Exxon Valdez Oil Spill
Restoration Project Annual Report

Harbor Seal Recovery: Application of New Technologies for Monitoring Health

Restoration Project 01558
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: Harbor seals were one of the resources that were injured by the 1989 *Exxon Valdez* oil spill (EVOS). To date this species is listed as 'not recovering'. Several studies have focused on the general health and metabolism of these seals as it relates to their diet, body condition and habitat (Restoration Projects xx001, xx341, xx371, and xx441). The present study complements these investigations as it is utilizing new techniques to enhance our understanding of the health and physiology of the species and incorporate the possible effects of environmental organochlorine contaminants. If the techniques can be combined to develop a concise indicator of a given animal's health, then these techniques should be incorporated into the routine assessment and monitoring of harbor seals in the Gulf of Alaska.

Abstract: This project is investigating the potential for new technologies to assess and monitor the endocrine and immune systems as diagnostic measures of the health of harbor seals. Analysis of thyroxine (T_4), triiodothyronine (T_3), and cortisol (primary metabolic and gluconeogenic hormones), and measurement of immunoglobulins (IgG, IgM, and IgA) and the body burden of organochlorine contaminants will provide a health assessment in both permanently captive seals as well as seals that are brought into the Alaska SeaLife Center for rehabilitation. This report covers the analysis of circadian patterns of cortisol and thyroid hormones from captive harbor seals at the Alaska SeaLife Center (ASLC), as well as from harbor seals admitted for rehabilitation.

Key Words: Circadian patterns, cortisol, harbor seal, immunoglobulins, *Phoca vitulina*, thyroid hormones, thyroxine, triiodothyronine.

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

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Executive Summary

The population of the Alaskan harbor seal has been declining over the last two to three decades. The purpose of this research project was to develop a suite of physiological parameters that may be used to monitor the overall health of free- ranging harbor seals. In the first year of this study, baseline data that reflect seasonal changes needed to be obtained. The suite of parameters that were chosen were the metabolic hormones, cortisol and total and free thyroxine (T_4) and triiodothyronine (T_3), as well as morphometric measurements. The first year focused on determining whether circadian patterns of cortisol and total and free T_4 and T_3 were present during the summer and winter seasons, and identifying how they might alter metabolic rate and/or maintenance of body reserves.

This study was carried out at the Alaska SeaLife Center in Seward, Alaska (60 N. latitude, 149 W. longitude) in June of 2000 and January of 2001. Blood samples were obtained every two to three hours over a twenty four period through in-dwelling catheters inserted the day before sampling. The hormones were analyzed using radioimmunoassays in the Endocrinology Lab at the Alaska SeaLife Center. The presence of a diurnal rhythm was determined comparing levels during *ante meridian* (12am –noon) and *post meridian* (noon to 12 midnight) time periods.

Cortisol levels were not different ($P \geq 0.1$) between the summer and winter, but cortisol displayed a circadian rhythm only during the summer. Mean levels of cortisol (51.5 ng/ml $\pm$ 20.3) in the summer hours of *ante meridian* were differed ($P \leq 0.05$) from levels in the hours of *post meridian* (28.5 ng/ml$\pm$17.4). Neither total and free T_4, nor T_3, displayed a diurnal rhythm in either season. However, total T_4, total T_3, and free T_3 levels were higher in the winter (total T_4, $P \leq 0.05$; total T_3, $P \leq 0.05$; free T_3, $P \leq 0.1$) than in the summer. There was no seasonal effect on free T_4 levels ($P \geq 0.1$).

The absence of a circadian rhythm of cortisol during the winter may have been a result of the limited amount of daylight during the winter season as well as the continued need to produce metabolic heat through gluconeogenesis. Higher levels of thyroid hormones in the winter indicate an adaptive mechanism to cope with the low temperatures of winter. In addition to the baseline data from captive seals, samples were collected from harbor seals that were admitted to rehabilitation at both Alaska SeaLife Center and The Marine Mammal Center (Sausalito, CA). Preliminary data suggest that cortisol is higher pre-weaning in seals that were successfully rehabilitated and compared to those seals that died during the rehabilitation process. These analyses are continuing in year two.

Introduction

The population of *Phoca vitulina richardsi*, the subspecies found in the eastern Pacific from Baja California, Mexico to Alaska, is estimated to be at 140,000 (Angliss *et al.*, 2001, Barlow *et al.*, 2001). Since the mid 1970's the population of the harbor seal in the Gulf of Alaska at Prince William Sound has continued to significantly decline. At Tugidak Island, near Kodiak, the Alaskan harbor seal population decreased by 90% since the mid 1970's (Kinkhart *et al.*, 1994). Exact causes of their decline are unknown; however, diseases, environmental instability, commercial fishing, and reduced prey availability have been suspected. The *Exxon Valdez* oil spill in Prince William Sound in 1989 also impacted the seal population; and the harbor seal is

still considered an injured species that has yet made little or no progress towards recovery over ten years after the incident (*Exxon Valdez* Oil spill Trustee Council, 2001). Because of the decline of the population of the harbor seal, it is important that we understand their physiological status and how they maintain their body reserves and regulate their metabolism during the different seasons. These physiological parameters likely contribute to the survival of individual seals during unfavorable environmental conditions.

The purpose of this research project is to develop a suite of physiological parameters to use in assessing the overall health of a given animal or subpopulation. To do this, baseline concentrations of cortisol and thyroid hormones must be measured and the presence of circadian patterns of cortisol, and total and free T_4 and T_3 in the harbor seal during the summer and winter seasons must be determined. Once the baselines have been identified for a group of captive harbor seals in a controlled environment, then these conclusions can be compared to those in seals admitted for rehabilitation. There have been few studies measuring the diurnal rhythm of hormones in seals through longitudinal sampling of individuals. Measuring these specific hormones in both summer and winter seasons in Alaska will also show how these seals physiologically adapt to extreme changes in their environment. Alaska has long days and short nights in the summer months, while *vice versa* in the winter months; therefore if circadian patterns are shown to be present, we could determine how seasons and photoperiod may alter the levels or secretion patterns of each hormone. Understanding the daily pattern of these hormones will enable the comparison of, and assess the metabolic state and overall well-being of, wild or rehabilitated harbor seals.

