THE SEASONAL NUTRITIONAL PHYSIOLOGY OF POLAR BEARS (*URSUS MARITIMUS*) UNDER HUMAN CARE

by

Veronica Ouellette

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Abstract

Despite the seasonal ecology of polar bears, zoos feed their captive bears a high-protein, high-calorie diet year-round. This approach to polar bear husbandry has been associated with poor welfare and death by liver/kidney disease. I assessed the effects of diet – calories and macronutrients – on the physiology and behaviour of 5 bears at The Toronto Zoo. Diet varied seasonally in caloric intake, and bears were fed a high-protein diet from January 2018 to February 2019, and a high-fat diet from January 2022 to June 2023. During both periods, the body mass and fat, blood chemistry, and body temperature of bears were recorded. Behavior was observed under the high-fat diet. Diet had significant effects on body and blood variables. Calories had significant effects on activity and body temperature, while macronutrients had no effect on these variables, suggesting thermal stress and laziness are not relevant welfare concerns for captive bears on this diet.

Acknowledgements

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The Toronto Zoo really is a village, and the zoo's commitment to collaboration has allowed me to work alongside experts in many fields of zoology. This project would not have been possible without the hard work of TZ staff in the wildlife nutrition, wildlife care, wildlife health, and welfare science divisions. Special thanks to Beth who has told me "Let me know if there's anything I can do to help" every day since she stepped into the role of interim wildlife nutrition manager. Thank you to all the polar bear keepers for giving me the best crash course in bear care and behavioural husbandry an aspiring zookeeper could ask for, and especially Erica, who continues to inspire me (and, I feel very confident saying, the entirety of the TZ wildlife care team) with her undying commitment to animal well-being and conservation. I hope I can be half the zookeeper she is one day.

They say to save the best for last, so finally, thank you to goofy Humphrey, sweet Aurora, bossy Nikita, inquisitive Juno, and *especially* big baby Hudson for being the cutest (and most bloodthirsty) study subjects a grad student could ask for. They will be thoroughly compensated for their participation in this project in the currency of fish and melon popsicles.

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1 INTRODUCTION

1.1 POLAR BEAR SEASONAL ECOLOGY

Each year Arctic sea ice undergoes a seasonal freeze-thaw cycle in accordance with environmental conditions. Beginning in late spring or early summer, depending on exact location, warmer air temperatures initiate sea ice melt and breakup (Paetkau et al. 1995, Derocher et al. 2004). Sea ice reflects light, but the exposed water absorbs solar heat from sunlight due to its dark color (Perovich & Polashenski 2012). As more water is exposed, more solar heat is absorbed, increasing water temperatures, and creating a positive feedback loop that further drives ice melt (Perovich & Polashenski 2012). Cooler temperatures in late fall and early winter trigger sea ice refreeze, where thick ice sheets develop on the ocean's surface until the following spring or summer (Perovich & Polashenski 2012).

This seasonal landscape effects the ecology of Arctic-dwelling animals – particularly polar bears (*Ursus maritimus*) who are reliant on sea ice to hunt their primary prey item, the ringed seal *(Pusa hispida*). When sea ice is present, adult polar bears have been observed hunting seals once every 5 days (Stirling 1974). However, the frequency of hunting depends on the size of the prey item obtained – it has been estimated that bears obtain the same energy from 1 adult seal as they do 3 sub-adult seals, or 19 pups, all of which should provide enough energy for 10-12 days (Pagano et al. 2018). The period of energy surplus that occurs when sea ice is present is known as the anabolic phase. When the annual ice melt occurs, bears are pushed onto land where they greatly reduce their caloric intake, entering an energy deficit period known as the catabolic phase. During this time bears do not fast, but rather consume small amounts of vegetation – including grasses, berries, shrubs, and algae – and scavenge from carcasses left behind by other predators (Russel 1975, Koettlitz 1989, Knudsen 1978). Energy intake is not halted entirely during the catabolic phase, but it is greatly reduced compared to the anabolic phase.

Polar bears withstand the catabolic phase by relying on their fat stores (Atkinson & Ramsey 1995, Atkinson et al. 1996). While hunting seals during the anabolic phase, bears target the seals' lipid-rich blubber (Best 1985, Beck et al. 2003, Rode et al. 2021). As fat is the most efficient form of stored energy (Best 1985), consuming copious amounts of seal blubber – up to 100 lbs in a single sitting (Polar Bears International 2021) – allows bears to quickly build ample fat stores within their bodies (Best 1985, Beck et al. 2003, Rode et al. 2021). These stores are then mobilized throughout the bloodstream when energy intake is low, acting as the primary source of energy during the catabolic phase (Atkinson & Ramsey 1995, Atkinson et al. 1996, Derocher et al. 1992). Being a polar bear is an energetically costly endeavor due to having a large body size, small body surface area:volume ratio, and a high metabolic rate (Whiteman et al. 2015, Gleason & Rode 2009, Kelly et al. 2015, Nelson et al. 1983), so sufficient fat stores are essential for surviving energy deficit. While polar bears also consume some protein from the muscle, viscera, and other lean tissue of seals, this protein cannot be stored in the same manner as fat, and relying on it for energy during the catabolic phase comes at a high energetic cost (Atkinson et al 1996, Ramsay et al 1989). Although dietary fats have higher digestive efficiency and assimilate energy more rapidly per food unit than protein, they also take longer to process (Best 1985). Though dietary proteins cannot be stored, they allow for more frequent food consumption, raising total potential energy intake (Best 1985). Thus, it has been suggested that bears regulate their diet intake so that protein requirements are met, but not exceeded (Atkinson et al 1996, Pond & Mattacks 1992).

On top of the unique seasonal ecology and subsequent energetic demands of the polar bear, the species' morphology also accommodates a high-fat diet. Compared to the highly omnivorous black bear or brown bear, the polar bear has a reduced surface area of the grinding molar teeth, hindering their ability to process tough plant material (Slater et al. 2010). Additionally, the longer, more slender head and snout of the polar bear compared to other bear species suggests that they are poorly suited to the large masticatory loads that come with the consumption of tough plant matter and fibrous protein-rich tissues, but better-suited to consuming fat-rich prey and scavenging carcasses (Slater et al. 2010, Petherick et al. 2021). Multiple studies that allowed polar bears to regulate their own macronutrient intake demonstrated that they will voluntarily consume at least 3 times more fat than protein on a dry-matter basis, prioritizing items such as isolated seal or beef fat (Best 1985, Robbins et al. 2022, Rode et al. 2021). One sub-adult male polar bear captured from the Western Hudson Bay subpopulation was offered a variety of ringed seal blubber, muscle, viscera, and skeleton, and selectively consumed 80% blubber and 20% muscle and viscera (Best 1985). More recently, a zoo-based study with a similar experimental design found that 5 adult male and 4 adult female polar bears chose to obtain 76 ±7% of their metabolizable energy from fat, and 24 ±7% from protein on a dry-matter basis (Rode et al. 2021, Robbins et al. 2022). Given the polar bears' seasonal ecology, morphology, and behavior, dietary fats are crucial for catabolic phase survivorship and overall well-being.

1.2 SEASONAL CHANGES IN PHYSIOLOGY AND BEHAVIOR

1.2.1 Body Mass and Composition

With the seasonal fluctuation in resource availability come a multitude of seasonal fluctuations in polar bear physique, physiology, and behavior. The body mass of wild bears is known to fluctuate seasonally – bears gain weight during the anabolic phase when resources are abundant (Pagano et al. 2018, Galicia et al. 2020), and lose on average 1 kg per day during the catabolic phase when resources are scarce (Pilfold et al. 2016). In fact, in the Western Hudson Bay subpopulation, it has been estimated that adults lose up to 43% of their total body mass throughout the catabolic phase (Atkinson & Ramsay 1995). This fluctuation in body mass comes largely in the form of a fluctuation in body fat (Atkinson and Ramsay 1995, Atkinson et al. 1996), which is thought to reflect stored energy (Best 1985). Given the high energetic cost of catabolizing body protein (Best 1985, Atkinson et al. 1996) bears are believed to rely primarily on their fat stores as a source of energy during the catabolic phase, meaning that increasing body mass and body fat reflect the accumulation of energy stores, and declining body mass and body fat reflect the subsequent use of those stores (Pagano et al. 2018, Galicia et al. 2020, Pilfold et al. 2016, Atkinson and Ramsay 1995). Measuring body mass alone gives insight as to food consumption, but coupling this with body fat measurements allows for better understanding of how and when bears are storing, using, and conserving energy.

1.2.2 Blood Chemistry

The measurement of blood metabolites can be interpreted as a reflection of energetic state in polar bears. Given the important role of dietary fat, one metabolite of particular interest are triglycerides, the form in which fat is stored and mobilized in the body (Ntambi et al. 2000, Tartu et al. 2017). Upon the consumption of dietary fats, triglycerides are emulsified, enzymatically digested, and absorbed through the jejunum and small intestine, before being transported, by lipoproteins, through the bloodstream to the adipose tissue for storage (Ros 2000). When energy intake is high, such as during the anabolic phase, triglycerides remain in the adipose tissue, leading to a low serum concentration indicative of energy storage (Ntambi et al. 2000, Tartu et al

2017, Hill 2013). Conversely, when energy intake is low, triglycerides are mobilized throughout the bloodstream to be used as a source of energy, leading to a higher serum concentration (Ntambi et al. 2000, Tartu et al 2017, Hill 2013).

Another blood indicator of change in energy storage and use is the ratio of serum urea to creatinine (hereafter UC ratio), whereby smaller UC ratios are common of catabolic bears (Derocher et al. 1990, Ramsay et al. 1991). Urea – the by-product of protein use – accumulates in the blood when resources are available, hence why serum urea concentrations are higher during the anabolic phase (Barboza et al. 1997, Whiteman et al. 2018). When resources are scarce, urea is recycled into new amino acids that replenish catabolized body protein, reducing circulating urea (Barboza et al. 1997, Whiteman et al. 2018). Conversely, creatinine – which cannot be metabolized – accumulates in the blood year-round (Whiteman et al. 2017). It has been proposed that UC ratios <10 indicate fasting (Nelson et al. 1984), while more recent studies have found that minimal food consumption can raise UC ratios slightly above 10 in bears who are still catabolic and losing mass (Rode et al. 2018, Pagano unpublished). Therefore, values <10 may indicate complete lack of caloric intake, however, relatively small values >10 are likely still indicative of energy deficit. Larger UC values indicate feeding – bears in Hudson Bay showed UC ratios as high as 61.2 within one day of feeding, however these ratios dropped significantly after as little as one day, indicating that a relatively large UC ratio is reflective of recent food intake (Derocher et al. 1990).

1.2.3 Body Temperature and Behavior

Wild bears are known to have lower body temperatures during the catabolic phase than the anabolic phase (Whiteman et al. 2015, Whiteman et al. 2017). It has been suggested that this reduction in body temperature is a means of conserving energy during catabolism (Whiteman et al. 2015) as heating the body requires energy (Hurst et al. 1982). While it is possible that bears reduce body temperature to conserve energy, this observed change in body temperature may also be driven by diet or environmental factors. Understanding exactly what drives the seasonal change in body temperature observed in wild bears is complicated further by the ways in which body temperature is tied to behavior. Activity is known to increase the body temperature of bears (Pagano et al. 2018), and the seasonal reduction in body temperature corresponds with an observed reduction in activity (Whiteman et al. 2015, Pagano et al. 2018, Robbins et al. 2012, Oritsland 1970). It has been suggested that bears reduce activity as a means of evading thermal stress (Whiteman et al. 2015) – something they are particularly susceptible to given the small surface area:volume ratio of their bodies, which minimizes heat loss, and their hollow fur that traps escaping body heat against the skin (Wang et al. 2006, Hurst et al 1982, Mathewson & Porter 2013). It has also been suggested that the concurrent reductions in body temperature and activity both occur in response to reduced caloric intake (Whiteman et al. 2015, Pagano 2018). While fluctuations in body temperature and activity appear to correspond with fluctuations in caloric intake, the exact combination of factors that drive these changes remain poorly understood.

1.3 CAPTIVE POLAR BEARS

Though polar bears are one of the earliest-recorded species ever kept in captivity – king Ptolemy II of Egypt kept one in his Alexandria Zoo (San Diego Zoo Library 2021) – there is little record of what these early captive bears were fed. A 1948 news article from the Oregonian reports that captive polar bears at the Washington Park Zoo were fed primarily lean, protein-rich horse meat (The Oregonian, 1948), and a 1963 image from the Chester Zoo shows polar bears being offered dry kibble, likely a feed manufactured for dogs (as bear-specific feeds were not widely available at this time), which tend to be high in protein (Williams 1963). As recently as 2015, the American Association of Zoos and Aquariums (AZA) – one of three primary accrediting bodies for North American zoological facilities – suggested captive polar bears be offered 25% of their metabolizable energy from protein on an as-fed basis, and only 5-20% from fat (AZA 2015). As research on captive polar bear diets is limited, the AZA makes diet recommendations based on existing research on the nutritional needs of canine species. This large range of recommended dietary fat values creates opportunity to offer bears a diet whereby protein drastically exceeds fat. While the AZA care manual for polar bears lists several suggested diet items such as restaurant-quality salt-water fish, whole prey items, dry feed, fresh produce, and bones, there is no specific mention of isolated animal fats or fat-rich kibble. While cornflower oil and cod liver oil are named in sample diets for juveniles and sub-adults, the AZA polar bear care manual does not identify sustainably sourced seal oil or other marine oils as suggested diet items for adult polar bears (AZA 2015). The AZA care manual mentions seasonal diets, suggesting that a seasonal fluctuation in calories is a diet strategy that may be used for captive polar bears, but not a necessity (AZA 2015). While many facilities follow the recommendation of offering seasonal diets, a 2006 survey of North American and European zoos found that 39% of zoos surveyed offered the same amount of calories year-round (Nutrition Advisory Group 2006). While specific macronutrient amounts were not quantified in this survey, only 35% of zoos surveyed offered lipid-rich oils at any point throughout the year (Nutrition Advisory Group 2006). As research on how best to feed polar bears in captivity is limited, and research supporting seasonal, high-fat diets in captivity are relatively new (Robbins et al. 2022, Rode et al. 2021), the AZA is limited in the guidance they can provide to zoos, creating inconsistencies in feeding across facilities.

The lack of peer-reviewed scientific evidence supporting seasonal, high-fat diets for captive polar bears raises concerns given that the species is uniquely adapted to consume a seasonal, fat-rich diet. A recent report examining captive polar bears in North American zoos found that liver and kidney disease accounted for 64% of deaths in bears under 30 years of age since 2005 (Robbins et al. 2022). While information about the effect of dietary protein on kidney and liver functions of polar bears is limited, both dogs (*Canis lupus familiaris*) (Polzin 2013, Ko et al. 2020) and cats (*Felis catus*) (Polzin 2013, Backlund et al. 2017) on high-protein diets are known to have a higher incidence of kidney and liver disease. The same trend has also been consistently observed in humans (*Homo sapiens*) (Bilsborough & Mann 2006). Given that polar bears evolved to consume fat-rich diets, and links between liver/kidney disease and high-protein diets were found in other species, the potential link between high-protein diets and early death in captive polar bears cannot be dismissed. Recent research suggests that a high-fat diet promotes species-specific behavior and metabolism in captive polar bears (Rode et al. 2021, Robbins et al. 2022), and thus increased welfare. As a result of these findings, new fat-rich commercial polar bear dry feeds are being manufactured (Robbins et al. 2022), making it easier for zoos to meet the newly suggested dietary requirements of the polar bears in their care.

1.4 IMPLICATIONS

Due to the challenges of gathering reliable and accurate data from wild bears, it has been suggested that studying captive bears can provide further insight as to how bears withstand the prolonged energy deficit period (Kelly et al. 2015). Difficulty locating bears in the wild makes repeated sampling of the same individuals a challenge, and arctic field conditions can negatively impact the quality of samples before they are analyzed. Gathering behavior data is particularly difficult in the wild as it requires the researcher to risk their safety by approaching unrestrained, non-sedated bears. However, furthering our understanding of how bears withstand prolonged energy deficit is especially important in the face of climate change, which is causing the ice-free period to lengthen, leading bears to go longer with minimal food intake (Stroeve & Noetz 2018). Studying bears in captivity allows for repeated sampling of the same individuals in a semi-controlled environment, increased precision in recording diet data, ease in transporting samples, and knowledge of each individual bears' health and life history. Additionally, captive bears at accredited zoos are trained to voluntarily participate in data collection using positive reinforcement, which makes sample collection less stressful for both the bear and the researcher. While the energetic differences between the wild and captivity limit the extrapolation potential of studies on captive bears, such studies are still important in furthering our understanding of polar bear seasonality. This study should be considered a pilot study – the potential for its immediate findings to be directly extrapolated to wild bears is limited, but it lays the groundwork for a seasonal management regime for captive polar bears that can easily be adopted by other zoos to facilitate similar studies.

Such studies would not only benefit polar bears in the wild, but those in captivity as well. As the captive environment becomes increasingly important in conserving this species, it is crucial that we learn how to provide polar bears with the best quality of life possible under human care. Quality of life in captivity is referred to as "welfare" – an animal's emotional, mental, and physical state at any given time – and exists on a scale from "very poor" to "positive" (Broom 1991). An animal's welfare is assessed by observing health, behavior, and physiology – for example, engaging in the natural, species-specific behaviors and physiological processes of their wild counterparts is thought to indicate that a captive animal is experiencing positive welfare (Held & Spinka 2011, Ohl & Van Der Staay 2012, Mellor 2015, Lawrence et al.

2019). Nutrition plays a crucial role in welfare (Kaliste 2007, Algers & Smulders 2009), especially for polar bears, who's seasonal ecology is believed to be driven largely by their diet. By studying this species in captivity, we can further our understanding of how best to manage the polar bears in our care in order to promote positive welfare.

1.5 HYPOTHESES AND PREDICTIONS

The objective of this study is to assess the effect of diet on the behavior and physiology of Toronto Zoo's five captive polar bears, while controlling for relevant environmental (air temperature, exhibit, etc.) and bear-specific (age, sex, etc.) variables.

1.5.1 Body mass and body fat

I hypothesize that *a)* total body mass (kg), *b)* total body fat (kg) and c) proportional body fat (%) will increase with increasing calories offered as bears store energy during the anabolic phase of their seasonal cycle. I hypothesize that *a)* total body mass (kg), *b)* total body fat (kg) and c) proportional body fat (%) will increase with increasing dietary fat composition because of the higher energy content and digestibility of dietary fat. Based on the documented sexual dimorphism of polar bears, I predict there will be an effect of sex, where male bears are heavier and fatter than female bears, and an effect of age where older bears are heavier and fatter.

1.5.2 Blood chemistry

I hypothesize that serum triglyceride concentration will decrease with increasing calories as small serum triglyceride values are associated with energy storage, and serum urea to creatinine ratio will increase with increasing calories as large UC values are associated with recent food consumption. I hypothesize that serum triglyceride concentration will increase with increasing dietary fat composition as triglycerides are the form in which dietary fat is stored within the body, while serum urea to creatinine ratios will decrease as urea is the biproduct of protein use. I predict age and sex will have minimal-to-no effect on these relationships. As body fat is stored in the form of triglycerides, I predict proportional body fat will have a positive effect on serum triglyceride concentration, but no effect on serum urea to creatinine ratios.

1.5.3 Body temperature

I hypothesize that body temperature will decrease with both decreasing calories and dietary fat composition as a means of energy conservation, as well as a result of decreased generation of metabolic heat through digestion. I predict ambient air temperature and humidity to have positive effects on body temperature, and windchill to have a negative effect as the gradient between internal temperature and environmental temperature effects thermoregulation. As polar bears' body fat is designed to store energy rather than to insulate the body, I predict that proportional body fat – as well as age and sex – will have no effect on body temperature.

1.5.4 Behavior

I hypothesize that activity will increase with both increasing calories due to the increase in energy being supplied, and increasing dietary fat due to the storage efficiency of fats. I predict air temperature and humidity will have positive effects on inactivity as activity produces body heat and thus increases the likelihood of thermal stress occurring. Proportional body fat is also predicted to have a positive effect on inactivity, as activity requires more energy in larger bears. I predict age to have an effect on inactivity, whereby geriatric bears are more inactive due to deteriorating muscle and joint condition as well as reduced energy. I also predict exhibit to have an effect on inactivity whereby bears are more active in the grass exhibit as it is largest and thus contains more enrichment and substrates. I predict time of day will also have an effect, where bears are more active in the morning as less time has passed since their last meal.

2 METHODS

2.1 STUDY AREA AND SUBJECTS

I investigated how dietary macronutrient content and gross caloric intake affect body mass and composition, behavior, body temperature, and blood chemistry of captive polar bears. Data were collected during two time periods: April 2018 to March 2019 and February 2022 to June 2023, with the exception of mass data which has been collected regularly for several years prior to the onset of the study (Figure 2.1). Research was conducted at The Toronto Zoo in Scarborough, Ontario, Canada (43.8207° N, 79.1815° W). Five polar bears participated in the study: Aurora (female, 22 years old at end of study), Nikita (female, 22 years), Hudson (male, 11 years), Humphrey (male, 9 years), Juno (female, 7 years). Presumed twins Aurora and Nikita were orphaned as cubs in Polar Bear Provincial Park (James Bay, Ontario, Canada), and came to The Toronto Zoo in 2001. Aurora gave birth to Hudson, Humphrey, and Juno at The Toronto Zoo, all fathered by the same adult male (Inukshuk, Cochrane Polar Bear Habitat (Cochrane, Ontario, Canada)) (Figure 2.2). All 3 cubs were hand-reared by Toronto Zoo Wildlife Care teams due to uncontrollable and unforeseen circumstances during early parturition, though Hudson received more direct human contact than Humphrey and Juno.

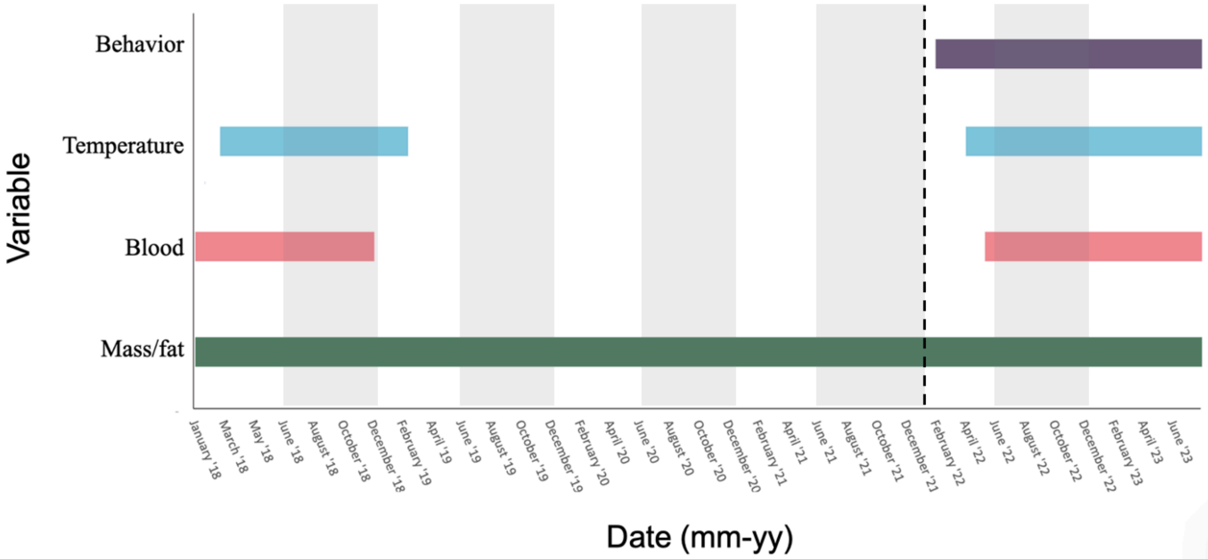

Figure 2.1: Timeline of data collection (behavior: purple, body temperature: blue, blood sampling: pink, and body mass and fat: green) for all 5 polar bears at the Toronto Zoo. Dashed line indicates the onset of the high-fat diet. Gray shaded areas represent the annual catabolic (low calorie) phases.

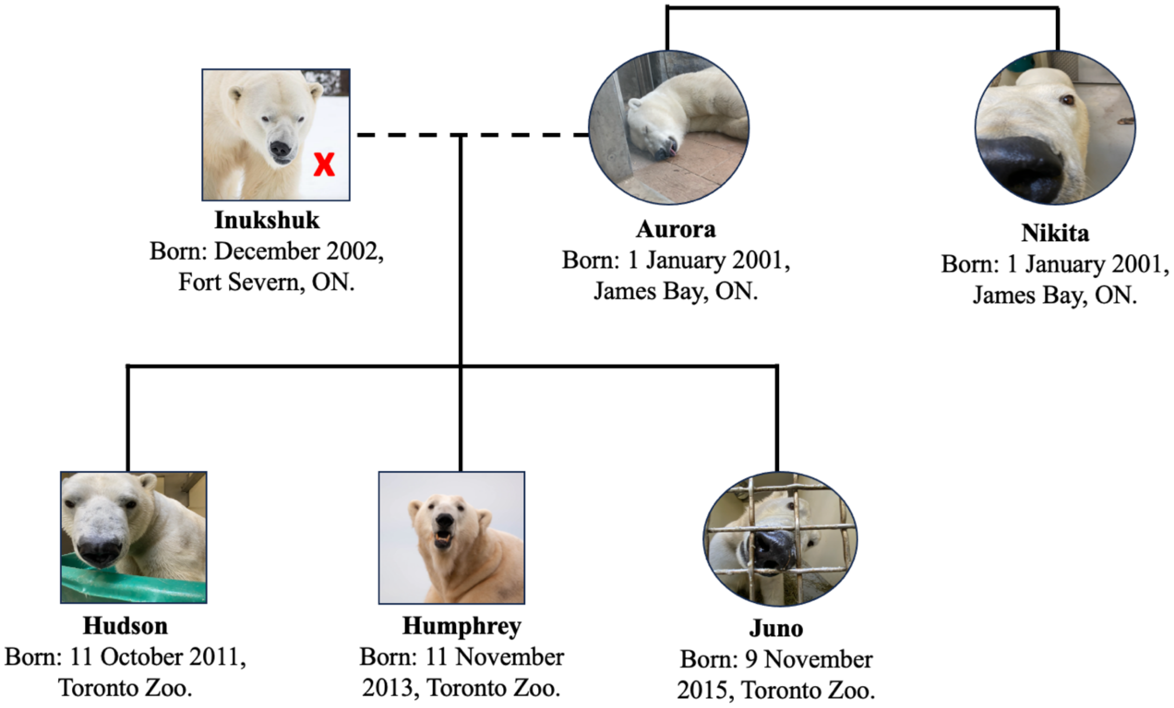

Figure 2.2: Family tree of all polar bears housed at Toronto Zoo involved in study. Solid lines represent familial relationship and dashed lines represent breeding pairs. Circular photos represent female bears, while square photos represent male bears. A red “X” indicates that the bear was not involved in the study.

