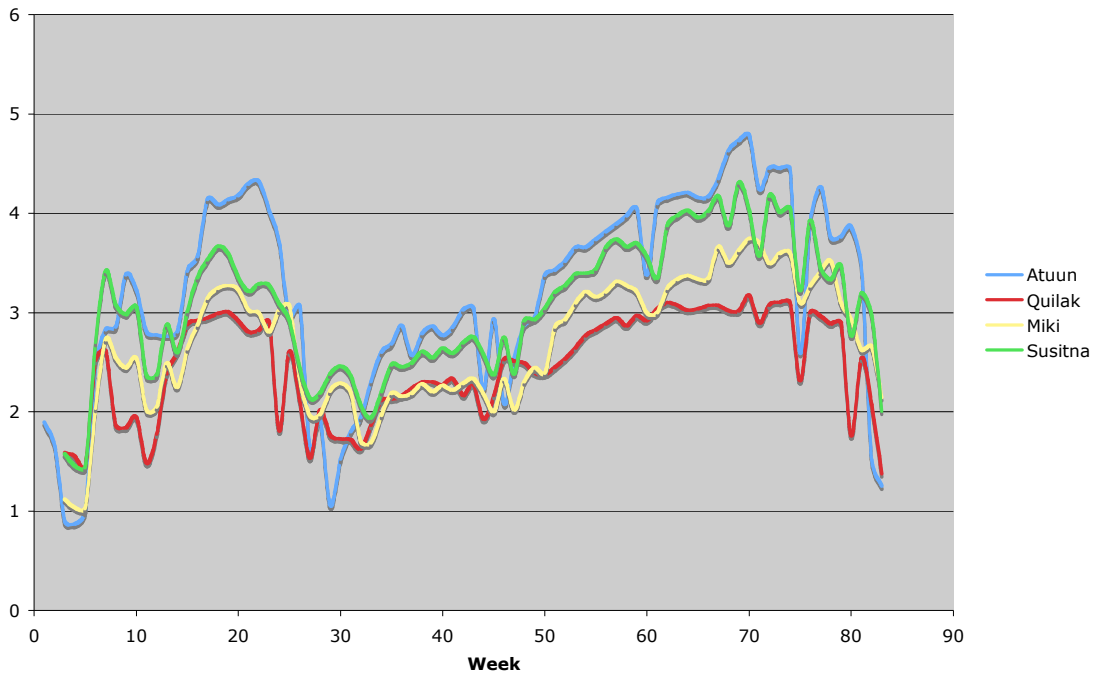


Table 1. Blood protein and enzyme concentrations of Harbor seals from weaning through 18 months of post-weaning growth (Means ± Std Dev.)