Neonatal marine mammals are more inclined to suffer from immunosuppression due to abandonment, malnourishment and anthropogenic contaminants. A combination of these factors can lead to young animals that fail to thrive in their natural environment. Analysis of hormone concentrations and body condition via morphometrics can give an indication of an animal's physiology. Hormones such as cortisol and thyroxine, can cause changes in metabolic rate, calcium absorption and blood pressure control. Immunocompetence in an individual animal or species is a complex series of metabolic and physiological parameters. It is dependent on environmental, nutritional and endocrinological factors. Hormones levels are one of the parameters that can be measured in an animal to produce a history of some of the above mentioned factors. Hormones, such as cortisol (an adrenal hormone) and the (thyroid hormones), are good indicators of metabolic demands or stress on an animal's physiological system. Spikes or depressions of these metabolic hormones can aid in determining the presence of a stressor in an animal's environment.

Objectives

The overall goal of this project is to develop and test new methods of monitoring the overall health and physiology of harbor seals. In doing so, the project has the following objectives:

1. Determine seasonal and circadian patterns of total and free triiodothyronine (T_3), thyroxine (T_4), and cortisol in healthy captive harbor seals (Yr 1).
2. Develop new antibodies specific to harbor seal immunoglobulin classes IgG, IgM and IgA (Yr 1).

3. Determine seasonal patterns of IgG, IgM, and IgA, in healthy captive harbor seals (Yrs 1 and 2).
4. Begin initial assessments in harbor seals admitted for rehabilitation (years 1 and 2).

Methods

Objective 1. Seven harbor seals (3 males, 4 females) housed at the ASLC had monthly blood samples collected to assay for total and free T_4, T_3, and cortisol. In addition, circadian patterns of these hormones had assessed from the five seals (2males and 3 females) during the seasonal extremes of the summer and winter solstices, with samples collected at 2 to 3 hourly intervals over a 24 hour period.

The analyses for these hormones have previously been validated for other pinniped species (Atkinson and Oki, 2002) and were validated for harbor seals during this project (Oki, 2001). Concentrations of cortisol were measured in unextracted plasma using a single-antibody radioimmunoassay (Atkinson and Oki, 2002; Atkinson and Adams, 1988). Samples were analyzed in batches to reduce inter-assay variation. Concentrations of total and free T_4 and T_3 were measured in unextracted plasma using solid phase radioimmunoassays (Diagnostic Products Corporation, Los Angeles, CA) that were specific to either total or free, T_4 or T_3. The standard curves of each assay were log-logit transformed, enabling extrapolation of sample concentration (Robard, 1974). Profiles of the variation in cortisol and total and free T_4 and T_3 were plotted against time.

Objective 2. The prerequisite for development of heavy chain specific antisera for the major immunoglobulin classes of the harbor seal is the production of purified preparations of each of these immunoglobulin classes. These purified immunoglobulin classes are being obtained from pooled sera from captive animals at ASLC and are being used as the source of the immunoglobulins to be purified. The first step toward purification of individual immunoglobulin isotypes (IgG, IgM, and IgA) from serum is to remove non-immunoglobulin proteins, leaving a mixture of all immunoglobulin isotypes present. Since the immunoglobulin isotypes being studied have molecular weights greater than 100,000 daltons, they will be retained after filtration, which has proven more satisfactory than other techniques involving differential precipitation of serum proteins. Antisera will be produced in rabbits against the precipitated immunoglobulins to permit preliminary analysis of the IgG, IgM, and IgA immunoglobulins in harbor seal serum. The titer and specificity of the antisera will be determined by (1) standard indirect ELISA (wells coated with purified harbor seal immunoglobulin heavy chain), followed by the rabbit anti-heavy chain antibody being tested, followed by enzyme-labeled anti-rabbit immunoglobulin, and finally by the indicator substrate) and (2) immunoelectrophoresis (IEP) methods including Grabar-Williams, Rocket IEP, Crossed IEP , and Tandem Crossed IEP. The antisera will be partially purified by use of the Millipore UltraFree®-15 centrifugal filter device followed by purification by Protein G Sepharose^R affinity chromatography to obtain the IgG fraction of this rabbit antisera. The purified antisera will be labeled with biotin or an enzyme (e.g. alkaline phosphatase or horseradish peroxidase) using standard labeling linkers (Pierce). The resulting antisera will be analyzed for specificity by several methods, including application of the antisera to Western blots of whole heavy chain preparations obtained by reduction/alkylation of the respective whole immunoglobulin isotype preparations. The sera to

develop this ELISA has been obtained and purified. The antibodies are currently being developed.

Objective 3. Once the antisera for each immunoglobulin's heavy chain isotype has been made, it will be possible to regularly monitor immunoglobulin levels as an indicator of immune status of a population of harbor seals. Monthly samples from captive harbor seals at Alaska SeaLife Center have been collected and are awaiting analysis for the immunoglobulins.

Objective 4. Harbor seal pups from both Alaska SeaLife Center and the Marine Mammal Center (Sausalito, California) had blood samples collected every 10 to 14 days as part of the routine veterinary care of the seals admitted to rehabilitation. All Marine Mammal Center samples were collected under the direction of Dr. Frances Gulland, DVM. Validation of the assays had been made prior to this using the captive harbor seals housed at the Alaska SeaLife Center. These seals were also sampled monthly and pools of sera have been run with the pup serum to act as control pools.

Results

Sex, age, winter and summer weights and blubber depths are presented in Table 1. Summer and winter seasons did not have a significant effect on mean cortisol levels ($P \geq 0.1$, Table 2) nor was there a significant difference in concentrations within the winter season ($P \geq 0.1$, Fig. 1). Mean cortisol levels in the hours of 12 am to noon during the winter was 51.6 ng/ml ± 14.3 while concentrations from noon to midnight in the winter season was 46.7 ng/ml ± 16.3. However, a diurnal rhythm was shown to be present in the summer season as mean concentrations in the hours of *ante meridian* (51.5 ng/ml ± 20.3) were higher ($P \leq 0.05$) than concentrations in the hours of *post meridian* (28.5 ng/ml ± 17.4). Concentrations in the *post meridian* of the summer days were significantly lower as levels dropped during the time block of 12 pm to 6 pm (Fig. 1). During the summer, each seal (n=5) showed similar diurnal rhythms of cortisol (Fig. 2) while each had different rhythms in the winter season (Fig .3).

