Impact of Health and Maternal Investment on Survival of Endangered Steller Sea Lion Pups

Pollock Conservation Cooperative Fisheries Management and Marine Research Fellowships

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Task Order Number:

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Abstract

There is a well-established link between the mammalian immune and endocrine systems. These finely tuned interactions are required for the survival and health of an individual and are regulated by circulating hormones, which are influenced by nutrition, body condition and a host of environmental factors. The overall goal of this project is to determine if changes in maternal investment, body condition, and circulating hormone levels result in measurable changes in the health and survival of Steller sea lion pups, as measured through the immune system.

As part of an ongoing long-term monitoring and research effort, researchers observe the Chiswell Island rookery via a remotely-operated video system, which provides an in-depth assessment of maternal investment as well as pup well-being and survival. During three breeding seasons (2005, 2007, and 2008) pups ranging in age from 5 to 38 days old were measured, weighed, and permanently marked allowing for identification of mother-pup pairs, determination of exact pup ages, and assessment of maternal investment. Blood samples were collected from 62 pups (24 female, 37 male, 1 unknown) for the measurements of a suite of blood-borne components, including complete blood cell counts, lymphocyte proliferation, serum chemistries, contaminants, and circulating levels of IL-6. Serum has been used to determine circulating levels of ghrelin, cortisol, aldosterone, leptin, and thyroid hormones. Further, to date two kits for adrenocorticotropic hormone (ACTH) have been tested for use with captive SSL serum.

Background

The immune system protects the body from potential infections and is critical for health and survival of an individual. At birth, the mammalian immune system is not fully developed requiring the temporary reliance on maternal antibodies received by consuming colostra until their immune system matures. A female under nutritional stress may produce a smaller pup or have reduced maternal investment due to more frequent or longer foraging trips (Harding et al., 2005; Bowen et al., 2001). An extreme example of this is the nearly complete mortality of the cohort of California sea lion born in an El Nino year (DeLong et al., 2008). Low birth weight in other mammals has been associated with impaired cell-mediated immune response, lasting months to years, including a reduced number of T cells and reduction in their response to mitogens (Chandra, 1991). T cells are an important component of the cell-mediated immune response whose function is driven by the presence of circulating antibodies. A compromised cell-mediated immune system may lead to an increase in susceptibility to disease and mortality and may be the result of low birth weight and/or inadequate transfer of immune protection from the mother. Thus maternal investment in offspring can be measured through focal behavioral sampling that quantify the time present on the rookery and devoted to maternal attendance and pups (Maniscalco et al., 2006).

There is a well-established bi-directional communication between the mammalian immune and endocrine systems (Haddad et al., 2002). The endocrine system is largely responsible for maintaining homeostasis and communicates its effectiveness in maintaining homeostasis to the immune system. The immune system in turn responds to the endocrine system by preparing for changes that are potentially detrimental to the animal. These finely tuned interactions are required for the survival and health of an individual and are regulated by circulating hormones and cytokines, which are in turn influenced by body condition, reproductive status, and stress. The potential impact of stress on an animals' ability to survive would be greatest during times of high energy demands such as pregnancy, lactation, and periods of development and rapid growth. Therefore, females and their pups have the highest potential within a population to be affected by nutritional stress or any form of metabolic impairment. Given that NMFS currently does not permit any research on adult female Steller sea lions (NMFS, 2007), assessing the health of pups may enable us to identify physiological indicators of food limitation and be a proxy for the health and fitness of the mother. Further, the health of pups has the potential to be used as an indication of the overall health of the population.

Objectives

The overall goal of this project is to determine if different levels of maternal investment, pup body condition, and circulating hormones result in measurable changes in the health and survival of SSL pups. Specifically, the objectives of this proposal are to:

1. Assess endocrine function by quantifying hormones previously shown to indicate body condition and/or stress including cortisol, aldosterone, leptin, ACTH, ghrelin, and thyroid hormones (T3 &T4).
2. Assess immune function by complete blood cell count, serum chemistries, lymphocyte proliferation assay and circulating IL-6 levels.
3. Combine measures of maternal investment, pup body condition and endocrine parameters to predict pup health and survival.