| | ---------- Seal Name ---------- | | | | |
|---|---|---|---|---|---|
| Variable | Atuun (HF*) | Miki (LF*) | Quilak (HF*) | Susitna (LF*) | P Value |
| (n) | 13 | 13 | 13 | 13 | N/A |
| ALB, g/dl | 3.24 ± 0.26 | 3.29 ± 0.19 | 3.57 ± 0.76 | 3.27 ± 0.28 | NS |
| ALKP, IU | 122.62 ± 27.78 | 87.46 ± 18.48 | 83.54 ± 11.49 | 86.77 ± 17.84 | P≤0.001 |
| ALT, IU | 45.62 ± 13.66 | 67.00 ± 20.21 | 62.92 ± 40.81 | 43.31 ± 16.87 | NS |
| AMYL, IU | 373.31 ± 53.21 | 193.62 ± 23.44 | 416.77 ± 59.69 | 433.85 ± 70.47 | P≤0.001 |
| AST, IU | 69.54 ± 25.49 | 81.00 ± 24.66 | 53.75 ± 11.64 | 57.08 ± 13.99 | NS |
| BUN, mg/dl | 37.33 ± 5.00 | 34.83 ± 4.39 | 35.49 ± 4.21 | 33.02 ± 4.74 | NS |
| CHOL, mg/dl | 257.23 ± 26.86 | 258.48 ± 25.04 | 275.28 ± 26.58 | 243.18 ± 21.68 | NS |
| CK, IU | 145.17 ± 111.2 | 302.8 ± 528.6 | 310.15 ± 531.2 | 351.9 ± 490.1 | NS |
| CREA, mg/dl | 0.75 ± 0.17 | 0.69 ± 0.14 | 0.69 ± 0.12 | 0.78 ± 0.13 | NS |
| GGT, IU | 26.69 ± 7.54 | 21.85 ± 4.88 | 21.42 ± 3.00 | 26.08 ± 11.46 | NS |
| GLU, mg/dl | 167.76 ± 26.59 | 158.91 ± 13.14 | 161.84 ± 15.15 | 154.46 ± 13.53 | NS |
| LDH, IU | 1780.5 ± 502.0 | 1772.1 ± 410.2 | 1548.2 ± 177.9 | 1742.9 ± 221.5 | NS |
| TBIL, mg/dl | 0.33 ± 0.11 | 0.33 ± 0.16 | 0.80 ± 1.58 | 0.33 ± 0.21 | NS |
| TP, g/dl | 6.48 ± 0.39 | 6.48 ± 0.22 | 7.21 ± 0.87 | 6.50 ± 0.37 | NS |
| TRIG, mg/dl | 47.42 ± 18.10 | 32.69 ± 16.58 | 31.69 ± 14.22 | 36.41 ± 16.62 | NS |
| GLOB, g/dl | 3.25 ± 0.30 | 3.21 ± 0.25 | 3.61 ± 0.24 | 3.22 ± 0.22 | NS |
| ALB GLOB | 1.00 ± 0.13 | 1.03 ± 0.12 | 0.94 ± 0.10 | 1.02 ± 0.11 | NS |

* HF = High-Fat diet; LF = Low-Fat diet

Table 2. Blood concentrations of Ca, Phos, electrolytes, fibrinogen and various blood cell types in Harbor seals from weaning through 18 months of post-weaning growth (Means ± Std Dev.)

| | ---------- Seal Name ---------- | | | | |
|---|---|---|---|---|---|
| Variable | Atuun (HF*) | Miki (LF*) | Quilak (HF*) | Susitna (LF*) | P Value |
| (n) | 13 | 13 | 13 | 13 | N/A |
| Ca, mg/dl | 9.69 ± 0.57 | 9.48 ± 0.49 | 9.48 ± 0.51 | 9.42 ± 0.98 | NS |
| Phos, mg/dl | 6.00 ± 0.77 | 5.71 ± 0.77 | 5.97 ± 1.71 | 5.73 ± 0.87 | NS |
| Na, mmol/l | 157.30 ± 3.04 | 158.41 ± 3.00 | 157.41 ± 5.14 | 158.57 ± 4.04 | NS |
| K, mmol/l | 3.94 ± 0.42 | 4.03 ± 0.30 | 4.00 ± 0.39 | 4.09 ± 0.37 | NS |
| Cl, mmol/l | 112.37 ± 1 .35 | 114.07 ± 1.62 | 112.18 ± 1.58 | 112.69 ± 1.61 | NS |
| Fibrin., mg/dl | 208.3 ± 274.6 | 200.0 ± 141.4 | 209.1 ± 137.5 | 250.0 ± 183.4 | NS |
| HCT | 58.50 ± 3.16 | 58.20 ± 2.75 | 59.40 ± 3.39 | 59.33 ± 3.19 | NS |
| PCV | 55.50 ± 3.06 | 55.33 ± 3.42 | 56.04 ± 3.53 | 55.50 ± 4.27 | NS |
| WBC 1000 | 8.03 ± 1.40 | 7.04 ± 2.96 | 9.92 ± 1.73 | 7.80 ± 1.91 | NS |
| Neutrophils | 59.75 ± 10.22 | 41.55 ± 16.95 | 55.38 ± 6.50 | 60.75 ± 8.82 | NS |
| Lymphocytes | 29.5 ± 12.00 | 39.54 ± 11.95 | 31.67 ± 5.65 | 26.25 ± 7.92 | NS |
| Monocytes | 7.25 ± 3.47 | 9.97 ± 6.82 | 7.75 ± 4.20 | 9.00 ± 4.82 | NS |
| Eosinophils | 1.67 ± 1.72 | 1.09 ± 1.01 | 3.25 ± 2.14 | 2.83 ± 2.44 | NS |
| Basophils | 1.42 ± 1.38 | 2.64 ± 2.62 | 1.25 ± 1.36 | 0.58 ± 1.00 | NS |
| Platelets | 3.45 ± 1.51 | 35.5 ± 112.0 | 3.18 ± 1.60 | 3.33 ± 1.50 | NS |

