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Contextual Factors Associated with Fecal Glucocorticoid Metabolites in Juvenile Polar Bears (*Ursus maritimus*) and a Cohabitating Juvenile Grizzly Bear (*Ursus arctos horribilis*) at the Detroit Zoo

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Abstract: Fecal glucocorticoid metabolites have been used to evaluate responses to stressors in captive adult polar (*Ursus maritimus*) and grizzly (*Ursus arctos horribilis*) bears. However, there is a lack of physiological information on juvenile bears in captivity that could help expand the current understanding of their development and welfare. To address these questions, we tracked fecal glucocorticoid metabolites (FGMs) and behavior for 15 months in two polar bear cubs born at the Detroit Zoo, one who was mother-reared (Astra) and one who was hand-reared (Laerke), and one rescued grizzly bear cub (Jeb) reared at the Zoo. To allow access to a social partner during key developmental stages, Laerke and Jeb were housed together for eight months. Daily opportunistic samples were analyzed for fecal cortisol metabolites using an enzyme immunoassay and compared against behavior, social proximity, and environmental data gathered from 15 min focal observations. Based on a combination of generalized linear mixed models and Wilcoxon and Kruskal–Wallis tests, we found no significant variation in mean FGMs between Astra and Laerke, but both had significantly different mean FGMs compared to Jeb. We found that Laerke had higher FGM concentrations when she spent more time engaged in all-occurrence social negative behaviors and lower FGMs when engaged in social positive behaviors. For Jeb, FGMs were lower when in social proximity and higher following separation from Laerke. These data provide novel insights into the physiological states of juvenile bears during key stages and contribute to the growing body of information on polar and grizzly bear development.

Keywords: fecal glucocorticoid metabolites; juvenile polar bear; juvenile grizzly bear; social conditions; zoo animal behavior

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1. Introduction

Fecal glucocorticoid metabolites (FGMs) are an established physiological measurement that can provide insight into individual animal welfare [1,2], although it is recognized that glucocorticoids in general do not show a straightforward relationship with physiological stress [3]. While FGMs can offer insight into the physiological experience of a particular animal, these measurements must be interpreted in relation to other contextual data, such as health measures, behavior, or environmental conditions. Animal welfare is defined by the Association of Zoos and Aquariums (AZA) as "an animal's collective physical, mental, and emotional states over a period of time, and is measured on a continuum from good to poor" [4]. Animal welfare data are often complicated by individual life

history, making cross-individual comparisons and population-level generalizations difficult. Habitat transitions and alterations, changes in available social partners, and involvement in breeding programs can all potentially affect an animal's FGMs [5–7]. There may even be significant variation in hormonal and behavioral stress responses between individual animals of the same species experiencing the same or similar social and environmental conditions [8], due to variations in genetics, temperament, or life history.

Cortisol enzyme immunoassays (EIAs) have been biologically validated for measuring FGMs in captive and wild adult polar (*Ursus maritimus*) and grizzly bears *(Ursus arctos horribilis*) and can be used to evaluate their responses to medium to long-term stressors [9–13]. While higher FGMs have been observed in concert with increased pacing and low interest in novel stimuli in adult polar bears, environmental variables, such as dry land availability, may also influence FGMs [14].

Despite the established methodology, the published literature exploring relationships between FGMs and other contextualizing variables in bears is relatively limited. Though age is often a variable of interest in welfare and captive husbandry, studies that include FGM measurements tend to focus on adult individuals [10,11,13–15]. While significant trends in relation to age have been recorded, such as differential responses to temperature based on age [16], the lack of published data detailing polar and grizzly bear physiological development leaves gaps in our current understanding of these animals [17]. As such, establishing physiological descriptions and trends for polar and grizzly bears during key developmental stages would be greatly beneficial to the existing body of work surrounding these ursids.

Here, we investigate FGM changes over time during development, as well as behavioral and environmental relationships with FGMs, in three juvenile bears. Our analysis explores physiological data in relation to differential rearing experiences of polar bear cubs, the use of steroid and anti-seizure medications on a polar bear cub, and the cohabitation of one polar and one grizzly bear cub. Given the challenges that can accompany captive polar and grizzly bear development and the scarcity of available information, the following physiological and behavioral data will help to progress the current understanding of bear development and could be a first step in establishing species-level trends in juvenile polar and grizzly bears.

2. Materials and Methods

2.1. Study Subjects and Location

The subjects of this study included two female polar bear cubs (Astra and Laerke) and one male grizzly bear cub (Jeb). The adult female, Suka, gave birth to Astra and Laerke at the Detroit Zoo in Royal Oak, MI on 17 November 2020. Laerke was removed from Suka's care a couple of days after birth due to medical necessity, while Astra remained with Suka to be mother-reared at the Detroit Zoo's Arctic Ring of Life (ARL) [18,19] (Figure 1). Laerke had four seizures of unknown origin on 16 March 2021, with an additional couple of seizures occurring on 17 and 18 March 2021. The management of this condition required the provision of medication under the direction and supervision of trained veterinary staff. Treatment included oral administration of the following medications: prednisone (targeting 1 mg per kg, gradually tapered off), phenobarbital (20–30 ug/mL circulating in serum), and keppra (250 mg) (Table 1). While Laerke did not have any seizures following the few she experienced in March 2021, she continued to be medicated out of an abundance of caution until February 2022. For full details regarding husbandry, veterinary care, and housing for all three cubs, please reference other publications [18,19].

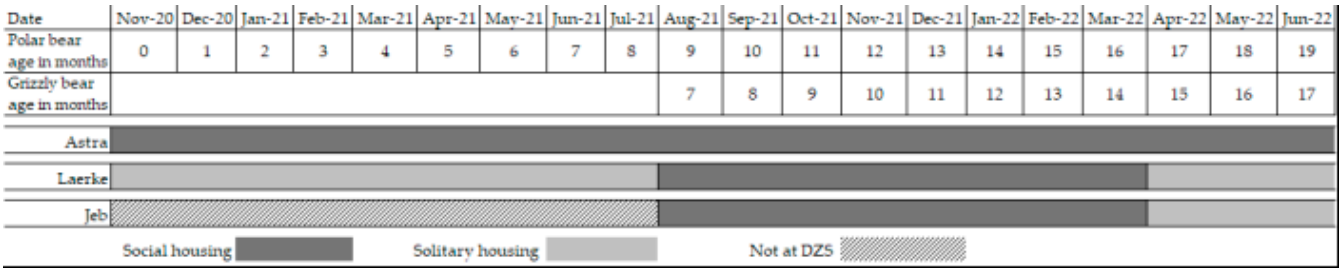

Figure 1. Social conditions for all three bear cubs.

Table 1. Laerke's medication and overlapping housing conditions.