Hypotheses

Given the interactions of the endocrine and immune systems and the reported effects of stress, body condition, and maternal investment, we hypothesize:

1. Cell-mediated immunity will change with pup age.
2. Smaller pups and/or pups with reduced maternal investment will have reduced cell-mediated immunity.
3. Smaller pups and/or pups with reduced maternal investment will have higher circulating stress hormone levels, specifically ACTH, cortisol and aldosterone.

Material and Methods

Animals and blood collection

Chiswell Island is home to a small SSL rookery, used by about 90 breeding animals producing up to 80 pups per year (Maniscalco et al., 2006) and is located 35 nautical miles south of Seward within the Alaska Maritime National Wildlife Refuge System. As part of a long-term monitoring and research effort, researchers at the Alaska SeaLife Center (ASLC; Seward, AK) have observed the Chiswell Island rookery via remotely-operated video monitoring equipment since 1998 (Maniscalco et al., 2002; 2006). The remotely operated video system was developed by SeeMore Wildlife Systems, Inc. (Homer, AK) and includes up to 6 cameras equipped with 12-18x optical and digital zoom functions mounted in fully weatherproof housing with remotely controlled pan, tilt, zoom, and windshield wiper-washer functions. Audio and video signals are transmitted to the ASLC via microwave transmission (Maniscalco et al., 2006). All SSL pups were continuously observed during daylight hours by the remote video camera monitoring program at the ASLC throughout the entire breeding season.

A total of 62 pups (Table 1) were sampled on June 30, 2005; July 3, 2007 and July 1, 2008 (Permit 881-1890-02; ASLC IACUC 07-001). Each pup was measured, weighed, and blood sampled. Body mass (BM) was measured to the nearest tenth of a kilogram using a hanging electronic scale (FWC series 7, FlexWeigh, Santa Rosa, Ca). Standard length (SL) was measured as a straight line from tip-of-nose to tip-of-tail while the pup was lying on a straight board. Axillary girth (AG) was measured using a tape measure after the pup had exhaled. Pups (n=53) were permanently marked by hot iron branding (Merrick et al., 1995) under isoflurane (USP; Halocarbon Industries, River Edge, NJ) anesthesia (Heath et al., 1997) with the remaining 9 pups having flipper tags attached in the axillary area of both fore-flippers. Branding pups allowed for the identification of mother-pup pairs and determination of pup ages (to within ± 4 hr) by association with naturally marked females that were tracked from the time they gave birth. Pups ranged in age from 5 to 38 days old at the time of blood collection. Identifiable individuals and the remote video camera monitoring program allowed researchers to track maternal attendance, and vital rates such as pup production, age-specific survival, and mortality rates, and often the specifics of mortality events (Maniscalco et al., 2006; 2008).

Blood samples (≤ 1ml/kg) were collected using standard aseptic techniques from the caudal gluteal vein with an 18 gauge needle directly into Vacuette® blood collection tubes. In 2005 and 2008 pups were anesthetized with isoflurane prior to blood collection and in 2007, pups were manually restrained during blood collection. Blood tubes included EDTA-treated tubes for total and differential leukocyte counts and Z serum clot activator tubes for serum chemistries and hormone analysis. Sodium heparin-treated tubes were collected in 2007 and 2008 for isolation of PBMC from 21 of the pups (10 female, 11 male). The serum separator tubes were collected in 2007 and 2008 were spun on the rookery within 2 hours of blood draw

while samples collected in 2005 were seperated upon return to the lab. All blood tubes were kept upright and chilled until further processing in the lab (< 12 hrs). Results from two pups were removed from the hematology dataset, before data analyses were conducted, due to several abnormal hematological parameters including extremely low platelet counts (<80) which were likely due to clotting.

Endocrine function

Blood collected into serum separator tubes was centrifuged for 10 minutes, aloquoted and serum frozen at -80°C until assayed with commercially available radioimmunoassay kits (RIA; Table 2) previously validated for SSL. Quantifying hormones previously shown to indicate body condition, stress or influence on immune function including cortisol (Mashburn and Atkinson, 2007; 2008), aldosterone, leptin (Mashburn and Atkinson, 2008), and thyroid hormones (T3 &T4; Myers et al., 2006) will be used to assess endocrine function. SSL serum has been validated following standard validation procedures. Two commercially available kits for adrenocorticotropic hormone (ACTH) have been used with SSL serum including one RIA (MP Biomedicals; Orangeburg, NY) and one ELISA (Alpco; Salem, NH).