The Toronto Zoo polar bears are housed in the “bear house” which is comprised of a keeper area and kitchen, maternity den, and six pens, with access to four outdoor exhibits (Figure 2.3). Each pen is roughly 9 m^2, however size varies slightly between pens, with pens 1 and 4 being the largest. Each pen is made of concrete with epoxy covering and contains a water bowl, a “hopper” – a metal tube to deliver food – and a metal door with external locks. Each pen is connected to at least 1 outdoor exhibit, as well as any adjacent pens, with hydraulic double doors (Figure 4.3). Pens 1 and 4 are connected by a transfer containing the scale, and a transfer can be set up to connect pens 3 and 6 (“O transfer”, figure 2.3). The 200 m^2 “mat” exhibit contains grass, concrete, a small, un-filtered pool, training wall, and one guest viewing area. The 1100 m^2 "pool" exhibit contains concrete, grass, rock, and mulch substrates alongside a large ozone-filtered pool with underwater viewing. Above-ground the exhibit has three additional guest viewing areas. The "grass" exhibit is comprised primarily of grass, but also contains rock, mulch, and straw, with three guest viewing areas. The 3127 m^2 exhibit features a large hill, a cave, a medium-sized ozone-filtered pool with a waterfall and "river", and a deep moat which the bears can access using a staircase. The bears also have access to a concrete "yard" exhibit, however there is no guest viewing areas, and bears were not observed in this exhibit. Each pair of adjacent exhibits can be connected by opening a hydraulic transfer door.

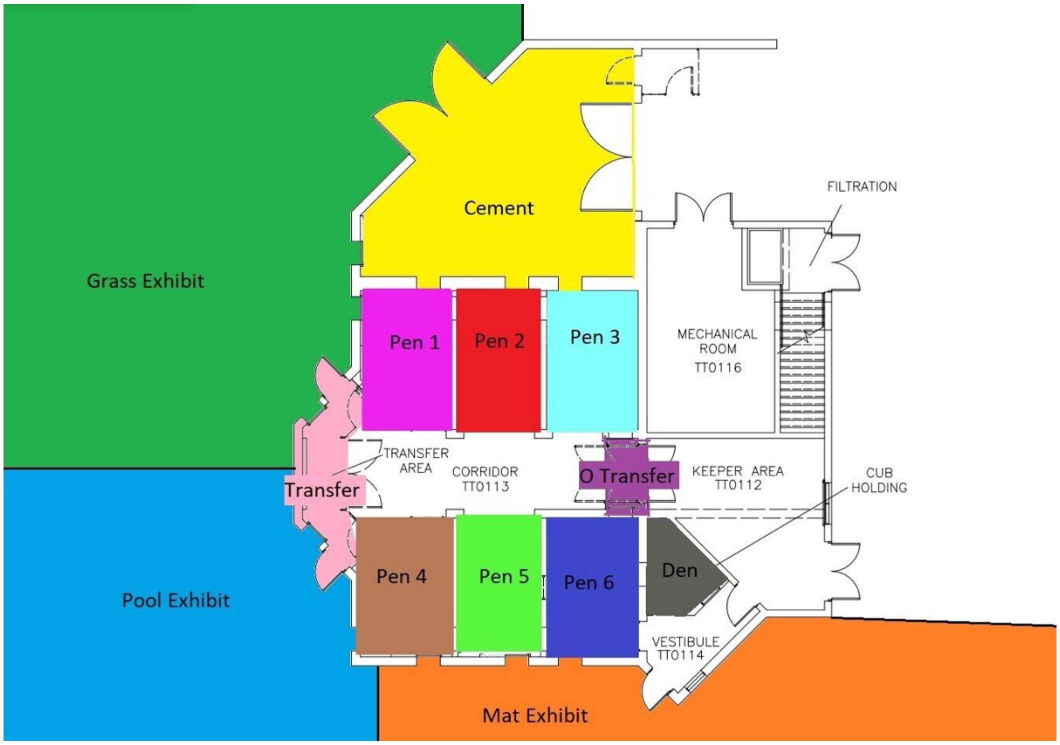

Figure 2.3: Illustration of Toronto Zoo polar bear habitat, including indoor holdings and exhibits. North is oriented upward, and the scale is 1:125. Illustration courtesy of Toronto Zoo exhibit design team (2013).

2.2 DIET

2.2.1 The Toronto Zoo Polar Bear Diet Program

Since early 2012 Toronto Zoo polar bears have been fed a seasonal diet meant to approximate that of the wild Western Hudson Bay subpopulation, with the anabolic phase ranging from January or February to June or July, and the catabolic phase from June or July to the following January or February, depending on the year and individual bear. Dietary changes were overseen by the Toronto Zoo wildlife nutritionists, veterinarians, welfare specialists, and wildlife care team based on bear body weights and behavioural cues such as changes to food intake (i.e. refusals or preferences). Baseline diets were established based on sex – male bears are larger and require more calories – and age, but keepers also communicated individual needs of specific bears to the nutrition team, and diets were adjusted accordingly. For example, Juno was noted to experience bouts of high-intensity stereotypy in the form of pattern swimming at high body fat, so her calories were decreased when this behavior was observed, regardless of time of year. Some individuals also showed diet-item preferences – for example, in winter 2022 Hudson began refusing apples, and was offered pears and melons instead.

Polar bears at the Toronto Zoo are fed a variety of diet items including fresh produce, fish, kibble, meat, and sustainably sourced seal oil. The Toronto Zoo works with commercial meat manufacturer, Milliken Meat Products Ltd. (Markham, ON), to produce specially designed meat diets that meet the nutritional needs of polar bears. The Toronto Zoo “feline diet” is a lean meat blend composed of horse and beef meat fortified with vitamins, contains 13% crude fat on an as-fed basis, and is fed primarily during the catabolic phase. The Toronto Zoo “polar bear diet” is composed primarily of pork fat, beef, and horse meat, contains 18% crude fat on an as-fed basis, and is fed primarily during the anabolic phase. Bears are offered large lake smelt

(*Osmerus mordax*) (Great lakes food company ltd., Chatham, ON), herring (*Clupea harengus*) (Allen's fisheries ltd., Benoit's Cove, NL), and capelin (*Mallotus villosus*) (Barry group inc., Corner Brook, NL) year-round, with specific amounts of each varying between dietary phases. Until early 2021, bears were fed *Orijen six fish dog chow* (Champion Petfoods, Auburn, KY), a commercial dry feed for dogs with a maximum of 37% crude protein and 17% crude fat on an as-fed basis. Beginning in 2022, bears were transitioned to *Mazuri Wild Carnivore Bear Plus Diet* (Land'O'Lakes Inc., Arden Hills, MN), a new commercial chow specifically for polar bears, with a maximum of 19% crude protein and 27% crude fat on an as-fed basis. The bears are fed a variety of fresh produce throughout the year; carrots, romaine lettuce, apples, bosc pears, butternut squash, corn on the cob, red and green grapes, yams, pumpkins, cantaloupe, and honeydew, with novel produce items such as whole watermelons and pumpkins being offered on special occasions. Beginning in January 2023 bears were also offered isolated beef fat. Bears were occasionally offered novel diet items including peanuts in-shell, peanut butter, apple juice, and Rice Krispie cereal (Kellogs, Battle Creek, MI), in order to provide more mental stimulation and elicit foraging behaviors and engagement with enrichment items.

2.2.2 Diet Calculations

Diets were calculated with a weekly allotment of calories and macronutrients, rather than daily. Certain diet items such as meat, fish, and kibble were fed based on specific daily amounts, while other items such as produce, novel items, and oil were fed irregularly throughout the week at the keepers' discretion (with limits predetermined by Toronto Zoo wildlife nutrition staff based on calorie and macronutrient composition). This was done to create spontaneity in the bears' daily routine and prevent repetitive anticipatory behaviors which can be associated with poor welfare (Tallo-Parra et al. 1996). Calories were calculated as the total KCal of metabolizable energy consumed per week, on an as-fed basis (equations 1-4). I performed calculations using the predictive equation for metabolizable energy in dog food and the Atwater values for dogs (National Research Council of the National Academies 2001), as such equations and constants have not been developed for any bear species.

$$GE = \left(5.7 \times g_{protein}\right) + \left(9.4 \times g_{fat}\right) + \left(4.1\left(g_{NFE} + g_{fiber}\right)\right) \tag{1}$$

$$\%\,energy\ digestibility = 91.2 - 1.43 \times \%\,fibre\ dry\ matter \tag{2}$$

$$DE = (GE \times \%\,energy\ digestibility) \div 100 \tag{3}$$

$$ME = DE - (1.04 \times g_{protein}) \tag{4}$$

Where *GE* represents gross energy, *DE* is digestible energy, *NFE* is nitrogen-free extract, $g_{macronutrient}$ is the grams of protein or fat, and ME is metabolizable energy. Protein, fat, fibre, and NFE values were obtained from the USDA FoodDataCentral database (United States department of agriculture 2023) for produce and commercial items, and from supplier labels for meat, fish, and chow. The metabolizable energy from protein was calculated using total protein per diet item (equation 5), and per total diet (equation 6) for every diet change that occurred throughout the study period. Metabolizable energy was then calculated from fat using the same equations, substituting fat values for protein values.

$$ME\ protein_{diet\ item} = ME_{diet\ item} \times (\%\,crude\ protein\ as\ fed\ \div 100) \tag{5}$$

$$\%ME\ protein_{diet} = \frac{\sum ME\ protein_{diet\ item}}{\sum ME_{diet\ item}} \times 100 \tag{6}$$

2.2.3 Diet administration and recording

Bears were fed in a variety of ways depending on the diet item, dietary phase, and daily schedule at the zoo. Bears were often fed a portion of meat and chow in their indoor pens via their individual hopper in the morning and again in the evening, with exact timeframes varying from day-to-day. Chow was often fed with a “topper”, such as apple juice, smelt slurry, or blended melons to increase acceptance and total intake. Bears were offered some combination of meat, chow, fish, fruits, nuts, cereal, and vegetables on exhibit by scattering, burying, and hiding diet items inside enrichment toys. All diet items excluding oil were occasionally fed in the form of a “polar bear popsicle”, where items were frozen in a large block of ice made from water and/or fruit juice. During training sessions keepers hand-fed small portions of meat, fish, and vegetables to individual bears. Oil was always hand-fed with a 10 mL plastic syringe with the tip cut off and sanded down, and was given during blood-draw training only. Nikita, Aurora, Hudson, and Humphrey received a daily 20 mL dosage of glucosamine to promote healthy joints and prevent cartilage damage. Aurora and Nikita also received a 4 mL dosage of liquid meloxicam – to treat their mild arthritis – thrice per week, and all bears received one 100 mg vitamin E tablet and one 200 mg thiamine tablet each morning, handfed by keepers in a meatball.

In the case that bears were put on exhibit together in a social group, their diets were combined and offered to the whole group. For example, if Aurora and Nikita were to each be offered 1 kg of meat outdoors, 2 kg of meat total would be placed in the exhibit, and both bears given access. Any diet refusals were re-offered, and if still refused, weighed so that metabolizable energy could be calculated. The metabolizable energy of the leftovers was then subtracted from the bear’s calories for that week. If refused diet was found in an exhibit shared by more than one bear, the metabolizable energy of the refusal was divided by the number of bears who had access to it, then subtracted from their individual intakes for the week. It should be noted that bears may have buried, hidden, or otherwise failed to remove diet from enrichment items. Keepers may also fail to find leftover diets on exhibit, and bears sharing exhibits may consume portions of each other’s diets. Therefore, all caloric and macronutrient values presented in this study represent diets offered rather than diets guaranteed to have been fully consumed.

2.2.4 Study Diets

Caloric intake fluctuated for each bear throughout the study period (Figure 2.4). Typically, diet changes and caloric increases were implemented in early-to-mid January, with caloric intake peaking in April or May and being maintained into June (Figure 2.4). Caloric decreases were usually implemented in late June or early July, with minimum caloric intake being achieved by late August or early September (Figure 2.4). While Aurora and Nikita’s maximum and minimum caloric intakes remained similar throughout the entirety of the study, Hudson and Humphrey saw an increase in caloric maximum during the winter and spring of 2022 to accommodate their transition into adulthood (Figure 2.4, Table 2.1). Juno occasionally had diet changes implemented at different times than the other bears to accommodate changes in her behavior and body weight. Toronto Zoo zookeepers observed an increase in stereotypic behavior displayed by Juno when she reached both her lowest and greatest body weights, therefore Juno’s diets were adjusted to minimize stereotypy regardless of time of year (Figure 2.4).

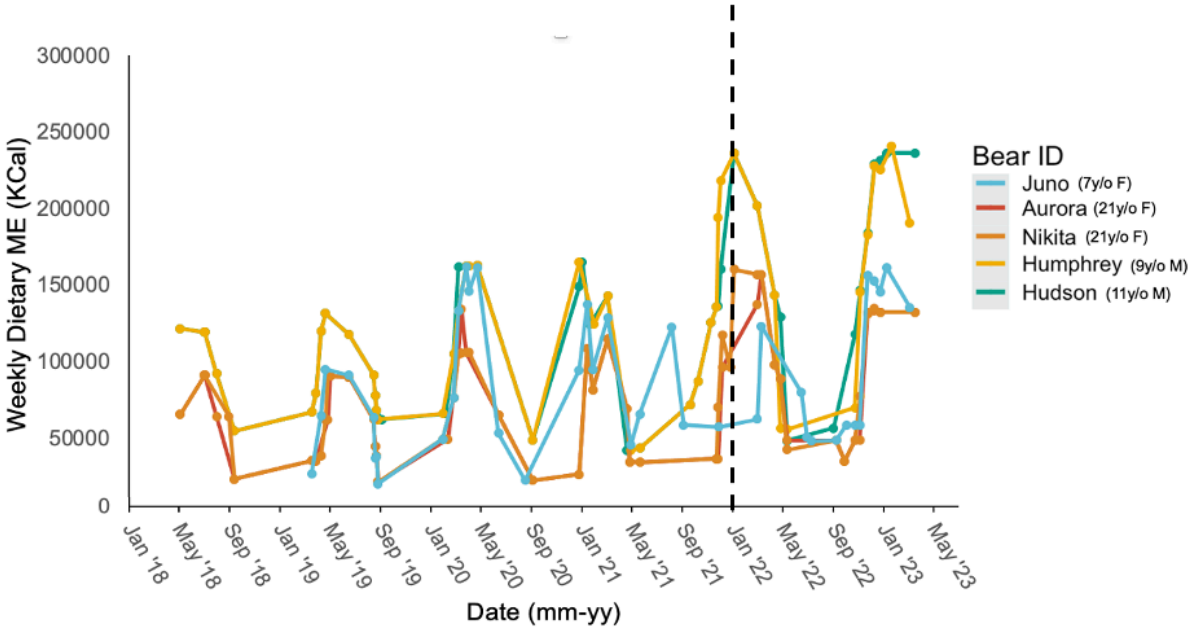

Figure 2.4: Changes in weekly dietary metabolizable energy (KCal) offered to 5 polar bears at The Toronto Zoo from January 2019 to August 2023. Dashed line indicates the onset of the high-fat diet. Insufficient data was recorded for Juno's diets until March 2019.

Table 2.1: Occurrence of the highest-calorie and lowest-calorie diet offered to each polar bear, per diet treatment. Calories and macronutrient composition are calculated on a weekly, as-fed, metabolizable energy basis. (where ME = metabolizable energy, HP = high protein, HF = high fat, and F:P = protein fat ratio).

Aurora & Nikita

Date (yy/mm/dd)	Phase	ME (KCal)	Diet	%ME fat	%ME protein	Fat:Protein Ratio
18/04/12	Anabolic	99042	HP	14.30	20.96	1:1.4
18/07/17	Catabolic	21367	HP	12.00	15.90	1:1.3
22/05/03	Anabolic	146928	HF	39.99	12.66	3:1
22/12/10	Catabolic	34961	HF	42.10	9.17	5:1

Hudson

Date (yy/mm/dd)	Phase	ME (Kcal)	Diet	%ME fat	%ME protein	F:P Ratio
18/02/06	Anabolic	121522	HP	14.15	17.63	1:1.2
18/06/30	Catabolic	54699	HP	13.93	18.60	1:1.3
22/02/19	Anabolic	236065	HF	34.50	15.20	2:1
22/11/11	Catabolic	48170	HF	36.55	12.20	3:1

Humphrey

Date (yy/mm/dd)	Phase	ME (Kcal)	Diet	%ME fat	%ME protein	F:P Ratio
18/02/06	Anabolic	121522	HP	14.15	17.63	1:1.2
18/06/30	Catabolic	54699	HP	13.93	18.60	1:1.3
22/02/08	Anabolic	227782	HF	29.73	16.25	2:1
22/11/15	Catabolic	55606	HF	36.05	14.70	2.51

Juno

Date (yy/mm/dd)	Phase	ME (Kcal)	Diet	%ME fat	%ME protein	F:P Ratio
19/02/27	Anabolic	94780	HP	15.50	15.45	1:1
19/01/22	Catabolic	26303	HP	8.85	12.84	1:1.3
22/04/01	Anabolic	161554	HF	23.61	14.63	1.6:1
22/09/12	Catabolic	47882	HF	34.95	11.64	3:1

The Toronto Zoo polar bears ate a low-fat diet until January 2022 (Figure 2.5). Prior to implementing the high-fat diet, all bears consumed fat:protein ratios of 1:1.3 on a weekly, as-fed, metabolizable energy basis during the catabolic phase (Table 2.1). During the anabolic phase, fat:protein ratios varied from 1:1 to 1:1.4, depending on the individual bear (Table 2.1). Following the implementation of the high-fat diet, catabolic phase fat:protein ratios reached 5:1 for Aurora and Nikita, 3:1 for Hudson and Juno, and 2.5:1 for Humphrey (Table 2.1). During the anabolic phase, these values changed to 3:1 for Aurora and Nikita, 2:1 for Hudson and Humphrey, and 1.6:1 for Juno (Table 2.1). While attempts were made to keep fat:protein ratios similar across dietary phases, this was not always possible due to availability of diet items, preferences/intakes, and behavioral cues of individual bears. In August of 2022, Juno saw a substantial increase in stereotypic behavior which was found to be associated with her large body weight. Juno's care team made the decision to begin decreasing her fat intake to encourage weight loss at this time (Figure 2.5).

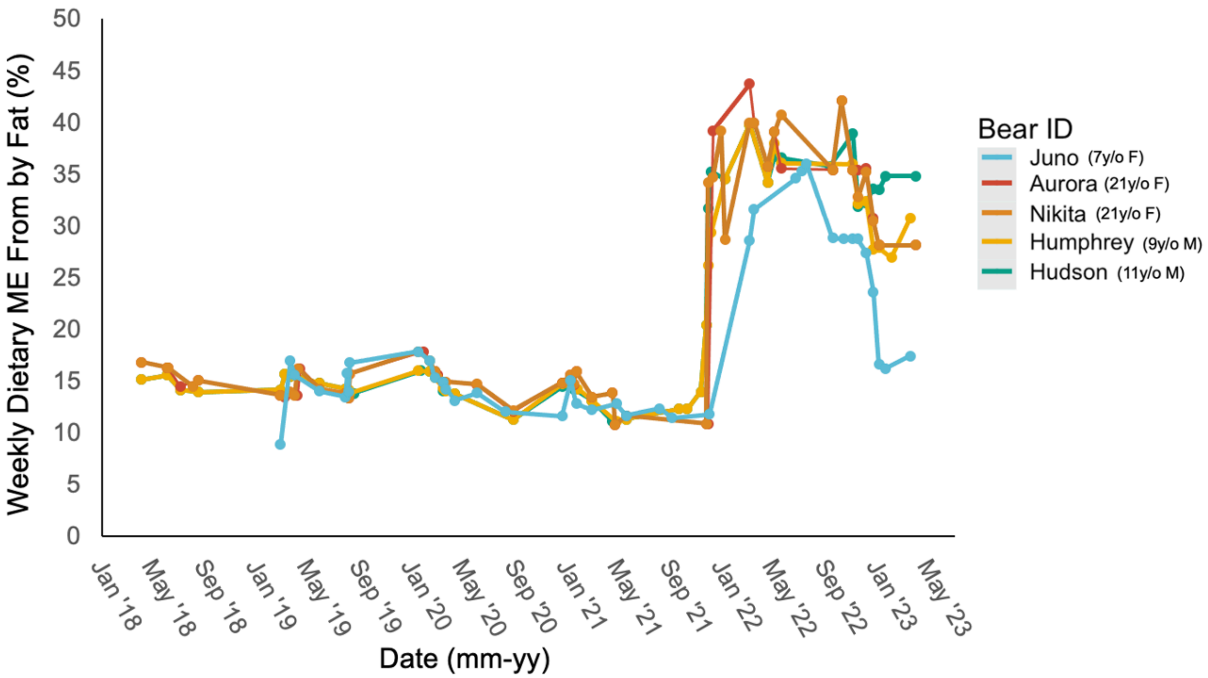

Figure 2.5: Weekly percentage of fat per KCal of metabolizable energy on an as-fed basis for 5 polar bears at The Toronto Zoo from January 2018 to August 2023. Insufficient data was recorded for Juno's diet until March 2019.

Diet variables – both calories and macronutrients – were presented continuously rather than categorically to account for the frequency of diet changes. Within one dietary phase (catabolic vs anabolic), bears were offered several diets – for example, Humphrey's calories were adjusted 9 times during the 2022 anabolic phase. Adjustments were made frequently for all bears throughout both dietary phases and calories also varied between sexes – for example, the male bears' mean weekly anabolic phase calories were 43700 KCal greater than Aurora and Nikita's mean weekly anabolic phase calories. In fact, male bears' mean weekly catabolic phase calories were only 35750 KCal less than Aurora and Nikita's mean anabolic calories, meaning that "catabolic phase" and "anabolic phase" categorical variables would encompass similar values across individual bears. Although changes to dietary fat were less drastic than changes to calories, macronutrient composition varied slightly with each diet change. Additionally, female bears tended to be offered more fat than male bears as a result of their dietary preferences and total caloric requirements. Thus, dietary fat was also presented continuously to account for the different dietary fat values encompassed by the "high fat" diet.

As ecology – and subsequently, energetic demands – differ greatly between wild and zoo bears, captive bears likely do not require as much stored fat as wild bears. The Toronto Zoo's approach to feeding polar bears balances the species' natural life history with the reduced energetic demands of captive bears by implementing a seasonal diet with frequent adjustments and a less-drastic caloric reduction than that experienced by wild bears. Additionally, the Toronto Zoo polar bear diets ensure that the metabolizable energy obtained from fat exceeds that obtained from protein. Consideration for both the diet-driven adaptations of the species as well as the unique needs of captive bears has led the Toronto Zoo to create diets that accommodate species-specific life history, prioritize welfare, and see to the needs of each individual bear under their care. This method of feeding captive polar bears also facilitates the study of behavior and physiology as it relates to a diet more closely aligned to that of their wild counterparts.

2.3 BODY MASS AND BODY FAT

Each bear was weighed once per week using a large industrial floor scale (InterWeigh Systems Inc., Markham, ON) located in a transfer hallway between pens 1 and 4 (Figure 2.3). Bears were trained using only positive reinforcement to voluntarily step onto the scale (Figure 2.6). To prevent the bears from moving, they were hand-fed meat, fish, or vegetables during weigh-ins. Attempts were made to weigh bears every Monday morning, and re-attempted later in the week if they did not want to participate.

Figure 2.6: Humphrey voluntarily steps on the scale located between pens 1 and 4 for a weekly weigh-in.

Figure 2.7: Toronto Zoo staff measure the straight-line body length of an immobilized bear.

Body mass measurements were used alongside body lengths to calculate body fat mass and percent body fat. To obtain body lengths, straight-line body length (from the tip of the snout to the end of the last tail vertebra) of bears was measured opportunistically when bears were immobilized for other medical procedures (Figure 2.7). The measurement was taken with the bear in sternal recumbency with the limbs extended away from the torso. A half-meter stick was held against the bear's snout and another against the tip of the tail while gently pulling the tail away from the body and measured the horizontal distance between the sticks. Using the von Bertalanffy length-at-age equation (equation 7) (Bertalanffy, 1938; Zullinger et al., 1984; Kingsley et al., 1988) I estimated body length to correspond with mass measurements that did not have accompanying lengths.

$$l_a = L\left(1 - e^{-k(a-A)}\right) \tag{7}$$

Where l_a is length at age, L is asymptotic body length, A is the theoretical age at which bears have 0 mass, a is age, and K is the mass growth rate constant. I used body weights and lengths to calculate storage mass (8a, 8b), storage fat mass (9a, 9b, 9c), and storage energy (10a, 10b, 10c) using Molnar's body composition model (Molnar et al. 2009).

$$M_{STO} = M - 15.14L^3 \tag{8a, female}$$

$$M_{STO} = M - 14.73L^3 \tag{8b, male}$$

$$M_{STOF} = 26.24\left(\frac{0.943}{E}\right)(M - 15.14L^3) \tag{9a, female}$$

$$M_{STOF} = 24.97\left(\frac{0.935}{E}\right)(M - 14.73L^3) \tag{9b, sub-adult male}$$

$$M_{STOF} = 19.50\left(\frac{0.885}{E}\right)(M - 14.73L^3) \tag{9c, adult male}$$

$$E = 26.24M - 390.53L^3 \tag{10a, female}$$

$$E = 24.97M - 373.05L^3 \tag{10b, sub-adult male}$$

$$E = 19.50M - 291.33L^3 \tag{10c, adult male}$$

Where M_{STO} is storage mass, M_{STOF} is storage (body) fat mass, E is energy, M is body mass, and L is body length. Body fat mass (M_{STOF}) is the total mass in kg of fat in the body, or a reflection of total fat stores. Body fat mass was divided by total body mass to obtain percent body fat.

2.4 BLOOD CHEMISTRY

Polar bears at the Toronto Zoo were trained to participate in blood draws using only positive reinforcement methods. All blood draws were performed by a Toronto Zoo veterinarian or veterinary technician, assisted by a Grade 4 zookeeper, either through free choice positive reinforcement training or when the bears were anesthetized for a medical procedure. Depending on the behaviour of individual bears and time of year, blood was drawn from either the front right or rear right dorsal pedal vein. For front dorsal pedal vein blood draws, the bear was instructed to lie down in pen 1 (Figure 2.3) and asked to offer the front right paw through the blood port (Figure 2.8). For rear dorsal pedal vein blood draws, bears voluntarily entered a transfer crate in the cement yard exhibit, connected to pen 2 (Figure 2.3), laid down, and extended the rear right paw through a port. The veterinary technicians located the vein by pressing along the bear's paw, between the 2nd and 3rd toes, then inserted a 20 gauge 1.5" needle into the vein. In the case that veterinary staff had difficulty locating a vein, a warm compress was used to increase blood flow. A 5 mL plastic syringe connected to the needle with plastic tubing was used to collect a maximum of 4 mL of blood. 1 mL of the sample was then transferred from the syringe to a 1 mL purple-top K2 EDTA test tube, and the remaining sample to a 5 mL glass test tube with a red top. The total amount of blood collected varied depending on the behaviour of the bear and the speed of blood flow, but never exceeded 4 mL.

Figure 2.8: Hudson is positively reinforced with sustainably sourced seal oil while receiving a hot compress on his front paw in preparation for a blood draw.

Blood in the 1 mL purple-top K2 EDTA test tube was used for complete blood count (CBC) analyses by the Toronto Zoo and were not used in this study. The sample in the glass test-tube was analyzed on-site in the Toronto Zoo Wildlife Health Center using an IDEXX Catalyst One machine (IDEXX Laboratories, Detroit, MI). For IDEXX analyses, including triglycerides, urea, and creatinine, the 5 mL glass test-tubes of blood were placed in the centrifuge (DRE Harmonic series 78105N, Louisville, KY), balanced with standards if necessary, and spun at 300 rpm for 10 minutes. A plastic disposable pipette was then used to add 0.5 mL of the newly separated serum to the sample well of the Catalyst One, where the IDEXX analysis was run for 10 minutes. Results were printed directly from the Catalyst One.