- HF = High-Fat diet; LF = Low-Fat diet

Table 3. Blood protein and enzyme concentrations of Harbor seals being fed high-fat or low-fat diets and in confined or unconfined holding areas (Means ± Std Dev.)

| Variable | High -Fat | Low-Fat | P Value | Confined | Unconfined | P Value |
|---|---|---|---|---|---|---|
| (n) | 30 | 22 | N/A | 22 | 32 | N/A |
| ALB, g/dl | 3.42± 0.54 | 3.23± 0.23 | NS | 3.29 ± 0.22 | 3.37 ± 0.54 | NS |
| ALKP, IU | 104.93± 27.7 | 81.68± 11.6 | NS | 91.0 ± 22.06 | 97.66 ± 26.8 | NS |
| ALT, IU | 52.47± 29.9 | 57.77± 21.7 | NS | 51.85 ± 19.75 | 56.50 ± 30.3 | NS |
| AMYL, IU | 375.0± 83.0 | 326.2± 135.6 | NS | 365.0 ± 118.5 | 347.8 ± 105.6 | NS |
| AST, IU | 64.10± 24.33 | 67.50± 19.7 | NS | 59.3 ± 21.64 | 69.61 ± 22.10 | NS |
| BUN, mg/dl | 35.40± 5.18 | 34.85± 4.11 | NS | 36.78 ± 5.32 | 34.16 ± 4.08 | NS |
| CHOL, mg/dl | 262.6± 27.9 | 253.0± 25.2 | NS | 261.1 ± 27.64 | 256.9 ± 26.82 | NS |
| CK, IU | 328.9± 537.7 | 215.8± 293.3 | NS | 170.7 ± 146.5 | 350.7 ± 555.5 | NS |
| CREA, mg/dl | 0.70± 0.15 | 0.76± 0.12 | NS | 0.83 ± 0.16 | 0.66 ± 0.09 | NS |
| GGT, IU | 24.14± 5.93 | 23.95± 9.63 | NS | 24.90 ± 10.97 | 23.52 ± 4.56 | NS |
| GLU, mg/dl | 166.11± 20.2 | 153.4± 11.55 | NS | 161.87± 13.1 | 160.04 ± 20.8 | NS |
| LDH, IU | 1735± 441.2 | 1694± 242.0 | NS | 1693 ± 382.7 | 1731± 355.2 | NS |
| TBIL, mg/dl | 0.53± 1.05 | 0.34± 0.20 | NS | 0.25 ± 0.12 | 0.57 ± 1.01 | NS |
| TP, g/dl | 6.80± 0.72 | 6.49± 0.32 | NS | 6.60 ± 0.40 | 6.71 ± 0.70 | NS |
| TRIG, mg/dl | 39.76± 17.2 | 33.35 ± 16.78 | NS | 28.70 ±11.73 | 42.27 ± 18.06 | NS |
| GLOB, g/dl | 3.36± 0.35 | 3.26± 0.21 | NS | 3.32 ± 0.28 | 3.32 ± 0.31 | NS |
| ALB GLOB | 1.00± 0.13 | 0.99± 0.10 | NS | 1.00 ± 0.10 | 1.00 ± 0.13 | NS |

