Physiological Responses of Polar Bear (*Ursus maritimus*) to a Changing Arctic Climate

By

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ABSTRACT

Understanding wildlife population health requires understanding how community and ecosystem changes affect physiologic function, which is especially important for species affected by climate change. Climate change affects polar bear (*Ursus maritimus*) habitat, diet, behavior, and population size, but the effects of climate change on their physiology is not well understood. In my dissertation research, I examine the effects of climate change on polar bears using blood-based biomarkers that reflect physiologic function and pathophysiologic processes related to organ system function, electrolytic balance, enzyme activity, protein abundance and nutrition. In Chapter 2, I define reference intervals for commonly accessed markers of physiologic function in polar bears providing a robust foundation from which to evaluate metabolic function based on age, sex, denning status, and season. In Chapter 3, I evaluate associations between 13 physiologic biomarkers and climate change as indexed through circumpolar and regional environmental processes and weather conditions while considering seasonal and demographic characteristics known to affect polar bear ecology. In addition, I evaluate whether behaviors, including those driven by climate change, had an additive effect on polar bear physiologic function. In Chapter 4, I characterize the serum virome of polar bears, providing a baseline inventory of viruses infecting polar bears during a time of extreme changes in climate and habitat use. As part of this characterization, I investigated if viruses from the family *Anelloviradae* might be used as a biomarker of immune function or physiologic stress in polar bears. This work advances our understanding of how climate change is affecting polar bear physiology and provides baseline data from which to measure changes in polar bear physiology and viral infection over time.

DEDICATION

To Addie and Tess

my beacons

my diversions

my inspirations

Thank you for always reminding me I can do anything.

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CHAPTER 1: INTRODUCTION

Introduction

The health of wildlife populations can be challenging to assess. Ideally, it requires understanding how environmental processes influence the physiologic function of individuals and, consequently, the vital rates of populations (Cooke and O'Connor, 2010; Ames *et al.*, 2020). It involves investigating the complexities associated with synergistic effects of abiotic and biotic changes including: climate and weather, habitat loss, changes in behavior and exposure to stressors, including pathogens, on the health of individuals and subsequently, populations. In short, assessing wildlife population health requires understanding how community and ecosystem changes affect physiologic function. This is especially important for species affected by climate change.

Polar bears from the southern Beaufort Sea off the coast of Alaska and western Canada are one of the most extensively studied sub-populations of polar bears. Using a longitudinal data set that spans nearly 40 years, this dissertation provides a unique and informative look at how a top predator's physiologic processes are responding to climate change. In 2008, polar bears were listed as threatened under the Endangered Species Act (ESA) due to the observed and predicted impacts of climate change on population persistence (U.S. Fish and Wildlife Service, 2008). Unlike most listings under the ESA, the decision to list polar bears was pre-emptive, given that models projected population declines driven by the loss of sea ice habitat for the circumpolar population (Amstrup *et al.*, 2008; U.S. Fish and Wildlife Service, 2008; Hunter *et al.*, 2010). The southern Beaufort Sea subpopulation of polar bears has experienced declines in abundance over the last two decades, including a precipitous decline in the mid-2000s followed by apparent stabilization from 2008-2010 (Bromaghin *et al.*, 2015, 2021). In addition, the effects of climate change have been documented in a variety of ways including decreases in body condition (Rode *et al.*, 2010, 2014), changes in habitat use (Durner *et al.*, 2009, 2011, 2019; Atwood *et al.*, 2016a, 2016b), risks related

to disease (Atwood *et al.*, 2015, 2017; Bowen *et al.*, 2015; Whiteman *et al.*, 2018), changes in toxicant exposure (Bentzen *et al.*, 2008; Cardona-Marek *et al.*, 2009; McKinney *et al.*, 2017b; Bourque *et al.*, 2020), changes in gut bacteria (Watson *et al.*, 2019) and diet (Rogers *et al.*, 2015; McKinney *et al.*, 2017a). Further, population level consequences from contaminants, disease and infectious agents can be an influential driver of population vital rates (e.g.,(Béland *et al.*, 1993; Cassirer *et al.*, 2013; Stenglein *et al.*, 2018). Monitoring such risks requires that we define effective biomarkers that allow us the ability to observe the cumulative effects of changing climate on polar bear health. My work quantifies this conceptual model described in Figure 1.

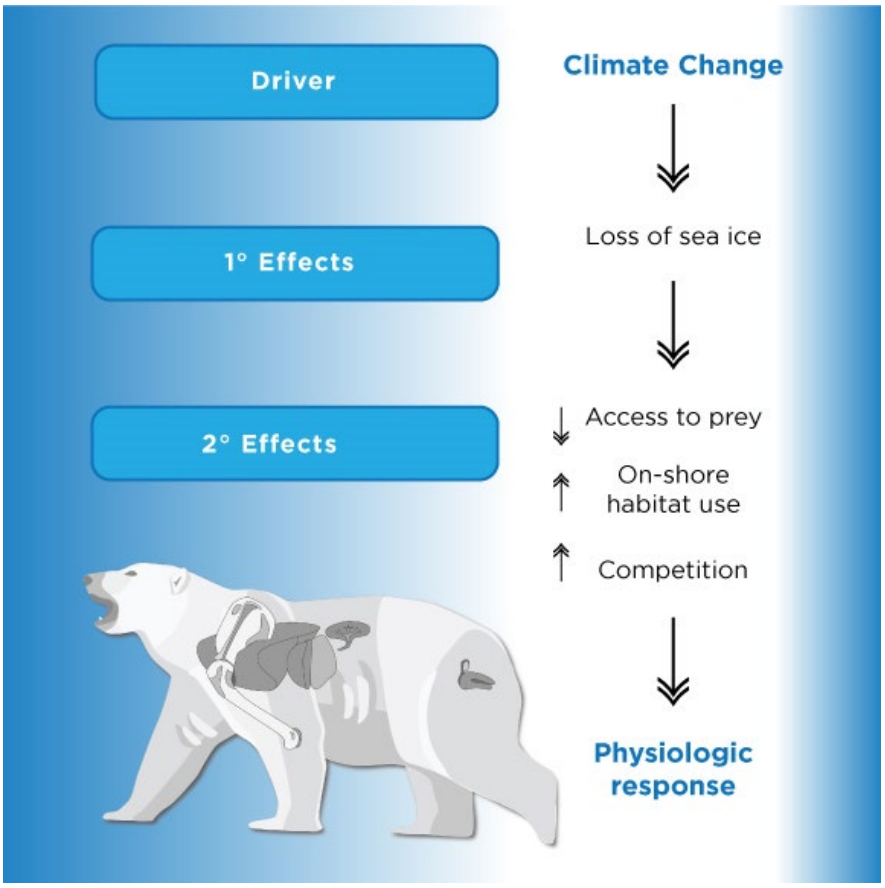

Figure 1: Conceptual model describing how changes in climate leads to physiologic changes in polar bears.

Dissertation Synopsis

In the dissertation research presented herein, I examine the physiologic effects of climate change on polar bears physiologic function through blood-based biomarkers. I did this through the establishment of reference intervals for commonly assessed biochemical analytes, examining the influence of circumpolar and regional climate driven conditions and the effects of weather on biomarkers of physiologic function, and characterizing and examining the effects of climate change on the polar bear virome.

Chapter 2 describes the reference intervals in polar bears for commonly assessed markers of physiologic function. A common method for assessing physiologic function and pathophysiologic processes in animals is the comparison of blood-based analyte point values to a value range which suggests normal, healthy organ system function, electrolytic balance, enzyme activity, protein abundance and nutrition. I identified serum samples from 651 polar bears collected between 1983 – 2016 to define reference intervals for polar bears. This sample size allowed for biologically appropriate subgrouping for the determination of reference intervals relative to age class, reproductive status, and season. This chapter provides a robust foundation from which to evaluate individual polar bear physiologic function, while allowing for temporal and spatial evaluation of the overall health of polar bears both within and among polar bear subpopulations as they respond to extensive ecological challenges.

In **Chapter 3,** I describe the influence of environmental conditions on physiologic functions of polar bears using data from blood samples collected from 1984 – 2018, a period marked by extensive environmental change. Biomarkers of physiologic function can contribute to the understanding of population health because physiology mechanistically connects an individual to its environment, which is key to understanding the effects of rapid environmental change on population health (Moore, 2008; Cooke *et al.*, 2014, 2020; Madliger and Love, 2015;

Madliger *et al.*, 2018). To better understand these effects, I evaluated associations between 13 physiologic biomarkers and circumpolar and regional climate processes and weather conditions, as well as seasonal and demographic characteristics known to affect polar bear ecology. In addition, I evaluated how behaviors driven by climate change further influence polar bear physiologic function. This investigation was aimed at assessing whether polar bears in the southern Beaufort Sea are showing physiologic plasticity or if climate change is resulting in pathophysiologic effects on immune and metabolic function.

Chapter 4 characterizes the polar bear serum virome and provides a baseline inventory of viruses infecting polar bears during a time of extreme changes in climate and behaviors. Such a catalogue will be invaluable to future conservation work as pathogens, including viruses, emerge with a warming climate. Previous unpublished etiologic work suggested the polar bear virome included small, commensal DNA viruses from the Anelloviradae family, which in humans have been hypothesized to vary with immune system function in humans (Thom and Petrik, 2007; Spandole *et al.*, 2015). With this in mind, I explored if anelloviruses could be used as an ecoimmunological markers of polar bear immune system function, and further, a surrogate for measuring a physiologic stress response in polar bears.

In **Chapter 5,** I conclude with a summary of the major impacts of my findings, including management implications and directions for future work.

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CHAPTER 2: REFERENCE INTERVALS FOR BLOOD-BASED BIOCHEMICAL ANALYTES OF SOUTHERN BEAUFORT SEA POLAR BEARS *(URSUS MARITIMUS*)

Fry, Tricia L., Kristen R Friedrichs, Todd C Atwood, Colleen Duncan, Kristin Simac, Tony Goldberg. (2019) Reference intervals for blood-based biochemical analytes of southern Beaufort Sea polar bears, *Conservation Physiology*, Volume 7, Issue 1 coz040, https://doi.org/10.1093/conphys/coz040

Abstract

Accurate reference intervals (RI) for commonly measured blood-based analytes are essential for health monitoring programs. Baseline values for a panel of analytes can be used to monitor physiologic and pathophysiologic processes such as organ function, electrolyte balance, and protein catabolism. Our reference population includes 651 serum samples from polar bears (*Ursus maritimus*) from the southern Beaufort Sea subpopulation sampled in Alaska, USA, between 1983 - 2016. To establish RI for 13 biochemical analytes, we defined specific criteria for characterizing the reference population and relevant subgroups. To account for differences in seasonal life history characteristics, we determined separate RI for the spring and fall seasons, when prey availability and energetic requirements of bears differ. We established RI for five subgroups in spring based on sex, age class, and denning status, and three subgroups in fall based on sex and age class in females. Alkaline phosphatase activities were twice as high in subadult as in adult polar bears in spring ($z_{males} = 4.08$, $P_{males} < 0.001$, $z_{females} = 3.90$, $P_{females} < 0.001$), and did not differ between seasons. Denning females had significantly higher glucose concentrations than non-denning females ($z = 4.94$, $P < 0.001$), possibly reflecting differences in energy expenditure during lactation. Ten of the 13 analytes differed significantly between seasons in either males or females; however, the physiologic importance of these differences may be minimal. Establishing these RI allows for temporal monitoring of polar bear health in the southern Beaufort Sea and may prove useful for assessing and monitoring additional polar bear subpopulations in a changing Arctic environment.

Introduction

Climate change is rapidly affecting the Arctic region. Arctic ocean temperatures have risen at over twice the average rate of global warming with models suggesting that the Beaufort Sea could increase 4°C above the 1981-2010 average by 2040 (Overland *et al.*, 2018), accelerating abiotic and biotic changes (IPCC 2018). With warming temperatures and changes in sea ice phenology, polar bears (*Ursus maritimus*) are being exposed to novel stressors related to changes in habitat, nutrition, competition, and pollutants (Burek *et al.*, 2008). Observed effects associated with environmental changes in polar bears include increased rates of fasting (Cherry *et al.*, 2009; Rode *et al.*, 2018a), declines in body condition and cub recruitment, (Rode *et al.*, 2010, 2012, 2014; Obbard *et al.*, 2016), and declines in survival and abundance (Regehr *et al.*, 2007; Bromaghin *et al.*, 2015, Obbard *et al.*, 2018). However, the effects of chronic environmental stressors on metabolic processes, physiologic function, and health are poorly understood (Atwood *et al.*, 2015; Bowen *et al.*, 2015; Fagre *et al.*, 2015; Patyk *et al.*, 2015). Thus, there is a critical need to describe biomarkers that can be used as a component in monitoring polar bear health (Friedrichs, 2009; Patyk *et al.*, 2015).

A common method for assessing physiologic function and pathology in animals is to measure blood-based analytes, which include measures of organ system function, electrolytic balance, enzyme activity, protein abundance, and nutrition. Deviations from expected values of blood-based analytes, are commonly used to ascertain pathologic states (Friedrichs *et al.*, 2012). A precursor to effectively using such indices is establishment of reference intervals (RI), which are baseline values for each analyte derived from a normal, healthy reference population. Grasbeck and Saris (1969) first introduced the concept of theoretical RI as values obtained under controlled conditions with ‘healthy, normal’ individuals as the reference population (Grasbeck

1990). A RI is mostly commonly delimited by the central 95% of the reference population with the low and high limits bounding the interval (Geffré *et al.*, 2009; Friedrichs *et al.*, 2012). Hanks (1981) outlined the usefulness of blood-based variables to assess physical condition and health status of wildlife as well as to assess disease status and changes in the environment. Friedrichs (2009) further suggested that RI could be used to assess the physiologic health of individuals, populations, or ecosystems.

When calculating RI, it is important to consider life history variables that may influence blood-based analytes of individuals. In wild, free-living animals this may include seasonal impacts. Seasonal fluctuations in blood biochemistry can result from a variety of factors, including diet and nutrition, reproduction, behavior, and metabolic requirements (Lathi, 2004; Friedrichs *et al.*, 2012). Ursids demonstrate substantial variation in biochemical values depending on habitat, behavior, and diet (Lee *et al.*, 1977; Matula *et al.*, 1980; Nelson *et al.*, 1983; Brannon, 1985; Schroeder, 1987; Franzmann and Schwartz, 1988; Ramsay *et al.*, 1991; Tryland *et al.*, 2002). Identifying seasonal changes in RI is especially important for polar bears, given the extreme seasonality of their life history and physiologic adaptations, such as hyperphagia in the spring and extended fasts in other seasons (Atkinson and Ramsay 1995; Cherry *et al.*, 2009; Rode *et al.*, 2018).

Our objective was to use the southern Beaufort Sea (SB) subpopulation of polar bears to define RI that can be used to monitor the health of the SB subpopulation and for comparisons to other subpopulations. Specifically, we used polar bear blood chemistry values collected over 34 years (1983 – 2016) to define RI for 13 common serum analytes that measure liver and kidney function and status, immune system activity, dietary intake, and electrolyte and mineral balance.

We also examined variation in analytes across sub-groups, such as denning status, age, and sex, in both spring and fall.

Methods

Polar bears were captured, sampled, and released on the sea ice of the southern Beaufort Sea, Alaska, as part of a long-term research program. Spring captures most commonly occurred on sea ice from 1983 to 2016 typically between March 20th to May 5th. Fall captures took place on sea ice and on land between August and November, intermittently from 1983 to 2009. The study area included the Alaska portion of the SB subpopulation, bounded by Icy Cape, Alaska, to the west and the United States - Canada border on the east and extended from the coast to approximately 90 km over sea ice in most years (Figure 1). Polar bears were located from a helicopter and immobilized with a rapid-injection dart (Palmer Cap-Chur Equipment, Douglasville, Georgia, USA) containing Sernylan or M-99 prior to 1987 and thereafter, zolazepam-tiletamine (Telazol® or Zoletil®, Stirling *et al.*, 1989). Immobilized bears were aged, weighed to the nearest kg, and marked with an ear tag number and a unique tattoo on the upper lip. Polar bears ≥5 years old were classed as adults, and three and four years old polar bears were classed as subadults. Denning status was ascertained when a female polar bear was captured with young of the year. Capture and handling of polar bears was conducted under appropriate research permits, including Marine Mammal Research Permit MA690038-17.

We collected blood into evacuated plain tubes (Vacutainer; BD Biosciences, Franklin Lanes, NJ) by venipuncture of the femoral vein. Whole blood was stored in a cooler with chemical heat packs to prevent freezing until returning from the field, at which point serum was separated from blood by centrifugation at 1,500 g for 5 min (TRIAC, Clay Adams, Parsippany, NJ) and frozen at -20°C. At the conclusion of the field season, sera were stored at -70°C until

analyzed. Sera were analyzed using a VetScan VS2 biochemistry analyzer (Abaxis, Union City, California) to measure the following analytes: alanine aminotransferase (ALT), alkaline phosphatase (ALP), albumin (ALB), blood urea nitrogen (BUN), calcium (CA), creatinine (CREA), glucose (GLU), phosphorus (PHOS), potassium (POT), sodium (NA), total bilirubin (TBIL), and total protein (TP). Globulin (GLOB) was calculated by subtracting ALB from TP. These analytes comprise the comprehensive diagnostic profile defined by Abaxis. The functional and interpretive characteristics of each analyte are summarized in Table 1 (Stockham and Scott 2013). We established RI based on the guidelines of the American Society of Veterinary Clinical Pathology (Friedrichs *et al.*, 2012). We calculated RI for each of the 13 serum analytes using the Excel macros Reference Value Advisor (Geffré *et al.*, 2011). Outliers were removed based on Dixon's range statistic (see Geffré *et al.*, 2011). In addition, individuals with two or more outliers in their analyte panel were excluded from the reference population under the assumption this may indicate a deviation from health. We defined subgroups based on age class, sex, and denning status, each of which may influence physiologic processes (Friedrichs *et al.*, 2012) as well as samples size. To reflect the life history traits of polar bears, spring RI were calculated for five subgroups (females: non-denning adults, denning adults, and subadults; males: adults and subadults (Table 2), and fall RI were calculated for three subgroups (female adults, female subadults, and males). Males were not further subdivided by age class in fall in order to maintain a sample size ≥ 20 (Friedrichs et al.2012). In fall, a single group for males was created because none of the analytes had a difference in means greater than 25% (Sinton *et al.*, 1986), and confidence intervals between the two age groups overlap for all analytes with the exception of BUN. All samples were independent; an individual polar bear was only in a sub-group once.

RI were calculated using non-parametric methods when samples sizes were adequate ($n \geq 40$). We used parametric analyses when $20 < n < 40$ and the distribution was Gaussian. We used a BoxCox transformation with parametric analysis when transformation to a Gaussian distribution was necessary (Daly *et al.*, 2017). Upper and lower confidence intervals were calculated using nonparametric bootstrap methods when $20 \leq n \leq 120$ and according to tables when $120 \leq n \leq 370$ (Wayne 2008; Geffré *et al.*, 2011). When data could not be transformed to a Gaussian distribution, RI were defined as the minimum and maximum values with lower and upper 90% confidence intervals excluded. To assess statistical differences between subgroups and season we compared the means of each analyte using a generalized linear model with Tukey's multiple comparison of the means. We assessed physiologic importance of differences in RI using the upper and lower confidence intervals between subgroups and seasons; if the upper or lower reference limit was bounded by the comparative subgroup confidence interval, the RI were considered to have limited physiologic difference.

Results

Our reference population included 651 polar bear serum samples (Table 2). Bears in the reference population had a body condition score ≥ 3 (Ranking 1 to 5, with 5 = obese; Stirling *et al.*, 2008) and had unremarkable physical exams. A summary of reference intervals, including sample size, summary statistics, and 90% upper and lower confidence intervals for each of the 13 analytes, is reported in Table 3 for female polar bears and Table 4 for male polar bears as well as statistically significant differences between subgroups. We report both statistical and physiologic differences in our results. Outliers were identified in the analysis of 32 out of 104 RI. In 20 RI calculations, the outliers represented < 9% of the reference population and in cases where

outliers represented a greater percentage of the reference population the sample size was small ($n < 8$). Outliers were distributed throughout the duration of the study.

Spring Reference Intervals

We partitioned females captured in spring into three subgroups: non-denning adults, denning adults, and subadults, consistent with expectations based on behavior and physiology. Mean ALP activities of subadult females were nearly twice that of adult female polar bears and significantly different from both denning and non-denning adult females (Table 3; $Z_{subadult/adult} = 5.64$, $p < 0.001$, $Z_{subadult/denning} = 6.24$, $p < 0.001$). Denning females had significantly lower mean concentrations of ALB, POT, TP, and mean ALT activity than non-denning adult females and significantly higher mean concentrations of GLU and CREA (Table 3).

Physiologic differences between females in spring based on the lower and upper confidence intervals of RI suggest limited differences in ALB levels, with denning females having a lower clinical decision interval (Friedrichs *et al.* 2012). Similarly, denning females had higher minimum GLU values than non-denning adults and subadult females in spring. GLOB levels showed physiologic difference within female bears based on age and denning status, with denning females having lower GLOB levels than both non-denning adults and subadults.

Males sampled in spring were partitioned into adult and subadult age classes. The means of ALT, ALP, CA, CREA, and GLOB were significantly different between the two age classes (Table 4). Similar to subadult females in spring, subadult males had significantly higher ALP activities, with an upper reference limit for subadults of 167 U/L, while the upper reference limit for adults was 89 U/L ($t=6.80$, $p < 0.001$). ALT activities in subadult males ($\overline{x} = 33.27$ U/L) were significantly lower than in adult males ($\overline{x} = 50.28$ U/L, $t= -3.69$, $p < 0.01$). For each of these

enzymes the upper limit of the confidence intervals suggests a potential physiologic difference between the age classes, with increased ALP activity in subadults compared to adults, and the inverse relationship with ALT, decreased activity in subadults compared to adults.

Fall Reference Intervals

Fall sample sizes were smaller than spring sample sizes, but still provided adequate numbers to calculate RI using an iterative (robust) statistical approach (Friedrichs et al. 2012). Females were grouped into adults and subadults (Table 3). Differences in ALP between age classes were consistent across seasons, with subadult females having significantly higher mean ALP activities ($\overline{x}$ = 64.02 U/L) than adult females ($\overline{x}$ = 31.65 U/L, t=5.69, p-value < 0.001). The higher upper confidence limit of ALP activity suggests a physiologic difference between the two age groups in fall. GLOB concentrations were the only other analyte where the mean differed significantly between subadult and adult females in fall (z=-2.68, p = 0.018). The upper limit of GLOB concentration in adult females suggests a physiologic difference between the two age classes. The single reference interval for each analyte for males in fall are reported in Table 4.