2.5 BODY TEMPERATURE

Gastrointestinal temperature – as proxy for core body temperature – of polar bears was tracked via 4k-Thermochron iButtons (DS1921G-F5#, iButtonLink Technologies, Whitewater, WI) (Figure 2.9). iButtons were programmed to take 1 temperature recording every 10 minutes using ThermoData temperature logging and reporting software (Embedded Data Systems, Lawrenceburg, KY). Each iButton was digitally labelled with the bear's name and the date it would be fed, then immediately placed in a paper bag with a matching label. iButtons were hidden in a variety of food items (most commonly meatballs, capelin, or herring) depending on the dietary phase and personal preferences of each bear. The chosen diet item containing the hidden iButton was hand fed, followed immediately by an additional diet item to act as a "chaser" to prevent bears from spitting the iButton out. The aim was to collect 5 days' worth of data per bear, per month. Because transit time varied and some iButtons got lost, temperature data collected varies from 50 hours to 90 hours' worth of data per bear, per month.

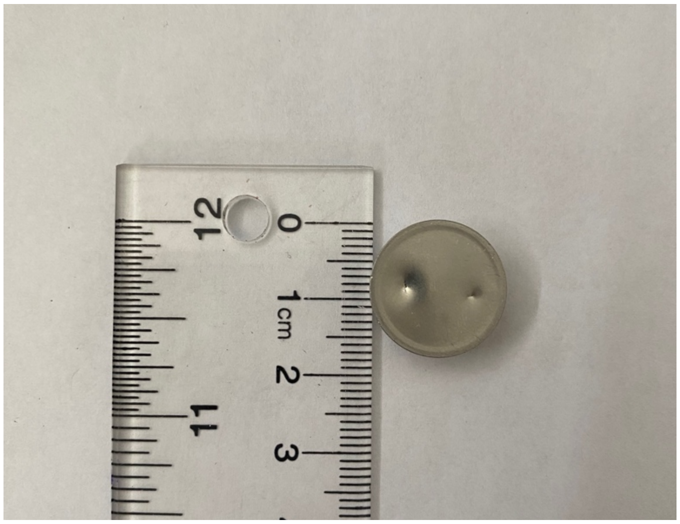

Figure 2.9: A 4k-thermocron iButton with bite marks placed next to a ruler (cm).

Feces were collected from exhibits and indoor pens each morning using a rake, shovel, and wheelbarrow. While wearing nitrile gloves, the feces were carefully searched one handful at a time, until all iButtons were retrieved. Each pool was drained for cleaning 2-4 times per year, at which time the catch basins were searched for iButtons as the bears were commonly observed defecating in the pools. Bedding material was also searched for iButtons each time it was changed. After each iButton was washed with warm soapy water and disinfected with a Lysol wipe, the ThermoData temperature logging and reporting software was used to export data. Mean daily body temperature, excluding the first 30 minutes after ingestion of the iButton to allow equilibration, was calculated and used in subsequent analyses.

2.6 BEHAVIOR

Behavioural observations were conducted from February 2022 to June 2023. A behavioural ethogram was developed based on preliminary observations, existing ethograms created by Toronto Zoo zookeepers, and existing literature (see appendix). Each specific behaviour was categorized as either active, inactive, or stereotypic. Active behaviours include any behaviour where the bear is engaged in locomotion or significant body movement leading to energy expenditure, including walking, running, playing, and swimming. Inactive behaviour was defined as any behaviour where the bear is engaged in little-to-no movement, including sitting, standing still, and laying. Stereotypic behaviour is defined as abnormal repetitive behaviour serving no purpose and displayed only in captivity (Mason 1991). In polar bears this includes pacing, pattern swimming, head swinging, and head bobbing. In the case that a bear was not visible, this was recorded as “out of view”, and later excluded from analyses.

A scan sample method (Altman 1974) was used with a 5-minute interval and 1-hour observation period. I set a timer to sound every 5 minutes, and beginning at minute zero, recorded the instantaneous behaviour of each bear when the timer sounded. When a bear was walking or swimming, I observed the bear for at least 10 seconds before recording the behaviour to differentiate between walking and pacing, and swimming and pattern swimming. For each scan I also recorded the number of guests at the viewing areas for each exhibit, excluding infants in strollers. At the start of each observation period I recorded the date, start time, air temperature, humidity, and wind speed, as well as which exhibit each bear was in. Behaviour data are presented as the proportion of in-view scans spent engaged in each behaviour category per observation period (Kelly 2015).

2.7 STATISTICAL METHODS

Statistical analyses were performed using R (version 4.2.3 for macOS) and RStudio (version 022.07.2+576 for macOS). Four datasets were compiled (blood analyses, behaviour, body mass and fat, body temperature) and preliminary visualization was conducted using the *ggplot2* package (Whickam 2016). There were two outliers removed in the blood dataset – one abnormally small UC ratio value (<5) and one abnormally large triglyceride value (>280) from Hudson, indicating increased urea recycling/stored fat mobilization, all of which corresponded with a period of time where he experienced mild vomiting and diarrhea. One abnormally large body mass measurement from Aurora was removed, which is assumed to have been human error during input.

Variables were standardized as necessary prior to conducting analyses, using either excel (version 16.77) or base R, and all variables reported as percentages were converted to proportions for statistical analyses. Base R was used to create a correlation matrix for each dataset. Explanatory variables that were highly correlated (>0.7) were examined, and only one was considered in model selection, based on preliminary visualization and biological reasoning (Akoglu 2018). If two response variables were highly correlated, they were analysed through separate, smaller univariate models as opposed to one larger multivariate model. Histograms were used to visualize distribution of response variables prior to commencing analyses. Several ANCOVAs were run to test for significant interactions. If any significant interactions were found, the interaction term was considered as a possible predictor during model selection. Any models excluding the interaction term contained only 1 of the 2 explanatory variables involved in the interaction.

The *lme4* package (Bates et al. 2015) was used to create linear mixed models. Dietary fat intake and calories consumed were coded as fixed factors, while all other possible explanatory variables were coded as random factors. Bear ID was always coded as a random factor in each possible model to account for repeated sampling of the same bears. In each analysis, calories, dietary fat, sex, age, and bear ID – or any relevant interaction terms, where applicable – were considered as possible predictors. For all analyses excluding body mass and body fat, proportional body fat was included as a predictor as well. For behaviour analyses, more explanatory variables were considered to account for the complexity of the bears' habitat and husbandry routine at the Toronto Zoo. These included exhibit, time of observation (categorical, morning (prior to 12:00PM) or afternoon (after 12:00PM)), air temperature, humidity, windspeed, and average number of visitors. For the body temperature analysis, air temperature, windspeed, and humidity were considered as explanatory variables as well. All data relating to weather was obtained from the Environment and Climate Change Canada website's hourly forecast (behavior) or daily forecast (body temperature) with data sourced from the Markham weather station.

For each dataset the *lme4* package was used to create a "full" model containing all fixed and random effects considered, and a "reduced fixed" model containing only fixed effects and bear ID, which were then compared with an ANOVA. The P-value was examined to determine if there was a significant difference between the models ($\alpha = 0.05$), and Aikake's Information Criterion (hereafter AIC_c) was used to determine the best model, whereby a smaller AIC_c indicates a better model, although all models with $\Delta AIC_c \leq 2$ were considered viable. In the case that the “full” model was significantly better, all random effects were considered going forward. This process was repeated for fixed effects, creating a “reduced random” model with only random effects, and comparing it against the full model. If the reduced model were significantly better, random effects would be dopped. Using the explanatory variables remaining after this process, several possible models were created based on preliminary visualization and biological reasoning (Hajduk & Gallois 2022, UCLA Statistical Consulting Group 2021). The models were compared using the *AICcmodavg* package (Barton 2023), and all models with a $\Delta AIC_c \leq 2$ were considered. The summaries of top models were produced using the *lme4* package, and effect size and direction were examined. The *R.squaredGLMM* function in the *MuMIn* package (Barton 2023) was used to produce standardized R^2 values for each model.

Each model with $\Delta AIC_c \leq 2$ was checked for multicollinearity and normality. I used the *VIF* function in the *MuMIn* package (Barton 2023) to assess variance inflation factors for each variable in each model, with $VIF \leq 3$ being considered acceptable. After calculating residuals, I used a Shapiro-Wilk test and qqnorm plot in base R to assess normality. Due to the nature of the data and small sample size, residuals were considered normal even when the Shapiro-wilk test found a significant difference if the plot passed visual inspection.

3. RESULTS

3.1 DIET ANALYSIS

I used Welch's t-tests to assess differences between diets due to unequal sample sizes. Aurora and Nikita were always offered identical or very similar diets, and Hudson and Humphrey's diets were pooled as the diets they were offered had minimal differences. Juno's diets were analyzed on their own. As expected, calories were significantly higher in the anabolic than catabolic phases for males (P = < 0.001, t = 11.41, df = 33.34), older females (P < 0.001, t = 11.13, df = 31.79) and Juno (P < 0.001, t = 6.45, df = 33.37). The difference in macronutrient composition between high-protein and high-fat diets was also found to be significant for males (P < 0.001, t = -21.08, df = 32.66), older females (P = < 0.001, t = -16.80, df = 14.83) and Juno (P = < 0.001, t = -5.85, df = 13.77) (Table 3.1).

Table 3.1: Results of Welch's T-tests comparing difference in KCal between the anabolic and catabolic phases and difference in metabolizable energy obtained from fat between the high-fat and high-protein diets for males, older females, and Juno.

Males						
	Difference	High mean (SD)	Low mean (SD)	P-Value	t-statistic	df
	Calories (ME Kcal)	143700(50302)	64262(16027)	< 0.001	11.41	33
	Dietary Fat (%)	33(4.82)	14(1.38)	< 0.001	-21.08	32
Females						
	Difference	High mean (SD)	Low mean (SD)	P-Value	t-statistic	df
	Calories (ME Kcal)	100014 (27319)	36282 (9243)	< 0.001	11.13	31
	Dietary Fat (%)	36(4.68)	14.2(1.77)	< 0.001	-16.80	15
Juno						
	Difference	High mean (SD)	Low mean (SD)	P-Value	t-statistic	df
	Calories (ME Kcal)	102495(44326)	40656(13926)	< 0.001	6.45	34
	Dietary Fat (%)	29(9.82)	13(2.30)	< 0.001	-5.85	14

3.2. BODY MASS AND COMPOSITION

3.2.1 Model Selection

Due to a high correlation between body mass and total body fat (0.72, correlation matrix), as well as body mass and proportional body fat (0.69, correlation matrix), each response variable was analyzed separately, with univariate models. Preliminary ANCOVAs were performed to assess interactions. A significant interaction (age x sex $p>0.05$) was found between age and sex in the body mass and body fat mass models. Thus, during the model selection process for body mass and body fat mass, no possible model contained both age and sex as random effects.

Table 3.2: Output of top models for body mass (kg) and proportional body fat (%) of 5 captive polar bears, as determined by AIC ranking. ΔAIC_c is the difference in Akaike's information criterion. See Tables 3.3-3.5 for p-values of fixed effects and estimates of fixed and random effects.

Body Mass	Rank	ΔAIC_c	Weight	R^2	log likelihood	Model
	1	0.00	1	80.90	-6155.66	*Calories* + *Fat_d* + *Bear ID* + *Age* x *Sex*
	2	40.62	0	70.02	-6175.97	*Calories* + *Fat_d* + *Bear ID* + *Age*
Body Fat Mass	Rank	ΔAIC^c	Weight	R^2	log likelihood	Model
	1	0.00	1	56.32	-5458.87	*Calories* + *Fat_d* + *Bear ID* + *Age x Sex*
	2	40.75	0	54.51	-5479.25	*Calories* + *Fat_d* + *Bear ID* + *Sex*
% Body Fat	Rank	ΔAIC^c	Weight	R^2	log likelihood	Model
	1	0.00	0.58	88.97	2860.88	*Calories* + *Fat_d* + *Bear ID* + *Age* + *Sex*
	2	0.62	0.42	88.34	2859.55	*Calories* + *Fat_d* + *Bear ID* + *Age*

3.2.2 BODY MASS

The best model for total body mass (kg) included caloric intake and dietary fat as fixed effects, and bear ID and the interaction term between age and sex as random effects, with a model R^2 of 80.90% (Table 3.2). The second-best model included bear ID and age as random effects alongside the aforementioned fixed effects, however this model will not be considered with a very large ΔAIC_c of 40.62 (Table 3.2). In the best model for body mass, calories and dietary fat each had a significant positive effect, with dietary fat having a slightly larger effect size (Table 3.3). The interaction between age and sex had a large, significant, positive effect, and other random effects cannot be interpreted due to this interaction (Table 3.3).

Table 3.3: Summary of best model for total body mass (kg) of 5 captive polar bears at The Toronto Zoo, as determined by AIC_c ranking (see Table 3.2). Significant p-values and effects are denotated by an asterisk (*). Random effects are deemed significant when the estimate is greater than the associated error.

Rank	Fixed Effects	Estimate	SE	df	t-value	Pr(>|t|)
1	(Intercept)*	393.220	28.43	5.240	13.83	<0.001*
	Calories*	5.33	1.20	1207.16	4.44	<0.001*
	Fat$_d$*	5.65	1.79	1190.44	3.12	0.002*
	Random Effects	Variance	SD	df		
	(Residual)*	1255	35.34	NA		
	Bear ID*	3603	60.03	4		
	Age:Sex*	1628	40.35	19		

All bears showed a positive relationship between total body mass and both caloric intake (Figure 3.1), and dietary fat (Figure 3.2), whereby mass increases as calories and dietary fat increased, respectively.

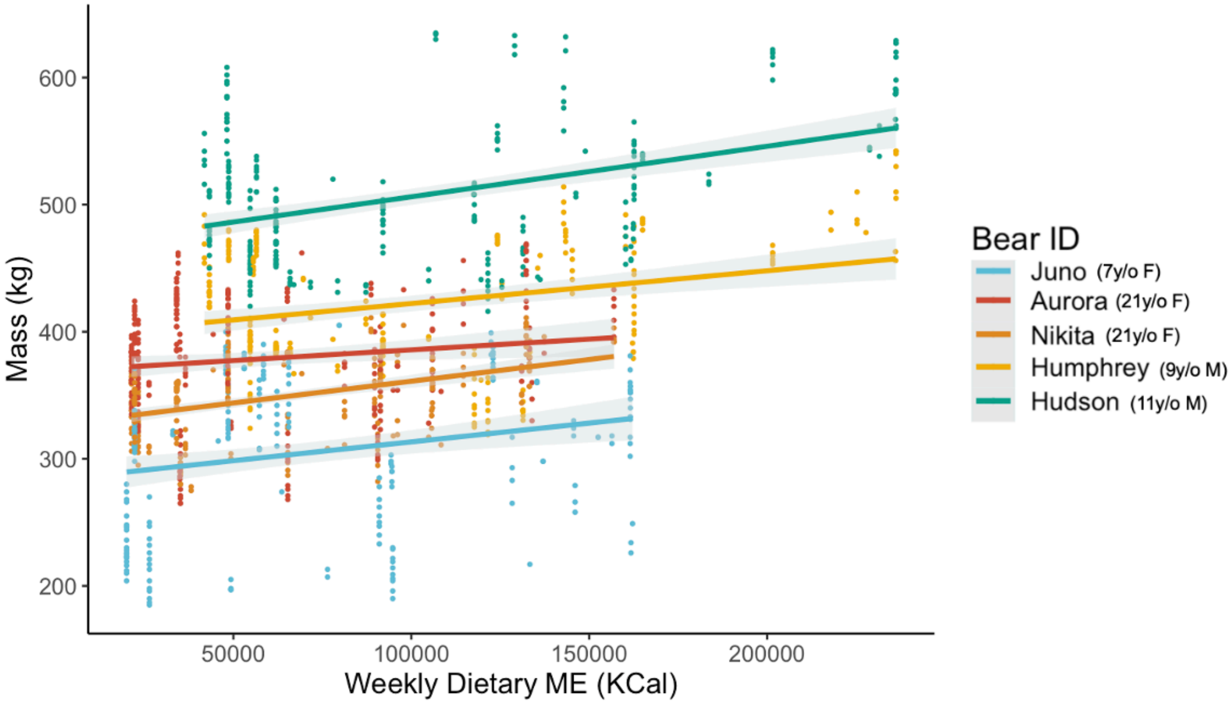

Figure 3.1: Total body mass (kg) of 5 captive polar bears at The Toronto Zoo with weekly metabolizable energy consumed (Kcal). Gray shading represents the 95% confidence intervals. Legend shows ages of bears at the end of the sample collection period. See Table 3.3 for effect size.

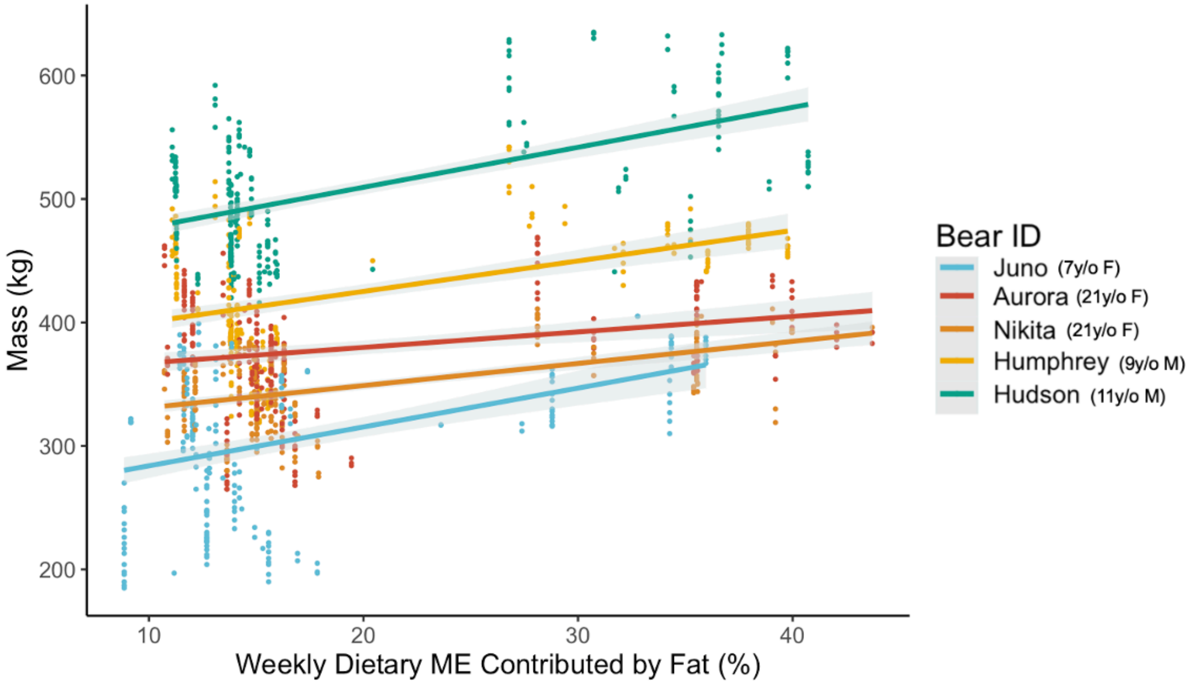

Figure 3.2: Total body mass (kg) of 5 captive polar bears at The Toronto Zoo with weekly metabolizable energy (Kcal) contributed by fat (%). Gray shading represents the 95% confidence intervals. See Table 2.3 for effect size.

A significant interaction was found between age and sex when body mass was the response variable (Table 2.3). When comparing total body mass across age and sex, males were found to have the greatest body mass, followed by adult-to-geriatric females (Aurora and Nikita), then juvenile-to-adult females (Juno) (Figure 2.3). All differences are significant, though the difference between the 2 female groups appears smaller than the difference between the males both female groups (Figure 2.3).

Figure 3.3: Total body mass (kg) of 2 male (left), 2 adult-to-geriatric female (center), and 1 juvenile-to-adult female (right) polar bears at the Toronto Zoo over 5.5 years. Boxes represent 1st (lower) and 3rd (upper) quartiles around the median line at box centers, and whiskers represent interquartile range. Points represent statistical outliers. A significant difference between groups is indicated by an asterisk (*).

3.2.3 Body Fat Mass

The best model for body fat mass included caloric intake and dietary fat as fixed effects, and bear ID and an interaction term between age and sex as random effects (Table 3.2). This model had an R^2 value of 56.32% (Table 3.2). The second-best model included bear ID and age as random affects alongside the aforementioned fixed effects, however this model had a $\Delta AIC_c > 2$ (40.75), therefore it will not be considered (Table 3.2). In the best model, both calories and dietary fat had significant positive effects, whereby dietary fat had a slightly larger effect size than calories (Table 3.4). The interaction between age and sex had a large, positive, significant effect (Table 3.4).

Table 3.4: Summary of best model for body fat mass (kg) of 5 captive polar bears at the Toronto Zoo, as determined by AIC$_c$ ranking (see Table 5.2). See Table 3.3 for interpretation.

Rank	Fixed Effects	Estimate	SE	df	t-value	Pr(>|t|)
1	(Intercept)*	393.22	28.43	5.24	13.83	< 0.001*
	Calories*	5.33	1.2	1207.16	4.44	<0.001*
	Fat$_{d}$*	5.65	1.79	1190.44	3.16	< 0.001*
	Random Effects	Variance	SD	df		
	(Residual)*	1255	35.43	NA		
	Bear ID*	2603	60.03	4		
	Sex *x* Age *	1628	40.35	19		

All bears were found to increase body fat mass with increasing caloric intake (Figure 3.4) and dietary fat composition (Figure 3.5), respectively, with the exception of Humphrey. In response to increasing caloric intake, Humphrey saw a slight decrease in body fat mass, while other bears saw a slight increase (Figure 3.4). In response to increasing dietary fat, Humphrey saw a large decrease in body fat mass, while the other bears saw a large increase in body fat mass (Figure 3.5).

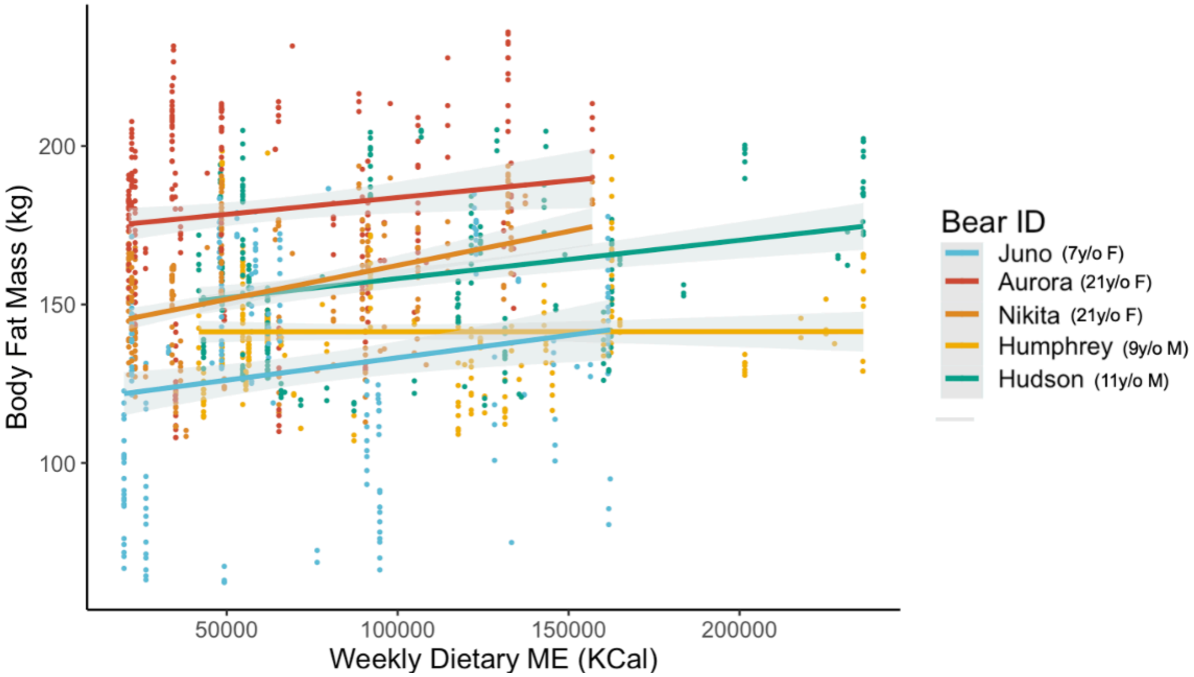

Figure 3.4: Body fat mass (kg) of 5 captive polar bears with weekly metabolizable energy consumed (Kcal). Gray shading represents the 95% confidence intervals. See Table 3.4 for effect size and p-value.

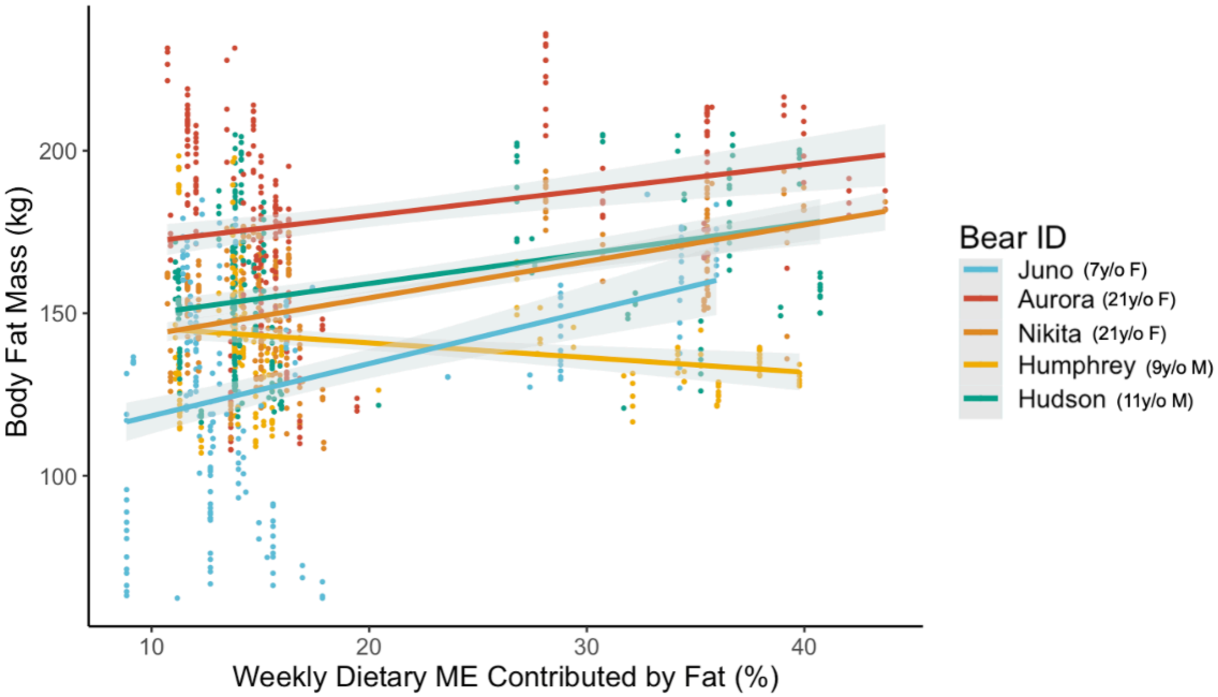

Figure 3.5: Body fat mass (kg) of 5 captive polar bears with weekly % of KCal of ME contributed by dietary fat. Gray shading represents the 95% confidence intervals. See Table 3.4 for effect size and p-value.

In terms of age and sex, adult-to-geriatric female bears (Aurora and Nikita) were found to have the greatest body fat mass, followed by sub-adult-to-adult males (Hudson and Humphrey), and finally juvenile-to-adult females (Juno) (Figure 3.6). The difference between males and both female groups is significant, as well as the difference between the 2 female groups (Figure 3.6). The difference between males and younger females was non-significant.