Table 4. Blood concentrations of Ca, Phos, electrolytes, fibrinogen and various blood cell types in Harbor seals being fed high-fat or low-fat diets and in confined or unconfined holding areas (Means ± Std Dev.)

| Variable | High -Fat | Low-Fat | P Value | Confined | Unconfined | P Value |
|---|---|---|---|---|---|---|
| (n) | 30 | 22 | N/A | 22 | 32 | N/A |
| Ca, mg/dl | 9.63 ± 0.53 | 9.37 ± 0.79 | NS | 9.80 ± 0.42 | 9.34 ± 0.72 | NS |
| Phos, mg/dl | 6.03 ± 1.23 | 5.61 ± 0.80 | NS | 6.06 ± 0.97 | 5.72 ± 1.34 | NS |
| Na, mmol/l | 157.5 ± 3.85 | 158.5± 3.8 | NS | 157.8 ± 5.78 | 158.0 ± 1.91 | NS |
| K, mmol/l | 4.01 ± 0.66 | 4.02 ± 0.30 | NS | 4.00 ± 0.26 | 4.03 ± 0.66 | NS |
| Cl, mmol/l | 112.5 ± 1.53 | 113.3 ± 1.77 | NS | 112.5 ± 1.91 | 113.0 ± 1.51 | NS |
| Fibrin., mg/dl | 207.4 ±197.9 | 233.3±181.5 | NS | 227.8 ± 152.6 | 211.1 ± 213.6 | NS |
| HCT | 59.10 ± 3.18 | 58.51± 2.97 | NS | 58.04 ± 3.82 | 59.44 ± 2.32 | NS |
| PCV | 56.16 ± 3.20 | 54.8 ± 3.80 | NS | 53.48 ± 3.76 | 57.11 ± 2.36 | NS |
| WBC 1000 | 8.78 ± 1.79 | 7.38 ± 2.67 | NS | 8.72 ± 2.67 | 7.83 ± 1.92 | NS |
| Neutrophils | 57.51 ± 8.11 | 49.93 ± 17.8 | NS | 56.68 ± 12.11 | 52.7 ± 14.24 | NS |
| Lymphocytes | 29.61 ± 9.04 | 34.73 ± 12.2 | NS | 32.45 ± 11.48 | 31.23 ± 10.23 | NS |
| Monocytes | 7.78 ± 3.71 | 9.48 ± 6.26 | NS | 7.50 ± 3.62 | 9.20 ± 5.66 | P≤0.05 |
| Eosinophils | 2.64 ± 2.25 | 1.61 ± 1.57 | NS | 1.60 ± 1.73 | 2.65 ± 2.16 | NS |
| Basophils | 1.71 ± 1.84 | 1.05 ± 1.65 | NS | 1.15 ± 1.76 | 1.66 ± 1.80 | NS |
| Platelets | 3.12 ± 1.48 | 22.90 ± 86.7 | P≤0.001 | 2.94 ± 1.51 | 17.36 ± 73.24 | P≤.01 |

Table 5. Blood protein and enzyme concentrations of Harbor seals during long (>12hr/d), intermediate (>8 and <12hr/d) and short (<8h/d) photoperiods (Means ± Std Dev.)