Medication	Dates	Age	Housing
Prednisone, Phenobarbital, Keppra	16 March 2021–30 June 2021	17–32 weeks	Solitary housing
Phenobarbital	1 July 2021–29 July 2021 30 July 2021–31 August 2021	32–36 weeks 36–41 weeks	Solitary housing Socially housed
First Phenobarbital Decrease	1 September 2021–31 October 2021	41–49 weeks	Socially housed
Second Phenobarbital Decrease	1 November 2021–31 December 2021	49–58 weeks	Socially housed
Third Phenobarbital Decrease	1 January 2022–28 February 2022	58–66 weeks	Socially housed
No Medications	1 March 2022–18 March 2022 19 March 2022–3 July 2022	66–69 weeks 69–84 weeks	Socially housed Solitary housing

By the time that Laerke was medically stable, she could not be re-introduced to Suka and Astra for parent rearing. Laerke was housed alone but with visual, olfactory, and auditory contact with the other polar bears at the ARL until August 2021, when an orphaned Alaskan grizzly bear (Jeb, born 2020) was identified as a social partner. She was managed with free contact (no barrier between her and care staff) until 18 June 2021, when she was moved to protected contact (interactions between her and care staff separated by a barrier). Laerke and Jeb were housed together to provide both cubs with crucial social companionship and skill development during the formative juvenile period (Figure 1, Table 1). Laerke was fed a diet of primarily fish and some produce, and Jeb was fed beef along with a significant amount of produce. The two cubs were co-housed from August 2021–March 2022, when Jeb became too big to safely live with Laerke and was ultimately transitioned to a new home to live with other grizzly bears. After their separation, Laerke was again provided with visual, olfactory, and auditory contact with other polar bears.

This study took place between 12 March 2021 and 3 July 2022. During this time, Astra, Laerke, and Jeb had access to behind-the-scenes spaces at the ARL, as well as outdoor habitats beginning on 26 April 2021 for Astra and 20 April 2021 for Laerke. Given the fluctuating number of bears and social arrangements at the ARL, there were periods in which more social units of bears were housed than there were available outdoor habitat spaces. Initially, the resident adult male (Nuka) resided on the Pack Ice side of the habitat, while access to the Tundra side of the habitat rotated between Suka/Astra and Laerke. When Jeb arrived, Suka/Astra were transitioned to primary access to the Pack Ice side of the habitat, while Nuka rotated access to the Tundra side of the habitat with Laerke/Jeb. This rotation continued until February 2022 when Nuka was temporarily transferred to another AZA-accredited institution on a breeding recommendation. From April 2021–February 2022, Laerke's (and later Laerke and Jeb's) access to the outdoor habitat, and thus visibility for observations, was limited by whether the other occupying unit willingly shifted off-habitat. The frequency with which either Suka/Astra or later Nuka refused to shift off

the Tundra habitat resulted in a notable disparity in completed behavioral observations between Astra, Laerke, and Jeb (Table 2).

Table 2. Total fecal samples with behavioral observations collected for each individual.

Individual	Total Fecal Samples	Fecal Samples with Behavioral Observations
Astra	124	60
Laerke	432	123
Jeb	234	58

2.2. Fecal Sample Collection and Processing

Daily fecal samples were opportunistically collected from each bear by animal care staff throughout the entire study period, resulting in a total of 790 samples (Table 2). In many cases, Suka and Astra did not come indoors overnight, meaning that staff could not collect a fresh verifiable sample from Astra. This, and the later date of Jeb's transfer to the Detroit Zoo, resulted in a disparity in total collected fecal samples between individuals (Table 2). Animal care staff utilized the most recent morning fecal sample available for collection. A 7 cm diameter ball of fecal material was collected and placed into a 7.62 × 17.78 cm^2 sample bag and stored at −20 °C until analysis.

Fecal samples were lyophilized for approximately 48 h. Dry samples were pulverized and double-sifted into a fine powder, removing hair and other particulates. For hormone extraction, 0.2 ± 0.01 g of fecal powder was combined with 2 mL of 80% ethanol, briefly vortexed, shaken on a multi-tube mixer for one hour, and centrifuged for 20 min at 2500× *g* at 4 °C. 1.0 mL of the supernatant was pipetted into a clean glass tube and dried under forced air at 37 °C. Dried samples were stored frozen at −20 °C. Samples were reconstituted in 1.0 mL of assay buffer, sonicated in a water bath for 20 min, and centrifuged for 10 min at 2500× *g* at 4 °C immediately prior to analysis.

FGM concentrations were measured using a multi-species enzyme immunoassay kit for cortisol (ISWE Cortisol mini-kit, Arbor Assays, Ann Arbor, MI, USA), using plates coated in-house with a goat anti-rabbit IgG antibody. According to the manufacturer, the assay cross-reactants at 50% binding are 100% for cortisol, 42.08% dehydrocortisol, 26.53% cortisone, 4.10% dexamethasone, 3.37% prednisone, 0.35% corticosterone, 0.18% desoxycorticosterone, and <0.16% tetrahydrocorticosterone. A 1:50 dilution was used for the assay antibody and conjugate. The assay was analytically validated for both species via parallelism and recovery values for pooled samples spiked with cortisol standards ranging in concentration from 100 pg/mL to 800 pg/mL (Table 3). In addition, we assessed the linearity for each parallelism following previously established methods [20] by calculating an expected value by dividing the concentration of the first dilution step with the dilution factor for each subsequent dilution step (Table 3). We statistically analyzed assay parallelism and linearity using linear regression tests (Table 3). All samples were analyzed in duplicate at a 1:8 dilution for Astra and Jeb, and a 1:8 to 1:40 dilution for Laerke, corresponding to approximately 50% binding on the standard curve. Laerke's samples were run at shifting dilutions to account for changes in her medication dosage and to ensure a consistent 50% binding on the standard curve. The inter- and intra-assay coefficients of variation based on percent binding were both below 10%. Final hormone concentrations are adjusted for dry weight and expressed in ng/g dry feces.

Table 3. Cortisol assay recovery, parallelism, and linearity validation results for each species.

Polar bear	Average Recovery	110%
	Parallelism	$y = 1.031x - 3.184$, $R^2 = 0.997$ $F_{1,7} = 1793.702$, $p < 0.001$
	Linearity	$y = 53.369x + 0.995$, $R^2 = 0.995$, $F_{1,5} = 972.989$, $p < 0.001$
Grizzly bear	Average Recovery	108%
	Parallelism	$y = 1.115x - 9.369$, $R^2 = 0.999$, $F_{1,4} = 3489.787$, $p < 0.001$
	Linearity	$y = 81.466x + 0.994$, $R^2 = 0.995$, $F_{1,6} = 1298.461$, $p < 0.001$

We conducted biological validations by measuring FGMs around planned animal management changes that could be defined as stressful. Fecal samples after a social separation between Suka and Astra, which occurred after the current study period when Astra was approximately 26 months old, showed an approximate six-fold increase in FGMs for Astra (Figure 2). An increase in FGMs following the separation was also detected in Laerke (Figure 2). Jeb's FGMs after the separation from Laerke showed an approximately two-fold increase (Figure 3).