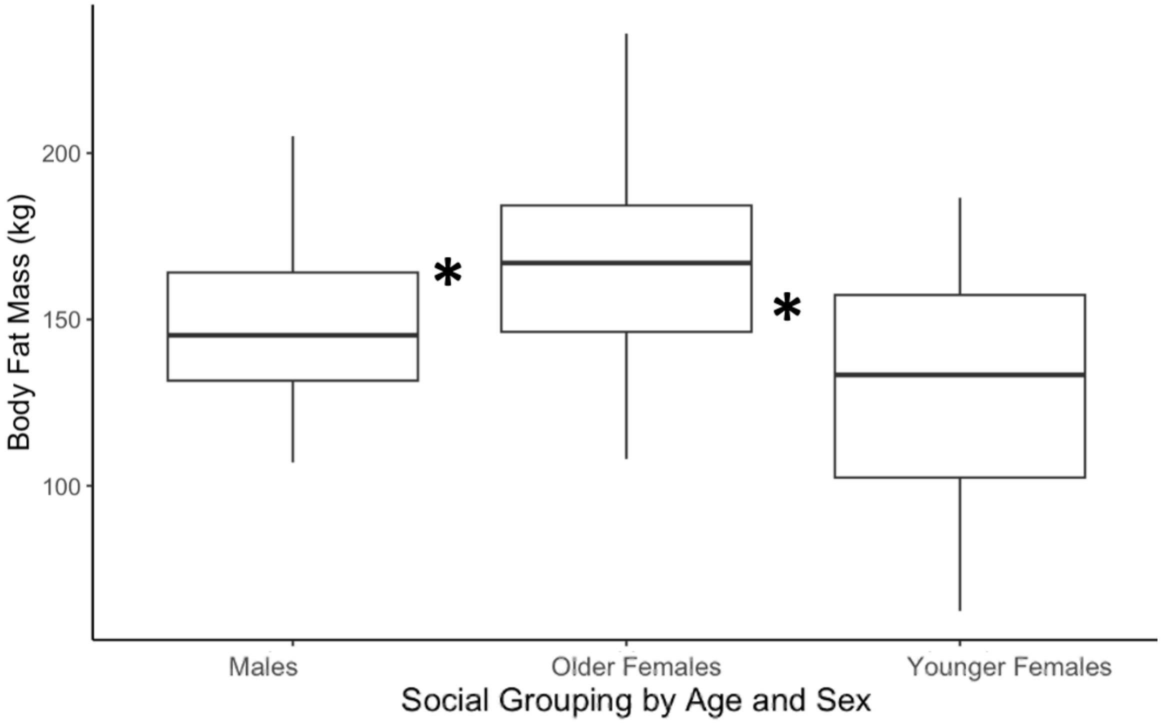

Figure 3.6: Body fat mass (kg) of sub-adult to adult males (left), adult to geriatric females (center), and sub-adult to adult female (right) polar bears at the Toronto Zoo. See Figure 3.4 for boxplot interpretation.

3.2.4 Proportional Body Fat

The best model for proportional body fat included caloric intake and dietary fat as fixed effects, and bear ID, age, and sex as random effects. The second-best model, which should be considered with a $\Delta AIC_c<2$, included only age as a predictor alongside bear ID, calories, and dietary fat (Table 3.2). In the best model, both calories and dietary fat had positive, significant, though small effects on proportional body fat, where dietary fat had a larger effect size than calories (Table 3.5). Age and sex each had equally small, positive, non-significant effects, and bear ID had the smallest, positive, non-significant effect (Table 3.5). This model had an R^2 value of 88.97% (Table 3.2). In the second-best model, calories and fat once again had small, positive, significant effects, with fat having a larger effect than calories. Bear ID and age both had small, positive, non-significant effects, where bear ID had a larger effect than age (Table 3.5). This model had an R^2 value of 88.34% (Table 3.2).

Table 3.5: Summary of best model for proportional body fat (%) of 5 captive polar bears, as determined by AIC$_c$ ranking (see Table 3.2). See Table 3.3 for interpretation.

Rank	Fixed Effects	Estimate	SE	df	t-value	Pr(>|t|)
1	(Intercept) *	0.39	0.032	2.16	12.04	0.005*
	Calories*	0.002	0.0007	1.21	2.95	0.003*
	Fat$_d$*	0.003	0.001	1.22	3.31	< 0.001*
	Random Effects	Variance	SD	df		
	(Residual)	0.0005	0.02	NA		
	Bear ID	0.0004	0.02	4		
	Age	0.001	0.04	15		
	Sex	0.001	0.04	1		
Rank	Fixed Effects	Estimate	SE	df	t-value	Pr(>|t|)
2	(Intercept)*	0.39	0.02	5.99	16.23	< 0.001*
	Calories*	0.002	0.0007	0.01	2.96	0.003*
	Fat$_{d*}$	0.003	0.001	0.01	3.32	< 0.001*
	Random Effects	Variance	SD	df		
	(Residual)	0.0005	0.02	NA		
	Bear ID	0.002	0.05	4		
	Age	0.001	0.04	15		

The relationship between percent body fat and caloric intake was found to vary based on sex, despite not finding a significant interaction between these variables. For female bears, proportional body fat increased slightly with increasing weekly caloric intake (Figure 3.7). The opposite trend was observed in male bears, whereby proportional body fat decreased with increasing calories (Figure 3.7).

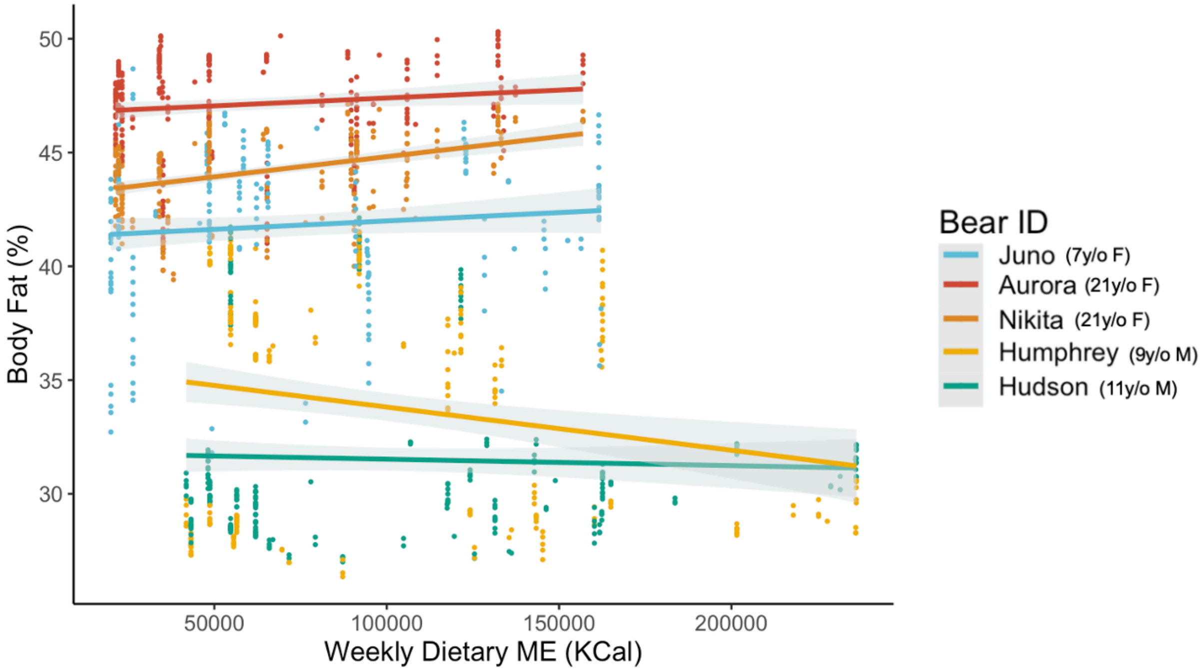

Figure 3.7: Proportional body fat (%) of 5 captive polar bears at The Toronto Zoo with weekly metabolizable energy offered (Kcal). Gray shading represents the 95% confidence intervals. See Table 3.5 for effect size and p-value.

Additionally, it was found that the relationship between proportional body fat and dietary fat was influenced by sex, despite not finding a significant interaction between these variables. For female bears, proportional body fat increased slightly with increasing dietary fat (Figure 3.8). The opposite was observed in male bears, where proportional body fat decreased with increasing dietary fat. Humphrey displayed a more severe reduction in body fat than Hudson (Figure 3.8).

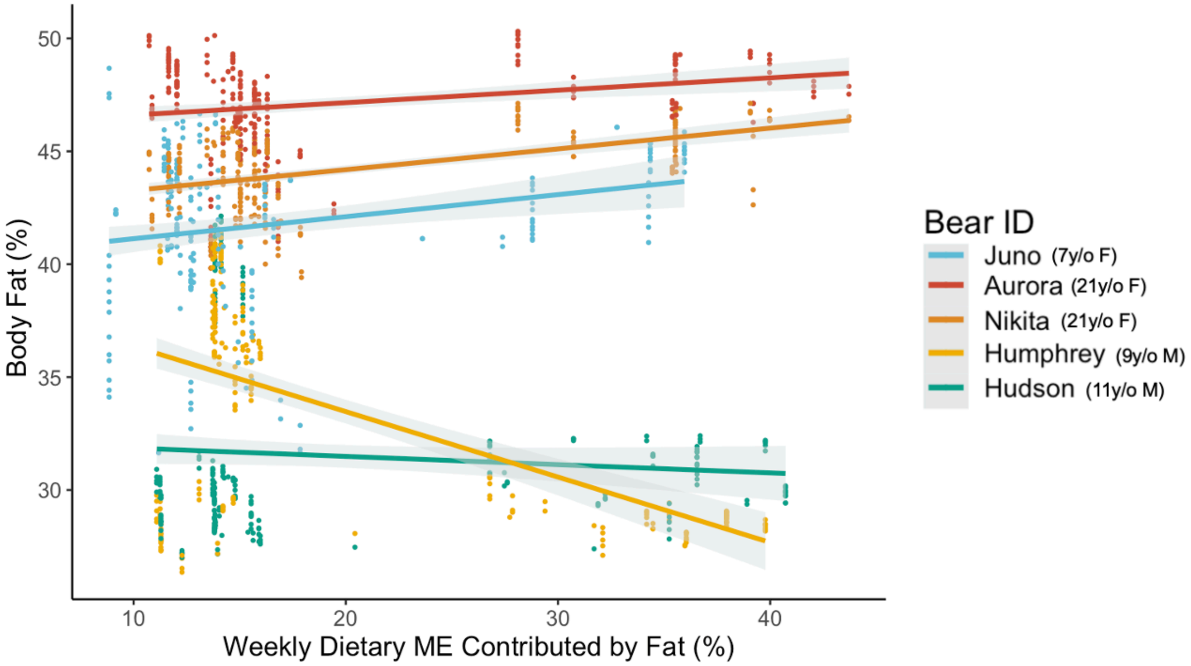

Figure 3.8: Proportional body fat (%) of 5 captive polar bears The Toronto Zoo with weekly metabolizable energy (Kcal) contributed by fat (%). Gray shading represents the 95% confidence intervals. See Table 3.5 for effect size and p-value.

Once again different trends were observed between sexes when examining the relationship between proportional body fat and age. Female bears saw an increase in proportional body fat as they aged, while male bears saw a decrease in proportional body fat with age (Figure 3.9). When comparing sexes, female bears overall had greater proportional body fat (Figure 3.10). Males had more variability in proportional body fat, though only females had outliers (Figure 3.10). The difference in proportional body fat between sexes was found to be significant, as the median line of either box falls outside of the upper and lower quartiles of the other box (Figure 3.10).

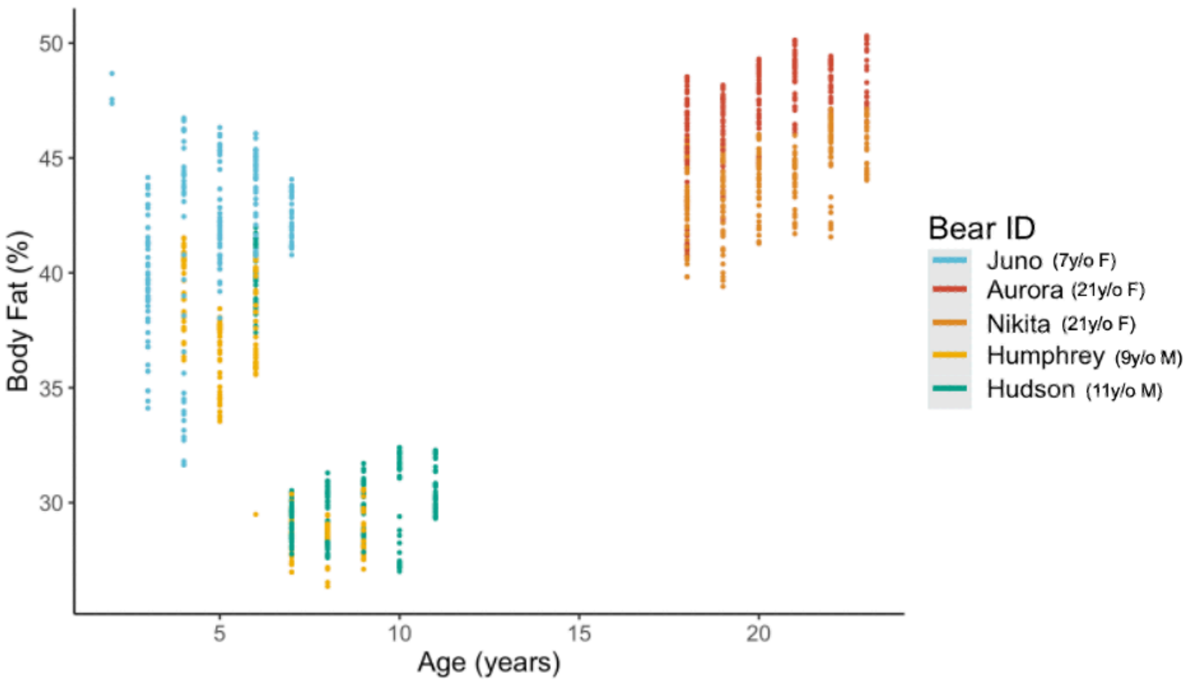

Figure 3.9: Proportional body fat (%) of 5 captive polar bears at the Toronto Zoo with age at date of sample. The variable was included in the best model, but with a non-significant effect. See Table 3.5 for effect size.

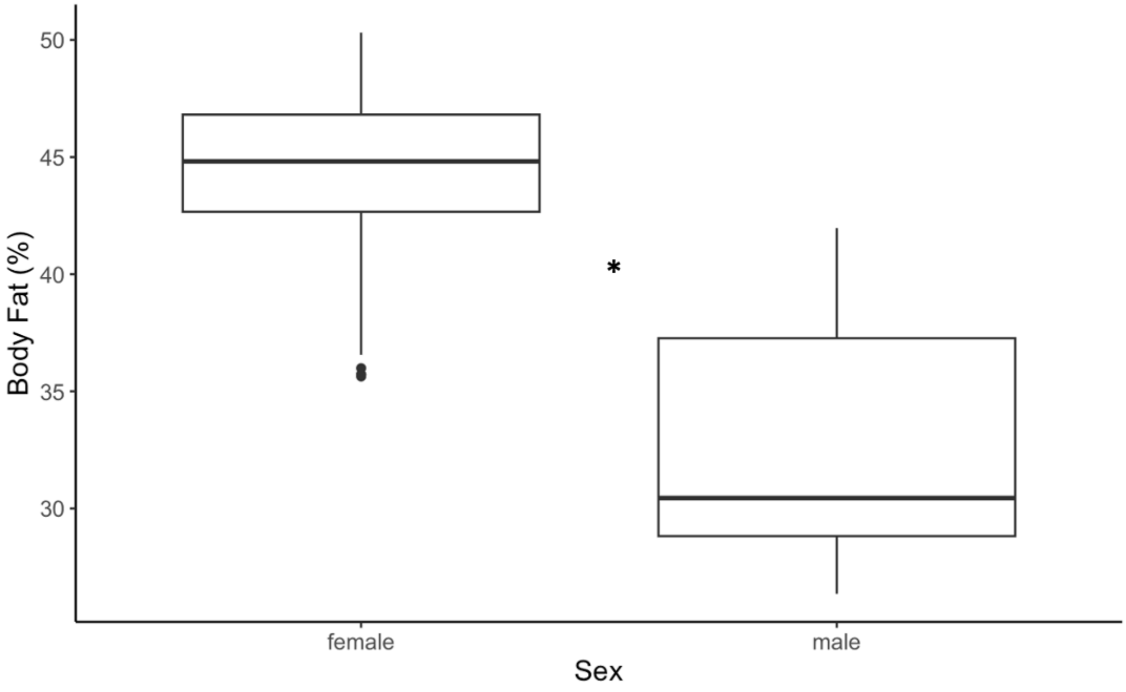

Figure 3.10: Proportional body fat (%) of female (left) and male (right) polar bears at the Toronto Zoo. See figure 3.3 for interpretation.

3.3 BLOOD CHEMISTRY

3.3.1 Model Selection

On occasions where insufficient blood was collected from a bear to test both serum triglyceride concentration and UC ratio, triglycerides were prioritized. Therefore, triglycerides and UC ratio were analyzed separately due to unequal sample size. A multivariate model would require some triglyceride samples to be dropped, which was undesirable due to the small overall sample size. To check interaction terms, preliminary ANCOVAs were run on triglyceride data and UC ratio data separately, finding no significant interactions ($p>0.05$).

Table 3.6: Top model outputs for serum triglyceride concentration and Urea: Creatinine ratio in 4 captive polar bears at The Toronto Zoo, as determined by AIC ranking. ΔAIC_c is the difference in Akaike's information criterion. See Tables 3.7-3.8 for p-values of fixed effects and estimates of fixed and random effects.

Trig

Rank	ΔAIC_c	Weight	R^2	Log likelihood	Model
1	0.00	0.75	94.9	-47.34	*Calories* + Fat_d + Bear ID + *Age* + Fat_b
2	2.36	0.23	94.8	-47.83	*Calories* + Fat_d + Bear ID + *Age* + Fat_b + *sex*

U:C

Rank	ΔAIC_c	Weight	R^2	Log likelihood	Model
1	0	0.33	54.31	-336.67	*Calories* + Fat_d + Bear ID
2	2.24	0.16	54.30	-336.77	*Calories* + Fat_d + Bear ID + Fat_b
2	2.24	0.16	54.30	-336.77	*Calories* + Fat_d + Bear ID + *Age*

3.3.2 Triglycerides

The best model to for serum triglyceride concentration included dietary fat and caloric intake as fixed effects, and bear ID, proportional body fat, and age as random effects (Table 3.6). The second-place model had a $\Delta AIC_c > 2$ (Table 3.6), therefore only the top model was considered. Calories had a significant negative effect, while dietary fat had a smaller, significant, positive effect (Table 3.7). Both age and proportional body fat had significant positive effects, as the effect size of both is larger than the associated error (Table 3.7). Proportional body fat accounted for more variance in the data than age (Table 3.7). There was no significant effect of bear ID, however it must be included in the model as a random effect to account for repeated sampling of the same individuals. This model had an R^2 value of 94.90% (Table 3.6).

Table 3.7: Summary of best model as determined by AIC_c ranking (see Table 3.6), showing effects of all predictors on serum triglyceride concentration of 4 captive polar bears at The Toronto Zoo. See Table 3.3 for interpretation.

Rank	Fixed Effects	Estimate	SE	df	t-value	Pr(>|t|)
1	(Intercept)*	201.522	7.91	6.84	25.21	<0.001*
	Calories*	-13.65	2.70	96.70	-5.10	<0.001*
	Fat_d*	7.90	5.17	28.22	1.53	0.013*
	Random Effects	Variance	SD	df		
	(Residual)*	65.24	8.07	NA		
	Bear ID	0.00	0.00	3		
	Age*	422.46	20.55	7		
	Fat_b*	570.69	23.90	96		

Serum triglyceride concentrations were found to decrease with increasing caloric intake (ME Kcal) for all bears (Figure 3.11). Additionally, serum triglyceride concentration increased with increasing dietary fat for all bears, though Humphrey saw a lesser increase than the other bears (Figure 3.12).

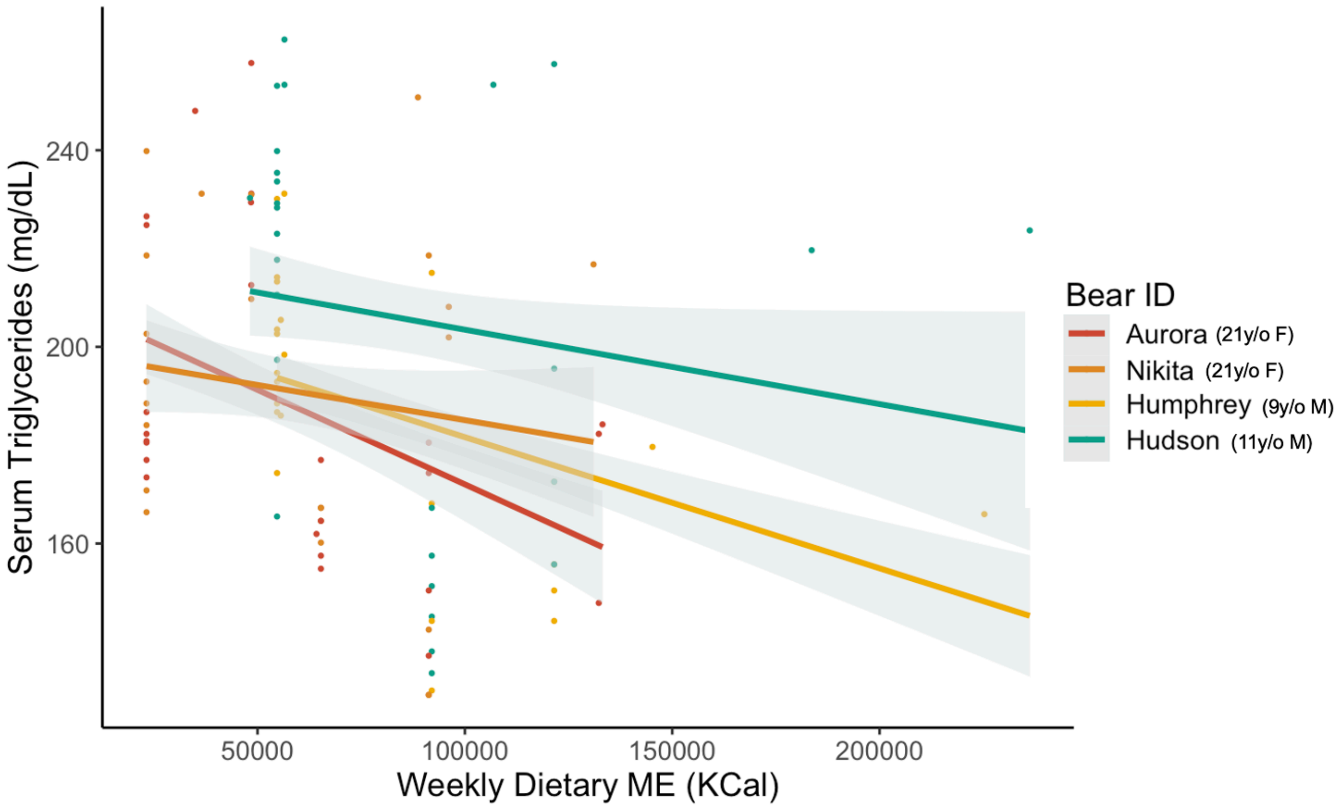

Figure 3.11: Serum triglyceride concentration (mg/dL) of 4 captive polar bears at The Toronto Zoo with metabolizable energy (ME) offered per week (Kcal). Gray shading represents the 95% confidence intervals. See Table 3.7 for effect size and p-value.

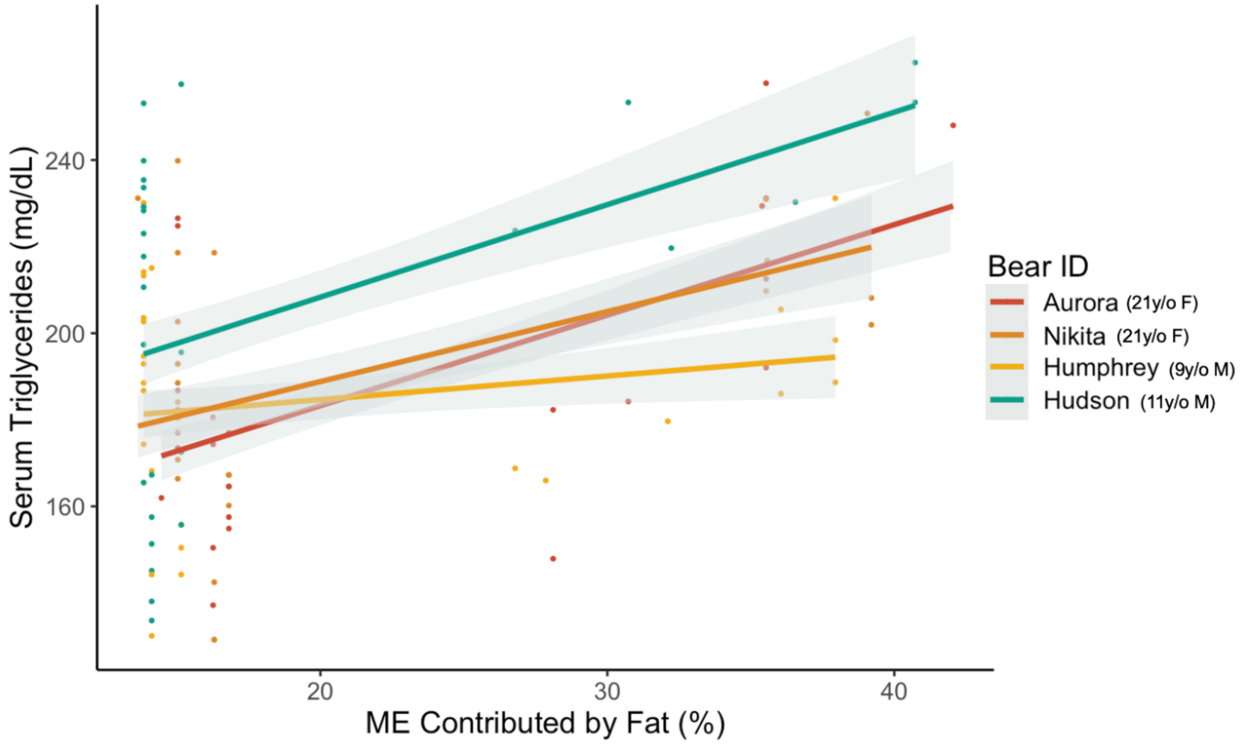

Figure 3.12: Serum triglyceride concentration (mg/dL) of 4 captive polar bears at The Toronto Zoo with metabolizable energy (ME) contributed from fat (%). Gray shading represents the 95% confidence intervals. See Table 3.7 for effect size and p-value.

The relationship between proportional body fat and serum triglyceride concentration was found to differ between sexes, despite no significant interaction between these variables. For male bears, serum triglyceride concentration decreased with increasing proportional body fat. For females, serum triglyceride concentration increased with increasing proportional body fat (Figure 3.13).