| Variable | Long | Intermediate | Short | P Value |
|---|---|---|---|---|
| (n) | 24 | 8 | 20 | N/A |
| ALB, g/dl | 3.36 ± 0.62 | 3.30 ± 0.16 | 3.34 ± 0.21 | NS |
| ALKP, IU | 99.92 ± 28.14 | 99.25 ± 22.88 | 87.65 ± 20.92 | NS |
| ALT, IU | 55.67 ± 34.49 | 54.88 ± 22.83 | 53.50 ± 16.36 | NS |
| AMYL, IU | 338.17 ± 101.28 | 326.75 ± 114.63 | 384.90 ± 116.37 | NS |
| AST, IU | 66.04 ± 24.28 | 73.25 ± 20.55 | 61.95 ± 20.75 | NS |
| BUN, mg/dl | 32.12 ± 4.08 | 35.86 ± 3.40 | 38.55 ± 3.39 | NS |
| CHOL, mg/dl | 252.06 ± 26.75 | 243.54 ± 17.00 | 272.33 ± 25.07 | NS |
| CK, IU | 359.00 ± 595.99 | 148.13 ± 108.42 | 242.15 ± 312.29 | NS |
| CREA, mg/dl | 0.65 ± 0.10 | 0.69 ± 0.06 | 0.83 ± 0.15 | NS |
| GGT, IU | 25.17 ± 9.97 | 24.63 ± 4.10 | 22.55 ± 5.40 | NS |
| GLU, mg/dl | 164.17 ± 22.32 | 154.38 ± 9.23 | 159.18 ± 14.54 | NS |
| LDH, IU | 1881.43 ± 383.19 | 1673.13 ± 470.36 | 1561.65 ± 200.15 | NS |
| TBIL, mg/dl | 0.60 ± 1.17 | 0.27 ± 0.14 | 0.34 ± 0.18 | NS |
| TP, g/dl | 6.69 ± 0.79 | 6.75 ± 0.38 | 6.60 ± 0.39 | NS |
| TRIG, mg/dl | 41.14 ± 15.96 | 42.14 ± 17.72 | 30.12 ± 16.81 | NS |
| GLOB, g/dl | 3.31 ± 0.32 | 3.46 ± 0.27 | 3.27 ± 0.28 | NS |
| ALB GLOB | 0.99 ± 0.15 | 0.96 ± 0.05 | 1.03 ± 0.10 | NS |

Table 6. Blood concentrations of Ca, Phos, electrolytes, fibrinogen and various blood cell types in Harbor seals during long (>12hr/d), intermediate (>8 and <12hr/d) and short (<8h/d) photoperiods (Means ± Std Dev.)

| Variable | Long | Intermediate | Short | P Value |
|---|---|---|---|---|
| (n) | 24 | 8 | 20 | N/A |
| Ca, mg/dl | 9.20 ± 0.76 | 9.82 ± 0.32 | 9.78 ± 0.44 | NS |
| Phos, mg/dl | 6.18 ± 1.41 | 5.92 ± 0.74 | 5.43 ± 0.42 | NS |
| Na, mmol/l | 157.87 ± 1.55 | 158.80 ± 0.96 | 157.63 ± 5.98 | NS |
| K, mmol/l | 4.12 ± 0.71 | 3.89 ± 0.32 | 3.94 ± 0.30 | NS |
| Cl, mmol/l | 112.80 ± 1.62 | 113.26 ± 1.12 | 112.69 ± 1.95 | NS |
| Fibrin., mg/dl | 247.62 ± 227.20 | 137.50 ± 118.77 | 218.75 ± 160.08 | NS |
| HCT | 57.82 ± 3.40 | 58.81 ± 2.49 | 60.43 ± 2.16 | NS |
| PCV | 55.46 ± 3.95 | 56.31 ± 1.44 | 55.44 ± 3.61 | NS |
| WBC 1000 | 7.84 ± 1.89 | 7.79 ± 2.00 | 8.95 ± 2.84 | NS |
| Neutrophils | 51.82 ± 14.78 | 54.75 ± 11.95 | 57.97 ± 11.73 | NS |
| Lymphocytes | 31.94 ± 10.88 | 29.50 ± 7.67 | 32.56 ± 12.01 | NS |
| Monocytes | 8.65 ± 4.33 | 11.75 ± 7.42 | 6.63 ± 3.56 | NS |
| Eosinophils | 2.55 ± 2.17 | 2.00 ± 2.00 | 1.81 ± 1.91 | NS |
| Basophils | 2.00 ± 1.81 | 1.75 ± 2.05 | 0.50 ± 1.21 | NS |
| Platelets | 20.35 ± 80.81 | 3.13 ± 1.55 | 3.07 ± 1.39 | NS |