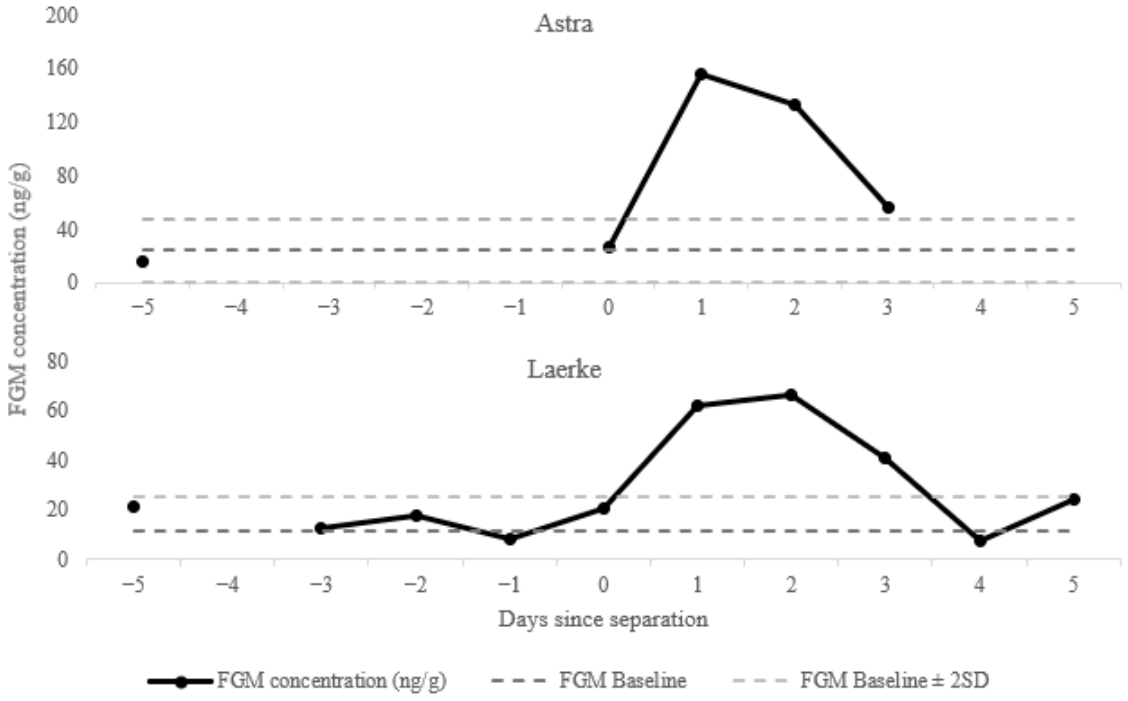

Figure 2. FGM concentrations in ng/g before and after a social separation in polar bears.

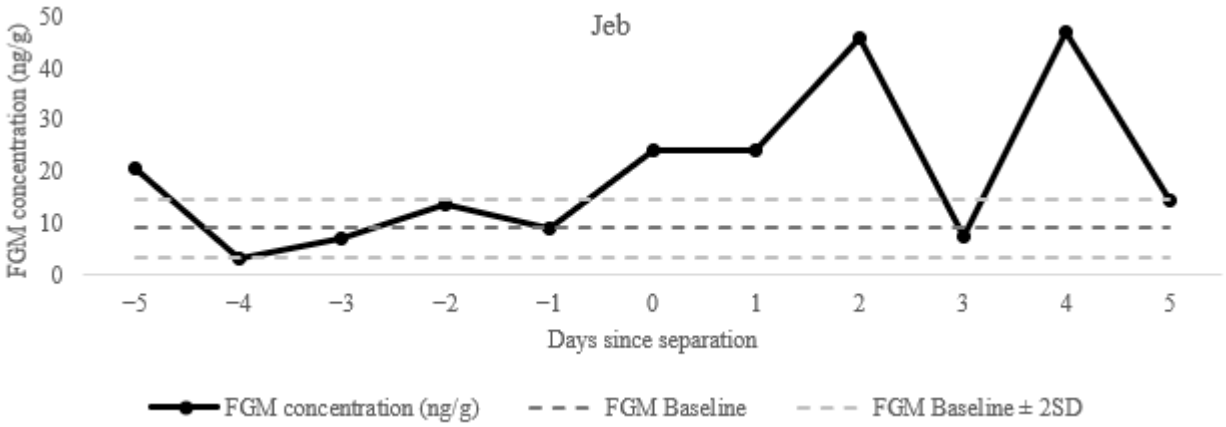

Figure 3. FGM concentrations in ng/g for Jeb before and after the separation from Laerke.

2.3. Behavioral Data Collection

Behavioral observations occurred when the cubs were in the outdoor habitat. We conducted 119 h of live behavioral observations via the ZooMonitor [21] program using 15 min focal observations with 1 min instantaneous scans to record behavior and social proximity (Table 4). We also recorded select all-occurrence behaviors. Observations occurred between 10:00 a.m. and 12:00 p.m., Monday through Friday. Observations could not be completed for a given individual if they did not have access to the outdoor habitat. Four observers collected data and maintained a minimum 90% inter-observer reliability rating. Given the length of the study period, observers were retested for inter-observer reliability every three months.

Table 4. Ethogram for use with polar and grizzly bear cubs [22,23].

Behavior	Modifier	Description
ALL-OCCURRENCE		
Swim		Cub interacts with pool when pool contains some amount of water. Swimming is locomotion within the water. Includes wading in water if water is not deep enough for full submersion. Record a new all-occurrence behavior every time the cub re-enters the water.
Social Positive		Cub gives or receives an affiliative interaction with another bear. These behaviors should include grooming, play behavior, or positive contact. Must be at least 5 s between instances to count multiple events.
Social Negative/Correction		Cub initiates or receives aggressive or ill-mannered behaviors towards another bear. This includes biting, swatting, or growling. Does NOT include play-wrestle-type biting. Must be at least 5 s between instances to count multiple events.
Abnormal		Cub engages in repetitive behaviors such as pacing and head-swinging.
SCAN SAMPLING		
Abnormal		Cub engages in repetitive behaviors such as pacing and head-swinging.
Nursing		Cub is actively drinking milk/formula either from Suka (Astra) or from a dish provided by keeper/veterinary staff (Laerke).
Eating solid food		Cub is chewing, licking, or otherwise ingesting a non-milk/formula food. This includes gruel and other cub-safe transitional foods provided by keepers/veterinary staff.
Drinking		Cub drinks water from bowl or other offered source. Does NOT include drinking of milk from dish or nursing from Suka.
Social Play		Cub is engaged in play behavior (such as chasing or wrestling) with another individual (keeper, Suka, Nuka).
Social Groom		Cub gives or receives grooming behaviors (licking or other pelt maintenance) from another individual.
Social Rest		Cub is inactive while in contact with another individual (keeper, Suka).
Social Other		Cub engages in a social behavior not otherwise covered by the other social categories.
Social Negative/Correction		Cub is directing aggressive or ill-mannered attention and behavior towards another individual (Suka or keeper/veterinary staff). This includes biting, swatting, and growling. Also score if Suka or keeper staff offers a correction (vocal, roll-away, etc.).
Scratch/Self-Groom		Cub itches themself either with a paw or by rubbing the body against a wall or other structure in the environment. Should also include self-directed grooming behavior like licking, picking, or scratching.
Object Interaction/Play		Cub investigates, plays with, or otherwise focuses attention on a human-made enrichment object such as a toy or boomer ball.
Investigation		Cub sniffs, paws at, plays with, examines, or otherwise engages with naturalistic aspects of their environment NOT including enrichment objects. This can include examination of substrates, natural structures, plant life, or other habitat features.