Total T_4, total T_3 and free T_3 levels were higher in the winter season (total T_4, $P \leq 0.05$, Fig. 4; total T_3, $P \leq 0.05$, Fig 6; free T_3, $P \leq 0.1$, Fig.7) than in the summer season. However, there was no seasonal effect on free T_4 levels ($P \geq 0.1$, Fig.5). Mean thyroid concentrations in each season are shown in Table 2. Samples collected in *ante meridian* and *post meridian* within each of the seasons were not different from each other for any of the thyroid hormones.

Each seal (n=5) gained a significant amount of blubber thickness at each body region during the winter (axillary, $P \leq 0.05$; mid-section, $P \leq 0.1$; hip-section, $P \leq 0.05$). There was also an increase in weight in the winter but it was not a significant gain ($P \geq 0.1$). There was a significant correlation with summer total T_4 levels with summer axillary blubber measurements ($r = 0.8158$, $P \leq 0.05$). Winter total T_4 and winter free T_4 levels both correlated with weight (total T_4: $r = 0.9540$, $P \leq 0.05$; free T_4: $r = 0.9097$, $P \leq 0.05$) and axillary blubber measurements (total T_4: $r = 0.8734$, $P \leq 0.05$; free T_4: $r = 0.9422$, $P \leq 0.05$) in the winter. Free T_3 levels in the summer correlated with summer blubber measurements at the mid-section ($r = 0.9024$, $P \leq 0.05$) while winter free T_3 levels showed a significant correlation with weight ($r = 0.8917$, $P \leq 0.05$), axillary blubber ($r = 0.9099$, $P \leq 0.05$), and mid-section blubber ($r = 0.8146$, $P \leq 0.05$). Winter

free T_3 levels also tended to be positively correlated with winter blubber measurements at the hip section ($r=0.7459$, $P \geq 0.05$).
The average daily gross energy intake of herring and pollock was greater for four (PO,PE,SK,SN) of the five seals during the winter months of our study (Table 3). However, seal SY had a higher daily gross energy intake in the summer, despite an increase in weight and blubber thickness in the winter. Seal SY had an average daily gross intake of 3.48 Mcal/d in the summer month compared to 1.87 Mcal/d in the winter month. The amount of total feed intake, in kilograms, by SY in the winter was approximately 52% less than what was consumed in the summer, although the proportions (70:30 of pollock and herring were similar.)

Air temperature in Seward on the days blood samplings occurred was averaged for each season. The average temperature on June 22nd and 23rd was 11.1° Celsius and the winter temperature, average in January 3rd to the 6th, was -1.1° Celsius. During the summer study, we had an average of 55% light that the photo cell read at 445 ohms; and during the winter, we had an average of 14% light that the photo cell read at 847 ohms. Light intensity during the summer remained particularly high between the periods of 7am and 7pm at approximately 67% light, while winter light intensity remained at its highest of 41% light between the time period of 12pm to 3pm. Samples are collected from harbor seals admitted for rehabilitation at the Alaska SeaLife Center and the Marine Mammal Center, (Sausalito, California). As these were typically neonatal seals, limited volumes of samples were obtained, and the decision was made to only analyze for total T_4 and cortisol. Preliminary results suggest that cortisol is higher pre-weaning in seals that were successfully rehabilitated and released as compared to seals who, were not released (died during rehabilitation), and whose cortisol concentrations remained high after weaning (Table 4). Analysis of the effects of weaning on total T_4 and cortisol are still being completed.

Discussion

The first year of this study resulted in significantly lower cortisol concentrations in harbor seals during the afternoon, however only in the summer season. Cortisol did not show a diurnal rhythm in the winter, indicating that photoperiod may play a role in regulating the hormonal rhythm. The higher intensity and longer duration of daylight during the summer may serve as the environmental cue for the suprachiasmatic nuclei (SCN) of the anterior hypothalamus, which controls the circadian rhythm of hormones. A study by Vondrasaova-Jelinkova *et.al.* (1999) suggested that the bright sunlight in the morning hours may be a signal that entrains the cortisol rhythm in humans. In humans, a desynchronization of biological rhythms can occur if they live in latitudes above 37° N and are not exposed to 25000 lux of light during winter seasons (Nelson, 1995). During the summer study, there was a longer duration and higher light intensity compared to the winter. The low intensity and shorter duration of daylight during the winter season may have been insufficient in entraining the seal's biological clock, and cortisol levels became free-running in the winter, indicating that it was not synchronized with environmental cues. Cortisol is classified as a glucocorticoid, and a by-product of gluconeogenesis is metabolic energy or heat. One of the functions that cortisol plays is to maintain body temperature in homeotherms as hypothermia can occur if the adrenal gland is removed (Hadley, 1992). Although not significant, cortisol concentrations were slightly higher in the winter season and levels remained steady throughout the 24-hour cycle. Concentrations did not decrease in the afternoon as they did in the summer season. This could indicate that cortisol needed to be

continually produced throughout the day in order to compensate for an overall higher level of stress on the body due to the colder environment in the winter. Air temperature during the winter study was approximately 22 degrees colder than the period of the summer study. Body temperatures in humans are known to follow a circadian rhythm as it peaks in the mid-afternoon (Moore-Ede *et al*, 1982). Assuming this is true in other animals, such as the harbor seal, it may explain the drop in summer cortisol concentrations during the mid-afternoon time period, when body temperatures are rising.