Complete Blood Cell Count

Complete blood cell (CBC) counts were determined using the Heska® CBC-Diff Veterinary Hematological System (Heska® Corporation, Loveland, CO). Blood smears were made from whole EDTA blood samples and stained with Wright-Giemsa (Dip Quick Stain, Jorgensen Laboratories, Loveland CO). WBC differentials, including neutrophils, lymphocytes, monocytes and eosinophils were counted manually. Percentage and absolute values of differentials are reported. To determine the PCV, EDTA blood samples were transferred into nonheparinized microhematocrit tubes and centrifuged for 3 minutes at 10,400 rpm (Clay Dams Brand TRIAC centrifuge) and PCV was determined by micro-capillary reader (International Equipment Company; MA) by measuring the length of the red blood cell layer compared to the length of the total sample held in the tube. Total solids were estimated using a hand-held refractometer (Reichert VET360).

Blood Chemistries

Serum or sodium heparin plasma was aliquoted, frozen and stored at -80°C until samples were submitted to Phoenix Central Laboratory (Everett, WA) for chemistries analysis. Table 3 includes a list of chemistry analysis included in the current study.

Isolation of peripheral blood lymphocytes and proliferation

Sodium heparin blood tubes were centrifuged at 200x g for 10 min within 12 hours following collection. The buffy coat was removed and resuspended in freezing media comprised of RPMI 1640, 20% FBS (Hyclone, Thermo Fisher Scientific Inc., Logan, Utah), 10% DMSO (Sigma Chemical Co., St. Louis, MO) and rate frozen in Nalgene cryojars (Nalgene, Rochester, NY) to -80°C, then transferred to liquid nitrogen 24 hours later. Cryopreserved WBCs were thawed and washed twice with PBS (without Ca^{2+}/Mg^{2+}) and re-suspended in RPMI 1640 supplemented with 10% FBS, 2 mM L-glutamine, penicillin (100 U/ml), and streptomycin (100 µg/ml). PBMC were isolated by density gradient centrifugation (Histopaque 1077; MP Biomedical, Solon, OH) for 20 min at 720 x g. The PBMC-containing band was isolated, washed twice, and re-suspended in RPMI 1640 supplemented with 10% FBS, 2 mM L-

glutamine, penicillin (100 U/ml), and streptomycin (100 μg/ml), 1 mM sodium pyruvate, 100 μM nonessential amino acids, 25 mM hepes and 0.05 mM 2-mercaptoethanol (Sigma Chemical Co., St. Louis, MO). All cell culture media and supplements, except for FBS, DMSO and 2-mercaptoethanol, were purchased from Gibco, Invitrogen Corp., Carlsbad, CA. Viability was assessed using the exclusion dye trypan blue (Sigma Chemical Co., St. Louis, MO).

Isolated PBMC were plated (2.0×10^5 cells/well) in 96-well flat-bottom tissue-culture plates (Falcon, Becton Dickinson, Franklin Lakes, NJ) and their proliferation assessed following exposure to one of two mitogens (conA or LPS 055:B5; Sigma Aldrich, St. Louis, MO). PBMC were cultured at 37°C with 5% CO2 in triplicate for each animal in each of the following treatment groups; 1) unexposed control, 2) ConA suboptimal (0.1μg/ml), 3) ConA optimal (1.0 μg/ml), 4) LPS suboptimal (1.0 μg/ml) and 5) LPS 055:B5 optimal (100 μg/ml). For each mitogen, two concentrations based upon preliminary experiments (data not shown), were used to represent suboptimal and optimal concentrations, respectively for ConA (0.1 and 1.0 μg/ml) and LPS (1.0 and 100 μg/ml). ConA is a plant-derived mitogen and preferentially stimulates T cells (Barta and Barta, 1993) while the LPS from Escherichia coli are a B-cell activator (Wechsler-Reya, 1996). Mitogens were reconstituted in RPMI 1640 supplemented with 10% FBS, 2 mM L-glutamine, penicillin (100 U/ml), and streptomycin (100 μg/ml). These conditions are similar to those previously used for pinniped species (Levin et al., 2005; Mori et al., 2006).