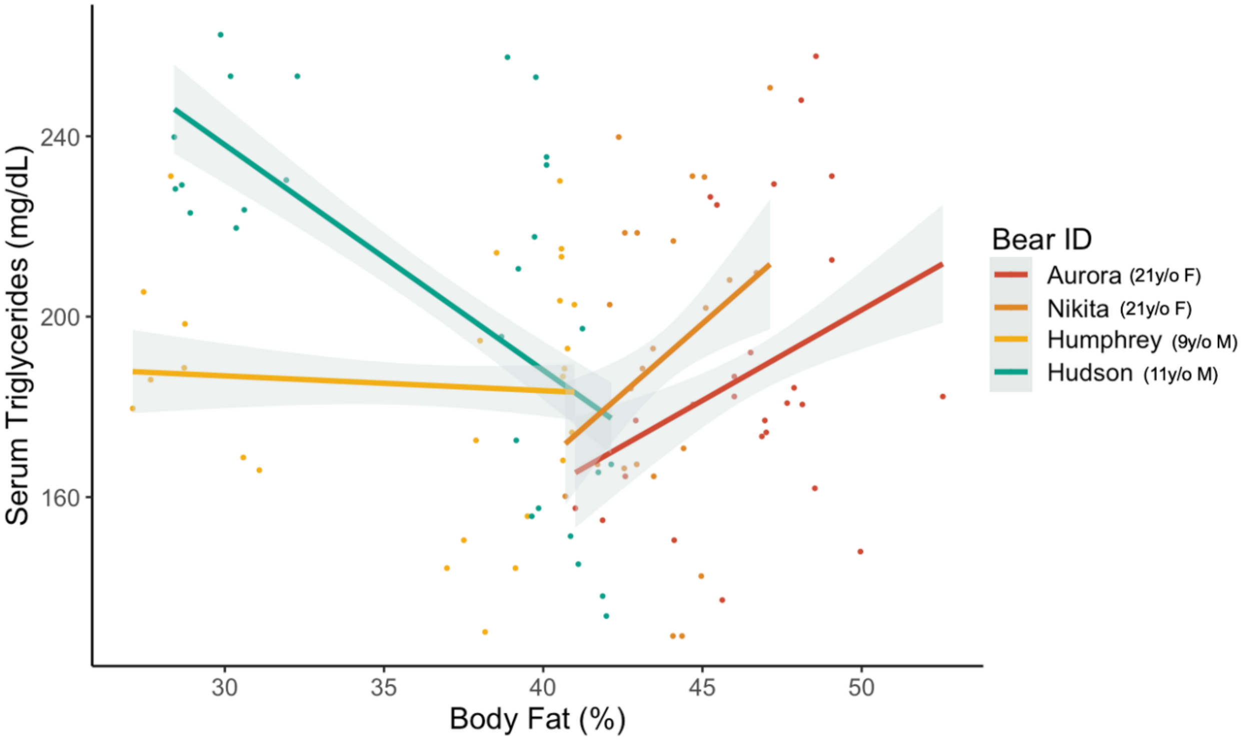

Figure 3.13: Serum triglyceride concentration (mg/dL) of 4 captive polar bears at The Toronto Zoo with proportional body fat (%). Gray shading represents the 95% confidence intervals. See Table 3.7 for effect size.

For all bears, serum triglyceride concentrations were found to increase with age, though Humphrey saw a lesser increase compared to the other bears (Figure 3.14).

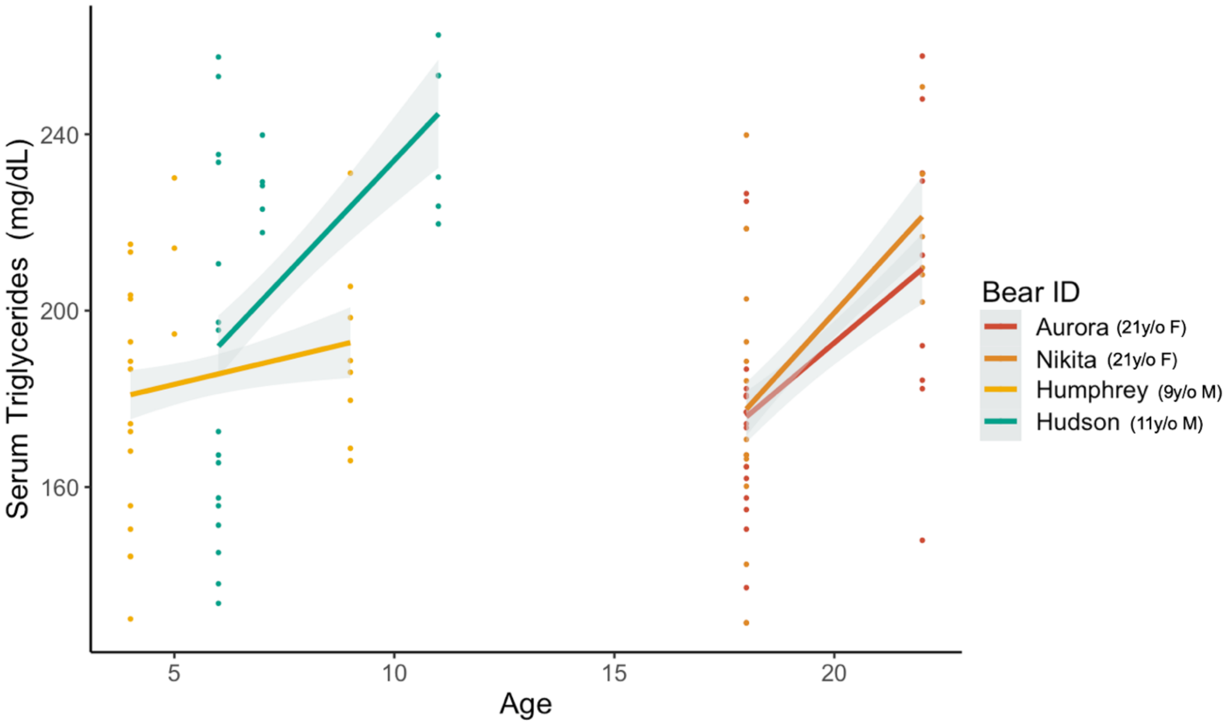

Figure 3.14: Serum triglyceride concentration (mg/dL) of 4 captive polar bears at The Toronto Zoo with age of bear at time of sample. Gray shading represents the 95% confidence intervals. See Table 3.7 for effect size.

3.3.3 Urea:Creatinine Ratio

The best model for serum UC ratio included only dietary fat, caloric intake, and bear ID (Table 3.6). The second-best models (tie) included either proportional body fat or age alongside the aforementioned predictors, however these models were not considered as $\Delta AIC_c > 2$. Calories had a significant, positive effect on UC ratio, and dietary fat a significant negative effect, where calories had a larger effect size than dietary fat (Table 3.8). Bear ID also had a significant positive effect (Table 3.8). This model had an R^2 value of 54.31% (Table 3.6).

Table 3.8: Summary of best model for serum UC ratio of 4 captive polar bears at The Toronto Zoo, as determined by AIC$_c$ ranking. See Table 3.3 for interpretation.

Rank	Fixed Effects	Estimate	SE	df	t-value	Pr(>|t|)
1	(Intercept) *	17.18	1.11	4.03	15.45	< 0.001*
	Calories*	5.23	0.53	108.77	9.84	< 0.001*
	Fat$_d$*	-1.85	0.51	106	-3.62	< 0.001*
	Random Effects	Variance	SD	df		
	(Residual) *	26.58	5.155	NA		
	Bear ID*	3.96	1.99	3		

Serum UC ratios were found to increase with increasing caloric intake for all bears (Figure 3.15). Serum UC ratios decreased with increasing dietary fat for all bears excluding Nikita, who saw a slight increase (Figure 3.16).

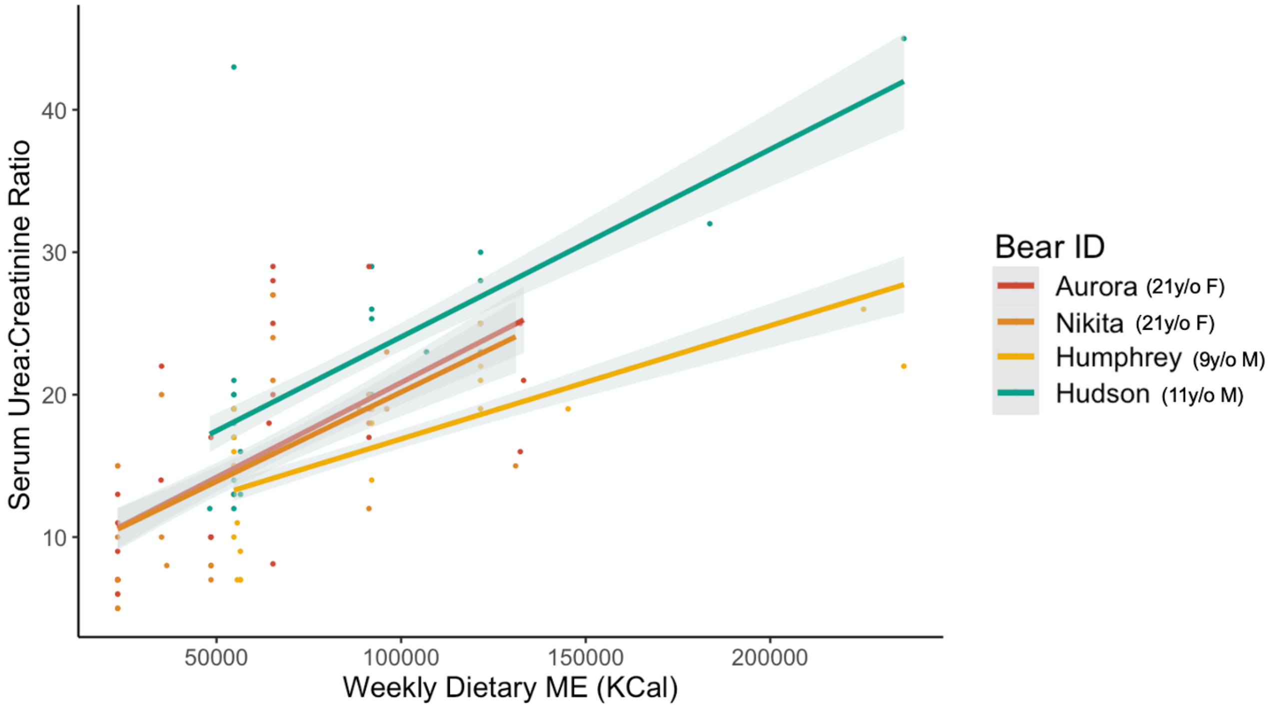

Figure 3.15: Serum UC ratios of 4 captive polar bears at The Toronto Zoo with metabolizable energy (ME) consumed per week (Kcal). Gray shading represents the 95% confidence intervals. See Table 3.8 for effect size and p-value.

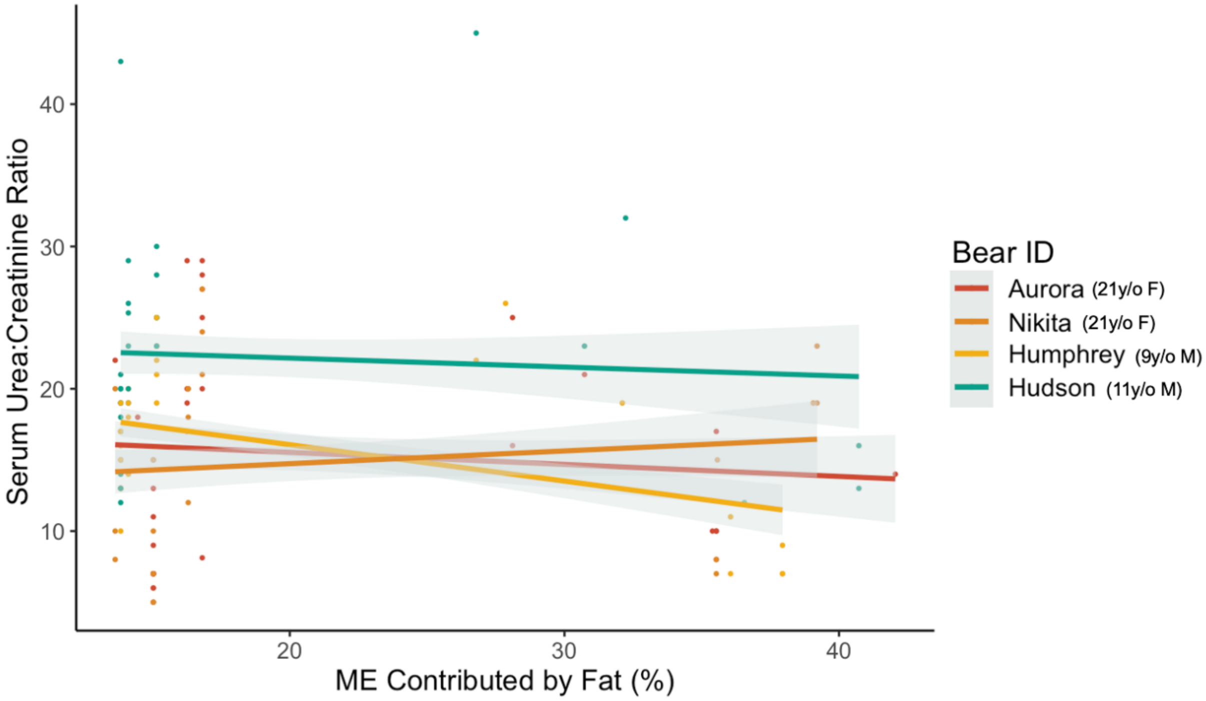

Figure 3.16: Serum UC ratio of 4 captive polar bears at The Toronto Zoo with metabolizable energy (ME) contributed from fat (%). Gray shading represents the 95% confidence intervals. See Table 3.8 for effect size and p-value.

3.4 BODY TEMPERATURE

3.4.1 Model Selection

Preliminary ANCOVAs were performed on body temperature data to check all possible interaction terms and found no significant interactions. The best model, with an R^2 of 44.41%, included calories and dietary fat as fixed effects, and bear ID and proportional body fat as random effects (Table 3.9). The second-best model is also considered as $\Delta AIC_c<2$. With an R^2 of 47.12%, the second-best model included air temperature as a random effect, alongside all aforementioned predictors (Table 3.9). Humidity, age, sex, and which exhibit bears were in were included as random effects in the model selection process but were not found to be included in any viable models.

Table 3.9: Output of top models for average daily body temperature (C°) of 5 captive polar bears, as determined by AIC ranking. See Table 3.2 for Table interpretation. Fixed effects of calories and dietary fat, as well as bear ID as a random effect, are included in all models. See Table 3.9 for p-values of fixed effects and estimates of fixed and random effects.

Rank	ΔAIC_c	Weight	R^2	LL	Model
1	0.00	0.51	44.41	-92.53	*Calories + Fat_d + Bear ID + Fat_b*
2	1.42	0.25	47.12	-92.17	*Calories + Fat_d + Bear ID + Airtemp*

3.4.2 Average Daily Body Temperature

In the best model, calories had a small, positive, significant effect on body temperature (Table 3.10). Dietary fat had a smaller, negative effect, which was non-significant despite being included in the best model. Both bear ID and proportional body fat had small, positive, non-significant effects, with body fat having a larger effect than bear ID (Table 3.10). In the second-best model, calories once again had a small, positive, significant effect, and dietary fat had a smaller, negative, non-significant effect. All random effects had small, positive, non-significant effects in this model, with body fat having the largest effect size, followed by air temperature, and finally bear ID (Table 3.10).

Table 3.10: Summary of best model for average daily body temperature (C°) of 5 captive polar bears at The Toronto Zoo, as determined by AIC$_c$ ranking (see Table 3.9). See Table 3.3 for interpretation.

Rank	Fixed Effects	Estimate	SE	df	t-value	Pr(>|t|)
1	(Intercept)*	36.06	0.04	4.97	919.58	< 0.001*
	Calories*	0.12	0.03	122.72	4.20	< 0.001*
	Fat$_d$	-0.03	0.02	142.41	-1.10	0.28
	Random Effects	Variance	SD	df		
	(Residual)	0.08	0.29	NA		
	Bear ID	0.004	0.056			
	Fat$_b$	0.05	0.22			
Rank	Fixed Effects	Estimate	SE	df	t-value	Pr(>|t|)
2	(Intercept)*	36.06	0.04	5.42	867.76	< 0.001*
	Calories*	0.13	0.03	120.65	4.21	< 0.001*
	Fat$_d$*	-0.03	0.02	140.64	-1.11	0.26
	Random Effects	Variance	SD	df		
	(Residual)	0.08	0.29	NA		
	Bear ID	0.003	0.05	4		
	Airtemp	0.004	0.06	40		
	Fat$_b$	0.05	0.23	133		

Average daily body ranged from 35.10 C° to 37.00 C° (mean 36 ± 0.4). Average daily body temperature was found to decrease with decreasing calories for all bears excluding Nikita, who displayed no observable relationship between body temperature and calories (Figure 3.17). No consistent trend was seen across bears for the relationship between average daily body temperature and dietary fat. Hudson, Juno, and Aurora's body temperatures increased with increasing dietary fat while Humphrey and Nikita's body temperatures decreased with increasing dietary fat (Figure 3.18), with the overall statistical trend being negative, and the effect size very small (Table 3.10)

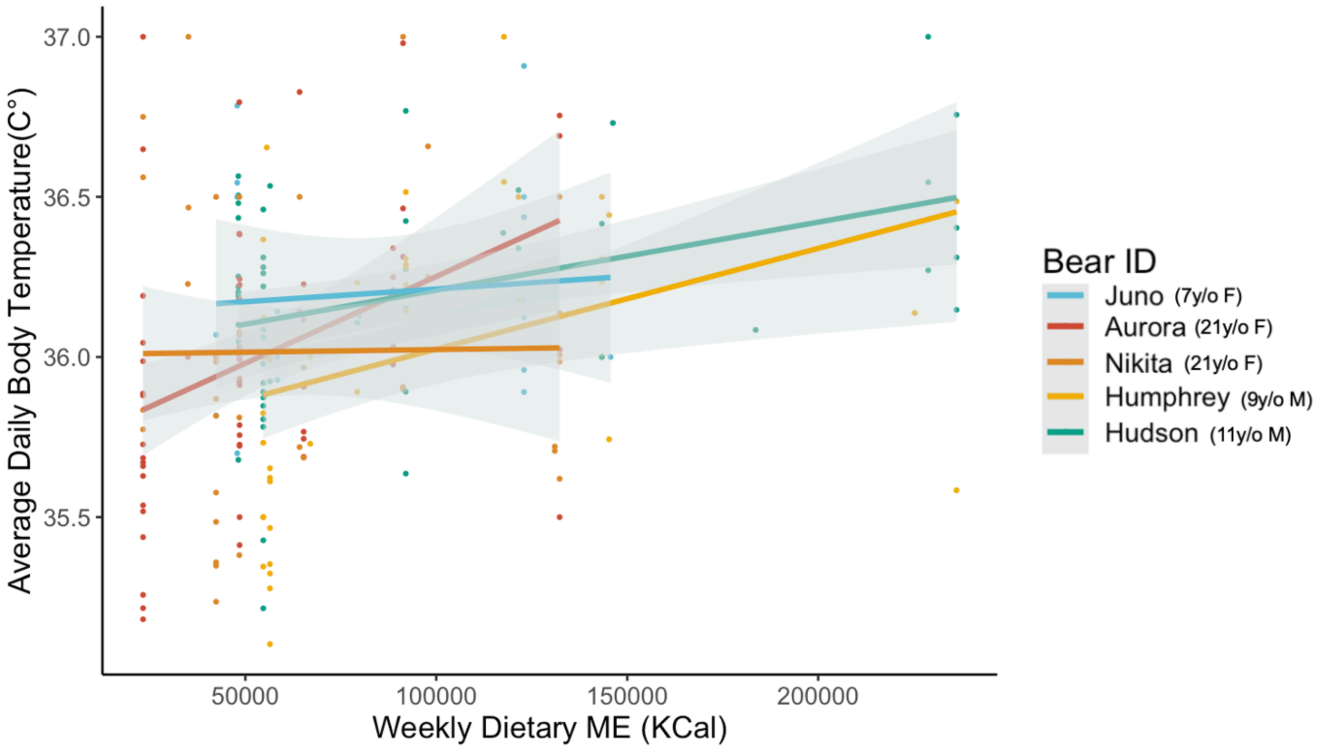

Figure 3.17: Average daily body temperature (°C) of 5 captive polar bears at The Toronto Zoo with weekly metabolizable energy (ME) offered (KCal). Gray shading represents the 95% confidence intervals. See Table 3.10 for effect size and p-value.

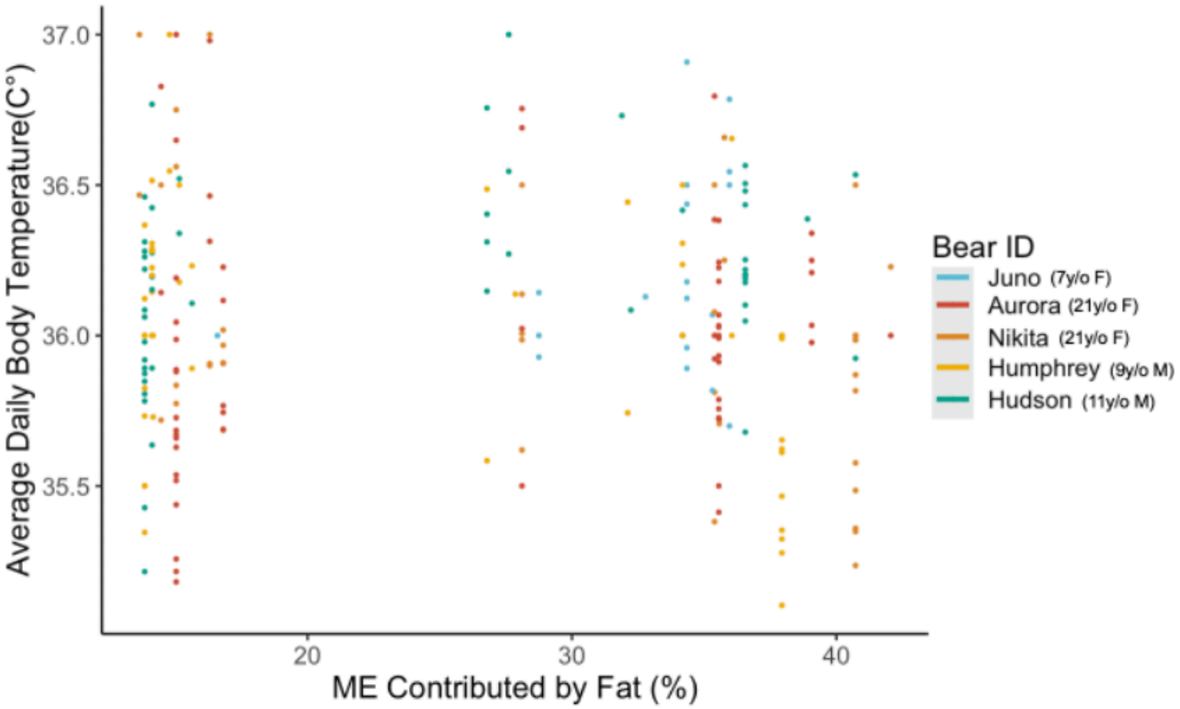

Figure 3.18: Average daily body temperature (°C) of 5 captive polar bears at The Toronto Zoo with weekly metabolizable energy (Kcal) contributed by dietary fat. The variable had a non-significant effect despite being included in the best model. See Table 3.10 for effect size and p-value.

All bears excluding Humphrey displayed a slight increase in body temperature with increasing air temperature, though the effect was non-significant (Figure 3.19). No consistent trend was found across bears for the relationship between proportional body fat and average daily body temperature. Humphrey, Nikita, and Juno all showed a decrease in body temperature with increasing proportional body fat while Aurora and Hudson's body temperatures increased with increasing proportional body fat, though the effect was non-significant overall (Figure 3.20).

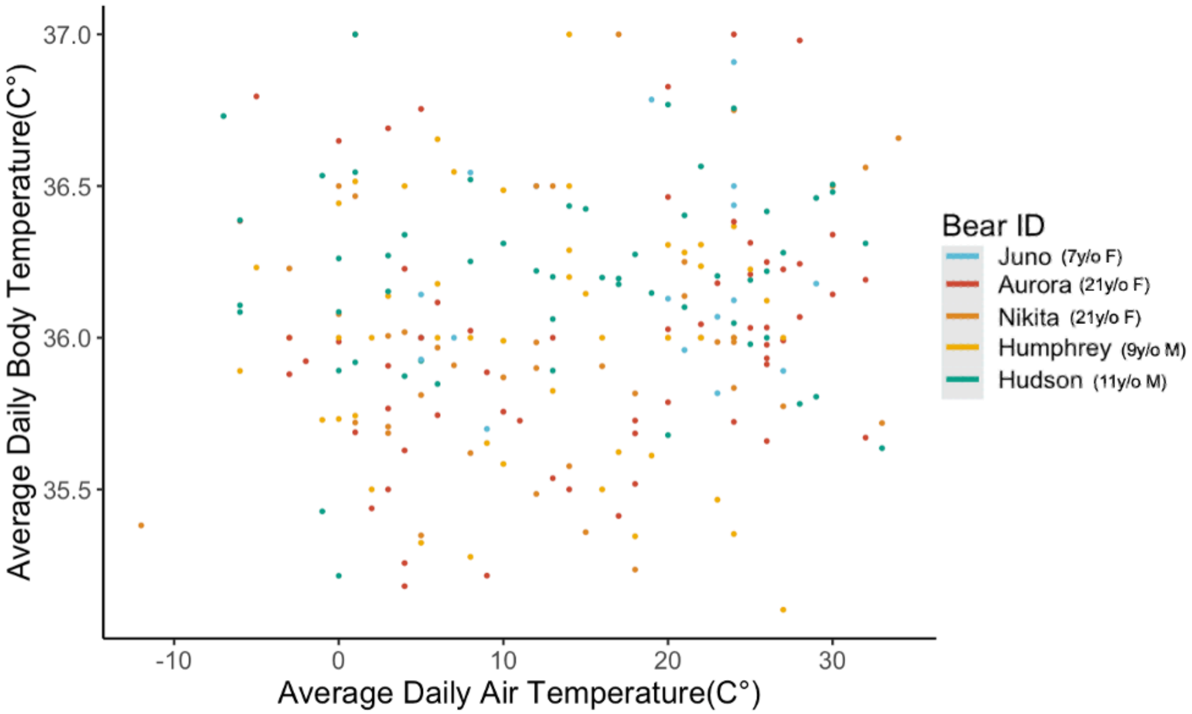

Figure 3.19: Average daily body temperature (°C) of 5 captive polar bears at The Toronto Zoo with average daily air temperature (°C). The variable was included in one of the best models, but with a non-significant effect. See Table 3.10 for effect size.