Table 4. *Cont.*

Behavior	Modifier	Description
Swim		Cub interacts with pool when pool contains some amount of water. Swimming is locomotion within the water. Includes wading in water if water is not deep enough for full submersion.
Solitary Play		Cub is amusing themselves through self-directed behaviors such as rolling around, pawing at the air, or playing with their own feet. Does not include any engagement without with an object/toy or participation from or interaction with Suka or keeper/veterinary staff.
Vocalization		Cub emits a whine, growl, chortle, or other recognizable sound.
Locomotion		Cub walks, runs, crawls, climbs or otherwise transports themselves from one location to another.
Excretion		Cub urinates or defecates.
Alert		Cub is stationary, but alert and awake. Cub may be taking a break from another activity, but still engaging with environment by looking around or sniffing.
Resting		Cub is resting or sleeping, very little body movement, eyes are likely closed.
Other		Cub is engaged in a behavior that does not fall under any of the previously outlined categories.
Not Visible		Cub is obscured by structures/items in habitat (or by conspecific) such that behavior cannot be reliably identified.
Not Visible—Presumed Swim		Cub has entered the pool but is not visible to the observer. Based on location, observer can infer contact or interaction with water.
Social Proximity	Contact	Cub is in contact with conspecific or keeper/staff.
	<1 m	Cub is less than 1 m from another bear or keeper/staff.
	<3 m	Cub is less than 3 m from another bear or keeper/staff.
	<5 m	Cub is less than 5 m from another bear or keeper/staff.
	>5 m	Cub is more than 5 m from another bear or keeper/staff.
	Unclear	Cub proximity to another individual cannot be determined due to obstruction.

The behavioral observations reported here were collected between April 2021 and June 2022 for Astra and Laerke. Jeb did not arrive at the Detroit Zoo until July 2021. Given the necessary period of quarantine and habituation post-arrival, as well as his social introduction to Laerke, data collection on Jeb did not begin until August 2021.

2.4. Data Analysis

Observations in which the focal individual was visible for less than half of the observation time were excluded from analyses to prevent bias. The previously mentioned housing challenges resulted in an unequal distribution of behavioral samples that could be matched to fecal samples between individuals (Table 2). Overall, observers collected 63 h of data for Astra (April 2021–June 2022), 36 h for Laerke (April 2021–June 2022), and 20 h for Jeb (August 2021–June 2022). Full behavioral analyses, including a consideration of fluctuating housing conditions and environmental variables, will be reported separately

FGM baselines were calculated in an iterative process in which values outside of the mean plus or minus two standard deviations were eliminated, the mean was recalculated, and the process repeated until no values fell outside of the range [10]. We calculated coefficients of variation (CV) and used them to compare variability between individuals and evaluate HPA activation [24].

All FGM data were included in profile comparisons between individuals and across Laerke's dosage conditions. For comparisons to behavior and environmental variables, we adjusted the dates for the FGM concentrations backwards by 24 h to account for

hormone processing and excretion based on other studies and our own previous tests of gut transit time using a fecal marker [9,10,25]. Using the adjusted dates, only FGM samples with matched live observation data were included in analyses against behavioral and environmental variables. Given the effects of synthetic glucocorticoids on FGMs, and the effects of epileptic discharges on hormone levels in humans, cats, and rats [26–29], data for Laerke were analyzed separated in two categories: a medication condition and a condition when there was no significant effect of medication on FGM levels, which includes the following categories: first phenobarbital decrease, second phenobarbital decrease, third phenobarbitall decrease, and no medication.

As the data were non-normally distributed, we elected to perform Kruskal–Wallis and Wilcoxon tests to establish significant variation between categorical variables. FGM values were log-transformed. All tests were performed with a Monte Carlo sampling method at 10,000 permutations for randomization to account for small sample size biasing [30,31]. Kruskal–Wallis tests with a significance of $p < 0.05$ were run with an added post hoc Dwass–Steel–Crichtlow–Fligner test.

Comparisons between FGM concentrations and behavioral frequencies were conducted using generalized linear mixed models (GLMMs). GLMMs may be applied to non-normally distributed datasets as well as case study datasets such as this one [31,32]. We ran four GLMMs for each individual: an environmental model, a proximity model, an all-occurrence behavior model, and an interval behavior model. Each model used the log-transformed FGM concentration as the outcome variable and was run with a Gaussian distribution, an identity link function, and the individual's age in weeks as the random intercept. Given the established influence of Laerke's medications on her FGM concentrations, the GLMMs only included data from when her medication dosage no longer had a significant influence on her FGM concentrations. The environmental model included temperature and crowd size as predictor variables. The proximity model included time spent in contact, within one meter, within five meters, and more than five meters from a social partner as predictor variables. The all-occurrence behavior model included hourly rate of swimming, social positive interactions, social negative interactions, and abnormal behaviors as predictor variables. The interval behavior model included time spent in nursing, eating, abnormal, social positive, social negative, self-grooming, object manipulation, investigation, swimming, solitary play, vocalization, locomotion, alert, resting, and other behaviors as predictor variables. All the proximity, all-occurrence, and interval predictor variables were adjusted for visibility. The final reported models have been trimmed for best fit. All statistical analyses were performed using SAS ©, 9.4.1 (Cary, NC, USA).

3. Results

As previously discussed, there is an established relationship between medications containing synthetic glucocorticoids and FGMs. As such, the table detailing results of the Wilcoxon and Dwass–Steel–Critchlow–Fligner tests comparing FGMs between individuals based on Laerke's medication dosages is provided as a supplement in Appendix A Table A1. Based on the Kruskal–Wallis results (Table 5), we can broadly separate Laerke's medication conditions into two larger categories: conditions a and b (hereafter referred to as Full Dosages) and conditions c-f (hereafter referred to as Decreasing Dosages). These two categories differed notably from each other, with Laerke displaying an average concentration of 68.49 ng/g $\pm$ 5.58 SE (average CV of 99%) in the combined Full Dosage conditions and an average concentration of 22.63 ng/g $\pm$ 1.09 SE (average CV of 79%) across the combined Decreasing Dosages conditions (Figure 4).

Table 5. Kruskal–Wallis results comparing FGMs between species, individuals, and medication conditions. The values for "Polar Bear" and "Laerke" include only data for Laerke after the first phenobarbital decrease when there was no effect of medication on her FGMs.