The presence of a diurnal rhythm in thyroid hormones is dependent on the species. In our captive harbor seals, neither total nor free T_4 and T_3 displayed circadian rhythms in either summer or winter seasons. However, seasons did have a significant effect on total T_4, total T_3 and free T_3 levels as mean concentrations were higher in the winter than in the summer. It is known that thyroid hormones are mainly involved in creating metabolic heat for the body in order to maintain a homeothermic state. Thyroid hormones increase the rate of glucose oxidation and thus increase the amount of metabolic heat that is produced. It is thought that the thyroid hormones can uncouple oxidative phosphorylation, which decreases the efficiency of ATP synthesis, allowing the release of more heat. Therefore a reduction in the efficiency of ATP synthesis increases the quantity of heat released per mole of glucose oxidized (Nelson, 1995).Triiodothyronine or T_3 is the physiologically active thyroid hormone, and T_4 is converted to T_3 by the loss of an iodine atom. Although most of the circulating thyroid hormones are bound to transport proteins, it is the free quantities of T_4 and T_3 that carry out their metabolic activity. Our data showed that there was no significant seasonal effect on free T_4 levels and this may have been due to the conversion of T_4 to T_3. Exposure to the cold have been associated with increased rates of deiodination, enhanced biliary excretion of T_4 and T_3 and increased conversion of T_4 to T_3 (Wartofsky and Burman, 1982). Decreased serum concentrations of TSH, T_3 and T_4 have resulted from acute exposure to heat in humans (Feldman and Nelson, 1987). Lower thyroid hormone concentrations during the summer indicate that the environmental temperature is one factor that can alter thyroid levels. In some hibernating animals, thyroid levels are higher in the winter during hibernation, while during the summer, the thyroid ranges from being low to inactive. Lower thyroid levels during the summer prevent overheating due to reduced endogenous heat production (Hudson, 1981). Total and free T_3 concentrations in the winter season of our study were significantly higher possibly due to the demand for elevated concentrations of this metabolically active thyroid hormone. Increased concentrations of T_3 allowed the seals to make the necessary physiological changes in order to effectively adapt to the colder environment.

The seals used in the study all had greater weight and blubber thickness at the auxiliary, mid- and hip-sections of the body in the winter than in the summer. Their blubber layer helps insulate them while in colder temperatures as well as serving as an energy source when fasting may occur. Animals who increase their fat stores seasonally must also decrease their metabolic rate for fat deposition (Hudson, 1981) During times of limited feed intake, metabolism is slower as fat reserves are utilized (Hudson, 1981).

Gross energy composition of the herring and pollock fed to the harbor seals at the Alaska SeaLife Center were analyzed by Castellini *et. al.* (2001) and Bando (unpublished data). This was used to calculate the average daily gross energy intake for both the summer and winter

season. On average, the herring and pollock fed to the seals in the winter season had a greater gross energy value than the summer feed. Four of the five harbor seals (PO, PE, SK, SN) consumed more in the winter season and had a greater average daily caloric intake during the winter. Interestingly, seal SY had a lower gross energy intake during the winter month despite a gain in weight and significant increase in its blubber thickness during that time of season. The average gross energy intake for seal SY in December of 2000 was approximately half of what was consumed during June of 2000, although the proportions of pollock to herring (70:30) were similar for both seasons. In a study by Renouf and Noseworthy (1990), captive harbor seals gained body mass during the winter despite a decrease in their feed intake. However, their increase in weight negatively correlated with water temperature (Renouf and Noseworthy, 1990). Follow-up studies by Renouf and Noseworthy's study (1991), their seals could have gained body mass when they were feeding on herring with a higher fat content, despite a total lower intake of herring. Although this was not expected in harbor seals, some hibernating animals will display similar physiological changes as they may continue to gain body mass even though their feed intake is decreased and food is not stored for the hibernation season (Musacchia and Deavers, 1981).

Measurements of free T_4 levels in Renouf and Noseworthy's (1991) male captive harbor seals were found to be significantly lower in the winter as feed intake decreased and blubber mass increased. However, in our harbor seals, total T_4, total T_3 and free T_3 levels were higher in the winter than in the summer, and there was no seasonal effect on free T_4 levels. For seal SY, our findings of higher thyroid levels in the winter is unusual considering the decrease in that particular seal's feed intake and its increased blubber layer and weight. Using these captive animals, food resources were adequate and the seals lived in stable conditions. Therefore, these captive seals may not have had to decrease their activity levels and depend on their fat for energy since there was not a limited supply. Increased blubber thickness in the winter may have been sufficient to maintain a homoethermic state and higher thyroid levels could have been associated with other functions such as protein and lipid deposition, molting, or growth and development.

Many factors can play a role in the secretion pattern and levels of hormones, such as environment, feed intake, and required metabolic energy. Each factor helps signal the animal's body to either release more or less of a hormone in order to compensate for external and internal effects that cause some physiological change. This study can be further investigated by monitoring other physiological mechanisms such as body temperature and respiration rate to understand how they might coordinate with hormone levels. These harbor seals used in this study were accustomed to a daily pattern of feeding and training and the activity level of these seals remained steady throughout the year. The data could lead to future studies that compare wild and rehabilitated seals to our captive data, helping us to understand the physiology of adult animals belonging to declining populations.

The immune system of marine vertebrates is a rapidly advancing area of study, both in the basic structure and immunodiagnostic reagents. Baseline information on the immune system of pinnipeds in general and rehabilitation animals in particular is critical to the long-term survival of treated animals. Presumably, animals are nutritionally and physiologically stressed when admitted for rehabilitation. Elevation of stress to a chronic level may occur as a consequence of rehabilitation techniques required to ensure the survival of the animal (Gulland et al., 1999). The resultant immunosuppressive effects may leave the animal vulnerable to disease. Existing data

for pinnipeds suggest that chronically elevated corticoid levels suppress the production of immunoglobulins by the B cells (Munck and Crabtree, 1981) and increase the production of immunosuppressive cytokines (Goodman, 1998; Dandona et al, 2001). A further consequence of chronic corticoid stimulation is metabolic compromise: loss of bone density, wasting of muscle, growth impairment and diabetes (Ezrin et al., 1973.). Baseline information on the immune system of pinniped species is critical to any future field assessment of immunocompetence. The lack of baseline immune system information of the harbor seal population in Europe hindered the assessment of the role of immunosuppression in the phocid distemper virus outbreak of 1988 (Dietz et al., 1989a). Studies of levels of immunoglobulins and of isotypes of those immunoglobulins have been reported for a few species of pinnipeds (northern fur seals: Cavagnolo and Vedros, 1979; grey seals: Baker, 1984; grey seals: Carter et al., 1990; harbor seals: Ross et al., 1993.) However, accuracy of immunoglobulin measurement can depend on species specificity of anti-bodies used in the assay systems and such antisera are not readily available for most species of pinnipeds.

