ASSESSING THE PHYSIOLOGICAL STATUS OF NORTHERN FUR SEAL POPULATIONS IN NORTH AMERICA WITH FECAL HORMONES

by

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Abstract

The core breeding population of northern fur seals (*Callorhinus ursinus*) in North America has declined significantly since the 1980s on St. Paul Island (one of the Pribilof Islands) while the smaller nearby population at Bogoslof Island (eastern Bering Sea) has increased exponentially. Further south, the population of northern fur seals on San Miguel Island off the coast of Southern California has fluctuated between exponential growth and catastrophic declines associated with re-occurring El Nino events. The goal of my thesis was to asses the physiological status of these three breeding populations of northern fur seals in North America to determine whether nutritional differences could explain the different population trajectories. I collected fecal samples (scats) in July 2009 from these three islands and measured the fecal metabolites of two hormones — a glucocorticoid associated with the stress response, and triiodothyronine (T3), a thyroid hormone associated with metabolic rate. I also assessed feeding conditions using diet and foraging data. I found that sub-adult males and lactating females on St. Paul Island experienced poorer feeding conditions (lower energy content food and longer feeding trips for lactating females) than at Bogoslof Island, but that only the females were nutritionally stressed. I also found that the San Miguel Island population differed physiologically compared to the northern populations in Alaska in terms of stress and nutritional status. The San Miguel fur seals were the most physiologically stressed of the North American fur seal populations (based on elevated levels of glucocorticoid metabolites). However, the stress was most likely related to heat stress and not food (based on low concentrations of T3 metabolites). The available hormone, diet, and foraging data from northern fur seals in North America suggest that lactating females were nutritionally stressed on St. Paul Island and heat stressed on San Miguel Island, and experienced better conditions on Bogoslof Island.

Preface

Sharon Melin and Robert DeLong (NOAA, Seattle, WA) provided scat samples from San Miguel Island. I performed radioimmunoassays in the laboratory of Joanne Weinberg (University of British Columbia). Brian Battaile and Chad Nordstrom (University of British Columbia) provided scat samples and the foraging data from Bogoslof and St. Paul. Hard parts were identified at Pacific Identification Inc. The Vancouver Aquarium provided scat samples for the field equivalency experiment. Scat Collection under NMFS Permit No. 715-1884 UBC Animal Care A09-0326

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Finally, thank you to all my friends and family who provided support in so many ways during my graduate studies at UBC. You are the ones who listened and supported me through all of the victories and defeats. There are no words to express how eternally grateful I am to have such amazing people in my life.

Dedication

To every person who helped make this thesis happen

Chapter 1: General introduction

Northern fur seals (*Callorhinus ursinus*) are the only member of the family otariidae in the northern hemisphere. They migrate throughout the North Pacific, and traditionally bred on the Pribilof Islands of Alaska, the Commander Islands in the Bering Sea, and Robben Island and the Kurile Islands of Russia (Figure 1.1). However, two new breeding populations were established in 1968 on San Miguel Island in California at the southern limit of their range, and in 1980 on Bogoslof Island near the Aleutian Islands in the Bering Sea. These six breeding populations of northern fur seals have different population trajectories.

The two new colonies of northern fur seals in North America have grown while the core breeding population on the Pribilof Islands has declined. The Pribilof Islands (St. Paul and St. George Islands) were once home to the majority of the world's breeding population of northern fur seals. However, an experimental harvest of females and pelagic scientific sampling of fur seals reduced the population to ~1.25 million by the early 1970s (Allen & Angliss 2009) — and an unexplained decline has continued since the 1980s to present (Allen & Angliss 2009). In 2010, the population on St. Paul totaled ~450,000 (Towell *et al.* 2011) and was only about 20% of its peak size in the 1950s of ~2.25 million (Kenyon & Scheffer 1984).

In contrast to the population declines on the Pribilof Islands, the smaller nearby population at Bogoslof Island (eastern Aleutian Islands) has increased exponentially in recent years. Births on Bogoslof increased from ~900 pups in 1992 to ~5,100 in 1997, ~12,600 in 2005 and ~17,600 in 2007 (Towell & Ream 2006). Much of the initial sharp increase in numbers is believed to be due to immigration from the Pribilof population (Ream *et al.* 1999).

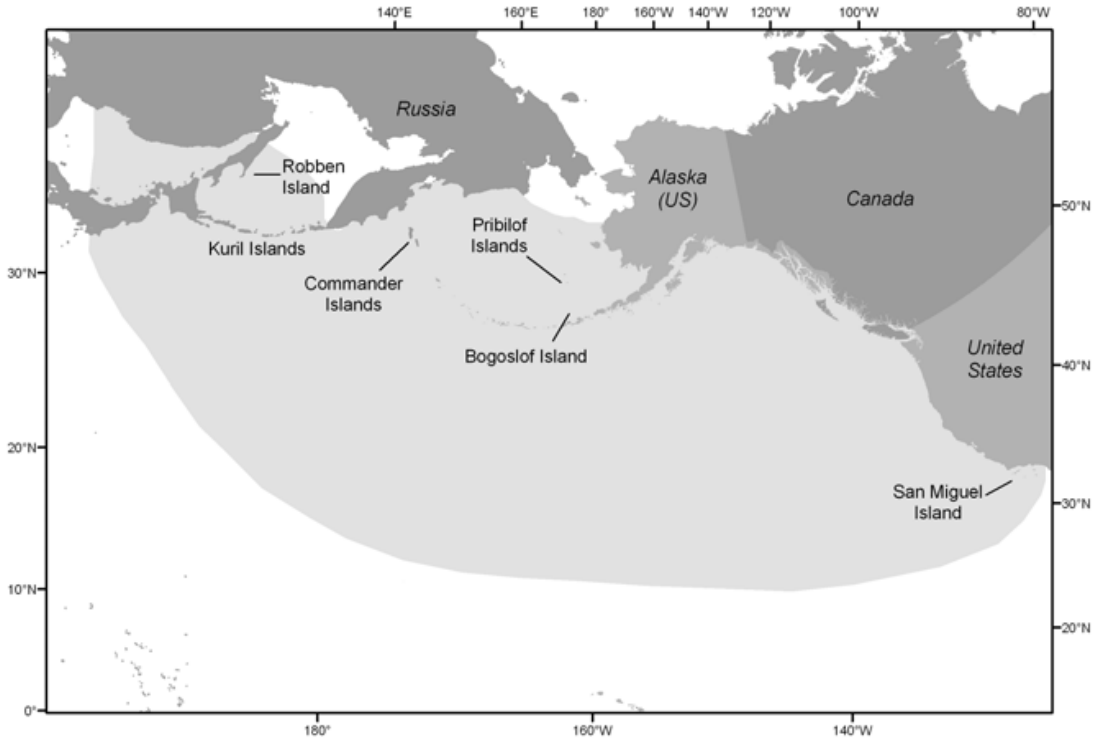

Figure 1.1 Breeding locations of the six populations of northern fur seals (NOAA 2010).

Further south, the population of northern fur seals on San Miguel Island off the coast of Southern California has fluctuated between exponential growth and catastrophic declines associated with re-occurring El Nino events (DeLong 1982, Melin *et al.* 2005, Allen & Angliss 2009). In 1997, total pup production was the highest recorded since the colony was first monitored. However, up to 87% of those pups died before weaning and production declined 80% from 1997 to 1998 (Melin *et al.* 2005). Although total production increased to 2,492 in 2007, the population has not yet recovered (Melin *et al.* 2008).

The leading hypotheses to explain the differing population trends of the three North American fur seal populations include climate differences (York 1995), interactions with commercial fisheries (Trites 1992), and predation (Springer *et al.* 2003). Of these hypotheses, changes in the availability of prey whether by fisheries or natural changes in the ecosystem are considered to be the most likely explanation for the differing population dynamics (NMFS 2007). Changes in food can alter body

size, reproductive rates, growth weights, survival of pups and juveniles, as well as foraging behavior (Trites & Donnelly 2003). Unfortunately, it has not yet been possible to apply these measures of nutritional status to assess whether nutritional differences are driving the observed changes in numbers at the three breeding islands in North America.

1.1 Nutritional stress and hormone analysis

Physiological methods to assess the nutritional status of an animal include assessing blood serum parameters (Castellini & Calkins 1993) and blubber thickness (Oki & Atkinson 2004). Such techniques are effective but can be costly, invasive, and stressful (Ortiz & Worthy 2000). An alternative means to assess nutritional status is by measuring the concentration of metabolites of two hormones contained in the feces of animals—glucocorticoids, which are associated with the stress response, and triiodothyronine (T3), a thyroid hormone associated with metabolic rate. Fecal glucocorticoid metabolites have been shown to increase in response to increased adrenocorticotropic hormone concentrations in the African elephant, sea otter, long-tailed macaque, northern spotted owl and 9 other species (Wasser *et al.* 2000), including Steller sea lions (Hunt *et al.* 2004). Elevated concentrations of glucocorticoid metabolites have also been found in feces from fur seals on St. Paul compared to Bogoslof, which may or may not reflect a difference between island in nutrition (Hunt *et al.* 2008).

Physiological stress in animals has been reliably detected in previous studies by measuring glucocorticoid levels. Glucocorticoids are secreted by the adrenal cortex in response to stimulation by adrenocorticotropic hormones (ACTH). Glucocorticoids are involved in metabolism (Zakrzewska *et al.* 1999, Khani & Tayek 2001, Mastorakos & Zapanti 2004) and stress responses (Gulland 1999, Harper 2000, Mashburn & Atkinson 2004, Cavigelli *et al.* 2005, Creel 2005, Kitaysky *et al.* 2005, Möstl *et al.* 2005). Both blood levels of cortisol and fecal corticosteroids have been shown to be an accurate indicator of adrenal activity in response to both laboratory induced acute stress, as well as in response to predation, in adult Steller

sea lions (Mashburn & Atkinson 2008). Previous studies have also demonstrated that cortisol increases during periods of fasting or reduced energy intake to provide energy by increasing fat oxidation and maintaining circulating glucose levels through increased gluconeogenesis (Exton *et al.* 1972).

Elevated levels of cortisol result in various physiological consequences. For example, glucocorticoids induce a catabolic effect on peripheral organs (skin, muscle, and adipose tissue), promoting protein and lipid degradation (Baxter & Forsham 1972). Chronic stress also causes hypertrophy and hyperplasia of the adrenal cortex and medulla (Harvey *et al.* 1984). Stress affects thyroid function and can cause morphological changes in the thyroid gland. In addition, glucocorticoids promote hepatic gluconeogenesis and the increased metabolic activities of the liver can lead to hyperplasia under conditions of chronic stress.