Seasonal Differences in Reference Intervals

Adult females were separated into non-denning and denning females in the spring and combined in the fall (Figure 2). We found significant differences in seasonal means for CREA ($\overline{x}_{fall}$ = 1.04 mg/dL, $\overline{x}_{spring}$ = 0.95 mg/dL; t = -2.96, p ≤ 0.01), POT ($\overline{x}_{fall}$ = 4.75 mmol/L, $\overline{x}_{spring}$ = 4.42 mmol/L; t = -5.01, p ≤ 0.001), TP ($\overline{x}_{fall}$ = 7.57 g/dL, $\overline{x}_{spring}$ = 6.96 g/dL; t = -7.45, p ≤ 0.001), NA ($\overline{x}_{fall}$ = 142.79 mmol/L, $\overline{x}_{spring}$ = 137.53 mmol/L; t = - 6.23, p ≤ 0.001), and GLOB ($\overline{x}_{fall}$ = 2.01 g/dL, $\overline{x}_{spring}$ = 1.43 g/dL; t = - 9.15, p ≤ 0.001) in adult females. Mean seasonal differences of analytes in subadult females were often statistically significant but minimal in magnitude, with the exception of BUN concentrations, suggesting limited seasonal differences on

physiologic function. Subadult females showed greater seasonal variation with significant differences between seasonal means for BUN ($\overline{x}_{fall}$ = 9.78 mg/dL, $\overline{x}_{spring}$ = 16.58 mg/dL; t = 2.60, $p \leq 0.01$), CREA ($\overline{x}_{fall}$ = 1.02 mg/dL, $\overline{x}_{spring}$ = 0.88 mg/dL; t = -2.70, $p \leq 0.01$), POT ($\overline{x}_{fall}$ = 4.81 mmol/L, $\overline{x}_{spring}$ = 4.51 mmol/L; t = - 3.44, $p \leq 0.001$), NA ($\overline{x}_{fall}$ = 143.14 mmol/L, $\overline{x}_{spring}$ = 139.73 mmol/L; t = - 3.38, $p \leq 0.01$), TP($\overline{x}_{fall}$ = 7.26 g/dL, $\overline{x}_{spring}$ = 6.81 g/dL; t = -5.72, $p \leq 0.001$), and GLOB ($\overline{x}_{fall}$ = 1.69 g/dL, $\overline{x}_{spring}$ = 1.29 g/dL; t = - 6.01, $p \leq 0.001$). Of these statistically different analytes, only GLOB concentration suggests a physiologic difference between spring and fall in both adults and subadults. BUN in subadult females was the only analyte to significantly increase in fall, all other significantly different analytes showed decreased activity and concentration in spring regardless of age.

Males were separated into subadults and adults in the spring and combined in the fall (Figure 3). For adult males, ALP was greater in fall ($\overline{x}$ = 59.17 U/L) than spring ($\overline{x}$ = 35.61 U/L, t = -3.69, $p < 0.01$), as was CA ($\overline{x}_{fall}$ = 10.26 mg/dL, $\overline{x}_{spring}$ = 9.70 mg/dL; t = -4.16, $p \leq 0.001$) and BUN ($\overline{x}_{fall}$ = 17.58 mg/dL, $\overline{x}_{spring}$ =11.70 mg/dL; t = -2.91, $p \leq 0.01$). In each of these cases, the RI shifted to the right in fall, suggesting a physiologic difference. ALT also showed a significant difference between spring and fall (($\overline{x}_{fall}$ = 26.51 U/L, $\overline{x}_{spring}$ = 47.87 U/L; t = 5.10, $p \leq 0.001$) with the upper limit of ALT activity in spring being more than twice as high as fall activity (Figure 3). Mean CREA concentration was significantly lower in fall ($\overline{x}_{fall}$ = 1.00 mg/dL, $\overline{x}_{spring}$ = 1.24 mg/dL; t = 4.09, $p \leq 0.001$); however, the physiologic importance of this difference is likely minimal.

Discussion

Although previous research has reported blood analyte values for polar bears, these reports have examined fewer analytes and smaller numbers of bears (e.g., Lee *et al.*, 1977;

Nelson *et al.*, 1983; Derocher *et al.*, 1990; Ramsay *et al.*, 1991; Tryland *et al.*, 2002; Rode *et al.*, 2014; Whiteman *et al.*, 2017, 2018). Our goal was to use a large dataset to create robust RI based on a well-studied subpopulation that can serve as a foundation for relating biochemical analytes and polar bear health in this and other subpopulations.

Assessment of health in reference subjects is of paramount importance is establishing RI and yet is challenging in free-living wildlife owing to a single point-in-time examination. Inclusion of unhealthy subjects has the potential to widen the RI rendering it less sensitive for detecting deviation from healthy analyte distributions (Johansen and Christensen, 2018). In order to minimize inclusion of potentially unhealthy subjects, specific criteria were defined in order to exclude potentially unhealthy subjects (see Methods). Our examination and exclusion of outliers from the reference population warranted our inclusion of samples from the last four decades despite accelerating rates of environmental change and habitat perturbation in the Arctic (Harr *et al.*, 2018).

Our results were consistent with related work on large carnivores that found higher ALP activity in subadult/juveniles than adults: wolves (*Canis lupus*, Thoresen *et al.*, 2009), grizzly bears (*Ursus arctos horribilis,* Brannon, 1985) and polar bears (Lee *et al.*, 1977; Tryland *et al.*, 2002). ALP is an enzyme in both liver and bone and is involved in bone growth and remodeling. ALP is thus expected to be higher in subadults regardless of season. BUN concentrations were lowest in denning females, which likely reflects extended fasting and the energetic demands of raising young. Females with cubs of the year are often captured shortly after leaving the den, leaving little time for hunting prior to capture (Derocher *et al.*, 1990). As access to food in spring increases, we would expect BUN concentrations to increase. Denning females also had significantly higher GLU concentrations than both adults and subadult females in spring. This

difference may be related to increased GLU requirements during lactation (Bell and Bauman, 1997). These results are inconsistent with the finding of Halloran and Pearson (1972) and Matula *et al.* (1980) in brown and black (*Ursus americanus*) bears respectively, but both authors note inconsistencies among published reports relating blood GLU concentration to denning and lactation (eg. Lee *et al.*, 1977; Franzmann and Schwartz, 1988; Stenvinkel *et al.*, 2013).

Seasonal differences in analytes are likely a response to changes in nutrition and behavior. In the Western Hudson Bay subpopulation, polar bears are forced on shore when the sea ice melts in summer and have little access to food until the ice re-forms in the fall (Atkinson and Ramsay 1995). Ramsay *et al.* (1991) reported a pronounced seasonal variation in BUN concentrations for western Hudson Bay bears, which averaged 48.4 ± 1.8 mg/mL for individuals captured on sea ice in spring and 19.1 ± 5.4 mg/mL for those captured on land in summer. In the SB, season-specific BUN and CREA RI were lower than those reported elsewhere (Nelson *et al.* 1983, Ramsay *et al.* 1991, Tyland *et al.* 2002). Our BUN RI for adult females in spring was 2.4 – 48.80 mg/dL with a mean of 16.7 mg/dL. Thus, our maximum spring value equaled the mean spring value reported for western Hudson Bay, while our mean spring value matched that reported for western Hudson Bay bears in the summer that had been fasting on land. Similarly, spring and fall CREA RI from our study were substantially lower than spring and summer CREA ranges and RI previously reported for the Western Hudson Bay and Barents Sea subpopulation (Nelson *et al.* 1980, Ramsay *et al.* 1991, Tyland *et al.* 2002) Explanations for these differences between the SB and other subpopulations could be due to disparate ice conditions during the respective study periods (Stroeve *et al.* 2012), or to differences in biological productivity between the subpopulations (Rode *et al.* 2018).

Many researchers have used BUN and CREA to assess fasting in polar bears. Recently, Rode *et al.* (2018) documented declines in the ratio of BUN to CREA, which is an index of feeding over the previous 7 days and found increased rates of fasting in SB polar bears between 1983-1999 and 2000-2016. Pagano *et al.* (2018) and Whiteman (2018) supported this finding noting increases in metabolic rates due to increased energy expenditure and declines in hunting opportunities related to deteriorating sea ice habitat. While not the goal of this research, our work provides a basis from which to continue investigations into physiologic adjustments resulting from a changing climate. Using deviations from RI we can better understand how abiotic and biotic conditions such as changes in sea ice are impacting polar bears and determine the best metrics for surveillance and monitoring.

Our work adds to the understanding of the blood biochemistry of polar bears. Our large sample size permitted biologically appropriate subgrouping, allowing us to examine differences in age class and reproductive status, the classifications used for managing polar bear populations. Nevertheless, our study has certain inherent limitations. For example, the declining availability of sea ice in the southern Beaufort Sea during summer and fall precluded the continuation of safe captures limiting our ability to calculate summer RI that included data beyond 2009. We caution that although we report a number of statistical differences for analytes across subgroups and between seasons, it is important to consider the functional importance of these differences. For example, mean total protein levels showed significant differences between all subgroups for females in spring. However, the calculated values suggest minimal influence on physiologic function and critical decision limits. To clarify the functional significance of the differences we have documented, it would be useful to determine how the analytes we measured vary with known disease states. To inform relationships between disease and blood biochemistry we

suggest examining zoo-managed polar bears as well as wild polar bears with known pathological conditions (Atwood et al. 2015) to establish critical values for these physiologic markers.

We also acknowledge that RI created for one subpopulation using one analytical system may not reflect the variability of values observed in other subpopulations or by other methods. However, we provide a well-documented and robust resource for comparisons within and across the circumpolar population of polar bears. Our work is therefore most relevant to monitoring the SB subpopulation of polar bears, including detecting changes in physiologic function that may reflect subclinical and clinical disease in individuals and populations. In general, reference intervals provide a baseline for assessing health, and deviation from these reference intervals may signal an adaptive physiologic response. The SB sub-population of polar bears is one of the most well studied; therefore, associations between stressors and physiologic responses documented for the SB sub-population can be used to inform monitoring and management decisions both for this population and potentially for other sub-populations even with different baseline values. Furthermore, combining baseline physiologic data such as ours with complementary data on hematology (Kirk et al. 2010), and transcriptomics (Bowen et al. 2015, 2015b), as well as data on diet and nutrition (McKinney et al. 2017), reproduction (Rode et al. 2010), behavior (Whiteman et al. 2015, Atwood et al. 2016, Lillie et al. 2018, Pagano et al. 2018), and pathogen exposure (Atwood et al. 2015, 2017) could help identify how polar bears might react and adapt to external stressors such as infectious diseases, environmental catastrophes, and climate change (Stroeve et al. 2012, Ladire et al. 2015). This set of RI for SB polar bears provides a robust foundation necessary to make temporal and spatial observations on the overall health of polar bears as well as comparisons both within and among subpopulations facing myriad ecological challenges.

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Figures

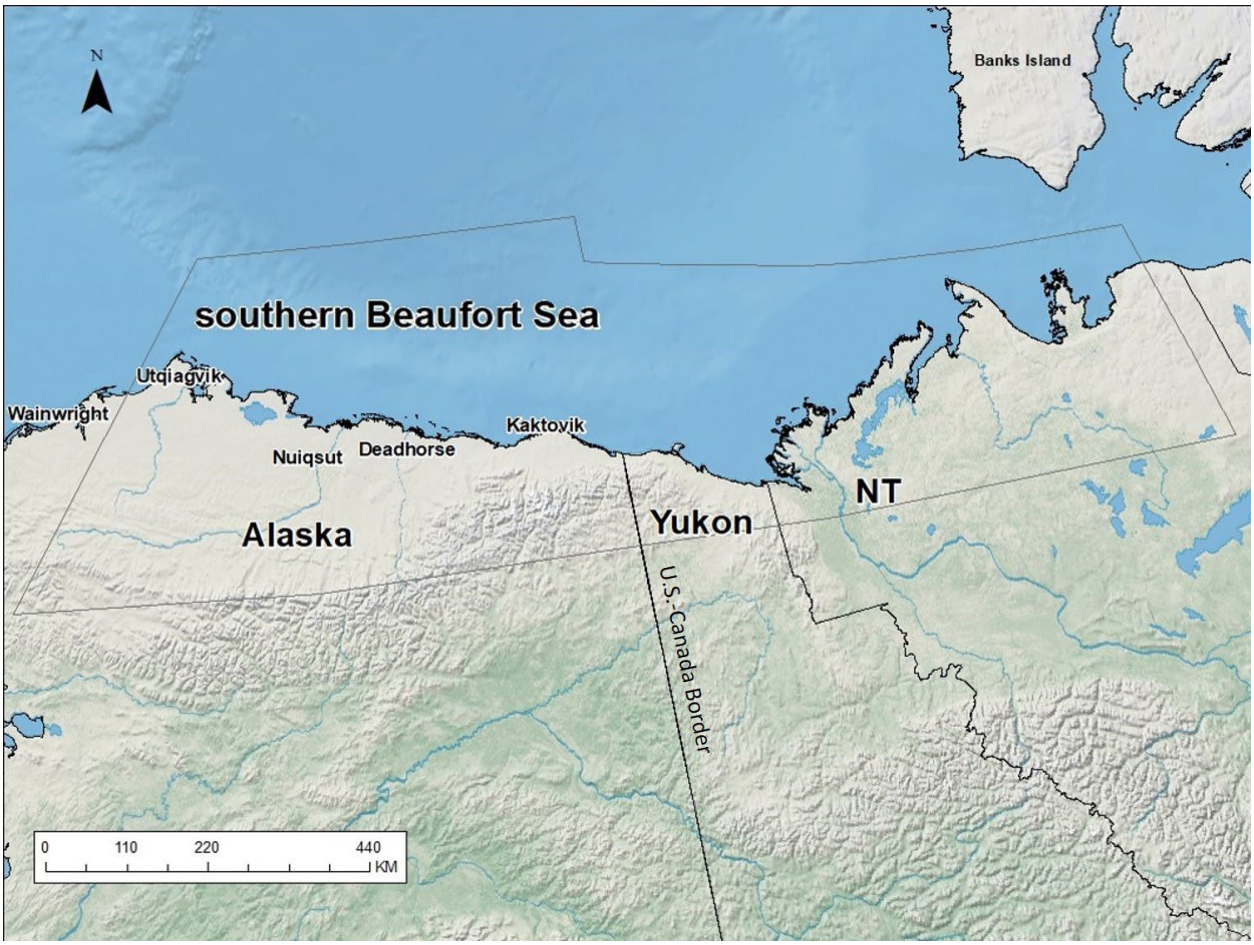

Figure 1: Between 1893 – 2016 polar bears were captured within the IUNC defined boundary (thin line) for the southern Beaufort Sea sub-population between Icy Cape, Alaska and the United States-Canada Border

Figure 2: Seasonal differences between biochemical analytes in female polar bears. Spring includes three subgroups and fall two subgroups. Significant differences are reported between like subgroups *p≤0.05, ** p≤0.01.

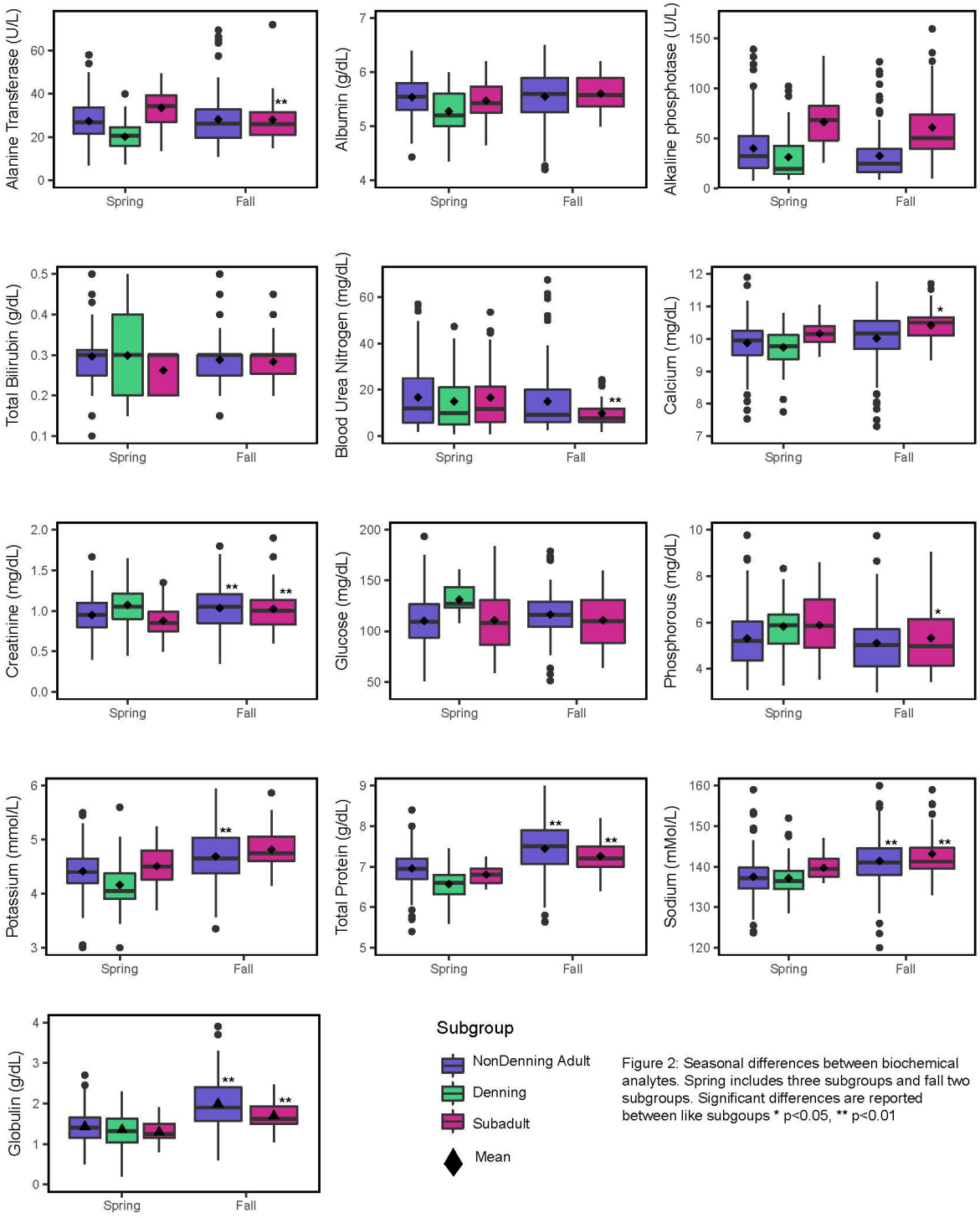

Figure 3: Seasonal differences between biochemical analytes in male polar bears. Spring includes two subgroups and fall a single subgroup. Significant differences are reported between like subgroups *p≤0.05, ** p≤0.01

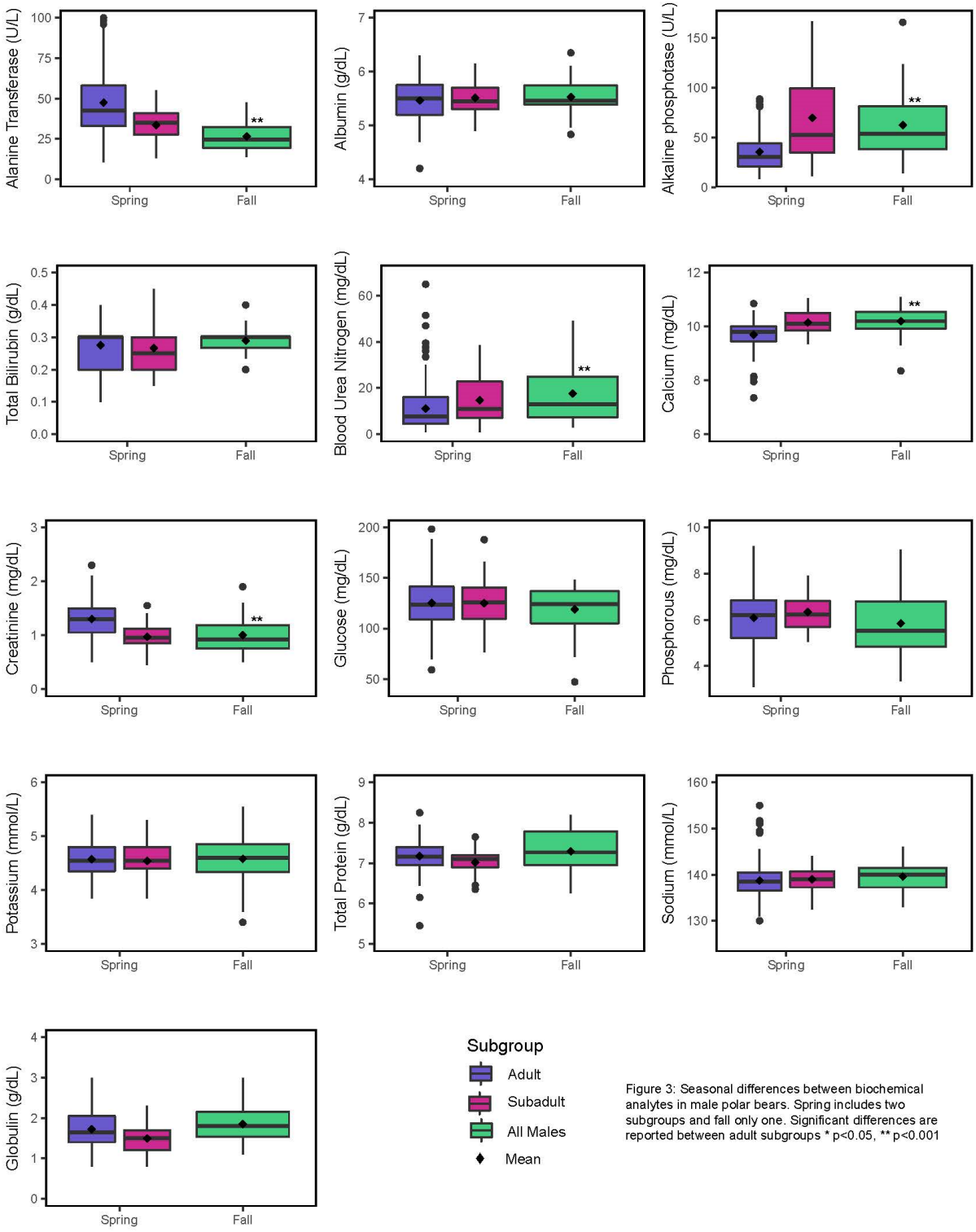

Figure 3: Seasonal differences between biochemical analytes in male polar bears. Spring includes two subgroups and fall only one. Significant differences are reported between adult subgroups * p<0.05, ** p<0.001