	Mean ± SE	Chi-Square	DF	Pr > ChiSq
Species				
Polar bear	22.76 ± 0.86	49.34	1	<0.0001
Grizzly bear	15.14 ± 0.90			
Individual				
Astra	22.71 ± 1.61	49.44	2	<0.0001
Laerke	22.63 ± 1.09			
Jeb	15.14 ± 0.90			
Medication conditions (Laerke only)				
a. All Medications (Prednisone, Phenobarbital, Keppra)	91.47 ± 8.47	225.3	7	<0.0001
b. Phenobarbital	38.62 ± 3.06			
c. First Phenobarbital Decrease	22.58 ± 1.87			
d. Second Phenobarbital Decrease	23.11 ± 2.33			
e. Third Phenobarbital Decrease	26.21 ± 3.02			
f. No Medications	21.01 ± 1.71			

We compared mean FGM parameters between the three cubs using the Decreasing Dosages condition of Laerke's samples (Table 5). Laerke and Astra had similar FGM baselines, mean levels, and variability despite being hand- and mother-reared, respectively. Jeb had significantly lower overall FGM measurements compared to the two polar bears (Table 5). Additionally, there was a high amount of variability in the FGM results for all three bear cubs, with CVs ranging from 78–99%.

Laerke's GLMMs indicated the random intercept (age in weeks) as the most significant predictor (as measured by *p*-value) of log-transformed FGM concentrations according to the environmental, all-occurrence behavior, and interval models (Table 6). In the environmental model, the intercept was the only significant predictor with log-transformed FGM concentrations increasing as Laerke aged, without an additional effect of temperature or crowd size. While the all-occurrence behavior model also suggests that Laerke's log-transformed FGM concentrations increased as her rate of social negative interactions increased and decreased as her rate of social positive interactions increased, the effect (as indicated by the estimate) is relatively negligible (Table 6). The interval model demonstrates that Laerke's time spent in abnormal behaviors had a greater effect on FGM concentrations, but at a lower significance value than age. Generally, Laerke's log-transformed FGM concentrations decreased as her time spent in abnormal behaviors and swimming increased (Table 6). Intriguingly, the proximity model was the only model in which the random intercept was not a significant predictor of log-transformed FGM concentrations. Rather, this model suggests that Laerke's concentrations increased as her time spent at distances of both greater than and less than five meters from Jeb increased. Her concentrations also trended towards increasing as her time spent in contact with Jeb increased. This is supported by the test of concentrations between housing conditions which demonstrated that Laerke's FGMs were significantly higher when she was housed with Jeb compared to when she was housed alone ($F_{1,272} = 4.63$, $p = 0.03$) (Figure 5). Across all models, we see a pattern in which Laerke's log-transformed FGM concentrations increased with age, some measures of social proximity, and hourly rates of social negative interactions, but decreased with hourly rate of social positive behaviors and time spent in abnormal and swimming behaviors (Table 6).

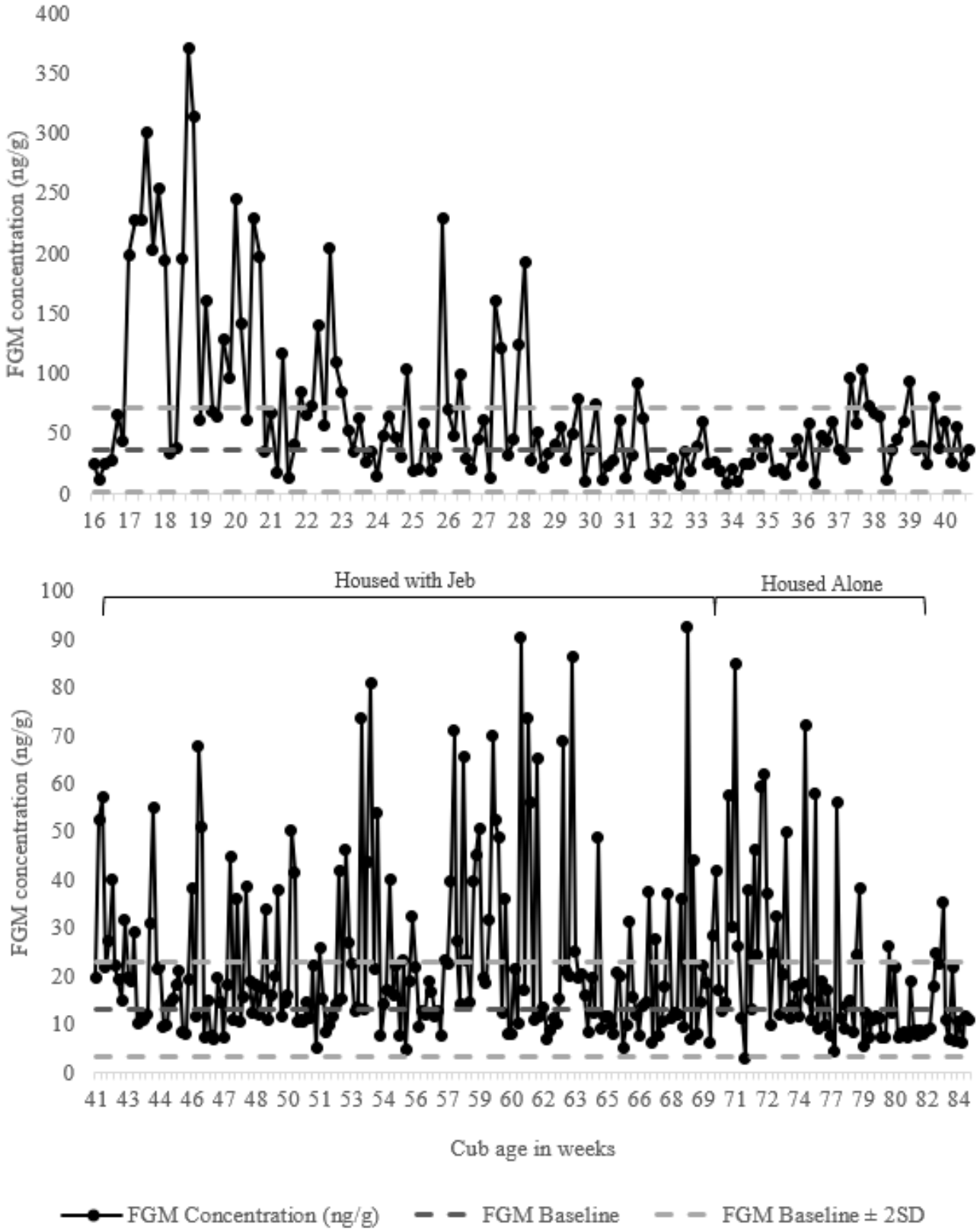

Figure 4. Longitudinal fecal glucocorticoid metabolite profile for Laerke. The top graph represents the combined Full Dosage conditions. The bottom graph represents the combined Decreasing Dosages conditions. FGM concentrations are expressed in ng/g of dry feces.

Table 6. Generalized linear mixed model (GLMM) results for the four models (environmental, proximity, all-occurrence behavior, and interval behavior) run for each study subject. Significant predictors in each model are indicated in bold. Trending predictors ($0.05 < p \leq 0.10$) in each model are indicated in italics.