As with corticosterone, thyroid hormones have similarly been successfully extracted and measured from the feces of monkeys, caribou, moose, wolf, killer whales and Steller sea lions (Wasser *et al.* 2010). T3 is one of the principle hormones released by the thyroid gland, and can affect various processes including metabolism, growth, and development. T3 generally acts to increase the basal metabolic rate and thus increases the body's oxygen and energy consumption. Small variations in thyroid hormone levels can have a large impact on protein and fat metabolism rates, even though thyroid hormone levels are generally very low in pinnipeds (Renouf & Noseworthy 1991). Previous experiments have demonstrated metabolic depression, and a corresponding decrease in T3, in captive Steller sea lions under many restricted feeding conditions (du Dot *et al.* 2009).

Detecting elevated stress levels by measuring glucocorticoids is fairly straightforward, but identifying whether elevated stress may be related to diet is more difficult. Concentrations of T3 metabolites should theoretically help determine whether an animal has altered its metabolism in response to a change in nutrition. Thus, measuring T3 concentrations in addition to glucocorticoids should help to determine if diet and nutrition contribute to elevated stress levels (Wasser *et al.* 2010).

1.2 Glucocorticoids and sex differences

Care must be taken with hormone analysis with regards to the sex and relative age of the animals being sampled because sex differences in the response of the hypothalamic-pituitary-axis (HPA) to a variety of stressors are known to occur in many animal species (Handa *et al.* 1994). For example, female rats react to physical and psychological stress with a response that is quantitatively greater than the response in males. Early studies demonstrating sexual dimorphism in adrenal function attributed the differences to changes in steroid synthesizing enzymes in the adrenal gland (Kitay 1963). However, recent demonstrations that sex differences in glucocorticoid secretion are accompanied by sex differences in ACTH (Handa *et al.* 1994) secretion suggests that sex differences in the HPA axis may originate in neuroendocrine systems in addition to the peripheral organs.

Sex differences also exist in several aspects of basal HPA axis function in rats. For example, there is a higher secretion and plasma concentration of glucocorticoids in females. Female rats exhibit a greater magnitude and duration of HPA response to stressors such as handling, ether, restraint, shocks, and high-conflict situations, suggesting that negative feedback may be less responsive than in males (Brett *et al.* 1986). Furthermore, testosterone can inhibit HPA axis function, and estrogen can enhance it (Handa *et al.* 1994) by binding to their respective receptors in the central nervous system. In both male and female rats as well as primates, estrogen administration increases basal glucocorticoid and ACTH secretion in response to both physical and psychological stressors (Handa *et al.* 1994).

1.3 Thesis goals, hypotheses and structure

The goal of my thesis was to assess the physiologic status of the three breeding populations of northern fur seals in North America. In order to do this, I collected fecal samples (scats) from Bogoslof, St. Paul, and San Miguel Islands in July of 2009. In addition to hormone metabolite concentrations, I assessed the nutritional status of male and female fur seals by identification of hard parts in scat

samples to determine what the fur seals on St. Paul, Bogoslof, and San Miguel Islands were eating. I also utilized foraging data, in particular foraging trip length for lactating females at each location. These data allowed me to 1) determine if there were sex differences in hormone metabolite concentrations; 2) compare hormones, diet, and foraging characteristics on St. Paul and Bogoslof to determine whether nutritional stress was contributing to the decline on St. Paul (Chapter 2); and 3) to compare the physiological status of the Alaskan populations (St. Paul and Bogoslof) to the San Miguel population using the same metrics (Chapter 3).

I hypothesized that declining fur seal population on St. Paul would have elevated glucocorticoid concentrations and elevated T3 concentrations compared to fur seals on Bogoslof as a result of increased foraging effort. I also predicted that glucocorticoid concentrations would be high in fur seals breeding on San Miguel due to heat, climate and possibly food—and hoped to distinguish between these possible stressors using T3 levels. Finally, I expected average glucocorticoid levels to be higher in female than in male fur seals.

My thesis is organized into four sections: Chapter 1 – general introduction; Chapter 2 – a comparison of physiologic parameters in the Bering Sea between St. Paul and Bogoslof Islands to understand why one population is declining, while the other is increasing; Chapter 3 – a comparison of physiologic parameters between the Alaskan populations and the San Miguel population to understand the physiological stresses posed on fur seals breeding at the northern and southern limits of their range; and Chapter 4 – a general conclusion. Data Chapters 2 and 3 are written as manuscripts and contain some necessary repetition of information.

Chapter 2: Fecal hormones indicate that northern fur seals are nutritionally stressed in the central Bering Sea during summer

2.1 Summary

Northern fur seals (*Callorhinus ursinus*) have declined on the Pribilof Islands (St. Paul and St. George) in the central Bering Sea, while a recently established population has increased exponentially on Bogoslof Island near the Aleutian Islands in the southern Bering Sea. The different population trajectories might be related to differences in food availability. We tested the hypothesis that the St. Paul Island population was nutritionally stressed by comparing the concentration of glucocorticoids (stress hormones) and T3-triiodothyronine (a thyroid hormone involved in metabolism) in fecal samples collected from St. Paul and Bogoslof Islands in July 2009. We found that concentrations of glucocorticoids were significantly higher for lactating females on St. Paul compared to Bogoslof. We also found that the concentrations of T3 for males and females were significantly higher on St. Paul than on Bogoslof, suggesting that both sexes on St. Paul were catabolizing more lipid or lean mass. One possible explanation for these differences between breeding populations is that seals on St. Paul needed to swim further and longer to meet their energy requirements compared those on Bogoslof. Both glucocorticoid and T3 concentrations were significantly lower overall on Bogoslof compared to St Paul Island. These inter-island differences in hormone concentrations are consistent with the different lengths of foraging trips made by lactating females in 2009 (foraging trips averaged 3.3 days from Bogoslof and 7.9 days from St. Paul). Collectively, our data suggest that fur seals from the Pribilof Islands are experiencing greater nutritional stress during summer compared to fur seals breeding on Bogoslof Island.

2.2 Introduction

Northern fur seal (*Callorhinus ursinus*) populations in Alaska are declining on St. Paul Island, and increasing exponentially on Bogoslof Island (Figure 2.1) but the cause or causes of these population trends is currently unknown (NMFS 2007). It has been hypothesized that fur seal condition, survival, or reproductive rates have been affected by factors such as climate (York 1995), interactions with commercial fisheries (Trites 1992) or predation by killer whales (Springer *et al.* 2003). Among the leading hypotheses to explain declining stocks is nutritional stress, a negative physiological and behavioral state resulting from sub-optimal quantity or quality of food available to an animal (Trites & Donnelly 2003).

Nutritional stress can have negative consequences for an individual animal as well as a population. Previous studies (Trites 1992, Castellini & Calkins 1993, Calkins *et al.* 1998, Donnelly *et al.* 2003) demonstrated this phenomenon in Steller sea lions by finding changes in body size in non-pups, reduced reproductive rates, changes in birth and growth weights, reduced survival of pups and juveniles, as well as behavioral modifications in declining populations that are consistent with changes in nutrition. Nutritional stress could have similar detrimental effects on the recovery of depleted populations of northern fur seals. Unfortunately, little research has been done on the nutritional status of northern fur seals, with the exception of one study that reported nutritional deficiencies on St. Paul Island based on elevated concentrations of glucocorticoid metabolites in feces from fur seals on St. Paul compared to Bogoslof Island (Hunt *et al.* 2008).

Methods to assess the nutritional status of an animal include assessing physiological parameters via blood chemistry (Castellini & Calkins 1993) and blubber thickness (Oki & Atkinson 2004). Such techniques are effective, but can be costly, invasive, and stressful (Ortiz & Worthy 2000). A simpler alternative means to assess nutritional status is to measure the concentration of hormone metabolites contained in the feces of animals (Harper 2000, Teskey-Gerstl *et al.* 2000, Wasser *et al.* 2000, Schatz & Palme 2001, Washburn *et al.* 2003, Hunt *et al.* 2004, Millspaugh & Washburn 2004, Rettenbacher *et al.* 2004, Touma *et al.* 2004, Weingrill *et al.* 2004,

Cavigelli *et al.* 2005, Deagle *et al.* 2005, Möstl *et al.* 2005). Fecal glucocorticoid metabolites have been shown to increase in response to increased adrenocorticotropic hormone concentrations in the African elephant, sea otter, long-tailed macaque, northern spotted owl and nine other species (Wasser *et al.* 2000). Thyroid hormones have similarly been successfully extracted and measured from the feces of monkeys, caribou, moose, wolf, killer whales and Steller sea lions (Wasser *et al.* 2010). It has thus been established that hormones excreted by animals can be measured in feces, and that it is a non-invasive method that can be used with a variety of species to assess stress in free-ranging populations.

I sought to measure hormone concentrations in feces from northern fur seals to test whether the declining population on St. Paul Island was nutritionally stressed compared to fur seals from the increasing population on Bogoslof Island. I collected fecal samples from rookeries and haulouts on both islands, and extracted hormones from sampled feces to measure glucocorticoids and T3 by radioimmunoassay. Based on the hypothesis that northern fur seals are nutritionally stressed on the Pribilof Islands, I predicted that glucocorticoid concentrations for St. Paul animals would be elevated and significantly different compared with those breeding on Bogoslof Island. I also expected to find high T3 levels on St. Paul (that would be indicative of animals that had been using energy and possibly mobilizing lipid and lean mass stores) and lower T3 levels on Bogoslof (indicating animals that were resting more). Findings from my study have implications for understanding the differing population trajectories of northern fur seals in the Bering Sea, and the role that nutritional differences may play.