Tables

Table 1. Summary of blood-based analytes

Analyte	Tissue source or function*	Brief interpretive use
Alanine transferase (ALT)	Liver and muscle	Increases in some hepatic and severe muscle disorders
Albumin (ALB)	Synthesized by liver, source of amino acids, acts as carrier protein.	Increased with dehydration; decreases in some liver, renal, and inflammatory disorders
Alkaline phosphate (ALP)	Primarily liver and bone	Increases in some liver and bone disorders, increases during active bone growth (juveniles)
Total Bilirubin (TBIL)	Product of erythrocyte catabolism, processed by the liver and eliminated in bile	Increases with hemolysis or in disease of the liver and biliary system
Blood Urea Nitrogen (BUN)	Product of protein catabolism, source of nitrogen for protein synthesis, eliminated primarily by kidney	Decreases with low protein intake and liver failure, increases with high protein meals and with decreased renal elimination (↓GFR)
Calcium (CA)	Structural component of bone, important cation for enzymatic, neurologic, and muscular function	~50% bound to albumin, may be altered by vitamin D disorders
Phosphorous (PHOS)	Structural component of bone, important anion for energy generation (ATP)	Increases with decreased renal elimination (↓GFR)
Creatinine (Crea)	Catabolic product of muscle, eliminated through kidney	Low muscle mass results in lower basal concentrations; increases with decreased renal elimination (↓GFR)
Glucose (GLU)	Energy metabolite derived from food intake and hepatic synthesis, stored as glycogen in the liver	Strictly regulated by insulin, glucagon, and other hormones; increased by glucocorticoid secretion (termed a stress response)
Sodium (NA)	Important cation for osmoregulation	Strictly regulated by several hormonal systems and renal function
Potassium (POT)	Important cation for neurologic and muscular activity	Strictly regulated by several hormonal systems and renal function
Total Protein (TP)	Comprised of albumin and many different globulin molecules	Changes in TP are reflected by changes in albumin, globulins, or both
Globulin (GLOB)	Comprised of many different protein molecules that function in immunity and coagulation and as carrier molecules	Increased globulins indicate an immune response of significant duration (several days or more), individual globulins can be measured for specific information

GFR = glomerular filtration rate, a measure of kidney function; GFR is decreased in dehydration, renal failure, and urinary bladder obstruction, *Functional and interpretive characteristics are described by Stockham and Scott 2008

Table 2: Number of polar bears sampled by season

Table 2: Number of polar bears sampled by season				
	Spring		Fall	
	Females	Males	Females	Males
Adults (Non-denning)	184	161	114	18
Subadult	43	30	38	15
Denning Adults	48	-	-	-

Table 3: Reference Intervals for Female Polar Bears from the Southern Beaufort Sea

Analytes	Units	Season	Subgroup	n	Mean	Standard Deviation	Median	Minimum	Maximum	Reference Interval	90% confidence interval for lower limit	90% confidence interval for upper limit
Alanine Transferase (ALT)	U/L	Spring	Adult[1,a]	162	50	25	43	11	160	16 - 126	11 - 25	96 - 160
			Denning[b]	32	33	9	35	13	55	14 - 53	9 - 18	48 - 58
			Sub-adult[c]	33	27	9	25	14	48	13 - 49	11 - 15	42 - 58
		Fall	Adult	110	28	12	27	11	70	12 - 65	11 - 13	50 - 70
			Sub-adult	37	27	8	26	15	43	14 - 45	12 - 16	40 - 51
Albumin	g/dL	Spring	Adult[a]	179	5.5	0.4	5.6	4.4	6.4	4.7 - 6.3	4.4 - 4.9	6.2 - 6.4
			Denning[b]	48	5.3	0.4	5.2	4.3	6.0	4.4 - 6	4.4 - 4.7	5.9 - 6
			Sub-adult[a,b]	42	5.5	0.4	5.4	4.7	6.2	4.7 - 6.2	4.7 - 5.1	6 - 6.2
		Fall	Adult	111	5.5	0.5	5.6	4.2	6.5	4.3 - 6.5	4.2 - 4.7	6.3 - 6.5
			Sub-adult	38	5.6	0.3	5.6	5.0	6.2	4.9 - 6.3	4.8 - 5.1	6.1 - 6.4
Alkaline Phosphotase (ALP)	U/L	Spring	Adult	165	40	27	32	6	139	8 - 116	6 - 10	91 - 139
			Denning	45	32	29	20	5	126	5 - 122	5 - 8	94 - 126
			Sub-adult[a]	38	66	26	68	26	132	12 - 120	1 - 24	108 - 132
		Fall	Adult[a]	106	32	25	24	3	127	6 - 115	3 - 9	88 - 127
			Sub-adult	36	64	41	50	10	173	11 - 181	7 - 18	141 - 229

Blood Urea Nitrogen (BUN)	mg/dL	Spring	Adult	187	16.7	13.1	12.0	2.0	57.0	2.4 - 48.8	2 - 2.7	45 - 57
			Denning	49	15.0	12.3	11.0	1.0	47.3	1.4 - 46	1 - 3	39.5 - 47.3
			Sub-adult	42	16.6	14.2	11.8	1.0	53.5	1 - 53.5	1 - 3	44 - 53.5
		Fall	Adult	113	16.6	18.0	9.5	2.7	114.5	3 - 70.3	2.7 - 3.9	53.2 - 114.5
			Sub-adult	32	10.2	5.9	8.0	4.0	24.3	4.1 - 28.9	3.9 - 4.6	20.2 - 38.3
Calcium	mg/dL	Spring	Adult	176	9.9	0.7	9.9	7.5	12.5	8 - 11.5	7.5 - 8.8	11.1 - 12.5
			Denning	48	9.8	0.7	9.8	7.8	12.4	7.8 - 12	7.8 - 8.8	10.7 - 12.4
			Sub-adult	42	10.2	0.4	10.1	9.4	11.1	9.5 - 11	9.5 - 9.6	10.7 - 11.1
		Fall	Adult	109	10.1	0.9	10.2	7.3	12.7	7.8 - 11.9	7.3 - 8.2	11.5 - 12.7
			Sub-adult	37	10.4	0.6	10.5	9.3	11.7	9.3 - 11.6	9.1 - 9.6	11.3 - 11.9
Creatinine	mg/dL	Spring	Adult	187	0.9	0.2	0.9	0.4	1.7	0.6 - 1.5	0.4 - 0.6	1.4 - 1.7
			Denning[a]	49	1.1	0.3	1.0	0.4	1.8	0.5 - 1.8	0.5 - 0.7	1.6 - 1.8
			Sub-adult	42	0.9	0.2	0.9	0.5	1.4	0.5 - 1.4	-0.5 - 0.6	1.2 - 1.4
		Fall	Adult	111	1.0	0.3	1.1	0.4	1.8	0.6 - 1.7	0.4 - 0.6	1.5 - 1.8
			Sub-adult	38	1.0	0.3	1.0	0.6	1.9	0.6 - 1.8	0.6 - 0.7	1.5 - 2.1
Globulin	g/dL	Spring	Adult	182	1.4	0.4	1.4	0.5	2.7	0.8 - 2.4	0.5 - 0.9	2.3 - 2.7
			Denning	48	1.3	0.5	1.3	0.2	2.3	0.3 - 2.3	0.2 - 0.8	2.2 - 2.3
			Sub-adult	41	1.3	0.3	1.2	0.8	1.9	0.8 - 1.9	0.8 - 1	1.8 - 1.9
		Fall	Adult[a]	109	2.0	0.7	1.9	0.6	4.4	0.8 - 3.8	0.6 - 1.1	3.2 - 4.4

			Sub-adult	38	1.7	0.3	1.6	1.0	2.5	1.1 - 2.3	0.9 - 1.2	2.2 - 2.5
Glucose	mg/dL	Spring	Adult	175	110	27	109	51	205	58 - 171	51 - 68	160 - 205
			Denning[a]	48	131	14	127	108	161	107 - 164	104 - 111	155 - 173
			Sub-adult	41	111	29	108	59	184	59 - 184	59 - 74	155 - 184
		Fall	Adult	108	117	25	116	31	201	56 - 175	31 - 81	154 - 201
			Sub-adult	38	111	26	110	64	160	57 - 164	46 - 71	151 - 176
Phosphorus	mg/dL	Spring	Adult[a]	180	5.3	1.3	5.2	2.4	9.8	3 - 8.3	2.4 - 3.3	7.6 - 9.8
			Denning[a,b]	49	5.8	1.1	5.8	3.3	8.3	3.4 - 8.2	3.3 - 4.1	7.5 - 8.3
			Sub-adult[b]	42	6.0	1.5	5.9	3.5	10.1	3.6 - 10	3.6 - 4.1	8.4 - 10.1
		Fall	Adult	111	5.2	1.5	5.0	2.5	10.6	3 - 9.9	2.5 - 3.3	7.8 - 10.6
			Sub-adult	37	5.2	1.6	4.9	2.6	9.0	2.7 - 9.1	2.3 - 3.1	7.9 - 10.4
Potassium	mmol/ L	Spring	Adult	176	4.4	0.4	4.4	3.0	5.5	3.6 - 5.2	3 - 3.8	5.1 - 5.5
			Denning[a]	48	4.2	0.5	4.1	3.0	6.1	3.1 - 6.0	3 - 3.7	5 - 6.1
			Sub-adult	42	4.5	0.4	4.5	3.7	5.3	3.7 - 5.2	3.7 - 4	5 - 5.3
		Fall	Adult	107	4.8	0.7	4.7	2.9	8.1	3.5 - 7.2	2.9 - 3.9	5.9 - 8.1
			Sub-adult	38	4.8	0.4	4.8	4.2	5.9	4.1 - 5.8	4 - 4.2	5.5 - 6.2
Sodium	mmol/ L	Spring	Adult	178	137	4	137	124	154	128 - 148	124 - 130	144 - 154
			Denning	44	136	4	137	129	145	129 - 145	129 - 130	141 - 145
			Sub-adult	40	140	2	139	136	146	136 - 146	136 - 137	143 - 146

		Fall	Adult	112	143	10	142	112	180	123 - 168	112 - 130	162 - 180
			Sub-adult	38	143	6	141	133	159	133 - 159	na	na
Total Bilirubin	g/dL	Spring	Adult	181	0.3	0.1	0.3	0.1	0.7	0.2 - 0.5	0.1 - 0.2	0.5 - 0.7
			Denning	49	0.3	0.1	0.3	0.2	0.5	0.2 - 0.5	0.2 - 0.2	0.4 - 0.5
			Sub-adult	41	0.3	0.0	0.3	0.2	0.3	0.2 - 0.3	0 - 0	0 - 0
		Fall	Adult	108	0.3	0.1	0.3	0.2	0.7	0.2 - 0.6	0.2 - 0.2	0.5 - 0.7
			Sub-adult	38	0.3	0.1	0.3	0.2	0.5	0.2 - 0.5	na	na
Total Protein (TP)	g/dL	Spring	Adult[a]	180	7.0	0.4	6.9	5.4	8.4	6 - 7.9	5.4 - 6.4	7.6 - 8.4
			Denning[b]	46	6.6	0.4	6.6	5.6	7.4	5.7 - 7.4	5.6 - 6	7 - 7.5
			Sub-adult[a,b]	41	6.8	0.2	6.8	6.4	7.3	6.5 - 7.3	6.5 - 6.6	7.2 - 7.3
		Fall	Adult	111	7.6	0.9	7.5	5.6	10.4	5.8 - 9.8	5.6 - 6.3	9.2 - 10.4
			Sub-adult	37	7.3	0.5	7.2	6.4	8.2	6.3 - 8.2	6.1 - 6.5	8 - 8.4

[1]Non-denning females are defined as 'Adult'

[a]Sub-groups with different letters are within the same season are significantly different ($p < 0.01$).

Table 3: Reference Intervals for Female Polar Bears from the Southern Beaufort Sea

Analytes	Units	Season	Subgroup	n	Mean	Standard Deviation	Median	Minimum	Maximum	Reference Interval	90% confidence interval for lower limit	90% confidence interval for upper limit
Alanine Transferase (ALT)	U/L	Spring	Adult[1,a]	162	50	25	43	11	160	16 - 126	11 - 25	96 - 160
			Denning[b]	32	33	9	35	13	55	14 - 53	9 - 18	48 - 58
			Sub-adult[c]	33	27	9	25	14	48	13 - 49	11 - 15	42 - 58
		Fall	Adult	110	28	12	27	11	70	12 - 65	11 - 13	50 - 70
			Sub-adult	37	27	8	26	15	43	14 - 45	12 - 16	40 - 51
Albumin	g/dL	Spring	Adult[a]	179	5.5	0.4	5.6	4.4	6.4	4.7 - 6.3	4.4 - 4.9	6.2 - 6.4
			Denning[b]	48	5.3	0.4	5.2	4.3	6.0	4.4 - 6	4.4 - 4.7	5.9 - 6
			Sub-adult[a,b]	42	5.5	0.4	5.4	4.7	6.2	4.7 - 6.2	4.7 - 5.1	6 - 6.2
		Fall	Adult	111	5.5	0.5	5.6	4.2	6.5	4.3 - 6.5	4.2 - 4.7	6.3 - 6.5
			Sub-adult	38	5.6	0.3	5.6	5.0	6.2	4.9 - 6.3	4.8 - 5.1	6.1 - 6.4
Alkaline Phosphotase (ALP)	U/L	Spring	Adult	165	40	27	32	6	139	8 - 116	6 - 10	91 - 139
			Denning	45	32	29	20	5	126	5 - 122	5 - 8	94 - 126
			Sub-adult[a]	38	66	26	68	26	132	12 - 120	1 - 24	108 - 132
		Fall	Adult[a]	106	32	25	24	3	127	6 - 115	3 - 9	88 - 127
			Sub-adult	36	64	41	50	10	173	11 - 181	7 - 18	141 - 229
Blood Urea Nitrogen (BUN)	mg/dL	Spring	Adult	187	16.7	13.1	12.0	2.0	57.0	2.4 - 48.8	2 - 2.7	45 - 57
			Denning	49	15.0	12.3	11.0	1.0	47.3	1.4 - 46	1 - 3	39.5 - 47.3
			Sub-adult	42	16.6	14.2	11.8	1.0	53.5	1 - 53.5	1 - 3	44 - 53.5
		Fall	Adult	113	16.6	18.0	9.5	2.7	114.5	3 - 70.3	2.7 - 3.9	53.2 - 114.5
			Sub-adult	32	10.2	5.9	8.0	4.0	24.3	4.1 - 28.9	3.9 - 4.6	20.2 - 38.3
Calcium	mg/dL	Spring	Adult	176	9.9	0.7	9.9	7.5	12.5	8 - 11.5	7.5 - 8.8	11.1 - 12.5
			Denning	48	9.8	0.7	9.8	7.8	12.4	7.8 - 12	7.8 - 8.8	10.7 - 12.4

			Sub-adult	42	10.2	0.4	10.1	9.4	11.1	9.5 - 11	9.5 - 9.6	10.7 - 11.1
		Fall	Adult	109	10.1	0.9	10.2	7.3	12.7	7.8 - 11.9	7.3 - 8.2	11.5 - 12.7
			Sub-adult	37	10.4	0.6	10.5	9.3	11.7	9.3 - 11.6	9.1 - 9.6	11.3 - 11.9
Creatinine	mg/dL	Spring	Adult	187	0.9	0.2	0.9	0.4	1.7	0.6 - 1.5	0.4 - 0.6	1.4 - 1.7
			Denning[a]	49	1.1	0.3	1.0	0.4	1.8	0.5 - 1.8	0.5 - 0.7	1.6 - 1.8
			Sub-adult	42	0.9	0.2	0.9	0.5	1.4	0.5 - 1.4	-0.5 - 0.6	1.2 - 1.4
		Fall	Adult	111	1.0	0.3	1.1	0.4	1.8	0.6 - 1.7	0.4 - 0.6	1.5 - 1.8
			Sub-adult	38	1.0	0.3	1.0	0.6	1.9	0.6 - 1.8	0.6 - 0.7	1.5 - 2.1
Globulin	g/dL	Spring	Adult	182	1.4	0.4	1.4	0.5	2.7	0.8 - 2.4	0.5 - 0.9	2.3 - 2.7
			Denning	48	1.3	0.5	1.3	0.2	2.3	0.3 - 2.3	0.2 - 0.8	2.2 - 2.3
			Sub-adult	41	1.3	0.3	1.2	0.8	1.9	0.8 - 1.9	0.8 - 1	1.8 - 1.9
		Fall	Adult[a]	109	2.0	0.7	1.9	0.6	4.4	0.8 - 3.8	0.6 - 1.1	3.2 - 4.4
			Sub-adult	38	1.7	0.3	1.6	1.0	2.5	1.1 - 2.3	0.9 - 1.2	2.2 - 2.5
Glucose	mg/dL	Spring	Adult	175	110	27	109	51	205	58 - 171	51 - 68	160 - 205
			Denning[a]	48	131	14	127	108	161	107 - 164	104 - 111	155 - 173
			Sub-adult	41	111	29	108	59	184	59 - 184	59 - 74	155 - 184
		Fall	Adult	108	117	25	116	31	201	56 - 175	31 - 81	154 - 201
			Sub-adult	38	111	26	110	64	160	57 - 164	46 - 71	151 - 176
Phosphorus	mg/dL	Spring	Adult[a]	180	5.3	1.3	5.2	2.4	9.8	3 - 8.3	2.4 - 3.3	7.6 - 9.8
			Denning[a,b]	49	5.8	1.1	5.8	3.3	8.3	3.4 - 8.2	3.3 - 4.1	7.5 - 8.3
			Sub-adult[b]	42	6.0	1.5	5.9	3.5	10.1	3.6 - 10	3.6 - 4.1	8.4 - 10.1
		Fall	Adult	111	5.2	1.5	5.0	2.5	10.6	3 - 9.9	2.5 - 3.3	7.8 - 10.6
			Sub-adult	37	5.2	1.6	4.9	2.6	9.0	2.7 - 9.1	2.3 - 3.1	7.9 - 10.4
Potassium	mmol/L	Spring	Adult	176	4.4	0.4	4.4	3.0	5.5	3.6 - 5.2	3 - 3.8	5.1 - 5.5
			Denning[a]	48	4.2	0.5	4.1	3.0	6.1	3.1 - 6.0	3 - 3.7	5 - 6.1
			Sub-adult	42	4.5	0.4	4.5	3.7	5.3	3.7 - 5.2	3.7 - 4	5 - 5.3
		Fall	Adult	107	4.8	0.7	4.7	2.9	8.1	3.5 - 7.2	2.9 - 3.9	5.9 - 8.1
			Sub-adult	38	4.8	0.4	4.8	4.2	5.9	4.1 - 5.8	4 - 4.2	5.5 - 6.2

Sodium	mmol /L	Spring	Adult	178	137	4	137	124	154	128 - 148	124 - 130	144 - 154
			Denning	44	136	4	137	129	145	129 - 145	129 - 130	141 - 145
			Sub-adult	40	140	2	139	136	146	136 - 146	136 - 137	143 - 146
		Fall	Adult	112	143	10	142	112	180	123 - 168	112 - 130	162 - 180
			Sub-adult	38	143	6	141	133	159	133 - 159	na	na
Total Bilirubin	g/dL	Spring	Adult	181	0.3	0.1	0.3	0.1	0.7	0.2 - 0.5	0.1 - 0.2	0.5 - 0.7
			Denning	49	0.3	0.1	0.3	0.2	0.5	0.2 - 0.5	0.2 - 0.2	0.4 - 0.5
			Sub-adult	41	0.3	0.0	0.3	0.2	0.3	0.2 - 0.3	0 - 0	0 - 0
		Fall	Adult	108	0.3	0.1	0.3	0.2	0.7	0.2 - 0.6	0.2 - 0.2	0.5 - 0.7
			Sub-adult	38	0.3	0.1	0.3	0.2	0.5	0.2 - 0.5	na	na
Total Protein (TP)	g/dL	Spring	Adult[a]	180	7.0	0.4	6.9	5.4	8.4	6 - 7.9	5.4 - 6.4	7.6 - 8.4
			Denning[b]	46	6.6	0.4	6.6	5.6	7.4	5.7 - 7.4	5.6 - 6	7 - 7.5
			Sub-adult[a,b]	41	6.8	0.2	6.8	6.4	7.3	6.5 - 7.3	6.5 - 6.6	7.2 - 7.3
		Fall	Adult	111	7.6	0.9	7.5	5.6	10.4	5.8 - 9.8	5.6 - 6.3	9.2 - 10.4
			Sub-adult	37	7.3	0.5	7.2	6.4	8.2	6.3 - 8.2	6.1 - 6.5	8 - 8.4

[1]Non-denning females are defined as 'Adult'

[a]Sub-groups with different letters are within the same season are significantly different ($p < 0.01$).