All PBMC collected during this project were assessed in 2 assays (one for each year 2007, 2008) and included PBMC collected from a captive adult SSL as an interassay control. No PBMC proliferation assays were conducted on 2005 blood samples. PBMC were incubated for 48 hours after which 20 μl of bromodioxyuridine (BrdU) was added to a final concentration of 10 μM BrdU and incubated for an additional 18 hours for a total incubation period of 66 hours. PBMC proliferation was assessed with the Cell Proliferation Biotrack ELISA System (version 2; GE Healthcare, Piscataway, NJ) per manufacturer's instructions using Dynatech MRX Revelation microplate reader (Dynatech Laboratories, Inc, Chantilly, VA) at 450 nm with a reference wavelength of 650 nm. Data are presented as mean of the triplicates for OD with standard deviation and as a stimulation index (SI; mean OD of cells exposed to mitogen/ mean OD of cells in media only).

Statistical analysis

Data were analyzed with Systat 10 (Systat Software, Inc, Point Richmond, CA). The best model for each parameter was selected using stepwise general linear model which uses an iterative process of comparing the full mixed effects model which included the categorical variables of sex and anesthesia with age as a continuous variable. The full model was compared to subsequent reduced models which included only significant grouping variables and interactions. If anesthesia was identified as a significant factor, then each treatment group (isofluorane vs. physical restraint) was analyzed separately for age and sex effects.

To evaluate the potential effect of the disturbance at the rookery associated with separation of pup and cow, and corralling the pups, each parameter was regressed against the time elapsed from the initial arrival on the rookery to the time when the individual pup was removed from the corral and taken for blood collection. If anesthesia or sex was identified as a significant factor, then each treatment group was analyzed separately. Values were considered significant if $p \leq 0.05$.

Preliminary Results

Body condition and morphometrics of pups

Based on mass and morphometrics all pups sampled were determined to be in good body condition for their respective age and sex at the time of sampling (Figure 1). Female pups were smaller than males in BM (female 26.7 ± 4.1 kg, male 32.2 ± 4.6 kg; $F_{3, 55}$=3.949, p=0.052), SL (female 101.6 ± 4.7, male 108.0 ± 4.8 cm; $F_{3, 55}$=5.666, p=0.021), and AG (female 71.0 ± 6.3 cm, male 74.2 ± 10.1 cm; $F_{3, 55}$=1.081, p=0.303). Age also significantly affected BM ($F_{3, 55}$=37.506, p<0.0001, r^2=0.613), SL ($F_{3, 55}$=25.851, p<0.0001, r^2=0.543) and AG ($F_{3, 55}$=11.293, p=0.001, r^2=0.334) of SSL pups. While female pups (14.7 ± 4.9 days) were on average younger than male pups (18.4 ± 7.2 days), there was not a significant interaction between age and sex for BM, SL, or AG.

Changes in total and differential leukocyte counts

Hematological results including total and differentials of WBC count from 42 pups under isoflurane anesthesia or physical restraint are presented in Table 1. There was not a significant effect of sex or anesthesia on total or differential WBC counts. Figure 2 displays total and differential WBC counts verses age or elapsed time. Total WBC count significantly decreased with increasing age of pups ($F_{1, 40}$=14.302, p<0.001, r^2=0.263). A similar pattern was found in the number of circulating neutrophils ($F_{1, 40}$=14.079, p=0.001, r^2=0.260) while there was no significant change in lymphocyte, monocyte, or eosinophil counts with age. The neutrophil to lymphocyte ratio also significantly decreased with age ($F_{1, 40}$=4.397, p=0.042, r2=0.099), primarily driven by the changes found in neutrophil counts. There was no effect of elapsed time between arrival on the rookery and removal of pup from the holding corral for blood collection on total WBC, neutrophil, or lymphocyte counts. However, monocyte counts increased ($F_{1, 40}$ =8.976, p=0.005, r^2=0.183) while eosinophils decreased ($F_{1, 40}$ =8.479, p=0.006, r^2=0.175) during the elapsed time.