**Discussion:** In studying both the photoperiod and temperature charts, it can be observed that the longest photoperiod for the year coincides with the warmest temperatures. After analyzing intake, it is difficult to make a concrete conclusion as to whether the animals consumed more or less due to photoperiod or temperature. The animals have only been observation since June 2004. Their initial records would most likely be unusual due to stress and adjustment period. When the animals were first captured they were also in their weaning period. During an animal's weaning period, its body is changing chemically and hormonally to adjust to becoming independent from its mother.

Because of the small number of animals sampled and large variation in values, there were few statistical differences. There have been relatively no significant statistical differences in the animals that were fed low fat diets versus high fat diets because they need to be monitored for a longer period. In the observations of animals that were confined during blood draws versus animals that were unconfined, no significant changes were noticed either. These findings in of themselves are interesting because they indicate that the animals may not in fact be physiologically stressed. Had the animals been stressed, their values would have indicated more differences. This finding is important because while we may feel bad seeing a seal being confined and may think it is uncomfortable, we are seeing that they are actually quite relaxed. The seals that were analyzed underwent extensive training throughout their captivity period from the time that they were captured. As shown, as time increased, it seems as though the animals have become more acclimated to the tests and contact with humans. It is hoped that with more evidence these observations may be used to help encourage the training and socialization to testing procedures and humans in research facilities prior to testing. It is interesting to note that

in both the Daily Lipid vs. Intake and Daily Protein vs. Intake charts, Susitna had values that seemed to be much different than the other seals. Susitna was clearly the largest animal at time of capture so probably because of her larger size she started on solid prey quicker and has continued to rank above her counterparts, both in growth and intake, throughout the study period.

This initial report is a precursor to the research that will be continuing over the next several years. Originally it was planned to observe the effects of the molt period on the seals' blood chemistry. Because blood was not taken from the yearling seals during their first molting period, it cannot be verified as to what exact blood parameters are affected by molt period. This variable will be monitored in future reports. Each bleed was performed roughly around the same time of day each day that it was taken to help stabilize the possibility for extra variables. The variation in blood parameters (maybe due to daily cyclicity or diurnal patterns) could potentially be due to the time of feeding and how the animals were fed and handled before the blood draws.

**Conclusion/Implications, and Future Research:** It is important to note that this study would be preferably continued in order to gain clearer and more accurate results. As this experiment monitored seals from capture when they were mere pups through their yearling state, it is difficult to make concrete conclusions as their adjustment period may have interfered with results initially. As the seals continue being watched over the next several years it will be much easier to obtain values and notice changes in their blood chemistry. We observed that the blood chemistry values in the harbor seals in this experiment were very similar to seals used in other experiments that used diet and constraint as variables. In future research it would be suggested to compare various averages for specific periods both before and after bleeding times. Several interesting variables to watch in the future in conjunction with the environmental stressors could be the molt period, water temperature and exercise. It is also hoped that the number of animals in each variable category can be increased in order to receive more accurate results. Future experiments should clearly document what time the blood is being taken so that it can correlate to the photoperiod and how that relates to the subsequent blood characteristics. In relation to the photoperiod, it would be interesting to see if the seals that were quarantined had a dramatic change from the animals that were kept outside.

In the future, we hope to use this data to be compared to values of Stellar sea lions which are endangered. By analyzing their similarities there may be hope at conserving their decreasing population. There is also a need to analyze metabolism to see how the animals use their energy.