Conclusions

1. Environmental cues, such as light intensity and air temperature, may be the signal for the presence of a diurnal rhythm of cortisol.
2. Thyroid hormones did not display a circadian rhythm in the captive harbor seals for both seasons.
3. Total T_4, Total T_3, and free T_3 concentrations were significantly higher in the winter months, which allow the seals to metabolically adapt to the colder temperatures.
4. Feed intake, blubber thickness, and weight gain are factors that play a significant role in the endocrine concentrations of the harbor seal.
5. Measurement of cortisol and thyroid hormones in both the summer and winter seasons in Alaska show how these seals physiologically adapt to extreme changes in their environment.

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Mrs. Danielle O'Neil is currently undertaking her Master's Degree at the University of Alaska Fairbanks, on the rehabilitation portion of this project. Samples for her work are being provided by the Alaska SeaLife Center and the Marine Mammal Center in California.

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Literature Cited

Angliss, R.P., Lopez, A., and DeMaster, D.P. 2001 *Draft Alaska Marine Mammal Stock Assessments*: 2001. National Marine Mammal Laboratory, Alaska Fisheries Science Center Seattle, WA.

Atkinson, S. and Adams, N.R., 1988 Adrenal glands alter the concentration of oestradiol – 17B and its receptor in the uterus of ovariectomized ewes. J. Endocrinol., 118: 375-380.

Atkinson S. and Oki, C. 2002. Body condition, cortisol and thyroxine concentrations in juvenile Hawaiian monk seals from a changing ecosystem. Comp. Biochem. Physiol. Accepted.

Baker, J.R. 1984. Mortality and morbidity in grey seal pups (*Halichoreus grypus*). Studies on it's causes, effects of environment, the natural sources of infectious agents, and the immunological status of pups. J. Zool. Lond. 203:23-48.

Bando, M. Unpublished data. Proximate compositions of steller sea lion prey items. M.S. Thesis. Alaska SeaLife Center, Seward, AK. and University of Alaska, Fairbanks, AK.

Barlow, J., Forney, K.A., Carretta, J.V., Muto, M.M., and Baker, J. 2001. Draft U.S. *Pacific Marine Mammal Stock Assessment*: 2001. U.S. Department of Commerce, NOAA, NMFS, Southwest Fisheries Science Center.

Carter, S.D., D.E. Hughes, and J.R. Baker. 1990. Characterization and measurement of immunoglobulin in the grey seal (Halichoreus grypus) J. Comp. Path. 102:13-23

Castellini, M.A., Castellini, J.M., and Trumble, S.J. 2001. Recovery of harbor seals. Phase II: Controlled studies of health and diet, *Exxon Valdez* oil spill restoration project final report (Restoration Project 01341), Alaska Department of Fish and Game, Habitat and Restoration Division, Anchorage, Alaska.

Cavagnolo, R.Z. and N.A. Vedros. 1979. Serum and colostrums immunoglobulin levels in the northern seal *Callorhinus ursinus*. Dev. Comp. Immunol. 3:139-146.

Dietz, R., Hansen, C.T., Have, P., Heide-Jorgensen, M-P 1989a. Clue to seal epizootic? Nature 338:627.

Exxon Valdez Oil Spill Trustee Council. 2001 Status Report. Alaska Department of Fish and Game. Anchorage, AK.

Ezrin, C., Godden, J.O., Volpe, R., Wilson, R. eds. 1973. "Systematic Endocrinology". pp 171. Harper and Row, New York, NY.

Feldman, E. and Nelson, R. Canine and Feline Endocrinology and Reproduction. 1987. Philadelphia, W.B. Saunders Company. p. 69-70.

Goodman, H.M., 1998. Adrenal glands. pp 537-563 in L.R. Johnson, ed. Essential medical physiology. 2nd edition. Lippencott-Raven, Philadelphia, PA.

Gulland, F.M.D., Lowenstine, L.J., Munro, C., Graham, P.A., Bauman, J., Harvey, J. 1999. Adrenal function in wild and rehabilitated Pacific harbor seals (*Phoca vitulina richardinii*) and in seals with phocine herpesvirus-associated adrenal necrosis. Mar. Mamm. Sci. 17: 835-861.

Hadley, M. Endocrinology. 1992. Prentice Hall. Englewood Cliffs, New Jersey. pp. 143,334-361,391-429.

Hudson, J.W. 1981 Role of the endocrine glands in hibernation with special reference to the thyroid gland. In: X.J. Musacchia and L. Jansky. *Survival in the Cold: Hibernation and Other Adaptations. Proceedings of the international Symposium for Survival in the Cold, Prague, Czecholosovakia, July* 2-5, 1980. Elsevier North Holland, Inc. New York, NY. pp. 33-54.

Kinkhart, E. and Pitcher, K. 1994. Alaska Department of Fish and Game Wildlife Notebook Series: Harbor Seal.

Musacchia, X.J. and Deavers, D.R. 1981. The regulation of carbohydrate metabolism in hibernators. In: X. J. Masacchia and L. Jansy. *Survival in the Cold: Hibernation and Other Adaptations. Proceedings of the International Symposium for Survival in the Cold, Prague Chechoslovakia*, July 2-5, 1980. Elsevier north Holland, Inc., New York, NY. pp 55-75.

Moore-Ede, M.C., Sulzman, F.M., and Fuller, C.A. 1982. *The Clocks That Time Us*. Harvard University Press, Cambridge, MA.