There was no difference between male and female pups, anesthesia treatment or age on Hct, RBC, Hb or PLT. There was a difference in mean corpuscular hemoglobin content (MCHC; $F_{1, 40}$= 49.126, p<0.001) between pups who were physically restrained (38.6 ±0.7 g/dl) versus those under isoflurane (35.5 ±1.4 g/dl) during blood collection. However, when MCHC was analyzed within each anesthesia treatment group there was no effect of age or sex. Similarly, there was an effect of anesthesia ($F_{1, 39}$=26.689, p<0.001) for mean corpuscular hemoglobin (MCH) with pups under isoflorane having lower MCH (35.8 ± 1.1 pg) compared to physically restrained pups (37.8 ± 1.3 pg), however there was no effect of age or sex on MCH within the two anesthesia groups. Mean corpuscular volume (MCV) was higher ($F_{1,39}$ =9.273, p=0.004) in pups under isoflorane (100.7 ± 3.7 fl) compared to physically restrained pups (97.9 ±2.3 fl). Further, MCV was higher ($F_{1,7}$=6.015, p=0.004) in male pups who were physically restrained during blood collection (98.8 ± 1.7 fl) compared to females (95.5 ± 1.8 fl). However, there was no difference between male and female pups under isoflorane ($F_{1,27}$=3.873, p=0.059) and there was no effect of age regardless of how the pup was restrained.

Changes in lymphocyte proliferation

No significant effects of sex or anesthesia were detected in PBMC response to ConA or LPS. The average response of PBMC to stimulation with ConA was 0.842 ± 0.784 OD (range 0.310-3.13 OD; optimal) and 0.313 ± 0.040 OD (range 0.240-0.370 OD; suboptimal). Stimulation with LPS at optimal and suboptimal doses was 0.636 ± 0.278 OD (range 0.238-1.21

OD) and 0.408 ± 0.131 OD (range 0.238-0.661 OD), respectively. Control PBMC proliferation was 0.300 ± 0.029 OD (range 0.240- 0.350 OD). The SI was also calculated for ConA at both the optimal (6.10 ± 2.49; range 1.33 – 10.44) and suboptimal dose (1.05 ± 0.11; range 0.89-1.28) and at the optimal (2.08 ± 0.85; range 0.92 – 3.74) and the suboptimal (1.36 ± 0.41; range 0.90 – 2.10) dose for LPS. A significant effect of age on the ability of PBMC to proliferate was found in cells exposed to the optimal dose of ConA. The effect of age was found whether the results were expressed as absorbency (OD; $F_{1, 19}$=4.941, p=0.039, r^2=0.206; Figure 3A) or SI ($F_{1, 19}$= 4.984, p=0.038, r^2=0.208; Figure 3B). Specifically, the ability of PBMC to proliferate when stimulated with optimal ConA was higher in young pups and decreased with age. There was not an effect of age on spontaneous proliferation of unstimulated PBMC or when exposed to the optimal LPS; nor was there a change in the proliferation of the suboptimal dose of ConA or LPS when expressed as absorbency or stimulation index.

Figures, tables, maps (in PDF or GIF format)

Table 1. Mean (± SD) for age, body mass (BM), standard length (SL), and axillary girth (AG) for 62 Steller sea lion pups by year and sex.

	Age (day)	BM (kg)	SL (cm)	AG (cm)
2005				
Male (20)	16.2 ± 5.3	31.1 ± 3.9	106.7 ± 3.7	71.1 ± 11.8
Female (8)	15.1 ± 4.5	27.5 ± 3.9	102.6 ± 3.7	70.7 ± 5.1
Unk (1)	19.8	33.6	108.0	79.0
2007				
Male (13)	21.6 ± 8.1	33.7 ± 4.5	110.4 ± 4.5	78.7 ± 4.9
Female (9)	16.3 ± 5.5	27.8 ± 4.5	102.8 ± 4.6	72.5 ± 5.2
2008				
Male (4)	18.7 ± 10.4	32.2 ± 7.9	106.5 ± 8.7	75.3 ± 10.0
Female (7)	12.8 ± 4.2	24.3 ± 3.2	99.0 ± 5.3	69.6 ± 8.8

Table 2. List of parameters quantified including abbreviations and units.