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**Literature Cited:**


BURNS, J.M., D.P. COSTA, K. FROST, J.T. HARVEY. 2005. Development of body oxygen stores in Harbor Seals: effects of age, mass, and body composition. Physiological and Biochemical Zoology. 78: 1057-1068

CASTELLINI, J.M, H.J. MEISELMAN, AND M.A. CASTELLINI. 1996. Understanding and interpreting hematocrit measurements in pinnipeds. Marine Mammal Science. 12: 251-264

DIERAUF, L.A. AND F.M.D. GULLAND. 2001. CRC Handbook of Marine Mammal Medicine, Second Edition. Boca Raton.

*EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL, 2001. 2001 Status Report: Alaska Department of Fish and Game. Anchorage, AK.

FADELY, B. S., J. M. CASTELLINI, AND M. A. CASTELLINI. 1998. Exxon Valdez oil spill restoration project final report; Recovery of harbor seals from EVOS: Condition and Health Status.

LANDER, M. E., J. T. HARVEY, AND F. M. D. GULLAND. 2003. Hematology and serum chemistry comparisons between free-ranging and rehabilitated harbor seal (*Phoca vitulina richardsi*) pups. Journal of Wildlife Diseases 39: 600-609

MAZARRO, L.M., J.L. DUNN, H.C. FURR AND R.M. CLARK. 1995. Study of vitamin A supplementation in captive northern fur seals and its effect on serum vitamin E. Marine Mammal Science, 11: 545-553

MCCONNELL, L.C. AND R.W. VAUGHN. 1983. Some blood values in captive and free-living common seals (*Phoca vitulina*). Aquatic Mammals. 10: 9-13

MO, G. AND C. GILI. 2000. Do photoperiod and temperature influence the molt cycle of *Phoca vitulina* in captivity? Marine Mammal Science. 16: 570-577

OKI, C. AND S. ATKINSON. 2004. Diurnal patterns of cortisol and thyroid hormones in the Harbor seal (Phoca vitulina) during summer and winter months. General and Comparative Endocrinology.

ROSEN, D.A.S. AND D. RENOUF. 1998. Correlates of seasonal changes in metabolism in Atlantic harbour seals (*Phoca vitulina concolor*). Canadian Journal of Zoology. 76: 1520-1528

SCHALM, O.W. 1965. Veterinary hematology. Lea and Febiger, Philadelphia, PA.

SCHWEIGERT, F.J. 1993. Effects of fasting and lactation on blood seal chemistry and urine composition in the grey seal (*Halichoerus grypus*). Comparative Biochemistry and Physiology. 105: 353-357

THOMPSON, P.M., D.J. TOLLIT, H.M. CORPE, *et al.* 1997. Changes in haematological parameters in relation to prey switching in a wild population of harbor seals. Functional Ecology. 11: 743-750

TRUMBLE, S. J., AND M. A. CASTELLINI. 2002. Blood chemistry, hematology, and morphology of wild harbor seal pups in Alaska. Journal of Wildlife Management 66:1197-1207

TRUMBLE, S. J. AND M. A. CASTELLINI. 2001. Blood chemistry and morphometric comparisons between harbor seal pups from Tugidak Island and within Prince William Sound, Alaska: using cluster analysis to assess health status. Alaska Department of Fish and Game.

WILLIAMS, E.E., B.S. STEWART, C.A. BEAUCHAT *et al.* 2001. Hydrostatic-pressure and temperature effects on the molecular order of erythrocyte membranes from deep-, shallow-, and non-diving mammals. Canadian Journal of Zoology. 79: 888-894

LAMMI, J. 2001. Online Photoperiod Calculator. http://www.saunalahti.fi/~benefon/sol.html. January 16, 2006.

National Climatic Data Center. http://www1.ncdc.noaa.gov/pub/orders/637828799259dat.html April 15, 2006.

US Naval Observatory. http://aa.usno.navy.mil/ April 4, 2006.