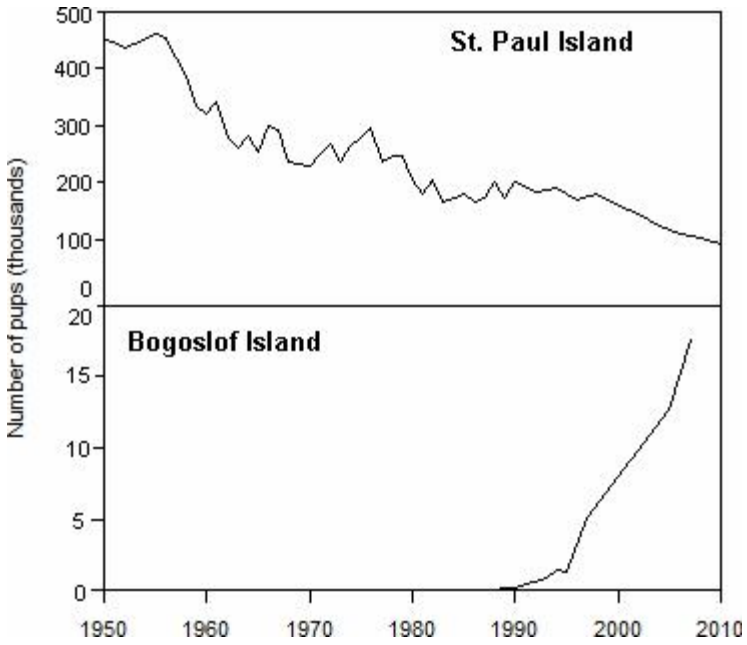

Figure 2.1 Number of northern fur seal pups born on St. Paul Island (Pribilof Islands) and Bogoslof Island from 1950-2000 (Allen & Angliss 2009).

2.3 Methodology

2.3.1 Field collection

Northern fur seal fecal samples (*n*=393 scats) were collected from Bogoslof and St. Paul in July 2009. Scats were collected from rookeries (used by breeding males and females), as well as from haulouts (used by adult and sub-adult males). Scat samples at all islands were stored in plastic bags. At St. Paul Island samples were frozen at -20°C immediately after collection and remained frozen until processing at the University of British Columbia (UBC). Samples collected at Bogoslof were stored at ambient temperature until they arrived at UBC where they were frozen at -20°C until processing.

We assessed the effects of different field storage methods of scat samples by collecting scat samples from captive northern fur seals at the Vancouver Aquarium (Vancouver, British Columbia, Canada). The samples from captive animals were collected from their haulouts and stored in plastic bags in either a -20°C freezer or a 4°C refrigerator for several weeks. We then transferred half of these samples from the aquarium for six weeks of storage in a -20°C freezer and kept the other half in an

outdoor environment that approximately simulated the July conditions on Bogoslof Island. At the end of 6 weeks, we processed the scat samples using the same techniques as for the field samples.

2.3.2 Laboratory processing

Before being sub-sampled for hormone analysis, the scats were thawed for 24 hours in a 4°C refrigerator. Samples were weighed and information regarding the consistency, color, collection date and location for each sample was recorded. Each scat sample was then passed through a sieve to prevent hard parts from passing through—and the resulting sub-sample was placed in a labeled plastic vial and stored at -20°C. The remaining scat sample, including hard parts, was subsequently stored in a 150 ml Nalgene jar and filled with water until cleaned to remove hard parts for prey identification.

All scat samples were soaked for a minimum of 24 hours before cleaning. The contents of each Nalgene jar were emptied into a sieve and the remaining soft matrix was carefully washed away until only hard parts remained. The collected hard parts were then dried for taxon identification (Trites, unpubl. data).

The sub-samples were lyophilized for 48 hours using a Labconco freeze-dryer. Each scat sample was then mixed with 70% ethanol (30% distilled water) and vortexed for 30 minutes, before being centrifuged at a speed of ~2200 rpm for 20 minutes. A subsample of the ethanol supernatant (2 ml containing hormones) was subsequently collected from each sample and stored in 2 ml Fisher plastic tubes until radioimmunoassays were performed.

Radioimmunoassays for glucocorticoid and T3 were performed on each fecal sample (i.e., on each 2 ml sample of ethanol supernatant). Standard curves for both hormones were produced using archived samples collected in 2008 from St. Paul and Bogoslof. Glucocorticoids were measured using the MP Biomedicals (07120102) Corticosterone 125I RIA Kit (Salon, Ohio) to determine the range of concentrations for each hormone. All assays were run in duplicate according to the

manufacturer's protocol with two exceptions: 1) all volumes were halved, and 2) we added three extra standards at the low end of the curve to increase sensitivity of the assay. To measure triiodothyronine, we used the MP Biomedicals (06b-254215) Total Triiodothyronine (total T3) Antibody Coated Tube 125I RIA Kit (Salon, Ohio). However, we failed to find a dilution using the manufacturer's protocol that would include the majority of the values in the standard curve regardless of the site or sex. We therefore used a dilution of 200:300 (sample: solution) for all the samples and gave any sample that was not detectable a value of 0.

Statistical analyses were undertaken using a Univariate Analysis of Variance with the statistical package SPSS 17. A Fisher's Least Significant Difference (LSD) post-hoc test was performed for each analysis, and an independent-samples *t*-test was used to analyze concentrations of hormone metabolites from scat samples collected from northern fur seals at the Vancouver Aquarium. All tests were considered statistically significant at the $p < 0.05$ level.

2.4 Results

Storage conditions (refrigerated versus non-refrigerated), expressed as mean, standard deviation, and *p* value) did not affect the concentrations of glucocorticoid metabolites or T3 metabolites (Table A.1). Glucocorticoid metabolites averaged 193.75 ng/g (± 118.88 SE, *n*=5) in refrigerated samples and 227.03 ng/g (±152.73 SE, *n*=5) in non-refrigerated fecal samples ($t = -0.17$, $p = 0.87$). There were also no significant differences for T3 metabolites in refrigerated 165.18 ng/g (± 158.34 SE, *n*=5) and non-refrigerated 8.31 ng/g (± 4.24 SE, *n*=5) fecal samples ($t = 0.99$, $p = 0.35$).

Hormone metabolite concentrations in fecal samples collected from St. Paul Island and Bogoslof Island ranged from 206.42 to 342.00 ng/g for glucocorticoids (Table A. 1) and from 210.31 to 697.04 ng/g for T3 (Table A. 2).

Glucocorticoid metabolite (Figure 2.2) concentrations of males averaged 233.12 ng/g (±33.96 SE, *n*=75) on Bogoslof and 207.08 ng/g (±34.40 SE, *n*=75) on St. Paul, while glucocorticoid concentrations of females averaged 207.43 ng/g

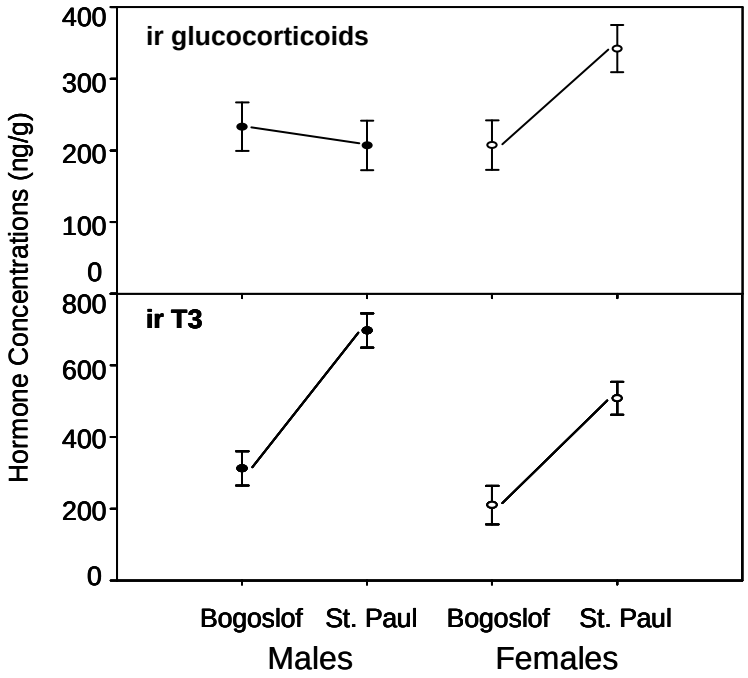

Figure 2.2 Mean and standard error of concentrations of immunoreactive glucocorticoids (n=307 scats) and T3 (n=294 scats) (nanograms/gram) in male and female northern fur seal scats from St. Paul Island and Bogoslof Island in July 2009. Data for St. Paul and Bogoslof are from Chapter 2. There was a significant interaction between location and sex for glucocorticoids, and main effects of sex and location for T3.

(±34.67 SE, *n*=74) on Bogoslof and 342.00 ng/g (±32.71 SE, *n*=83) on St. Paul. There was no main effect of location ($F_{1, 305} = 2.56$, $p = 0.11$) or sex ($F_{1, 305} = 2.59$, $p = 0.11$). However, the high concentration of glucocorticoid metabolites in the scats of female fur seals on St. Paul (compared with females on Bogoslof and males on either island) resulted in a significant statistical interaction between sex and location ($F_{1, 305} = 5.60$, $p < 0.01$) (Table A. 3).

T3 metabolite concentrations (Figure 2.2) of males averaged 312.81 ng/g (±47.68 SE, *n*=76) on Bogoslof and 697.04 ng/g (±48.00 SE, *n*=75) on St. Paul, while T3 metabolite concentrations of females averaged 210.31 ng/g (±53.66, *n*=60) on Bogoslof and 508.21 ng/g (±45.63 SE, *n*=83) on St. Paul. Mean T3 concentrations differed significantly between islands ($F_{1, 290} = 48.78$, $p < 0.01$), and between males and females ($F_{1, 290} = 9.00$, $p < 0.01$). However, unlike glucocorticoids, there was no interaction between sex and location ($F_{1, 290} = 0.78$, $p = 0.38$) (Table A. 4).

2.5 Discussion

The concentrations of glucocorticoid and T3 metabolites I found in the feces of northern fur seals are consistent with the hypothesis that northern fur seals are experiencing nutritional stress on St. Paul Island, but not on Bogoslof Island. Most notably, I found that lactating females on St. Paul Island had higher levels of glucocorticoid metabolites compared to lactating females on Bogoslof Island. I also found that the St. Paul females also had higher levels of T3 metabolites that were indicative of having higher metabolic rates, which might be associated with increased foraging effort. In contrast to the lactating females, male fur seals on both islands had comparable levels of glucocorticoid metabolites. However, the T3 metabolite levels were significantly higher among sub-adult males on St. Paul Island suggesting they were spending more energy to acquire adequate nutrition compared to the males using the haulouts on Bogoslof Island.