	Abbrevation	Units
Albumin	ALB	g/dl
Alkaline phosphates	ALKP	U/L
Alanine transaminase	ALT	U/L
Amylase	AMYL	U/L
Aspartate transaminase	AST	U/L
Blood urea nitrogen	BUN	mg/dl
Calcium	Ca^{2+}	mg/dl
Cholesterol	CHOL	mg/dl
Creatine kinase	CK	U/L
Creatinine	CREA	mg/dl
Gamma glutamyl transferase	GGT	U/L
Glucose	GLU	mg/dl
Lactate dehydrogenase	LDH	U/L
Phosphate	PHOS	mg/dl
Total Bilirubin	TBIL	mg/dl
Total Protein	TP	g/dl

Triglycerides	TRIG	mg/dl
Globulin	GLOB	g/dl
Sodium	Na^+	mmol/l
Potassium	K^+	mmol/l
Chloride	Cl^-	mmol/l

Table 3. Summary of commercially available RIA used on the current study to quantify circulating hormone levels.

Hormone	Manufacturer	Catalog	Range	Sensitivity
Cortisol	Siemens	TK01	1-50 μg/dL	0.2 μg/dL
Alodsterone	Siemens	PITKAL-4	25-1200 pg/ml	11 pg/ml
Triiodothyronine (TT3)	Siemens	TKT31	20-600 ng/dL	7 ng/dL
Thyroxin (TT4)	Siemens	TKT41	0.5-24 μg/dL	
Free thyroxin (FT4)	Siemens	PITKF4-5	0.1-10 ng/dL	0.01 ng/dL
Leptin	Linco	XL-85K	1-50 ng/ml	1.0 ng/ml
Ghrelin	Linco	CHRT-89HK	109 -3500 pg/ml	93 pg/ml

Table 4. Mean (± SD) hematological values Steller sea lion pups under isoflurane anesthesia (n=31) or physical restraint (n=11).

	Isoflurane			Physical Restraint		
Parameter	Mean ± sd	Min.	Max.	Mean ± sd	Min.	Max.
WBC (10^3/μl)	13.3 ± 2.7	8.8	19.6	12.7 ± 3.1	7.3	16.3
Neutrophil (10^3/μl)	8.7 ± 2.7	4.4	16.1	8.2 ± 1.9	4.8	10.7
(% of WBC)	64.9 ± 10.3	45.0	84.0	65.0 ± 5.6	55	73
Lymphocyte (10^3/μl)	2.6 ± 1.2	1.1	5.6	2.4 ± 1.2	1.1	5.5
(% of WBC)	23.1 ± 8.8	8.0	42	19.3 ± 7.1	10.0	35.0
Monocytes (10^3/μl)	1.4 ± 0.9	0	3.8	1.8 ± 0.9	2.9	3.3
(% of WBC)	11.0 ± 6.5	0	24	14.1 ± 5.6	4.0	21.0
Eosinophil (10^3/μl)	0.5 ± 0.4	0	1.2	0.2 ± 0.2	0	0.8
(% of WBC)	4.2 ± 3.1	0	10	1.5 ± 2.1	0	6.0
Neutrophil:lymphocyte ratio	4.2 ± 2.3	1.1	9.7	3.9 ± 1.7	1.7	7.3
Hct (%)	34.7 ± 3.5	29.7	43.8	34.4 ± 1.8	31.9	36.6
MCV (fl)*	100.7 ± 102.1	94.9	108.2	97.9 ± 2.3	93.4	101.8
RBC (10^6/μl)	3.5 ± 0.3	3.0	4.3	3.5 ± 0.2	3.2	3.9
Hb (g/dl)	12.3 ± 1.2	10.7	15.0	13.2 ± 0.7	12.2	14.3
MCH (pg) *	35.8 ± 1.1	32.5	39.2	37.8 ± 1.3	35.6	40.0
MCHC (g/dl) *	35.5 ± 1.4	34.0	38.3	38.6 ± 0.7	36.8	39.4
PLT (10^3/μl)	382 ± 119	93	639	327 ± 119	104	477

* denotes parameters with a significant difference between restraint methods.