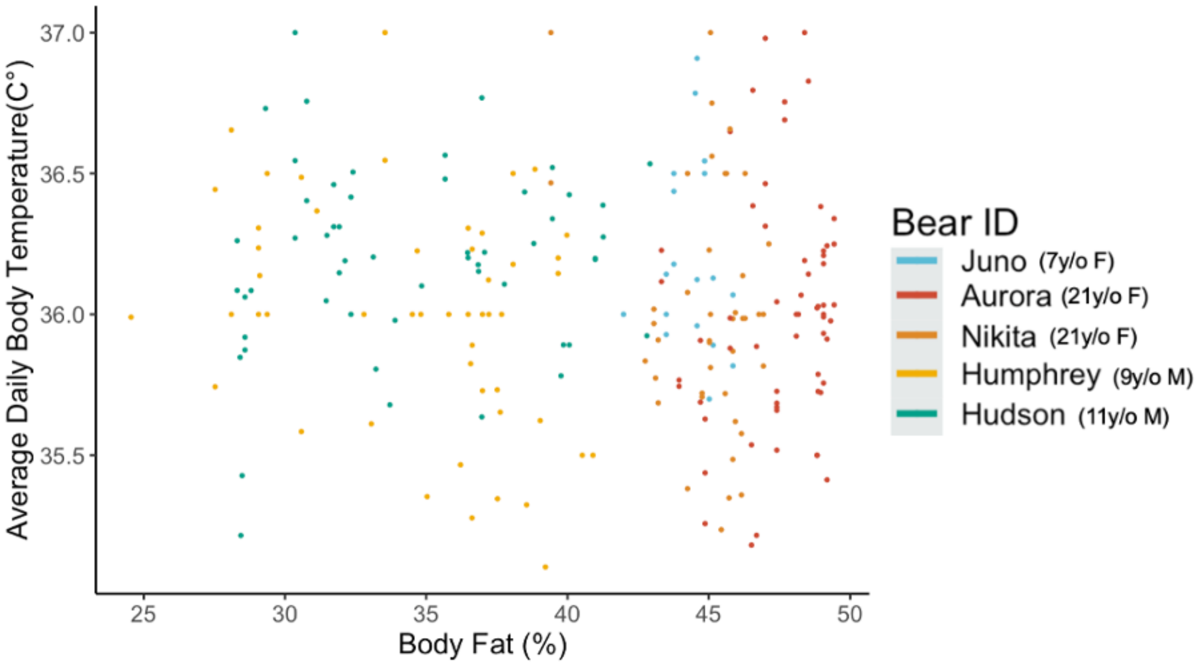

Figure 3.20: Average daily body temperature (°C) of 5 captive polar bears at The Toronto Zoo with metabolizable energy (ME) contributed from fat (%).The variable was included in one of the best models, but with a non-significant effect. See Table 3.10 for effect size.

3.5 BEHAVIOR

3.5.1 Model Selection

Preliminary ANCOVAs were performed to test for interactions between predictor variables and found no significant interactions. The best model for inactivity included caloric intake and dietary fat as fixed effects, bear ID as a random effect to account for repeated sampling of the same individuals, and body fat, time of day (categorical, morning or afternoon), and air temperature as random effects (Table 3.11). This model had an R^2 value of 29.77% (Table 3.11). Humidity, age, which exhibit bears were in, and average number of visitors were considered in the model selection process but were not found to be included in the best model. Windchill was recorded, but not considered in the analysis due to poor distribution. Humphrey was removed from the analysis due to a very high occurrence of stereotypic behavior during the breeding season, which prevented analysis of his activity budgeting in response to diet. Therefore, sex was not considered as a possible predictor in this model as only 1 male was analyzed.

Table 3.11: Top models for inactivity (proportion) of 4 captive polar bears at The Toronto Zoo, as determined by AIC_c ranking. See Table 3.12 for p-values of fixed effects and estimates of fixed and random effects.

Rank	ΔAIC_c	Weight	R^2	LL	Model
1	0.00	0.99	29.77	-172.34	*Calories* + Fat_d + *Bear ID* + Fat_b + *Time* + *Airtemp*
2	9.69	0.01	18.45	-179.25	*Calories* + Fat_d + *Bear ID* + Fat_b + *Time*

3.5.2 Inactivity

In the best model, caloric intake had a small but significant negative effect on inactivity, while dietary fat had a smaller, positive effect. Although dietary fat was included in the best model, the effect was non-significant (Table 3.12). All random effects were found to be non-significant despite being included in the model. Body fat and time of day had small, positive effects with equal magnitude, followed by air temperature which had a smaller positive effect (Table 3.12). Bear ID was found to have no effect (Table 3.12).

Table 3.12: Summary of best model for inactivity of 4 captive polar bears at The Toronto Zoo, as determined by AIC_c ranking (see Table 3.20). See Table 3.3 for interpretation.

Rank	Fixed Effects	Estimate	SE	df	t-value	Pr(>|t|)
1	(Intercept)*	0.57	0.16	29.66	3.67	< 0.001*
	Calories*	-0.06	0.01	214.10	-3.56	< 0.001*
	Fat_d	0.04	0.40	134.20	0.092	0.93
	Random Effects	Variance	SD	df		
	(Residual)	0.09	0.31	NA		
	Bear ID	0.00	0.00	3		
	Fat_b	0.01	0.11	220		
	Time	0.01	0.10	1		
	Airtemp	0.002	0.04	38		

All bears were found to have a negative relationship between inactivity and caloric intake, whereby bears became more active with increasing caloric intake (Figure 3.21). Hudson appears to display a more rapid decrease in inactivity than the other bears (Figure 3.21). The non-significant relationship between inactivity and dietary fat was not consistent across bears (Figure 3.22). I found that Juno, Hudson, and Nikita's inactivity increased slightly with increasing dietary fat, while Humphrey (excluded from model) and Aurora's inactivity decreased slightly, with the effect being small, positive, and non-significant overall (Figure 3.22, Table 3.12).

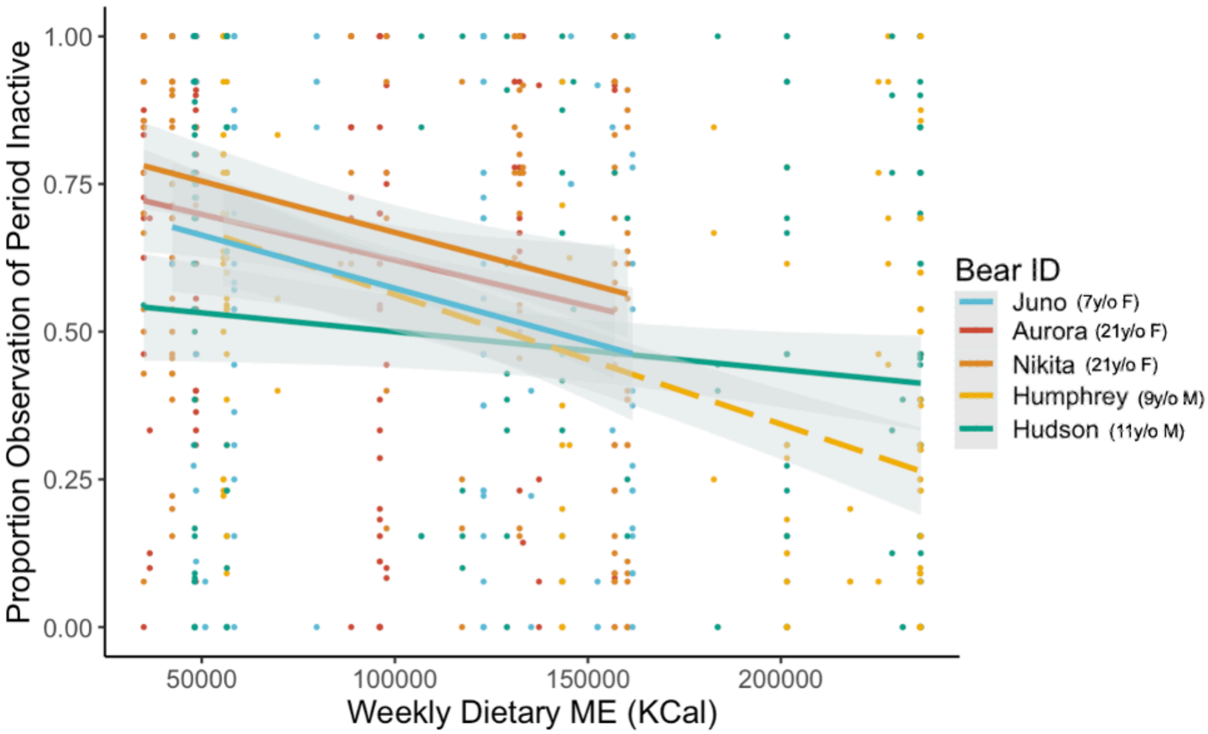

Figure 3.21: Proportion of 1-hour long observation periods spent inactive for 5 captive polar bears at the Toronto Zoo with metabolizable energy (ME) consumed per week (KCal). Gray shading represents the 95% confidence intervals. Dashed lines indicates that the individual was excluded from the model. See Table 3.12 for effect size and p-value.

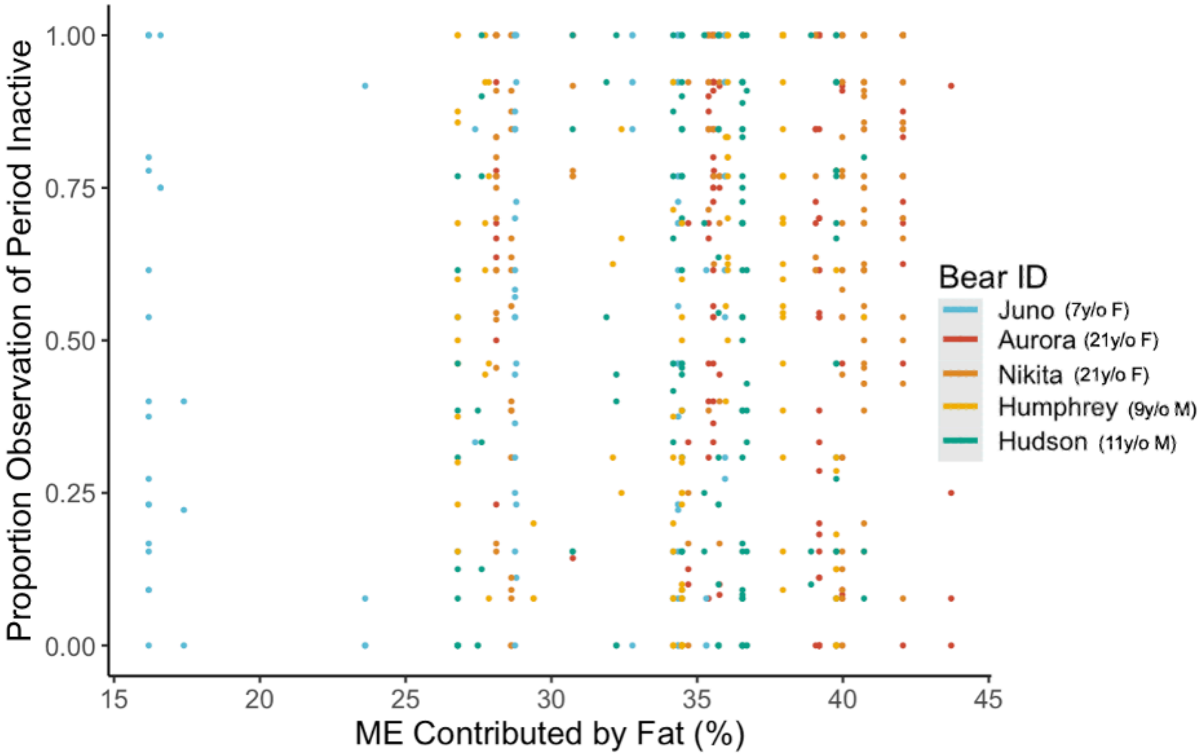

Figure 3.22: Proportion of 1-hour long observation periods spent inactive for 5 captive polar bears at the Toronto Zoo with metabolizable energy contributed by fat per week (%). Humphrey (yellow) was excluded from the model. See Table 3.12 for effect size and p-value.

Hudson and Humphrey had a reduction in inactivity with increasing proportional body fat, while the 3 female bears displayed the opposite relationship (Figure 3.23)., though the overall effect was positive and non-significant (Table 3.12).

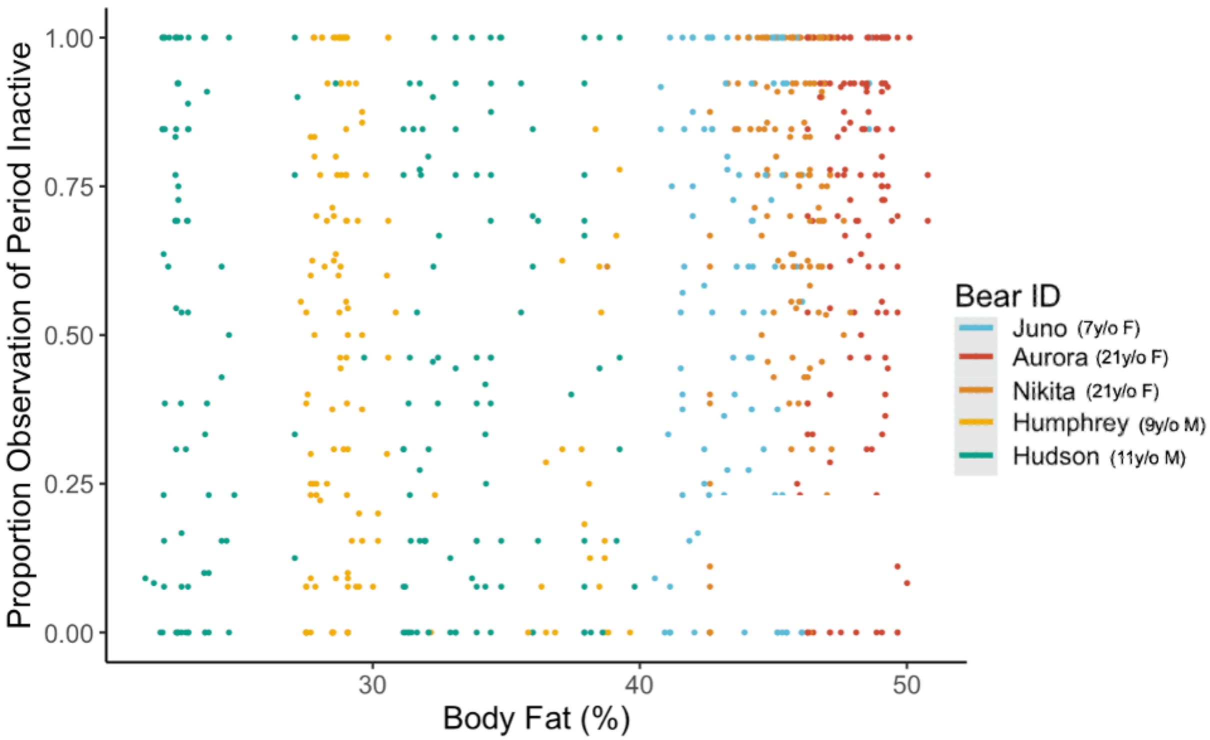

Figure 3.23: Proportion of 1-hour long observation periods spent inactive for 5 captive polar bears at the Toronto Zoo with proportional body fat (%). Humphrey (yellow) was excluded from the model. See Table 3.12 for effect size and p-value.

The relationship between inactivity and air temperature was consistent across all bears excluding Juno. Juno notwithstanding, all bears showed a slight increase in inactivity with increasing air temperature (Figure 3.24). Conversely, Juno showed a slight decrease in inactivity with increasing air temperature (Figure 3.24), though the effect was non-significant (Table 3.12). All bears were more inactive in the afternoon compared to the morning, but the difference in inactivity was non-significant, though borderline (Figure 3.25).

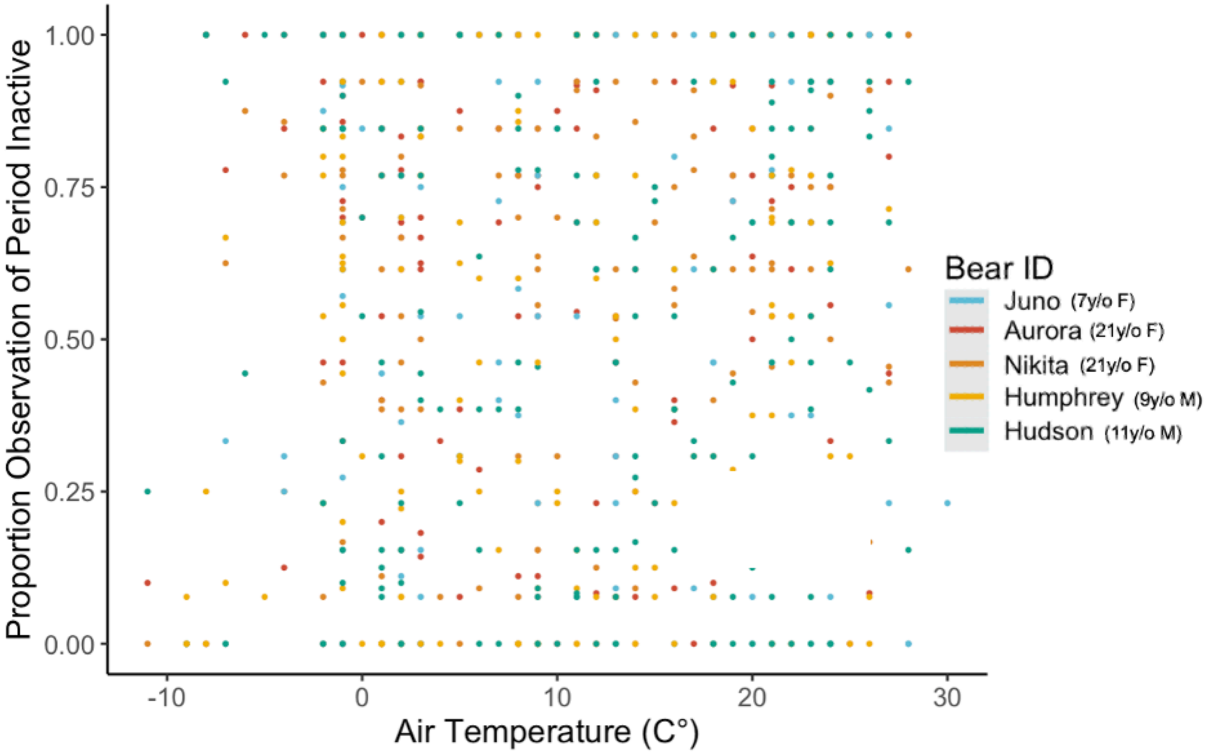

Figure 3.24: Proportion of 1-hour long observation periods spent inactive for 5 captive polar bears at the Toronto Zoo with air temperature at start of observation period (°C). Short-dash lines indicate the trend was non-significant, and long-dash line indicates that the individual was excluded from the model. See Table 3.12 for effect size and p-value.

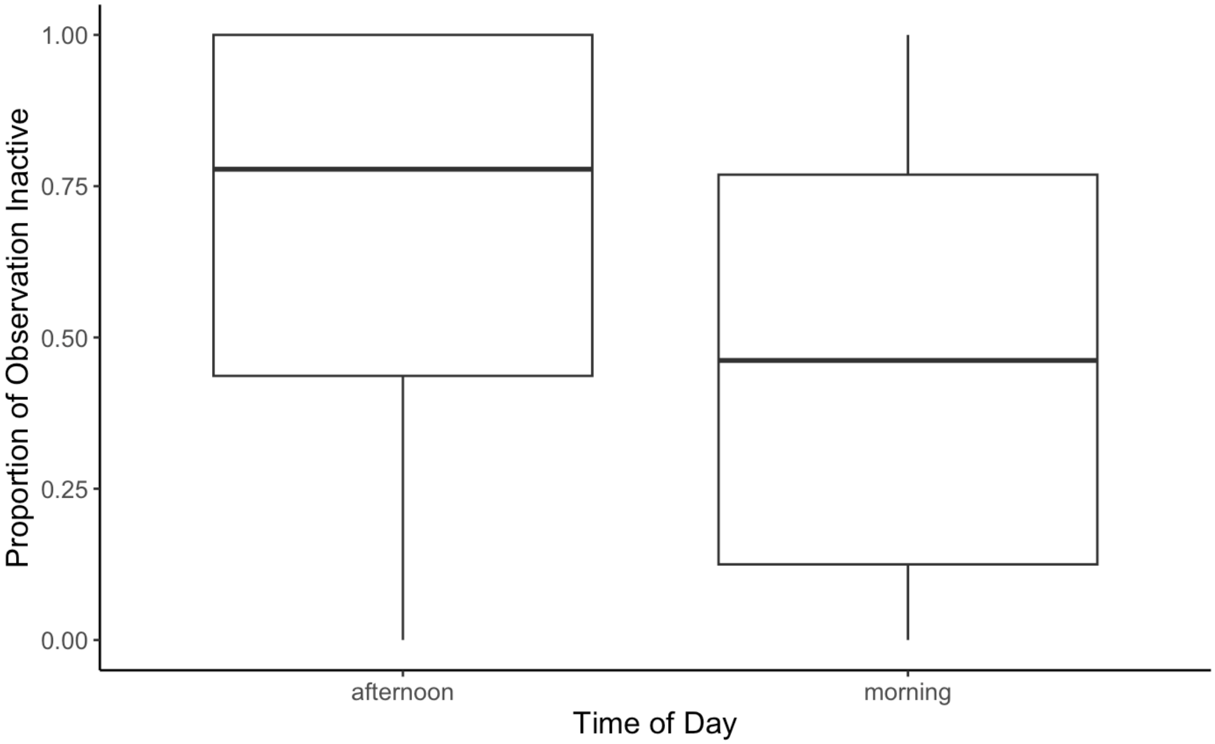

Figure 3.25: Proportion of observation period spent inactive for 5 polar bears at the Toronto Zoo in the afternoon (left) and morning (right). See Figure 3.7 for boxplot interpretation.

3.6 SUMMARY

In some cases, individual bears showed different relationships between response variables and fixed effects (Table 3.13). The outlying bears were most often Humphrey and Nikita. Inconsistencies across bears were seen more often in response to change in dietary fat than change in calories (Table 3.13).

Table 3.13: Summary of the relationship between fixed effects and response variables for individual bears. Upward arrows indicate a positive relationship, downward arrows indicate a negative relationship, and dashes indicate no relationship was observed for that bear. “NA” indicates that the bear was not sampled.

Calories

Bear	Trig	U:C	Mass	Fat$_b$	Fat$_b$ mass	Temp	Inactivity
Aurora	⇓	⇑	⇑	⇑	⇑	⇑	⇓
Hudson	⇓	⇑	⇑	⇓	⇑	⇑	⇓
Humphrey	⇓	⇑	⇑	⇓	-	⇑	⇓
Nikita	⇓	⇑	⇑	⇑	⇑	-	⇓
Juno	NA	NA	⇑	⇑	⇑	⇑	⇓

Fat$_d$

Bear	Trig	U:C	Mass	Fat$_b$	Fat$_b$ mass	Temp	Inactivity
Aurora	⇑	⇓	⇑	⇑	⇑	⇑	⇓
Hudson	⇑	⇓	⇑	⇓	⇑	⇑	⇑
Humphrey	⇑	⇓	⇑	⇓	⇓	⇓	⇓
Nikita	⇑	⇑	⇑	⇑	⇑	⇓	⇑
Juno	NA	NA	⇑	⇑	⇑	⇑	⇑

The strongest model was that predicting change in serum triglyceride concentration, with an R^2 of 94.90% (Table 4.1). This was followed by proportional body fat with an R^2 of 88.97%, and body mass with an R^2 of 80.90% (Table 4.1). All 3 of these models appear to be strong fits, with minimal unexplained variability remaining in the data. Unlike other body response variables, the best model to for body fat mass had an R^2 of 56.32% (Table 4.1), indicating that the model fits adequately, but that change in body mass and proportional body fat are predicted by diet better than body fat mass is. The best model for UC ratio had a R^2 of 54.31% (Table 4.1), indicating that the model is an appropriate fit, with the included predictors explaining over half of the variability in the data. However, it seems serum UC ratio is not predicted by diet as efficiently as serum triglyceride concentration. The best model for body temperature had a R^2 of

47.12% (Table 4.1), a sub-par fit that explains close to half of the variability in the data. With a R^2 of 29.77% the weakest model was that for inactivity (Table 4.1).

Table 3.14 Summary of best model for each response variable as determined by ΔAIC_c ranking and R^2. "Y" indicates that the predictor was included in the model, "N" indicates that it was considered but not included, and "NA" indicators the predictor was not considered. Significant effects based are denoted by asterisk (*). Note that all models included bear ID as a random effect to account for repeated sampling of the same individuals.

Response Variable	R2 best model (%)	Calories	Fat_d	Fat_b	Age	Sex	Age:Sex	Air Temp	Time
Body Mass	80.90	Y*	Y*	NA	N	N	Y*	NA	NA
Fat Mass	56.32	Y*	Y*	NA	N	N	Y*	NA	NA
% Fat	88.97	Y*	Y*	NA	Y	Y	NA	NA	NA
Triglycerides	94.9	Y*	Y*	Y*	Y*	N	NA	NA	NA
UC ratio	54.31	Y*	Y*	N	N	N	NA	NA	NA
Body Temp	47.12	Y*	Y	Y	N	Y	NA	Y	N
Inactivity	29.77	Y*	Y	Y	N	NA	NA	Y	Y

4 DISCUSSION

Polar bears are known to exhibit seasonal changes in behavior, body size, and blood chemistry in accordance with their diet (Tartu et al. 2017, Pagano et al. 2018, Robbins et al. 2012, Derocher et al. 1980, Ramsay et al. 1991, Whiteman et al. 2015, Gleason & Rode 2009, Kelly et al. 2014, Nelson et al. 1983). In the wild, bears consume substantially more calories while on sea ice (anabolic phase) compared to the ice-free season (catabolic phase) (Stirling & McEwan 1975). Sea ice facilitates the hunting of seals, the primary fat-rich diet item bears consume to accumulate fat stores to accommodate their seasonal lifestyle (Stirling & McEwan 1975). Although the AZA does not mandate a seasonal diet regime for captive polar bears, it is a method used by many accredited facilities, including the Toronto Zoo. However, several zoos continue to feed their polar bears a high-calorie diet year-round, often with minimal dietary fat and an abundance of protein. Due to health and welfare concerns associated with a high-protein diet, it has recently been suggested that captive polar bears should be transitioned to a lower-protein, high-fat diet to improve welfare and encourage a more natural lifestyle (Rode et al. 2021, Robbins et al. 2021). With the participation of 5 captive polar bears at The Toronto Zoo, I assessed the effects of caloric intake and dietary macronutrient composition on body mass and body fat, blood metabolite concentrations, body temperature, and inactivity. While many of my findings align with trends observed in wild bears, I also highlight observed differences between captive and wild individuals, as well as unexpected sex-based differences. Diet was found to affect some response variables more than others, and these findings highlight areas where further research is required to better explain the observed changes in response variables over time.

4.1 BODY MASS AND BODY FAT

4.1.1 Body Mass

Calories were found to have a positive effect on total body mass for all bears (Figure 3.1, Table 3.3). It has been shown repeatedly in wild bears that the seasonal fluctuation in resource availability and subsequent caloric intake is associated with a proportional fluctuation in total body mass (Atkinson and Ramsay 1995, Yurkowski et al 2020, Rode et al. 2020, Pagano et al. 2018, Robbins et al. 2012). Bears increase their body mass when resources are available (Pagano et al. 2018, Galicia et al. 2020), reflecting the accumulation of energy (Best 1985). However, when resource availability is limited during the ice-free season, bears lose on average 1 kg of body mass per day as the previously built energy stores are expended (Pilfold et al. 2016). A positive relationship between body mass and calories has also been observed in mice (*Mus musculus*) (Diaz et al. 2006) and marmots (*Marmota vancouverensis*) (Carrier et al. 2022) fed a seasonal diet. The same relationship has been observed in humans, though this was found to be affected by exercise, overall body size, metabolic rates, and health conditions of individuals as well (Fang et al. 2003). Given the strong positive, significant effect of calories on body mass, as well as the large R^2 of the best model, the hypothesis that body mass increases with calories is supported.

As fat is the most efficient form of stored energy (Atkinson et al. 1996, Ramsay et al. 1989), consuming more dietary fats allows bears to assimilate more energy per food unit, and subsequently increase their body mass (Best 1985). Marmots, hibernating mammals that also eat a seasonal diet, are known to increase their body mass with increasing dietary fat (Florant 1998). The same trend has been observed in both human children (Naude et al. 2018) and mice (Wu et al. 2022), though in mice, the rate and amount of mass gained were found to be – at least partially – influenced by genetics (Wu et al. 2002). Although all bears in this study gained body mass with increasing dietary fat, it should be considered that all bears in this study were related. Given the small sample size, it is possible that the mass gain associated with increasing dietary fat may occur at different rates and/or magnitudes in other bears. Regardless, the R^2 value for the model and highly significant effect of dietary fat support the hypothesis that body mass increases with dietary fat.