Table 4: Reference Intervals for Male Polar Bears from the Southern Beaufort Sea

Analytes	Units	Season	Subgroup	n	Mean	Standard Deviation	Median	Minimum	Maximum	Reference Interval	90% confidence interval for lower limit	90% confidence interval for upper limit
Alanine Transferase (ALT)	U/L	Spring	Adult	162	50	25	43	11	160	16 - 126	11 - 25	96 - 160
			Sub-adult[a]	32	33	9	35	13	55	14 - 53	9 - 18	48 - 58
		Fall	Adult	33	27	9	25	14	48	13 - 49	11 - 15	42 - 58
Albumin	g/dL	Spring	Adult	162	5.5	0.4	5.5	3.8	6.4	4.7 - 6.3	3.8 - 4.8	6.1 - 6.4
			Sub-adult	31	5.5	0.3	5.4	4.9	6.2	4.9 - 6.2	4.7 - 5	6 - 6.3
		Fall	Adult	32	5.5	0.3	5.5	4.8	6.4	4.9 - 6.2	4.7 - 5	6 - 6.4
Alkaline Phosphotase (ALP)	U/L	Spring	Adult	143	35.7	19.2	30.5	8.3	88.5	10.3 - 86.7	8.3 - 13	76.5 - 88.5
			Sub-adult[a]	32	68.7	43.3	48.8	11.5	166.5	12 - 167	na	na
		Fall	Adult	32	59.2	29.6	53.3	14.0	123.7	14 - 123.7	na	na
Blood Urea Nitrogen (BUN)	mg/dL	Spring	Adult	162	11.3	10.4	7.8	1.0	65.0	1 - 39.4	1 - 2	30 - 65
			Sub-adult	31	14.5	10.2	11.0	1.0	38.7	1 - 38.7	0.4 - 3	33.1 - 62
		Fall	Adult	32	17.6	12.4	13.0	3.0	49.0	2.6 - 53.1	2.3 - 3.9	39 - 66.6
Calcium	mg/dL	Spring	Adult	161	9.7	0.5	9.8	7.3	10.9	8.2 - 10.6	7.4 - 8.9	10.4 - 10.9

			Sub-adult[a]	31	10.1	0.5	10.1	9.4	11.1	9.2 - 11.2	8.9 - 9.4	10.9 - 11.4
		Fall	Adult	30	10.3	0.5	10.2	9.3	11.1	9.3 - 11.3	9.1 - 9.5	11 - 11.5
Creatinine	mg/dL	Spring	Adult	162	1.3	0.3	1.3	0.5	2.3	0.7 - 1.9	0.5 - 0.8	1.8 - 2.3
			Sub-adult[a]	32	1.0	0.2	0.9	0.4	1.5	0.5 - 1.4	0.4 - 0.6	1.3 - 1.5
		Fall	Adult	32	1.0	0.3	0.9	0.5	1.9	0.3 - 1.7	0.2 - 0.5	1.5 - 1.8
Globulin	g/dL	Spring	Adult	162	1.7	0.5	1.7	0.8	3.0	1 - 2.7	0.8 - 1.1	2.5 - 3
			Sub-adult[a]	32	1.5	0.4	1.5	0.5	2.3	0.6 - 2.3	0.4 - 0.8	2.1 - 2.5
		Fall	Adult	33	1.8	0.5	1.8	1.1	3.0	0.9 - 2.8	0.7 - 1.1	2.6 - 3
Glucose	mg/dL	Spring	Adult	161	125	25	124	59	199	80 - 177	59 - 86	167 - 199
			Sub-adult	31	123	26	124	72	188	69 - 178	56 - 82	164 - 192
		Fall	Adult	30	119	24	124	47	148	51 - 155	47 - 82	148 - 162
Phosphorus	mg/dL	Spring	Adult	162	6.1	1.2	6.2	3.1	9.4	3.7 - 8.6	3.1 - 4.1	8.2 - 9.4
			Sub-adult	28	6.3	0.8	6.3	5.1	7.9	4.8 - 7.9	4.4 - 5.2	7.5 - 8.3
		Fall	Adult	33	5.8	1.6	5.5	3.4	9.1	3.2 - 9.6	2.8 - 3.7	8.5 - 10.8
Potassium	mmol/L	Spring	Adult	158	4.6	0.3	4.6	3.8	5.4	4 - 5.2	3.9 - 4.1	5.1 - 5.4
			Sub-adult	31	4.5	0.4	4.6	3.6	5.3	3.7 - 5.3	3.5 - 3.9	5.1 - 5.5
		Fall	Adult	33	4.6	0.5	4.6	3.4	5.5	3.6 - 5.5	3.4 - 3.9	5.3 - 5.7

Sodium	mmol/L	Spring	Adult	162	139	4	139	119	155	132 - 150	119 - 133	145 - 155
			Sub-adult	31	139	3	139	133	144	133 - 145	132 - 135	143 - 146
		Fall	Adult	32	140	3	140	133	146	133 - 146	132 - 135	144 - 148
Total Bilirubin	g/dL	Spring	Adult	161	0.3	0.1	0.3	0.1	0.4	0.2 - 0.4	0.1 - 0.2	0.4 - 0.4
			Sub-adult	32	0.3	0.1	0.3	0.2	0.4	0.2 - 0.5	na	na
		Fall	Adult	33	0.3	0.1	0.3	0.2	0.6	na	na	na
Total protein (TP)	g/dL	Spring	Adult	162	7.2	0.4	7.2	5.5	8.3	6.5 - 7.9	5.5 - 6.7	7.8 - 8.3
			Sub-adult[a]	30	7.0	0.3	7.1	6.3	7.8	6.3 - 7.8	6.2 - 6.5	7.6 - 8
		Fall	Adult	33	7.3	0.6	7.3	6.3	8.9	6.1 - 8.7	5.9 - 6.4	8.3 - 9

[a]Sub-groups within the same season are significantly different ($p < 0.01$)

CHAPTER 3: LONGITUDINAL ASSESSMENT OF RELATIONSHIPS BETWEEN CHANGING ENVIRONMENTAL CONDITIONS AND THE PHYSIOLOGY OF SOUTHERN BEAUFORT SEA POLAR BEARS (*URSUS MARITIMUS*)

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Abstract

Climate change is influencing polar bear (*Ursus maritimus*) habitat, diet, and behavior, but the effects of these changes on their physiology is not well understood. Blood-based biomarkers are used to assess the physiologic health of individuals but their usefulness for evaluating population health, especially as it relates to changing environmental conditions, has rarely been explored. We describe links between environmental conditions and physiologic functions of southern Beaufort Sea polar bears using data from blood samples collected from 1984 – 2018, a period marked by extensive environmental change. We evaluated associations between 13 physiologic biomarkers and circumpolar (Arctic oscillation index) and regional (wind patterns and ice-free days) environmental metrics and seasonal and demographic co-variates (age, sex, season, year) known to affect polar bear ecology. We observed signs of dysregulation of water balance in polar bears following years with a lower annual Arctic oscillation index. In addition, liver enzyme values increased over time suggestive of potential hepatocyte damage as the Arctic has warmed. Biomarkers of immune function increased with regional-scale wind patterns and the number of ice-free days over the Beaufort Sea continental shelf and were lower in years with a lower winter Arctic oscillation index, signifying increased allocation of energetic resources for immune processes under these conditions. We propose the variation in polar bear immune and metabolic function is likely indicative of physiologic plasticity, a response that allows polar bears to remain in homeostasis even as they experience changes in nutrition and habitat in response to changing environments.

Introduction

Biomarkers of physiologic function can be powerful in contributing to the understanding of population health because physiology mechanistically connects an individual to its environment (Cooke et al., 2014; Madliger et al., 2018). Blood-based physiologic biomarkers provide dynamic measures of organ system function, electrolyte balance, protein quantity, nutrition, and immune function, and can be used to infer pathologic states in individuals (Gånheim et al., 2007; Friedrichs et al., 2012; Cooke et al., 2020; Madliger et al., 2021). Linking individual physiologic changes with abiotic factors is key to understanding the effects of rapid environmental change on population health. (Moore, 2008; Madliger and Love, 2015; Cooke et al., 2020). Thus far, most eco-physiological investigations have associated environmental changes with stress hormones in wildlife (see Möstl and Palme, 2002; Busch and Hayward, 2009; Sheriff et al., 2011; Narayan et al., 2019; Boonstra et al., 2020). Studies of other physiological measures, including those measuring immune and metabolic function, are less common (Cosgrove et al., 2017; Wilson et al., 2021).

The Arctic is warming substantially faster than the rest of the planet (Cohen et al., 2014; IPCC, 2018; DeRepentigny et al., 2020). Environmental changes to Arctic ecosystems include loss of sea ice, increased air and sea temperatures, altered precipitation patterns, and the thawing of permafrost (Frey et al., 2014; Stroeve and Notz, 2018; Box et al., 2019; Serreze and Meier, 2019). Climate-induced environmental changes have resulted in measurable effects on the habitat use, fitness, or health of many Arctic species, including polar bears (*Ursus maritimus*) (Derocher, 2005), sea birds (Irons et al., 2008), reindeer (*Rangifer tarandus platyrhynchus)* (Descamps et al., 2017), ringed-seals (*Pusa hispida*) (Ferguson et al. 2017), and beluga whales (*Delphinapterus leucas*) (Hauser et al., 2016). For polar bears, the loss of sea ice habitat is

considered the ultimate threat to their long-term persistence (Moore and Huntington, 2008; Atwood et al., 2016a; Hamilton and Derocher, 2018; Laidre et al., 2020).

Polar bears exist throughout ice-covered areas of the circumpolar Arctic in 19 subpopulations (Obbard et al., 2010). Research suggests that climate change may redistribute the polar bears (Wiig et al. 2008), alter predator-prey dynamics (Hamilton et al., 2017), and change diet and energetics (Molnár et al., 2009; Blanchet et al., 2020; Pagano and Williams, 2021. While these effects may ultimately influence subpopulation vital rates (Hamilton and Derocher, 2018; Bromaghin et al., 2021), the mechanistic links between environmental change and population dynamics remain unclear. A large part of the challenge in identifying mechanistic links between environmental and population-level change is the lack of comprehensive longitudinal data on species experiencing the effects of a changing environment.

The southern Beaufort Sea (SB) subpopulation of polar bears provides an opportunity to investigate the connections between environmental processes and physiologic function. Research on the SB spans over 40 years and has documented changes in habitat use (Rode et al., 2010, 2012; Atwood et al., 2016b), diet (McKinney et al., 2017), aspects of health (Kirk et al., 2010a, 2010b; Atwood et al., 2015, 2017; Whiteman et al., 2018), and population dynamics, including declines in survival and abundance (Regehr et al., 2010; Bromaghin et al., 2015, 2021)–all concomitant with the loss of sea ice habitat. In this study, we sought to identify mechanistic relationships between environmental processes, abiotic conditions, and polar bear physiology (Figure 1). We used a longitudinal data set of blood-based measurements that spans several decades to examine associations between environmental change and biomarkers of physiologic function. Our first objective was to (i) investigate relationships between regional- and circumpolar-scale environmental processes (i.e., sea ice phenology and wind speed, and the

Arctic Oscillation, respectively) and a suite of blood-based biomarkers that index metabolic and immune function. Using models developed for objective (i), we then (ii) considered if behaviors including habitat selection, short-term fasting, and reproductive status (for adult females) further influenced variation in analyte values. Our research addresses how biomarkers of polar bear physiologic function are affected by a changing climate and contributes to our understanding of the usefulness of blood-based biomarkers as a tool for assessing the health of wildlife populations experiencing environmental changes (Burek et al., 2008; Cooke et al., 2014; Ames et al., 2020; Madliger et al., 2021).

Methods

Field and Diagnostic

We used blood samples from polar bears captured and sampled on land and on the sea ice of Alaska's southern Beaufort Sea (USA) from 1983 to 2018. Spring captures (March–May) occurred on sea ice, whereas fall captures (October and November) occurred on either the sea ice or land (Figure 2). Polar bears were located by helicopter and immobilized with a rapid injection dart (Palmer Cap-Chur Equipment, Douglasville, Georgia, USA) containing Sernylan or M-99 prior to 1987 and, thereafter, zolazepam-tiletamine (Telazol® or Zoletil®, Stirling et al., 1989). Immobilized bears were aged, weighed (kg), and marked with a unique ear tag number and tattoo on the upper lip. Beginning in 2012, they were implanted with an AVID® subcutaneous microchip. Age was determined by direct observation (cubs of the year and yearlings), by extraction of a vestigial premolar and analysis of cementum annuli on initial capture for bears >1 year old and based on prior capture history (Ramsay and Stirling, 1988; Atwood et al., 2016b). We classified polar bears >4 years old as adults and bears 1 – 4 years old as young. Capture and handling of polar bears was conducted under appropriate research permits and animal care and

use approvals, including most recently Marine Mammal Research Permit MA690038-17 and USGS IACUC approval 2017-03.

Blood was collected into evacuated plain tubes (Vacutainer; BD Biosciences, Franklin Lanes, NJ) by venipuncture of the femoral vein and stored in a cooler with chemical heat packs to prevent freezing. Upon returning from the field each day, serum was separated from blood by centrifugation at 1,500 g for 5 min (TRIAC, Clay Adams, Parsippany, NJ) and frozen at -20°C. Frozen sera were transported to the laboratory and stored at -70°C until analyzed. Archived sera samples were analyzed on a VetScan VS2 biochemistry analyzer using the comprehensive diagnostic panel (Abaxis, Union City, California), which includes measurements of the following analytes: alanine aminotransferase (ALT), alkaline phosphatase (ALP), albumin (ALB), blood urea nitrogen (BUN), calcium (Ca), creatinine (CREA), phosphorus, sodium, and total protein (TP). Globulin (GLOB) was calculated by subtracting ALB from TP; however, for some samples only a subset of these analytes was available.

Between 2005 and 2018 a complete blood count (CBC) was performed on whole blood samples on the day of capture. Blood for CBC analysis was collected into vacutainers containing potassium EDTA (Vacutainer; BD Biosciences, Franklin Lanes, NJ) and transported as described above. Prior to 2008, CBC included total white blood cell count (WBC), differential blood cell counts, and packed cell volume (PCV) conducted manually as described by Kirk et al. (2008). Beginning in 2008, CBC and hematocrit were obtained from whole blood using a diagnostic analyzer (HM5, Abaxis, Union City, CA). Differential blood cell counts included counts of neutrophils and lymphocytes, which enabled us to evaluate their ratio as a measure of acute stress. The functional and interpretive characteristics of the biomarkers evaluated are summarized in Table 1 (recreated from Fry et al., 2019).

Statistical Analysis

Our primary goal was to evaluate the influence of environmental processes occurring at different temporal and spatial scales on biomarkers of physiology (Table 1). We evaluated how biomarker values responded to variation in circumpolar- and/or regional-scale atmospheric circulation and weather, which are known to influence sea ice phenology, habitat quality, and access to and condition of prey (Rigor et al., 2002; Stroeve et al., 2011; Pilfold et al., 2015; McKinney et al., 2017; Atwood et al., 2021; Rode et al., 2021a). We evaluated the effects of these processes using the Arctic Oscillation (AO) index, the number of ice-free days over the continental shelf, and wind speed. We further explored the extent to which differences in summer habitat selection (i.e., use of land versus sea ice), breeding status of females, and short-term fasting status influenced physiologic biomarkers. Because demographic characteristics of polar bears and season affect physiology (Derocher et al., 1990; Kirk et al., 2010a; Whiteman et al., 2015, 2018; Atwood et al., 2016b; Rode et al., 2017, 2021a; Fry et al., 2019), we included sex, age, and denning status, as well as season of capture and year as co-variates in our analysis.

The AO index is a measure of the variability in sea-level atmospheric pressure, surface air temperature, and surface winds over the Arctic (Thompson and Wallace, 1998; Rigor et al., 2002; Ogi et al., 2016) and has been shown to affect polar bear diet (McKinney et al., 2017), body condition and behavior (Pilfold et al., 2015; Rode et al., 2018), and ringed seal (*Pusa hispida*) condition and vital rates (Ferguson et al., 2017, 2020; Harwood et al., 2020). A lower AO is associated with sea ice persisting longer during spring, a higher proportion of multi-year ice present, and fall freeze-up occurring earlier across the Arctic basin (i.e., the annual ice-free season is shorter) (Rigor et al. 2002). In years with higher AO, the opposite effects prevail, including delayed sea ice formation in the fall, which extends the number of ice-free days during late summer and early fall (Rigor et al., 2002; Stroeve et al., 2011). We included two measures of

the AO index in our model, the average annual AO (AAO) and the winter AO (WAO) to evaluate the influence of circumpolar-scale environmental processes on polar bear physiology. AAO was calculated by averaging monthly AO in the calendar year prior to capture. This metric, which ranges from -2 to 2, captures annual atmospheric circulation patterns that may affect sea ice conditions experienced by SB polar bears prior to capture. The winter AO is a more proximate measure of the AO index as it relates to spring sea ice conditions. The WAO was calculated as the mean AO for the months of January – March in the year of capture. We expected that during years with a higher WAO sea ice would be thinner and break up more easily, resulting in increased lead formation, which provides hunting habitat for polar bears (Rigor et al., 2002). We calculated AAO and WAO using data reported by the Climate Prediction Center, National Weather Service, National Oceanic and Atmospheric Administration (https://www.cpc.ncep.noaa.gov/products/precip/CWlink/).

We used indices of sea ice phenology (ice-free days) and sea surface windspeed (mean and standard deviation in the 14 days prior to capture) to assess relationships between regional-scale environmental conditions and physiologic function. Sea ice data were obtained from the National Snow and Ice Data Center (NSIDC; Boulder, Colorado, USA) and processed as described in Atwood et al. (2021). We considered the number of ice-free days over the continental shelf in the SB based on sea ice concentrations of ≤50% and ≤15%. Polar bears generally prefer sea ice concentrations >50%, although SB polar bears have been shown to tolerate lower sea ice concentrations (Durner et al., 2009; Pagano and Williams, 2021). Further, 15% sea ice is the minimum concentration reliably detected from imagery (Stern and Laidre, 2016). Sea ice concentration data were obtained using 25 x 25 km resolution raster of passive microwave satellite imagery (Cavalieri et al., 2006).

Wind speeds influence sea ice movement, affecting the formation and closure of leads and, subsequently, the distribution of sea ice foraging habitat (Carlens et al., 2006; Pilfold et al., 2015; Rode et al., 2017). We extracted offshore wind speed from the North American Regional Reanalysis (NARR) at 11 NARR grid points distributed longitudinally from Utqiaġvik, Alaska, to the MacKenzie River Delta, Northwest Territories, Canada. NARR wind estimates were disseminated as u, v vector components with 3-hr periodicity. Briefly, we derived wind speeds (m/s) from the u, v components, averaged them daily, and then for each capture date we calculated the 14-day average and standard deviation from daily means (Atwood *et al*. 2021). Table 2 summarizes the climate variables and co-variates used to model changes in blood-based biomarkers.

We analyzed data for male and female polar bears separately using linear model selection procedure for all possible models, with each biomarker as the dependent variable using the leaps package (v3.1; (Miller, 2020)) in R 3.5.0 (R Core Team, 2018). Exploratory analysis did not show interactions between variables, so we did not include interaction terms in models. We report all models within Δ2BIC (Bayesian Information Criterion) of the model with the lowest BIC (Burnham and Anderson, 2004; Tredennick et al., 2021). For each significant parameter ($P \leq 0.05$), we calculated 85% confidence intervals to identify potentially uninformative parameters (Arnold, 2010) and checked for multicollinearity of variables in the top models using variance inflation factors. We used the natural log transformation of ALP, BUN and WBC to standardize their distributions and calculated z-scores to standardize the ice-free days and wind variables.

We successively added each of three behavior variables (summer onshore habitat-use, recent fasting condition, and breeding status), individually, to each model with the lowest BIC to evaluate the behavior variable's influence on the biomarker. Bowhead whales are available to SB

bears in summer and fall through scavenging whale remains left on land by subsistence hunters (Herreman and Peacock, 2013; Rogers et al., 2015). To evaluate the influence of on-shore/off-shore habitat use we used dietary data from a subset of polar bears sampled between 2005 and 2016 as part of another study (McKinney et al., 2017; Bourque et al., 2020) to assign bears to summer habitat use categories (onshore or sea ice). Bears with >5 % bowhead whale (*Balaena mysticetus*) in their diet were considered to have used on-shore habitat during the summer prior to capture (see Atwood et al., 2016a). To ascertain fasting status, we calculated the BUN:CREA ratio ((BUN x 0.466)/CREA) and considered individuals with serum BUN:CREA ≤ 12.7 to have been fasting during the 10 days prior to capture (Nelson et al., 1984; Cherry et al., 2009; Rode et al., 2017). We considered all adult females without cubs to be capable of breeding in the spring prior to capture, while females captured in spring with cubs of any age were not in breeding condition. We compared the log likelihood ratio for the nested models, the model with the lowest BIC with the behavior added as a parameter, and the same model without the behavior, using a chi-squared test.

Results

We analyzed blood samples from 1,258 polar bears captured between 1983 and 2018. Sample sizes for biomarkers varied by demographic class and sampling season (Table 3) with subsets of this population to evaluate each of the behaviors (Table 4). The two measures of ice-free days (ice-free [50], ice-free [15]) and the wind variables (mean wind speed and variability of wind speed) were correlated, and thus, not included in the same models (R_{corr}=0.89, R_{corr}=0.79, respectively). No other predictor variables were correlated. We report the coefficients of significant variables ($P \leq 0.05$) and coefficients of determination of all models within $\Delta BIC \leq 2$ for females (Table 5) and males (Table 6).

Model Selection: Female SB Polar Bears

Demographic and seasonal covariates were included in top models for all analytes, whereas associations between analytes and environmental parameters varied (Table 5). BUN declined in years with a positive WAO for denning females, and with capture year, and increased with the number of ice-free days based on 15% sea ice concentration. CREA levels were lower for individuals captured during spring, declined with mean wind speed and age, and increased for denning females. Sodium levels were lower for bears captured during spring and significantly higher in years with a lower AAO in the year prior to capture. Calcium and phosphorous levels were higher in young females and had opposite responses to season and capture year, with calcium levels lower in spring and increasing with year, and phosphorus levels higher in spring and decreasing with year.

The liver enzymes ALT and ALP were not associated with any of the environmental parameters; however, activity of these enzymes increased annually and was lower in females with cubs of the year. ALT activity in female polar bears was lower in spring than in fall. ALP activity was higher in young individuals.

Associations between environmental processes and markers of immune function varied considerably among models. GLOB increased with the number of ice-free days (15% sea ice concentration) and in years with a lower WAO. ALB decreased during years with a higher WAO, while WBC increased when wind speeds were more variable. GLOB, ALB, TP, WBC, and N:L ratio varied with capture season and demographic co-variates (age class and denning status). GLOB and TP concentrations and N:L ratios were lower during spring and in young bears. Markers of immune function (WBC, TP, ALB) declined over time in females with cubs of the year.

The addition of behavior parameters improved model fit for ALP, phosphorous, and WBC. ALP activity increased in female bears that used onshore habitat, were breeding, and that had eaten within the 12 days prior to capture. Phosphorus levels were significantly higher in bears that appeared to have recently eaten (i.e., BUN:CREA >12.7). WBC concentration was significantly higher in mating females (Table 7).

Model Selection: Male SB Polar Bears

For male polar bears, environmental parameters were more often associated with biomarkers than for female bears (Table 6). BUN increased with variation in wind speed and was significantly lower during spring than during fall. CREA levels increased with capture year and declined as ice-free days and mean wind speed increased. Sodium increased with lower AAO and was lower for bears captured during the spring, following the same patterns as female polar bears. The top model for phosphorous included a negative relationship with ice-free days. Influence of the AAO on ALB levels was mitigated by the WAO during the year of capture. ALB and GLOB showed opposite responses to ice-free days, with ALB levels declining and GLOB increasing. These opposing trends resulted in TP levels remaining unchanged.