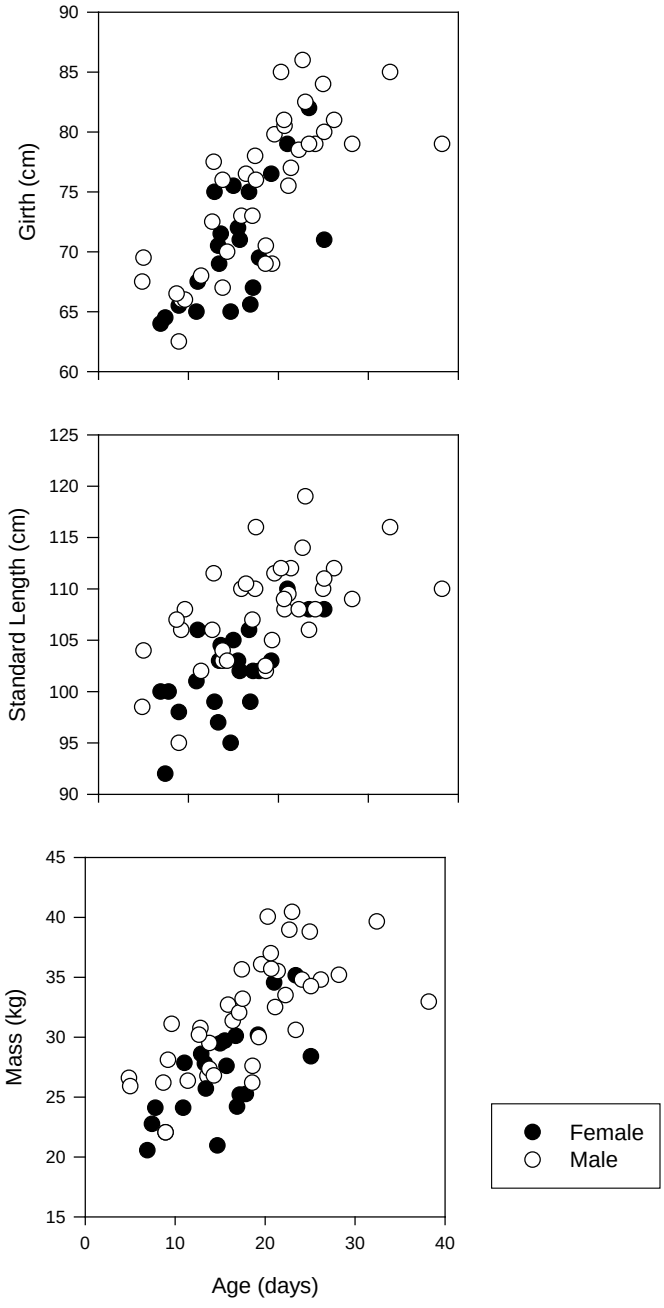
Captions to figures

Figure 1. A total of 61 pups (male=37, female=24) were sampled during 2005, 2007, and 2008. Morphometerics for individual pups at age are presented in (A) body mass axillary girth, (B) standard length, and (C) axillary girth.

Figure 2. (A) Total WBC counts from 42 pups during the 38 days following birth and differential counts for (B) neutrophils; (C) lymphocytes; (D) monocytes; and (E) eosinophils. (F) Total WBC counts (G) neutrophils; (H) lymphocytes; (I) monocytes; and (J) eosinophils are also presented against the elapsed time from arrival on rookery and pup handling for sample collection. Only significant trend lines are presented.

Figure 3. Lymphocyte proliferation response from 21 SSL pups exposed to 1.0 □g/ml ConA. Results presented as (A) absorbancy (OD) and (B) stimulation index.