My presumption that the differing concentrations of hormone metabolites contained in the fur seal scats reflect differences between the two breeding islands in nutritional status is based on controlled studies of other species. It is therefore important to understand the nature of these studies and the caveats associated with age and sex to ensure that sound conclusions are drawn about the physiological and ecological meaning of hormone metabolite concentrations shed in the feces of northern fur seals and other animals.

2.5.1 Study limitations

A growing number of studies have measured the concentration of hormones in the feces of free-ranging animals to gain insights into how environmental stressors such as season, age, stage of reproduction, and human interaction affect excreted adrenal and thyroid hormones (Harper 2000, Huber *et al.* 2003, Mashburn & Atkinson 2004, Möstl *et al.* 2005, Touma & Palme 2005, Wasser *et al.* 2010). Collecting fecal samples is generally seen as a non-invasive means from which to assess the physiological status of an individual or population compared to capturing an animal to draw blood. However, there are a number of caveats that can

complicate interpreting the meaning of hormone concentrations in feces. For instance, only the metabolites of certain hormones are present in feces, and assay kits do not specifically measure these fecal metabolites. Basal hormone production is also a function of the sex and age of an individual, which is not necessarily known for fecal samples. Such limitations need to be considered when drawing conclusions about fecal hormone measurements.

It has been well-established that baseline plasma glucocorticoid concentrations vary according to sex (Handa *et al.* 1994, Hall *et al.* 1998, von Holst 1998, Teskey-Gerstl *et al.* 2000). These differences are also observed in fecal metabolites. Several studies have reported that females have higher concentrations of fecal glucocorticoids than males. This has been demonstrated for the common marmoset (Raminelli *et al.* 2001), northern muriqui (Strier *et al.* 2003), laboratory mouse (Touma *et al.* 2004), European hare (Teskey-Gerstl *et al.* 2000), domestic dog (Schatz & Palme 2001), African wild dog (Creel 2005), domestic cat (Schatz & Palme 2001), and cheetah (Wielebnowski *et al.* 2002). However, other studies have reported that males have higher fecal glucocorticoids than females in laboratory rats (Cavigelli *et al.* 2005), Steller sea lions (Hunt *et al.* 2004), and domestic chickens (Rettenbacher *et al.* 2004). Some studies have failed to find any significant differences between the sexes; in the wolf (Creel 2005), black rhinoceros, white rhinoceros (Brown *et al.* 2001), elk and red deer (Huber *et al.* 2003), and mourning dove (Washburn *et al.* 2003). It is not known whether the glucocorticoid concentrations differ between male and female northern fur seals, although the differences reported for Steller sea lions suggest they probably are sex specific.

I collected my fur seal fecal samples from rookeries and haulouts but was unable to confirm the sex associated with the individual scat samples. Uncertainty in the sex of the animal that produced the sample may alter the interpretation of both glucocorticoid and T3 metabolite concentrations. During summer, northern fur seals breed on rookeries that are populated by pups, females and territorial males. Each rookery tends to have a nearby haulout used primarily by immature males and non-breeding bulls. Pup scat is easily distinguished from adult scats—and breeding bulls tend to fast without defecating—while resting areas (haulouts) are generally

populated by males alone. Thus the consistent patterns of breeding behavior of northern fur seals combined with my relatively large sample of adult fur seal scat gives me confidence that most if not all of the scats I collected from rookeries were from lactating females while those from haulouts were from male fur seals based on where the samples were collected.

Age relative to sexual maturity and reproductive status may also influence hormone concentrations in plasma and fecal samples. For example, basal levels of plasma glucocorticoids have been shown to increase with age in rats (Sapolsky 1992), and fecal glucocorticoid metabolites are higher in males of species that compete for females such as wolves (Creel 2005), and capuchin monkeys (Lynch *et al.* 2002). Lactation, stage of pregnancy and the phase of estrous cycle have similarly been shown to influence fecal glucocorticoids in female chacma baboons (Weingrill *et al.* 2004), rats (Cavigelli *et al.* 2005), and spotted hyenas (Goymann *et al.* 2001). These factors have also been shown to significantly impact fecal T3 metabolites in the rat (Fukuda *et al.* 1980) and harbour seal (Haulena 1998). Serum T3 is also reported to be higher in young animals such as Steller sea lion pups (Myers *et al.* 2006) and juvenile captive gray seals (Boily 1996) compared to adults. Thus it appears that serum and fecal T3 decrease with age—and that plasma and fecal glucocorticoids tend to be higher among males during the breeding season, and vary (along with T3) among females according the phase of estrous cycle, stage of pregnancy, and lactation.

The scats I analyzed from rookeries can all be considered to have come from sexually mature females that were making regular trips to sea to feed and produce the milk needed by their pups on shore. However, I am less certain about the ages of the male fur seals on the haulouts and how they may have differed between islands. It is conceivable that the age structure of the two male populations may differ given that the St. Paul population has been declining for the past 30 years on St. Paul Island, while the Bogoslof Island population has been increasing at an exponential rate since the rookeries were established (Trites 1992, Towell & Ream 2006, NMFS 2007) . However, the age structures of sub-adult males (ages 2-8y) should not differ between the two islands if the population decline on St. Paul

continues to be caused by a higher mortality of juveniles (4 mo – 2 y) (Trites & Larkin 1989, NMFS 2007). Thus, it seems reasonable to assume that the two islands contained similar proportions of 2–8 y old northern fur seals on the bachelor beaches.

Despite the complex effects that age, gender, and reproductive status can have on hormone concentrations, the typical physiologic processes associated with glucocorticoids and T3 are generally well understood in mammals. For example, an animal that encounters stressors (environmental or psychological) will experience a cascade of physiological events, including the secretion of cortisol from the adrenal gland (Sapolsky *et al.* 2000). This will in turn increase the concentration of glucocorticoids in blood, and be later expelled as glucocorticoid metabolites in feces. Thyroid hormones are secreted from the thyroid gland, and help to regulate metabolism. Thyroxine (T4) is the primary thyroid hormone present in blood, but it is converted to active T3 by deiodinase (Bernal & Refetoff 1977) and is later shed as T3 metabolites in feces. An increase in T3 is known to be associated with an increase in the consumption of oxygen and energy (Peterson 1965). This is effectively an increase in the basal metabolic rate which could occur if an animal increased its energy expenditure. A drop in T3 on the other hand reflects a drop in energy expenditure as might occur if an animal was fasting (Spencer *et al.* 1983, Reyns *et al.* 2002). Taken together, glucocorticoids and thyroid hormones such as T3 can help identify whether elevated stress in an animal is related to nutrition, or some other factor.

Increasing numbers of studies have measured glucocorticoids metabolites in feces to assess the relative levels of stress experienced by wild, captive and domesticated animals (Harper 2000, Teskey-Gerstl *et al.* 2000, Schatz & Palme 2001, Washburn *et al.* 2003, Hunt *et al.* 2004, Millspaugh & Washburn 2004, Cavigelli *et al.* 2005, Deagle *et al.* 2005, Touma & Palme 2005, Wasser *et al.* 2010). Other studies have measured the metabolites of thyroid hormones (T3) in feces to identify nutritional deficits (van der Heyden *et al.* 1986, Eales 1988, Wasser *et al.* 2010). Measuring hormone concentrations shed in feces is a non-invasive means to monitor the physiological health of individuals (compared with capturing and

restraining animals to measuring circulating levels of hormones in blood). Simultaneously measuring hormone metabolites of T3 and glucocorticoids in feces should therefore identify populations that are stressed and determine whether the stress is nutritionally related.

Measuring hormone metabolites in feces is a good way to monitor the typical and dysfunctional physiological processes in an animal, and has been successfully done in several studies (Harper 2000, Huber *et al.* 2003, Hunt *et al.* 2004, Mashburn & Atkinson 2004, Millspaugh & Washburn 2004, Möstl *et al.* 2005, Wasser *et al.* 2010). However, careful experimental design and interpretation of results is necessary when analyzing fecal samples (Palme 2005). One reason for this is that it is generally the metabolites of hormones rather than the hormones themselves that tend to be found in feces. Measuring these metabolites can be challenging considering commercial kits are designed to measure un-metabolized steroid in plasma, not metabolized hormone in feces (Touma & Palme 2005). Furthermore, unlike analyzing plasma, measuring these metabolites in feces does not reflect hormone concentrations in an organism at a particular time, but represents instead a longer-term hormonal profile.

I appreciate that age, gender, and reproductive status are associated with changes in both glucocorticoids and thyroid hormone levels in many animals, and that careful interpretation of hormone data is necessary. I am confident, however, that samples collected form rookeries came from females that were reproductively mature and at about the same reproductive status. I am equally confident that the samples collected from haulouts came from sub-adult males (2-8 y), and believe that the age structures should be similar between islands (based on the assumption that the decline on St. Paul is associated with a higher mortality of juveniles (<2 y old). And finally, I feel that hormone concentrations shed by fur seals in feces can help to quantify stress in an animal (from glucocorticoids), and can help reveal whether that stress may be related to nutrition (from thyroid hormones such as T3). Thus, given careful design and interpretation, measuring hormone concentrations in fecal samples can provide insights into the physiological status of the two northern fur seal populations and possible explanations for the different population trajectories.

2.5.2 Nutritional status of northern fur seals in Alaska

The concentrations of glucocorticoid and T3 metabolites I measured in feces of northern fur seals suggest that the declining population of lactating females breeding on St. Paul Island was experiencing nutritional stress compared to the increasing population breeding on Bogoslof Island. However, I did not detect a significant difference in glucocorticoid metabolites among males using haulouts on the two islands. Instead I found a difference in T3 concentrations suggesting that the declining population of males on St. Paul Island was working harder for their food compared to males on Bogoslof Island. These results suggest that sub-adult males and lactating females at St. Paul Island experienced poorer feeding conditions than at Bogoslof Island, but that only the females were nutritionally stressed. The elevated concentrations of glucocorticoids on St. Paul Island is consistent with the early study that reported elevated concentrations of glucocorticoid metabolites in feces from fur seals on St. Paul compared to Bogoslof, and that these increased stress levels were associated with nutrition (Hunt *et al.* 2008).