Muck, A., Crabree, G.R. 1981 Glucocorticoid-induced lymphocyte death. In "Cell Death in Biology and Pathology" (I.D. Brower and R.A. lockshin, eds.), pp. 329-357. Chapman and hall, London and New York.

Nelson, R.J. 1995. An Introduction to Behavioral Endocrinology. Sinauer Associates, Inc. Massachusetts. pp. 65-68, 337-442.

Oki, C.E. 2001. Cortisol and thyroid hormones secretion, patterns and concentrations in the harbor seal (Phoca Vitulina) in summer and winter seasons. Masters Thesis animal Sciences Department, University of Hawaii. December 2001.

Renouf, D. and Noseworthy, E. 1990. Feeding cycles in harbor seals: weight gain in spite of reduced food intake and increased thermal stress. *Mar. Begav, Physiol.* 17:203-212.

Renouf, D. and Noseworthy, E. 1991. Changes in food intake, mass, and fat accumulation in association with variations in thyroid hormone levels of harbor seals (*Phoca vitulina). Can. J. Zool.* 69(9): 2470-2479.

Rodbard, D. 1974. Statistical quality control and routine data processing for radioimmunoassay and immunoradiometric assays. *Clin. Chem.* 20: 1255-1270.

Vondrasova-Jelinkova, D., Hajek, I., Illnervova, H. 1999. Adjustment of the human melatonin and cortisol rhythms to shortening of the natural summer photoperiod. *Brain Res.* 816: 249-253.

Wartofsky, L. and Burman, K.D. 1982 Alterations in thyroid function in patients with systemic illness: the "euthyroid sick syndrome". *Endocrinol Rev.* 3: 164-217.

Tables

Table 1. Sex, age, mean (± s.e.) seasonal weight, and blubber measurements (taken during week of June 19-25, 2000 and January 1-7, 2001), of five harbor seals used in the study.

SEAL ID	Sex	Age (yrs)	Weight (kg)		Blubber Axillary (mm)		Blubber Mid (mm)		Blubber Hip (mm)	
		*	*Summer*	*Winter*	*Summer*	*Winter*	*Summer*	*Winter*	*Summer*	*Winter*
SK	Female	25	60.5 ± 0.0	81.0 ± 0.0	16.0	30.0	22.0	33.0	21.0	38.0
PO	Female	5	56.6 ± 1.0	70.5 ± 0.0	16.0	23.0	20.5	29.0	18.0	28.0
SY	Female	4	45.8 ± 0.4	55.3 ± 1.8	17.0	25.0	22.0	31.0	23.0	34.0
SN	Male	16	84.0 ± 0.0	95.5 ± 0.0	20.0	33.0	21.0	30.0	22.0	34.0
PE	Male	4	42.9 ± 0.1	56.0 ± 0.0	12.0	18.0	13.0	19.0	15.0	23.0

* Approximate age in year of 2000

Table 2. Comparison of mean hormone levels in summer and winter season (Mean ± S.E.)

	SUMMER	*WINTER*	*P-value*
Cortisol (ng/ml)	41.4 ± 22.2	49.4 ± 15.3	> 0.1
Total T_4 (ng/ml)	5.2 ± 4.1	8.5 ± 4.2	< 0.05
Free T_4 (pg/ml)	1.9 ± 0.9	1.9 ± 1.1	> 0.1
Total T_3 (ng/ml)	0.37 ± 0.06	0.55 ± 0.07	< 0.05
Free T_3 (pg/ml)	0.25 ± 0.18	0.63 ± 0.24	< 0.1

Table 3. Monthly feed intake and average daily gross energy intake of herring and Pollock in June (summer) and December (winter) 2000.

SEAL ID	**Total Pollock Consumed (kg)**		**Total Herring Consumed (kg)**		**Average Daily Gross Energy Intake (Mcal/day wet basis)**	
	Summer	***Winter***	***Summer***	***Winter***	***Summer***	***Winter***
PO	17.90	16.50	45.00	53.10	3.88	5.10
PE	52.60	20.60	21.60	60.30	3.50	5.87
SK	19.10	15.50	38.60	43.70	3.47	4.28
SN	42.40	40.00	43.00	40.20	4.65	5.02
SY	50.90	26.10	22.20	9.20	3.48	1.87

Table 4. Mean (+/- SE) thyroxine, cortisol, weight and length in harbor seal pup serum

		TT4 (µg/dL)		**Cortisol (µg/dL)**		**Weight (kg)**		**Length (cm)**	
	n	**mean**	**SE**	**mean**	**SE**	**mean**	**SE**	**mean**	**SE**
Pre-wean released	16	4.60	0.64	15.13	2.59	10.54	0.63	75.53	1.33
Post-wean released	16	3.61	0.30	9.80	1.78	19.02	1.19	81.87	1.27
Pre-wean non-released	3	5.44	1.27	18.27	5.81	11.85	2.11	73.60	7.86
Post-wean non released	3	3.45	0.82	13.70	10.91	21.89	2.98	85.13	3.45

Figures

Figure 1. Mean cortisol levels during summer (○) and winter season (□)