ALT activity was not associated with environmental variables but increased significantly with capture year and was lower in young bears. ALP activity increased as the number of ice-free days increased and declined with WAO. PCV increased with a declining AAO. WBC and the N:L ratio were lower in younger individuals and WBC count declined with WAO.

The addition of behavior parameters in the top models significantly improved model fit for six analytes. Males that had been fasting prior to capture showed increased liver enzyme activity (ALT and ALP) and phosphorous levels, whereas use of onshore habitat resulted in declines in ALB, CREA, and calcium levels (Table 6).

Discussion

We show that measures of atmospheric conditions, sea ice availability, and wind are associated with biomarkers of metabolic and immune function of SB polar bears. Responses of some analytes (sodium, phosphorous, and PCV) varied with circumpolar-scale parameters; whereas others (WBC, BUN, ALB, GLOB, CREA) varied with regional-scale parameters (i.e., wind speed and ice-free days). Biomarkers of acute and chronic immune function (e.g., WBC and ALB, GLOB, respectively) varied with the time-lagged effects of the AAO and sea ice conditions. Collectively, these results provide evidence of associations between polar bear physiology and climate-driven changes to the Arctic ecosystem (McKinney et al., 2013, 2017; Atwood et al., 2016b; Pagano et al., 2021).

Several of the biomarkers we evaluated (e.g., sodium, CREA, BUN, proteins, and PCV) are used, in part, to assess water balance, which is linked to diet in polar bears (Table 1). Polar bears showed increases in sodium (males and females) and PCV (males only) in the spring following a year when the AAO was in a negative phase. Sodium is an important and narrowly regulated cation of osmoregulation that is hormonally maintained through intake and excretion. Such fluctuations in sodium suggest hemoconcentration, a response that may be driven by changes in diet. The significant changes we observed in sodium and PCV are likely linked to the ability of polar bears to maintain consistent access to a fat-rich diet. Polar bears primarily obtain water by catabolizing fat from marine mammal prey (Nelson, 1987). Ringed seals, the primary prey of polar bears, showed declines in blubber thickness in years with a lower WAO (Harwood et al. 2020) and reduced reproductive rates with a lower AAO (Nguyen et al. 2017). Changes in the abundance and/or condition of prey could result in decreased fat consumption and increased protein consumption (Nelson, 1987; Cherry et al., 2009; Ferguson et al., 2017; Pagano et al.,

2018; Rode et al., 2021b), resulting in dysregulation of water balance. If polar bears increase the proportion of protein in their diet, the need for external sources of water would increase and fat accumulation could decrease, requiring muscle catabolism for gluconeogenesis potentially amplifying this effect (Nelson 1987). In addition, we found that in years with lower WAO, female polar bears had higher BUN levels, a response that occurs when dietary protein exceeds anabolic requirements. Such changes in the nutritional makeup of diet are likely to result in synergistic effects on polar bear physiology (Ferguson et al. 2017).

We also observed annual changes in biomarkers linked to liver function. In clinical veterinary medicine, small increases in liver enzyme activity are often masked by wide reference intervals (Fry et al., 2019), with pathologic concerns indicated by at least a 2-fold or greater change in enzyme activity. Although we did not see multifold increases in ALT, it significantly increased with capture year in both male and female bears. Increases in ALT activity can indicate hepatocyte injury caused by liver disease. We cannot confirm pathophysiology associated with this increasing ALT activity, but several hypotheses warrant further investigation, including whether increases in ALT could indicate liver damage caused by pathogens, pollutants, or diet.

Biomarkers of immune function varied with circumpolar and regional-scale environmental indices for male and female polar bears. Monitoring changes in WBC allow for immediate evaluation of immune system activity; whereas changes in serum proteins, ALB and GLOB, reflect adaptive immune response. WBC increased in females when winds were more variable in the two weeks prior to capture and with higher WAO in males. In previous studies, wind speed and the higher WAO resulted in reduced polar bear movement and foraging (Rode et al., 2017; Togunov et al., 2017), but also can create optimal hunting habitat. Such conditions may trigger increased opportunities for injuries from contact with conspecifics or interactions

with prey that could potentially increase WBC (Ovsyanikov, 1995). To determine whether these WBC changes were suggestive of acute versus chronic inflammatory responses, we evaluated the ratio of neutrophils to lymphocytes. We expected that acute infections would exhibit higher neutrophils relative to lymphocytes, whereas chronic infection would have higher lymphocytes relative to neutrophils (Thrall et al., 2012). However, we were unable to differentiate acute versus chronic inflammatory responses using the N:L ratio. We did see that breeding females showed increased WBC, which may be a direct response to interactions with males and the risks associated with breeding, including injury and infection (Ramsay and Stirling, 1986; Derocher et al., 2010).

The association between environmental conditions and ALB and GLOB (which collectively reflect TP) are further evidence of an effect on immune function, specifically the inflammatory response (Thrall et al., 2012). Individually, both ALB and GLOB were significantly associated with environmental covariates. For both males and females, ALB increased with higher WAO and in males declined with the AAO and ice-free days. GLOB increased with ice-free days and in years with a lower WAO, while TP remained unchanged across all environmental conditions. The magnitude of changes in GLOB and ALB were small, with decreases in ALB offset by increases in GLOB. These changes, paired with increases in WBC under the same conditions, suggest an increased energetic allocation for immune activity in years with a lower WAO. These results may be indicative of the cumulative effects of changing environmental processes on polar bear physiology; however, determining if these environmental parameters cause disease would require extensive diagnostics to ascertain the source of the inflammatory response.

Previous studies have shown differences in pathogen exposure and immune system function based on habitat use for SB polar bears. For example, use of on-shore habitat led to higher WBC counts and GLOB levels than in bears that used sea ice year-round (Whiteman et al. 2018). Declines in persistent organic pollutants (Atwood et al. 2017) and methyl mercury exposure (McKinney et al. 2017) were observed in on-shore bears, a response that was attributed to an increase in bears feeding on lower trophic position foods while on land. Atwood et al. (2017), using antibody seroprevalence, demonstrated that the diversity of pathogen exposure varied based on summer habitat use. However, we found limited evidence that on-shore habitat use influenced metabolic and immune function, with significant differences between on-shore and on-ice bears observed in only a few analytes (Table 6). It is important to note that the previously mentioned studies differed from ours relative to objectives, hypotheses, and study designs (including types of samples collected) and are not directly comparable. The majority of SB polar bears still use sea ice year-round (Atwood et al. 2016), which may explain the limited effect of summer habitat use on the blood-based biomarkers used in this study.

Extrapolating clinical pathologic data intended to answer questions about individual animal health to population health is complex and is generally limited to animal husbandry in agricultural settings (Cook et al., 2006; Gånheim et al., 2007; Huzzey et al., 2014). A key challenge in evaluating physiologic function relative to environmental conditions is accounting for processes that occur at different temporal and spatial scales. For example, biochemical analytes represent cellular-level activity days and weeks prior to sampling, whereas the environmental conditions reflect processes occurring over time scales ranging from several days to a year. Similarly, climate indices (e.g. WAO, AO) which are used to describe Arctic-wide conditions have been shown to result in opposite effects at smaller spatial scales. For example, a

lower WAO is expected to result in the maintenance of spring sea ice in the southern Beaufort Sea has instead been found to enhance sea ice divergence in the eastern Beaufort Sea (adjacent to our study area) resulting in the early formation of polar bear habitat (Rigor *et al*. 2002). Inference is further complicated because physiologic profiles of individuals at a single point in time provides a "snapshot" of an individual's physiologic function, which may be muted at the population level, making it difficult to assess whether changes represent physiologic plasticity or pathology. Nevertheless, we found significant relationships between select physiologic biomarkers and environmental processes in the southern Beaufort Sea, which highlights their usefulness for monitoring the health of wildlife populations vulnerable to environmental change (Cooke and O'Connor, 2010).

Polar bear life history characteristics and population dynamics are being influenced by global warming (Rode et al., 2010; Bromaghin et al., 2015, 2021; Atwood et al., 2016b; Pagano et al., 2018), and the effects of these changes are expressed at the physiologic level. Our research demonstrates that physiologic biomarkers varied with ecosystem and demographic parameters and are likely plastic responses to changes in diet and nutrition resulting from environmental change (Boonstra, 2013). Our findings also indicate it may be beneficial to prioritize the evaluation of tightly regulated blood-based biomarkers such as sodium and PCV, as opposed to more widely regulated analytes (e.g., ALT, ALP, BUN, CREA, TP) to inform population-level physiologic perturbations related to climate change. Additionally, biobanking blood and serum samples for -omics research (e.g., metagenomics, metabolomics, microbiomics, viromics, epigenetics and transcriptomics), may be valuable for clarifying mechanistic relationships between physiologic and environmental processes (Breithoff and Harrison, 2020). Continuing to explore the physiologic effects of climate change, including the role of clinical pathology in

conjunction with community and ecosystem conditions, will further our understanding of the health of wildlife populations. (Patyk et al., 2015; Wittrock et al., 2018).

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Conflict of Interest

The authors have no conflicts of interest to declare. All co-authors have seen and agree with the contents of the manuscript. We certify that the submission is original work and is not under review at any other publication.

Data Availability

The data that support the findings of this study are openly available in USGS Alaska Science Center data repository at www.usgs.gov/centers/alaska-science-center/science/polar-bear-research#data.

Figures

Figure 1: Graphical Abstract

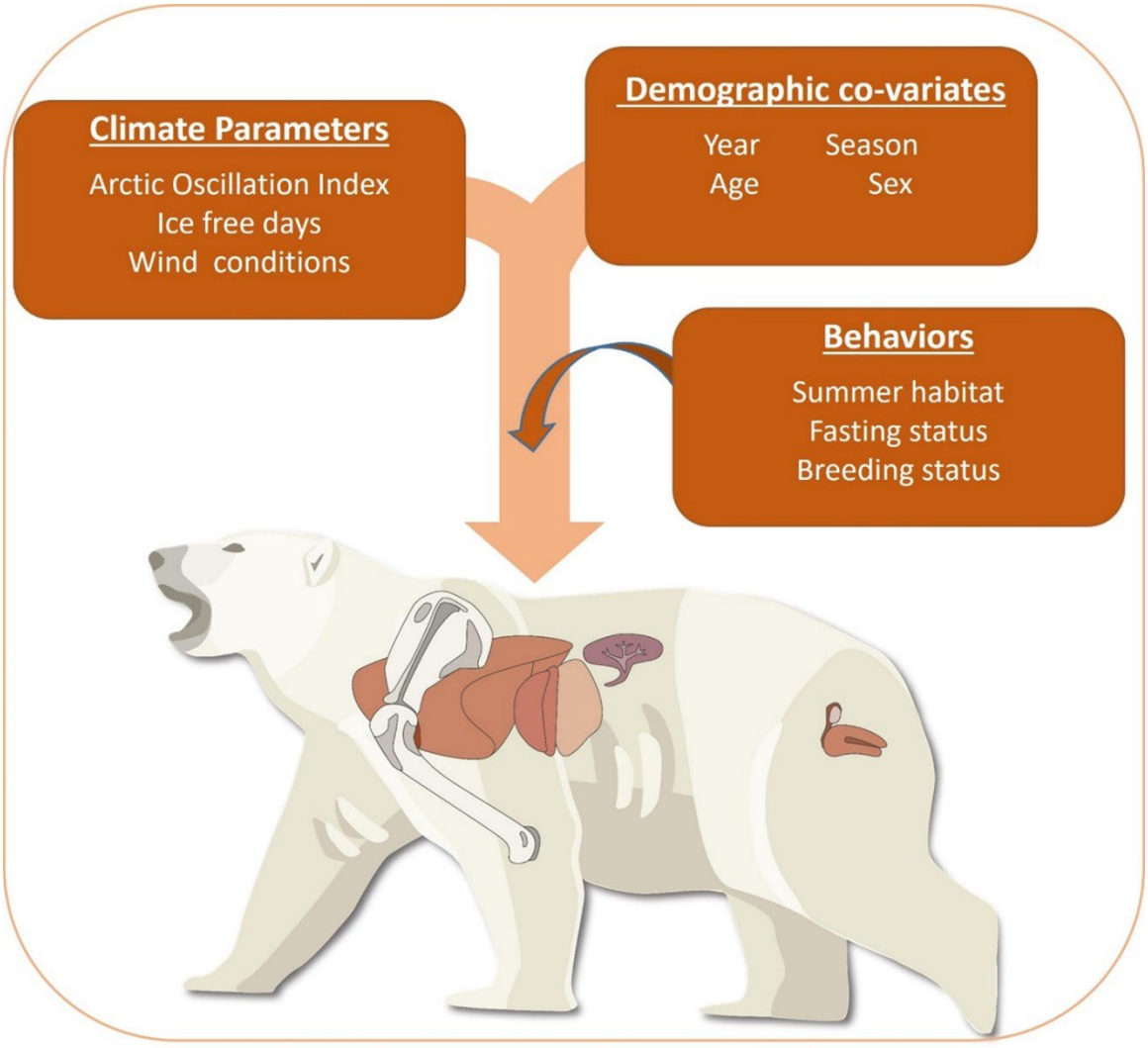
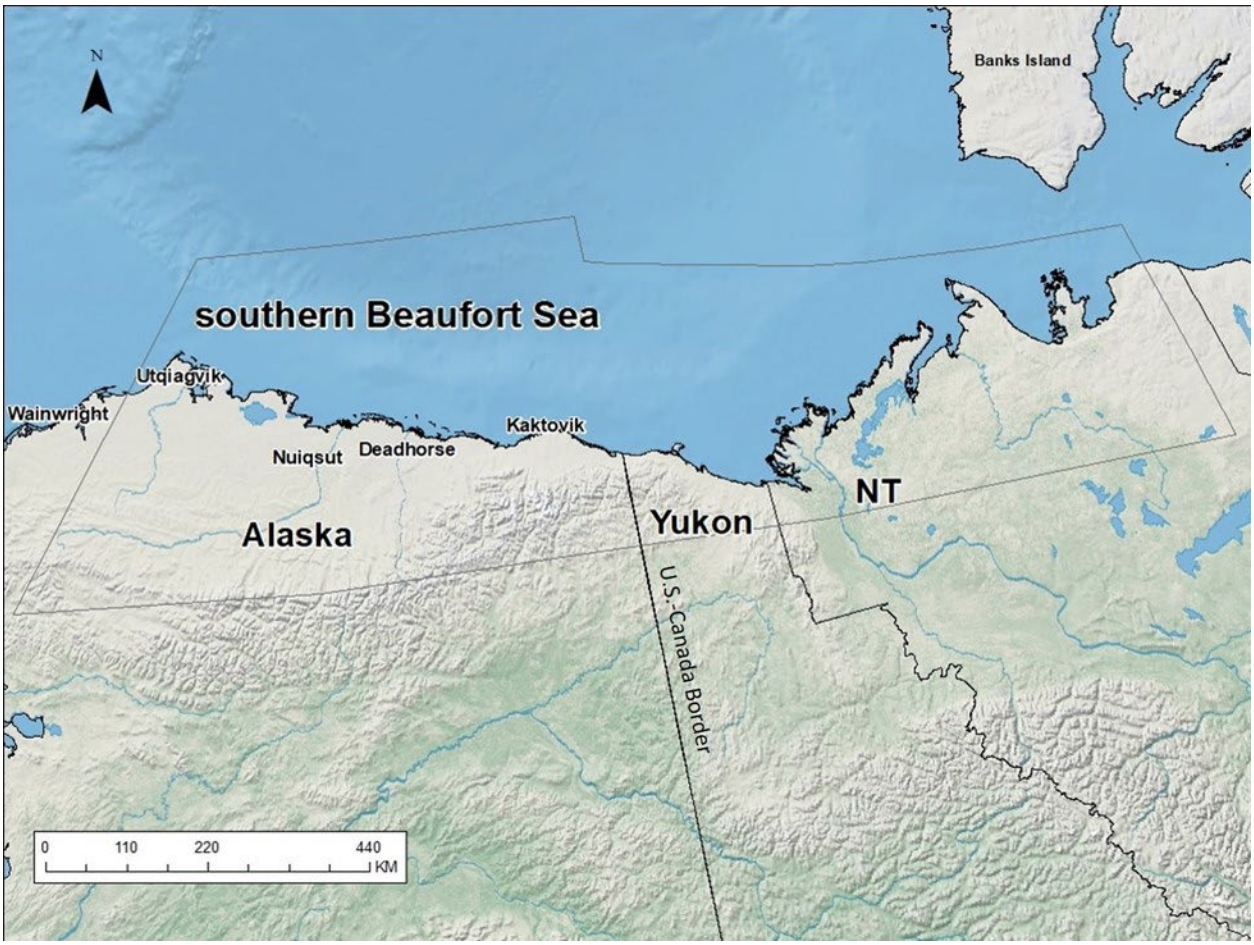


Figure 2: Study Area

Between 1983 and 2018, polar bears were captured within the IUCN defined boundary (thin line) for the southern Beaufort Sea sub-population between Icy Cape, Alaska and the United States-Canada border.



Tables

Table 1: Summary of blood-based analytes

	Analyte (dependent variable)	Brief interpretive use	Metabolic	Immune
Liver Enzyme	Alanine transferase (ALT)	Increases with some hepatic and severe muscle disorders	*	
	Alkaline phosphate (ALP)	Increases in some liver and bone disorders, increases during active bone growth (juveniles)	*	
Proteins	Albumin (ALB)	Synthesized by liver, source of amino acids, acts as carrier protein, increases with dehydration; a negative acute phase protein that decreases with inflammation as globulin increases; decreases with some liver and renal disorders	*	*
	Globulin (GLOB)	A positive phase protein that when increased indicates an immune response of significant duration (several days or more), individual globulins can be measured for specific information		*
	Total Protein (TP)	Changes in TP are reflected by changes in albumin, globulins, or both	*	*
Kidney Function	Blood Urea Nitrogen (BUN)	Product of protein catabolism, source of nitrogen for protein synthesis, eliminated primarily by kidney; decreases with low protein intake and liver failure, increases with high protein meals and with decreased renal elimination (↓ Glomerular filtration rate (GFR))	*	
	Creatinine (CREA)	Catabolic product of muscle, eliminated through kidney, low muscle mass results in lower basal concentrations; increases with decreased renal elimination (↓GFR)	*	
Electrolytes	Phosphorous	Structural component of bone, important anion for energy generation (ATP), Increases with decreased renal elimination (↓GFR)	*	
	Sodium	Important cation for osmoregulation, strictly regulated by renal and hormonal function	*	
	Calcium	Structural component of bone, hormonally- regulated	*	
Complete Blood Count	White blood cell count (WBC)	Generally, a measure of immune function including acute infection resulting from injury, infection, inflammation or general pathology		*
	Neutrophil:Lymphocyte Ratio(N:L ratio)	Increased ratio suggests acute stress		*
	Packed Cell Volume (PCV)	Decreased PCV indicates anemia associated with hemorrhage or inflammation, increases suggest dehydration	*	

Table 2: Description of Model Parameters

	Variable	Description
Environmental Processes	Mean Wind Speed[1]	Mean wind speed in SB 14 days prior to capture
	Variability of Wind Speed[1]	Standard deviation of wind speed in SB 14 days prior to capture
	Ice-free days 15%[2]	Number of ice-free days over the continental shelf in the SB in year prior to capture as determined by 15% sea ice concentration (Cavalieri et al. 2006)
	Ice-free days 50%[2]	Number of ice-free days over the continental shelf in the SB (two concentrations) as determined by 50% sea ice concentration (Cavalieri et al. 2006)
	Arctic Oscillation (AO)[3]	Mean of monthly Artic oscillation index in the year prior to capture
	Winter Arctic Oscillation (WAO)[3]	Mean monthly AO for January – March of year of capture
Co-variates	Capture year	Calendar year of capture
	Season	Spring (March – May) / Fall (October – November)
	Denning status	Captured with cubs of the year
	Age Class	Young (1-4 years old)/ Adult > 4 years old
Behavior	On/off-shore Status	Polar bears with >5% bowhead in diet were considered onshore bears
	Fasting Status	Polar bears with a blood urea nitrogen:creatinine ratio ≤ 12.7 were considered to be fasting for the 10 days prior to capture
	Breeding Status	Females captured without cubs in the spring prior to capture
[1, 2] variables were not included in the same models, [3] index ranges from -2 to 2		

Table 3: Maximum (minimum) sample sizes for model analysis of blood-based biomarkers

Table 3: Maximum (minimum) sample sizes for model analysis of blood-based biomarkers										
	Females						Males			
	Spring			Fall			Spring		Fall	
	Adults	Adults with cubs of the year	Young	Adults	Adults with cubs of the year	Young	Adult	Young	Adult	Young
Serum Based Analytes	368 (354)	100 (92)	69 (65)	110 (108)	9 (9)	41 (41)	252 (546)	64 (63)	21 (20)	17 (17)
Complete Blood Counts	154 (141)	31 (19)	32 (32)	17 (9)	7 (5)	5 (5)	150 (149)	34 (34)	No data	No data

Table 4: Number of polar bear samples available to assess the influence of summer behavior, fasting, and breeding on blood biomarkers

	Sample size[1]			
	Females		Males	
	0	1	0	1
Shore status (0=offshore, 1=onshore)	94	123	60	145
Fasting (0=fasting, 1=feeding)	552	153	312	42
Breeding (0=not breeding 1=breeding)	219	586		
[1]Maximum sample size varied by analyte				

Table 5: Summary of the significant coefficients (standard error, *P*-value) in the top models (within 2.0 ΔBIC from the top model) describing the influence of climate and demography on blood-based biomarkers in female SB polar bears.