Female fur seals have distinct diets and foraging strategies that differ between St. Paul and Bogoslof Islands. During my study, the fur seals on St. Paul ate mostly pollock, while those on Bogoslof ate mostly northern smooth-tongue (*Leuroglossus schmidti*) and squid (Trites, unpublished data). Lengths of feeding trips for lactating females averaged 7.9 days on St. Paul only 3.3 days on Bogoslof Island (Battaile, Nordstrom & Trites, unpubl. data). Consequently, lactating northern fur seals on St. Paul Island traveled twice as far and spent twice as much time obtaining a diet that was less energetically dense than that of fur seals on Bogoslof Island. Longer foraging trips could have a negative effect on nursing pups which would have to fast for longer periods on St. Paul than pups on Bogoslof.

The diet of sub-adult males (ages 2-8 y) on St. Paul Island appears to be similar to that of lactating females (Call & Ream 2012). As with females, sub-adult males primarily consumed small walleye pollock followed by cephalopods, Pacific salmon, sand lance, and Pacific herring. Sub-adult males also traveled far from St.

Paul to feed, but spent more time at sea compared with lactating females (Ream *et al.* 2005). Similar tracking data is not available for sub-adult males from Bogoslof Island, but diet data from 2009 showed male fur seals on St. Paul ate mostly pollock, while those on Bogoslof ate mostly northern smooth-tongue and squid (Trites, unpubl. data). Thus it appears that males have the luxury of staying longer at sea to meet their energetic needs, while females are constrained by the need to return to the rookery to nurse their pups.

Coupling my hormone data with the ecological data on diets and foraging behavior provides a physiological perspective on the condition of fur seals breeding on St. Paul and Bogoslof Islands. The elevated T3 metabolites for example suggest that lactating females on St. Paul were catabolizing their lipid and lean mass stores, resulting in poor body condition. The elevated T3 metabolites in sub-adult males hauling out on St. Paul may indicate that males were also expending more energy than sub-adults on Bogoslof, likely as a result of longer feeding trips. All told, the combined elevated levels of T3 and glucocorticoid metabolites suggest that lactating females were experiencing nutritional stress during summer on St. Paul Island, but not at Bogoslof Island; while males appeared to be working harder on average to acquire food at St. Paul compared to males at Bogoslof, but cannot be considered to have been stressed on either island based on their glucocorticoid metabolite levels.

2.5.3 Consistency of hormone concentrations among species

My interpretations of hormone concentrations for fur seals are consistent with those of other species for glucocorticoids, but not for T3. I found higher levels of T3 metabolites in lactating females and assumed they were indicative of higher metabolic rate that might be associated with a hunger response and increased foraging effort. However, others have shown that T3 levels decrease when animals fast. No previous study has explored the response of T3 in animals that are food-restricted *and* highly metabolically active.

T3 is a thyroid hormone that influences metabolism and is in theory an indirect proxy of nutritional status. It has been shown that glucocorticoid increases

and T3 decreases in response to fasting in tufted puffin chicks (Kitaysky *et al.* 2005), chickens (Geris *et al.* 1999), and white-tailed deer (Bahnak *et al.* 1981). All of these studies manipulated food alone and did not consider the confounding influence of activity.

There are few studies that demonstrate elevated T3 levels after exercise, and even fewer that document this phenomenon during nutritional stress. A human study reported an increase in free T3 when subjects were involved in exercise using 45% to 70% of maximum heart-rate (Ciloglu *et al.* 2005). Another study in humans found that T3 rose significantly during maximal physical exercise in medium and long distance runners and divers (Schmid *et al.* 1982). I therefore believe that elevated T3 among lactating northern fur seals is due to intensive foraging; however the lack of evidence in non-humans leaves some uncertainty.

A wild animal that is undernourished (as opposed to starved) is likely to increase its metabolism and undergo a hunger response as it attempts to acquire additional energy to meet shortfalls. A fasting response on the other hand would result in an animal reducing its metabolism to save what remaining energy it has. I thus suspect that the elevated T3 in lactating northern fur seals reflects active foraging and the need to find food and produce the milk required by their pups waiting for them onshore. Reducing metabolism in order to conserve energy should result in a decrease in thyroid hormones, but such a strategy would not make sense given the need of the mother to provision for her pup. Unfortunately there is little or no evidence in previous literature concerning animals that are food-restricted, but still have high activity levels. In the aforementioned T3 studies, the tufted puffins were nest bound, and thus not using much energy (Kitaysky *et al.* 2005), and the chickens were caged and again did not have a particularly high activity level (Geris *et al.* 1999) in comparison to a foraging fur seal. In my study, lactating northern fur seals that have nursing pups cannot reduce their activity level to conserve energy. Therefore the high T3 I found in female fur seals on St. Paul Island is likely due to the increased energy expended for long foraging trips.

2.5.4 Conclusion

The National Marine Fisheries Service issued a conservation plan for northern fur seals (NMFS 2007) that outlined several non-natural potential causes for the decline of the Pribilof population. Among the long list of possible suspects are environmental change, commercial harvest, subsistence harvest, commercial fishing, human presence and coastal development, and other human-related disturbances. Environmental change and commercial fishing are the largest factors that are likely to contribute to nutritional stress in northern fur seals. However, there has not been any tangible data to test whether these fur seals were indeed nutritionally stressed.

My study demonstrates that relevant biological information about the physiological status of northern fur seals can be attained from measuring the concentration of hormone metabolites shed in the feces of free-ranging animals (when proper methodology is adhered to and limitations considered). My physiological data are consistent with the hypothesis that lactating females from St. Paul Island have been experiencing nutritional stress during summer. This could contribute to the population decline of northern fur seals on St. Paul Island if it means that dependent pups ultimately acquire insufficient milk and wean in less than optimum body condition.

Chapter 3: Northern fur seals differ physiologically in terms of stress and nutritional status in California compared to northern populations in Alaska

3.1 Summary

Northern fur seals (*Callorhinus ursinus*) migrate throughout the North Pacific, but breed on offshore islands located at the northern limits of their range in Alaska and Russia, with one exception in California. This southern population on San Miguel Island has grown in a hot arid climate since discovered in 1968, despite incurring major population crashes during El Nino events. The goal of our study was to determine whether the San Miguel Island population breeding at the southern limit of the fur seal range differed physiologically in terms of stress and nutrition compared to the northern populations in Alaska. We collected 88 fecal samples from male and female northern fur seals in July 2009 on San Miguel Island to measure their concentrations of glucocorticoids (stress hormones) and triiodothyronine-T3 (a thyroid hormone involved in metabolism). We compared these concentrations with similar measures from St. Paul and Bogoslof Islands, and found that adult females had higher levels of glucocorticoids on San Miguel Island compared to either male or female fur seals in Alaska. We suspect the high glucocorticoid levels reflect heat stress associated with bulls preventing breeding females free access to the water to cool off. Forced confinement is consistent with the females also having low T3 levels (comparable to those seen on Bogoslof Island) that are indicative of resting animals. Hormone concentrations of males were also higher on San Miguel than on the Alaskan islands and also imply heat stress. Overall, our data suggest that northern fur seals in California experience greater stress (as reflected by glucocorticoids) associated with hot air temperatures rather than the possible nutritional limitations experienced by the declining population on St. Paul Island (as reflected by T3).

3.2 Introduction

Historically, northern fur seals (*Callorhinus* ursinus) were known to migrate throughout the North Pacific but breed only at the northern limits of their range on the Pribilof Islands of Alaska and the Kuril and Commander Islands in Russia (Allen & Angliss 2009). In the 1950's or early 1960's, a small breeding colony was established at San Miguel Island, California, that defined the southern limit of the northern fur seal breeding range (DeLong 1982). In 1980, another new breeding colony of northern fur seals was discovered on Bogoslof Island, Alaska located about 300 km southeast of the Pribilof Islands (Lloyd *et al.* 1981). Today, the Pribilof populations continue a 3-decade long decline, while the smaller Bogoslof population increases exponentially and the San Miguel population fluctuates between exponential growth and catastrophic declines associated with re-occurring El Nino events (Figure 3.1) (DeLong 1982, Melin *et al.* 2005, Allen & Angliss 2009). Explanations for the differing population trends include differences in terrestrial climate, ocean conditions, and the quantities and qualities of food available to animals (NMFS 2007).

Northern fur seals are adapted to cold environments with fur that acts as an insulator against cold air and water temperatures. Thermoregulation is an important physiological process that allows them to regulate their body temperature. Most of their terrestrial habitats are characterized by cool air temperatures, frequent fog or rain, and rocky substrate that keep the animals cool during breeding season. The exception is San Miguel Island where mean air temperatures during the fur seal breeding season are warmer (12.6 – 21.4º C) than the Alaska or Russian colonies (6.1 – 10.1º C for St. Paul). Fur seals easily overheat when exposed to direct sunlight and warm air temperatures, and can either enter the water to cool down, or can pant and wave their hind flippers to regulate their body temperature without going into the water (DeLong 1982).

Fur seals can be physiologically stressed by weather conditions or changes in the distribution or availability of their prey in their foraging range. Breeding fur seals

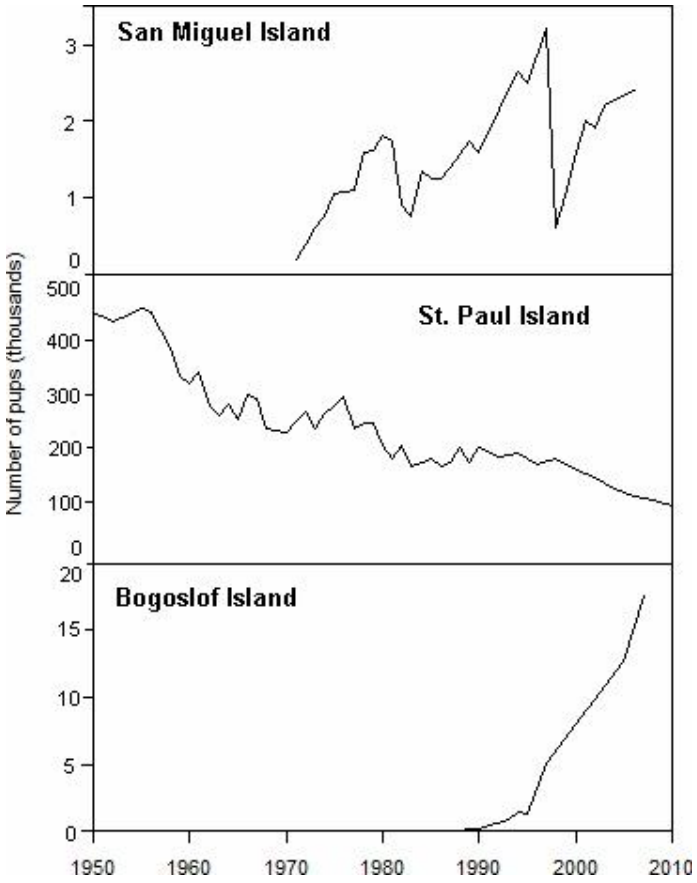

Figure 3.1 Number of northern fur seal pups born on San Miguel, St. Paul, and Bogoslof Islands from 1950-2000 (Allen & Angliss 2009).

in Alaska feed mostly in the Bering Sea ecosystem which is dominated by seasonally predictable prey resources and cold sea surface temperatures whereas San Miguel Island fur seals feed in the California Current, a seasonally variable boundary current that is influenced strongly by the El Niño Southern Oscillation (ENSO) that results in temporary declines in fur seal prey availability for a year or longer. Diets differ between the breeding colonies in terms of species consumed and energy densities of their primary prey species (Antoneli & Perez 1984, Antonelis *et al.* 1997, Zeppelin & Ream 2006).