The interaction term between age and sex had a significant positive effect on change in body mass (Table 3.3). Polar bears are sexually size dimorphic, whereby males are significantly larger than females (Ramsay and Stirling 1986). Being large increases the overall fitness of male bears by strengthening their ability to compete for resources (Ramsay & Stirling 1986) and mates (Rode et al. 2020), thus furthering their ability to contribute to the gene pool. While both males included in this study are of similar age, Juno is 14 years younger than Aurora and Nikita. Juno's body mass remained below that of Aurora and Nikita (Figure 3.3), consistent with trends observed in wild bears whereby adult females had greater body mass than sub-adult and yearling females (Molnar et al. 2009). It should be noted that the sample period began when Juno was a juvenile – only two years old – and concluded once she was an adult – age seven – which likely accounts for her greater variability in overall body mass (Figure 3.3). The interaction between age and sex prevents the effects of age and sex from being interpreted separately, therefore predictions regarding the individual effects of age and sex on body mass can neither be supported nor refuted. However, the literature suggests that a positive effect of age, and a display of sexual dimorphism whereby males are heavier than females, are to be expected in this species. The findings of this study demonstrate that sub-adult-to-adult males are heavier than adult-to-geriatric females, who are heavier than juvenile-to-adult females.

4.1.2 Body Fat Mass

All bears excluding Humphrey saw an increase in body fat mass with increasing calories (Figure 3.4). The seasonal fluctuation in body mass experienced by wild bears is known to occur largely in the form of body fat (Atkinson & Ramsay 1995, Atkinson et al. 1996, Derocher et al. 1992), the primary energetic currency of polar bears. In the Western Hudson Bay subpopulation, it has been found that bears drew, on average, 93% of the energy expended during the catabolic phase from fat stores (Atkinson and Ramsay 1995). Therefore, it is logical that body fat mass is greater when resources are available, and lower when resources are scarce. A positive effect of calories on body fat has also been observed in American black bears of both sexes and various ages (Ayers et al. 2013), as well as adult female grizzly bears from four different American subpopulations (Hilderbrand, Gustine & Mangipane et al. 2018). In both cases, differences in amount of body fat gained were observed between individuals within a population, but the overall trend of body fat increasing with calories was supported (Hilderbrand, Gustine, Mangipane et al. 2018, Ayers et al. 2013). Given the significant positive effect of calories in the best model, the hypothesis that body fat mass increases with calories is supported.

Dietary fat was also found to have a significant effect on body fat mass, with a very similar effect size to that of calories (Table 3.3). It is known that polar bears are capable of efficiently storing fat within their bodies (Pond 1981), therefore it is logical that bears would amass more body fat stores when consuming more dietary fat. An increase in body fat with increasing dietary fat is not unique to polar bears – a 1998 review of various zoo and farm-based studies found positive relationships between body fat mass and dietary fat in dogs (*Canis lupus familiaris)*, cats (*Felis catus*), pigs (*Sus scrofa domesticus*), hamsters (*Cricetinae spp.)* , squirrels (*Sciuridae spp*.) , rats (*Rattus spp*.), mice (*Mus musculus*), macaques (*Macaca fascicularis*), and rhesus monkeys (*Indochinese rhesus macaque*) (West & York 1998). A similar trend has been observed in humans, whereby body fat mass increased with dietary fat regardless of age and sex (Tucker & Kano 1992). In dogs, body fat mass was found to increase with dietary fat, even when controlling for total caloric intake (Romsos et al. 1978). Given the large, significant effect of dietary fat on body fat mass within the best model, the hypothesis that body fat mass increases with dietary fat is supported.

Unlike the other bears in the study, Humphrey saw a negative relationship between body fat mass and calories (Figure 3.4), and dietary fat (Figure 3.5), respectively. As Humphrey is not castrated (while Hudson is) he experiences an increase in stereotypic breeding behaviors during the breeding season which ranges from mid-February to early-June. This period of time coincides with the anabolic phase when calories are increased. Furthermore, keepers noted that Humphrey's stereotypic breeding behaviors increased as he entered adulthood (Toronto Zoo unpublished), the timing of which coincided with the implementation of the high-fat diet. It is likely that the energetic demands of performing stereotypic behaviors prevented Humphrey from building fat stores. Activity, whether normal or stereotypic, requires an output of energy (Watts et al. 1991, Pagano et al. 2019), especially for bears with a large body size (Pagano et al. 2018), as Humphrey was shown to have (Figures 3.1-3.3). The timing of his stereotypic breeding behaviors in relation to the implemented dietary changes likely drives the negative relationship he experienced between body fat mass and dietary predictors. Despite Humphrey's inconsistencies, the statistical effects of calories and dietary fat on body fat mass remained positive and significant.

The interaction term between age and sex had a significant effect on body fat mass, with older females having the greatest body fat mass overall, followed by males, and finally younger females, with the differences between older females and both other groups being significant (Figure 3.6). It is likely that adult female bears build more fat stores due to the important role body fat plays in pregnancy. It has been shown in the wild that females entering their dens with more fat stores rear larger litter sizes and larger individual cubs, who thereby have an increased chance of survival (Atkinson and Ramsay 1995). While male bears require substantial stored fat to maintain their large bodies (Rode et al. 2020, Pagano et al. 2018) and survive the ice-free period (Molnar et al. 2010), their overall fitness may be more closely associated with total body size. The sampling period saw Juno at ages 2-7, and wild female polar bears do not reproduce until ages 4-6 (Sonne et al. 2007), meaning younger female bears have less need for an abundance of fat stores. The significant interaction term hinders the interpretation of individual random effects, thus no predictions relating to the individual effects of sex or age on body fat mass can be concluded upon. However, it appears likely that some combination of sexual dimorphism, sex-based biological roles, and age play a role in driving body fat mass. In this study, it was shown that adult-to-geriatric females had the greatest body fat mass, followed by sub-adult-to-adult males, and finally juvenile-to-adult females.

4.1.3 Proportional Body Fat

The relationships between proportional body fat and both caloric intake (Figure 3.7) and dietary fat (Figure 3.8) varied between sexes, with both effects being significant. Female bears were found to have positive relationships between proportional body fat and dietary predictors, while male bears had negative relationships (Figures 3.7-3.8). However, I found that male bears had positive relationships between *body mass* and dietary predictors (Figures 3.1-3.2), and Hudson showed positive relationships between *body fat mass* and dietary predictors (Figures 3.4-3.5). Thus it appears that male bears in this study continued to gain body mass – and in the case of Hudson, body fat – as calories and dietary fat increased, but lost proportional body fat, meaning the rate of mass gain exceeded the rate of fat gain. This is possibly related to the way in which male fitness is benefitted by large body size for competition, while female fitness is benefitted by an abundance of fat stores for a successful pregnancy (Atkinson and Ramsay 1995, Rode et al. 2020). In wild bears, adipose tissue adipocyte volume is positively correlated with body mass in females only (Ramsay, Mattacks, & Pond 1992), further suggesting that males – especially those of breeding age – prioritize structural body size over fat abundance. It should be noted that caloric increases coincided with the breeding season, when females require an abundance of fat stores and males make use of their large overall body size to attract mates (Ramsay & Stirling 1986, Rode et al. 2020). These sex-based approaches to reproduction likely play some role in driving the opposing relationships observed with proportional body fat and dietary predictors, especially given the timing of the breeding season in relation to resource availability. The hypotheses that proportional body fat increases with *a)* calories and *b)* dietary fat can be partially supported, for females only, and refuted for males in this study. However, given the small sample size and poor age-sex distribution of this study, further work is needed to draw stronger conclusions (with greater extrapolation potential) about the effect of calories and dietary fat on proportional body fat in male and female polar bears.

I found that male and female bears again displayed opposing relationships between proportional body fat and age, with females showing a positive relationship and males a negative, although this effect was non-significant (Figure 3.9). Additionally, overall proportional body fat of female bears exceeded that of male bears (Figure 3.10). Studies on proportional body fat of wild bears found significant effects of both age and sex (Molnar et al. 2009), whereby proportional body fat was greatest in adult females, followed by yearlings/juveniles of both sexes, sub-adult males, cubs-of-year of both sexes, and finally adult males, further supporting that males have more proportional body fat when they are younger, and females when they are older. Given that the effect of age was small and non-significant, and considering the small sample size of this study, predictions about the effect of age on proportional body fat cannot be statistically supported. However, the trend of proportional body fat increasing in females over time and decreasing in males is worth noting, as it is supported by the literature. The effect of sex was non-significant, but the difference in proportional body fat between sexes was significant, suggesting the prediction that male bears have greater proportional body fat than females should be refuted. Before extrapolating these findings, it should be considered that the 2 male bears sampled in this study are of similar age. Furthers studies, with better age-sex distribution, are needed before drawing firm conclusions as to how sex and age effect the fat storage of all polar bears.

4.1.4 A Note on Bertalanffy's Equation and Molnar's Model

I used Bertalanffy's length-at-age equation (Von Bertalanffy 1938, Derocher & Wiig 2002) and Molnar's body fat mass model (Molnar et al. 2009) to estimate body fat mass for each total body mass measurement obtained. The output from Molnar's model was then used to calculate proportional body fat as the body fat mass over the total body mass, multiplied by 100%. Bertalanffy's length-at-age equation, which has previously been successfully validated on polar bears (Kingsley 1979, Derocher and Wiig 2002), estimates the length of an individual bear as a function of their mass, sex, and age (see section 4.2, equation 7). Bertalanffy's equation includes an asymptotic body length constant and a fitting constant (theoretical age at which bear was 0 length), both of which vary between sexes (Derocher & Wiig 2001). Molnar's model includes several constants that vary based on age and sex, with bears being classified as female (all ages), sub-adult male (5.9 years of age and under) and adult male (6 years of age and over) (see section 2.2, equations 8a-10c). These different constants were derived from sampling wild bears belonging to each age/sex group, and thus reflect the sexual dimorphism displayed by the species. However, it should be acknowledged that using these models rather than directly measuring body length introduces error. Body length could not be measured on a weekly basis like mass as it would require bears to be chemically immobilized, a procedure prohibited by The Toronto Zoo's animal care protocols if not for medical or husbandry reasons. Thus, all body length measurements – and subsequently all body fat mass and proportional body fat measurements – presented in this study are estimates and may not accurately reflect the fat stores of each bear. Special consideration should be taken when considering Hudson and Humphrey's body fat as the sampling period saw their transition into adulthood, meaning they each have body fat estimates calculated with 2 different sets of constants. While the constants were derived based on biological differences between male bears of different ages, in reality, changes in metabolism do not occur instantaneously when male bears turn 6 years old.

4.1.5 Sources of Error and Other Considerations

While the models for body mass and proportional body mass have large R^2 values indicating a good fit, the body fat mass model has an R^2 of 56.32% (Table 6.1). While this R^2 value still warrants consideration of the model, it is considerably smaller than that of the other 2 models, indicating that body fat mass is not as well-predicted by diet, age, and sex as body mass and proportional body fat are. Time spent active was not included as a predictor for body fat mass, which may account for some of the unexplained variability, given that exercise uses energy in the form of fat stores (Atkinson et al. 1996, Ramsay et al. 1989). It should also be considered that all diet data presented in the study represent the diets offered, not necessarily the diets consumed. While a portion of the diet can be fed indoors with strict regulation, the portion of the diet fed on exhibit has potential for measurement error. Although leftover diet items were weighed and subtracted from weekly intake, there remains the possibility that bears sharing exhibits consumed portions of each other's diets, or that zookeepers failed to find leftover diet items. When considering the implications of these findings for other captive polar bear populations it should also be considered that there is some genetic component to metabolism and lipid mobilization (Tartu et al. 2017), and that all bears sampled in this study are related (Figure 2.2). Therefore, it is possible that polar bears bearing no genetic relation to the bears in this study may display different trends in body mass and body fat.

4.2 BLOOD ANALYSES

4.2.1 Triglyceride Concentration

All bears showed a negative relationship between serum triglyceride concentration and caloric intake (Figure 3.11). Triglycerides are the primary form of lipids stored within the adipose tissue; they accumulate when energy intake is high and are mobilized through the bloodstream when energy intake is reduced (Ntambi et al. 2000). The negative relationship between calories and serum triglycerides has been observed in various species, including dogs (Yi et al. 2023), rats (Thomas-Moya et al. 2006), and squirrel monkeys (*Saimiri sciureus*) (Lane et al. 2001), regardless of sex. In humans, a study with a sample size of over 2000 adults of both sexes found a large, significant, negative effect of calories on serum triglyceride concentration (Nestel et al. 1970). This phenomenon is also well-documented in various species of bear who eat a seasonal diet, as stored fat is their primary source of energy during the catabolic phase. Serum triglyceride concentrations have been found to be negatively associated with calories in wild polar bears (Nelson et al. 1983, Tartu et al. 2017) as well as wild and captive grizzly (Hill

2016, Nelson et al. 1983) and black bears (Hill 2016). Given the significant effect of calories found in this study, the hypothesis that serum triglyceride concentration decreases with increasing calories is supported.

All bears showed an increase in serum triglyceride concentration with increasing dietary fat (Figure 3.12). Following consumption, triglycerides are emulsified and enzymatically digested before being absorbed through the intestines and transported to the adipose tissue (Alvez-Bazerra et al. 2017). As triglycerides are a form of fat, consuming more fat allows for more triglycerides to be absorbed, transported, stored, and subsequently mobilized, leading to an increased concentration in the blood. It is possible for the high serum triglyceride concentrations observed at high dietary fat intake to be associated with transport from the intestines, or mobilization (Alvez-Bazerra et al. 2017). Serum triglyceride concentrations are known to increase with increasing dietary fat in healthy, overweight, and obese dogs (Verkest et al. 2012), as well as healthy and overweight adult humans (Nestel et al. 1970, Cooling et al. 1998), although this was also found to be affected by the type of fat consumed. Further classifying dietary fats was beyond the scope of this project, however the hypothesis that total dietary fat intake positively effects serum triglyceride concentration is supported.

The relationship between percent body fat and serum triglyceride concentration was found to vary between sexes (Figure 3.13). Female bears experienced an increase in triglyceride concentration with increasing body fat, while males experienced a decrease (Figure 3.13), similar to the trends observed between proportional body fat and calories, dietary fat, and age (Figures 3.4-3.6). There are many possible explanations as to what drives this sex-based difference, but given the small sample size of this study, it is not possible to draw conclusions as to the root cause of this sex-based difference. Sex has not been found to affect the relationship between proportional body fat and triglyceride concentrations in other species; in dogs (José Lahm Cardoso et al. 2016) and humans (Shimokata et al. 1989, Freedman et al. 1990), serum triglyceride concentration increased with proportional body fat in both sexes. One study on humans found the positive relationship between body fat and serum triglyceride concentration to be stronger in women than in men, but observed a significant positive relationship in men nonetheless (Despres et al. 1985). It should be considered that these species do not exhibit the same degree of sexual dimorphism as polar bears, so while it is possible that the biological differences between male and female bears lead to sex-based differences in fat storage and utilization, further studies are required to investigate this. In wild polar bears, adipose tissue adipocyte volume was greater in adult females than adult males (Ramsay, Pond, & Mattacks 1992), suggesting some degree of sexual dimorphism in fat storage, although this has not yet been tied to blood chemistry. Given the significant effect of proportional body fat on triglycerides, the prediction that proportional body fat has a positive effect on serum triglyceride concentration can be partially supported, in females only. Further work is needed to understand how sex affects the relationship between proportional body fat and serum triglyceride concentrations.

Serum triglyceride concentrations of all bears to increased with age (Figure 3.14), suggesting the prediction that age would have no effect on serum triglyceride concentration should be refuted. Although sex was not found to be included in the best model, the opposing relationships between serum triglyceride concentration and proportional body fat across sexes cannot be ignored, and the prediction that sex has no effect on serum triglyceride concentration cannot fully be supported. Given the small sample size of this study, poor age-sex distribution, and lack of supporting literature on polar bear sex and age in relation blood chemistry, further work is required to accurately assess the way in which age and sex effect serum triglyceride concentrations of polar bears as a species, if at all.

4.2.2 Urea: Creatinine Ratio

I found all bears displayed an increase in serum UC ratio with increasing caloric intake (Figure 3.15). Urea is the biproduct of protein use – it accumulates in the blood when energy intake is high, leading to an increased concentration, and is later recycled out of the blood to replenish catabolized body protein, leading to a reduced serum concentration (Barboza et al. 1997). A positive relationship between calories and serum UC ratio has been observed in humans (Chen, Ohashi & Kasai 1974, Porikos & Van Itallie 1983), dogs (Kronfeld 1984), and cats (Frantz, Yamka & Friesen 2007). In cats, this positive relationship was found to be significant regardless of macronutrient composition (Frantz, Yamka & Friesen 2007). In polar bears, a significant difference in UC ratios between the anabolic and catabolic phases has been observed in several subpopulations, whereby serum UC ratios are greater during the anabolic phase (Nelson et al. 1984, Derocher et al. 1990). In the Southern Beaufort Sea subpopulation, bears were found to have the lowest UC ratios in October, toward the end of the catabolic phase (Whiteman et al. 2017). In the Western Hudson Bay subpopulation, bears experiencing energy surplus were found to have UC ratios > 20, whereas those in deficit were found to have UC ratios < 10. Such small UC ratios were rarely but occasionally observed in this study, however, the overall trend of UC ratios increasing with calories is apparent. Thus the hypothesis that serum UC ratios increase with increasing calories is supported.

All bears excluding Nikita saw a decrease in serum UC ratios with increasing dietary fat (Figure 3.16). Although macronutrients in this study were only measured through the proportion of metabolizable energy obtained from fat, increases in dietary fat were associated with reductions in dietary protein. When there is less protein available in the diet, it is logical that less urea will accumulate in the blood (Barboza et al. 1997). The negative relationship between dietary fat and serum UC ratio has also been observed in humans (Kesteloot & Joosens 1993) and pigs (Freire et al. 1998). This is consistent with trends observed in wild black and grizzly bears, who were found to have lower UC ratios when consuming fat-rich diets than when consuming protein-rich diets (Hellgren 1998). Despite these consistent trends across species, it has been suggested that UC values are not an overly reliable method of gaging endogenous protein use and conservation in polar bears (Atkinson 1996, Whiteman et al. 2017). In Western Hudson Bay, adult bears lost up to 18% body mass from muscle and lean tissue – indicative of body protein catabolism – but this was only weakly correlated with UC ratios (Whiteman et al. 2017). In humans, it has been found that UC ratios can change significantly within two hours of eating, or immediately following urination (Kestteloot & Joosens 1993). While the hypothesis that serum UC ratio decreases with increasing dietary fat is supported, it is unclear how accurately this trend reflects energy use and conservation. Body fat, age, and sex, were not found to be included in any of the best models for serum UC ratio, meaning that the predictions that these variables will have no effect on UC ratios are supported.

Unlike the other bears sampled, Nikita's serum UC ratios were found to increase slightly with increasing dietary fat (Figure 3.16). Nikita chose to participate in the least amount of blood draws, giving only 7 blood samples while on the high-fat diet. It should also be noted that Nikita was frequently sampled for blood first, meaning that at the time of the sample collection it had not been long since her last meal, and recent food consumption is known to cause elevated urea in wild polar bears (Whiteman et al. 2017). There is also a possibility that Nikita has some metabolic difference to Aurora – who is her same age and sex – given that Nikita is consistently lighter and less fat than her despite being on the same diet (Table 2.1). It can also be considered that Nikita is simply naturally skinnier than her sister and accumulates more urea in the blood as her reduced activity levels (Figure 3.21-3.24) are less energetically demanding. It is unlikely that Nikita's UC ratios are driven by her age or sex, given that neither were included in the best model, and have also been found to have no effect on UC ratios in wild bears (Nelson 1984, Ramsay and Nelson 1989).

4.2.3 Sources of Error and Other Considerations

The 54.31% R^2 value associated with the UC ratio model compared to the 94.91% R^2 of the triglyceride model indicates that triglycerides are better predicted by diet and bear-related variables than UC ratios are. Urea is known to be time-sensitive and changes hourly in bears (Whiteman et al. 2014, Schroeder 2014), meaning that the failure to include time since last meal as a predictor likely accounts for a portion of the unexplained variability in the data. Additionally, I included a direct measurement of dietary fat only, and not dietary protein, further accounting for the small R^2 of the UC ratio model.

Juno was not included in either blood analysis, as she was not interested in voluntarily participating in blood draws. Removing Juno from the analysis effectively removes any representation of a young female bear, given that Aurora and Nikita are geriatric. All inferences drawn about blood chemistry in relation to age and sex must be considered in the context of this study. It is possible that a young female bear like Juno may display different trends than older females like Aurora and Nikita.

4.3 BODY TEMPERATURE

4.3.1 Average Daily Body Temperature

In both of the best models, calories had a small, positive, significant effect on average daily body temperature (Table 3.10). Excluding Nikita, all bears were found to have a slight decrease in body temperature with decreasing calories (Figure 3.17). Similar trends have been noted in the wild, where bears in Western Hudson Bay exhibited their lowest body temperatures from June to October, during the catabolic phase (Whiteman et al. 2015). In the Southern Beaufort Sea, bear body temperature has been found to decline slightly throughout the entirety of the catabolic phase (Whiteman et al. 2015), a trend typical of food-deprived mammals (McCue et al. 1979). It has been suggested that polar bears reduce body temperature during catabolism as a means of energy conservation (Whiteman et al. 2015). Additionally, metabolic heat generated within the body increases with calories consumed (Ramsey et al. 2000), it is logical that bears would be warmer when consuming more food. Given the statistically significant effect of calories in the best model, the hypothesis that body temperature increases with calories is supported. However, the small effect size and small sample size should be considered when extrapolating these findings to other polar bears.

Though the models containing dietary fat were stronger than those excluding it, the effect of dietary fat was non-significant (Table 3.10), and inconsistencies were observed across bears. Hudson, Juno, and Aurora displayed increasing body temperatures with increasing dietary fat, whereas Nikita and Humphrey displayed the opposite relationship (Figure 3.18). As dietary fat is an efficient source of energy, it is possible bears consuming more fat have less need to conserve energy through lowering their body temperature, leading to the positive relationship observed in Hudson, Juno, and Aurora. Additionally, in humans, increased dietary fat is associated with increased metabolic activity which leads to the generation of more body heat (Neeves et al. 2017, El-Zayat, Sibaii, & El-Shamy 2019). However, this was found to be affected type of fat consumed, where saturated fats were associated with greater body temperatures (El-Zayat, Sibaii, & El-Shamy 2019). Similar findings have been noted in rats, where individuals consuming a diet rich in saturated fats had a significantly greater average body temperature than those consuming a diet rich in poly-unsaturated fats (Geiser & Kenagy 1987, Geiser et al. 1994). While the dietary fats in this study were not further classified by saturation, Nikita was offered an identical or very similar diet to Aurora, and Humphrey to Hudson, so it is unlikely that fat type drives these observed differences, unless there was some difference in the offered diet items actually consumed by each individual. The small and negative effect of dietary fat on body temperature, inconsistencies across bears, and relatively small R^2 of the model suggest that minimal conclusions can be drawn from these findings about the effect of dietary fat on body temperature. In this study, the overall negative and non-significant effect of dietary fat indicates that the hypothesis of body temperature increasing with dietary fat should be refuted.

Although proportional body fat was included as a predictor in both of the best models, it did not have a significant effect (Table 3.10). Once again, inconsistencies were observed across bears, where Hudson and Aurora showed an increase in body temperature with body fat, and Nikita, Juno, and Humphrey a decrease (Figure 3.18). A study on hibernating black bears found that fatter bears had higher body temperature during catabolism, but skinnier bears had greater body temperature during anabolism, suggesting that the role of body fat in thermoregulation may be dependent upon caloric intake (Toein et al. 2015). As calories were found to be the most significant predictor for body temperature, it is possible that the relationships observed between body fat and body temperature are simply a function of fluctuating calories. It is known that polar bears possess non-specialized adipose tissue as opposed to specialized adipose tissue (such as blubber) (Thiemann, Iverson, & Stirling 2006), the primary purpose of which is to store energy rather than to insulate the body (Thiemann, Iverson, & Stirling 2006). Therefore, it is understandable that an increase in body fat is not necessarily associated with an increase in body temperature. Although some bears in this study showed a slight increase in body temperature with increasing proportional body fat, the small and non-significant effect of proportional body fat indicates that the prediction of body fat having no effect on body temperature can be supported.

Air temperature was only included in the second-place model by ΔAIC_c ranking, where it had a non-significant positive effect, with an effect size smaller than that of body fat (Table 3.10). All bears excluding Humphrey displayed an increase in body temperature with increasing air temperature (Figure 3.20). The loss of body heat to the environment is dependent upon the temperature gradient between the air and the body (Hurst et al. 1982) – when the air is warmer, it is more difficult to lose body heat and reduce body temperature. A positive relationship between air temperature and body temperature has been observed in the wild, in both cubs (Oritsland 1969) and adult polar bears (Tributsch et al. 1990). However, other studies on wild polar bears found that the effect of air temperature on body temperature interacts with activity (Whiteman et al. 2015), an explanatory variable not considered in this analysis. Furthermore, calories had the most significant effect on body temperature, and bears tended to be offered their lowest-calorie diets in late fall and early winter (just prior to the onset of the anabolic phase), when air temperatures are low. Given that the effect of air temperature was non-significant, only included in the second-place model, and interconnects with other explanatory variables, the prediction that body temperature increases with air temperature is refuted, although the trend observed in this study warrants further consideration.

The additional prediction that humidity would positively effect body temperature must also be refuted as the predictor was considered but not found to be present in any appropriate models. The prediction that windchill has a negative effect on body temperature can be neither supported nor refuted, as poor windchill distribution prevented the predictor from being considered in models.

4.3.2 Variability Unaccounted for

With an R^2 of 47.12% for the 2nd place model by ΔAIC_c ranking, the majority of variability in body temperature data is unaccounted for by the model. One area where inconsistencies were observed across bears was the relationship between body temperature and proportional body fat (Figure 3.19). While I only considered the effect of proportional fat stores, a study on body temperature in black bears found effects of fat density and fat conductivity (Cottrell et al. 2003). It should be noted that both Hudson and Aurora – who saw positive relationships between body fat and body temperature – are fatter (Figures 3.4, 3.8) but shorter (Toronto Zoo unpublished) than their respective counterparts, Humphrey and Nikita, who saw negative relationships between body fat and body temperature (Figure 3.19). It can be speculated that body length may drive some difference in body fat distribution, and subsequent density and/or conductivity, thus effecting body temperature. However, calculating fat density or conductivity in this study would require entering the body lengths estimated via Bertalanffy's equation into another set of equations, increasing the chance of error occurring. The relationships between body temperature and fat density and conductivity would be better explored by a study where bears can frequently be measured, or where body fat is quantified with a more direct method like bioelectrical impedance.

No variable related to fur density was included, which likely accounts for unexplained variability in the data. Each strand of the polar bear's fur is largely hollow, with a labyrinth-like structure in the center. Both the labyrinth and the hollow structure of the fur work to trap heat against the body, and this process is further abetted by the fur's translucent colour, which allows heat from sunlight to penetrate to the skin (Wang et al. 2006). If dense fur traps both heat from sunlight and escaping body heat against the skin, it would likely increase skin surface temperature, thus reducing the air temperature-bear temperature gradient and reducing heat loss. It is known that captive bears in temperate environments "blow coat" – a process by which large clumps of fur are shed at once – on the chest, back, and legs (Toronto Zoo unpublished). Toronto Zoo zookeepers reported that all the bears blew coat during the summer and early fall, but that Nikita tends to lose the most fur, followed by Aurora. As less fur should facilitate more heat loss to the environment, this may explain, in-part, Nikita's low body temperatures (Figure 3.17-3.18). Additionally, polar bear fur has been said to lose 90% of its heat retention when wet (Scholander et al. 1950), meaning that time spent in water – a variable not considered in this study – likely had some effect on body temperature as well.