	Climate and Weather Parameters					Demographic Parameters				Model Fit		
Analyte	AAO[1]	WAO	Number ice-free days[2a,b]	Mean Wind Speed[3]	Standard deviation Wind Speed[3]	Year	Season[4]	Age class[6]	Denning[5]	adjusted R^2	BIC	ΔBIC
ALT						0.64(.04)***	-3.17(.83)***		-6.81(.94)***	0.32	-243.70	0
				0.92(.38)**		0.63(.04)***	-2.43(.88)**		-6.95(.93)***	0.33	-242.83	0.89
				1.27(.35)***		0.6(.04)***			-7.31(.93)***	0.32	-241.90	1.79
ALP						0.01(.01)***		0.65(.09)***	-0.37 (.09)***	0.12	-62.14	na[7]
			0.11(.03) [2a]***					0.63(.09)***	-0.36(.09)***	0.12	-61.57	0.57
ALB		0.06(.02)***							-0.19 (.05)***	0.03	-4.18	na
GLOB		-0.05(.02)**	0.09(.02)[2a]***				-0.58(.04)***	-0.19(.05)***		0.22	-144.91	0
			0.10(.02) [2a]***				-0.57(.04)***	-0.18(.05)***		0.22	-143.60	1.31
TP						0.01 (0)***	-0.66 (.06)**	-0.20 (.07)**	-0.28 (.07)	0.16	-89.49	0
							-0.6(.06)***	-0.2(.07)**	-0.25(.07)***	0.15	-88.40	1.09

BUN		-0.07(.03)**	0.14(.05)** 2a**		`	-0.02 (0)***			-0.22(.08)**	0.04	3.11	0.00
			0.14(.05)2a**			-0.02 (0)***			-0.23(.08)**	0.03	3.44	0.33
									-0.24(.08)**	0.01	3.73	0.62
		-0.07(.03)**							-0.23(.08)**	0.02	4.04	0.93
		-0.08(.03)**	0.14(.05)2a**			-0.02(0)***				0.03	4.56	1.45
		-0.06(.03)**	0.12(.06)2b**			-0.02(0)***				0.03	5.11	2.01
CREA				-0.03(.01)**			-0.09 (.03)**	-0.09 (.03)***	0.09 (.03)**	0.04	-2.46	na
Sodium	-2.39 (.69)***						-4.52 (.64)***			0.09	-51.4	na
Calcium						0.02 (0)***	-0.37 (.09)***	0.32 (.1)**		0.07	-24.34	na
Phos						-0.02 (.01)***	0.40 (.13)**	0.40 (.15)**		0.03	1.34	0.00
						-0.02(.01)***	0.37(.13)**			0.02	2.03	0.69
	0.38(.14)**					-0.02(.01)**				0.02	2.15	0.81
		0.11(.05)*				-0.02(.01)***	0.44(.13)***	0.42(.15)**		0.04	2.72	1.38
						-0.02(.01)**				0.01	2.75	1.41
	0.31(.14)*					-0.02(.01)***	0.35(.13)**	0.4(.15)**		0.04	2.94	1.60
	0.37(.14)**					-0.02(.01)**	0.35(.15)*			0.03	3.14	1.81
	0.38(.13)*			-0.13(.06)*		-0.02(.01)**				0.03	3.21	1.87
WBC					0.14 (.04)***			0.15(.06)**	-0.25(.06)***	0.15	-21.61	0
					0.14(.04)***				-0.28(.05)***	0.13	-19.89	1.72

N:L Ratio							-3.26(.77)***	-2.50(.7)***		0.11	-12.2	na
PCV							-8.47(1.19)***	2.44(.9)**	-2.80(1.05)**	0.22	-33.88	0
			0.74(.36)[2a*]				-7.93(1.21)***	2.38(.89)**	-2.84(1.04)**	0.23	-32.69	1.19
							-7.72(1.17)***	2.85(0.9)**		0.2	-32.1	1.78

[1] year prior to capture, [2] sea ice concentration in year prior to capture, [2a]15 percent cover, [2b] 50 percent cover, [3] 14 days prior to capture, reference is [4]Fall, [5]not denning, [6]adult, [7]na= no model within ΔBIC of the presented model. P-value: <.05*. <.01**, <.001***.

Table 6: Summary of the coefficients (standard error) in the top modes (within 2.0 ΔBIC from the top model) describing the influence of climate and demography on blood-based biomarkers in male SB polar bears

	Climate and Weather Parameters					Demographic Parameters			Model Fit		
Analyte	AAO[1]	WAO	Number ice-free days[2a,b]	Mean Wind Speed[3]	Standard deviation Wind Speed[3]	Year	Season[4]	Age class[5]	adjusted R^2	BIC	ΔBIC
ALT						1.04(.15)***		-13.41(3)*	0.18	-54.68	na[6]
ALP		0.15(.04)***	0.21(.04)[2b]***				-0.50(.13)***	0.45(.09)***	0.16	-35.16	0
		0.1(.04)*	0.22(.04)[2a]***				-0.44(.13)***	0.45(.09)***	0.16	-34.07	1.09
			0.22(.04)[2a]***				-0.42(.13) **	0.45(.09)***	0.15	-33.98	1.18
ALB	-0.16(.06)**	0.1 (.02)***	-0.1(.02) [2a]***						0.09	-14.68	0
	-0.16(.06) *	0.09(.02)***	-0.1(.02) [2a]***		0.05(.02) *				0.11	-14.51	0.17
		0.08 (.02)***	-0.11(.02) [2a]***						0.08	-13.84	0.84
		0.08(.02)***	-0.11(.02) [2a]***		0.05(.02) **				0.09	-13.73	0.95
GLOB			0.13 (.03) [2a]***				-0.27 (.08)**	-0.32 (.06)***	0.13	-26.85	0
	0.16(.07)*		0.12(.03)[2a]***				-0.28(.08)***	-0.31(.06)***	0.14	-26.22	0.63

	0.2(.07)**	-0.06(.03)*	0.12(.03)[2a]***				-0.27(.08)**	-0.31(.06)***	0.15	-26.2	0.65
TP							-0.25 (.09)**	-0.23 (.06)***	0.05	-0.99	0
					0.06(.02)**			-0.22(0.06)***	0.04	0.56	1.55
BUN					0.1 (.04)*		-0.41 (.15)**		0.05	-1.05	0
							-0.51(.14)***		0.03	-1	-1
				0.1(.05)*			-0.39(.15)*		0.04	-0.18	0.87
					0.14(.04)***				0.03	0.48	1.53
				0.15(.04)***					0.03	0.58	1.63
CREA			-0.13(.04)[2a]***	-0.05(.01) **		0.01(0)***		-0.31(.04) ***	0.28	-88.63	0
Sodium	-1.99(.77)**						-3.37 (.89)***		0.06	-4.45	0
	-2.02(.76)**			-0.65(.29)*			-4.12(.95)***		0.07	-3.63	0.82
							-3.55(.9)***		0.04	-3.58	0.87
Calcium								0.43(.09)***	0.06	-9.97	0.00
					0.07(.04)*			0.41(.09) ***	0.07	-8.52	1.46
						0.01(0)*		0.45(.09)***	0.06	-8.35	1.62
						0.01(0)**	-0.32(.14)*	0.42(.09)***	0.08	-8.02	1.96
					0.08(.04)*	0.01(0)*		0.43(.09)***	0.08	-7.99	1.99
Phosphorous			-0.17(.07) [2a]*						0.01	6.5	0
WBC		0.07(.03)*						-0.25(.07)***	0.08	-1.07	0
								-0.23(.07)**	0.05	0.27	1.34
N:L Ratio								-2.36(.81)*	0.04	2.03	na

PCV	-2.53(.90)**								0.04	-2.69	na

[1] year prior to capture, [2] sea ice concentration in year prior to capture, [2a]15 percent cover, [2b] 50 percent cover, [3] 14 days prior to capture, reference is [4]Fall, [5]not denning, [6]adult, [7]na= no model within ΔBIC of the presented model. P-value: <.05*. <.01**, <.001***.

Table 7: Directional influence of behavior parameters that significantly impact blood-based analytes ($P \leq .05$)

	On/Off Shore[1]	Fasting Status[2]	Breeding Status[3]
Analyte	Females		
ALP	+	+	+
Phos		+	
WBC			+
	Males		
ALT		+	reference is [1]off shore, [2]fasting, [3]non-breeding bears

CHAPTER 4: SERUM VIROME OF SOUTHERN BEAUFORT SEA POLAR BEARS (*URSUS MARITIMUS*)

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Abstract

Climate change can affect the behavior, physiology, and life history of many Arctic wildlife species. It can also influence the ecology of infectious diseases across the spectrum of infectious agents. The southern Beaufort Sea (SB) sub-population of polar bears (*Ursus maritimus*) has experienced dramatic behavioral changes due to retreating sea ice and other climate-related factors, but the effects of these changes on physiology and infection remain poorly understood. Using serum from polar bears sampled between 2004-2015 and metagenomic DNA sequencing, we identified 48 viruses, all of the family *Anelloviridae*,. Anelloviruses are small, ubiquitous infectious agents with circular single-stranded DNA genomes that are not known to cause disease but, in humans, covary in diversity and load with immunological compromise. We therefore examined the usefulness of anelloviruses as biomarkers of polar bear physiological stress related to climate and habitat use. Polar bear anelloviruses sorted into two distinct clades on a phylogenetic tree, both of which also contained anelloviruses of giant pandas (*Ailuropoda melanoleuca*), another ursid. Neither anellovirus diversity nor load were associated with any demographic variables, behavioral factors, or direct physiological measures. However, pairwise genetic distances between anelloviruses were positively correlated with pairwise differences in sampling date, suggesting that the polar bear "anellome" is evolving over time. These findings suggest that anelloviruses are not a sensitive indicator of polar physiological stress, but they do provide a baseline for evaluating future changes to polar bear viromes.

Introduction

Host-associated microbiota, including bacteria, fungi, protists, and viruses, play an important role in health by influencing physiological processes. In some cases, microbiota composition can alter susceptibility to infectious disease through the presence or absence of specific microorganisms (Hernández-Gómez, 2020), yet baseline characterization of wildlife microbiomes is often lacking (Smith *et al.*, 2009; Stephen *et al.*, 2019; Watson *et al.*, 2019). Like other microbiota, viruses may be commensal or parasitic (Trevelline *et al.*, 2019; Plyusnin *et al.*, 2020; Zhu *et al.*, 2021); however, viruses are more likely than other classes of microbes to emerge and cause epidemics in wildlife populations (Dobson and Foufopoulos, 2001). Climate change is affecting viral disease emergence risk through the behavior and physiology of wildlife hosts and vectors (Caminade *et al.*, 2019; Baker *et al.*, 2022; Carlson *et al.*, 2022). These effects are particularly important for threatened or endangered wildlife populations (Le Roux and McGeoch, 2008; Moore and Huntington, 2008; Thomas, 2010).

The Arctic is experiencing the effects of global warming at a significantly faster rate than other regions of the world. (Cohen *et al.*, 2014; IPCC, 2018; DeRepentigny *et al.*, 2020). With a warming climate come changes in the behavior and life history for many Arctic wildlife, including polar bears (*Ursus maritimus*). The southern Beaufort Sea (SB) sub-population of polar bears is experiencing the effects of climate change. These include changes in abundance (Bromaghin *et al.*, 2015, 2021), recruitment (Rode *et al.*, 2010), behavior (Atwood *et al.*, 2016b), physiology (Pagano *et al*., 2020; Fry et al, in review), diet and toxicant load (Atwood *et al.*, 2016a; McKinney *et al.*, 2017; Bourque *et al.*, 2018; Watson *et al.*, 2021) and bacterial microbiome diversity (Watson *et al.*, 2019). Yet little is known about the pathogens of polar bears. A review of infectious agents in polar bears identified few reports of viral infection (Fagre

et al., 2015). Serological studies have revealed exposure of wild polar bears to canine morbillivirus (Philippa *et al.*, 2004), phocine morbillivirus and dolphin morbillivirus (Cattet *et al.*, 2004; Philippa *et al.*, 2004), calicivirus, (Tryland *et al.*, 2005), dolphin rhabdovirus (Philippa *et al.*, 2004), canine adenovirus (Philippa *et al.*, 2004), and a single report of rabies virus (Taylor *et al.*, 1991). Additional viruses have been identified in captive polar bears either serologically or through health effects and pathology, including West Nile Virus (Dutton *et al.*, 2009) and herpesviruses (e.g., equine herpesvirus-1, equine herpesvirus-9, suid herpes virus-1) (Greenwood *et al.*, 2012).

A 14-year epizootic of alopecia syndrome in SB polar bears described by Atwood *et al.* (2015), led to a broad investigation into potential etiological causes including viruses (unpublished). This investigation revealed the presence of viruses of the family *Anelloviridae* in the serum of ten SB polar bears. Anelloviruses are small (3.9-4 kilobases), single-stranded circular DNA viruses with two main open reading frames (ORFs), with the largest, ORF 1, encoding the capsid protein (Takahashi *et al.*, 1998; Arze *et al.*, 2021). These small, highly genetically diverse viruses appear to be commensal and omnipresent in humans (Kaczorowska and van der Hoek, 2020; Arze *et al.*, 2021). Human anelloviruses (also known as "torque teno viruses," or TTVs) infect healthy individuals, occur at high prevalence, and may be the most abundant eukaryotic virus in the human virome (Virgin *et al.*, 2009). Anelloviruses have also been identified in wildlife species, including non-human primates (Romeo *et al.*, 2000), a number of felids (Kraberger *et al.*, 2021), palm civets, *Paguma larvata*, (Nishizawa *et al.*, 2018), bats, rodents, marsupials (de Souza *et al.*, 2018), and marine mammals such as Pacific harbor seals (*Phoca vitulina richardsii*) (Ng *et al.*, 2011), fur seals (*Arctocephalus gazella*) (Crane *et al.*, 2018), California sea lions *(Zalophus californianus)* (Ng *et al.*, 2009), Weddell seals

(*Leptonychotes weddellii)* and Risso's dolphins (*Grampus griseus)* (Fahsbender *et al.*, 2017). In nearly every described instance, individuals appear to be infected with multiple anelloviruses. See Varsani *et al.* (2021) for a complete list of known mammalian hosts. Anelloviruses have not been shown to cause disease. In humans, anelloviruses appear to be an infectious biomarker of immunological function, with diversity and load increasing with immune system suppression (Thom and Petrik, 2007; Spandole *et al.*, 2015), although the mechanisms for infection and replication remain unknown because no cell culture system nor animal model has been identified (Nasser *et al.*, 2009; Kaczorowska and van der Hoek, 2020).

Our goal was to characterize the serum virome of SB polar bears collected over 11 years, including anellovirus diversity and load. We were especially interested in evaluating whether infection and viral load covaried with demographic, physiological and ecological factors, including internal measures of physiological stress and habitat use driven by climate change. Should anelloviruses covary with these factors, they could represent a novel ecoimmunological tool for monitoring polar bear populations for immunological "health."

Methods

We examined serum samples from 24 polar bears collected as part of ongoing population monitoring studies by the United State Geological Survey (Table 1). Polar bears were captured on land and on the sea ice of Alaska's southern Beaufort Sea (USA) from 2004 to 2015 (Figure 1). Briefly, helicopters were used to locate polar bears, which were chemically immobilized, weighed (kg), marked, and aged using visual measures and dental analyses (see Atwood *et al.*, 2016b). Blood samples were collected by venipuncture of the femoral vein, evacuated into plain tubes (Vacutainer; BD Biosciences, Franklin Lanes, NJ) and stored to prevent freezing. Serum was separated by centrifugation at 1,500 x g for 5 min (TRIAC, Clay Adams, Parsippany, NJ),

frozen at -20°C immediately, then transferred to -80°C for long term storage. All animal research was conducted under appropriate permits, including animal care and use approvals (Marine Mammal Research Permit MA690038-17 and USGS IACUC approval 2017-03).

We identified viruses in the serum of polar bears following previously described methods (Sibley *et al.*, 2016; Bennett *et al.*, 2020; Campbell *et al.*, 2022). Briefly, we centrifuged polar bear serum for 10 minutes at 10,000 x g to pellet cellular debris, and total nucleic acids were extracted from 200 µl of supernatant using the QIAmp MinElute Virus Spin Kit (Qiagen, Hilden, Germany). We used the Superscript IV system (Thermo Fisher, Waltham, MA, USA) with random hexamers to reverse transcribe RNA to cDNA, and prepared cDNA libraries using the Nextera XT DNA sample preparation kit (Illumina, San Diego, CA, USA). We sequenced libraries on a MiSeq instrument using 150 x 150 cycle V2 paired-end sequencing chemistry (Illumina, San Diego, CA, USA), and sequencing adapters were removed from the resulting reads by on-board Illumina processing software.

We analyzed viral sequences using CLC Genomics Workbench v. 20.0.4 (QIAGEN, Aarhus, Denmark) trimming low-quality bases (Phred quality score <30), discarding short reads (<75 bp), and subjecting the remaining reads to *de novo* assembly using the CLC assembler with automatic word and bubble size selection and a minimum contiguous sequence (contig) length of 500. We analyzed contigs for nucleotide- (blastn) and protein-level (blastx) similarity to known viruses in GenBank. For blastx, we applied the BLASTX algorithm with the BLOSUM62 matrix to sequences translated into all 6 frames. We analyzed all sequence data at the individual read level by mapping reads to viruses in the GenBank database using the CLC mapping tool at low stringency (length fraction of 0.5, similarity fraction of 0.6). We disregarded contigs matching viruses of known non-mammalian hosts (e.g., bacteria, invertebrates, plants, fungi), then mapped

reads back to viral contigs to calculate the proportion of reads mapping to each virus (for virus-specific load) or the proportion of reads mapping to any virus (for total viral load). We normalized these measures for sequencing depth and target sequence length, expressing viral loads as $\log_{10}$ viral reads per million per kilobase of target ($\log_{10}$ vRPM/kb), which has been shown to correlate with quantitative real-time polymerase chain reaction data (Toohey-Kurth *et al.*, 2017).

Phylogenetic relationships among viruses were inferred from ORF 1 nucleotide sequences. We first aligned sequences of newly identified viruses with published sequences of related viruses in the GenBank database using the Prank algorithm (Löytynoja, 2014) in TranslatorX (Abascal *et al.*, 2010), with the Gblocks algorithm (Castresana, 2000) to remove poorly aligned regions. We inferred maximum-likelihood phylogenetic trees from the alignments using PhyML 3.0 with smart model selection (Lefort *et al.*, 2017) and 1000 bootstrap replicates to assess statistical confidence in clades. We used Figtree v. 1.4.4 to display final phylogenetic trees (Rambaut, 2018).

We assessed whether viral richness (number of viruses in each bear), viral load ($\log_{10}$vRPM/kb) and presence of individual viruses (> 0.01 $\log_{10}$vRPM/kb) were related to demographic characteristics (sex, age (years), age*sex, and capture year), and physiologic biomarkers of immune function. We included two markers of immune function: globulin, a protein that bridges the adaptive and innate immune response and leukocyte count, a measure of the innate immune response (see Fry *et al.*, 2019 methods). We included hair cortisol levels as a measure of chronic stress (Meyer and Novak, 2012; Manenschijn *et al.*, 2013; Karlén *et al.*, 2015) (see Van der Walt *et al.*, 2021 for cortisol methods).

We assessed the effect of climate driven changes in summer habitat use by polar bears on virus richness and total viral load. Some SB polar bears opportunistically scavenge bowhead whale (*Balaena mysticetus*) carcasses left by subsistence hunters in summer and fall (Herreman and Peacock, 2013; Rogers *et al.*, 2015). Using on-shore habitat, versus on-ice habitat leads to increased risks associated with contact with humans, polar bears and other wildlife. These behaviors have been shown to affect exposure to toxicant load and bacterial pathogens; and therefore may influence viral load and richness (Atwood *et al.*, 2016b, 2017; McKinney *et al.*, 2017; Bourque *et al.*, 2018).

We examined these relationships statistically with generalized linear models using the Wald method to test for significance at an alpha level of 0.05. For viral presence/absence we conducted logistic regression. To examine possible genetic changes in anelloviruses over time, we computed pairwise patristic distances between ORF 1 nucleotide sequences and compared them to pairwise differences in the year of anellovirus detection using the Mantel tests of matrix correlation (Mantel, 1967) with 10,000 permutations (APE package in R (Paradis *et al.*, 2004). We conducted all analyses using R 4.2.1 (R Core Team, 2021).

Results

We identified 48 distinct anelloviruses in the serum of 24 polar bears (Table 1) and no other viruses associated with eukaryotic hosts. All polar bear anelloviruses identified shared the typical genome architecture for this type of virus (Supplement A Figure 1). Amino acid similarity of ORF 1 to known viruses was low, as expected from published results (Varsani et al. 2021) and ranged from 29.21% - 60.00%. (Table 2*)*. A maximum likelihood phylogenetic tree based on ORF 1 nucleotide sequences (final alignment = 2,163 positions) of newly-discovered anelloviruses (n = 48) and the closest BLAST matches in Genbank (reference sequences, n = 8)

consisted of two clades. Clade A is comprised of 26 polar bear anelloviruses and clade B includes 22 polar bear anelloviruses (Figure 2). In all cases, sequences clustered most closely with viruses from the same host species indicating that the newly identified polar bear anelloviruses are more similar to each other than to previously-described anelloviruses. Of the polar bears infected by anelloviruses (n=23) all (100%) were infected with viruses from clade A. Polar bear 7 was the only individual that was not infected by a virus from clade B. Clade B contained three divergent sequences in addition to sequences from giant pandas (*Ailuropoda melanoleuca*) and a tree shrew (*Tupaia belangeri*) (Figure 2). Pairwise genetic and temporal distances between polar bear anelloviruses were positively correlated, indicating that anelloviruses from samples collected closer together in time were also more genetically similar (Figure 3; $r = 0.16$; $P=0.028$; slope = 0.007 % change per year of detection).

Average anellovirus richness in SB polar bears was 20 (Figure 4a, range: 0-38, median=22, SD =11.1,). One individual (PB#15), a year-old cub, did not have any detectable anelloviruses, while the only other year-old cub in our sample (PB#22) had the highest anellovirus richness of 38 (Table 1, Supplement B, Figure 1). The prevalence of each virus in the populations ranged from 8% − 83% (Supplement B, Figure 2). The mean total anellovirus load from the serum of our sample population was 0.61 $\log_{10}$ vRPM/kb (Figure 4b, range 0:2.01, median = 0.49, SD = 0.52).

Anellovirus load and richness increased slightly with age for females and declined slightly with age for males, but these relationships were not statistically significant (Table 3). Similarly, year of capture did not significantly influence viral load or richness (Table 3). Physiological biomarkers were also not significantly correlated with richness or load of anelloviruses (Table 3). Whether polar bears spent the summer using on-shore habitat or off-

shore habitat was not significantly related to anellovirus richness or load (Table 3, Supplement C).