Comment [sa1]: ??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Standard Length (cm)
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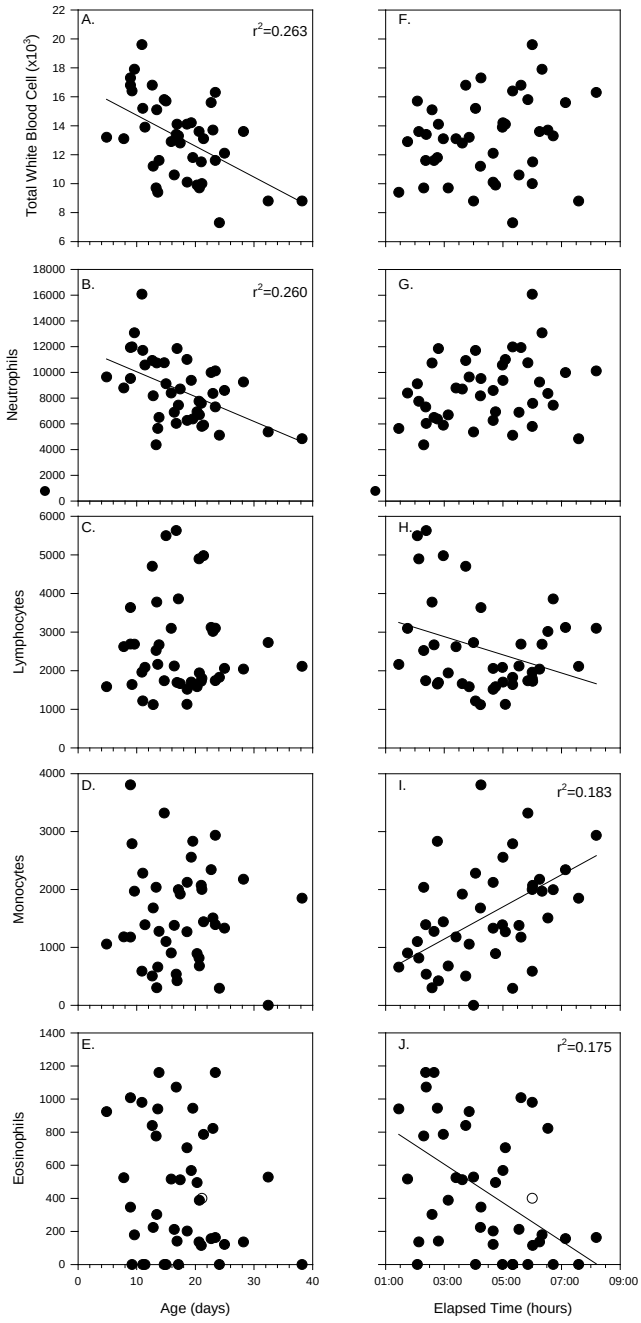
Age (days)
Female
Male


A.
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Total White Blood Cell (x10^3)
B.
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G.
Neutrophils
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Lymphocytes
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I.
r^2=0.183
Monocytes
E.
J.
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Eosinophils
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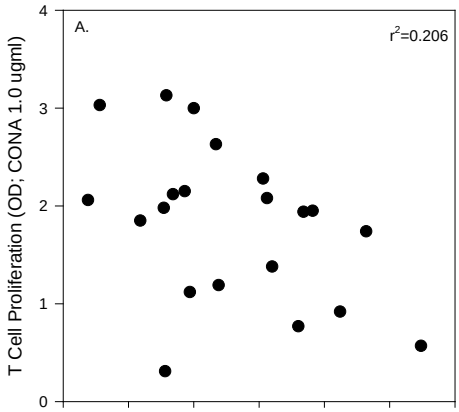
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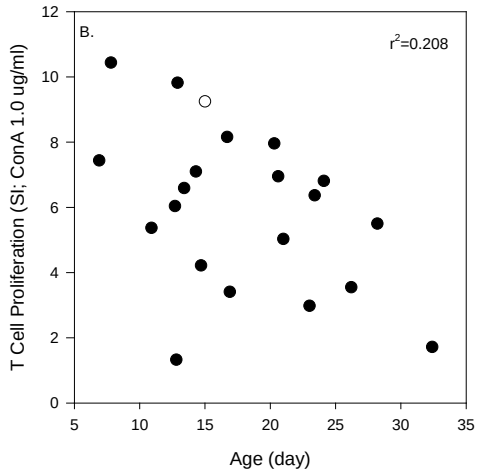
B.
r^2=0.208
T Cell Proliferation (SI; ConA 1.0 ug/ml)
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25
30
35
Age (day)

Future plans

The first manuscript entitled "Steller sea lion pups (*Eumetopias jubatus*) undergo a reduction in circulating white blood cells and the ability of T cells to proliferate during the 38 days following birth" is currently in review by co-authors following which it will be submitted to the journal of Veterinary Immunology and Immunopathology. Data analysis for the second manuscript is currently underway.

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