Understanding and predicting changes in numbers and limits to distributions of marine mammals such as northern fur seals cannot be done from simple counts alone and requires additional information about the diet, health and physiological condition of individuals. One means of gaining such insight into the status of

individuals relative to the size and distribution of their populations is by measuring the concentrations of hormone metabolites that individual animals shed in their feces (scats) in response to changes in their immediate environments. This technique has been widely applied to terrestrial species (Harper 2000, Wasser *et al.* 2000, Raminelli *et al.* 2001, Huber *et al.* 2003, Lynch *et al.* 2003, Möstl *et al.* 2005) and is being applied with increasingly frequency to marine mammals (Wasser *et al.* 2000, Hunt *et al.* 2004, Mashburn & Atkinson 2004, Deagle *et al.* 2005).

Two hormones that can provide insight into the physiological status of an individual are glucocorticoids (stress hormones) and T3 (a thyroid hormone). Glucocorticoids are released by animals in response to changes in such things as social status, dominance, aggression, and reproductive status — and have been previously measured in fecal samples to assess stress in free-ranging and captive animals (Minton 1994, Hunt *et al.* 2004, Mashburn & Atkinson 2004, Creel 2005, Palme 2005, Touma & Palme 2005, Keech 2008). Thyroid hormones have also been measured in feces, and are involved in maintaining basal metabolic rate as well as oxygen and energy consumption (Keech 2008, Wasser *et al.* 2010). Concentrations of these hormone metabolites shed by animals in feces can provide information on the physiological condition of an individual relative to diet, stress, climate, nutrition, and other important physiological processes — and can be used to help understand changes in population numbers and distributions.

The goal of our study was to assess the physiological states of fur seals that breed on San Miguel Island relative to those that breed in Alaska. In particular, we wanted to know whether the San Miguel Island population breeding at the southern limit of the fur seal range differed physiologically in terms of stress and nutrition compared to the breeding populations near the northern limits of their range. We collected fecal samples from male and female northern fur seals on San Miguel Island to measure and compare with the concentration of glucocorticoids and T3 hormones shed by northern fur seals at St. Paul Island and Bogoslof Island. We predicted that glucocorticoid concentrations would be higher in fur seals breeding on San Miguel due to heat and climate. We also expected to find lower T3 levels on San Miguel consistent with fur seals reducing their metabolic expenditure to prevent

overheating. Findings from this study have implications for understanding important physiological characteristics that distinguish three populations that span the geographic and demographic range of northern fur seals in North America. They also provide baseline measures of physiological status that can be used in future assessments of population status and management.

3.3 Methodology

Methods used to collect and process the San Miguel fecal samples are as described in Chapter 2 for scats collected on St. Paul and Bogoslof Islands. In brief, 88 northern fur seal fecal samples (scats) were collected on San Miguel Islands in July 2009 from the rookery (used by breeding males and females) and the adjoining haulout (used by adult and sub-adult males). The scats were sealed in plastic bags and stored in ambient conditions until shipment to NOAA, Seattle, where they were stored at -20°C. Scats were sub-sampled and fecal metabolite hormones were measured by radioimmunoassay. The remaining scat sample was cleaned to identify prey items from recovered hard parts.

Sub-samples were freeze-dried for 48 hours and mixed with 70% ethanol, then vortexed and centrifuged. Radioimmunoassays for glucocorticoid and T3 were performed on a subsample of the ethanol supernatant, and standard curves were produced using archived fecal samples collected in 2008 from St. Paul and Bogoslof. MP Biomedicals (07120102) Corticosterone 125I RIA Kit (Salon, Ohio) was used to measure glucocorticoids, and all assays were run in duplicate. MP Biomedicals (06b-254215) Total Triiodothyronine (total T3) Antibody Coated Tube 125I RIA Kit (Salon, Ohio) was used to measure triiodothyronine with a dilution of 200:300 (sample: solution) (See Chapter 2 for further details).

We compared the concentrations of hormone metabolites from San Miguel Island with those previously reported from fur seals breeding in 2009 on Bogoslof Island and St. Paul Island (Chapter 2). Statistical comparisons were undertaken using a Univariate Analysis of Variance (SPSS 17), and post hoc tests were done using Fisher's Least Significant Difference (LSD). All tests were considered statistically significant at the $p < 0.05$ level.

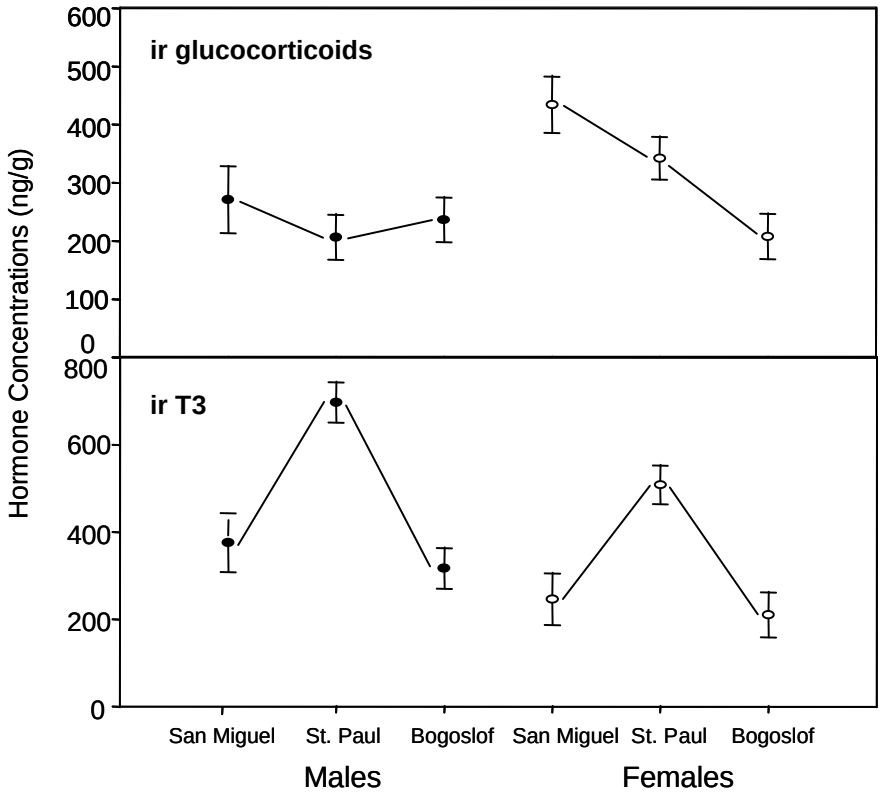

Figure 3.2 Mean and standard error concentrations (nanograms/gram) of immunoreactive glucocorticoids (n =393) (Top panel) and T3 (n = 277) (Lower panel) in male and female northern fur seal scats collected from St. Paul, Bogoslof, and San Miguel Islands in July, 2009. There was a main effect of location for glucocorticoids, and main effects of sex and location for T3.

3.4 Results

Mean T3 concentrations did not differ significantly between males and females on San Miguel Island, however glucocorticoids of females were significantly higher than males (Figure 3.2, Table 3.1). Overall, the mean glucocorticoid metabolite concentrations of males and females was significantly different between St. Paul, Bogoslof, and San Miguel Islands ($F_{2, 385} = 3.56$, p=0.03). There was also a significant difference between male and female fur seals ($F_{1, 385} = 5.64$, p=0.02), but no significant statistical interaction between sex and location ($F_{2, 385} = 2.79$, p = 0.06) (Table A. 7). Mean T3 metabolite concentrations differed significantly between islands ($F_{2, 371} = 22.09$, $p < 0.01$), and between males and females ($F_{1, 371} = 4.44$, p=0.036). Again, there was no interaction between sex and location ($F_{2, 371} = 1.08$, p=0.342) (Table A. 8).

Table 3.1 Mean glucocorticoid (n = 393 scats) and T3 (n = 277) concentrations (nanograms/gram) of male (M) and female (F) northern fur seal fecal samples from San Miguel, St. Paul, and Bogoslof Islands with associated samples sizes (n) and standard errors (SE).

Location	Sex	*n*	Mean (ng/g) glucocorticoids	SE	*n*	Mean (ng/g) T3	SE
San Miguel	M	34	270.81	57.18	35	375.8	75.45
San Miguel	F	52	414	48.12	49	362.85	64.42
St. Paul	M	75	206.42	38.49	75	697.04	51.54
St. Paul	F	83	342	36.6	83	508.21	49
Bogoslof	M	75	233.12	38	76	312.81	51.2
Bogoslof	F	74	207.43	38.75	60	210.31	57.62

3.5 Discussion

The concentrations of glucocorticoid and T3 metabolites I found in the feces of northern fur seals suggest that the three populations of northern fur seals differed physiologically. Most notably, the San Miguel fur seals had higher glucocorticoid metabolite levels than either population in Alaska — with San Miguel females having the highest stress levels of any other group. In contrast to glucocorticoids, the T3 metabolite concentrations of males and females were similar on San Miguel and were comparable to levels on Bogoslof Island. However, T3 concentrations on San Miguel and Bogoslof were significantly lower than on St. Paul Island.