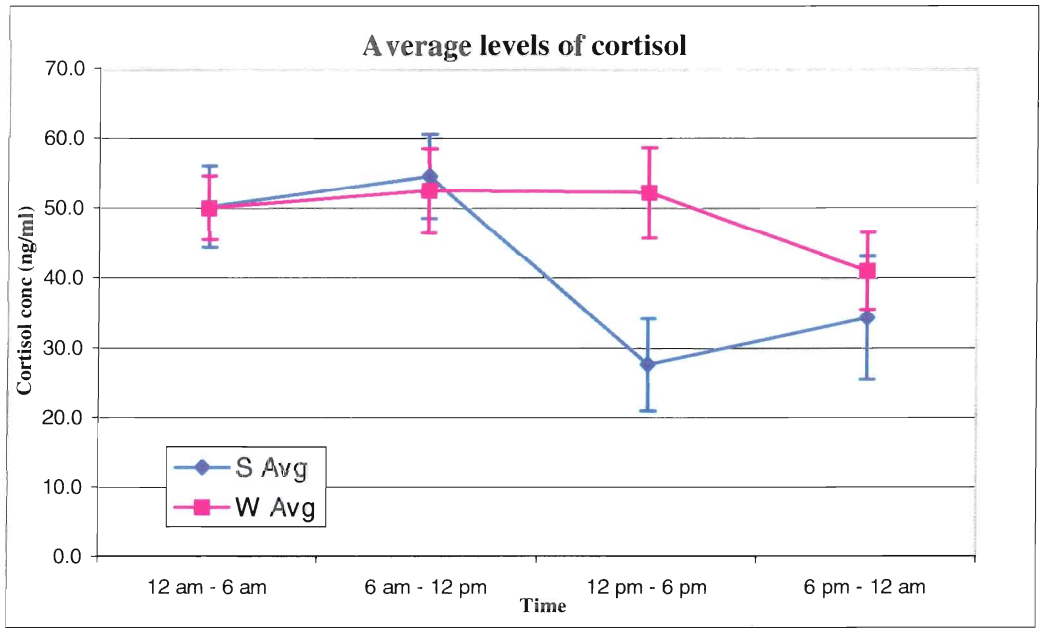

Figure 2. Cortisol levels of each seal (n=5) during summer season

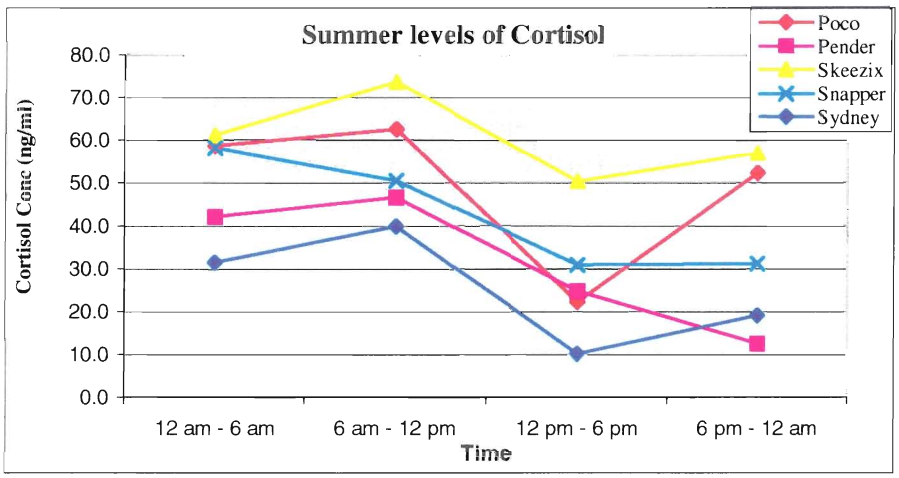

Figure 3. Cortisol levels of each seal (n=5) during winter season

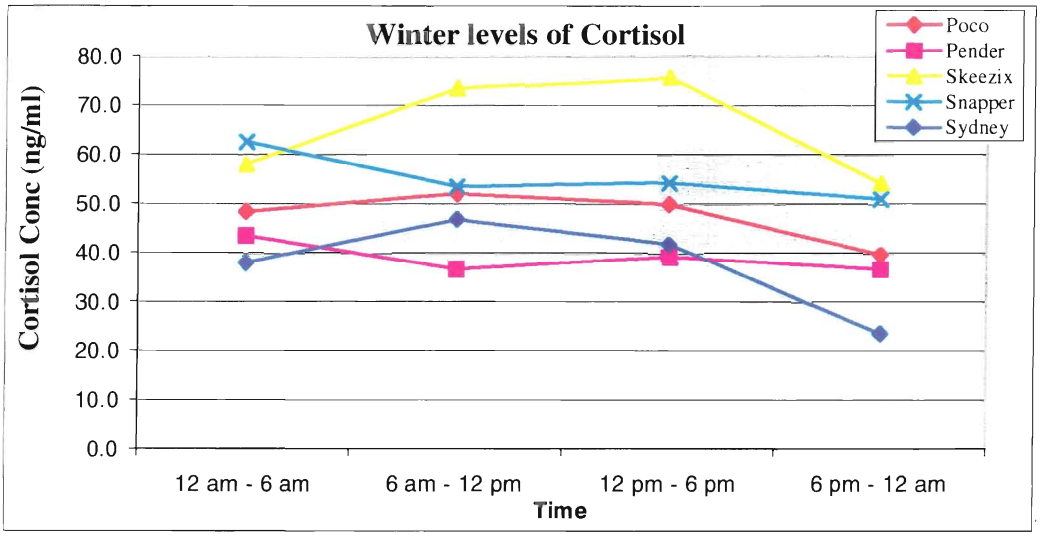

Figure 4. Mean Total T_4 levels during summer (○) and winter season (□)