Model	Predictor	Est.	SE	DF	t	Pr > |t|	Lower	Upper
Astra Results								
Environmental	**Intercept**	0.80	0.20	43	4.09	<0.001	0.41	1.19
	Temperature	0.01	0.00	14	2.48	0.03	0.00	0.01
	Crowd Size	−0.02	0.05	14	−0.40	0.69	−0.13	0.09
Proximity	**Intercept**	1.16	0.13	43	9.01	<0.0001	0.90	1.43
	Contact	0.10	0.18	13	0.58	0.57	−0.28	0.49
	Less than 1 m	0.12	0.19	13	0.65	0.53	−0.29	0.54
	Less than 5 m	0.15	0.23	13	0.64	0.53	−0.35	0.65
All-Occurrence Behavior	**Intercept**	1.26	0.07	43	17.97	<0.0001	1.12	1.40
	Swimming	0.00	0.00	13	0.34	0.74	−0.01	0.01
	Social Positive	−0.00	0.00	13	−0.46	0.65	−0.01	0.01
	Social Negative	0.01	0.02	13	0.39	0.71	−0.03	0.04
Interval Behavior	**Intercept**	0.82	0.15	43	5.42	<0.0001	0.52	1.12
	Eating	0.89	0.46	10	1.96	0.08	−0.12	1.91
	Social Positive	0.53	0.21	10	2.51	0.03	0.06	1.00
	Object Manipulation	0.61	0.30	10	2.04	0.07	−0.05	1.27
	Investigation	0.68	0.26	10	2.63	0.03	0.10	1.26
	Swimming	0.38	0.19	10	1.97	0.08	−0.05	0.82
	Locomotion	0.74	0.29	10	2.59	0.03	0.10	1.37
Laerke Results								
Environmental	**Intercept**	1.41	0.11	31	12.88	<0.0001	1.19	1.63
	Temperature	−0.00	0.00	35	−1.46	0.15	−0.01	0.00
	Crowd Size	−0.03	0.06	35	−0.56	0.58	−0.15	0.08
Proximity	Intercept	0.43	0.27	31	1.60	0.12	−0.12	0.99
	Contact	0.91	0.47	34	1.93	0.06	−0.05	1.87
	Less than 5 m	0.80	0.37	34	2.16	0.04	0.05	1.56
	More than 5 m	0.86	0.28	34	3.09	<0.01	0.29	1.42
All-Occurrence Behavior	**Intercept**	1.27	0.05	31	27.57	<0.0001	1.17	1.36
	Social Positive	−0.00	0.00	35	−2.54	0.02	−0.01	−0.00
	Social Negative	0.04	0.02	35	2.46	0.02	0.01	0.07
Interval Behavior	**Intercept**	1.61	0.13	31	12.81	<0.0001	1.35	1.87
	Abnormal	−2.03	0.90	33	−2.25	0.03	−3.87	−0.19
	Investigation	−0.63	0.40	33	−1.57	0.13	−1.45	0.19
	Swimming	−0.58	0.15	33	−3.73	<0.001	−0.89	−0.26
	Alert	−0.55	0.35	33	−1.58	0.12	−1.26	0.16
Jeb Results								
Environmental	**Intercept**	1.15	0.11	45	10.05	<0.0001	0.92	1.38
	Temperature	0.00	0.00	10	0.14	0.89	−0.00	0.01
	Crowd Size	0.00	0.05	10	0.00	1.00	−0.10	0.10
Proximity	**Intercept**	1.22	0.04	45	31.97	<0.0001	1.14	1.30
	Less than 1 m	−0.98	0.32	11	−3.04	0.01	−1.68	−0.27
All-Occurrence Behavior	**Intercept**	1.23	0.04	45	27.42	<0.0001	1.14	1.32
	Swimming	−0.01	0.00	11	−2.58	0.03	−0.02	−0.00
Interval Behavior	**Intercept**	1.15	0.04	44	31.88	<0.0001	1.08	1.22
	Vocalizations	4.75	2.52	12	1.89	0.08	−0.74	10.25

The mean FGM concentration for Jeb was 15.14 ng/g ± 0.86 SE (Figure 6) with a CV of 86%. According to the GLMMs, the random intercept (age in weeks) was the best predictor of Jeb's log-transformed FGM concentrations according both to significance (*p*-value) and

effect size (as measured by the estimate) (Table 6). Jeb's FGM concentrations demonstrated no relationship with predictors in the environmental model (temperature and crowd size) and only a trending relationship with vocalizations in the interval behavior model such that his long-transformed FGM concentrations trended towards increasing as his time spent in vocalizations increased (Table 6). We saw significance in the all-occurrence model such that FGM concentrations decreased as the hourly rate of swimming increased. However, the estimate for this is almost negligible. The proximity model demonstrated that Jeb's FGM concentrations decreased as his time spent within one meter of Laerke increased. This supports results comparing Jeb's housing conditions, as following the separation from Laerke Jeb's FGMs were significantly higher ($F_{1,229} = 10.72$, $p = 0.001$) (Figure 5).

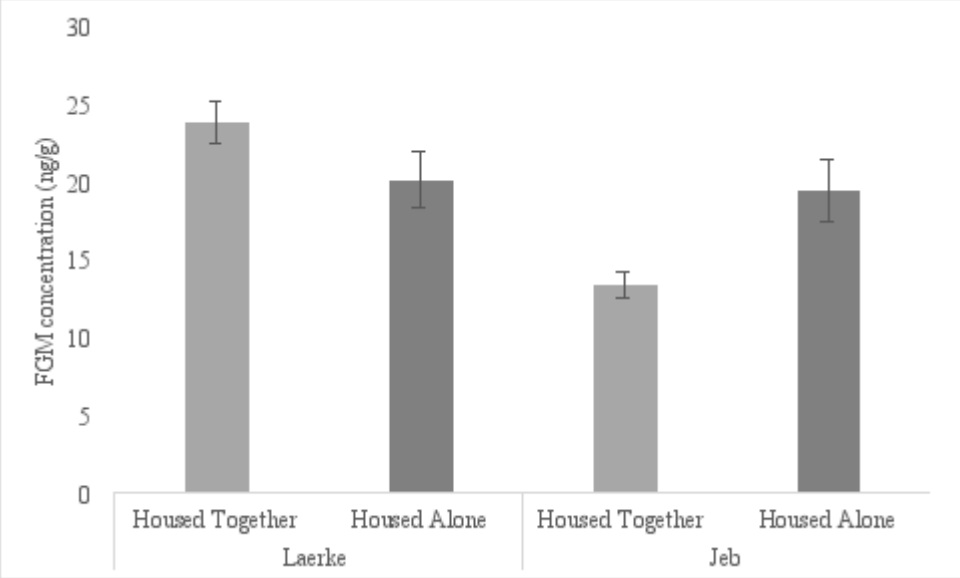

Figure 5. FGM concentration (Mean ± SE) for Laerke and Jeb between housing conditions.