All told, my data suggest that fur seals on San Miguel are the most physiologically stressed of the North American fur seal populations (based on elevated levels of glucocorticoid metabolites), but that the stress is unlikely related to food (based on their low T3 metabolites). Instead, my data suggest that fur seals (particularly females) reduced their metabolic expenditure to prevent overheating on San Miguel. Heat stress is known to increase glucocorticoids in farm animals (Minton 1994) and laboratory animals (Palme 2005). I believe that heat stress similarly explains the high glucocorticoid levels recorded in northern fur seals on San Miguel Island and is consistent with regional differences in climate between California and Alaska.

Climate conditions in 2009 were not typical at San Miguel. There was an anomalous oceanographic event that included the strongest negative upwelling observed in 40 years as well as increased sea surface temperatures (Melin *et al.* 2010). While northern fur seals seemed to be mostly unaffected (Melin, pers. comm.), there was unprecedented mortality among California sea lion (*Zalophus californianus*) pups on San Miguel. It is therefore possible that the high concentrations of glucocorticoids I observed in this population in 2009 were associated with the unusually warm conditions that year. With the exception of El Niño years, the typical difference in air temperatures between Alaska and California result in different thermoregulation responses, but are not believed to have population level effects and are managed behaviorally (DeLong 1982).

It is important to recognize that measuring hormone metabolites in feces is a technique that merits careful interpretation. For instance, season, sex, age, stage of reproduction, and human interaction can all influence excreted adrenal and thyroid hormones in mammals and other species (Sapolsky 1992, Handa *et al.* 1994, Hall *et al.* 1998, von Holst 1998, Harper 2000, Teskey-Gerstl *et al.* 2000, Huber *et al.* 2003, Mashburn & Atkinson 2004, Cavigelli *et al.* 2005, Creel 2005, Möstl *et al.* 2005, Touma & Palme 2005, Wasser *et al.* 2010). However, northern fur seals breed on rookeries that are populated by pups, adult females and territorial adult males, and each rookery tends to have a nearby haulout used primarily by immature males and non-breeding adult males. Pup scat is easily distinguished from adult scats by size and color, and territorial adult males fast while holding territory and rarely defecate—so it is unlikely that the samples were from territorial adult males. Thus, I am confident that most if not all of the scats collected from rookeries were from adult females and those from haulouts were from immature or non-breeding adult male fur seals based on where the samples were collected. Despite the complex effects that age, gender, and reproductive status can have on hormone concentrations and the challenge associated with extraction from feces, I believe my data reflect an accurate representation of the physiological parameters assessed in northern fur seals breeding in Alaska and California in summer 2009.

3.5.1 Physiological status of fur seals in Alaska vs. California

Northern fur seals breeding at the southern limit of their range on San Miguel appear to experience higher levels of stress than those breeding in Alaska based on the concentrations of hormone metabolites I measured in feces collected during the summer breeding season. However, unlike the Pribilof population in Alaska (Chapter 2), I suspect the stress on San Miguel is related to high on-shore air temperatures (i.e., thermal stress) rather than poor nutrition (based on T3 metabolite concentrations).

The climate in southern California during the breeding season of northern fur seals is drastically different from than that of Alaska. During July when pups are born and adult males are holding territories (Peterson 1965, DeLong 1982, Gentry 1998), air temperatures on San Miguel Island may be twice as warm as on St. Paul Island. Average July air temperatures ranged 12.6 – 21.4º C on San Miguel Island (1971-2000; NOAA Western Regional Climate Center http://www.wrcc.dri.edu/cgi-bin/cliMAIN.pl?ca7803+sca) compared with just 6.1 – 10.1º C on St. Paul Island (1950-2005; NOAA Western Regional Climate Center). Summer weather on St. Paul Island is also typified by fog and drizzle, which reduces thermal stress on adults. In 2009, mean air temperature (June – August) at San Miguel Island was 18.8°C (SD=1.5°C) (http://www.wrcc.dri.edu/cgi-bin/cliMAIN.pl?ca7803+sca) compared to 9.9°C (SD=3.1°C) at St. Paul Island (http://weather-warehouse.com/WeatherHistory/PastWeatherData_StPaulIslandArpt_SaintPaulIsland_AK_August.html) supporting the idea that the higher glucocorticoid levels in San Miguel fur seals was related to thermoregulatory challenges of living in a warmer climate.

Fur seals that are stressed by heat can compensate to some extent by making behavioral and physiological adjustments. They can lower their metabolism, increase blood flow to their limbs and body surface, begin panting, and fan their hind flippers. They can also stay cool by entering the surf or tide pools to wet their fur (Bartholomew & Wilke 1956, Ohata & Miller 1977, DeLong 1982). Air temperatures of 20º C can be lethal to pups unless they can cool themselves, such as by wetting their fur (Trites & Antonelis 1994) as observed on San Miguel Island where pups that

stayed on the hot sand began to convulse and died (DeLong 1982). Behavioral adjustments indicative of heat stress such as panting and flipper-fanning are regularly observed among adult males and females at all the rookeries but at San Miguel Island animals spend a greater amount of time in these behaviors (Melin pers. comm.).

The limited dietary data available from northern fur seals on San Miguel are also consistent with the idea that animals were stressed on San Miguel because of heat and not diet. Prey remains recovered from fecal samples suggest that diets of young males and lactating females on San Miguel have consistently been dominated by Pacific hake (*Merluccius productus)*, northern anchovy (*Engraulis mordax*), Pacific sardine (*Sadinops sagax*) , and market squid (*Loligo opalescens*) (Melin pers. comm.). In terms of relative energy density content of their primary prey species (Logerwell & Schaufler 2005, Whitman 2010, Zeppelin & Orr 2010, Vollenweider *et al.* 2011), this diet is similar to that of fur seals on Bogoslof Island (that ate primarily northern smoothtongue *Leuroglossus schmidti* and squid; Trites unpubl. data), but superior to that of fur seals on St. Paul Island (that ate primarily juvenile walleye pollock *Theragra chalcogramma*; Trites unpubl. data).

Males and females do not need to feed with equal frequency. Males can presumably spend longer at sea than females because they do not have a dependent pup to return to, and they do not need to build up lipid stores for lactation. Little is known about male foraging cycles, but one study reported that juveniles males from St. Paul spent an average of 17.0 days (SD = 1.22 d) at sea in 1999 and 2000 (Sterling & Ream 2004). This was over twice as long as feeding trips recorded for lactating females that shared the same island (Nordstrom & Trites unpubl. data).

While diets differed significantly between St. Paul and San Miguel in terms of species and energy density, the duration of feeding trips by lactating females was similar (an average of 7.5 days for San Miguel and 7.9 days ± 2.25 for St. Paul; Battaile & Trites unpubl. data, Melin, pers. comm.). However, female foraging trips appear to have increased on St. Paul from an average of 5.7 days (SD = 2.5 d, *n*=173 trips) in the 1980s (Loughlin *et al.* 1987) to 7.5 days in the 2000s, whereas

trip length has not changed on San Miguel (Melin, pers. comm.). This, combined with the concentrations of T3, suggests that the high levels of stress expressed by fur seals reflects a nutritionally poorer diet on St. Paul and greater thermal stress on San Miguel. Only on Bogoslof do conditions appear to be optimal for northern fur seals, which had the lowest T3 and glucocorticoid levels, as well as the shortest feeding trips (3.3 days ± 1.90) and nutritionally superior diet (Battaile & Trites, unpubl. data).

3.6 Conclusions

We sought to compare the physiological status of a population of northern fur seals breeding at the southern extreme of their range to two populations in the Bering Sea. All told, the available hormone, diet, and foraging data from northern fur seals in the North Pacific are consistent with female northern fur seals on San Miguel Island experiencing heat stress, and female northern fur seals on St. Paul experiencing nutritional stress. Our data further demonstrate that relevant biological information can be attained from free-ranging animals using fecal samples. Diet and hormone analysis from feces can be used to gain insight into the status of other seal and sea lion populations between years and across geographic ranges. Northern fur seals, like other top predators, contribute to maintaining a healthy, balanced marine ecosystem. Assessing their populations in terms of stress and diet could provide insight into the relative health of their ecosystems.

Chapter 4: General conclusions

The overall goal of my thesis was to assess the physiologic status of three breeding populations of northern fur seals in North America in July 2009 using hormone, diet and foraging data. I was particularly interested in gaining insight into why the Pribilof population was declining while the Bogoslof population was increasing. I also wanted to explore how the population breeding on San Miguel has managed to survive despite the extreme and variable climate they experience there. I thus collected fecal samples from rookeries and haulouts on all three islands and extracted hormones from sampled feces to measure glucocorticoids and T3 by radioimmunoassay. I also used hard parts from scat samples to characterize the relative energy density of prey consumed by males and females on each Island. Finally, I compared foraging behavior between lactating females on the three islands.

The concentrations of glucocorticoid and T3 metabolites in feces of northern fur seals revealed that the declining population of lactating females breeding on St. Paul Island was likely experiencing nutritional stress compared to the increasing population breeding on Bogoslof Island. However, I did not detect a significant difference in glucocorticoid metabolites among males using haulouts on these two islands. Instead I found a difference in T3 concentrations suggesting that the declining population of males on St. Paul Island was working harder for their food compared to males on Bogoslof Island. These results suggest that sub-adult males and lactating females on St. Paul Island experienced poorer feeding conditions than at Bogoslof Island, but that only the females were nutritionally stressed.

Dietary and foraging data were consistent with hormone data in supporting the idea that female northern fur seals were nutritionally stressed in July 2009. During my study, the fur seals on St. Paul ate mostly pollock, while those on Bogoslof ate mostly northern smooth-tongue and squid. Lengths of feeding trips for lactating females averaged 7.9 days on St. Paul, and only 3.3 days on Bogoslof Island. Consequently, lactating northern fur seals on St. Paul Island traveled twice

as far and spent twice as much time obtaining a diet that was less energetically dense than that of fur seals on Bogoslof Island. Longer foraging trips could have a negative effect on nursing pups which would have to fast for longer periods on St. Paul than pups on Bogoslof.

The concentrations of glucocorticoid and T3 metabolites I found in the feces of northern fur seals on San Miguel revealed that this population differs physiologically in terms of stress and nutritional status compared to the northern populations in Alaska. Most notably, the San Miguel fur seals had higher glucocorticoid metabolite levels than either population in Alaska— with San Miguel females having the highest stress levels of any other group. In contrast to glucocorticoids, the T3 metabolite concentrations of males and females were similar on San Miguel and were comparable to levels on Bogoslof Island. However, T3 concentrations on San Miguel and Bogoslof were significantly lower than on St. Paul Island. Thus, San Miguel were the most physiologically stressed of the North American fur seal populations (based on elevated levels of glucocorticoid metabolites), but that the stress was unlikely related to food (based on their low T3 metabolites). Instead, the data are consistent with regional differences in climate and suggest that fur seals (particularly females) were heat stressed on San Miguel and reduced their metabolic expenditure to prevent overheating.