While inactivity was analyzed as a response variable in a separate analysis, no measure of behavior was considered as an explanatory variable for body temperature. Given that activity raises the core body temperature in polar bears (Best 1985), behavior likely had some effect on body temperature in this study. Further studies considering the effect of activity on body temperature must also carefully consider the amount of time spent swimming, and the temperature of the water. Other thermoregulatory behaviors such as panting and shivering are known to effect body temperature (Mathewson & Porter 2013). Such behaviors were not recorded in this study, however, by personal observation, bears were sometimes observed panting. It is possible that engaging in this behavior had some effect on body temperature.

4.3.4 Sources of Error and Considerations

It should be considered that obtaining variables relating to weather from Environment and Climate Change Canada may have introduced error because the method of obtaining temperature differed – body temperature was obtained directly with an iButton, and air temperature was recorded from a third party. Temperatures recorded from Environment and Climate Change Canada were available for the Markham area, not the zoo itself, and certainly not specific areas in each exhibit. For example, in the pool exhibit, the cave – which is obstructed from sunlight – likely has a lower ambient air temperature than the elevated, open area on the rocks. Additionally, air temperatures inside the bear house were often significantly cooler than outside. This means that the air temperatures recorded may not reflect the air temperature actually experienced by that bear at the moment of body temperature recording. For this reason, interpretation of our findings on air temperature should be made with careful consideration. It should also be considered that interactions between air temperature and other variables were not picked up on for this reason.

4.4 BEHAVIOUR

4.4.1 Inactivity

Calories were found to be the only significant predictor in the best model, and all bears displayed an increase in activity with increasing caloric intake (Figure 3.21). Given that consuming more calories supplies the bears with more energy, it is logical that they are more active under high caloric intake. Several studies on wild polar bears have found that mean activity levels were lower during the catabolic phase compared to the anabolic phase (Whiteman et al. 2017, Whiteman et al. 2015, Pagano et al. 2018, Robbins et al. 2012, Shimodaira 2011, Meiser et al. 1990). The same relationship has previously been observed in both Aurora and Nikita, as a 2014 Toronto Zoo study found them to be most inactive during the summer and fall, when caloric intake was lowest (Kelly 2014). This is expected, as inactivity is typical of food deprived mammals (McCue et al 1979), being observed in mice (Overton & Williams 2006), elephants (*Loxodonta 95fricana*) (Rees 2009), gelada monkeys (*Theropithecus gelada*) (Christin 2022), dogs, and chimpanzees (*Pan troglodytes*) (Koene 1999). In polar bears, it has been suggested that inactivity can compose 70-90% of an individual's activity budget during the catabolic phase (Knudsen 1978, Latour 1981, Lunn & Stirling 1985). It has also been suggested that an increase in activity is associated with an increase in food availability, not just food intake (Whiteman et al. 2015). Though acquiring food in captivity requires far less energy expenditure than acquiring food in the wild, it should still be considered that an increase in the number of diet items put on exhibit could motivate the bears to engage in more active behaviors such as searching, digging, and manipulating enrichment items. Subsequently, finding and consuming the food items would provide fuel for further activity, creating a positive feedback loop motivated by diet. As calories were the only significant predictor in the best inactivity model, with a strong negative effect, the hypothesis that activity increases with increasing calories is supported.

Though dietary fat was included in the best model, it had a non-significant effect, and inconsistencies were found across bears (Figure 3.22). Nikita, Juno, and Hudson displayed an increase in inactivity with increasing dietary fat, and Humphrey and Aurora displayed a decrease. For most of the bears involved in the study, the relationship between dietary fat and inactivity may be partially driven by the effect of fat intake on body size. Larger bodies require more energy to move (Pagano et al. 2018), so bears may be resting as a means of energy conservation. However, captive bears need to expend less energy than wild bears due to the different environment and motivators (Rode et al. 2010). Although a high-fat diet provides the bears with more energy, they may feel little motivation to expend that energy in a captive environment, particularly when they have access to several comfortable resting areas. In other zoo-based studies it was found that dietary fat had no effect on the activity budgets of gorillas (*Gorilla gorilla*) (who were instead affected primarily by calories) (Less 2012) and lorises (*Lorisinae lydekkerianus*) (Williams, Cabana & Nekaris 2015). However, other studies on mice (Keleher et al. 2015) and orangutans (*Pongo pygmaeus*) (Dierenfeld 1997) found that individuals were more inactive on a high-fat diet. Given the small R^2 of the model, inconsistencies across individuals, and non-significant effect of dietary fat, the hypothesis that activity decreases with dietary fat is refuted, with no discernable relationship being observed between dietary fat and inactivity. The inconsistencies observed across individual bears and across species as documented in the literature suggest that further studies are needed to more accurately assess how dietary fat affects the behavior of polar bears.

The effect of body fat was once again found to differ depending on sex, with males displaying an increase in activity – and females a decrease – with increasing proportional body fat (Figure 5.33). Again, this may be related to the sex-based lifestyle differences of polar bears, whereby females require an abundance of fat stores for successful pregnancy (Atkinson & Ramsay 1995) and males require a larger overall body size for increased fitness (Ramsay & Stirling 1986, Rode et al. 2020). When female bears are at high proportional body fat, it is crucial that they conserve their fat stores – through inactivity – to later be expended on pregnancy and cub-rearing. Conversely, when males are at high-body fat, there are less repercussions to expending fat stores so long as loss of total body size is minimized. In the wild, it has been found that females are less active than males during the breeding season (Stirling et al 1997), though it must be noted that Hudson, being castrated, does not engage in any of the energetically costly breeding behaviors seen in wild male bears. However, Humphrey, who was not included in the statistical analysis but whose trend is depicted in Figure 3.23, did engage in some breeding behaviors in 2022 and early 2023. Even when breeding behaviors are not displayed, it is possible that biological differences between sexes are enough to drive the opposing relationships between body fat and inactivity, though further studies are needed to investigate this. As the effect of body fat was non-significant, the prediction that inactivity increases with increasing proportional body fat is refuted, although the positive trend for females is worth noting.

Time of day was considered as a categorical predictor, whereby observations taken before 12:00 PM were classified as “morning”, and observations after 12:00 PM as “afternoon”. Although the effect of time of day was non-significant, the difference in median inactivity between the two time periods is borderline, with activity being greater in the morning (Figure 3.25). While this has been refuted by studies on wild polar bears that found activity peaked in the early afternoon (Ware et al. 2020) or evening (Togunov et al. 2002), those findings were associated with food availability. At The Toronto Zoo, bears are fed a portion of their meals indoors at roughly 8:30AM and 4:00PM each day, then released into the exhibit where they finish the remainder of their meals at roughly 9:00AM and 4:30PM. Given that sampling always concluded by 3:30PM, it is possible that I observed bears being most active in the morning as less time had passed since their last meal. Additionally, when bears are first put on exhibit in the morning, the presence of new-to-them enrichment items may incite activity (Forthman et al. 1992). Given the non-significant and small effect of time of day, and non-significant difference in inactivity between time periods, the prediction that inactivity is greater in the afternoon is refuted. However, the trend of inactivity being greater in the afternoon is worth noting, as it relates to enrichment and feeding times. This trend should not be extrapolated to wild bears, but further zoo-based studies considering explanatory variables related to husbandry schedules would provide a better understanding as to how time of day effects inactivity.

Excluding Juno (who experienced a bout of high-intensity stereotypic behavior in the form of pattern-swimming during July and August 2022, coinciding with warm air temperatures), I found that all bears displayed an increase in inactivity with increasing air temperature (Figure 3.24). This trend has been observed in wild grizzly bears (Heard, Ciarniello, & Seip 2008), but studies on wild polar bears have found that air temperature does not significantly affect behavior (Eckard 2016). Other studies have suggested that polar bears may reduce activity under high air temperatures to avoid thermal stress (Whiteman et al. 2015). Given that activity generates heat and raises the core body temperature (Best 1982), it is logical that bears would be inactive under hot air temperatures as a method of avoiding overheating. However, the positive effect of air temperature on inactivity was found to be non-significant, with the smallest effect size of all random effects excluding bear ID. Therefore, the prediction that air temperature positively effects inactivity must be refuted, although the observed trends suggest that further research is warranted. Additionally, the predictions that humidity and age positively effect inactivity are refuted, as both were considered during model selection, but not included in the best model. Due to a poor distribution of windchill values the predictor was excluded, thus the prediction that windchill negatively effects inactivity cannot be assessed.

4.4.2 Variability Unaccounted For

The best model for inactivity had a R^2 of 29.77% (Table 3.11) indicating that much of the variability in the data is unexplained by the model. No variables related to enrichment were recorded, which may account for some unexplained variability. The presence of enrichment items is known to have an effect on the behavior of zoo animals (Forthman et al. 1992), and different items can elicit different behavioural responses in captive bears (Altman 1999, Canino & Powell 2010). A study examining the activity budgets of captive polar bears at the Bronx Zoo found that bears were more active when they had access to enrichment items that they did not have access to the previous day (Canino & Powell 2010). Considering the number and type of enrichment items offered, as well as the date of their offering, may help to explain more variability in the data.

I also did not consider any variables related to social grouping. Bears at The Toronto Zoo have multiple social groupings – Juno is always alone, Aurora and Nikita are always together but sometimes joined by Hudson, and Hudson is either with Humphrey, alone, or with Aurora and Nikita. Based on personal observation and notes from zookeepers, both males are more active when together than when isolated, and Aurora is more active when with Hudson. Furthermore, it appears the bears' behavior can be affected by who is in the neighboring exhibit. Nikita and Juno have been observed displaying aggression towards one another from adjacent exhibits, and Humphrey often directed his stereotypic behaviors toward the 3 female bears in adjacent exhibits during the breeding season.

While visitor numbers were recorded and found to have no effect, noise levels were not measured. Toronto Zoo zookeepers report that Hudson is particularly sensitive to noise, so there is a possibility that increased noise from visitors may affect his or the other bears' behavior.

Lastly, there is always a degree of uncertainty in animal behavior data due to the inherent variation in behavioral predictability that exists across individuals (Hertel et al. 2020). Animal behavior relies, at least in-part, on the decision-making of the animal (Budaev et al. 2019). While we can make informed predictions about which variables elicit certain decisions, animals may make choices that seem unprecedented to the researcher simply because they do not possess human cognition. While much of polar bear cognition, and animal cognition in general, remains poorly understood, it has been suggested that individual polar bears have both unique personalities (Chant 2021) and the capacity to make independent behavioral decisions (Renner & Kelly 2006). It must be considered that some unexplained variability will always exist in polar bear behavior data on account of the personalities, free-will, and decision-making capabilities of the individuals being studied.

4.4.3 Sources of Error and Other Considerations

As diet availability – not just consumption – is known to affect activity, the amount of food offered likely had some effect on behavior. When individuals were put on exhibit together, enough food was put on exhibit for all individuals. For example, if Aurora, Nikita, and Hudson were on exhibit together in the morning, three bear breakfasts worth of food were put on exhibit. Even if each bear ate exactly 1/3 of the diet offered, it must be considered that the presence of more food elicited more activity. It should also be noted that behavior data was only collected while bears were on the high fat diet – the proportion of metabolizable energy from fat still varies, but no behavior observations were conducted while bears were on a diet where protein exceeded fat. Therefore, it is possible that the observed relationships with dietary fat would change if samples from a lower-fat, higher-protein diet were included.

All variables related to weather were obtained from Environment and Climate Change Canada at the start of the observation period. Given that observation periods lasted up to 1 hour, it is possible that the weather changed throughout the sample period, meaning that a behavior recorded at the end of an observation period may have been associated with inaccurate weather variables. This is true not only of air temperature, but also humidity, which was recorded and considered but found not to be included in the best model. Furthermore, it is possible that exhibits may vary in temperature, or that specific areas within each exhibit vary in temperature due to differences in sun and wind exposure.

4.5 CONCLUSIONS

My data supported the hypotheses that body mass and body fat mass increase with both calories and dietary fat. Additionally, I found that both body mass and body fat mass are affected by an interaction between age and sex. Adult male bears were heaviest, but adult female bears were fattest. Proportional body fat was found to increase with calories, dietary fat, and age in female bears only, and decrease in males, which was not expected. These observed sex-based differences in fat storage warrant further investigation, as only 5 bears were sampled in this study.

These sex-based differences were observed again in relation to blood chemistry. The hypotheses that serum triglyceride concentration has a negative relationship with calories and positive one with dietary fat were supported, but the prediction of a positive relationship with dietary fat was refuted in males. Therefore, the prediction that sex would not affect serum triglyceride concentration was refuted, as was the prediction that age would have no effect. The model for serum UC ratio contained fewer predictors, supporting the hypotheses that serum UC ratio increases with calories but decreases with fat. Body fat, age, and sex were not included in the model, therefore the predictions that they would have no effect were supported.

The hypothesis that body temperature increases with calories was supported, while the hypothesis that body temperature increases with dietary fat was refuted. While three bears had positive relationships between dietary fat and body temperature, the remaining two bears had negative relationships, and the overall statistical effect was negative, small, and non-significant, therefor the hypothesis was refuted. It was supported that age, sex, and proportional body fat have no effect on body temperature. While no significant effect of air temperature was observed, the predictor was included in the model with a non-significant positive trend worth noting.

The only significant predictor for inactivity was calories, supporting the hypothesis that inactivity decreases with increasing calories. The hypothesis that inactivity increases with dietary fat was refuted, as were predictions regarding an effect of air temperature, age, humidity, and exhibit. Although the effect of time of day was non-significant, and the prediction that inactivity would be greater in the afternoon refuted, the trend of increased activity in the mornings is worth noting, as it relates the bears' husbandry schedules.

5 APPLICATIONS AND IMPLICATIONS

5.1 Key findings

My findings in regards to the effects of feeding a seasonal diet in captivity largely support the existing literature on wild polar bears. Captive polar bears appear to be able to undergo fluctuations in body mass, body fat, blood metabolites, body temperature, and activity following the same trends as wild bears, though to a lesser extent given their reduced energetic requirements. Therefore implementing a seasonal diet regime with captive polar bears, although not required by the AZA, allows bears to engage in natural, species-specific processes. The effects of dietary macronutrients were most notable in regard to body mass and body fat. With increasing dietary fat intake, body mass and body fat mass increased for all bears excluding Humphrey, thereby contributing to enhanced fitness as polar bears benefit from having large bodies with an abundance of fat stores. Although that fitness is not utilized in captivity to the extent it is in the wild, amassing fat stores and thus increasing overall body size remains a natural, species-specific process for polar bears, which should be encouraged in captivity to promote increased welfare. Additionally, a high-fat diet also benefits bears by containing less protein, and thus reducing their risk of developing liver and kidney disease (Rode et al. 2022, Robbins et al. 2021). While further work is needed to determine which exact macronutrient ratios best suit captive polar bears, this study presents welfare-based evidence for feeding a seasonal diet whereby fat at least exceeds protein.

While dietary fat had large effects on body mass, body fat, and blood chemistry, it had little-to-no effect on body temperature and behavior. Although it has previously been suggested that increased dietary fat and an abundance of body fat limit heat loss (Younge 1967), our findings align with more recent suggestions that polar bear macronutrient consumption and subsequent amounts of body fat play little-to-no role in thermoregulation (Rchida et al. 2023, Bissonnette et al. 2023). Despite the scientific evidence to the contrary, many easily accessible articles on laymen websites such as the Public Broadcasting Service and the British Broadcasting Corporation’s Science Stands make claims of polar bears being kept warm by thick blubber (PBS 2020, BBC 2023). This circulation of misinformation leads zoo-goers to develop concerns as to the welfare of zoo bears housed in temperate environments, often becoming upset and angry as they worry zoo bears are suffering thermal stress. My findings indicate that thermal stress is not a primary concern for zoo bears housed in temperate environments (at least under the climate conditions experienced in the Toronto area), even when they are fed a high-fat diet that leads them to build ample fat stores. Given that the purpose of non-specialized adipose tissue of polar bears is to store energy in the form of fat, feeding a high-fat diet facilitates this natural fat-building process without putting bears at risk of thermal stress.

Zoo guests often express concern that captive polar bears, especially when fat, are inactive due to “laziness” or “sadness”. While accurately quantifying the emotions and thoughts of polar bears is not possible, I found dietary fat – and subsequently, body fat – to have no effect on activity. Polar bears are naturally rather inactive animals due to their large bodies and high energetic demands (Gleason & Rode 2009, Kelly et al. 2015), and feeding a high-fat, low-protein diet does not appear to exacerbate this. Additionally, the implementation of a high-fat diet also did not appear to dissuade bears from engaging in typical captive behaviors associated with positive welfare such as moving around their exhibit, swimming, engaging with guests, and engaging with enrichment items. Thus, it appears that feeding a high-fat diet does not negatively impact the behavioral welfare of captive polar bears.

However, the possibility of over-feeding fat must be considered before implementing a high-fat diet for captive bears. While a high-fat diet facilitates natural behaviours and physiological processes to promote positive welfare, the welfare detriments of over-feeding fat for captive polar bears are largely unknown – gaining such understanding would require further studies that directly quantify health. It is known that feeding a diet whereby protein exceeds fat is associated with early death by liver and kidney disease (Robbins et al. 2021), but little is known about the effects of feeding a diet whereby fat exceeds protein by too wide a margin. Although having more fat stores than necessary may induce unexpected behavioral trends, we cannot necessarily say that this diminishes welfare, nor can we say that it promotes positive welfare.

While a high-fat diet certainly seems to be a more natural, welfare-based choice for captive polar bears than a high-protein diet, it remains unclear the extent to which fat should exceed protein. It is possible future studies may find sufficient evidence to adjust the exact macronutrient ratios fed in this study, so that fat exceeds protein by a different margin.

5.2 Future Studies

Any future studies pertaining to captive polar bear nutrition should carefully consider diet administration and recording procedures. In this study, a portion of the diet was fed indoors and isolated, to carefully observe and record consumption, but the remainder of the diet was fed on-exhibit, often to multiple bears at once. While such a method of diet administration provides enrichment by allowing bears to forage for a portion of their diet, it also introduces ample room for error. Future studies may consider feeding full diets in isolation whenever possible. However, such a decision would also require increased behavioural monitoring, to ensure welfare is not being negatively impacted by a lack of on-exhibit diet offerings.

Future studies pertaining to captive bear behavior should also record more exhibit-specific variables, such as enrichment items offered and date of last enrichment change. Following behavior, the lowest R^2 value I found was associated with body temperature. As it has been suggested that fur plays a large role in thermoregulation, future studies should address the role of fur density on body temperature. One possible approach would be to use photographic and thermal imaging to assess how much of the bear's body surface area has blown coat. As zoo bears are trained to lie down to display their dorsal side and stand bipedally to display their ventral side for veterinary checks, it would be possible to have the bears assume these helpful and consistent body positions for fur density assessment. Another method of assessing fur density would require the development of a visual key, such as the existing bear body condition index, which uses a 1-5 scale with accompanying scientific illustrations. As fluctuation in coat density is less severe than fluctuation in body condition, a 3-level key may be more appropriate.

In general, future captive polar bear nutrition studies should aim for a larger sample size than the 5 bears included in this study. Additionally, studies should aim to sample non-related bears with different combinations of age and sex. This is exceedingly difficult to accomplish in captivity, as most zoological facilities will have no more than 5 bears in their care at once, and those bears are often related. Additionally, animal care guidelines make it difficult to transport bears across provincial borders, and creating a research facility to house a large number of bears would require an abundance of space, time, and financial resources. However, there is great potential for zoos across the world to collaborate and contribute to a shared database. Regular diet-tracking, behavior observations, weigh-ins, and blood draws are already commonplace at most accredited zoos, and temperature tracking can easily be implemented using iButtons. Collaboration and data sharing would allow for increased sample size, comparisons of bears of different ages, further investigation of sex-based trends, comparison of unrelated individuals, comparison of different diets, and a wider range of environmental factors represented. Despite its benefits, this approach would also introduce sampler error, and would require the sharing of and stringent adherence to methodological protocols. Accredited zoos often focus on using animal science to connect people with animals, but there is also great potential for zoos to use animal science to connect with one another.

5.3 Climate Change and welfare

Although polar bears have evolved to withstand a prolonged energy deficit period each year, the length of that deficit period is increasing as a result of climate change and is projected to continue increasing going forward (Stroeve et al. 2007, Molnar et al. 2010, Holland et al.

2006). Due to warmer air temperatures, sea-ice breakup is occurring earlier in the year, and sea-ice freeze-up later (Markus et al. 2009, Crawford et al. 2021, Pagano & Williams 2021), meaning polar bears are going longer without access to their primary food sources. An increased ice-free period also leads bears to swim or walk longer distances in search of food or denning sites, meaning more energy stores are being expended (Mauritzen et al. 2003). The effects of the lengthened ice-free period bear negative consequences for wild polar bear populations, especially given that the relationship between length of time without ice and mortality is non-linear. Statistical models have predicted that the benchmark for adult survivorship lies at 180 days without ice, after which point the rate of mortality increases drastically (Molnar et al. 2010). However, even prior to this benchmark, smaller-bodied individuals with insufficient fat stores risk starvation (Molnar et al. 2010). The lengthened ice-free period also has negative consequences for reproduction, as females entering the breeding season with insufficient fat stores are less likely to have a successful pregnancy, and those who do successfully gestate with reduced fat stores rear fewer cubs, who are also less likely to survive (Atkinson and Ramsay 1995, Rode et al. 2020). Numbers are already declining in some sub-populations such as the Western Hudson Bay and Southern Beaufort Sea (Lunn et al. 2016), and declines are projected for others such as the Chukchi Sea population which is currently stable but projected to be in decline by 2040 (Molnar 2020).

While the small sample size of this study and reduced energetic requirements of captive bears limit the potential for extrapolation of results to wild populations, this methodology has the potential to be used to better understand the physiological and behavioural processes underlying the polar bear’s ability to withstand prolonged energy deficit. Repeatedly sampling the same individuals provides a level of precision and accuracy extraordinarily difficult to achieve through sampling wild bears due to difficulty locating and relocating individuals, researcher safety concerns, and samples being affected by Arctic field conditions. Through the collaboration of accredited zoos, the creation of a shared captive polar bear database would create the potential for statistical analysis with greater power and greater possibility of being applied to wild populations. While the differences in environmental conditions experienced by wild and captive bears must always be considered, further studying the energy budgeting of captive polar bears in greater numbers can help us to better understand how wild bears are managing prolonged energy deficits now, as well as to predict how they will do so in the future.

As climate change continues to threaten the natural habitat of polar bears, the captive environment will become increasingly important in conserving this species. It is crucial that we learn how best to care for polar bears in captivity, including how we feed them. The risks associated with high-protein diets were only recently addressed (Rode et al. 2021, Robbins et al. 2021), and the most recent AZA polar bear care manual suggests a diet whereby metabolizable energy obtained from protein exceeds that obtained from fat (AZA 2015). While I present a framework and pilot study for assessing the effects of diet on physiology and behavior, further work is needed to determine the macronutrient ratios best suited to promoting positive welfare, especially as it pertains to bears of different ages and sexes. If the captive environment is to become fundamental to the conservation of polar bears in the future, it is crucial that accredited zoos gain more understanding as to what and how they should be feeding the bears under their care in order to support their needs and encourage natural, species-specific behaviors and physiological processes. While our ability to provide wild polar bears with positive welfare is limited by irreversible changes to the global climate, we are fully capable of using science to provide captive polar bears with the best quality of life possible. It is important that we continue to further our understanding of both wild bears’ ability to withstand energy deficits in the face of climate change, and captive bears’ dietary needs and subsequent wellbeing.

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APPENDIX

Table 1: Ethogram used for polar bear behavior observations at The Toronto Zoo.

Category	Behaviour	Ab.	Description
Active	Walking	W	Bear engaged in non-repetitive locomotion with a slow or medium-speed gait.
	Running	R	Bear engaged in non-repetitive locomotion with a fast gait.
	Swimming	SWIM	Bear is moving through the water. Includes movement of the legs, with none of the paws in contact with the base or sides of pool.
Inactive	Sitting	S	Bear is not engaged in locomotion, stagnant with the rear end in contact with the ground. The line of the spine is often rounded.
	Standing	ST	Bear is not engaged in locomotion, with the bottoms of all 4 paws in contact with the ground, length of body roughly parallel to but not touching ground.
	Laying (alert)	LA	Parallel to the ground, with all or most of the length of the body in contact with the ground. The bear may be laying dorsally, ventrally, or on its side. The eyes are open and the bear may move the head, limbs, or adjust its laying position.
	Laying (rest)	LR	Laying as described previously, instead with the eyes closed. Bear engages in little-to-no movement, and the body lacks rigidity.
	Inactive in Pool	IP	Bear is at least partially submerged in water, but does not appear to be swimming. May include sitting or standing in the pool.
	Standing on hind legs	StH	Standing on bipedally on the hind legs.
Social (Active)	With other Bears	ScB	Actively watching or engaging with other bears in the enclosure. May include sniffing, nuzzling, apparent play, sparing, or other apparently “amicable” interactions. The focal bear may initiate the interaction or respond to another bear. Not considered when

			the focal bear is unresponsive to the social behaviour of another bear.
	With Keeper	ScK	Actively watching or following a keeper.
	With Public	ScP	Actively watching or following zoo guests.
	Aggression	A	May include displays such as paw swiping, barring teeth, lunging, growling, or lurching toward other bears. (Renner & Lussier 2002)
	With researcher	ScR	Actively watching or following the researcher performing behaviour observations.
	Training	T	Actively participating in a training session.
Stereotypic	Head Swinging	HS	Repeatedly moving the head right-to-left (transverse). The bear alternates through movements of cervical flexion and cervical side-bending, often with an undulating quality. The bear may engage the upper body in this movement, and may “throw” a hind leg periodically. Often combined with pacing (ie: P+HS).
	Pacing	P	Cyclic, invariant walking of the same path for at least 3 repetitions (Fraser & Broom 1990, Ross 2006).
	Suckling	SU	Includes taking a portion of any object / environmental item into the mouth, closing the front of the lips, and performing a sucking motion. May include periodic lapping of the tongue. Bears may also suckle the air.
	Suckling on paw	SUC	Suckling behaviour performed on the bear’s own paw, or part of the paw.
	Pattern Swimming	PS	Cyclic, invariant swimming of the same path, for at least 3 repetitions.
	Repeated Digging	SD	Repeated, trance-like digging behaviour. Likely to be observed in Juno only.
Object/Environment (Active)	Play with Toy	PT	Any interaction with an enrichment item provided by keepers. May include mouthing, swatting, sniffing, or pushing the object (suckling excluded, to be recorded separately). May be combined

			with eating when food Is placed inside toys (ie: E+PT).
	Digging	D	Movement of at least 1 paw against the ground or an object, often repeated.
	Play in pool	PP	Apparently playful behaviour with at least 2 paws in the pool. With or without toy. May include pouncing or submerging the head in the water.
	Rubbing	Rb	Rolling or rubbing the body on the ground, in a laying position.
	Scratching	Sc	Scratching any part of the body on the walls or other parts of the enclosure. The bear is in a standing or sitting position.
	Eating	E	Bear consumes food items.
	Grazing	GZ	Bear consumes grass from their enclosure, not provided by keepers.
	Drinking	Dr	Bear consumes water.
Other	Grooming	GM	May include scratching or rubbing the paws over another body part, using the tongue to lick any part of the body. Often repeated.
	Out of View	OOV	The bear is out of view of the researcher.