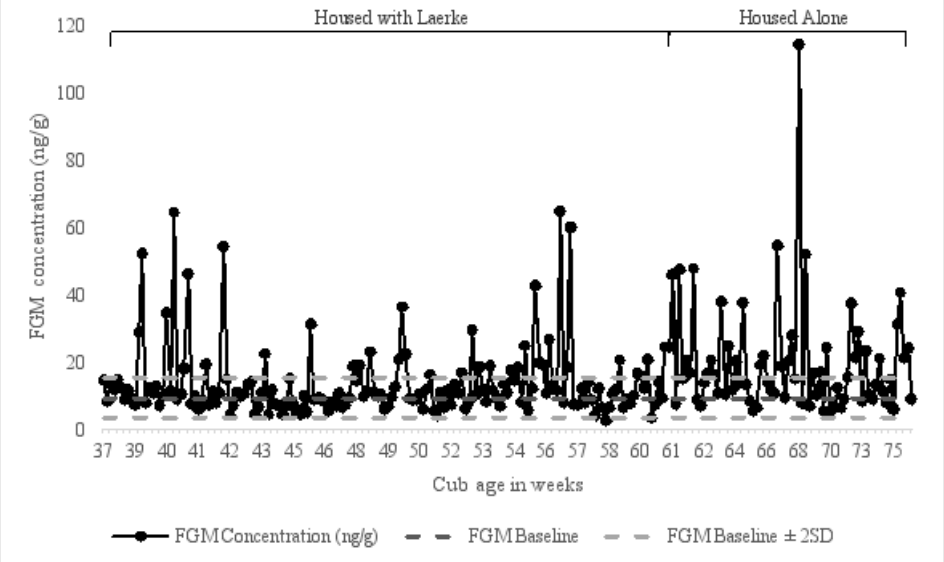

Figure 6. Longitudinal fecal glucocorticoid metabolite profile for Jeb. FGM concentrations are expressed in ng/g of dry feces.

The mean FGM concentration for Astra was 22.71 ng/g ± 1.61 SE (Figure 7) with a CV of 79%. The strongest predictor for log-transformed FGM concentrations for Astra in each model was the random intercept (age in weeks) such that Astra's concentrations increased as she aged (Table 6). While the proximity and all-occurrence behavior models

demonstrated no other trending or significant predictor variables outside of the intercept, we did see other significant predictors in the environmental and interval behavior models. According to the environmental model, Astra's log-transformed FGM concentrations increased as the temperature increased, but the effect size (as measured by estimates) was almost negligible (Table 6). We saw more significant/trending results, along with more meaningful effect sizes, in the interval behavior predictors. Specifically, the model indicated that Astra's log-transformed FGM concentrations increased as her time spent in social positive, investigation, and locomotory behaviors increased. Her concentrations also trended towards increasing as her time spent in eating, object manipulation, and swimming behaviors increased (Table 6).

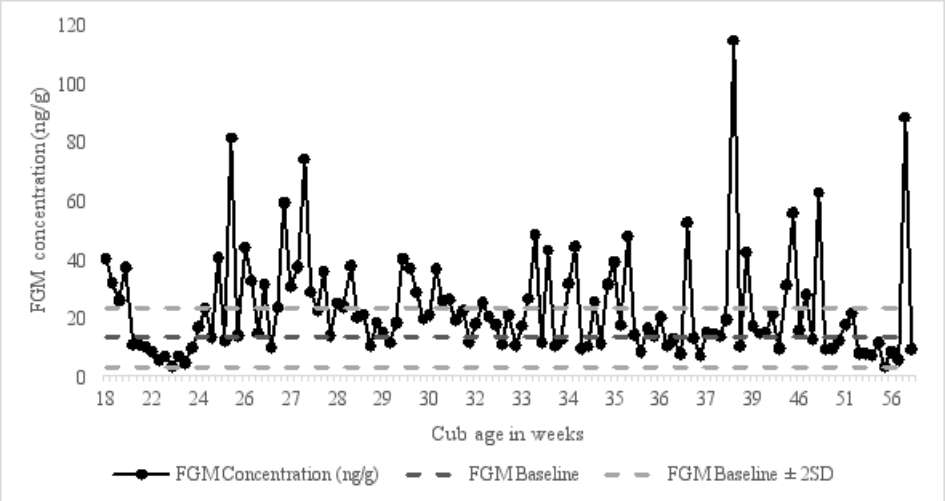

Figure 7. Longitudinal fecal glucocorticoid metabolite profile for Astra. FGM concentrations are expressed in ng/g of dry feces.

4. Discussion

Although the circumstances surrounding the individual subjects of this study were rather unique, there are many valuable insights that can be drawn from the available combined physiological, behavioral, and environmental data. The similarity in the FGM baselines between Astra and Laerke, after accounting for the effects of the latter's medication, suggests that the two polar bear cubs likely experienced similar levels of adrenocortical activity regardless of their distinctive rearing experiences, potentially due to canalization [33,34]. Although this is certainly a significant finding, this inference should be interpreted with caution due to the small sample size (both the number of bears and the dearth of fecal samples from Astra in the latter months of the study), as well as several external factors such as Laerke's shifting social conditions. We expected an increased variability in Laerke's FGMs while receiving prednisone, given the potential for cross-reaction within the EIA and the established endocrine side effects of synthetic glucocorticoids in humans [29]. However, the influence of lower doses of phenobarbital and any epileptic episodes on FGM variability, or whether they had any detectable impact at all, is unknown.

The high amount of FGM variability was consistent across species and individuals. Jeb showed higher CVs than the three adult male grizzly bears in our previous long-term study [10]. The variability in glucocorticoids for Astra and Laerke follow a demonstrated trend of increased variability in the FGMs of juvenile polar bears as compared to adults [16]. The variability of FGMs can provide information on how frequently the HPA axis is activated [35,36]. Multiple studies have found higher FGMs in juveniles compared to adults in different species, evidence of a difference in adrenal activity, perhaps due to a higher sensitivity to external stressors [37,38]. Juvenile polar and grizzly bears may perhaps

have higher HPA activation, due to increased activity compared to adults, or different physiological responses to external stressors and conditions. This is consistent with our GLMM results, which demonstrated a significant relationship between log-transformed FGM concentrations and individual age in weeks (as our random effect) across multiple models in all three bears. Given that this study spanned a formative developmental period, we expect that the increased activity and independence of the bears influenced this result. Continued long-term monitoring into adulthood may see this effect lessen, as has been observed in the previously mentioned studies [37,38].

Broadly, FGMs have been demonstrated to be influenced by variables such as sex, species, and diet [35,39,40]. Our sample demographics restrict us from drawing any conclusions regarding potential sex-based differences in FGM trends. Instead, we can cautiously make some inferences about species differences, recognizing that individual differences could also underlie any apparent trends detected in our small sample size. While significantly different than the two polar bear cubs, Jeb's FGM baselines were similar to those of three other adult male grizzly bears housed at the Detroit Zoo, individuals who were also found orphaned in the wild, when measured using the same EIA [10], supporting a potential species-specific trend. Though no literature directly comparing the two bear species was found, previous work on FGMs in wild grizzly bears found significant differences in FGMs based on season and dietary differences, but not sex or age [13]. In other mammals, increased dietary fiber was associated with greater fecal cortisol metabolite concentrations [41]. However, in the aforementioned von der Ohe et al. [13] study, this relationship was not so clear, as wild grizzlies that consumed relatively high-fiber diets had the lowest FGMs measured when the greatest number of samples were analyzed. While the relationship between dietary fiber and FGMs in ursids is unclear, the dissimilar diets of these two species, and how their diets changed over time, may nevertheless be influential factors in their respective overall FGM measurements. Therefore, we cautiously suspect that this finding reflects a difference between the two species of bear, resulting either from innate biological differences, their divergent diets, or both.