The limited dietary data available from northern fur seals on San Miguel are also consistent with the idea that animals were stressed on San Miguel because of heat and not diet. Prey remains recovered from fecal samples suggest that diets of young males and lactating females on San Miguel have consistently been dominated by Pacific hake, northern anchovy, and market squid (Melin, pers. comm.). In terms of relative energy density content (Logerwell & Schaufler 2005, Whitman 2010, Vollenweider *et al.* 2011), this diet was similar to that of fur seals on Bogoslof Island (that ate primarily northern smoothtongue and squid; Trites unpubl. data), but superior to that of fur seals on St. Paul Island (that ate primarily juvenile walleye pollock; Trites unpublished data).

While diets differed significantly between St. Paul and San Miguel in terms of species and energy density, the duration of feeding trips by lactating females was similar (approximately a week) (Battaile & Trites, unpubl. data, Melin, pers. comm.). However, female foraging trips appear to have increased on St. Paul from an average of 5.7 days in the 1980s (Loughlin *et al.* 1987) to 7 days in the 2000s, whereas trip length has not changed on San Miguel (Melin, pers. comm.). This, combined with the concentrations of T3, suggests that the high levels of stress in fur seals reflected a nutritionally poorer diet on St. Paul and greater thermal stress on San Miguel. Only on Bogoslof did conditions appear to be optimal for northern fur seals, which had the lowest T3 and glucocorticoid levels, as well as the shortest feeding trips (3 days) and nutritionally superior diet (Battaile & Trites unpubl. data).

4.1 Study limitations

The kits I used for both glucocorticoid and T3 radioimmunoassays had cross-reactivity with other compounds (besides glucocorticoids and T3) which could have bound with the antibody and counted towards the hormone metabolite concentrations. Ideally, any such compounds would have been identified and quantified using a technique such as high-performance chromatography, or HPLC. Unfortunately I did not have access to the equipment to perform this analysis, and this factor may have influenced the hormone data.

Although there were no significant differences in hormone metabolites between storage methods (refrigerated vs. non-refrigerated) in the fecal samples from female northern fur seals from the Vancouver Aquarium, it would have been ideal to have consistent storage of my samples collected from St. Paul, Bogoslof, and San Miguel. It was not possible to refrigerate fecal samples at Bogoslof and San Miguel, which could confound directly comparing them to scats that had previously been frozen. Until refrigeration is available at these study sites, a consistent method, such as storage in ethanol or methanol, should be considered.

The female foraging data collected by Brian Battaile and Chad Nordstrom as well as the diet analysis for animals on St. Paul and Bogoslof were both critical

pieces of data that supported the hormone data and the conclusions that were drawn about the physiological status of northern fur seals from these populations. I was fortunate to have Sharon Melin to provide diet and foraging data from San Miguel. Unfortunately, detailed dietary information from the scats collected in 2009 was not available to make a detailed comparison with diets of fur seals breeding on St. Paul and Bogoslof Islands.

4.2 Research applications and future directions

Monitoring the concentrations of hormones shed by marine mammals can provide information to resource managers about environmental change and the effects of commercial fishing — the two largest factors that are likely to contribute to nutritional stress in northern fur seals (NMFS 2007). As such, my data provide a background of hormone, foraging, and diet information to compare with future studies of these and other populations of northern fur seals, and might help reveal changes and assess the overall health of the ecosystems where they live. The methodology I used supports the idea that relevant physiological information can be obtained non-invasively from wild animals, and that the source of stress—whether from nutritional limitation or something else—can be distinguished by measuring T3 along with glucocorticoids in feces.

In my study, confirmation of ages and sexes were limiting factors, and could be addressed in future studies by DNA and further hormone analysis. Uncertainties in the measurement of concentrations of hormone and hormone metabolites were also limiting factors, and could be addressed in the future by using high-performance liquid chromatography (HPLC) to confirm the presence of the compounds that are being measured.

Continual collection of hormone, diet and foraging data would allow the physiological status of northern fur seals to be monitored while their population dynamics continue to fluctuate. Comparisons of North American populations of northern fur seals with their counterparts in Asia may also reveal environmental differences between regions. Taking measurements such as weight and length from

pups as well as tagging individuals to estimate survival rates would also help to create a more complete picture of what is happening to northern fur seals in Alaska. Data on hormone concentrations, diets and foraging trips are more readily available for lactating females than for non-lactating females and immature males. Gathering more data from males could further expand understanding of the complex lives of individuals in these populations. Lastly, behavioral observations, especially for San Miguel fur seals that may be experiencing heat stress, may be an important addition to physiological and foraging data.

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Appendix: Statistical tables

Table A.1 Differences in storage methods (refrigerated vs. not refrigerated) of fecal samples (*n* = 20) from female northern fur seals at the Vancouver Aquarium. Mean and standard error for glucocorticoids and T3 in refrigerated and not refrigerated fecal samples.

	Storage Condition	*n*	Mean ng/g	SE	*t*	df	*p*
Corticosterone	Refrigerated	5	193.75	118.88	-0.17	8	0.868
	Not refrigerated	5	227.03	152.73			
T3	Refrigerated	5	165.18	158.34	0.99	8	0.351
	Not refrigerated	5	8.31	4.27			

Table A.2 Mean glucocorticoid metabolite concentrations (nanograms/gram) of male (M) and female (F) northern fur seal fecal samples (n=307 scats) from Bogoslof and St. Paul Islands with associated samples sizes (n) and standard errors (SE).

Location	Sex	*n*	Mean (ng/g)	SE
Bogoslof	M & F	149	220.27	24.25
St. Paul	M & F	158	274.52	23.73
Bogoslof & St. Paul	M	150	220.10	24.17
Bogoslof & St. Paul	F	157	274.70	23.82
Bogoslof	M	75	233.12	33.96
Bogoslof	F	74	207.43	34.67
St. Paul	M	75	207.08	34.40
St. Paul	F	83	342.00	32.71

Table A.3 Mean T3 metabolite concentrations (nanograms/gram) of male (M) and female (F) northern fur seal fecal samples (n=294 scats) from Bogoslof and St. Paul Islands with associated samples sizes (n) and standard errors (SE).

Location	Sex	*n*	Mean (ng/g)	SE
Bogoslof	M & F	136	261.56	36.00
St. Paul	M & F	158	602.63	33.11
Bogoslof & St. Paul	M	151	504.93	33.83
Bogoslof & St. Paul	F	143	359.26	35.22
Bogoslof	M	76	312.81	47.68
Bogoslof	F	60	210.31	53.66
St. Paul	M	75	697.04	48.00
St. Paul	F	83	508.21	45.63

Table A.4 Source, degrees of freedom, *F* and *p* of a 2-way analysis of variance with an interaction between location and sex for glucocorticoid metabolites in scats from Bogoslof and St. Paul Islands.

Source	df	*F*	*p*
Corrected model	3	3.74	0.012
Intercept	1	212.63	<0.001
Location	1	2.56	0.111
Sex	1	2.59	0.109
Location * Sex	1	5.60	0.0019

Table A.5 Source, degrees of freedom, *F* and *p* of a 2-way analysis of variance with a main effect of sex and a main effect of location for T3 metabolites in scats from Bogoslof and St. Paul Islands.

Source	df	*F*	*p*
Corrected model	3	18.77	<0.001
Intercept	1	313.18	<0.001
Location	1	48.78	<0.001
Sex	1	9.00	0.003
Location * Sex	1	0.78	0.377

Table A.6 Mean glucocorticoid concentrations (nanograms/gram) of male (M) and female (F) northern fur seal fecal samples (n = 393 scats) from San Miguel, St. Paul, and Bogoslof Islands with associated samples sizes (n) and standard errors (SE).

Location	Sex	*n*	Mean (ng/g)	SE
San Miguel	M & F	86	342.38	37.36
St. Paul	M & F	158	274.19	26.56
Bogoslof	M & F	149	220.27	27.13
San Miguel, St. Paul, Bogoslof	M	184	236.79	26.23
San Miguel, St. Paul, Bogoslof	F	209	321.11	23.93
San Miguel	M	34	270.81	57.18
San Miguel	F	52	414.00	48.12
St. Paul	M	75	206.42	38.49
St. Paul	F	83	342.00	36.60
Bogoslof	M	75	233.12	38.00
Bogoslof	F	74	207.43	38.75

Table A.7 Mean T3 metabolite concentrations (nanograms/gram) of male (M) and female (F) northern fur seal fecal samples (n = 277 scats) from San Miguel, St. Paul, and Bogoslof Islands with associated samples sizes (n) and standard errors (SE).

Location	Sex	*n*	Mean (ng/g)	SE
San Miguel	M & F	84	369.32	49.60
St. Paul	M & F	158	602.63	35.60
Bogoslof	M & F	136	261.56	38.58
San Miguel, St. Paul, Bogoslof	M	186	461.90	34.91
San Miguel, St. Paul, Bogoslof	F	191	360.46	33.18
San Miguel	M	35	375.80	75.45
San Miguel	F	49	362.85	64.42
St. Paul	M	75	697.04	51.54
St. Paul	F	83	508.21	49.00
Bogoslof	M	76	312.81	51.20
Bogoslof	F	60	210.31	57.62

Table A.8 Source, degrees of freedom, *F* and *p* with main effects of location and sex as well as a significant interaction for glucocorticoid metabolites in scats from and San Miguel, St. Paul, and Bogoslof Islands.

Source	df	*F*	*p*
Corrected model	5	4.24	0.002
Intercept	1	246.82	0.000
Location	2	3.56	0.030
Sex	1	5.64	0.018
Location * Sex	2	2.79	0.063

Table A.9 Source, degrees of freedom, *F* and *p* with main effects of location and sex for T3 metabolites in scats from and San Miguel, St. Paul, and Bogoslof Islands.

Source	df	*F*	*p*
Corrected model	5	10.15	0.000
Intercept	1	292.04	0.000
Location	2	22.09	0.000
Sex	1	4.44	0.036
Location * Sex	2	1.08	0.342