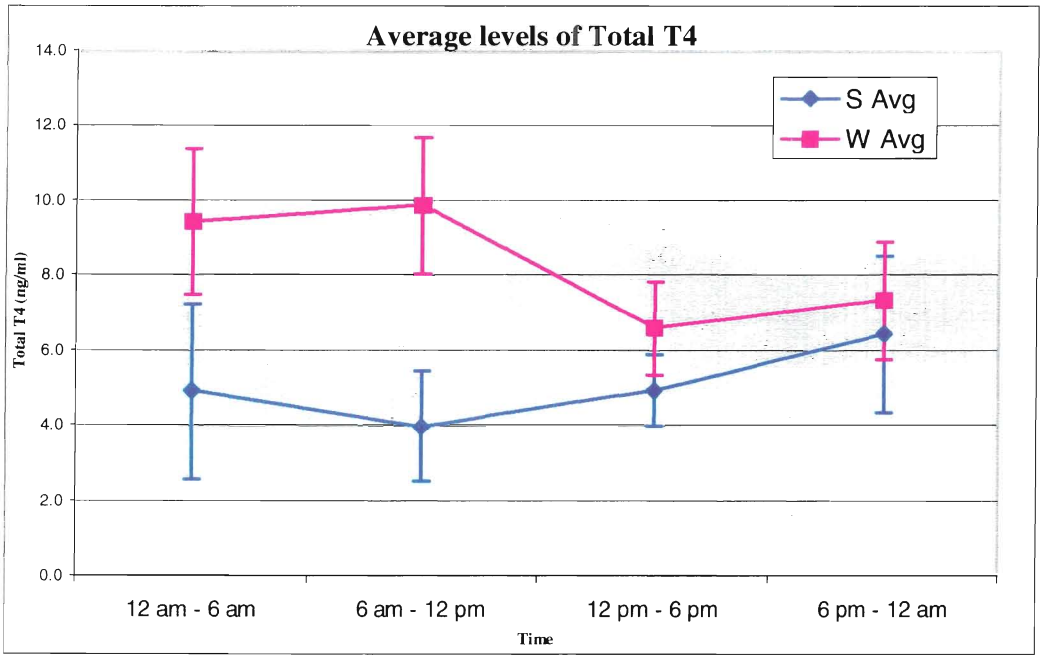

Figure 5. Mean Free T_4 levels during summer (○) and winter season (□)

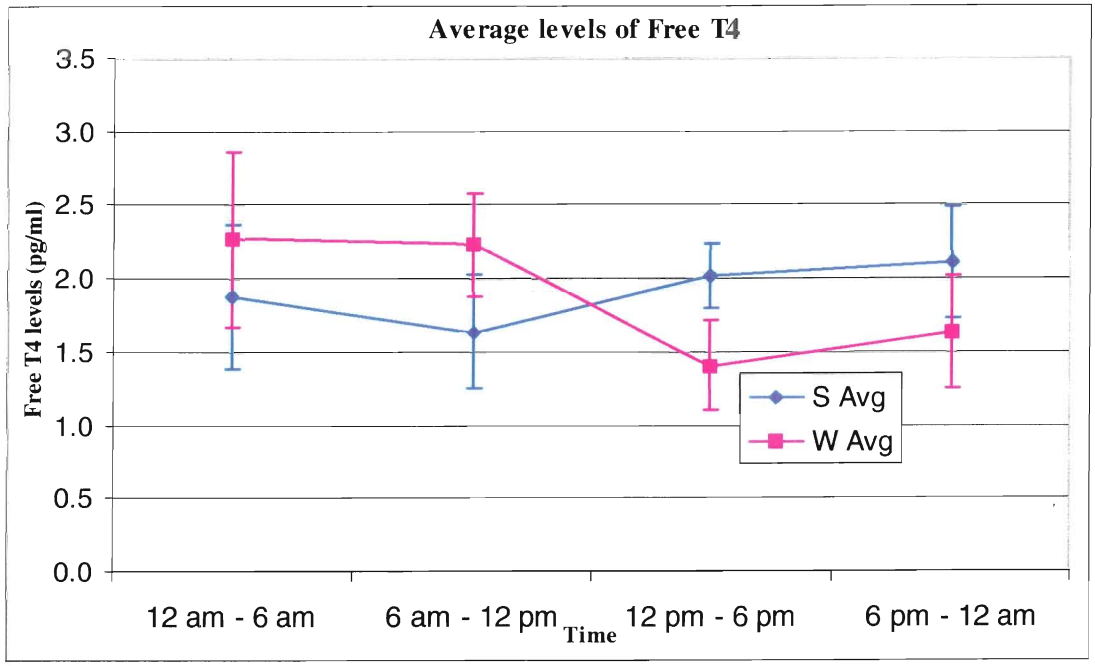

Figure 6. Mean Total T_3 levels during summer (○) and winter season (□)

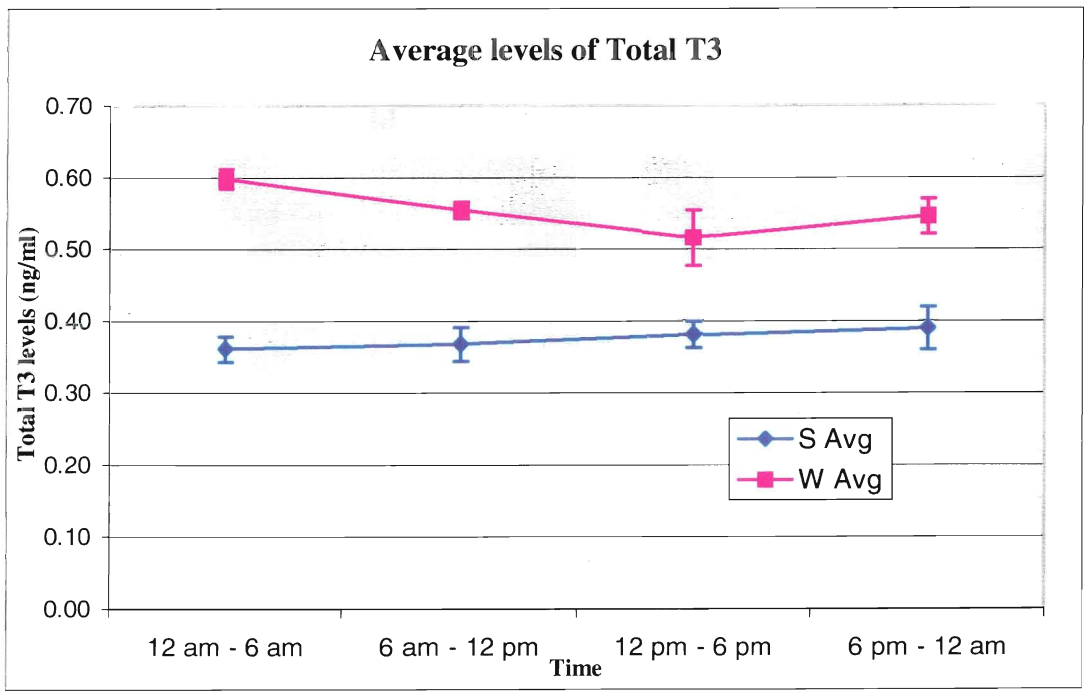

Figure 7. Mean Free T_3 levels during summer (○) and winter season (□)