Discussion

We characterized the serum virome component of the microbiome of 24 polar bears from the SB subpopulation and identified 48 new anelloviruses. The anelloviruses we identified sort into two clades together with anelloviruses of another ursid, the giant panda (Varsani *et al.*, 2021). These results are similar to reports of host-associated anelloviruses in other wildlife species (Fahsbender *et al.*, 2017; Kraberger *et al.*, 2021; Varsani *et al.*, 2021). Similarly, our finding of a large number of anellovirues in individual bears aligns with data from felids (Kraberger et al. 2021), palm civets (Nishizawa *et al.*, 2018), primates (Hrazdilová *et al.*, 2016) and suids (Huang *et al.*, 2010) and makes recombination likely (Fahsbender *et al.*, 2017; Arze *et al.*, 2021; Kraberger *et al.*, 2021). This pattern was consistent over time, supporting the notion that anelloviruses persistently infect hosts and are likely controlled by the immune system (Arze *et al.*, 2021). Interestingly, we identified a weak but statistically significant trend of increasing genetic differentiation over time in SB polar bear anelloviruses. We caution that this trend should not be interpreted as an evolutionary rate, because our analysis was not lineage specific (due to very limited numbers of viruses from the same lineages over time). Rather, we speculate that this trend reflects a combination of anellovirus community turnover and molecular evolution of the ORF 1 gene.

Persistent infection is a hallmark of the anelloviruses (Arze *et al.*, 2021; Kraberger *et al.*, 2021). The polar bear “anellome” appears to be commensal and to vary by individual, consistent with results from other species, (Crane *et al.*, 2018). Similar to anelloviruses of other species, polar bear anelloviruses are diverse but host-specific (Nishizawa *et al.*, 2018; Kraberger *et al.*,

2021). Contrary to our predictions, viral richness and load did not correlate with the demographic, physiologic or behavioral parameters we assessed. We do, however, show that anelloviruses identified more closely in time have shorter genetic distances between them than those identified further apart suggesting that the polar bear anellome is evolving, likely through a combination of point substitution and haplotype turnover (Arze *et al.*, 2021).

Anelloviruses have been found in blood, serum, feces, semen, and the oral cavity and tissues of their hosts (Kaczorowska and van der Hoek, 2020). Mechanisms for virus transmission have been hypothesized to occur including through diet, sexual, fecal-oral, respiratory, and through blood transfusion and organ transplant (Arze et al. 2021). A dietary route of infection for polar bear anelloviruses is possible but difficult to ascertain. The viromes of ringed seals (*Pusa hispida*), the primary prey of polar bears, and bowhead whales (*Balaena mysticetus)*, the primary on-shore diet of polar bears, have yet to be investigated. Other modes of transmission, such as airborne or sexual, are also possible (Kaczorowska *et al.*, 2022b). Anelloviruses have been detected in human infants as young as 6 weeks-old but were unrelated to maternal anelloviruses (Kaczorowska *et al.*, 2022a), making vertical transmission unlikely.

Our sample population was selected to maximize representation of bears across demographic characteristics such as sex, age, and summer habitat use over 11 years during which polar bears underwent marked changes in habitat availability. Longitudinal studies of individual bears over longer time periods could reveal associations between anellovirus richness and load and physiological and ecological factors. Our findings, in this regard, are similar to Watson et al.'s (2019) investigation of the fecal microbiota of polar bears, which showed that neither sex nor age significantly influenced microbiota richness. Further, lack of a relationship between anellovirus load or richness and physiological biomarkers suggests that, unlike in humans,

anelloviruses in polar bears do not appear to respond to physiological stress, at least within the range of physiological parameters we were able to examine, suggesting that immune function in these polar bears is competent in controlling anellovirus load (Arze et al. 2021). Overall, anelloviruses are unlikely to be an effective ecoimmunological marker of immune function in polar bears. Nevertheless, our findings of a relatively naïve virome in polar bears provide a baseline against which to evaluate changes over time.

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Tables

Table 1: Summary of demography, anellovirus richness and relative total anellovirus load for 24 polar bears from the Southern Beaufort Sea.

Polar Bear ID	Capture Year	Sex	Age (years)	Summer Habitat Use	Anellovirus Richness	Total Anellovirus load (log_{10} vRPM/kb)
1	2004	M	19	On-shore	25	0.98
2	2013	F	3	On-shore	28	0.54
3	2009	F	22	On-shore	34	0.87
4	2013	M	6	On-shore	35	1.18
5	2014	F	4	Off-shore	19	0.17
6	2014	M	3	On-shore	5	0.06
7	2015	F	17	Off-shore	3	0.02
8	2015	F	2	Off-shore	27	0.54
9	2013	M	23	On-shore	22	0.39
10	2006	M	4	On-shore	14	0.12
11	2005	M	8	On-shore	19	0.43
12	2006	F	3	On-shore	13	0.45
13	2009	M	11	Off-shore	20	0.44
14	2011	M	10	Off-shore	12	0.08
15	2011	F	1	On-shore	0	0.00
16	2013	F	5	Off-shore	22	0.92
17	2005	F	23	On-shore	34	1.49
18	2005	F	16	Off-shore	7	0.10
19	2010	M	10	On-shore	37	1.21
20	2004	M	4	Off-shore	23	0.99
21	2004	F	5	On-shore	6	0.13
22	2009	M	1	On-shore	38	2.01
23	2011	M	2	On-shore	25	0.56
24	2010	F	14	On-shore	25	1.02

Table 2: ORF1 characteristic of viruses identified in 24 serum samples from southern Beaufort Sea polar bears.

Virus ID	Year Detected	Size (nt)[a]	Closest nt match accession[b]	Closest nt match taxon(year)[c]	% nt ID to closest match[e]	Clade	GenBank accession number
PbV-1	2004	1,653	ASH99133	Gpan20684 (2017)	50.28	B	OP970915
PbV-2	2004	2,064	ASH99133	Gpan20684(2017)	53.64	B	OP970916
PbV-3	2004	1,872	ASH99133	Gpan20684(2017)	57.98	B	OP970917
PbV-4	2004	2,031	ASH99133	Gpan20684(2017)	60.00	B	OP970918
PbV-5	2006	1,431	ASH99109	Gpan20681(2017)	41.63	A	OP970919
PbV-6	2005	1,464	ASH99109	Gpan20681(2017)	44.04	A	OP970920
PbV-7	2004	1,404	ASH99085	Gpan20859(2017)	38.42	A	OP970921
PbV-8	2005	1,398	ASH99079	Gpan21094(2017)	43.36	A	OP970922
PbV-9	2004	1,626	ASH99133	Gpan20684(2017)	42.56	B	OP970923
PbV-10	2009	1,269	ASH99085	Gpan20859(2017)	36.46	A	OP970924
PbV-11	2004	1,413	ASH99079	Gpan21094(2017)	39.95	A	OP970925
PbV-12	2004	2,298	ASH99133	Gpan20684(2017)	56.11	B	OP970926
PbV-13	2006	930	ASH99085	Gpan20859(2017)	46.05	A	OP970927
PbV-14	2005	1,509	ASH99079	Gpan21094(2017)	39.82	A	OP970928
PbV-15	2004	2,061	ASH99106	Gpan21094(2017)	59.96	B	OP970929
PbV-16	2004	1,485	ASH99133	Gpan20684(2017)	44.03	A	OP970930
PbV-17	2004	1,485	ASH99109	Gpan20681(2017)	54.30	B	OP970931
PbV-18	2004	1,494	ASH99109	Gpan20681(2017)	36.04	A	OP970932
PbV-19	2004	1,416	ASH99109	Gpan20681(2017)	41.33	A	OP970933

PbV-20	2005	1,416	ASH99109	Gpan20681(2017)	38.20	A	OP970934
PbV-21	2005	1,407	ASH99085	Gpan20859(2017)	39.25	A	OP970935
PbV-22	2004	1,416	ASH99109	Gpan20681(2017)	40.15	A	OP970936
PbV-23	2004	2,133	ASH99133	Gpan20684(2017)	54.54	B	OP970937
PbV-24	2004	2,091	ASH99133	Gpan20684(2017)	56.32	B	OP970938
PbV-25	2004	1,455	ASH99079	Gpan21094(2017)	38.24	A	OP970939
PbV-26	2004	2,133	ASH99133	Gpan20684(2017)	54.44	B	OP970940
PbV-27	2004	1,413	ASH99133	Gpan20684(2017)	38.18	A	OP970941
PbV-28	2004	1,416	ASH99109	Gpan20681(2017)	41.61	A	OP970942
PbV-29	2004	1,416	ASH99079	Gpan21094(2017)	41.40	A	OP970943
PbV-30	2004	1,425	ASH99085	Gpan20859(2017)	38.78	A	OP970944
PbV-31	2004	1,374	ASH99079	Gpan21094(2017)	53.76	B	OP970945
PbV-32	2004	771	ASH99109	Gpan20681(2017)	44.91	A	OP970946
PbV-33	2004	1,566	ASH99133	Gpan20684(2017)	58.79	B	OP970947
PbV-34	2004	2,067	ASH99133	Gpan20684(2017)	55.68	B	OP970948
PbV-35	2004	1,569	QZE11967	Gpb08AV03-5(2022)	45.38	B	OP970949
PbV-36	2004	1,443	ASH99106	Gpan21066(2017)	37.33	A	OP970950
PbV-37	2004	2,064	ASH99133	Gpan20684(2017)	55.09	B	OP970951
PbV-38	2004	2,133	ASH99133	Gpan20684(2017)	53.74	B	OP970952
PbV-39	2004	1,410	ASH99085	Gpan20859(2017)	36.29	A	OP970953
PbV-40	2004	2,094	ASH99133	Gpan20684(2017)	55.65	B	OP970954
PbV-41	2009	1,323	YP_009505746	Tbc-TTV14(2001) [d]	29.21	B	OP970955

PbV-42	2004	1,467	ASH99079	Gpan21094(2017)	45.89	A	OP970956
PbV-43	2005	1,476	ASH99106	Gpan21066(2017)	41.82	A	OP970957
PbV-44	2004	1,410	ASH99133	Gpan20684(2017)	57.79	B	OP970958
PbV-45	2006	1,413	ASH99079	Gpan21094(2017)	41.50	A	OP970959
PbV-46	2004	2,007	ASH99133	Gpan20684(2017)	59.58	B	OP970960
PbV-47	2009	1,545	QZE11973	Gpb08AV05-5(2022)	39.90	B	OP970961
PbV-48	2004	1,005	ASH99106	Gpan21066(2017)	39.62	A	OP970962

[a] Length refers to the length of the nucleotide sequence for ORF1, used for phylogenetic and viral load analyses; [b]GenBank accession number of closest match using BLASTx is shown [c]all Gp reference viruses are giant panda from China in (year), except [d]Tupasis, Japan, 2001, [e] % identity refers to percent nucleotide identity of ORF1 to the closest match in GenBank.

Table 3: Summary of model covariates and their relationship with anellovirus richness and load in 24 polar bears (See Supplement C Figures 1 and 2).

			Richness		Relative Load (log_{10} vRPM/kb)	
Covariate	Description	Sample size	t value	P -value	t value	P -value
Sex	Male/Female	24	1.05	0.31	1.20	0.25
Age	Range: 0.5-23 years	24	0.76	0.46	0.57	0.58
Age*Sex	Interaction of age and sex	24	-0.75	0.46	-1.37	0.18
Year	Year of capture (Range: 2004-2015)	24	0.03	0.98	-0.83	0.41
Globulin	Measure of sustained immune response	16	2.00	0.07	1.11	0.29
Leukocyte Count	Measure of acute immune response	15	0.56	0.59	0.02	0.98
Hair Cortisol	Glucocorticoid hormone elevated during periods of stress	17	0.20	0.84	0.68	0.51
Summer Habitat Use	Polar bears with >5% bowhead in diet considered on-shore bears	24	1.23	0.23	1.37	0.19

Figures

Figure 1: Study area and capture location of 24 polar bears from the southern Beaufort Sea subpopulation, 2004-2015**.**

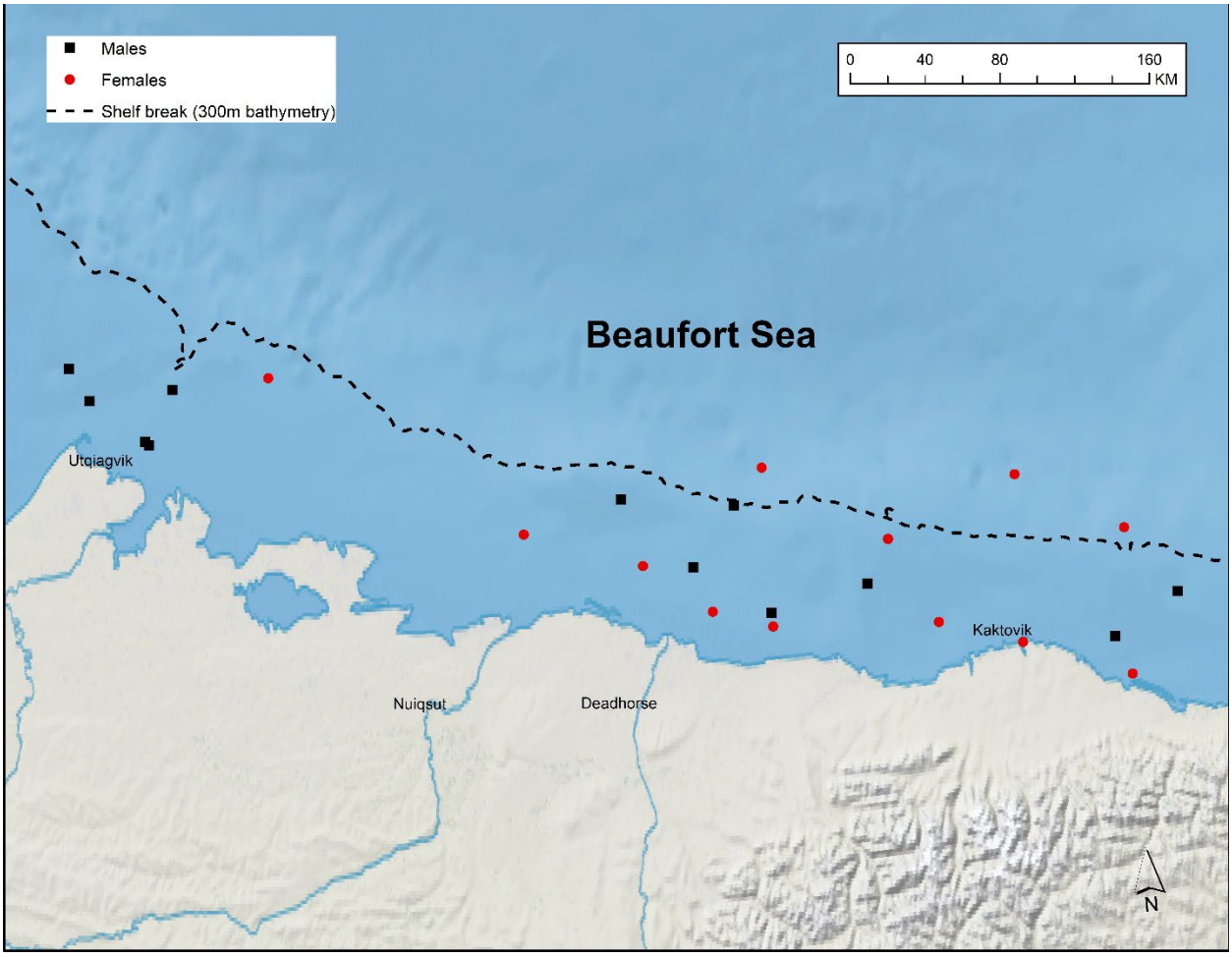


Figure 2: Maximum-likelihood phylogenetic tree of polar bear anelloviruses. Bootstrap values (%) are based on 1000 replicates, bootstrap values <50% are not labeled. All Gp taxon are from China in year referenced. All PbV sequences are from the USA with year of detection. Sequence Tbc-TTV14 is from a tree shrew from Japan. Scale bar indicates nucleotide substitutions per site. Clades A and B are indicated. See Table 2 for GenBank accession numbers.

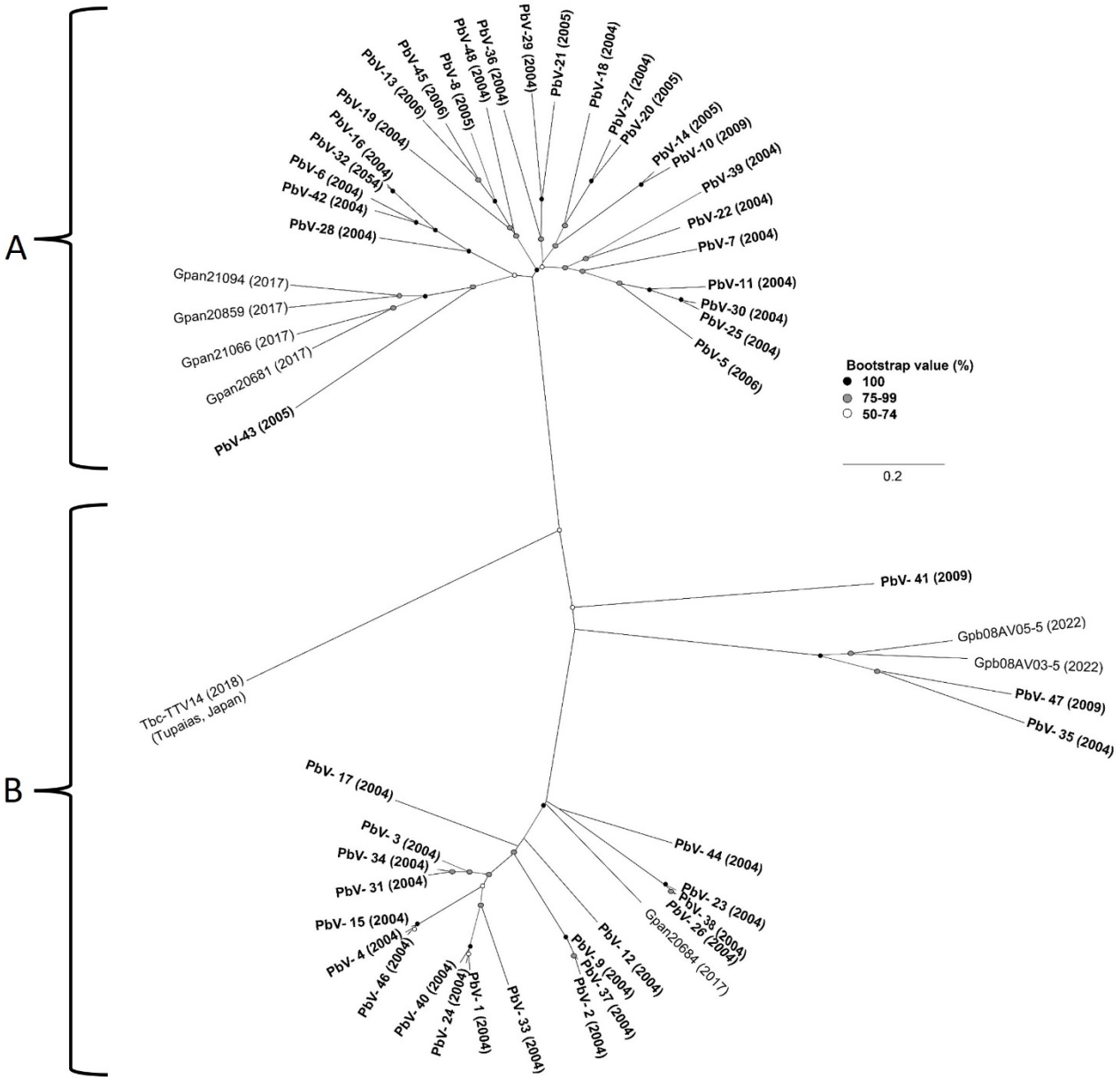


Figure 3: Pairwise genetic and temporal distance of polar bear anelloviruses. Solid line is the least squares lines (pairwise distance = 0.007x+70.03, $r = 0.1596$).