Shepherdson, Carlstead, and Wielebnowski [24] evaluated variability in polar bear FGMs, finding that bears with higher FGM CVs demonstrated less stereotypic behavior, suggesting that healthy adrenal reactivity could be associated with a more positive behavioral profile. Our results for Laerke partially support this observation, as she did demonstrate decreased FGMs with higher rates of social positive behaviors. However, we also observed decreased FGMs with increased time spent in abnormal or stereotypic behaviors. While this may appear to be in direct conflict with results from Shepherdson and colleagues [24], Laerke's abnormal behavior was primarily a suckling behavior rather than other typical stereotypies in bears such as pacing or head-swinging. We suspect this behavior served a self-soothing purpose, resulting in the observed decreases in FGM concentrations. Laerke also demonstrated a negative relationship between FGMs and social positive behaviors, suggesting that positive social interactions may have mitigated adverse responses to other stressors, a trend observed in adult grizzly bears housed at the Detroit Zoo [10].

Broom and Johnson [42] observed positive correlations between FGMs and active behaviors such as swimming and locomotion. Astra demonstrated the same relationship between FGMs and locomotion, as well as a trending relationship with swimming. However, our results for Laerke and Jeb showed that their FGMs decreased when they engaged in swimming behaviors. This may be due to limitations in our behavioral dataset or obstruction of results by the shifting medical and social conditions experienced by Laerke and Jeb. In addition, a previous study found a positive correlation between FGMs and increasing temperatures in adult polar bears but not in juveniles [16]. They hypothesized that juveniles have additional physiological challenges compared to adults that impact adrenal

cortisol production. We found similar results in that there was no relationship between FGMs and temperature for Laerke and Jeb, and Astra's positive relationship between FGMs and temperature had a relatively negligible effect size. In addition to small sample sizes, there was limited variation in FGMs for Astra, and shifting conditions for Laerke could have masked temperature results, making direct comparisons between studies difficult. However, continuing to explore potential differential responses to temperatures between adult and juvenile polar bears could be useful.

Laerke and Jeb showed different physiological reactions to their social separation, in that Jeb demonstrated significantly higher FGMs following the separation, while Laerke's FGMs were significantly lower. This is supported by results from both bears' proximity models. Jeb demonstrated decreased FGM concentrations with increased time spent within one meter of Laerke. The proximity results for Laerke appear to be more conflicting, in that she had higher FGM concentrations while both less than five meters and greater than five meters from Jeb. However, this could be a result of how their relationship changed over time. When they were introduced, it appeared to be beneficial for her to have a social companion. After several months, Jeb grew larger than Laerke, resulting in more social negative interactions and thus potentially higher FGMs while they were closer together. For both grizzly and polar bears, dispersal typically occurs around two and half years of age, demonstrating that bear cubs of both species would not typically be solitary at under two years of age [43,44]. While Jeb and Laerke showed opposite FGM trends between social conditions, it is difficult to attribute their responses solely to the separation, as there could also be influences of seasonality or activity level. In addition, Laerke was given more opportunities to interact with conspecifics than Jeb. Although these were inconsistent and opportunistic at this stage of Laerke's development, perhaps she experienced less social isolation than Jeb. Social isolation has been found to impact FGMs [45]. Maybe the different responses to their separation were also influenced by the ability to have even limited social interaction. Due to the small sample size and number of confounding variables in the dataset (age, temperature, crowd size), we acknowledge these findings are preliminary and warrant further investigation. Still, the physiological results of Jeb and Laerke for social proximity and behavior may suggest that access to a social partner was beneficial to their development, regardless of species.

Overall, the information presented here contributes to the growing body of knowledge on polar and grizzly bear development. In particular, the FGM measurements, in conjunction with other contextual data, provide novel insights into the physiological state of these ursids during key developmental stages. We found physiological evidence of the value of social companionship in early development for both polar and grizzly bears. Despite the difference in rearing, Astra and Laerke (once she was weaned off medication) demonstrated similar FGM baselines and ranges, which could be the results of a species-typical FGM range or could be due to canalization. We also found evidence that certain behaviors, such as social positive interactions, demonstrated by polar bears could be coping behaviors for stressors. With such a scarcity of available data, these findings may be greatly beneficial in aiding animal care professionals with challenging management decisions, while simultaneously helping to inform future research efforts.

5. Conclusions

1. Despite differences in rearing, both polar bear cubs showed similar baseline levels and patterns of variability in FGMs when there was no effect of medication. FGMs also differed between the bear species.
2. There is evidence that a change in housing and access to a social partner had physiological effects for cubs of both species.

3. Fecal glucocorticoid metabolites were related to some behaviors, although there were individual differences.

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Appendix A

Table A1. Results of Wilcoxon and Dwass–Steel–Critchlow–Fligner tests comparing FGMs between individuals and Laerke's medication conditions. Significant results are in bold. Phenobarbital is abbreviated to Pheno.

Individual/Medication Condition		Wilcoxon Z	DSCF Value	*p* Value
Laerke No Medications	Astra	−1.4760	2.0874	0.8205
	Jeb	**3.4445**	**4.8713**	**0.0133**
	All Medications	**−9.2357**	**13.0613**	**<0.0001**
	Phenobarbital	**−5.5865**	**7.9005**	**<0.0001**
	First Pheno decrease	−1.8448	2.6089	0.5892
	Second Pheno decrease	−1.5478	2.1890	0.7813
	Third Pheno decrease	−1.4958	2.1154	0.8101
Laerke All Medications (Prednisone, Phenobarbital, Keppra)	**Astra**	**9.1354**	**12.9194**	**<0.0001**
	Jeb	**12.1194**	**17.1394**	**<0.0001**
	Phenobarbital	**4.4431**	**6.2835**	**0.0002**
	First Pheno decrease	**7.5417**	**10.6656**	**<0.0001**
	Second Pheno decrease	**7.4198**	**10.4932**	**<0.0001**
	Third Pheno decrease	**6.6850**	**9.4541**	**<0.0001**
Phenobarbital	**Astra**	**5.0275**	**7.1099**	**<0.0001**
	Jeb	**8.3203**	**11.7666**	**<0.0001**
	First Pheno decrease	**4.3701**	**6.1803**	**0.0003**
	Second Pheno decrease	**4.3134**	**6.1000**	**0.0004**
	Third Pheno decrease	**3.5277**	**4.9889**	**0.0100**
First Phenobarbital Decrease	Astra	0.5937	0.8396	0.9990
	Jeb	4.9653	7.0219	1.0000
	Second Pheno decrease	0.2562	0.3623	1.0000
	Third Pheno decrease	**0.0402**	**0.0569**	**<0.0001**
Second Phenobarbital Decrease	Astra	0.2371	0.3353	1.0000
	Jeb	**4.5482**	**6.4321**	**0.0001**
	Third Pheno decrease	0.0116	0.0165	1.0000
Third Phenobarbital Decrease	Astra	0.3632	0.5136	1.0000
	Jeb	**4.2128**	**5.9577**	**0.0007**
Astra	**Jeb**	**5.3702**	**7.5946**	**<0.0001**

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