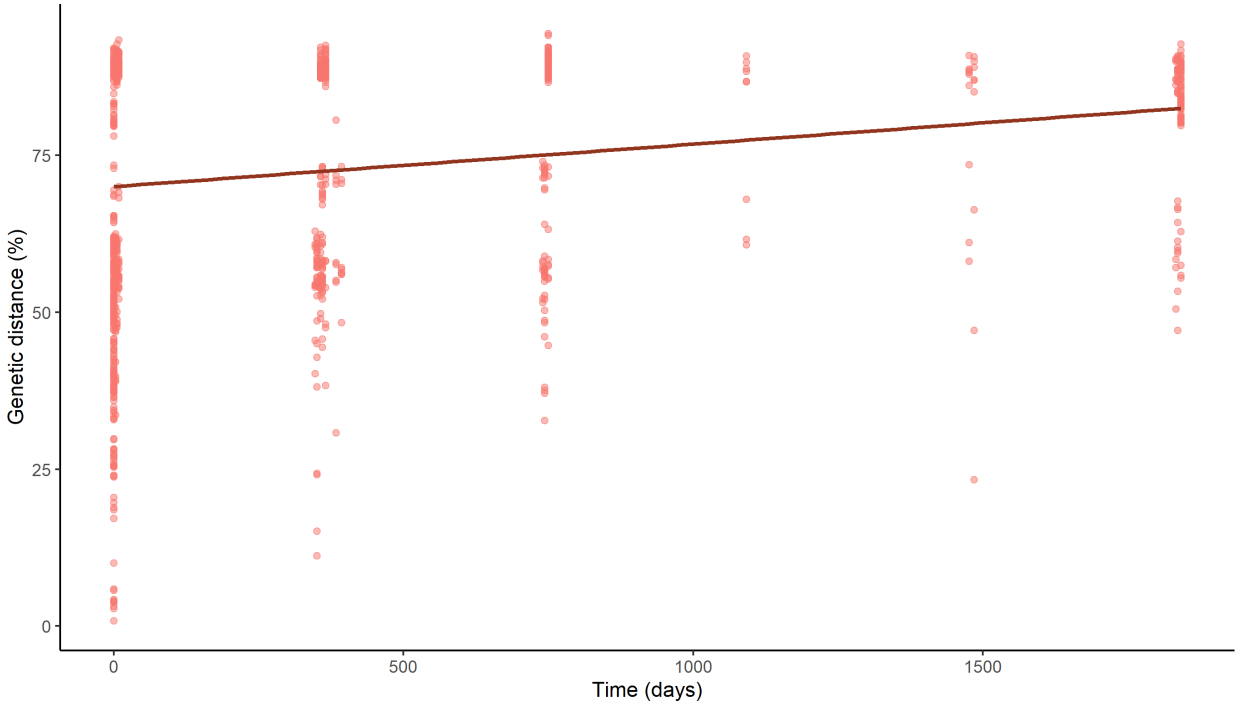
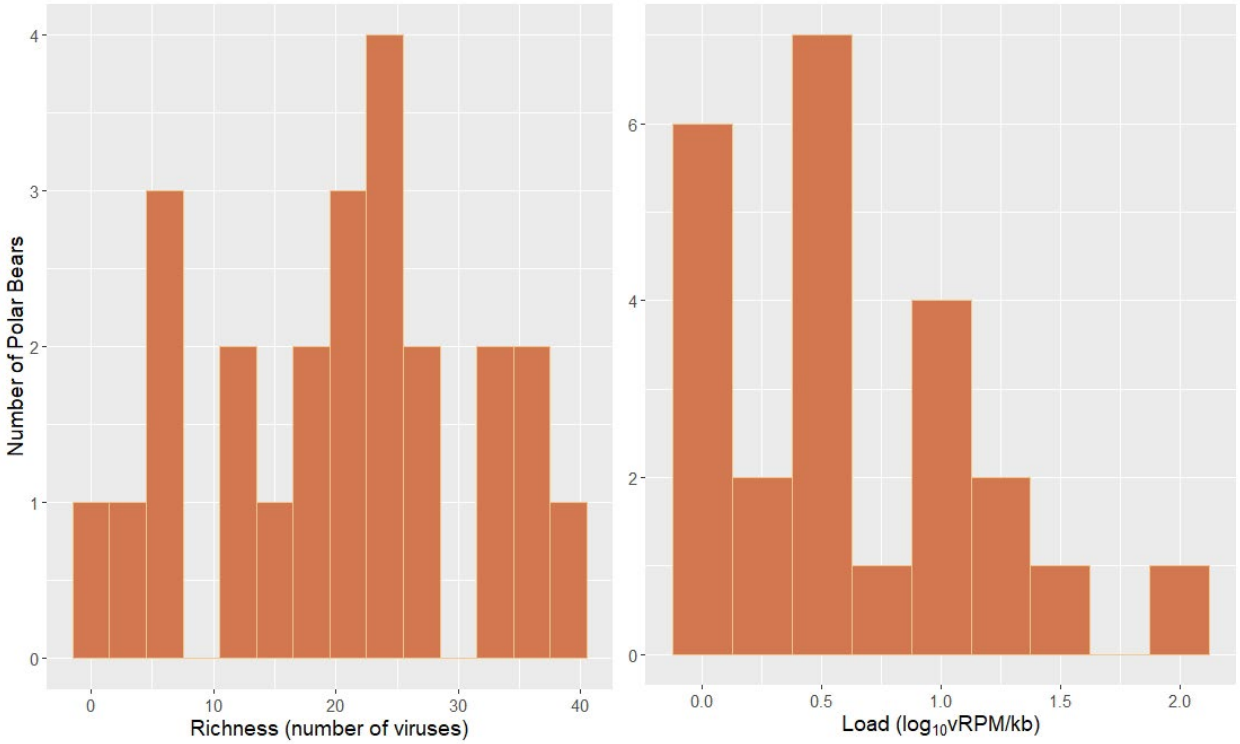


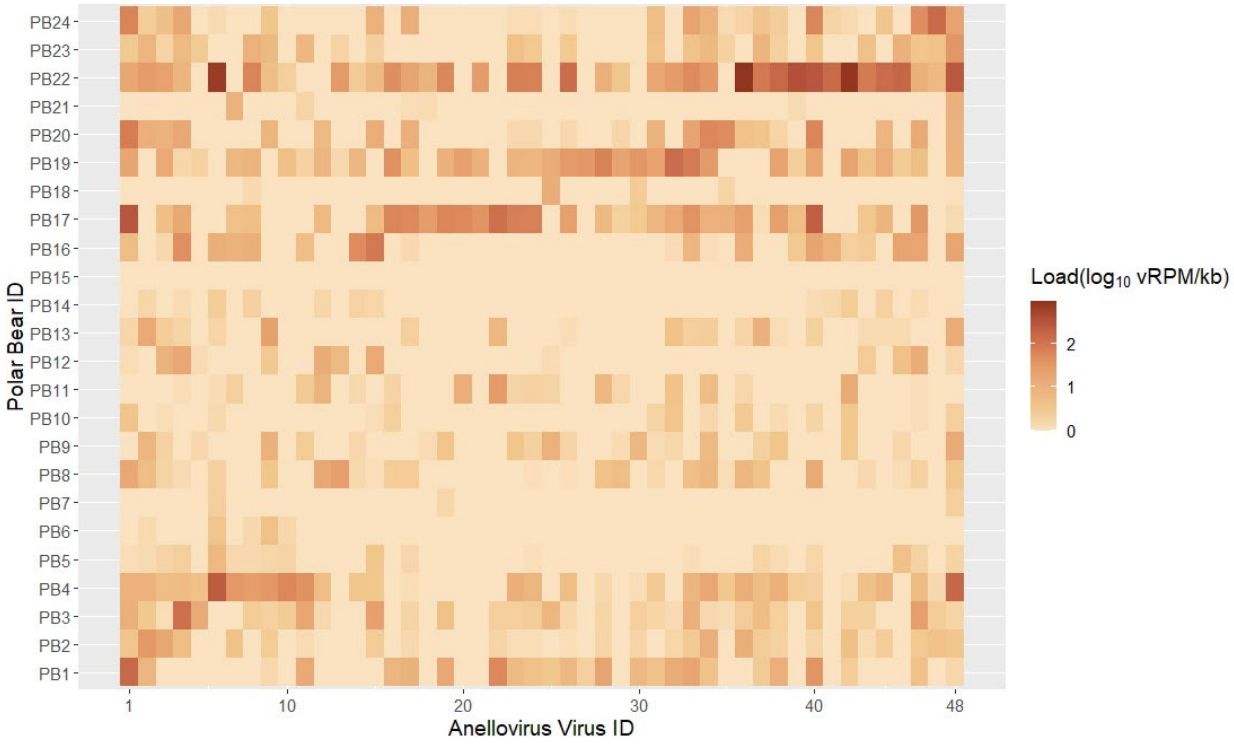
Figure 4: Histograms of (a) anellovirus richness and (b) relative total anellovirus loads in 24 SB polar bears.



Supplements

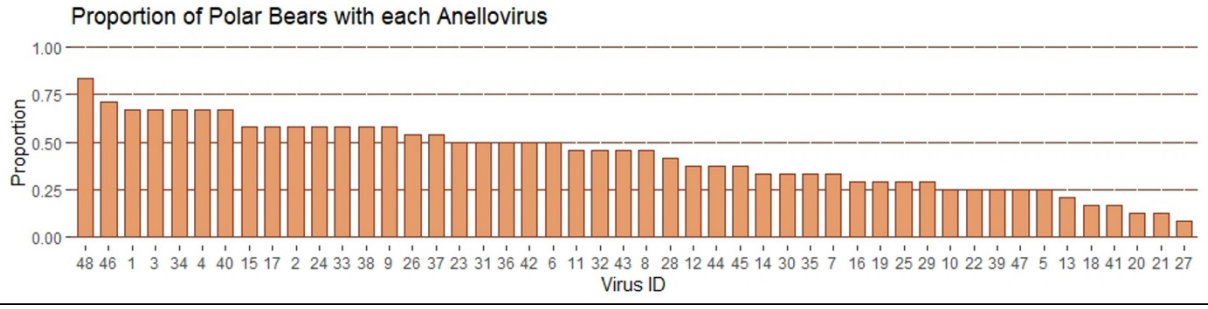
Supplement A Figure 1: Genome maps of anelloviruses found in southern Beaufort Sea polar bears. Numbers = virus id (top) and genome length (base pairs, bottom). Gray arrow – GC Rich Box, Yellow = ORF1, Green = ORF2, Blue = ORF3, Red = ORF4

Supplement B Figure 1: Heat map of viral loads for 48 anelloviruses in the serum of 24 southern Beaufort Sea polar bears**.**

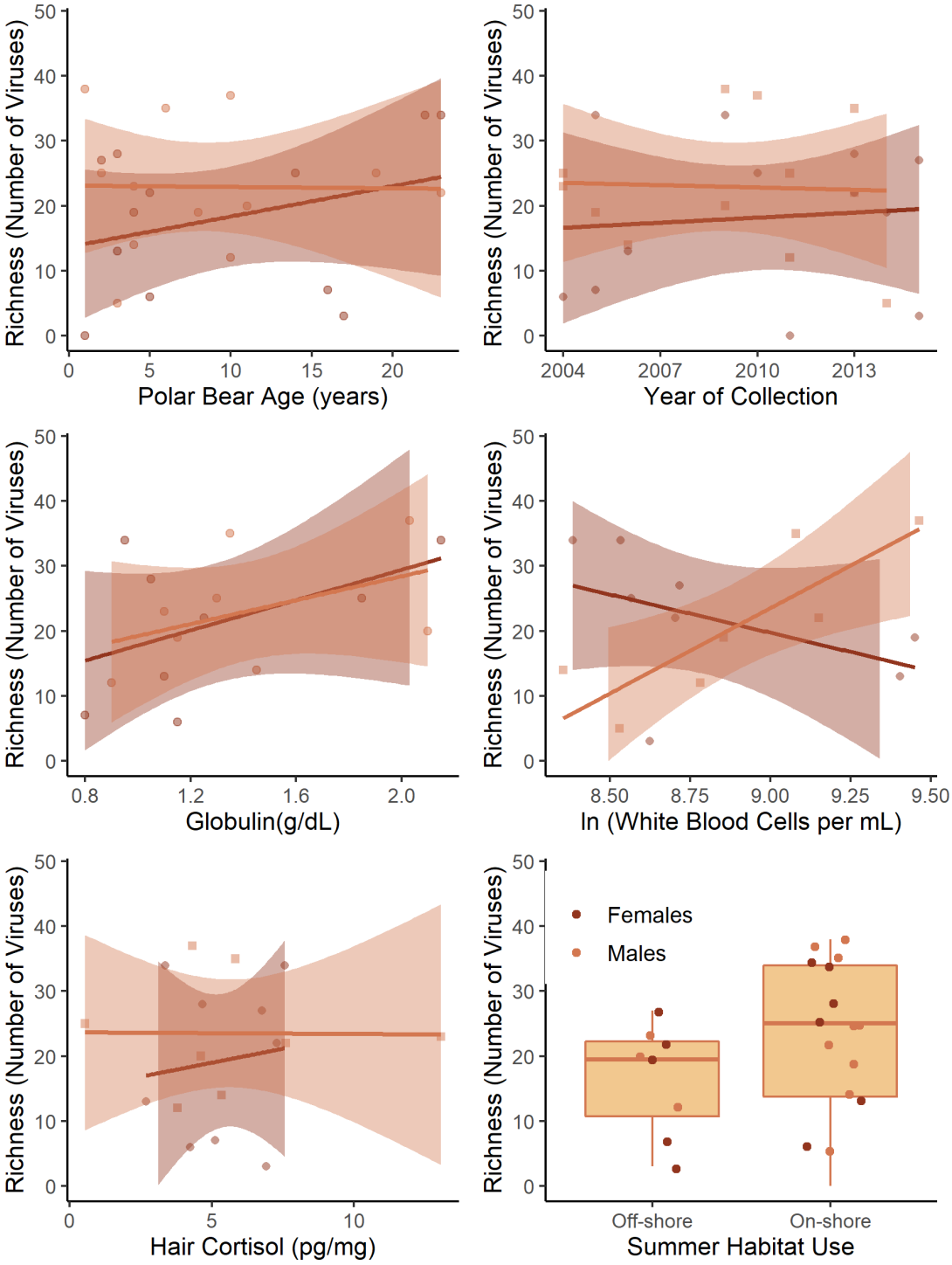


Supplement B Figure 2: Prevalence (%) of each anellovirus in the sampled population (24 polar bears) with upper and lower 95% confidence intervals using the Wald Method. B. Proportion of polar bears with each anellovirus.

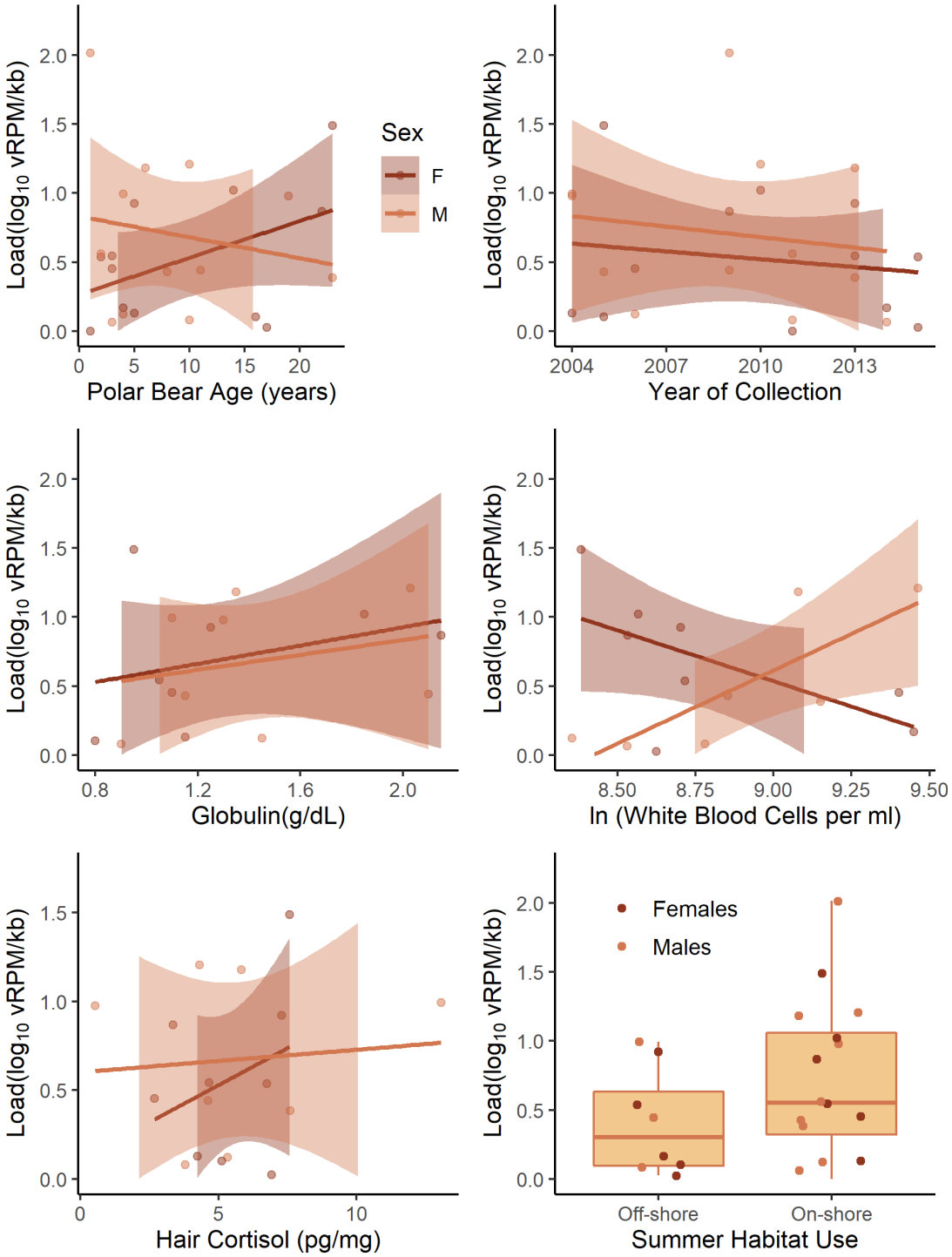
Virus ID	n*	Prevalence (%)	lower	upper	Virus ID	n	Prevalence (%)	lower	upper
1	16	66.7	0.48	0.86	25	7	29.2	0.11	0.47
2	14	58.3	0.39	0.78	26	13	54.2	0.34	0.74
3	16	66.7	0.48	0.86	27	2	8.3	-0.03	0.19
4	16	66.7	0.48	0.86	28	10	41.7	0.22	0.61
5	6	25.0	0.08	0.42	29	7	29.2	0.11	0.47
6	12	50.0	0.30	0.70	30	8	33.3	0.14	0.52
7	8	33.3	0.14	0.52	31	12	50.0	0.30	0.70
8	11	45.8	0.26	0.66	32	11	45.8	0.26	0.66
9	14	58.3	0.39	0.78	33	14	58.3	0.39	0.78
10	6	25.0	0.08	0.42	34	16	66.7	0.48	0.86
11	11	45.8	0.26	0.66	35	8	33.3	0.14	0.52
12	9	37.5	0.18	0.57	36	14	58.3	0.39	0.78
13	5	20.8	0.05	0.37	37	13	54.2	0.34	0.74
14	8	33.3	0.14	0.52	38	12	50.0	0.30	0.70
15	14	58.3	0.39	0.78	39	6	25.0	0.08	0.42
16	7	29.2	0.11	0.47	40	16	66.7	0.48	0.86
17	14	58.3	0.39	0.78	41	4	16.7	0.02	0.32
18	4	16.7	0.02	0.32	42	12	50.0	0.30	0.70
19	7	29.2	0.11	0.47	43	11	45.8	0.26	0.66
20	3	12.5	-0.01	0.26	44	9	37.5	0.18	0.57
21	3	12.5	-0.01	0.26	45	9	37.5	0.18	0.57
22	6	25.0	0.08	0.42	46	17	70.8	0.53	0.89
23	12	50.0	0.30	0.70	47	6	25.0	0.08	0.42
24	14	58.3	0.39	0.78	48	20	83.3	0.68	0.98



Supplement C Figure 1: Model covariates as a function of anellovirus richness in 24 southern Beaufort Sea polar bears



Supplement C Figure 2: Model covariates as a function of total anellovirus load in 24 southern Beaufort Sea polar bears.



CHAPTER 5: CONCLUSION

Conclusion

Using samples archived as part of long-term population monitoring program this research used blood-based biomarkers to assess changes to metabolic and immune system function of a wildlife population. This includes establishing reference intervals of blood-based biomarkers (Chapter 2) and characterizing the serum virome of polar bears (Chapter 4). In addition, this dissertation explores a longitudinal dataset, spanning 40 years, to explore the influence of climate change on measures of physiologic function of polar bears in the southern Beaufort Sea subpopulation (Chapter 3). Together, these chapters offer a robust and comprehensive look into polar bear health and the effects of climate change on polar bear physiology, as well as elucidating the usefulness of clinical pathology as a tool to monitor wildlife population health.

Key findings include, in **Chapter 2**, creating a robust description of reference intervals from which to evaluate individual polar bear physiologic function. These reference intervals are optimized for the southern Beaufort Sea subpopulation, yet useful for comparisons between other subpopulations of polar bears. Along with providing a reference for health, these reference intervals provide a means from which to detect physiologic responses to external stressors. They also provide a means from which to define decision limits, the points at which disease can be identified (Friedrichs *et al.*, 2012). Decision limits for commonly assessed biomarkers remain unknown for polar bears. Collaborations that allow for the comparison of zoo-owned and wild populations would be advantageous. Although, they may not be ideal comparisons, such collaborations could inform our understanding of clinical pathology associated with disease and senescence as well as identify pathophysiologic limits of polar bears.

In **Chapter 3**, I show that environmental processes, including Arctic wide indices of climate, regional effects of climate change (sea ice loss) and weather (wind), are influencing

polar bear physiology. The environmental processes I evaluated, which function on spatial and temporal scales incongruent to physiologic processes, link abiotic conditions with biotic function and reveal the resiliency of polar bears in a rapidly changing landscape. I show that critical metabolic functions are tied to changes in prey, which are concurrently affected by some of the same environmental processes. Future research aimed at understanding the additive effect of changes across multiple tropic levels could lead to increased understanding of the additive effects of climate change in wildlife populations.

There remain hurdles for assessing and understanding population health from blood-based biomarkers of physiologic function. It has been a practice of wildlife research and monitoring to collect blood, archive serum and even ascertain analyte levels; yet the information used from these practices often remains unrealized. Through this work, I used archived samples to assess their usefulness to monitor population health. In doing so, I identified a subset of biomarkers that may be most helpful in understanding the physiologic effects of changes to habitat, behavior and diet. Future work would benefit from using blood-based biomarkers of physiologic function in concert with other diagnostic methods including, transcriptomics or metabolomics, with abiotic measures of change and a population's genetic structure. Information gleaned from such research could be used to evaluate wildlife populations forced to respond to change that outpaces their ability to adapt. In addition, future research should include collaborative efforts to establish biobanking databases and facilities. Collaborative monitoring endeavors would maximize the use of samples, help define future sample type and preservation techniques and protect biodiversity (Castelhano *et al.*, 2018; Coppola *et al.*, 2019).

My characterization of the polar bear serum virome (**Chapter 4)** reveals the limited diversity of viruses in the microbiota of polar bears. The serum virome is currently limited to the

Anelloviradae family of viruses, known to be ubiquitous and commensal in humans, and unaffected by age, sex, climate and climate driven changes in behavior. With little dispute regarding the effects of climate change to alter the distribution and evolution of pathogens, it is likely that the virome of polar bears will change (Gallana *et al.*, 2013; Parkinson *et al.*, 2014; Omazic *et al.*, 2019). This chapter offers a baseline to measure this change. From this baseline we can monitor novel pathogens and spillover, and when possible, mitigate the conditions under which infection thrives. This includes continuing to understand what is 'normal' from what is extraordinary.

Climate change remains the ultimate threat to the circumpolar population of polar bears, and will continue to threaten the circumpolar population. Many aspects of polar bear life history have been affected by the changing Arctic climate (Rode *et al.*, 2010, 2022; Atwood *et al.*, 2016b, 2016a, 2017; McKinney *et al.*, 2017; Bromaghin *et al.*, 2021), including physiology, (Whiteman *et al.*, 2017; Pagano *et al.*, 2020, Fry et al., in review). It is anticipated that future changes to the Arctic environment will require polar bears to exhibit continued plasticity. In terms of physiology, this means tradeoffs, with one system benefiting to the detriment of another. It is when physiologic tradeoffs become insufficient that disease occurs, and populations can be affected (Boonstra, 2013). Being able to identify and respond to physiologic sentinels will aid in the conservation of polar bears especially if the effects of climate change outpace physiologic plasticity. As such, continuing to explore the role of ecosystem level processes on physiology and incorporating this information in a broader understanding of wildlife health (Wittrock *et al.*, 2019) is integral to conservation.

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